The Chemical Analysis of Fresh Water

THIRD EDITION

IISD Experimental Lakes Area

Sonya Havens
Blake Cooney
Michael Stainton

October 2024

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Head Office

111 Lombard Avenue, Suite 325
Winnipeg, Manitoba
Canada R3B 0T4

Tel: +1 (204) 958-7700
Website: iisd.org
𝕏: @IISD_news

IISD Experimental Lakes Area

IISD Experimental Lakes Area is the world's freshwater laboratory. A series of 58 lakes and their watersheds in northwestern Ontario, Canada, IISD-ELA is the only place in the world where scientists can research on and manipulate real lakes to build a more accurate and complete picture of what human activity is doing to freshwater lakes. The findings from over 50 years of ground-breaking research have rewritten environmental policy around the world—from mitigating algal blooms to reducing how much mercury gets into our waterways—and aim to keep fresh water clean around the world for generations to come.

IISD-ELA

111 Lombard Avenue, Suite 325
Winnipeg, Manitoba
Canada R3B 0T4

Tel: +1 (204) 958-7700
Website: iisd.org/ela
𝕏: @IISD_ELA

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Written by Sonya Havens, Blake Cooney, and Michael Stainton

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Abstract

This publication describes the protocols used in the IISD Experimental Lakes Area Analytical Service Laboratory for the measurement of chemical constituents in freshwater samples. The chemical constituents measured include anions (Cl and SO_4), cations (Na, Mg, K, Ca, Mn, and Fe), carbon speciation (dissolved inorganic carbon, dissolved organic carbon, absorbance scan to assess dissolved organic carbon quality, and particulate carbon), chlorophyll-a, conductivity, dissolved oxygen, gran alkalinity, nitrogen speciation (nitrite, nitrate, ammonia, total dissolved nitrogen, and particulate nitrogen), particulate iron, pH, phosphorus speciation (total dissolved phosphorus and particulate phosphorus), soluble reactive silicon, and turbidity. In addition to the analytical methods used, quality assurance and quality control (QA/QC) protocols used for labware cleaning, calibration, and verifications, sample tracking and data recording, and sample preparation, filtration, and storage are also provided.

Table of Contents

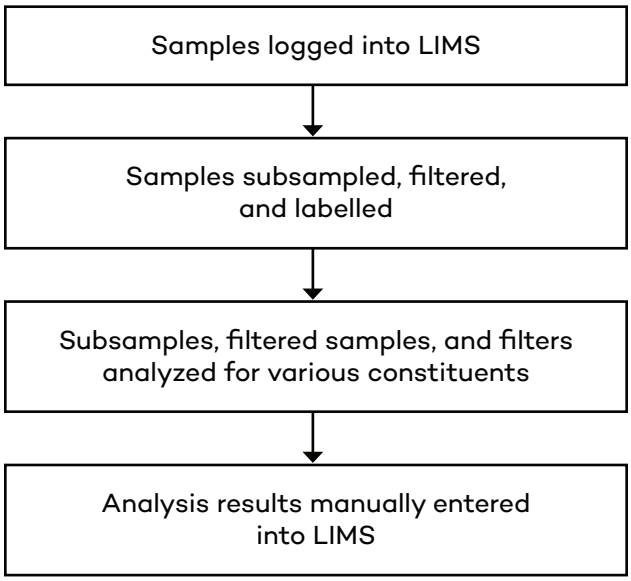
1.0 Introduction

The International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA) Analytical Services Laboratory (ASL) has conducted chemical analyses of various constituents in fresh water for projects occurring at IISD-ELA since 1969. This document outlines how samples are processed (e.g. tracked, filtered, stored, etc.) and analyzed for various chemical constituents in freshwater samples, mostly of low solute concentrations, that are submitted to the IISD-ELA ASL. These methods have mainly been used in studies of aquatic productivity and thus are designed for the measurement of micronutrients rather than for pollutants or water quality. Care should be taken when applying them to alkaline, hard, or saline waters, though many are adaptations of methods designed for seawater.

The chemical constituents measured include major nutrients (C, N, P) in their dissolved and particulate phase, soluble reactive silicon, anions (Cl and SO_4), cations (Na, Mg, K, Ca, Mn, and Fe), chlorophyll-a, conductivity, dissolved oxygen, Gran alkalinity, particulate iron, pH, and turbidity. Analyses for the speciation of C (dissolved inorganic carbon, dissolved organic carbon, absorbance scans to assess dissolved organic carbon characteristics, and particulate carbon), N (nitrite, nitrate, ammonia, total dissolved nitrogen, and particulate nitrogen), and P (total dissolved phosphorus and particulate phosphorus) are described.

Dissolved and particulate fractions are operationally defined by the filters used (Whatman GF/C, 1.2 µm), which are measured separately (filter and filtrate) and summed for total concentrations (e.g. total phosphorus equals total dissolved phosphorus plus particulate phosphorus). The dividing line, in particulate size, between particulate and dissolved phases could reasonably be put between 0.1 and 1.0 µm, where the smaller particles might be kept from settling by Brownian motion. Many researchers use 0.45 µm membrane filters, but most cellulose ester membrane filters of this porosity are slow and apt to clog. The IISD-ELA ASL has used Whatman GF/C filters since the early 1970s because they are faster; in addition, because they are inorganic, they can be ignited before use and thus may be used for carbon and nitrogen determination. Although the pore size is greater and not well defined, the manufacturers state that 98% of particles between 0.5 and 1.0 µm are retained. There is very little difference in the quantity of particulate matter retained by the GF/C filters compared to 0.45 µm membrane filters.

Figure 1.1. Sample processing flow chart



Samples submitted to the IISD-ELA ASL are logged into the Laboratory Information Management System (LIMS), Sample Master®, wherein sample metadata is recorded, Sample IDs are assigned, and labels are generated. Samples are subsampled, labelled, and filtered, then subsequently analyzed for chemical constituents. At summer temperatures, changes in nutrient speciation and concentration in stored samples can be rapid due to increased bacteria and phytoplankton abundance. As such, strict storage holding times need to be adhered to. Sample storage holding times adhered to by the IISD-ELA ASL for filtering and analyses are included for each applicable method. Quality control (QC) and quality assurance protocols are provided for each analysis. Details for how labware is cleaned, calibrated, and validated and how reference samples are prepared are also provided.

The analysis results, QC results, and associated metadata are manually entered into the LIMS. Three quality statuses are applied to IISD-ELA ASL data. Data that has passed the analytical QC requirements are designated “Entered,” data that has been assessed for outliers is designated “Validated,” and data that has undergone peer review is designated “Approved,” though a process for peer review has not yet been developed.

2.0 Labware Cleaning

2.1 Introduction and scope

The following methods are used in the IISD Experimental Lakes Area Analytical Service Laboratory to clean the various labware.

2.2 Occupational health and safety

The concentrated hydrochloric acid (HCl) used for reagent preparation is corrosive and reacts violently with water. Handle in a fume hood to avoid HCl vapours. Avoid contact with skin. Wear goggles and gloves when handling and diluting. Always pour acid into water slowly while stirring. Never pour water into acid.

The use of extremely hot water could lead to burns. Wear rubber gloves when washing dishes.

2.3 Equipment

- dishwasher
- scrub brushes of various sizes
- drying racks
- shallow bins lined with absorbent pads or mats
- trays lined with absorbent pads or mats
- large acid-resistant bin with a lid

2.4 Prepared reagents

2.4.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix® 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

2.4.2 Hydrochloric acid – 10%

Dilute 100 mL of concentrated ACS-grade HCl in 800 mL of DRO and make to 1 litre.

2.5 Procedure

2.5.1 General use labware

General use labware (beakers, cylinders, sample bottles, etc.) is cleaned by rinsing three times with extremely hot running tap water followed by three rinses with DRO water. Rubber gloves are worn in order to utilize the hottest water possible and to ensure that finger marks are not left on the equipment.

After rinsing, the residual water is shaken out of the labware, and the labware is set upside down to drain and dry. Caps and small items are dried in a shallow bin lined with an absorbent pad. When the labware is dry, it is coupled with its appropriate cap, if needed, and is returned to its storage place. Aluminum foil is placed over the opening of any labware that does not have a cap/lid (e.g. beakers, cylinders, etc.) to prevent dust contamination.

2.5.2 Volumetric flasks

Volumetric flasks are cleaned in the same manner as general labware, but the water is warm rather than as hot as possible since heat can affect the accuracy of the volumetric glassware.

2.5.3 Particulate (Part) P and Part Fe vials

The 40 mL glass Part P vials, polytetrafluoroethylene (PTFE)-lined caps, 20 mL high-density polyethylene (HDPE) Part Fe vials, and HDPE caps are rinsed three times with hot water, soaked in a 10% HCl bath for at least four hours, and then rinsed three times with DRO water. The vials and caps are dried in a shallow bin lined with an absorbent pad.

2.5.4 Dissolved organic carbon (DOC) and alkalinity bottles

The 175 mL HDPE DOC bottles and caps and the 125 mL HDPE alkalinity bottles and caps are inspected for adhered particulates and cleaned with a scrub brush as needed. The bottles are then washed, without detergent, in the dishwasher, and rinsed three times with DRO water. The 175 mL polyethylene terephthalate glycol alkalinity bottles will melt in the dishwasher, so they are filled with hot water and soaked overnight, cleaned with a scrub brush as needed, rinsed three times with hot water, and then rinsed three times with DRO water.

After rinsing, the residual water is shaken out of the bottles, and the bottles are set upside down to drain and dry. Caps are dried in a shallow bin lined with an absorbent pad. Once the bottles are dry, they are capped and returned to their storage place.

2.5.5 Petri dishes

The tops and bottoms of the 50 mm Petri dishes are washed separately so that they do not attach to each other during the cleaning process. Labels are removed and then the tops or bottoms of the Petri dishes are placed in a bucket with a lid, rinsed three times with hot water, and rinsed three times with DRO. The Petri dishes are laid out upside down on a tray lined with an absorbent pad. Once the tops and bottoms are dry, they are reassembled and stored in a large low-density polyethylene bag.

Appendix 2A. 10% Hydrochloric Acid Preparation Record

iisd ela experimental lakes area

10% HCl
Preparation Record

Stable for two years

Preparation date	Manufacturer	Manufacturer lot number	Grade	HCl vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

3.0 Labware Calibration and Verification

3.1 Introduction and scope

Volumetric flasks, graduated cylinders, displacement pipettes, and lab balances are calibrated and/or verified using the following procedures.

3.2 Occupational health and safety

There are no occupational health and safety concerns associated with these procedures.

3.3 Equipment

- balances
- displacement pipettes (fixed, variable, and repeater)
- *Pipette Calibration* logbook

3.3.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

3.3.2 Effluent from an Elix® 20 Water Purification System (> 5 MΩ·cm purity)

The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

3.3.3 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q® Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

3.4 Laboratory balances

This procedure is based on the ASTM directive E898-88 (1993) *Standard Method of testing Top-Loading, Direct-Reading Laboratory Scales and Balances* approved September 30, 1988.

This calibration checks for off-centre loading errors, repeatability, hysteresis, precision, accuracy, and linearity. It is conducted when a new balance has been procured and when the balance has been recalibrated due to a daily calibration verification failure. All calibrations are performed under typical operating conditions (e.g. temperature, humidity). Additional tests are described in

ASTM directive E898-88 (1993). Results of calibration checks are recorded in a logbook for each balance.

Turn on the instrument and warm it up to the manufacturer's specifications. Calibrate the balance using the manufacturer's specifications, then run the internal calibration, if available, and perform the following tests.

3.4.1 Off-centre error (eccentricity)

1. Zero the balance.
2. Place a weight that is 90–100% of the capacity of the balance in five different locations on the balance pan and record the readings:
   - centre
   - front edge
   - back edge
   - left edge
   - right edge
3. Calculate the eccentricity, which is the maximum difference from the centre. If the eccentricity exceeds 0.01 g, the balance is recalibrated.

3.4.2 Repeatability

1. Zero the balance.
2. Repeatedly (at least 8 times) measure a constant load, which is approximately 50% of capacity. The standard deviation, σ, of this series is the repeatability. If the repeatability exceeds 0.01 g, the balance is recalibrated.

3.4.3 Hysteresis

1. The balance reading depends on the direction from which it is approached.
2. Zero the balance and record it as Z_1.
3. Place a weight of about 50% capacity on the balance and, when stable, record the reading as W_1.
4. Add weights to about 90% of capacity.
5. Remove weights added in the previous step to return to a mass of 50% capacity and record the reading as W_2.
6. Remove all weights and record the reading as Z_2.

The hysteresis effect is calculated from equation 3.1:

Equation 3.1

$$h = \frac{(W_1 - W_2) + (Z_1 - Z_2)}{2}$$

If the hysteresis exceeds 0.01 g, the balance is recalibrated.

3.4.4 Precision

Precision is the sum of h + σ.

3.4.5 Accuracy and linearity

1. Zero the balance and, if available, set the full-scale indication.
2. Place weights on the pan in increments of approximately 10% of capacity and record the readings. Plot the nominal weight versus the observed weight.
3. The difference at any point is the inaccuracy, which by definition cannot be less than the precision since every observation has an uncertainty of as much as 3σ.

3.4.6 Daily calibration verification

The balance calibration is verified on a daily basis, prior to use. To verify the balance calibration, weigh a 50 g weight and 1.0 g weight and record the results in the balance's logbook. If the weight obtained exceeds a difference of 0.01 g from the expected result, the balance is recalibrated.

3.5 Displacement pipettes

Fixed volume, variable volume, and repeater pipettes are calibrated annually, and the calibrations are verified weekly to ensure that they maintain a delivery volume and a coefficient of variation that are within the limits specified by the manufacturer.

Pipettes are individually identified with a unique number and are marked as "Calibrated" with the calibration date.

Different operators should conduct the pipette calibration verifications to ensure that all are using them identically.

3.5.1 Calibration verification

Variable pipettes are tested at three volumes:

- nominal volume (largest volume),
- 50% of the nominal volume, and
- 10% of the nominal volume.

A table of weight ranges and target values is provided in the *Pipette Calibration* logbook (Appendix 3A) to determine if the weights obtained are acceptable.

All results are recorded directly in the *Pipette Calibration* logbook.

1. Place a covered beaker of Milli-Q water on the bench overnight so that the water can equilibrate to room temperature before use.
2. Confirm the balance accuracy by weighing 1 g and 5 g weights. Record the results in the balance's logbook. If the weights obtained exceed the expected value by 0.0005 g, recalibrate the balance before proceeding to the pipette verification.
3. Place a weighing vessel (e.g. plastic weighing boat) on the balance and tare the balance.
4. Place the appropriate tip on the pipette to be verified, and pre-wet it five times to produce a humidity balance in the "dead" air volume.
5. Hold the pipette vertically and immerse the pipette tip a few millimetres into the water (2 to 4 mm below the surface for volumes 1 to 1000 µL and 3 to 6 mm for volumes greater than 1000 µL). Slowly and evenly aspirate the appropriate volume of water. The filled tip is slanted against the wall of the weighing vessel and dispensed into the weighing vessel.
6. Once the balance has stabilized, record the weight in the *Pipette Calibration* logbook.
7. Re-zero the balance and repeat steps 5 and 6. At least three readings are required for adequate verification.
8. Repeat steps 3–7 for each pipette.

Pipettes that do not "Pass" both accuracy and precision requirements as outlined in the *Pipette Calibration* logbook must be taken out of service and have the "Calibrated" label removed. A "failed" pipette may have its calibration rechecked to verify that it has "failed" or show that the "failure" was in error. "Failed" pipettes must be repaired and/or recalibrated and "Pass" a calibration verification before being put back into service.

3.6 References

ASTM directive E898-88. (1993). *Standard method of testing top-loading, direct-reading laboratory scales and balances* (approved September 30, 1988).

Appendix 3A. Pipette Calibration Logbook

Date

Balance ID

Variable pipette (µL)

100 - 1000

				Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C
	0.6% Accuracy												
100	0.3% Imprecision												
Min wt	0.09907	Max diff between extremes	1										
Ideal Mean wt	**0.09967**		2										
Max wt	0.10027	**0.04229**	3										
	0.6% Accuracy												
500	0.2% Imprecision												
Min wt	0.49537	Max diff between extremes	1										
Ideal Mean wt	**0.49836**		2										
Max wt	0.50135	**0.04229**	3										
	0.6% Accuracy												
1000	0.2% Imprecision												
Min wt	9.90739	Max diff between extremes	1										
Ideal Mean wt	**9.96719**		2										
Max wt	10.02699	**0.04229**	3										

Date

Balance ID

Pipette (µL)

				Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C
	0.6% Accuracy												
500	0.2% Imprecision												
Min wt	0.49537	Max diff between extremes	1										
Ideal Mean wt	**0.49836**		2										
Max wt	0.50135	**0.00212**	3										
	0.6% Accuracy			Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C
1000	0.2% Imprecision												
Min wt	0.99074	Max diff between extremes	1										
Ideal Mean wt	**0.99672**		2										
Max wt	1.00270	**0.00424**	3										

Date

Balance ID

Variable pipette (µL)

10 - 100

			Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C
	1.0%											
10	0.4%											
Min wt	0.00987	Max diff between extremes										
Ideal Mean wt	**0.00997**											
Max wt	0.01007	**0.00006**										
	0.7%											
50	0.3%											
Min wt	0.04949	Max diff between extremes										
Ideal Mean wt	**0.04984**											
Max wt	0.05018	**0.00021**										
	0.6%	Accuracy										
100	0.3%	Imprecision										
Min wt	0.09907	Max diff between extremes										
Ideal Mean wt	**0.09967**											
Max wt	0.10027	**0.00042**										

Date

Balance ID

Variable pipette (µL)

20 - 200

			Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C
	1.0%	Accuracy										
20	0.4%	Imprecision										
Min wt	0.01974	Max diff between extremes										
Ideal Mean wt	**0.01993**											
Max wt	0.02013	**0.00085**										
	0.6%	Accuracy										
100	0.3%	Imprecision										
Min wt	0.09907	Max diff between extremes										
Ideal Mean wt	**0.09967**											
Max wt	0.10027	**0.00085**										
	0.6%	Accuracy										
200	0.2%	Imprecision										
Min wt	0.19815	Max diff between extremes										
Ideal Mean wt	**0.19934**											
Max wt	0.20054	**0.00085**										

Date

Balance ID

Variable pipette (µL)

100 - 1000

Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C

0.6% Accuracy

100 0.3% Imprecision

Min wt	0.09907	Max diff between extremes	1						
Ideal Mean wt	**0.09967**		2						
Max wt	0.10027	**0.04229**	3						

0.6% Accuracy

500 0.2% Imprecision

Min wt	0.49537	Max diff between extremes	1						
Ideal Mean wt	**0.49836**		2						
Max wt	0.50135	**0.04229**	3						

0.6% Accuracy

1000 0.2% Imprecision

Min wt	9.90739	Max diff between extremes	1						
Ideal Mean wt	**9.96719**		2						
Max wt	10.02699	**0.04229**	3						

Date

Balance ID

Variable pipette (µL)

500 - 5000

Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C

0.6% Accuracy

500 0.2% Imprecision

Min wt	0.49537	Max diff between extremes	1						
Ideal Mean wt	**0.49836**		2						
Max wt	0.50135	**0.04229**	3						

0.6% Accuracy

2500 0.2% Imprecision

Min wt	2.47685	Max diff between extremes	1						
Ideal Mean wt	**2.49180**		2						
Max wt	2.50675	**0.04229**	3						

0.6% Accuracy

5000 0.2% Imprecision

Min wt	9.90739	Max diff between extremes	1						
Ideal Mean wt	**9.96719**		2						
Max wt	10.02699	**0.04229**	3						

Date										
Balance ID										

Variable pipette (µL)

1000 - 10000				Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C	Analyst	°C
	0.6%	Accuracy											
1000	0.2%	Imprecision											
Min wt	0.99074	Max diff between extremes	1										
Ideal Mean wt	**0.99672**		2										
Max wt	1.00270	**0.04229**	3										
	0.6%	Accuracy											
5000	0.2%	Imprecision											
Min wt	4.95369	Max diff between extremes	1										
Ideal Mean wt	**4.98359**		2										
Max wt	5.01349	**0.04229**	3										
	0.6%	Accuracy											
10000	0.2%	Imprecision											
Min wt	9.90739	Max diff between extremes	1										
Ideal Mean wt	**9.96719**		2										
Max wt	10.02699	**0.04229**	3										

4.0 Reference Sample Preparation

4.1 Introduction and scope

Reference samples are included in every analytical run to assess inter-run variability, verify that the calibration standards have been prepared properly, and ensure that the method is providing accurate results.

4.2 Occupational health and safety

The muffle furnace, used to bake GF/C filters, is set to 500 °C and thus presents a risk for burns when handling the furnace and items baked therein. To reduce the risk of burns, vials and trays are placed into the muffle furnace prior to turning it on. Allow the vials and trays to cool down and use protective gloves and/or tongs when removing them. Do not leave the muffle furnace door open to cool. Post a **HOT** warning to caution other lab staff about the risk of burns from the muffle furnace or items baked in the muffle furnace.

4.3 Equipment

- filtration apparatus with a 47-mm diameter magnetic filter funnel
- vacuum desiccator
- 42.5 mm GF/C filters, baked for 14 hours at 500 °C
- 50-mm Petri dishes
- flat-ended filter forceps

4.4 Purchased reagents

4.4.1 $PO4^{-3}$ calibration standard stock

Certified commercial $PO4^{-3}$-P standard

1.0 mL = 50 ± 1 µg $PO4^{-3}$-P

Store at 2–6°C until the expiry date on the manufacturer's label.

4.4.2 NO_2 calibration standard stock

Certified commercial NO_2-N standard

1.0 mL = 0.1 ± 0.01 mg NO_2-N

Standard is stable until the expiry date on the manufacturer's label.

4.4.3 NH_3 calibration standard stock

Certified commercial NH_3-N standard

1.0 mL = 0.1 ± 0.01 mg NH_3-N

Standard is stable until the expiry date on the manufacturer's label.

4.5 Prepared reagents

4.5.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

4.5.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

4.5.3 Desiccant

The silica gel desiccant is baked at 120 °C for at least 24 hours prior to use.

4.6 Procedures

4.6.1 Collection and aging

The reference sample water is collected by pumping Lake 239 surface water into a black 55-gallon drum and aged for at least one year at 4 °C to ensure that the analytes have reached equilibrium and will no longer change over time.

4.6.2 Bottling

Once the reference sample water has been aged for at least one year, it is homogenized as best as possible by rolling the barrel on its side. The reference sample is then pumped into pre-rinsed 2-L glass bottles and stored at 4 °C. Each reference sample bottle is assigned a lot number, in the format "Byyyymmdd-#," wherein yyyymmdd represents the date it was bottled and -# represents

each bottle's unique ID. The collection date, bottling date, assigned lot numbers, and analyst's initials are recorded in the *Reagent Preparation* logbook.

The aged and bottled reference sample is used for all analyses that are performed on unfiltered samples, namely pH, conductivity, turbidity, and dissolved inorganic carbon (DIC). The aged and bottled reference sample is also used to prepare the soluble reactive silica reference sample.

4.6.3 Filtering

Reference sample water that has been aged for at least one year is filtered through 1.2 µm GF/C filters that had been baked at 500 °C for 14 hours and stored in pre-rinsed 2-L glass bottles at 4 °C. Each filtered reference sample bottle is assigned a lot number, in the format "Fyyyymmdd-#," wherein yyyymmdd represents the date it was filtered and -# represents each bottle's unique ID. The filtration date, bottling date, filtration volume, filter type, filter pore size, assigned lot numbers, and analyst's initials are recorded in the *Reagent Preparation* logbook.

The filtered reference sample is used for total dissolved nitrogen, absorbance scans, and the preparation of the total dissolved phosphorus, nitrite, nitrate, and ammonia reference sample preparation. The filtered reference sample for absorbance scans is aliquoted into an amber bottle to reduce light exposure.

4.6.4 Soluble reactive silica

The concentration of soluble reactive silica (SRSi) in the unfiltered reference sample is above the concentration range of the SRSi analysis (i.e. 2.5 mg/L; ELA-SRSi_v3.1) and thus requires dilution. The reference sample for SRSi is prepared by diluting the unfiltered reference sample by a factor of two with Milli-Q water. The SRSi reference sample is assigned a lot number in the format SRSiyyyymmdd-#, wherein yyyymmdd represents the date it was filtered and -# represents each bottle's unique ID. The preparation date, filtered reference sample lot number, reference sample volume, final volume, dilution factor, assigned lot number, and analyst's initials are recorded in the *Reagent Preparation* logbook. The SRSi reference sample is stored at room temperature.

4.6.5 Total dissolved phosphorus

The concentration of total dissolved phosphorus (TDP) in the filtered reference sample is below the detection limit of the TDP analysis (ELA-TDP_v3.0). As such, the filtered reference sample is spiked with 0.5 mL of the 50 µg/mL PO4-3-P calibration standard stock per litre of filtered reference sample (final concentration of 25 µg/L). The TDP reference sample is assigned a lot number in the format TDPyyyymmdd-#, wherein yyyymmdd represents the date it was filtered and -# represents each bottle's unique ID. The preparation date, filtered reference sample lot number, concentration, volume, manufacturer material lot number of the calibration standard,

final volume, final concentration, assigned lot number, and analyst's initials are all recorded in the *Reagent Preparation* logbook. The TDP reference sample is stored at 4 °C.

4.6.6 Nitrite, nitrate, and ammonia

The concentration of nitrate in the filtered reference sample is above the concentration range of the nitrate analysis (i.e. 100 µg/L; ELA-NOxNH3_v2.0) and the nitrite and ammonia concentrations are below the detection limit (i.e. 0.5 and 10.6 µg/L, respectively). As such, the reference sample for the ELA-NOxNH3_v2.0 analysis is prepared by diluting the filtered reference sample by a factor of two with Milli-Q water and spiked with 0.25 mL of the 0.1 mg/mL NO2-N calibration standard stock and with 2.5 mL of the 0.1 mg/mL NH_3-N calibration standard stock per litre of diluted filtered reference sample (final concentration of 25 µg/L and 250 µg/L for NO_2-N and NH3-N, respectively). This NO_xNH_3 reference sample is then preserved with chloroform at a concentration of 1 mL/L and stored at 4 °C. The NO_xNH_3 reference sample is assigned a lot number in the format "NOxNH3yyyymmdd-#," wherein yyyymmdd represents the date it was filtered and -# represents each bottle's unique ID. The preparation date, filtered reference sample lot number, dilution factor, concentration, volume, manufacturer material lot numbers of the NO_2 and NH_3 calibration standards, final volume, final concentrations, assigned lot number, and analyst's initials are all recorded in the *Reagent Preparation* logbook.

4.6.7 Chlorophyll-*a*, particulate phosphorus, and particulate iron

The chlorophyll-*a* (Chl-*a*), particulate phosphorus (Part P), and particulate iron (Part Fe) reference samples are comprised of GF/C filters with retained seston. A water sample is collected from the epilimnion of Lake 227 during peak phytoplankton biomass. The collected water sample is transferred to a container with a spigot and continuously homogenized with a stir bar and stir plate. Fifty-millilitre aliquots are filtered through baked GF/C filters that were pre-rinsed with 50 mL of Milli-Q water. Each reference sample filter is placed in a labelled 50-mm Petri dish, desiccated in the dark in a vacuum desiccator, and stored in the dark at -20 °C. The Chl-a and Part Fe reference samples are assigned a lot number in the format "Partyyyymmdd-##", wherein the yyyymmdd represents the date the samples were prepared and the -## represents each filter. The sample description (e.g. 227 LA CB 5m), date sampled, filtration date, volume filtered, GF/C lot number, assigned lot numbers, and analyst's initials are all recorded in the *Reagent Preparation* logbook.

Appendix 4A. Reference Sample Bottling Preparation Record

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L239 Reference Sample
Bottling Record

Bottling date	L239 reference sample collection date	Assigned lot number	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 4B. Reference Sample Filtration Preparation Record

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L239 Reference Sample
Filtration Record

Filtration date	Check sample bottling lot number	Filtration vol (mL)	Filter type	Filter pore size	Assigned lot number	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 4C. Soluble Reactive Silica Reference Sample Preparation Record

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SRSi Reference Sample
Preparation Record

Preparation date	Filtered reference sample lot number	Reference sample vol (mL)	Final vol (mL)	Dilution factor	Assigned lot number	Analyst initials

Authorized by Sonya Havens
Authorization date: September 2016

Appendix 4D. Total Dissolved Phosphorus Reference Sample Preparation Record

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TDP Reference Sample
Preparation Record

Preparation date	Filtered reference sample lot number	Spike				Final vol (mL)	Final conc (µg/L)	Assigned lot number	Analyst initials
		conc (µg/mL)	Vol (mL)	Manufacturer	Material lot number				

Authorized by Sonya Havens
Authorization date: October 2016

Appendix 4E. Nitrite, Nitrate, and Ammonia Reference Sample Preparation Record

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experimental lakes area

AA3 NOx/NH$_3$ Reference Sample
Preparation Record

				Spike							
Preparation date	Filtered reference sample lot number	Analyte	DF	conc (µg/mL)	Vol (mL)	Manufacturer	Material lot number	Final vol (mL)	Final conc (µg/L)	Assigned lot number	Analyst initials
		NO$_3$									
		NO$_2$									
		NH$_3$									
		NO$_3$									
		NO$_2$									
		NH$_3$									
		NO$_3$									
		NO$_2$									
		NH$_3$									
		NO$_3$									
		NO$_2$									
		NH$_3$									
		NO$_3$									
		NO$_2$									
		NH$_3$									
		NO$_3$									
		NO$_2$									
		NH$_3$									

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 4F. Chlorophyll-a and Particulate Iron Reference Sample Preparation Record

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Chla and Part Fe Reference Sample
Preparation Record

Filtration date	Sample description	Date sampled	DF	Vol filtered (mL)	GFC lot number	Assigned reference sample lot number

Authorized by: Sonya Havens
Authorization date: October 2016

5.0 Laboratory Information Management System – Sample Master®

5.1 Introduction and scope

Laboratory information management software (Sample Master®) is used to track samples and record data and metadata for each sample. Each sample that is processed by the IISD Experimental Lakes Area Analytical Service Laboratory is logged into Sample Master with its requested tests and collection metadata (e.g. Site, Collect Date, etc.) and is assigned a Sample ID. Sample Master is also used to generate sample labels, record and track quality control, and retrieve data.

5.2 Equipment

- computer with Windows Server operating system, SQL Server Express, SQL Server Management Studio, Sample Master version 10 (Web), and Crystal Reports installed
- Honeywell Intermec Easy Coder PD41 printer equipped with Honeywell Intermec Duratherm II Thermal Labels, 1" × 2" (p/n E25762)
- HP Color LaserJet MFP M283 printer.

5.3 Sample login

Samples for each project (e.g. L239 profile) are logged in as an "order," which is assigned an Order ID in the format "yyyymmdd##" wherein the yyyymmdd represents the date that the sample was logged in. Each sample in the order is assigned a Sample ID in the format "yyyymmdd##-##" wherein the yyyymmdd## represents the Order ID. Blanks should be the first order logged in each day.

Any sample that is attempted to be collected is logged in. If that sample is not available (e.g. 239 NE@LA is not flowing), that sample is cancelled (not deleted) in the Order Details page *(Edit > Cancel Sample)*, and the reason for the cancellation is recorded. This provides a record that the collection was attempted but that the sample was not available for the given reason.

5.3.1 Login order

1. Login to Sample Master with username and password and navigate to *Sample Tracking* > *Sample Login*

   This page provides the metadata for the order, which includes the Order ID, Order date, user that signed it in, Customer ID, Customer Contact, Billing ID, Billing Contact, and Project ID.

2. Click *New Order ID,* select *Login*, and click *Submit*

   Select appropriate Customer ID (use the "Requested Analyses" spreadsheet) and appropriate Project ID (e.g. 239 Profile). Click *Yes* when prompted by "Would you like to add samples and Tests associated with [Project ID]?"

3. Click *Samples* to retrieve the Order Details page

   This page lists all of the samples and the associated metadata for each sample, which includes the Sample ID, Customer Sample ID, Site, Matrix, Collector, Date Collected, Time Collected, Date Received, Start Depth and End Depth (for depth-integrated lake samples), and Start Collect Date, End Collect Date, Rain Gauge, and Observed Precipitation Volume (for precipitation samples).

   a. Ensure that all of the appropriate samples (i.e. correct sites) were added and enter all relevant metadata.

   b. If the order includes a field duplicate, highlight the sample to be duplicated, hover over *Edit*, and click *Copy Sample*. Ensure that all of the boxes are clicked, except Cust. Sample ID, and click OK.

4. Verify that the appropriate tests are requested for each sample by selecting a sample and clicking *Tests* to retrieve the Sample Details page, which includes all of the requested tests, as well as the metadata for each requested test (i.e. Method, Prep Method, Due Date, Preservative, Bottle Type, # Hauls, Equipment Code, Net Diameter, and Total Depth).

5.3.2 Save Login Report

The Login Report provides a concise record of the details of each order.

Return to the Sample Login page, hover over *Edit*, and click *Calculate Due Dates*.

Retrieve the Login Report by hovering over *Print* and clicking *Login Report*. Review it to make sure everything is correct. Click the download icon and save the Login Report in \\ela-lab.iisd.ca\shared\Chem Lab\Sample Master\Login Reports in the format "yyyymmdd##_LoginReport" wherein the yyyymmdd## represents the Order ID.

Figure 5.1. Example login report

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Login Report
19-Oct-2020

Order ID: 2020092202
Order Date: 22-Sep-2020
Project ID: 239 Streams
Customer ID: LTER
Customer Name: Scott Higgins

Sample #: 2020092202-01
Site: 239 EIF
Matrix: Stream Water
Date Collected: 22-Sep-2020
Date Received: 22-Sep-2020

Test	Due Date
Alk	03/21/2021
Anions	03/21/2021
Archive	09/22/2021
Cations	09/22/2021
Cond	09/23/2020
DIC	09/23/2020
DOC	01/20/2021
DOC Quality	09/22/2021
Filtration	09/22/2021
NH3	09/24/2020
NO2	09/24/2020
NO3	09/24/2020
pH	09/23/2020
SRSi	03/21/2021
Susp C/N	09/23/2020
Susp P	09/22/2020
TDN	09/29/2020
TDP	09/29/2020

Sample #: 2020092202-02
Site: 239 NEIF
Matrix: Stream Water
Date Collected: 22-Sep-2020
Date Received: 22-Sep-2020

Test	Due Date
Alk	03/21/2021
Anions	03/21/2021
Archive	09/22/2021
Cations	09/22/2021
Cond	09/23/2020
DIC	09/23/2020
DOC	01/20/2021
DOC Quality	09/22/2021
Filtration	09/22/2021
NH3	09/24/2020
NO2	09/24/2020
NO3	09/24/2020

5.3.3 Print sample labels

Return to the Sample Login page, hover over *Print*, and click *Sample Labels* to retrieve the sample labels. Click the *print icon*, ensure that EasyCoder PD41 (203 dpi) – IPL is selected, and click *Print*.

Figure 5.2. Example sample label

5.3.4 Sample summary

Continue to log in orders, as outlined above, until all the samples for the day have been logged in.

Retrieve the daily Sample Summary by hovering over *Print*, clicking *Summary*, selecting the appropriate Start of Range and End of Range, and clicking *OK*. Click the *print icon*, ensure that HP LaserJet 4050 is selected, and click *Print*. The Sample Summary is then used to record filtration volumes, start depths, end depths, start dates, end dates, rain gauges, observed precipitation volumes, and any other metadata (e.g. notes/comments) for each sample, which are later recorded in Sample Master.

Figure 5.3. Example sample summary

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Sample Summary

Date: 19-Oct-2020

			Filtration volumes (mL)				
Collect Date	**Sample Number**	**Site**	**Susp P**	**Chl-a**	**Susp C/N**	**Archive**	**Notes**
22-Sep-2020	2020092202-01	239 EIF					
22-Sep-2020	2020092202-02	239 NEIF					
22-Sep-2020	2020092202-03	239 NWIF					
22-Sep-2020	2020092202-04	239 OF					
22-Sep-2020	2020092202-05	239 NWIF					

5.3.5 Order signoff

The orders are signed off by hovering over *Edit* and clicking *Order Signoff*. Mark all of the orders and click *Signoff*.

5.4 Sample preparation

The lot numbers of the GF/C filters that are used for each test sample are recorded in Sample Master.

To enter GF/C lot numbers, navigate to *Sample Tracking* > *Sample Preparation*, ensure that "Unassigned Samples" is checked, and query the samples by filtering the test for Archive, Chl-*a*, Part C/N, Part Fe, and Part P. Click the check box to mark the appropriate sample(s), select the appropriate GF/C lot number from the drop-down menu (if available), and click *Add to Batch*.

Table 5.1. Default filtration volumes

Test	Default Vol. (mL)
Archive	200
Chl-*a*	200
Part C/N	200
Part Fe	100
Part P	100

If not available, add the GF/C lot number by clicking *New…*, entering the GFC lot number in the Prep Filter Lot field and the date the first GF/C from that lot was used in the Date Prepared field (leave Matrix, Test, Method, and Supply Name blank), and clicking *OK*.

5.4.1 Enter prep volumes

The Analysis Volumes recorded in Sample Master are configured with the default volumes for the Archive, Chl-*a*, Part C/N, Part Fe, and Part P tests. If the actual volume filtered is adjusted from the default filtration volume, the Analysis Volume needs to be revised to the adjusted filtration volume.

To revise the Analysis Volume, navigate to *Sample Tracking* > *Enter Prep Volumes*, and query the samples by filtering by the appropriate Order ID(s) or Sample ID(s).

Revise the "AnalysisVolume" of any test samples that had filtration volumes that were adjusted from the default and click *Update*.

5.4.2 Create QC Batch

A QC Batch is created prior to data entry to assign quality control (QC) samples (e.g. reference standards, duplicates, etc.) to the batch of samples in the analytical run.

To create a QC Batch, navigate to *Sample Tracking* > *Create QC Batch*, ensure that "Unassigned Samples" is checked, select the appropriate Test from the drop-down menu, and click *Retrieve*.

Mark the samples to be included in the batch and click *New…* to create a new QC Batch ID.

> **Note:** To mark all of the samples, click the box in the header between "Test" QC Batch ID." Alternatively, batches of samples can be marked by typing or selecting a number in the drop-down menu next to *Mark* and clicking *Mark*.

Ensure that the appropriate Instrument, Matrix, and Test are selected and select the appropriate Method from the drop-down menu.

Ensure that the appropriate QC Types are marked and click *Create Run Sequence* (do not click *OK*) to reach the *QC Batch – Edit Sequence* page, wherein the sample sequence order and number of QC Types (e.g. Method Blank, Instrument Duplicate, etc.) can be adjusted.

Adjust the number of each QC Type by choosing the QC Type from the "Add QC:" menu and clicking the right-arrow to add it to the Batch Sequence.

To adjust the sequence order, highlight the sample or QC Type to be adjusted and use the up arrow or down arrow to move it to the desired sequence number.

Once the Batch Sequence includes all of the samples, the appropriate number of each QC Type, and the desired sequence order, click *OK*.

> **Note:** If *Create Run Sequence* is not clicked, the Run Sequence is not created, and the run sequence order and QC Samples can never be adjusted. If this occurs, a new QC Batch ID needs to be created. To return to the Run Sequence page, hover over *Edit* and click *Edit Run Sequence*.

For each required duplicate, select the appropriate Order ID and Sample ID to be duplicated from the drop-down menus.

To print the Run Sequence BenchSheet, ensure that the samples are still marked, hover over *Print*, and click *Run Sequence BenchSheet*. Click the *print icon*, ensure that HP LaserJet 4050 is selected, and click *Print*.

Figure 5.4. Drop-down menus for duplicates

QC Type	Order ID	Sample ID
Instrument Duplicate	2020102002	2020102002-01

5.4.3 Enter data

Once the analysis is complete and a QC Batch has been generated, navigate to *Data Entry* > *Result Entry* to record the results of the analysis.

Samples that have passed the analytical quality control parameters are Entered into Sample Master along with the results of the associated quality control parameters. Samples that have passed the trend analysis are Validated. Samples that have undergone external peer review are Approved. A process for external peer review has not yet been developed.

Query the appropriate QC Batch ID and click *Retrieve*. Select *Results to Enter*.

In the "Sample Results" tab, enter the "Result" for each sample as well as the "Analysis Employee," "ParamAnalyst," "Analysis Date," and "ParamAnalysisDate."

In the "Spike Results" tab, enter the results of the reference standards, reference samples, nitrate conversions (if applicable), urea conversions (if applicable), and duplicates (e.g. instrument duplicate, method duplicate, etc.) as well as the "ParamAnalyst" and the "ParamAnalysisDate."

In the "Blank Results" tab, enter the results of the blanks (e.g. reagent blanks, method blanks, etc.), as well as the "ParamAnalyst" and "ParamAnalysisDate."

Once all of the data and metadata have been entered, click *Enter*. The samples will now be transferred to "Results to Validate."

5.5 Trend analysis

Trend analysis is conducted on each site/parameter combination using historical data to assess data for outliers while taking seasonal dynamics into account. Outliers are investigated and either retained or rejected in Sample Master depending on the revelations of the investigation. All of the samples that have undergone trend analysis are "Validated" for that site/parameter combination.

To conduct a trend analysis, navigate to *Data Entry* > *Trend Analysis*, query for the appropriate site (e.g. L239 EIF) and parameter (e.g. NO_2-N), and click *Retrieve*. Mark all of the samples by clicking the box between "Parameter" and "Results" and click *Chart*. Assess the chart for and record any outliers, as well as any information about the outlier (e.g. result is high) in the cell that represents the site/parameter combination in the Trend Analysis spreadsheet. If no outliers exist, put an "X" in the cell that represents the site/parameter combination.

Continue conducting the trend analysis for all site/parameter combinations.

To "Validate" samples, navigate to *Data Entry* > *Result Entry*, query for the samples by filtering by the site and parameter combination, and click *Retrieve*. Click *Results to Validate*, mark all of the samples, except for the outliers, and click *Validate*.

Figure 5.5. Subsection of Trend Analysis spreadsheet

Site	Alk	Cl	SO4	Ca
Filter blank				
114 LA CB Epi				
114 LA CB Meta				
224 LA CB Epi				
224 LA CB Meta				
224 LA CB 5m				
224 LA CB 10m				

5.6 Data retrieval

All nutrient chemistry data and associated metadata for samples collected after May 2017 can be retrieved from Sample Master.

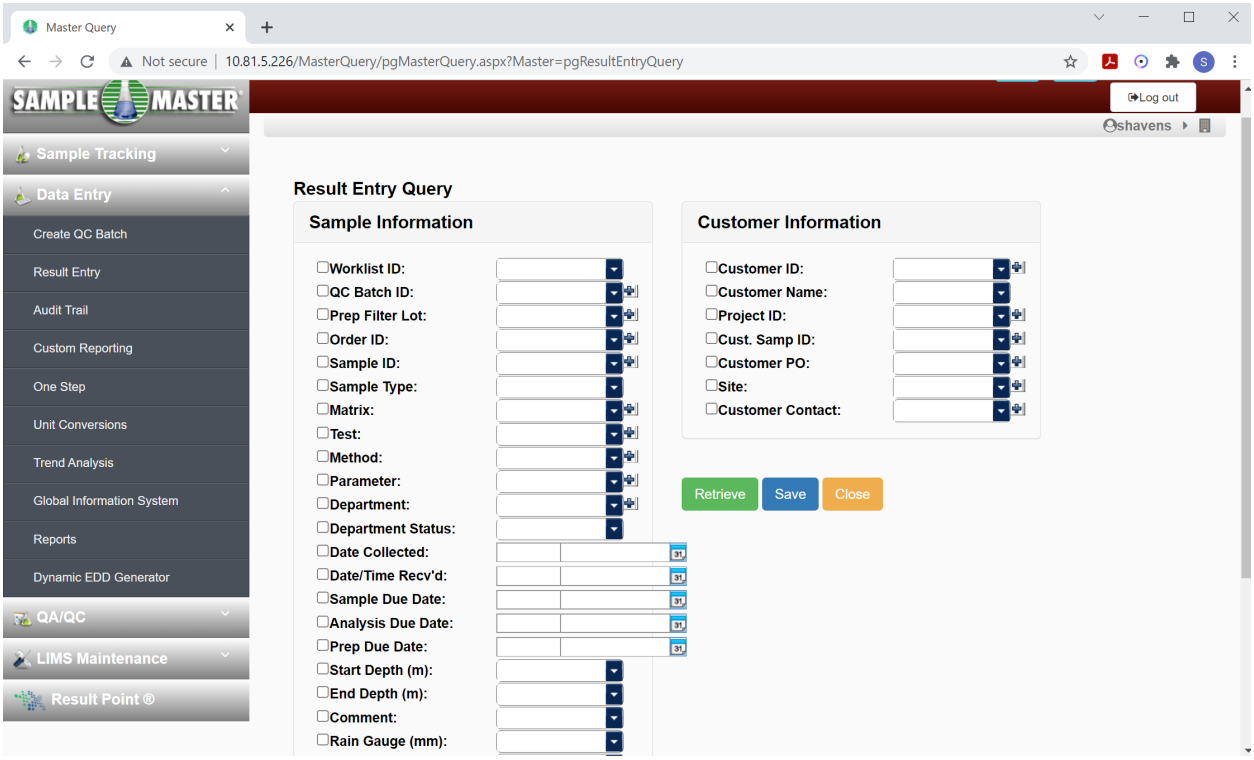
There are two ways you can retrieve data using Sample Master: *Data Entry* > *Result Entry* and *Data Entry* > *Dynamic EDD Generator*. Using *Result Entry* retrieves the analytical results and analysis-based metadata (e.g. analysis employee, analysis date, method, instrument, etc.); it

does not include sample-based metadata (e.g. start depth, end depth, etc.). The *Dynamic EDD Generator* is used when sample-based metadata is required as it provides metadata from any of the background tables (e.g. Sample Login, Enter Prep Volumes, etc.).

5.6.1 Result Entry

Results can be retrieved by navigating to *Data Entry* > *Result Entry* and querying for content in various fields (e.g. Customer ID, Test, Site, Project ID, Collect Date, etc.). Select the desired content you would like to filter for from each of the desired fields using their associated drop-down menus and/or date fields and click *Retrieve*.

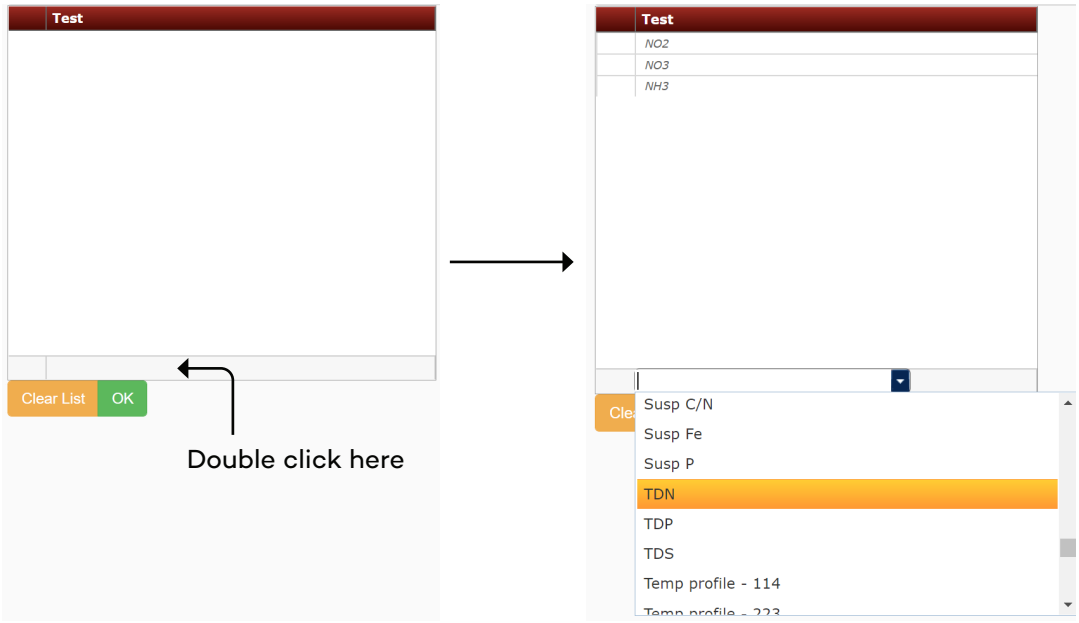
Figure 5.6. Result Entry query page



Example: To query for all chlorophyll-*a* (Chl-*a*) collected from 239 LA CB Epi from January 1, 2020, to December 31, 2021, select "Chl-a" in the Test drop-down menu, "239 LA CB Epi" in the Site drop-down menu, input 01/01/2020 in the start collect date and 12/31/2020 in the end collect date fields, and click *Retrieve*.

To query for multiple contents within a field (e.g. NO_2, NO_3, and NH_3 in the Test field), click the "+" button next to the field to retrieve a list page. Double-click on the empty field below the list section to reveal a hidden drop-down menu. Select the desired content to filter for and hit *Enter* to add it to the list. Continue selecting and adding desired content to the list. Once all of the desired contents are added to the list, click *OK*. Continue to add filters to additional fields and then click *Retrieve*.

Figure 5.7. List page to select multiple tests for addition to the Result Entry query



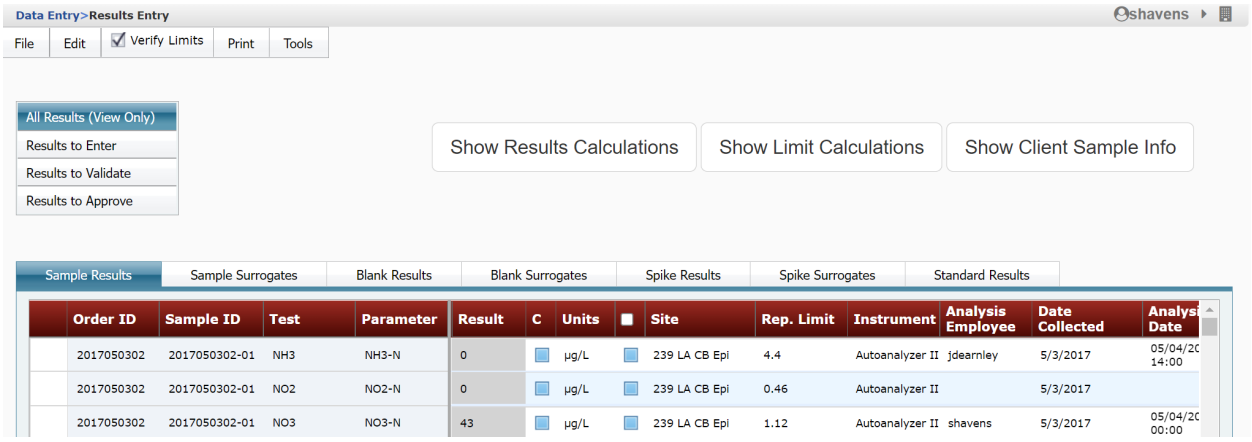
The retrieved *Results Entry* page lists the Test Parameter result for each sample, along with the associated metadata listed in Table 5.2. When "All Results" is selected, all samples collected, regardless of Status (i.e. Awaiting Entry, Awaiting Validation, or Awaiting Approval), are listed. When "Results to Enter" is selected, all of the samples that are awaiting entry are listed. When "Results to Validate" is selected, all of the samples that have been entered but have not yet undergone trend analysis are listed. When "Results to Approve" is selected, all of the samples that have been Validated but have not yet been peer-reviewed are listed.

Table 5.2. Sample metadata available in Sample Master in Result Entry

Order ID	Rep. Limit (LOD)	Validated By
Sample ID	Instrument	Validated Date
Site (e.g. 239 LA CB Epi)	Analysis Employee	Approved By
Date Collected	Analysis Date	Approved Date
Test	Method	QC Status (i.e. Awaiting Entry, Awaiting Validation, or Awaiting Approval)
Parameter	Matrix	
Units	Entered By	
	Entered Date	

To export results to Excel, hover over *File* > *Export to Excel*. The exported Excel file will pop up and automatically download to the Downloads folder.

Figure 5.8. Example Result Entry page



5.6.2 Dynamic EDD Generator

The Dynamic EDD Generator is used to retrieve results and metadata from any background table. Navigate to *Data Entry* > *Dynamic EDD Generator*, query for desired samples in the same manner as the *Result Entry* query, and click *Retrieve*. A list of samples will appear. Select each desired sample by marking the box next to it. To select all samples, click the check box at the top of the table. Click *EDD Generator*.

Select desired fields from the "FieldName" list by clicking its associated box in the Mark column. Please note that the Dynamic EDD Generator lists the default caption for each field rather than the user-defined custom caption. Table 5.3 can be used as a guide for selecting the proper default caption field.

Once all of the desired fields have been added to the list, click either the .csv or .xls button and click *Export*. The exported Excel file will pop up and automatically download to the Downloads folder.

The list of desired fields can be saved as a template. Provide a name for the template in the Save Template field and click *Save Template*. This template is now available for future use.

Table 5.3. Custom captions legend

Default caption	Custom caption
OrderDetails_User1	Start Depth (m)
OrderDetails_User2	End Depth (m)
OrderDetails_User3	Comment
OrderDetails_User4	Rain Gauge (mm)
OrderDetails_User5	Observed Precip. Vol. (L)
SampleDetails_User1	# of Hauls
SampleDetails_User2	Equipment code
SampleDetails_User3	Net diameter (cm)
SampleDetails_User4	Total depth (m)

6.0 Sample Preparation and Filtration

6.1 Introduction and scope

Samples are collected in the field in polyethylene bottles and insulated during transport to prevent them from heating in the summer and freezing in the winter. Laboratory information management software (Sample Master) is used to track samples and record data and metadata for each sample. Subsamples are aliquoted into labelled containers for analyses conducted on unfiltered water. Additional subsamples are filtered through a series of 1.2-µm glass microfibre filters. The filtrate and each filter are retained to conduct analyses on the dissolved and particulate fractions, respectively.

Table 6.1. Chemical constituents analyzed in unfiltered filtered and particulate samples

Unfiltered	Filtered	Particulate
conductivity	absorbance scan (200–800 nm)	chlorophyll-*a*
dissolved inorganic carbon	anions (Cl and SO_4)	particulate carbon
Gran alkalinity	ammonia (as N)	particulate iron
pH	cations (Na, Mg, K, Ca, Mn, and Fe)	particulate nitrogen
phytoplankton taxonomy	dissolved organic carbon	particulate phosphorus
soluble reactive silica	nitrate (as N)	
	nitrite (as N)	
	total dissolved nitrogen	
	total dissolved phosphorus	

6.2 Method principle

It is essential to filter samples as soon as possible (within hours) after collection. This permits the distinction between dissolved and particulate phases and minimizes changes in sample composition arising from biological uptake and excretion. Since parameters differ in their storage condition and time limits, it is useful to subdivide a sample into different containers, each receiving appropriate storage treatment. The sample handling scheme found useful in our laboratory appears in Figure 6.1.

Particulate samples are obtained for chlorophyll-*a*, particulate carbon and nitrogen, particulate phosphorus, and particulate iron when needed. An archive particulate sample is also obtained as a backup for any of these analyses. Filtrate is divided into four portions (Figure 6.1): one for nutrients that have short-term storage requirements (Table 6.1); one for absorbance scan, which is stored in low light conditions; one for anions and cations, which are outsourced to an external laboratory; and one for dissolved organic carbon. The unfiltered sample is sub-divided

into dissolved inorganic carbon, which is stored at 4 °C and analyzed within 24 hours; pH and conductivity, which is equilibrated to 25 °C in a water bath prior to analysis; soluble reactive silica, which is stored at room temperature; phytoplankton taxonomy, which is preserved with Lugol's solution; and alkalinity, which is outsourced to an external laboratory. Dissolved and particulate phases are operationally defined by what passes through or is retained on a pre-ignited (14 hours at 500 °C) Whatman GF/C glass fibre filter.

While several preservation methods are widely used (Brezonik & Lee, 1966; Hellwig, 1964; Moore & Locke, 2013), none are satisfactory for preserving samples for all analyses. Freezing samples inevitably produces a freeze concentration causing precipitation of silicon in neutral to acidic waters and meta salts in alkaline waters. These precipitates often do not quantitively redissolve on thawing. For nutrient analysis, we have found no substitute for rapid analysis. The permissible storage times listed in Table 6.1 are a compromise between desirable and logistically possible time limits.

6.3 Occupational health and safety

The muffle furnace, used to bake GF/C filters, is set to 500 °C and thus presents a risk for burns when handling the furnace and items baked in the furnace. To reduce the risk of burns, vials and trays are placed into the muffle furnace prior to turning it on. Allow the vials and trays to cool down and use protective gloves and/or tongs when removing them. Do not leave the muffle furnace door open to cool. Post a **HOT** warning to caution other lab staff about the risk of burns from the muffle furnace or items baked therein.

6.4 Sample conditions

The processing and holding times described are designed for dilute Canadian Shield waters (e.g. IISD-ELA samples); samples from other sources (e.g. marine) may have other requirements. This is discussed with clients who bring samples from other sources. Sampling and adherence to IISD-ELA sampling protocols are the client's responsibility.

Samples are stored at 4 ± 2 °C and filtered within 24 hours of sample collection.

Default filtration volumes are 200 mL for chlorophyll-*a*, particulate carbon and nitrogen, and archive samples and 100 mL for particulate phosphorus samples. If the filter clogs prior to reaching these volumes, the volume filtered is reduced accordingly and the actual filtered volume is recorded on the sample label, in the Sample Summary, and in Sample Master. Alternative filtration volumes are available upon request but are contingent on sufficient sample volume being supplied by the client.

6.5 Equipment

- filtration apparatus with two 47-mm diameter magnetic filter funnels, one 25-mm diameter filter funnel, and three rinse water collection bottles. The filtration apparatus is set up to collect both the filter and the filtrate.
- vacuum desiccator
- 25-mm GF/C glass fibre filters, baked for 14 hours at 500 °C
- 42.5-mm GF/C glass fibre filters, baked for 14 hours at 500 °C
- flat-ended filter forceps
- 250-mL graduated cylinder
- 500-mL narrow-mouth polyethylene terephthalate glycol (PETG) bottles
- 175-mL square wide-mouth high-density polyethylene (HDPE) bottles
- 125-mL narrow-mouth HDPE bottles with polyethylene caps
- 125-mL narrow-mouth HDPE bottles conical caps
- 50-mL conical tubes
- 20-mL HDPE vials with polypropylene caps
- 20-mL HDPE vials with polypropylene caps – acid washed
- 40-mL glass vials with conical caps
- 15-mL glass vials with polytetrafluoroethylene (PTFE)-lined polypropylene caps – acid washed
- 4 × 50-mm Petri dishes
- 500 µL pipette and 1,000 µL pipette tips

6.6 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of the new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.
- Reagents and standards are stable at room temperature unless otherwise specified.

6.6.1 Type 2 ultrapure deionized reverse osmosis water (DRO)

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

6.6.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

6.6.3 Lugol's solution

- 100 g potassium iodide
- 50 g iodine
- 100 mL glacial acetic acid

Dissolve the potassium iodide, iodine, and glacial acetic acid into ~700 mL Milli-Q water and dilute to 1 L with Milli-Q water. Store at room temperature in an amber glass bottle with a cap and a conical insert.

6.6.4 Desiccant

The silica gel desiccant is baked at 120 °C for at least two hours prior to use.

6.7 Quality control

Several provisions are taken to reduce and quantify contamination at various stages of sample preparation, as well as to provide an estimation of sample duplication—from sampling through analysis.

6.7.1 GF/C procurement and preparation

Whatman GF/C glass microfibre filters, 42.5 mm, are procured in quantities of at least 40 boxes (100 filters/box) from the same lot. The GF/C filters are baked at 500 °C for 14 hours and then placed into acid-washed wide-mouth glass jars using forceps. The lot numbers of the GF/C used for each test sample are recorded in Sample Master.

6.7.2 Filter blanks

A filter blank, consisting of a baked 42.5-mm GF/C filter, is collected for particulate phosphorus each day that samples are filtered.

6.7.3 Method blanks

Filter method blanks are prepared by filtering 100 mL of Milli-Q water through 42.5-mm GF/C filters for particulate iron and particulate phosphorus and by filtering 200 mL of Milli-Q water through a 25-mm GF/C filter for particulate carbon and nitrogen each day that samples are filtered for these analyses.

Method blanks, consisting of Milli-Q water, are collected each day that samples are prepared for tests that have greater than one-month storage holding times—i.e. alkalinity, anions, cations, dissolved organic carbon, and soluble reactive silica.

6.7.4 Field duplicate

A field duplicate is collected each day that samples are submitted to the lab and is analyzed for the full suite of analyses.

6.7.5 Duplicate filtration

Duplicate filtrations to collect method duplicates for particulate tests are generally not conducted. However, duplicate filtrations are available upon request, provided a sufficient volume of sample is supplied.

6.8 Logging samples into Sample Master

Samples for each project (e.g. L239 profile) are logged in as an "order," which is assigned an Order ID in the format "yyyymmdd##" wherein the yyyymmdd represents the date that the sample was logged in. Each sample in the order is assigned a Sample ID in the format "yyyymmdd##-##" wherein the yyyymmdd## represents the Order ID. Blanks should be the first order logged in each day.

Any sample that is attempted to be collected is logged in. If that sample is not available (e.g. 239 NE@LA is not flowing), that sample is cancelled (not deleted) in the Order Details page *(Edit > Cancel Sample)*, and the reason for the cancellation is recorded. This provides a record that the collection was attempted but that the sample was not available for a given reason.

1. Login to Sample Master® and navigate to *Sample Tracking > Sample Login.*

   This page provides the metadata for the order, which includes the Order ID, Order date, user that signed it in, Customer ID, Customer Contact, Billing ID, Billing Contact, and Project ID.

2. Click *New Order ID*, select Login, and click *Submit.*

   Select the appropriate Customer ID (use *Requested Analyses* spreadsheet) and appropriate Project ID (e.g. 239 Profile). Click *Yes* when prompted with "Would you like to add samples and Tests associated with [Project ID]?"

3. Click *Samples* to retrieve the Order Details page.

   This page lists all of the samples and the associated metadata for each sample, which includes Sample ID, Customer Sample ID, Site, Matrix, Collector, Date Collected, Time Collected, Date Received, Start Depth, End Depth, Start Collect Date, End Collect Date, Rain Gauge, and Observed Precipitation Volume. Ensure that all of the appropriate samples (i.e. correct sites) were added and enter all relevant metadata.

   If the order includes a field duplicate, highlight the sample to be duplicated, hover over *Edit*, and click *Copy Sample*. Ensure that all of the boxes are clicked, except Cust. Sample ID, and click *OK*.

4. Verify that the appropriate tests are requested for each sample by selecting a sample and clicking *Tests* to retrieve the Sample Details page, which includes all of the requested tests as well as the metadata for each requested test (i.e. Method, Prep Method, Due Date, Preservative, Bottle Type, # Hauls, Equipment code, Net Diameter, and Total Depth).

5. Return to the Sample Login page, hover over *Edit*, and click *Calculate Due Dates*.

   Retrieve the Login Report by hovering over *Print* and clicking *Login Report*. Review it to make sure everything is correct. Click the download icon and save the Login Report in \\ela-camp.iisd.ca\shared\Chem Lab\Sample Master\Login Reports in the format "yyyymmdd##_LoginReport" wherein the yyyymmdd## represents the Order ID.

   Return to the Sample Login page, hover over Print, and click *Sample Labels* to retrieve the sample labels. Click the *print icon*, ensure that EasyCoder PD41 (203 dpi) – IPL is selected, and click *Print*.

6. Once all of the Orders have been logged in, retrieve the daily Sample Summary by hovering over *Print*, clicking *Summary*, selecting the appropriate Start of Range and End of Range, and clicking *OK*. Click the *print icon*, ensure that HP LaserJet 4050 is selected, and click *Print*. The Sample Summary is then used to record filtration volumes, start depths, end depths, start dates, end dates, rain gauges, observed precipitation volumes, and any other metadata (e.g. notes/comments) for each sample, which are later recorded in Sample Master.

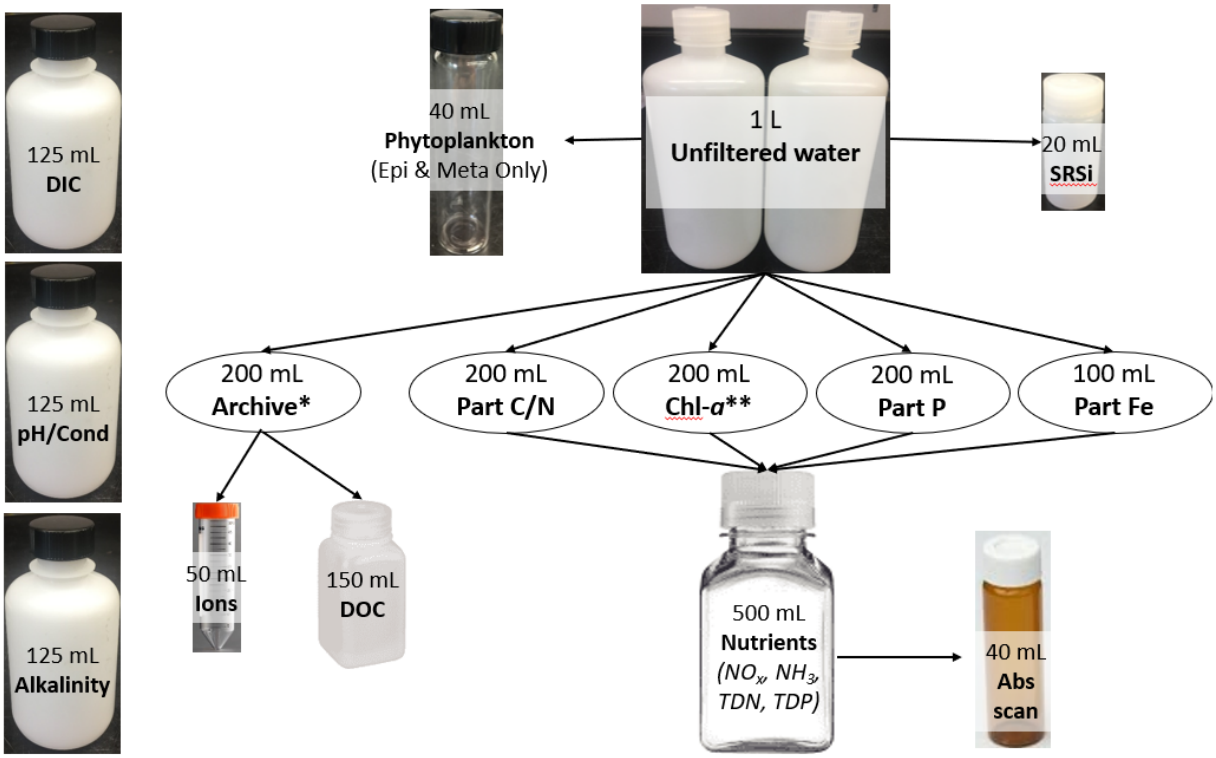
6.9 Sample preparation and filtration

1. Clients are provided with three 125-mL narrow-mouth HDPE bottles, two of which should have conical inserts in the caps, and two 500-mL narrow-mouth HDPE bottles for each sample. If the sample is anaerobic, a PETG bottle is used instead of the 125-mL HDPE bottle with the regular cap to prevent oxygen diffusion into the sample.

2. Using the labels generated by Sample Master, label each of the bottles, vials, and Petri dishes according to Figure 6.1. The three 125-mL bottles and the two 500-mL bottles filled with sample are labelled once they are received from the client.

3. Once the samples arrive, label the dissolved inorganic carbon (DIC), pH/Cond, and alkalinity samples according to Figure 6.1. Store the pH/Cond sample in the 25 °C water bath and store the DIC and alkalinity samples at 4 °C. Label the two 500-mL unfiltered water samples with "Filtration" labels and retain for subsequent filtration.
4. Turn on the vacuum line and ensure that the pressure is less than 15 ppm. Rinse each filtration apparatus with DRO water.

Figure 6.1. Sample processing and filtering flow chart



* Archive filter rinsed 100 mL Milli-Q water prior to filtering sample
**Chl-*a* not conducted on stream or precipitation sample

5. Prepare the filter blank by placing a baked, unused filter into a pre-labelled Petri dish.

   Prepare the filter method blanks for particulate iron (Part Fe) and particulate phosphorus (Part P) by filtering 100 mL of Milli-Q water through each 42.5-mm GF/C filter and for particulate carbon and nitrogen (Part C/N) by filtering 200 mL of Milli-Q water through a 25-mm GF/C filter.

   Prepare method blanks for alkalinity, anions and cations (ions), dissolved organic carbon (DOC), and soluble reactive silica (SRSi) by rinsing and filling a 125-mL HDPE bottle, a 50-mL conical tube, a 175-mL square HDPE bottle, and a 20-mL HDPE vial, respectively, with Milli-Q water.

6. Using Figure 6.1 as a guide, process each 1 L unfiltered water sample as follows:

    a. To prepare the phytoplankton taxonomy sample, invert the sample several times, rinse and fill the labelled 40-mL glass vial with the sample and preserve with 500 µL of Lugol's solution.

    b. To prepare the SRSi sample, invert the sample several times, rinse and fill the labelled 20-mL HDPE vial with the sample.

    c. To prepare the Part P sample, place a 42.5-mm GF/C filter onto the filtration apparatus and rinse with 50–100 mL of Milli-Q water into a rinse bottle. Switch the rinse bottle to the labelled 500-mL PETG bottle (Nutrients), invert the sample several times, and then filter 100 mL of sample through the filter and into the Nutrients bottle. Use this filtrate to rinse the Nutrients bottle. Using forceps, fold the GF/C filter twice lengthwise and transfer it to the labelled 15 mL vial.

    d. To prepare the Part Fe sample (if requested), place a 42.5-mm GF/C filter onto the filtration apparatus and rinse with 50–100 mL of Milli-Q water into a rinse bottle. Switch the rinse bottle to the Nutrients bottle, invert the sample several times, and filter 100 mL of the sample through the filter and into the Nutrients bottle. Retain this filtrate. Using forceps, transfer the GF/C filter to a labelled acid-washed 20-mL HDPE vial.

    e. To prepare the chlorophyll-*a* (Chl-*a*) sample, place a 42.5-mm GF/C filter onto the filtration apparatus and rinse with 50–100 mL of Milli-Q water into a rinse bottle. Switch the rinse bottle to the Nutrients bottle, invert the sample several times, and filter 200 mL of sample through the filter and into the Nutrients bottle. Retain this combined filtrate. Using forceps, transfer the GF/C filter to a labelled 50-mm Petri dish.

    f. To prepare the Part C/N sample, place a 25-mm GF/C filter onto the filtration apparatus and rinse with 50–100 mL of Milli-Q water into a rinse bottle. Switch the rinse bottle to the Nutrients bottle, invert the sample several times, and filter 200 mL of sample through the filter and into the Nutrients bottle. This combined filtrate is the Nutrients (NO_2, NO_3, NH_3, TDN, and TDP) sample. Using forceps, transfer the GF/C filter to a labelled 50-mm Petri dish.

    g. To prepare the absorbance scan sample, rinse and fill the labelled 20-mL HDPE vial with the filtered Nutrients sample.

    h. To prepare the archive filter, ions, and DOC samples, place a 42.5-mm GF/C filter onto the filtration apparatus and rinse with at least 100 mL of Milli-Q water into a rinse bottle. Invert the sample several times and measure 200 mL of sample in a graduated cylinder. Switch the rinse bottle to the labelled 50-mL conical tube (ions), filter ~10 mL of sample, and use this filtrate to rinse the ions bottle. Filter ~45 mL of sample into the ions tube. Switch the ions tube to the 175-mL square HDPE bottle (DOC), filter ~20 mL of sample, and use this filtrate to rinse the DOC bottle. Filter

the remaining sample into the DOC bottle. Using forceps, transfer the GF/C filter to a labelled 50-mm Petri dish.

7. Rinse the filtration apparatus and graduated cylinder(s) with DRO water.
8. Repeat steps 6 and 7 for all subsequent samples.
9. The Chl-*a*, Part C/N, and Archive samples all need to be dried in a vacuum desiccator prior to being stored at -20 °C.

   Prepare the vacuum desiccator by placing baked desiccant into the glass desiccator and applying vacuum grease to the rim, if needed. Place each Chl-*a*, Part C/N, and Archive sample into the desiccator by placing the lid of each Petri dish onto the bottom of the Petri dish and then stacking them in the desiccator.

   Evacuate the desiccator with the vacuum line and place it in a dark cabinet until the filters have dried (at least 24 hours).

6.10 Sample storage

Samples are analyzed within the storage holding times listed in Table 6.2, and the analysis dates are recorded in Sample Master.

The GF/C filters are dried for at least 72 hours and then the Petri dishes are reassembled.

Table 6.2. Containers, storage holding times, and storage temperature of each analytical test

Test	Container	Holding time	Storage Temp.
absorbance scan	40 amber glass vial	7 days	4°C
alkalinity	125 mL HDPE[a]	6 months	4 °C
conductivity	125 mL HDPE with conical cap	24 hours	25 °C
dissolved inorganic carbon	125 mL HDPE with conical cap	24 hours	4 °C
particulate carbon	50 mm Petri dish	12 months	-20 °C
particulate iron	20 mL HDPE	12 months	room temperature
particulate nitrogen	50 mm Petri dish	12 months	-20 °C
particulate phosphorus	15 mL glass vial with PTFE[b]-lined cap	12 months	room temperature
pH	125 mL HDPE with conical cap	24 hours	25 °C

Test	Container	Holding time	Storage Temp.
phytoplankton taxonomy	40 mL glass vial with conical cap	Indefinite[c]	room temperature
soluble reactive silica	20 mL HDPE	6 months	room temperature
anions (Cl and SO_4)	50 mL conical tube	6 months	4 °C
ammonia (as N)	500 mL PETG	48 hours	4 °C
cations (Na, Mg, K, Ca, Mn, Fe)	50 mL conical tube	6 months	4 °C
dissolved organic carbon	175 mL HDPE	6 months	4 °C
nitrate (as N)	500 mL PETG	48 hours	4 °C
nitrite (as N)	500 mL PETG	48 hours	4 °C
total dissolved nitrogen	500 mL PETG	7 days	4 °C
total dissolved phosphorus	500 mL PETG	7 days	4 °C
chlorophyll-*a*	50 mm Petri dish	12 months	-20 °C

[a] High-density polyethylene (HDPE); Anaerobic samples are stored in polyethylene terephthalate glycol (PETG) to prevent oxygen diffusion into the sample.
[b] Polytetrafluoroethylene (PTFE)
[c] Post Lugol's preservation

Archive and Chl-*a* samples are grouped together by test and date collected using white vinyl tape with the range of Sample IDs written on the tape.

Samples for Part C/N are placed in individual polyethylene bags. The samples are then grouped together in polyethylene bags, the test and range of Sample IDs are written on the outside of the bag, and the samples are stored at -20 °C.

Absorbance scan samples are stored at 4 °C inside a black polyethylene bag to eliminate light exposure.

6.11 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning.* Briefly, all equipment is cleaned by rinsing it three times with hot water and three times with DRO water.

6.12 Data entry

1. For each Order ID, Navigate to *Sample Login* > *Order Details* and enter the time collected, collector and any comments for each sample, and the start depth and end depth for integrated epilimnion and metalimnion samples, as well as the start collect date, end collect date, rain gauge, and observed precipitation volume for precipitation samples.
2. To enter GF/C lot numbers, navigate to *Sample Tracking* > *Sample Preparation*, ensure that "Unassigned Samples" is checked, and query for samples by filtering the test for Archive, Chl-*a*, Part C/N, Part Fe, and Part P.

   Click the check box to mark the appropriate sample(s), select the appropriate GF/C lot number from the drop-down menu (if available), and click *Add to Batch*.

   If not available, add the GF/C lot number by clicking *New*, entering the GFC lot number in the Prep Filter Lot field and the date the first GF/C from that lot was used in the Date Prepared field (leave Matrix, Test, Method, and Supply Name blank), and clicking *OK*.
3. The Analysis Volumes recorded in Sample Master are configured with the default volumes for the Archive, Chl-*a*, Part C/N, Part Fe, and Part P tests. If the actual volume filtered is adjusted from the default filtration volume, the Analysis Volume needs to be revised to the adjusted filtration volume.

   To revise the Analysis Volume, navigate to *Sample Tracking* > *Enter Prep Volumes* and query the samples by filtering by the appropriate Order ID(s) or Sample ID(s).

   Revise the "Analysis Volume" of any test samples that had filtration volumes that were adjusted from the default and click *Update*.

6.13 References

Brezonik, P. L., & Lee, G. F. (1966) Preservation of water samples for inorganic nitrogen analysis with mercuric chloride. *Air and Water Pollution, 10*(8), 549–553. https://www.researchgate.net/publication/17237785_Preservation_of_water_samples_for_inorganic_nitrogen_analysis_with_mercuric_chloride

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Kotlash, A.R., & Chessman, B.C. (1998). Effects of water sample preservation and storage on nitrogen and phosphorus determinations: Implications for the use of automated sampling equipment. *Water Research, 32*(12), 3731-3737. https://doi.org/10.1016/S0043-1354(98)00145-6

Moore, M. T., & Locke, M. A. (2013). Effect of storage method and associated holding time on nitrogen and phosphorus concentrations in surface water samples. *Bulletin of Environmental Contamination and Toxicology, 91,* 493–498. https://doi.org/10.1007/s00128-013-1084-6

Appendix 6A. Lugol's Solution Preparation Record

Lugol's Solution
Preparation Record

Stable for two years

Preparation date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Vol (L)	Final vol (L)	Expiry date	Analyst initials
	Potassium iodide								
	Iodine								
	Glacial acetic acid								
	Potassium iodide								
	Iodine								
	Glacial acetic acid								
	Potassium iodide								
	Iodine								
	Glacial acetic acid								
	Potassium iodide								
	Iodine								
	Glacial acetic acid								
	Potassium iodide								
	Iodine								
	Glacial acetic acid								
	Potassium iodide								
	Iodine								
	Glacial acetic acid								
	Potassium iodide								
	Iodine								
	Glacial acetic acid								
	Potassium iodide								
	Iodine								
	Glacial acetic acid								

Authorized by: Sonya Havens
Authorization date: May 2019

7.0 Outsourced Analyses

7.1 Introduction and scope

This method outlines sample storage, transport, and data management for outsourced analyses. The following analyses (Table 7.1) are outsourced to the Biogeochemical Analytical Service Laboratory (BASL) at the University of Alberta, which is accredited to ISO/IEC 17025 by the Canadian Association for Laboratory Accreditation.

Table 7.1. Analyses outsourced to the University of Alberta

alkalinity
anions (Cl and SO_4)
cations (Na, Mg, K, Ca, Mn, Fe)
particulate carbon and nitrogen
total dissolved solids
total suspended solids

7.2 Occupational health and safety

There are no occupational health and safety concerns associated with the storage and transport of the outsourced analyses.

7.3 Sample conditions and storage

Samples are logged into Sample Master®, where they are assigned a Sample ID, and all relevant metadata is recorded (see Section 6, *Sample Preparation and Filtration*). The processing and holding times described are designed for dilute Canadian Shield waters (e.g. IISD-ELA samples); samples from other sources (e.g. marine) may have other requirements. This is discussed with clients who bring samples from other sources. Sampling and adherence to IISD-ELA sampling protocols are the client's responsibility.

Samples are stored at 4 ± 2 °C and filtered within 24 hours of sample collection.

The default filtration volume for particulate carbon and nitrogen is 200 mL. If the filter clogs prior to reaching this volume, the volume filtered is reduced accordingly, and the actual filtered volume is recorded on the sample label, the Sample Summary, and in Sample Master®. Alternative filtration volumes are available upon request but are contingent on a sufficient sample being supplied by the client.

Table 7.2. Containers, storage holding times, and storage temperature of each analytical test

Test	Container	Holding time	Storage Temp.
alkalinity	125 mL HDPE[a]	6 months	4 °C
anions (Cl and SO_4)	50 mL conical tube	6 months	4 °C
cations (Na, Mg, K, Ca, Mn, Fe)	50 mL conical tube	6 months	4 °C
particulate carbon and nitrogen	50 mm Petri dish	12 months	-20 °C
total dissolved solids	500 mL HDPE	6 months	4 °C
total suspended solids	500 mL HDPE	6 months	4 °C

[a] HDPE = high density polyethylene; Anaerobic samples are stored in polyethylene terephthalate glycol (PETG) to prevent oxygen diffusion into the sample

7.4 Equipment

- refrigerator set to 4 °C for sample storage
- coolers for sample transport
- ice packs
- 25 mm GF/C glass fibre filters, baked at 500 °C for 14 hours
- 50 mm Petri dishes
- silica gel desiccant
- 50 mL conical tubes
- 500 mL high-density polyethylene (HDPE) narrow-mouth bottles
- 125 mL HDPE narrow-mouth bottles

7.5 Prepared reagents

7.5.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

7.5.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

7.6 Quality control

7.6.1 Blanks

A method blank is collected for alkalinity, anions and cations, and particulate carbon and nitrogen (Part C/N) each day that a sample is received and processed for these analyses. The alkalinity method blank consists of Milli-Q water stored in a 125 mL HDPE narrow-mouth bottle. The anions and cations method blank consists of Milli-Q water that has been filtered through a baked and rinsed 42.5 mm GF/C filter. The Part C/N method blank is a baked 25 mm GF/C filter that had 200 mL of Milli-Q water filtered through it. See Section 6, *Sample Preparation and Filtration*, for additional details on sample filtration.

7.6.2 Field duplicates

A field duplicate, wherein a sample is collected in duplicate, is collected and processed for alkalinity, anions and cations, and Part C/N each day that a sample is received and processed for these analyses.

7.7 Alkalinity

Alkalinity samples from aerobic water are collected in 125 mL HDPE narrow-mouth bottles. Alkalinity samples from anaerobic water are collected in 125 mL polyethylene terephthalate glycol bottles to prevent oxygen diffusion into the sample. Samples are stored at 4 °C until they are shipped to the BASL at the University of Alberta.

Alkalinity samples are analyzed using U.S. Geological Study *Method 09-A6.6 Alkalinity and acid neutralizing capacity* (Rounds & Wilde, 2012), and the American Public Health Association *4500-H+ B pH Value – Electrometric Method* (Baird & Bridgewater, 2017) with the Mantech PC-Titration Plus System (Mantech Inc.) and PC-Titrate instrumentation software (Mantech Inc.).

7.8 Anions and cations

Anion and cation samples are processed according to *Sample preparation and filtration*. Briefly, samples collected in 500 mL HDPE narrow-mouth bottles are filtered through a baked and rinsed 42.5 mm GF/C filter into a 50 mL conical tube with a screw-cap lid. Samples are stored at 4 °C until they are shipped to the BASL at the University of Alberta.

The anions chloride (Cl^-) and sulfate (SO_4^{2-}) are analyzed using U.S. Environmental Protection Agency (EPA) *Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography* (Pfaf et al., 1997) with the Dionex™ DX-600 Ion Chromatograph (ThermoFisher™ Dionex™).

The cations sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), manganese (Mn), and iron (Fe) are analyzed using the U.S. EPA *Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry* (Martin et al., 1994) with the Thermo Scientific ICAP-6300 Inductively Coupled Argon Plasma – Optical Emission Spectrometer (ThermoFisher™ Scientific).

7.9 Particulate carbon and nitrogen

The Part C/N samples are processed according to Section 6, *Sample Preparation and Filtration*. Briefly, samples collected in 500 mL HDPE narrow-mouth bottles are filtered through a baked and rinsed 25 mm GF/C filter. The filter with retained particulates is placed in a Petri dish and desiccated in the dark in a glass vacuum desiccator with silica gel desiccant. Once the filters are dry, the lids are attached to the Petri dish and the Petri dishes are then placed into polypropylene zipper-top bags, which are subsequently consolidated into polyethylene Whirl-Pak® bags and stored at -20 °C until they are shipped to the BASL at the University of Alberta.

Part C/N is analyzed using U.S. EPA *Method 440.0, Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis* (Zimmerman et al., 1997) with the Exeter CE 440 Elemental Analyzer (Exeter Analytical, Inc.).

7.10 Total dissolved solids and total suspended solids

Samples for total dissolved solids (TDS) and total suspended solids (TSS) are collected in 500 mL HDPE narrow-mouth bottles and stored at 4 °C until they are shipped to the BASL at the University of Alberta.

The TDS is analyzed using US EPA *Method 160.1: Total Dissolved Solids (Gravimetric, Dried at 180 °C)* (U.S. EPA, 1983a) and TSS is analyzed using US EPA *Method 160.3: Residue, Total (Gravimetric, Dried at 103-105 °C)* (U.S. EPA, 1983b).

7.11 Data entry

The lab manager at BASL will email an Excel report that includes the analytical data, the detection limit, the data analyzed, and the instrument used. The data and metadata are entered into Sample Master (see Section 5, *Laboratory Information Management System – Sample Master*). A QC Batch is not created for outsourced analyses. To enter outsourced analytical data, navigate to *Data Entry* > *Result Entry*, query for the appropriate "Parameter" and range of "Date Collected," and click *Retrieve*. Select *Results to Enter*. In the "Sample Results" tab, enter

the analytical result into Result field, the detection limit into the Report Limit field, the analysis date into both the Analysis Date and ParamAnalysisDate fields, and enter "BASL – UofAlberta" into both the Analysis Employee and ParamAnalyst fields. Ensure that the instrument and method listed in Sample Master are correct (see Table 7.3 for methods of outsourced analyses).

Once all of the data and metadata have been entered, click *Enter*. The samples will now be transferred to "Results to Validate."

Table 7.3. Method recorded in Sample Master for each outsourced analysis

Analysis	Method
gran alkalinity	UA-Alk
anions	UA-ClSO4
cations	UA-ICP
particulate carbon and nitrogen	UA-C/N
total dissolved solids	UA-TDS
total suspended solids	UA-TSS

7.12 Trend analysis

Trend analysis is conducted on each site/parameter combination to assess the data for outliers. A request is submitted to BASL to reanalyze any samples with outliers. The outlier data are either updated with the reanalysis data, retained, or rejected in Sample Master depending on the revelations of the reanalysis and subsequent investigation. All of the samples that have undergone trend analysis are "Validated" for that site/parameter combination.

To conduct a trend analysis, navigate to *Data Entry* > *Trend Analysis*, query for the appropriate site (e.g. L239 EIF) and parameter (e.g. Na), and click *Retrieve*. Mark all of the samples by clicking the box between "Parameter" and "Results" and click *Chart*. Assess the chart for outliers; record any outliers and any information about them (e.g. result is high) in the cell that represents the site/parameter combination in the Trend Analysis spreadsheet. If no outliers exist, put an "X" in the cell that represents the site/parameter combination.

Continue conducting the trend analysis for all site/parameter combinations.

Figure 7.1. Subsection of Trend Analysis spreadsheet

Site	Alk	Cl	SO4	Ca
Filter blank				
114 LA CB Epi				
114 LA CB Meta				
224 LA CB Epi				
224 LA CB Meta				
224 LA CB 5m				
224 LA CB 10m				

To "Validate" samples, navigate to *Data Entry* > *Result Entry*, query for the samples by filtering by the site and parameter combination, and click *Retrieve*. Click *Results to Validate*, mark all of the samples, except for the outliers, and click *Validate*.

7.13 Clean up

The HDPE bottles used for alkalinity, TDS, and TSS samples are scrubbed of any adhered particulate and washed at a high temperature in a dishwasher without detergent, rinsed three times with DRO water, and air dried.

7.14 References

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8.0 Dissolved Oxygen

8.1 Introduction and scope

This method is used to determine dissolved oxygen in freshwater and precipitation samples. *In situ* samples are stored at the original temperature of the sample and analyzed within three hours of sample collection using the Winkler titration method.

Dissolved oxygen concentrations are generally measured using *in situ* dissolved oxygen sensors (e.g. XRX-620, RBR Ltd., etc.), which are managed by the Lake Sampling department and are not included in Sample Master. Winkler titrations are performed on *in situ* Lake 239 profile samples monthly and used to verify the calibration of the dissolved oxygen sensors.

8.2 Method principle

The lab measurements of dissolved oxygen are based on the classic Winkler titration of oxygen that was first developed by Lajos Winkler in 1888 and later modified and validated by James Carpenter (Carpenter, 1965a, 1965b; Carritt & Carpenter, 1966). Manganous sulfate reacts with potassium or sodium hydroxide to give a white precipitate of manganous hydroxide. In the presence of oxygen, basic brown manganic oxide is formed, i.e.:

$$Mn^{+2}SO_4 + 2\ KOH = Mn^{+2}(OH)_2 + K_2SO_4$$

$$2Mn^{+2}(OH)_2 + O_2 = 2Mn^{+4}O(OH)_2$$

The addition of sulfuric acid dissolves the brown manganic oxide, yielding manganic sulfate, which reacts instantly with iodide to yield iodine, i.e.:

$$Mn^{+4}O(OH)_2 + 2H_2SO_4 = Mn^{+4}(SO_4)_2 + 3H_2O$$

$$Mn^{+4}(SO_4)_2 + 2KI = Mn^{+2}(SO_4) + K_2SO_4 + I_2$$

In effect, oxygen oxidizes Mn^{+2} to Mn^{+4}, which oxidizes I^- to I_2. Iodine is then determined titrimetrically using thiosulfate. The reaction of thiosulfate with iodine in acidic conditions is

$$2S_2O_3^{-2} + I_2 = S_4O_6^{-2} + 2I^-$$

Overall, the relation between O_2 and thiosulfate is

$$4Na_2S_2O_3 = O_2$$

Four moles of thiosulfate are titrated for each mole of molecular oxygen (O_2). Thus 1 mL of 0.025 N thiosulfate (normality is the same as molarity for thiosulfate) is equivalent to 0.025 meq of oxygen. This value is multiplied by 8 mg/meq to convert to mg O_2. When 200 mL of the original 300 mL sample is titrated, then 1 mL of 0.025 N thiosulfate equals 1 mg/L O_2.

$$200 \text{ mL}/1{,}000 \text{ mL} = 0.2 \text{ mg } O_2 \text{ and } 8 \text{ mg/meq} \times 0.025 = 0.2 \text{ mg } O_2$$

Interferences occur from other oxidants in the sample that can liberate or absorb iodine (e.g. brown precipitate in alkaline iodide and manganous reagents or dissolved organic carbon in water samples). The effect of nitrite is eliminated with the addition of sodium azide.

The thiosulfate solution is not stable and therefore must be standardized with a standard, specifically potassium iodate (KIO_3). Standardization is based on the co-proportionation reaction of iodide with iodate, thereby forming iodide:

$$KIO_3 + 5NaI + 6H^+ = 3I_2 + 3H_2O + K^+ + 5Na^+$$

One mole of iodate produces 3 moles of iodine, which are consumed by 6 moles of thiosulfate:

$$KIO_3 = 6S_2O_3^{-2}$$

Interferences occur from other oxidants in the sample that will liberate iodine. The effect of nitrite is eliminated with the addition of sodium azide.

8.3 Occupational health and safety

The NaI reagent is very alkaline and must be handled with care.

NaN_3 is very poisonous—keep away from acids.

The concentrated sulfuric acid (H_2SO_4) is very corrosive and must be handled carefully. Avoid contact with skin. Wear gloves, goggles, and a lab coat when handling. Always pour acid into water slowly while stirring. **Never pour water into acid.**

8.4 Validation

8.4.1 Operating range

0 – 20 mg/L O_2

8.4.2 Detection limit

Method detection limit	0.2 mg/L O_2

8.5 Sample conditions

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. Sampling technique is critical for this method. The sample analyzed must be

- collected in specially designed glass or polyethylene terephthalate glycol (bottles with a capacity of approximately 300 mL and ground glass stoppers with conical ends to prevent trapping air bubbles,
- collected without exposure to atmosphere,
- stored in a sealed bottle at the temperature sampled,
- analyzed within three hours of sample collection, and
- free of chemical reductants and oxidants (other than oxygen).

8.6 Equipment

- manufacturer-calibrated glass or PETG dissolved oxygen bottles with a 300-mL capacity
- burette with 0.1-mL readability
- magnetic stir plate and stir bars
- balance with 0.01-g readability
- repeater pipette, set to 4
- three 25 mL capacity Eppendorf™ Combitips—one each for manganese sulfate, alkaline iodide, and sulfuric acid
- 200-mL volumetric flask
- QC Bach Run Sequence BenchSheet

Note: It is important that each Combitip is only used for one reagent and that the caps for the reagent bottles are not mixed up. A brown precipitate in the reagents indicates that the alkaline iodide and the manganous reagents have become cross-contaminated. Reagents must be replaced if cross-contamination is suspected.

8.7 Purchased reagents

8.7.1 Sulfuric acid (H_2SO_4), ACS grade

Store in an acid cabinet at room temperature. Stable as per manufacturer's specifications.

8.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.
- Reagents and standards are stable at room temperature unless otherwise specified.

8.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

8.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

8.8.3 Manganese sulfate or manganese chloride

Dissolve

- 480 g MnSO4 4H2O, (2.15M), **or**
- 400 g MnSO4 2H2O, (2.14M), **or**
- 364 g MnSO4 H2O, (2.15M), **or**
- 425 g MnCl2 4H2O, (2.15M)

in ~500 mL Milli-Q water and dilute to 1 L with Milli-Q water. Stable for two years, or until contaminated, at room temperature.

8.8.4 Alkaline iodide azide

Dissolve either

- 500 g sodium hydroxide and 135 g sodium iodide, **or**
- 700 g potassium hydroxide and 150 g potassium iodide

In ~500 mL of Milli-Q water and dilute to 1 L with Milli-Q water. To this, add 10 g sodium azide that has been dissolved in 40 mL of Milli-Q water. Stable for two years, or until contaminated, at room temperature.

8.8.5 10% H_2SO_4

Dilute 10 mL of sulfuric acid into 100 mL of Milli-Q water. Stable indefinitely at room temperature.

8.8.6 Starch indicator

Prepare a paste of 1 g potato starch in 5 mL of Milli-Q water and add 100 mL of boiling Milli-Q water. Stable until the solution turns grey/violet at 5 ± 3 °C.

8.8.7 Sodium thiosulfate – 0.025 N

Commercially available concentrated standard solution is diluted to 0.025 N with Milli-Q water. Stable for one year.

8.8.8 Calibration standard

Add

- 12 g KI
- 12 ml 10% H_2SO_4
- 8 ml potassium iodate (KIO_3) solution.

to ~150 mL of Milli-Q water and dilute to 200 mL with Milli-Q water. Prepared daily.

8.9 Quality control

This method utilizes a calibration standard and a field duplicate. If the result of the calibration standard does not meet expectations, the thiosulfate and/or calibration standard are remade and re-tested. The quality control (QC) results are recorded in Sample Master.

8.9.1 Calibration standard

A calibration standard, consisting of a known concentration of iodine, is used to validate the thiosulfate concentration.

8.9.2 Field duplicate

A random duplicate is included in every sampling event.

8.9.3 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in *Labware Calibrations and Verifications*. Glassware is cleaned according to Section 2, *Labware Cleaning*.

8.10 Quality assurance

Proficiency testing for the dissolved oxygen analysis is not currently conducted.

8.11 Sample preparation

1. Create a QC Batch in Sample Master (see Section 5.4.2. *Laboratory Information Management System – Sample Master: Create QC Batch*) for the samples and print the Run Sequence BenchSheet.
2. Ensure that there are no air bubbles in the sample bottle and that the sample is less than three hours old.

8.12 Analysis

8.12.1 Calibration

1. Weigh 200 ± 0.1 g of the calibration standard into a 250 mL Erlenmeyer flask. Create a gentle vortex using the stir bar and stir plate and titrate with thiosulfate until colour is reduced to a pale straw yellow.
2. Add several drops of starch solution and titrate with thiosulfate until the first disappearance of the blue colour.

   Note: Adding the starch to an excessive concentration of I_2 degrades the starch. Hence it is added after the I_2 concentration has been reduced.

   Note: Iodine is present in the form of the I_3- complex. This complex decomposes to release I_2, which regenerates the blue colour with the starch indicator.

3. Record the volume of thiosulfate used, as well as any other pertinent comments, on the Run Sequence BenchSheet.

8.12.2 Analysis

1. To each water sample within a calibrated dissolved oxygen bottle, add 2 mL of manganese sulfate, followed by 2 mL of alkaline iodide azide, immediately re-stoppering the bottle after each addition and taking care not to entrap any air bubbles. Pour off the 4 mL of displaced O_2 containing sample water and invert several times to mix reagents and sample.

   Note: These reagents should be injected well below the liquid surface, with sample bottles being un-stoppered for as short a time as possible. It is at this stage that these two reagents can become cross-contaminated.

2. Permit the precipitate to settle and then mix the samples again.
3. After the precipitate has settled to the bottom of each sample a second time, inject 2 mL of sulfuric acid into each sample, immediately re-stoppering the bottle after each addition. Pour off the 2 mL of displaced O_2-free water (all of the O_2 is bound in the precipitate) and invert several times to mix.

   Note: It is essential in the above manipulations that none of the precipitate or iodine arising from acidification of the precipitate escapes from the bottle.

4. Weigh 200 ± 0.1 g of I_2 solution from a sample bottle into a 250 mL Erlenmeyer flask.
5. If the colour of the sample is pale yellow to begin with, as with low O_2 waters, continue to step 7.

   If the colour of the sample is deep orange, titrate the sample with thiosulfate until the colour is reduced to a pale straw yellow.

   Note: Some water samples are naturally yellow coloured due to organic compounds in the water. Iodine is present in the form of the I_3^- complex. When exposed to starch, this complex decomposes to release I_2, which regenerates the blue colour and provides the distinction between iodine and natural water colouring.

6. Add several drops of starch solution and titrate until the first disappearance of the blue colour.

   Note: Adding the starch to an excessive concentration of I_2 degrades the starch. Hence, it is added after the I_2 concentration has been reduced by thiosulfate.

7. Record the volume of thiosulfate used. Record any other pertinent comments on the sample or the titration. Record the volume of thiosulfate used, as well as any other pertinent comments, on the Run Sequence BenchSheet.
8. Repeat steps 4 through 7 for the remaining samples.

8.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning*. Briefly, all of the labware is cleaned by rinsing three times with hot water and three times with DRO water.

8.14 Calculations

The calculation of O_2 in samples is performed by using the "O_2 Calculation Template.xls" located at \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\O2\O2 Calculation Template.xls.

Concentrations of O_2 are calculated using equation 8.1:

Equation 8.1.

$$CO_2 = \left(\frac{V_T}{CF}\right) \times \left(\frac{V_S}{200\text{mL}}\right)$$

where V_T is the volume, in mL, of the thiosulfate titrated, V_S is the volume of sample that was titrated, and CF is the conversion factor of the standardized 0.025N thiosulfate:

Equation 8.2.

$$CF = \left(\frac{8}{CF}\right) \times \left(\frac{V_{Std}}{200\text{mL}}\right)$$

where V_{Std} is the volume of KIO_3 standard that was titrated.

This spreadsheet template should be saved to a new file name to record the data. The file name should be in the format of yyyymmdd_O2.xls (e.g. 20201203_O2.xls) and saved in the directory \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\O2.

To calculate the O_2 concentrations in the samples, fill in each of the yellow fields with the Sample ID, sample volume (i.e. weight), and volume of thiosulfate titrated.

The analysis date, analyst, and QC Batch ID are also recorded in the yellow fields of this spreadsheet.

8.15 Data entry

The O_2 concentrations of each sample, which are calculated using the "O_2 Calculation Template.xls", as well as the QC data, are entered into the Sample Master®LIMS. The completed *O2 Calculation Template* and Run Sequence BenchSheet are filed into the O2 folder.

8.16 References

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Appendix 8A. Manganese Sulfate/Chloride Preparation Record

iisd|ela experimental lakes area

Manganese Sulfate/Chloride
Preparation Record

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 8B. Alkaline Iodide Azide Preparation Record

iisd ela experimental lakes area

Alkaline Iodide Azide
Preparation Record

Stable for two years

Preparation date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Expiry date	Analyst initials
	NaOH							
	Sodium iodide							
	Sodium azide							
	NaOH							
	Sodium iodide							
	Sodium azide							
	NaOH							
	Sodium iodide							
	Sodium azide							
	NaOH							
	Sodium iodide							
	Sodium azide							
	NaOH							
	Sodium iodide							
	Sodium azide							
	NaOH							
	Sodium iodide							
	Sodium azide							
	NaOH							
	Sodium iodide							
	Sodium azide							
	NaOH							
	Sodium iodide							
	Sodium azide							

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 8C. 10% Sulfuric Acid Preparation Record

iisd|ela experimental lakes area

10% H_2SO_4
Preparation Record

Stable for two years

Preparation date	Manufacturer	Manufacturer lot number	Grade	H_2SO_4 vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 8D. Starch Indicator Preparation Record

iisd|ela experimental lakes area

Starch Indicator
Preparation Record

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 8E. Sodium Thiosulfate Preparation Record

iisd|ela experimental lakes area

0.025 N Sodium Thiosulphate
Preparation Record

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Grade	Vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 8F. Calibration Standard Preparation Record

iisd ela
experimental lakes area

Oxygen Standard
Preparation Record

Stable for one week

Preparation date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Vol (mL)	Final vol (mL)	Expiry date	Analyst initials
	KI								
	H_2SO_4								
	K bi-iodate								
	KI								
	H_2SO_4								
	K bi-iodate								
	KI								
	H_2SO_4								
	K bi-iodate								
	KI								
	H_2SO_4								
	K bi-iodate								
	KI								
	H_2SO_4								
	K bi-iodate								
	KI								
	H_2SO_4								
	K bi-iodate								

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 8G. QC Batch Run Sequence Benchsheet

Analytical Benchsheet

QC Batch ID: QC2019091805

Created Date: 09/18/2019
Created By: shavens

Test: DO
Method: ELA-DO
Instrument: Burette
Analysis Date: 09/18/2019
Analysis Employee: shavens

Sample ID	Result	Notes
2019091802-03		
2019091802-04		
2019091802-05		
2019091802-06		
2019091802-07		
2019091802-08		
2019091802-09		
Ref Standard		

9.0 Specific Conductance

9.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of specific conductance of freshwater and precipitation samples.

9.2 Method principle

Solutions of dissociated ions can conduct an electrical current. The resistance, R, of electrolyte solutions is a function of electrode surface area, A; the distance between electrodes, l; the solution temperature; and the concentration and nature of the ion in solution. For a given solution and temperature:

Equation 9.1.

$$R \alpha \frac{l}{A}$$

if l is in cm and A is in cm^2 then the resistance, R, is in ohms.

Resistance is measured with platinum electrodes using a bridge circuit with an alternating current supply at 60-3999 Hz to minimize polarization effects. Under these conditions, Ohm's law is obeyed.

A more commonly used measurement than resistance is conductance, L, measured in Siemens, S, where

Equation 9.2.

$$L = R^{-1}$$

From equations 9.1 and 9.2 we get

Equation 9.3.

$$L \alpha \frac{l}{A}$$

or

Equation 9.4.

$$L = K \frac{l}{A}$$

where K is a proportionality constant termed the specific conductance and is the observed conductance of a solution measured with a cell of unspecified geometry (A and l) normalized to the conductance one would obtain with a cell having A = 1 cm^2 and l = 1 cm. K is usually measured at 25 °C.

From equation 9.4, setting l = 1 cm, we get

Equation 9.5.

$$K = L\frac{1}{A}$$

1/A is the cell constant, which varies with the geometry of the cell (i.e. the area of electrode plates) and their separation. Commercially prepared cells are labelled with an approximate cell constant but must be calibrated before use by measuring the conductance *L* of a KCl solution of known specific conductance *K* at 25 °C and calculating from equation 9.5. Specific conductance of samples is obtained by measuring *L* for samples at 25 °C and calculating *K* using equation 9.5 and the calibrated cell constant.

In low ionic strength waters, typical of Precambrian Shield lakes, conductance is highly influenced by dissolved CO_2 levels. This method is designed to measure *in situ* conductance.

9.3 Occupational health and safety

There are no occupational health and safety concerns associated with this analysis.

9.4 Validation

9.4.1 Operating range

This is dependent on the instrument used and the cell constant of the electrode.

Cell constant = 0.1 cm^{-1}, range = 0.1 µS – 150 mS

Cell constant = 1.0 cm^{-1}, range = 1 µS – 1500 mS

9.4.2 Detection limit

Method detection limit	0.05 µS/cm
Limit of quantitation	0.16 µS/cm

9.5 Sample conditions

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must be

– collected in a manner that avoids contact with the atmosphere (i.e. an *in situ* sample) using a 125-ml polyethylene bottle with a conical cap insert that is completely filled without air bubbles,

- unfiltered,
- equilibrated to 25 ± 1 °C in a water bath,
- analyzed within 24 hours of sample collection, and
- not acidified or frozen.

9.6 Equipment

- Radiometer CDM 3
- Radiometer CDC 314 flow-through conductivity cell
- cell constant 0.316
- peristaltic pump fitted with 1.85 mm I.D. tubing
- water bath to maintain samples at 25 ± 1 °C

9.7 Prepared reagents

- Preparation information for all reagents and standards are recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.
- Reagents and standards are stable at room temperature unless otherwise specified.

9.7.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

9.7.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

9.7.3 Potassium chloride calibration standard – 0.2 N

Dissolve 14.912 g of dry KCl into 1 L of Milli-Q water. Stable at room temperature indefinitely.

9.7.4 Potassium chloride calibration standard – 0.01 N

Dilute 50 ml of 0.2 N KCl to 1 L with Milli-Q water. Specific conductance at 25 °C = 1411µS/cm (Kortum & Bockris, 1951). Stable at room temperature indefinitely.

9.7.5 Potassium chloride calibration standard – 0.001 N (L_{KCl})

Dilute 1.0 ml of 0.2N KCl to 200 ml with Milli-Q water. Specific conductance at 25 °C = 147 µS/cm (Kortum & Bockris, 1951). Stable at room temperature indefinitely.

9.7.6 Potassium chloride reference standard – 0.001 N KCl

Dissolve 14.912 g of dry KCl from a different manufacturer than the calibration standard into 1 L of Mill-Q water to make 0.2N KCl. Dilute 1.0 mL of this 0.2N KCl to 200 mL with Milli-Q water.

9.7.7 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference Sample Preparation*. The conductivity reference sample consists of water from Lake 239 that has been aged for at least one year and equilibrated to 25 ± 1 °C in a water bath.

9.8 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective action is taken before testing is resumed.

9.8.1 Blanks

A Milli-Q water blank and a DRO water blank are included at the beginning of a run. The specific conductance for blanks should be < 10 µS/cm.

9.8.2 Potassium chloride calibration and reference standards – 0.001 N

The 0.001 N KCl calibration and reference standards should have a specific conductance in the 147 µS/cm ± 5%. The calibration and reference standards should agree to within 5.0% of each other.

9.8.3 Potassium chloride calibration standard – 0.01 N

The 0.01 N KCl standard is not normally required for IISD-ELA samples. However, occasionally, a sample requiring verification at the higher range is encountered. This calibration standard is also included as a routine standard to gain experience with it. The specific conductance should be 1412 µS/cm ± 5%.

9.8.4 Reference sample

A reference sample is included at the beginning of every run. The conductivity must be within 5.0% of the expected value. Results for check sample performance are charted and assessed for drift and bias. The conductivity range for the L239 reference sample is between 30–40 µS/cm.

9.8.5 Duplicates

A random duplicate is included in each run and after every 15 samples. Duplicates must agree to within 5.0% of each other. If duplication is inadequate, samples bracketed by the duplicates are reanalyzed.

9.9 Quality assurance

Quality assurance for conductivity is maintained through annual participation in the Environment and Climate Change Canada Proficiency Testing Program (ECCC-PT). Non-compliance is defined by ECCC-PT and is reported directly to the Research Chemist. Samples analyzed during the period of non-compliance are flagged in the database.

9.10 Sample preparation

1. Place calibration standards, reference standard, blanks, and samples in the 25 ± 1 °C water bath for at least 30 minutes.

   Note: Conductivity analysis must be conducted prior to pH analysis since 200 µl of 3N KCl is added for the pH analysis.

2. Create a QC Batch in Sample Master (see Section 5, *Laboratory Information Management System – Sample Master*) for the samples and print the Run Sequence BenchSheet.
3. Secure the tubing on the peristaltic pump and tighten the clamp. Place the tubing into a beaker filled with Milli-Q water and turn the pump on.

9.11 Analysis

9.11.1 Calibration

The following steps are used to verify that the Radiometer CDM 3 is providing accurate conductivity measurements prior to analyzing samples.

1. Place the tubing into and pump the 0.001 N KCl (L_{KCl}) standard until the Radiometer stabilizes and record the conductance. The conductance should be in the 147 µS/cm ± 5%. Return the tubing to the Milli-Q water and pump until the conductance is stabilized.
2. Repeat step 1 two more times and record the average conductance in the Conductivity logbook.
3. Pump 0.001 N reference standard until the conductivity is stable and record the conductance in the *Conductivity* logbook. Return the tubing to the Milli-Q water and pump until the conductance is stabilized.
4. Repeat step 3 for the 0.01 N KCl, the reference sample, Milli-Q, and DRO. Record the conductivity of the reference sample, Milli-Q, and DRO on the Run Sequence BenchSheet in addition to the *Conductivity* logbook.

9.11.2 Analysis

1. Pump the sample into the Radiometer until the conductivity is stable and record the conductance on the Run Sequence BenchSheet.
2. Pump Milli-Q water into the Radiometer until the conductivity is stable.
3. Repeat steps 1 and 2 for each sample. Run at least one sample in duplicate and additional duplicates every 15 samples.

9.12 Clean up

The tubing is removed from the peristaltic pump and the equipment is cleaned according to Section 2, *Labware Cleaning*. Briefly, the 125 mL bottles and conical caps are cleaned by rinsing three times with hot water and three times with DRO water.

9.13 Calculations

Calculate the conductivity cell constant, K, from equation 9.6:

Equation 9.6.

$$K = \frac{1411}{L_{KCl}}$$

where 1411 is the accepted specific conductance of a 0.01 N KCl solution at 25 °C and L_{KCl} is the observed conductance of a 0.01 N KCl solution at 25 °C.

Multiply all sample conductance readings (L_s) by the cell constant obtained above to give specific conductance values for samples.

9.14 Data entry

The conductivity of each sample, as well as QC samples, are entered into the Sample Master LIMS. The completed Run Sequence BenchSheet is filed into the conductivity folder.

9.15 References

Barrow, G. M. (1966). *Physical chemistry.* McGraw-Hill.

Kortum, G., & J. O'M. (1951). *Textbook of electrochemistry* (vol. 1). Elsevier Press, Inc.

Radiometer Copenhagen. (n.d.) *CDM3 Conductivity Meter: Operating Instructions.*

Appendix 9A. 0.2 N Potassium Chloride Standard Preparation Record

iisd ela experimental lakes area

0.2 N KCl
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (L)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 9B. 0.01 N Potassium Chloride Standard Preparation Record

iisd|ela experimental lakes area

0.01 N KCl
Preparation Record

Stable indefinitely

Preparation date	Stock lab lot number	Grade	0.2 N KCl vol (mL)	Final vol (L)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 9C. 0.001 N Potassium Chloride Standard Preparation Record

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0.001 N KCl
Preparation Record

Stable indefinitely

Preparation date	Stock lab lot number	Grade	0.2 N KCl vol (mL)	Final vol (L)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 9D. 0.001 N Potassium Chloride Reference Standard Preparation Record

iisd ela experimental lakes area

0.001 N KCl Reference
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (L) 0.2 N KCl	0.2 N KCl vol (mL)	Final vol (L) 0.001 N KCl	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

10.0 Hydrogen Ion Concentration – pH

10.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory to determine the pH of fresh water and atmospheric precipitation.

10.2 Method principle

The pH of an aqueous solution is defined operationally by equation 10.1:

Equation 10.1.

$$pH = pH_s + \frac{(E - E_s)}{0.000198T}$$

Here, pH_s is the assigned pH of a buffer used to standardize the system. E and E_s are the electromotive force (EMF) of a suitable cell arrangement with unknown and buffer, respectively, and T is the temperature (Kelvin). Usually, the cell arrangement consists of a glass electrode with an electrolyte bridge to a saturated calomel electrode. Cell EMF is measured with a high-impedance voltmeter, with the usual response being 59 mV per pH unit at 25 °C.

The glass electrode assembly is fragile and requires careful use and frequent calibration to obtain meaningful results. Since electrode response may be non-linear, it is important to standardize using buffers that have a pH close to and preferably bracket the pH of samples. Suitable time must be allowed for the electrode to come to equilibrium with solutions before a reading is taken.

pH values are influenced by several variables that must be controlled to give meaningful results. These variables include temperature, CO_2, ionic strength, and the condition of the reference junction in the combination electrodes.

Temperature affects both the electrode performance (the millivolt value produced by a given hydrogen ion concentration) and the actual pH of the sample (influence on carbonate equilibrium). Most modern pH electrode/meter combinations correct for the temperature response of the electrode. However, the sample temperature must be controlled (usually to 25 °C) to provide comparable data.

CO_2 content significantly affects pH in most natural waters (pH > 4.5). Natural water samples can be oversaturated or undersaturated with respect to atmospheric CO_2 levels. Hence any gas exchange that allows samples to equilibrate with the atmosphere will change the observed pH from the "true" *in situ* value. Gain in CO_2 will lower the pH, while a loss of CO_2 will raise the pH. This problem is addressed by collecting, storing, and analyzing samples with minimal exposure to the atmosphere.

Ionic strength influences the stability of pH electrode performance. Low ionic strength water, typical of lakes of the Precambrian Shield, can be a particularly difficult medium to measure pH. Changes in streaming potential generated at the reference junction through stirring or turbulence produce an unstable electrode response. This problem is addressed through the addition of KCl, which raises the ionic strength of the sample without changing the pH. Electrode performance in dilute waters is validated by measuring the pH of a 100 mL Milli-Q water sample containing 200 µl of 3N KCl and equilibrated with the atmosphere. For an atmospheric CO_2 concentration of 350 ppm, the pH should be 5.65.

Essentially, pH electrode problems can be traced to problems with their reference junction, the semi-permeable linkage between the reference electrode filling solution, and the glass electrode filling solution. This junction can be either ineffective (poor electrical contact causing a slow or erratic response) or can change following a calibration, essentially establishing a new reference electrode potential (electrode slope remains the same but different absolute voltage and hence pH). The reference junction may be affected by the growth of biological material (algae, bacteria, or fungi) or contaminated by samples or buffers. Problems with reference junctions have been largely eliminated by electrode designs that allow for quick cleaning and the re-establishment of a "new" reference junction (e.g. the Orion "Sur Flo" electrode).

10.3 Occupational health and safety

There are no occupational health and safety concerns associated with this analysis.

10.4 Validation

10.4.1 Operating range

1–14 pH units

10.4.2 Detection limit

Method detection limit	N/A
Limit of quantitation	N/A

10.5 Sample condition

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must be

- collected in a manner that avoids contact with the atmosphere (i.e. an *in situ* sample),
- stored in a 125-ml polyethylene bottle with a conical cap that is filled completely without air bubbles,
- unfiltered,
- equilibrated to 25 ± 1 °C in a water bath,
- analyzed within 24 hours of sample collection, and
- not acidified or frozen.

10.6 Equipment

- Orion™ Ross™ type "Sur Flow" electrode. This electrode is fitted with a silicon stopper that allows the electrode to be inserted into the sample bottles while maintaining an effective seal against atmospheric invasion. This stopper must be equipped with a vent hole through which the sample is allowed to escape when the electrode is inserted into the sample bottles. Without this vent hole, a positive pressure would develop inside the sample bottle, forcing some of the sample into the electrode reference junction. This would invalidate the electrode calibration.
- Fisher Scientific "Accumet" meter with multi-point calibration
- 200 µl pipette
- water bath set to 25 ± 1 °C
- stir plate and small Teflon stir bars
- Run Sequence BenchSheet

10.7 Purchased reagents

10.7.1 Calibration buffer solution, pH 4.00

Stable at room temperature until the expiry date on the manufacturer's label.

10.7.2 Calibration buffer solution, pH 6.00

Stable at room temperature until the expiry date on the manufacturer's label.

10.7.3 Calibration buffer solution, pH 8.00

Stable at room temperature until the expiry date on the manufacturer's label.

10.7.4 Electrode storage buffer solution, pH 7.00

Stable at room temperature until the expiry date on the manufacturer's label.

10.7.5 Orion Ross pH electrode filling solution

Stable at room temperature indefinitely.

10.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.
- Reagents and standards are stable at room temperature unless specified otherwise.

10.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

10.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

10.8.3 3N KCl solution

Dissolve 223.68 g of dry KCl into 1 L of Milli-Q water. Stable at room temperature indefinitely.

10.8.4 Electrode storage solution

Dilute 100 mL of pH 4.00 buffer into 400 mL of Milli-Q water.

10.8.5 Reference sample

Reference samples are prepared according to Section 4, *Reference Sample Preparation*. Briefly, the pH reference sample consists of water from Lake 239 that has been aged for at least one year, equilibrated to 25 ± 1 °C, and spiked with 200 µl 3N KCl.

10.9 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective action is taken before testing is resumed.

10.9.1 Blanks

A Milli-Q water blank and a DRO water blank are included at the beginning of a run.

10.9.2 Reference sample

A reference sample is included at the beginning of every run. The pH must be within 5.0% of the expected value. Results for check sample performance are charted and assessed for drift and bias.

10.9.3 Duplicates

A random duplicate is included in each run and after every 15 samples. Duplicates must agree to within 5.0% of each other. If duplication is inadequate, samples bracketed by the duplicates are reanalyzed.

10.10 Quality assurance

Quality assurance for pH is maintained through annual participation in the Environment and Climate Change Canada Proficiency Testing Program (ECCC-PT). Non-compliance is defined by ECCC-PT and is reported directly to the Research Chemist. Samples analyzed during the period of non-compliance are flagged in the database.

10.11 Sample preparation

1. Place calibration buffers, blanks, and samples in the 25 ± 1 °C water bath for at least 30 minutes.
2. Blanks and samples are spiked with 200 µl of 3N KCl prior to pH measurement.

   Note: Conductivity analysis must be conducted prior to adding KCl to the sample.
3. Create a QC Batch in Sample Master® (see Section 5, *Laboratory Information Management System – Sample Master*) for the samples and print the Run Sequence BenchSheet.

10.12 Analysis

10.12.1 Calibration

1. Flush the reference junction of the electrode with electrode filling solution by pressing the spring-loaded top of the electrode (see Section 10.16 for additional information).
2. Fill the reference chamber to just below the fill hole with electrode filling solution, rinse the outside of the electrode with DRO water, and wipe dry with a Kimwipe™. The fill hole must be uncovered during measurements.
3. Touch the screen to wake it up. Click the *Std* button. Click *Clear* to clear the previous standards.
4. Place a stir bar and the electrode into the pH 8.00 buffer and turn on the stir plate. Click *Std* twice to start calibrating the first standard (pH 8.00). Enter the standard's pH (e.g. 8.000) when prompted.
5. Record the mV, slope (%), and standard pH (i.e. what it reads once you enter in 8.000) in the pH logbook (Table 10.1).
6. Repeat steps 4 and 5 for pH 6.00 and pH 4.00, rinsing the electrode with DRO water and drying with a Kimwipe in between standards. The mV for pH 8.00, 6.00, and 4.00 should be around -59, 59, and 177 mV, respectively, but more importantly, the slope should be between 92-102%.
7. Once the pH meter has been calibrated with the three standards, measure each of the buffers by clicking *Measure*. Record the measured reading in the pH logbook. The measured reading must be ± 0.02 of the standard. If it is not, clear the standards and start over.
8. Record the water bath temperature (°C) in the pH logbook (Table 10.1).

Table 10.1. Calibration parameters to record in the pH logbook

	mV	Slope (%)	Standard	Measured reading	QA/QC	Water bath temp (°C)
pH 8.00					DRO:	
pH 6.00					MQ:	
pH 4.00					L239:	

10.12.2 Analysis

1. Place a stir bar in the KCl-spiked DRO water and measure the pH by placing the electrode into the bottle and clicking *Measure*. Once stabilized, record the measured pH in the pH logbook and in the Run Sequence BenchSheet.
2. Repeat step 1 for Milli-Q water and the reference sample (L239), rinsing the electrode with DRO water and drying with a Kimwipe in between samples.
3. Place a stir bar into the KCl-spiked sample and measure the pH by placing the electrode into the bottle and clicking *Measure*. Once stabilized, record the measured pH on the Run Sequence BenchSheet.
4. Repeat step 3 for each sample, rinsing the electrode with DRO water and drying with a Kimwipe in between samples.
5. Duplicate the measurement of at least one sample in the run and make additional duplicate measurements every 15 samples.

10.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning.* Briefly, the 125 mL bottles and conical caps are cleaned by rinsing three times with hot water and three times with DRO water. The outside of the electrode is rinsed with DRO water, dried with a Kimwipe, and stored in the electrode storage solution with the fill hole sealed.

10.14 Calculations

No additional calculations are required.

10.15 Data entry

The pH of each sample, as well as QC samples, are entered into the Sample Master LIMS by querying for the QC Batch ID. The completed Run Sequence BenchSheet is filed into the pH folder.

10.16 Maintenance

Use caution when handling the Orion "Sur Flow" pH electrode to avoid damaging the surface of the reference junction. Scratches or KCl deposits on the junction surface can affect the instrument's ability to equilibrate with the sample solution and may influence results. Prior to starting up each day, carefully inspect the electrode for scratches or salt buildup. Use a Kimwipe to clear away any salt or bacterial matter that may be accumulating on the reference junction. Press the top of the electrode to open the seal of the junction and flush it with electrode filling solution. With the top of the electrode held down, a Kimwipe can be used to clean the inner surface of the electrode thoroughly. Once the reference junction has been properly cleaned, the electrode must be filled with electrode filling solution.

To prevent bacteria from growing on the electrode, regularly change the pH 7.00 buffer solution in which the electrode is stored. Periodic soaking in a weak acid solution followed by thorough washing with DRO water will help to alleviate problems with bacterial growth on the reference junction.

Appendix 10A. 3 N Potassium Chloride Solution Preparation Record

iisd|ela experimental lakes area

3 N KCl
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (L)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 10B. Electrode Storage Solution Preparation Record

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Electrode Storage Solution
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	pH 4 buffer vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 10C. QC Batch Run Sequence Benchsheet

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Analytical Benchsheet

QC Batch ID: QC2019091805

Created Date: 09/18/2019
Created By: shavens

Test: DO
Method: ELA-DO
Instrument: Burette
Analysis Date: 09/18/2019
Analysis Employee: shavens

Sample ID	Result	Notes
2019091802-03		
2019091802-04		
2019091802-05		
2019091802-06		
2019091802-07		
2019091802-08		
2019091802-09		
Ref Standard		

11.0 Dissolved Inorganic Carbon

11.1 Introduction and scope

This method is used to determine dissolved inorganic carbon (DIC) in freshwater and precipitation samples. *In situ* samples are stored at 4 °C and analyzed within 24 hours of sample collection using a Li-Cor 850 CO_2/H_2O gas analyzer.

11.2 Method principle

Samples containing H_2CO_3, HCO^{-3}, CO_2 and CO_3^{-2} are acidified using a non-volatile acid converting carbonate and bicarbonate to CO_2. The CO_2 is extracted into a nitrogen stream and measured as CO_2 on an infrared carbon dioxide analyzer.

11.3 Occupational health and safety

The concentrated sulfuric acid (H_2SO_4) is very corrosive and must be handled carefully. Avoid contact with skin. Wear gloves, goggles, and a lab coat when handling and diluting. Always pour acid into water slowly while stirring. Never pour water into acid.

11.4 Validation

11.4.1 Operating range

0–3,000 µM

11.4.2 Detection limit and limit of quantitation

The detection limit, which is based on the residuals along the standard curve, is calculated for each run using equations 11.1 through 11.3:

Equation 11.1

$$C_{dl} = \frac{y_d - b}{m}$$

where b is the y-intercept, m is the slope, and y_d is the instrument response detection limit, which is calculated using equation 11.2:

Equation 11.2.

$$y_d = 3s_y + b$$

Where s_y is based on the residuals between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration and is calculated using equation 11.3:

Equation 11.3.

$$s_y = \sqrt{\frac{\sum di^2}{n-2}}$$

where n is the number of standards in the calibration curve, and di is the difference between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration.

The limit of quantitation is calculated in the same manner as the detection limit, except equation 11.2 is replaced with equation 11.4:

Equation 11.4.

$$y_q = 10s_y + b$$

The averages of the detection limits and limits of quantitation for samples run between 2019 and 2021 were as follows:

Detection limit	7.6 ± 3.8 µmol/L
Limit of quantitation	25.2 ± 12.8 µmol/L

11.5 Sample conditions

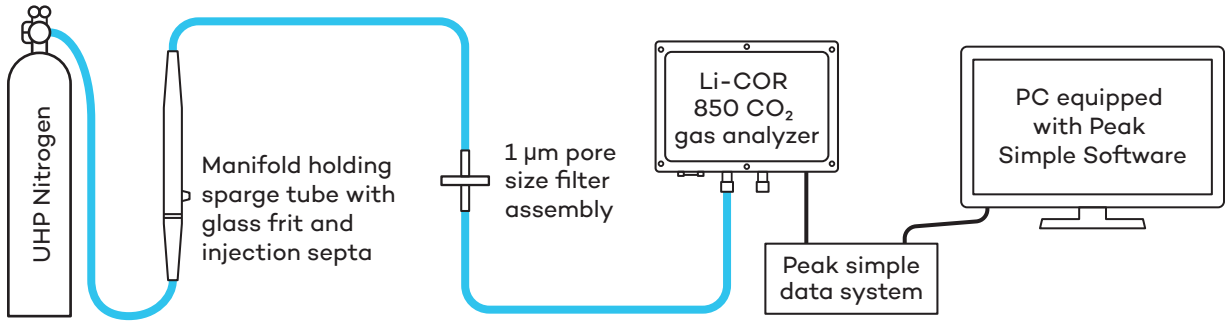
Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must be

- collected in a manner that avoids contact with the atmosphere (i.e. an *in situ* sample),
- stored in a 125-ml polyethylene bottle with a conical cap insert that is filled completely without air bubbles,
- unfiltered,
- stored at 4 ± 2 °C,
- analyzed within 24 hours of sample collection, and
- not acidified or frozen.

11.6 Equipment

- LI-850 Li-Cor CO_2 analyzer
- manifold with sparge tube plumbed to ultra-high-purity (UHP) nitrogen gas and CO_2 analyzer (Figure 11.1)
- a tank of UHP nitrogen (carrier gas) and regulator (CGA 580)
- a tank of CO_2 balanced with nitrogen and regulator (CGA 320) with an outlet line fitted with a septum for collecting CO_2 gas standards with a syringe
- 1 µm filter assembly
- several syringes (1.0 ml to 3.0 ml); one set is used for gas standards, and the other set is used for the liquid samples
- Peak Simple integration system to measure peak areas

Figure 11.1. Schematic of dissolved inorganic carbon analytical system



11.7 Purchased reagents

11.7.1 Calibration CO_2 gas standard

Tank of 1700–2000 ppm CO_2, balanced with N_2. Stable at room temperature indefinitely.

11.7.2 UHP nitrogen

A tank of UHP nitrogen gas. Stable at room temperature indefinitely.

11.7.3 Sulfuric acid, ACS grade

Store in an acid cabinet at room temperature. Stable as per manufacturer's specifications.

11.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.
- Reagents and standards are stable at room temperature unless otherwise specified.

11.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

11.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

11.8.3 0.01 N Sulfuric acid

Caution: ALWAYS pour acid into water slowly while stirring. NEVER pour water into acid.

Slowly pour 8.3 mL of concentrated ACS-grade sulfuric acid into ~975 mL of Milli-Q water. Cool and dilute to 1 L with Milli-Q water. Store at room temperature. Stable indefinitely.

11.8.4 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference Sample Preparation*. Briefly, the DIC reference sample consists of water from Lake 239 that has been aged for at least one year. The reference sample is stored at 4 ± 2 °C.

11.9 Quality control

This method has a number of conditions for calibration standards, reference standards, and reference samples that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective

action is taken before testing is resumed. If reanalysis is not possible, the samples are cancelled in Sample Master, with the non-conformance issue recorded during cancellation.

11.9.1 Calibration curve

Calibration standards are run at the beginning and end of the run. Each standard is injected in duplicate, which must be within 5.0%. The linear correlation coefficients obtained from the calibration standards must have an $R^2 \geq 0.995$ with a first order fit.

11.9.2 Reference sample

A reference sample is included at the beginning of every run. The concentration obtained must be within 5.0% of the expected value. Results for check sample performance are charted and assessed for drift and bias.

11.9.3 Duplicates

Each standard and sample is injected in duplicate, and the resulting peak areas must be within 5.0%. A random duplicate, wherein two duplicate injections are conducted, is included in each run and after every 15 samples. Duplicates must agree to within 5.0% of each other. If duplication is inadequate, samples bracketed by the duplicates are reanalyzed.

11.9.4 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verifications*. Glassware is cleaned according to Section 2, *Labware Cleaning*.

11.10 Quality assurance

Quality assurance for DIC is maintained through annual participation in the Environment and Climate Change Canada Proficiency Testing Program (ECCC-PT). Non-compliance is defined by ECCC-PT and is reported directly to the Research Chemist.

11.11 Sample preparation

Ensure that there are no air bubbles in the sample bottle and that the sample is less than 24 hours old.

11.12 Analysis

1. Ensure the septum is sealed properly on the manifold and the LI-850 has been on for at least 30 minutes.
2. Create a new folder in the \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\DIC directory with the yyyymmdd_DIC format (e.g. 20200129_DIC).
3. Open the *PeakSimple* icon on the desktop
4. Open the control file (*File* > *Open control file*…) and open the DIC.con file located either on the desktop or at \\ela-lab.ca\shared\Chem Lab\Analysis Results\DIC.
5. Edit the default data file path field (*Edit* > *Overall*…) to equal the new directory name created in step 2. This tells the software where to save the files.
6. Edit the Post Run settings (*Edit* > *Channels* > *PostRun* (of channel1):
    a. Edit the *Save file* as field to yyyymmdd_DIC01.chr.
    b. Edit the *Results log* field to yyyymmdd_DIC
7. Save control file (*File* > *Save control file*…) as DIC.con (yes on overwrite).
8. Turn on N_2 and CO_2 gases. The manifold should read ~100 psi, and N_2 should read ~11 psi.
9. Open the Excel template ("DIC calculation template.xls") on the desktop. Enter the lab barometric pressure (inHg × 25.4 = mmHg) and lab air temperature. Then save the file as yyyymmdd_DIC.xls in the folder you created for that day.
10. Click the spacebar to start the run.
11. Inject 0.5 mL 0.01N H_2SO_4 into the column.
12. Using a syringe, inject a series of CO_2 gas standard volumes (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL) in duplicate into the manifold, ensuring that the standard is not warmed by hands/fingers and that each standard volume is replicated within 5.0%. Label the components as "0.5 std", etc. Copy the results (*View* > *Results*) to the calculation template. Ensure that the standard curve has an $R^2 \geq 0.995$.
13. Inject 0.25 mL of the L239 reference sample, in duplicate. Ensure that the replication is within 5.0% and the L239 reference sample concentration is within 5.0% of the expected value. Record the L239 reference sample concentration in the *Reference Sample* log \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Reference sample log.xls.
14. Inject 0.25 mL of each sample, in duplicate, with replication within 5.0%. Label the components with the sample number (e.g. 2020012902-01). If the peak area of the samples is not within the range of the calibration curve, adjust the injection volume accordingly and add the volume injected to the component label (e.g. 2020012902-01 1.0 mL). **The sparging column can only handle a maximum cumulative volume of 2.0 mL of liquid.** Once 2.0 mL of liquid has been injected into the column, remove the liquid through the septum using a syringe and inject another 0.5 mL 0.01N H_2SO_4 into the column.

15. Analyze at least one sample in duplicate (i.e. two duplicate injections) for every 15 samples.

Table 11.1. Typical sequence of standards, reference samples, and samples. A sample is duplicated at least every 15 samples, and a standard curve is run at the beginning and end of the run

Inject. #	Sample description
1–2	0.25 mL calibration standard
3–4	0.5 mL calibration standard
5–6	1.0 mL calibration standard
7–8	1.5 mL calibration standard
9–10	2.0 mL calibration standard
11–12	2.5 mL calibration standard
13–14	3.0 mL calibration standard
15–16	reference sample
17–18	sample 1
19–20	sample 2
21–22	sample 3
23–24	sample 4
25–26	sample 5
27–28	sample 6
29–30	sample 7
31–32	sample 8
33–34	sample 9
35–36	sample 10
37-38	sample 11
39-40	sample 12
41-42	sample 13
43-44	sample 14
45-46	sample 15
47-48	random duplicate from sample 1-15
49-50	sample 16
51-52	sample 17
n-n+1	...etc., running a duplicate every 15 samples
n-n_{+1}	reruns of problem samples
n-n_{+1}	0.25 mL calibration standard
n-n_{+1}	0.5 mL calibration standard
n-n_{+1}	1.0 mL calibration standard
n-n_{+1}	1.5 mL calibration standard
n-n_{+1}	2.0 mL calibration standard
n-n_{+1}	2.5 mL calibration standard
n-n_{+1}	3.0 mL calibration standard

16. Remove the liquid from the column and inject another 0.5 mL 0.01N H_2SO_4 into the column. Repeat step 12 with gas standards.
17. End the PeakSimple run by pressing *ctrl* + *end* on the keyboard.
18. Ensure that the files are saved.
19. Remove the acid from the column and turn off the gases.

11.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning.* Briefly, the sample bottles, volumetric flasks, etc. are cleaned by rinsing three times with hot water and three times with DRO water. Do not dispose of samples until the results are deemed satisfactory or the sample is more than 24 hours old.

11.14 Calculations

The calculation of DIC in samples is performed by using the "DIC Calculation Template.xls" worksheet located at \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\DIC\DIC Calculation Template.xls or on the Desktop.

The concentration of CO_2 in the DIC standards is calculated using equation 11.5, which utilizes the ideal gas law (i.e. PV = *n*RT):

Equation 11.5.

$$C = \left(\frac{PV}{RT}\right)$$

where C is the concentration of CO_2 in the standard, P is the barometric pressure (atm) of the room, V is the volume (L) of gas injected, R is the ideal gas constant (L·atm/mol·K), and T is the room temperature (K).

The concentration of DIC in the samples is calculated using equation 11.6:

Equation 11.6.

$$C = \left(\frac{A_{samp} - b}{m}\right) \times \left(\frac{V_{samp}}{0.5}\right)$$

Where C is the concentration of the sample, A_{samp} is the sample peak area, V_{samp} is the volume of sample injected into the column, b is the intercept, and m is the slope of the calibration curve. The sample concentration is reported to the nearest integer.

11.15 Data entry

The DIC concentrations for each sample, which are calculated using the *DIC Calculation Template*, as well as the QC data, are entered into the Sample Master LIMS to the nearest integer. The completed *DIC Calculation Template* is printed and filed into the DIC folder.

Appendix 11A. 0.01 N Sulfuric Acid Preparation Record

iisd|ela experimental lakes area

0.01 N H_2SO_4
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	H_2SO_4 vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

12.0 Dissolved Organic Carbon – Non-Purgative Organic Carbon (NPOC)

12.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of dissolved organic carbon (DOC) in fresh water and precipitation.

12.2 Method principle

Filtered water samples (Whatman GF/C filters or otherwise specified) are acidified with HCl to a pH of 2–3. The inorganic carbon component is removed by sparging with hydrocarbon-free ultra-high-purity (UHP) compressed air. The remaining carbon in the sample is oxidized at 720 °C in a combustion tube to carbon dioxide. The UHP air carries the combustion products to a dehumidifier to cool and dehydrate. Subsequently, a halogen scrubber removes chlorine and other halogens. A non-dispersive infrared analyzer then measures the carbon dioxide content in the gas and an analog detection signal (peak) is produced.

12.3 Occupational health and safety

12.3.1 Acids and oxidants

This method requires the use of strong acids and oxidants. Hand and eye protection must be used when mixing and handling these reagents. Mixing should be performed in the fume hood.

12.3.2 Compressed gas

This method requires the use of compressed air to remove dissolved inorganic carbon (DIC) from samples and carry CO_2 from DOC oxidation into the infrared detector. The cylinder in use must be properly secured vertically at all times.

12.4 Validation

This method is fit for the determination of DOC in fresh water. The method is still under validation in terms of the recovery of carbon from various DOC sources.

12.4.1 Operating range

40.8–2,000 µM

12.4.2 Detection limit and limit of quantitation

The detection limit, which is based on the residuals along the standard curve, is calculated for each run using equations 12.1 through 12.3:

Equation 12.1.

$$C_{dl} = \frac{y_d - b}{m}$$

where b is the y-intercept, m is the slope, and y_d is the instrument response detection limit, which is calculated using equation 12.2:

Equation 12.2.

$$y_d = 3s_y + b$$

where s_y is based on the residuals between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration and is calculated using equation 12.3:

Equation 12.3.

$$s_y = \sqrt{\frac{\sum di^2}{n-2}}$$

where n is the number of standards in the calibration curve, and di is the difference between the measured instrument reponse for each standard concentration and the calibration curve-predicted response for each standard concentration.

The limit of quantitation is calculated in the same manner as the detection limit, except equation 12.2 is replaced with equation 12.4:

Equation 12.4.

$$y_q = 10s_y + b$$

The averages of the detection limits and limits of quantitation for samples run between 2018 and 2018 were

Detection limit	40.8 ± 9 µmol/L
Limit of quantitation	136 ± 30 µmol/L

12.5 Sample conditions

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must be

- field sampled in a Nalgene polyethylene bottle,
- filtered within 24 hours,
- stored in pre-washed polyethylene bottles (see Section 2, *Labware Cleaning*),

- stored at 4 ± 2 °C, and
- stored for no more than six months after filtration.

The alkaline buffering capacity of the sample must not exceed the acid buffering capacity of the hydrochloric acid added. An ideal pH of 2–3 must be achieved to purge inorganic carbon out of the sample before analysis. Failure to add adequate acid will result in false high values for DOC. DOC is converted to CO_2 within the combustion tube of the system. Hence, any remaining inorganic carbon within the sample is accounted for as the total DOC of the sample.

12.6 Equipment

- Shimadzu TOC-VCPH + TNM-1 Total Organic Carbon analyzer (TOC)
- Shimadzu ASI-V auto sampler connected to the TOC-VCPH analyzer through a proprietary cable (ASI)
- compressed hydrocarbon and CO_2-free Air cylinder (UN 1002). *(There is too much oil vapour in the filtered air lines installed in the building.)*
- compressed air regulator (CGA-590, dual-stage, stainless steel diaphragm)
- a computer running Windows XP, or higher, with TOC Control-V installed. The computer is connected to the analyzer via a standard serial cable (RS232)
- 24-mL glass vials
- vial racks for auto sampler
- polytetrafluoroethylene-coated septa and plastic caps for vials
- *DOC Batch Identification Worksheet* (see Appendix 12F *DOC Worksheet Shimadzu Full Run*).

12.7 Purchased reagents

12.7.1 Compressed air, UHP grade

A tank of ultra-high-purity compressed air. Stable at room temperature indefinitely.

12.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.

- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.
- Reagents and standards are stable at 4 °C unless otherwise specified.

12.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from a Thermo Scientific Barnstead D8902 Ion Exchange Cartridge, Mixed-Bed (> 15 MΩ·cm purity). The feedwater for the D8902 Ion Exchange Cartridge is effluent from a Culligan Reverse Osmosis System.

12.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

12.8.3 2 N Hydrochloric acid

Dilute 85 mL of concentrated HCl to a final volume of 500 mL with Milli-Q water. Stable indefinitely at room temperature.

12.8.4 Calibration standard stock – 0.1 M C

Dry potassium hydrogen phthalate ($KC_8H_4O_4$) for four hours at 100 °C in an oven. Place in a desiccator after drying and cool for at least five minutes. Dissolve 2.54022 g of $KC_8H_4O_4$ into 1 L of Milli-Q water. Stable for one year.

12.8.5 Reference standard stock – 0.1 M C

Dry urea (NH_2CONH_2) for four hours at 100 °C in an oven. Place in a desiccator after drying and cool for at least five minutes. Dissolve 6.05526 g of NH_2CONH_2 into 1 L of Milli-Q water. Stable for one year.

12.8.6 DOC calibration standard curve

Seven DOC standards are prepared according to Table 12.1 in 250 mL quantities, with the exception of the 1000 µM standard, which is prepared in a 500 mL quantity. Stable for one week.

Table 12.1. Dissolved organic carbon calibration standards

Cal std vol (µL)	Final vol (mL)	DOC conc. (µM)
0	250	0
250	250	100
750	250	300
1250	250	500
5000	500	1000
3750	250	1500
5000	250	2000

12.8.7 Reference standard – 1000 µM C

The reference standard is prepared by diluting 2.5 mL of the 0.1 M reference standard stock to 250 mL with Milli-Q. Stable for one year.

12.8.8 Reference sample

Reference samples are prepared according to Section 4, *Reference Sample Preparation*. Briefly, the reference sample consists of water from Lake 239 that has been aged for at least one year at 4 °C.

12.9 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective action is taken before testing is resumed.

12.9.1 Physical inspection

At the beginning of each run, all sample and reagent tubing, as well as the syringe, will be visually inspected for obstructions, residue, etc.

12.9.2 Blanks

Three blanks, consisting of Milli-Q water, are included at the end of the beginning of a run to clear out any residual carbon in the system. Additional blanks are included after the reference standard every 15–20 samples and immediately preceding the second calibration curve.

12.9.3 Reference standard

A reference standard is included at the end of the first calibration curve. These must be within 5.0% of the calibration standard of the same concentration.

12.9.4 Calibration curve

The linear correlation coefficients obtained from the calibration standards must have an $R^2 \geq 0.995$ with a first order fit. If necessary, new calibration standards should be prepared.

12.9.5 Reference sample

A reference sample is included toward the beginning of every run. The DOC concentration obtained must be within 5.0% of the expected value. Results for check sample performance are charted and assessed for drift and bias.

12.9.6 Drift standard

A 1000 µM standard is run every 15–20 samples to assess drift. All 1000 µM standards, including those in the calibration curve, must agree within 5.0%.

12.9.7 Duplicates

Random duplicates are run every 15–20 samples. Samples for duplicate analysis are chosen at the beginning of the analysis run and must agree to within 5.0% of each other. If duplication is inadequate, samples bracketed by the duplicates are re-tested. When dilutions are required, a duplicate of each dilution factor is included.

12.9.8 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verifications*. All glassware is cleaned according to Section 2, *Labware Cleaning*.

12.10 Quality assurance

Quality assurance for DOC is maintained through bi-annual participation in the Environment and Climate Change Canada Proficiency Testing Program (ECCC-PT), as well as the Canadian Association for Laboratory Accreditation Inc. (CALA). Non-compliance is defined by these external agencies and is reported directly to the Research Chemist. Samples analyzed during the period of non-compliance are flagged in the database.

12.11 Sample preparation

1. Turn on the Shimadzu TOC-VCPH + TNM−1 instrument using the power button on the front of the machine at the bottom right and allow the instrument to warm up for 45–60 minutes.
2. Fill out the DOC Batch Identification Worksheet with the appropriate vial positions of each sample. Be sure to include the date and analyst's name. See Appendix 12F, *DOC Worksheet Shimadzu Full Run* for a typical full run sequence.

Figure 12.1. TOC-Control V program options

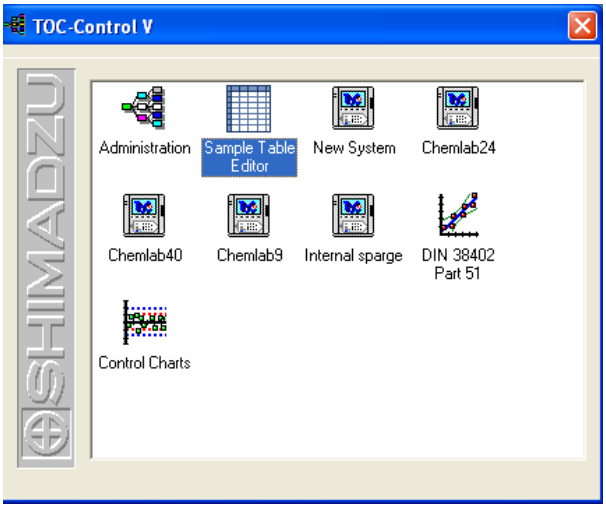
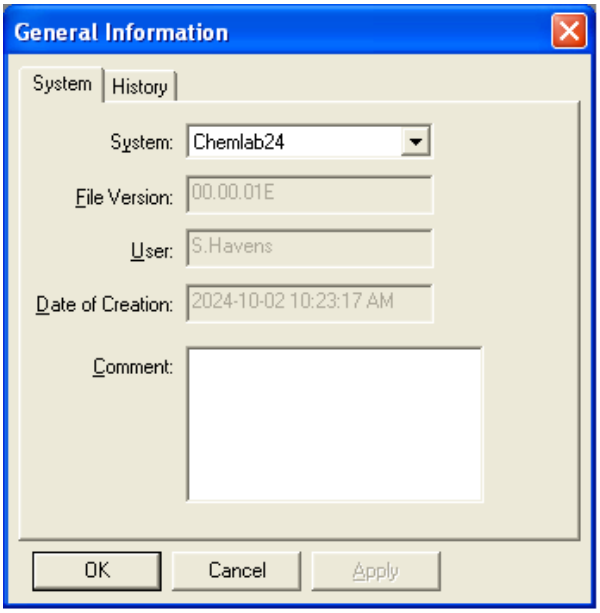


Figure 12.2. Vial type selection



3. Set up the rack on the auto-sampler according to the DOC Batch Identification Worksheet as follows:

    a. Rinse each 24-mL glass vial with its respective standard or sample.

    b. Fill vials to approximately 20 mL with standard or sample (just above the height of the rack). Filling the vial too full can result in splashing when the standard or sample is sparged.

    c. With the use of forceps or sterile nitrile gloves, place a Teflon-coated septum in the cap with the Teflon side facing the sample. The Teflon septa can be reused even after having been perforated, provided the perforation is of reasonable size (less than 4 mm in diameter).

        i. **Do not use parafilm to seal the vials.** Parafilm sticks to the needle upon acid addition, causing the vials to lift from position and break when the needle ascends.

Figure 12.3. Method selection

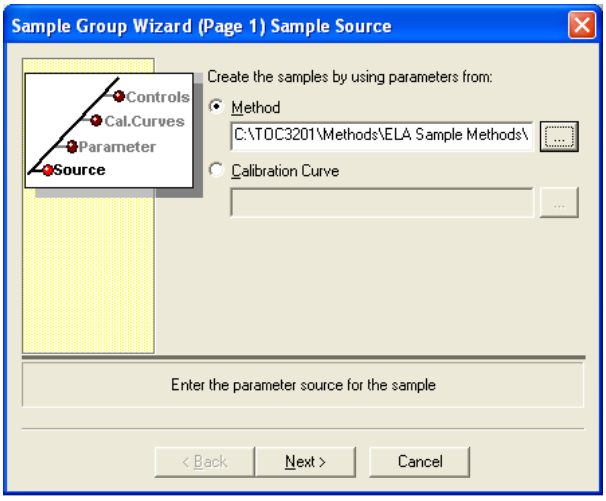


Figure 12.4. Sample Parameters window

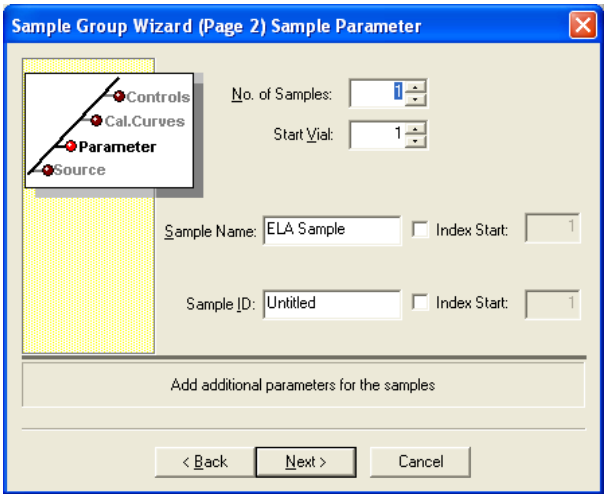


Figure 12.5. Calibration Curves window

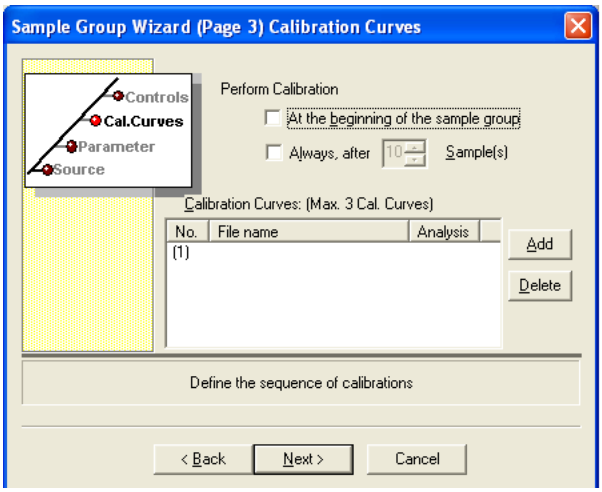


Figure 12.6. Calibration Curve Check window

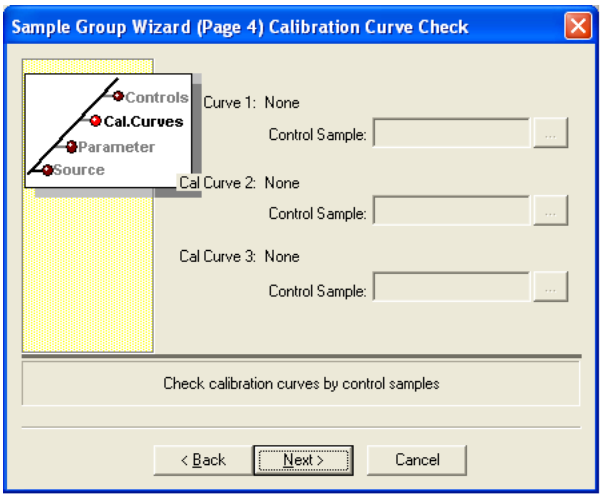
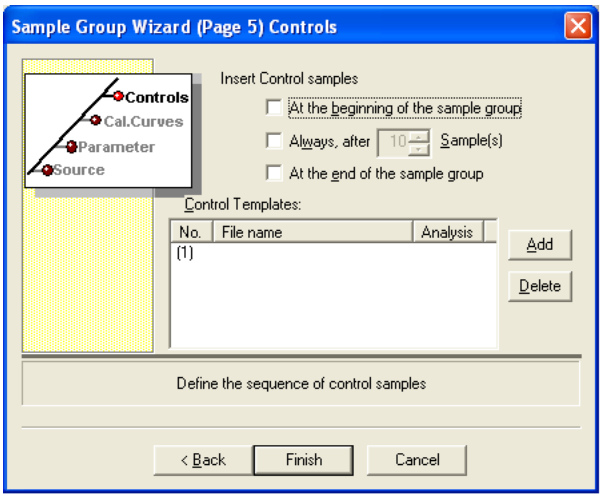


Figure 12.7. Controls window



d. Screw the white plastic caps provided onto the vials to secure the septum in place (hand tight) and place the vial in the appropriate position in the rack.

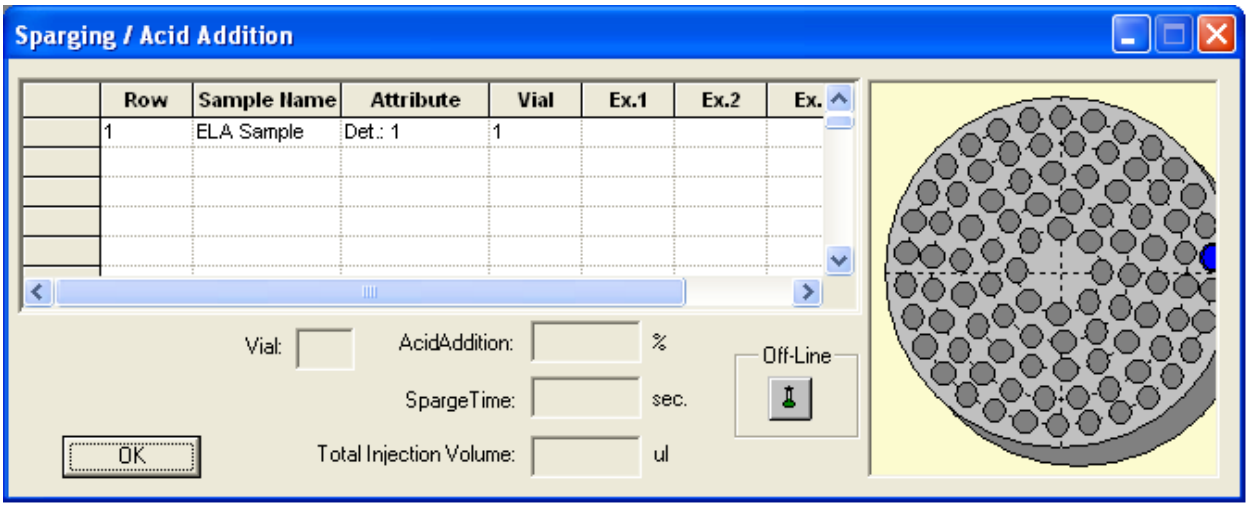
e. Once all the standards and samples are in the rack, place the rack into the auto-sampler and close the lid. The instrument will not function with the lid removed or sample rack missing.

12.12 Sample analysis

1. Open the compressed air source and set the pressure between 44 and 88 psi. Check to ensure there is enough compressed air left in the cylinder (~500 psi) for the complete run.

2. Fill up the two water reservoirs with Milli-Q water (amber bottle beside ASI, and small plastic container beside TOC).
3. Ensure that there is sufficient 2N HCl in the plastic bottle.
4. Ensure there is room in the waste container.
5. Fill the humidifier and cooler drain reservoirs to the "Hi" marking. Open the front lid of the TOC using the latch on the left side at the bottom. The humidifier reservoir is found in the front of the machine and can be accessed by simply removing the reservoir cap and filling it up with Milli-Q. A 50-cc syringe and a clear plastic tube that leads to the outside of the machine are used to fill the cooler drain (located at the back). Inspect these reservoirs for mould or mildew growth and clean them with Milli-Q and brushes or Kimwipes™ if necessary.
6. Turn on the PC and login to Windows.
   - Username: chemlab
   - Password: Fisheries1
7. Double-click the *TOC Control-V* icon on the desktop to open the software. The software can also be accessed by clicking *Start* > *Programs* > *TOC-Control*.
8. A pop-up box will appear; select *Sample Table Editor* (Figure 12.1). The program will ask for a username; enter your initials and click *Enter*.
9. To create a new run, click *File* > *New*. A pop-up box will appear; select *Sample Run*.
10. The program will ask where you want data saved. Create a folder in C:\TOC 3201\Data\ year (i.e. 2019), and save as yyyymmdd_DOC.
11. A drop-down box appears requesting which vials are to be used. Select Chemlab24 for 24-mL vials (Figure 12.2). Click *OK*.
12. From the toolbar, click *Insert* > *Auto Generate*. Click the "…" button, navigate to the ELA Sample Methods folder, select the "ELA Sample Methods.met" file, and click *Next* (Figure 12.3). This ensures the same parameters are always used for each run.
13. In the Sample Parameters window (Figure 12.4), enter the total number of samples (including reference and calibration standards, duplicates, and samples) to be included in the run in the No. of Samples field, and the number of the first vial in the Start Vial field. Leave the Sample Name and Sample ID blank and click *Next*.
14. The calibration is conducted externally from the software using the "DOC Calculation Template.xls." Leave the check boxes blank in the *Calibration Curves* window and click *Next* (Figure 12.5).
15. Leave everything blank in the *Calibration Curve Check* window and click *Next* (Figure 12.6.
16. Leave everything blank in the *Controls* window and click *Next* (Figure 12.7).

Figure 12.8. Sparging / Acid Addition window for verification of vials



17. In the *Sparging / Acid Addition* window, check to see that the diagram indicating which vial positions are used matches the samples in the rack and click *OK* (Figure 12.8).
18. Before you change the sample names, connect the instrument so that it can stabilize while you enter the sample names. Click on the *lightning bolt icon* in the toolbar. A pop-up window will appear. Select the *Use PC Settings* option.
19. Open the front lid of the Shimadzu TOC-VCPH + TNM-1 instrument and ensure the carrier gas flow rate is set to 150 mL/min and the TOC instrument pressure is 200 kPa. Adjust if necessary by turning the appropriate knobs. Let the system stabilize for a minimum of one hour (flow rate/ pressure gauges will not be active until the flow has been started by the software after the lightning bolt icon is clicked and the computer is connected to the machine).
20. In the toolbar select *Instrument* > *Background Monitor*. The Background Monitor will appear, giving details on the machine's status (Figure 12.9). Wait until all options display a check mark before starting the run. If a checkmark does not appear within 45 minutes in all options, the catalyst might need to be regenerated (see Section 12.16).

Figure 12.9. Background Monitor window

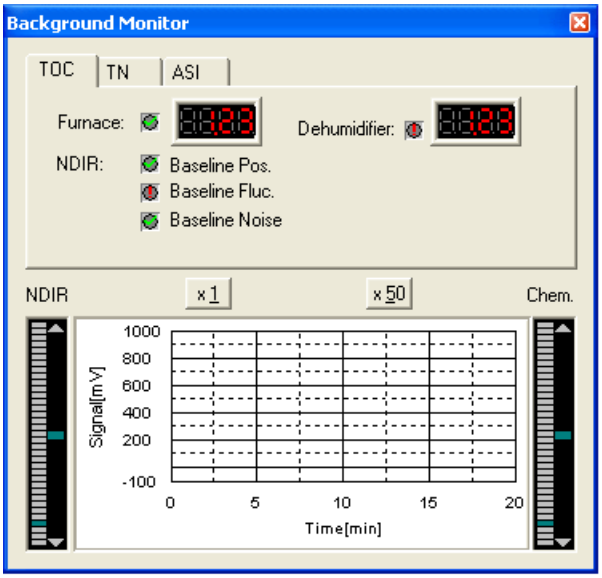
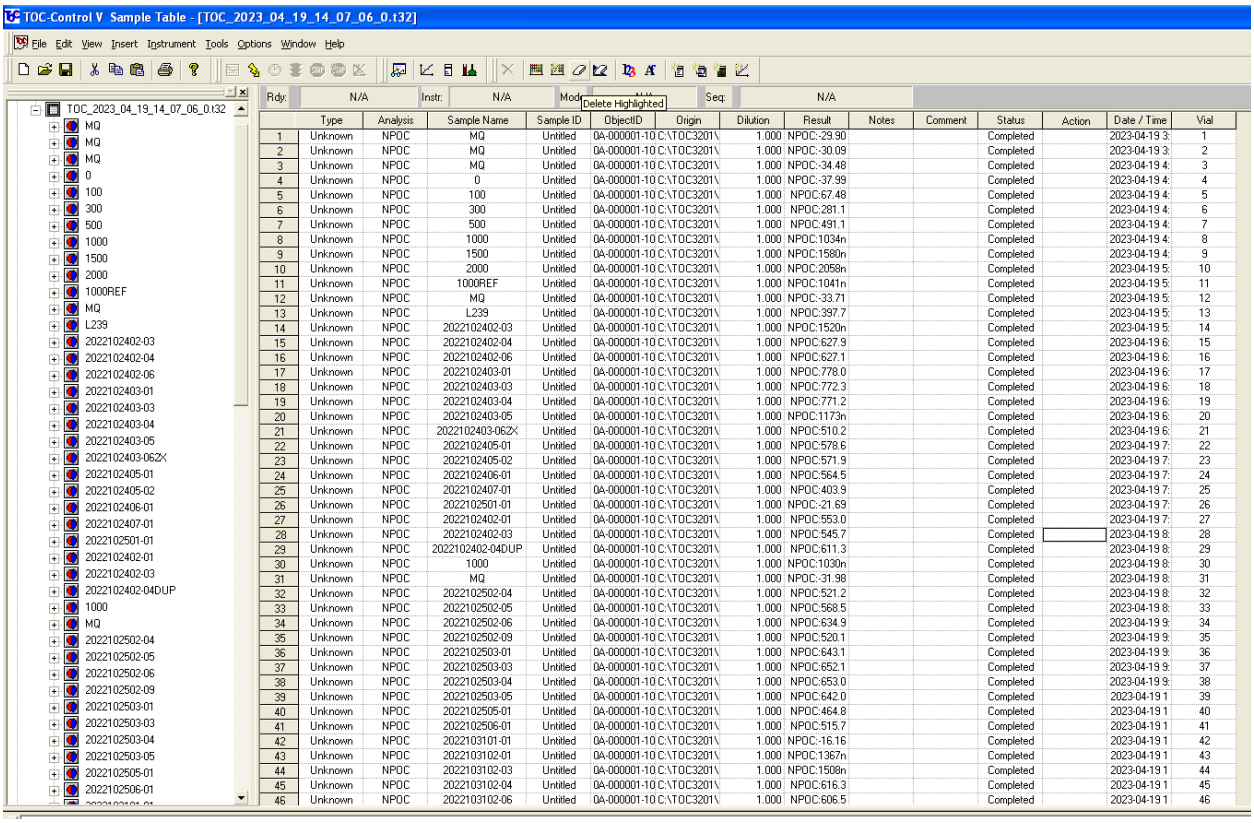


Figure 12.10. Main Sample window



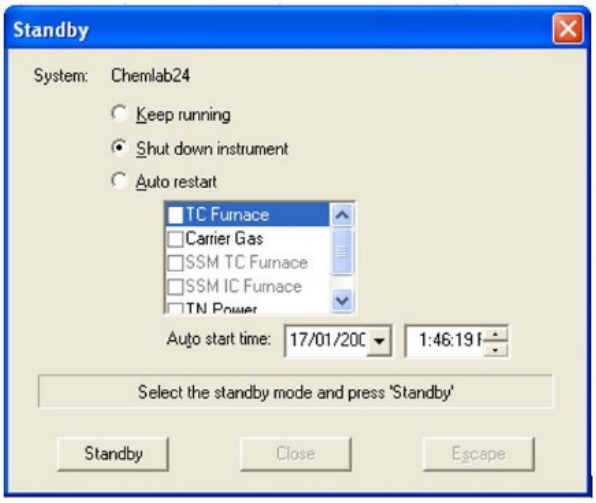
21. While waiting for the furnace to heat up and the background noise to stabilize, enter the Sample ID for each sample in the "Sample Name" column (Figure 12.10) of the Main Sample window.

22. Inspect all flow lines for any precipitates or residues. From the toolbar, select *Instrument* > *Maintenance* > *Washing*. Ensure that there is water in the reservoirs before starting this procedure. Select TC Port, ASI Port, and IC Port.

    Note: The maintenance options are only available when the Background Monitor window is closed.

23. Wash all other components of the system from residues by selecting *Instrument* > *Maintenance* > *Residue*

Figure 12.11. Standby window



Removal in the toolbar. Ensure that there is enough acid in the reagent containers before starting this procedure.

24. Once the Background Monitor indicates the machine has stabilized and the Sample Names are entered in the Main Sample window, click the green traffic light button in the toolbar to start the analysis. The Standby pop-up window will appear (Figure 12.11) asking which components to shut down once the run is complete. Select *Shut Down Instrument*. This shuts down the furnace before the fan to ensure that the instrument does not melt. Click *Standby*.

    Note: Do not shut down the instrument using the power button on the Shimadzu TOC-VCPH + TNM-1 instrument. This will turn off the cooling fan at the inlet to the combustion column, which keeps the plastic components of the injection mechanism from melting.

25. The sparging/acid addition window will appear. Check the box for acid addition and click *OK*. The analysis will now begin. Samples are processed at a rate of ~11 min/sample.

26. If you want to watch or check the peak profile or area as samples are analyzed, click *View* > *Sample Window*. This is a good way to check that the samples are running normally before you leave for the night. Expected values for various standards are provided in Table 12.2.

Table 12.2. Approximate peak areas expected with some of the standards analyzed

Standard	Peak area
1000 ref	~195
1000 cal	~190
2000 cal	~380
Milli-Q	~2-4
Lake 239	~80-90 (conc. = ~460)

27. Once the run is complete, turn off the gas valve on the compressed air.

12.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning*. Briefly, the vials, caps, and septa are cleaned by rinsing three times with hot water and three times with DRO water. Vials are placed upside down in the rack and air dried. Lids and septa are air dried in a metal mesh container with aluminum foil placed on top to prevent exposure to dust particles. Visually inspect the vials, lids, and septa for cracks, large perforations, residues, etc., and discard them if compromised.

12.14 Calculations

The calculation of DOC in samples is performed by using the "DOC Calculation Template-updated 2019Apr.xls" located at C:\TOC 3201\Data\DOC Calculation Template.xls.

Concentrations of DOC are calculated using equation 12.5:

Equation 12.5.

$$C = \frac{A_{samp} - A_{blk} - b}{m}$$

where C is the concentration of the sample, A_{samp} is the sample absorbance, A_{blk} is the reagent blank absorbance, m is the slope, and b is the intercept of the calibration curve after correcting for the reagent blank absorbance. The sample concentration is reported to three decimals.

If a dilution has been made, the concentration of the sample needs to be corrected for the concentration of DOC in the dilution water using equation 12.6:

Equation 12.6.

$$C = (C_0 \times D) - ((C_{dil} \times (D - 1))$$

where C_0 is the concentration of DOC in the diluted sample, C_{dil} is the concentration of DOC in the dilution water, and D is the dilution factor, which is calculated using equation 12.7:

Equation 12.7

$$D = \frac{V_1}{V_0}$$

where V_1 is the volume of the diluted sample, and V_0 is the volume of the undiluted sample.

To retrieve the data:

1. In TOC Control V Sample Table window, export the data by selecting *File* > *Export to ASCII* > *Sample Table and Each Injection*.
2. Navigate to the folder you created in Section 12.12, step 11 (C:\TOC 3201\Data\year\yyyymmdd_DOC), and save the file in the yyyymmdd_DOC_Raw format (where yyyymmdd is the analysis date; e.g. 20201207_DOC_Raw.txt). Right-click on the yyyymmdd_DOC_Raw.txt file and select *Open with Excel*. Move the .pkt and .t32 files into this folder as well.
3. Copy the "Sample Name" and "Mean Area" columns to the "RAW" and "dup removal" tabs in the "DOC Calculation Template.xls."
4. In the "dup removal" tab, remove the duplicates by selecting *Data* > *Filter* > *Advanced*, then place a check mark in the "Unique Values Only" box and click OK.
5. Use the contents of the "dup removal" tab to populate the yellow cells in the "Calc" tab with the Sample ID, dilution factor and mean peak areas of each of the blanks, calibration standards, reference standards, reference samples, and samples.
6. Information on the date, analyst's initials, standard lot numbers, expiry date and stock concentrations, number of calibration standards *(n)*, reference standard preparation date, and reference sample check batch number are also recorded in this spreadsheet in the yellow fields.

Note: Do not use the first three Milli-Q samples, as these are used to flush the system prior to the run.

Save the completed "DOC Calculation Template.xls" as yyyymmdd_DOC.xls (e.g. 20201207_DOC.xls) in the directory C:\TOC 3201\Data\year\yyyymmdd_DOC. There should be four files in each sample run folder (i.e. a .pkt, a .t32, a .txt, and a .xls).

12.15 Data entry

The DOC concentrations for each sample, which are calculated using the DOC Calculation Template and QC data are entered into the Sample Master LIMS. The completed DOC Calculation Template is printed and filed into the DOC folder.

12.16 Troubleshooting and maintenance

If the instrument needs to be shut down due to an error (e.g. Vertical Arm Position Error), follow these steps:

1. **Briefly** (~5 seconds) turn off the Shimadzu TOC-VCPH + TNM-1 instrument using the power button on the front right-hand side.
2. **Immediately** turn the instrument back on using the same power button. This will shut off the alarm, but the fans are not off long enough to melt the inside of the instrument.
3. Close the *TOC-Control V* software and save whatever data was processed before the error occurred.
4. Re-launch the program and follow steps 7–19 in Section 12.12.
5. Connect the PC to the instrument using the lightning bolt icon.
6. Ensure no samples are listed in the main sample window. Select *Instrument* > *Standby* and select *Shut Down Instrument*. This will shut down the instrument over a period of 30 minutes in the appropriate order ensuring the safety of the instrument.

   Note: If the instrument will not connect properly, repeat steps 7–19 of the 12.12 Sample analysis section again after the program has been closed.

If the reproducibility of the machine is poor, the catalyst might need to be regenerated. From the toolbar select, *Instrument* > *Maintenance* > *Regeneration of Catalyst*. Ensure that there is enough acid and water (at least half full) in the reservoirs before performing this step. If the problem is still not resolved after performing this step, the catalyst may need to be replaced. See TOC-V User's Manual for the replacement of the catalyst.

If the PC loses connection with the system for any reason, both the PC and the machine must be reset. Shut down and restart the PC, then turn off the power on the TOC/TN system, wait 15 seconds, and turn the power on again.

If the needle of the autosampler does not return to its home position, go to *Instrument* > *Maintenance* > *Mechanical Check* > *ASI / 8Port Sampler (Tab)* > *Arm Vertical Reset.*

The halogen scrubber must be changed once half of the column changes colour from blue to white. The halogen scrubber's main function is to absorb HCl gas within the sample lines to prevent corrosion within the system.

If a stable baseline cannot be achieved after one hour from the time the carrier gas was turned on, the CO_2 absorber of the instrument must be replaced (the aluminum column found on the outside of the machine). The CO_2 absorber is typically good for one year after its first use.

Appendix 12A. 2 N Hydrochloric Acid Preparation Record

2 N HCl
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	HCl vol (mL)	Final vol (mL)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2018

Appendix 12B. 0.1 M Carbon Calibration Standard Stock Preparation Record

iisd|ela experimental lakes area

0.1 M Carbon Calibration Standard Stock (DOC)
Preparation Record

Target is 2.43022 g/L $KC_8H_4O_4$ to obtain 0.1 M C

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (L)	Conc (M)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 12C. 0.1 M Carbon Reference Standard Stock Preparation Record

iisd|ela experimental lakes area

0.1 M Carbon Reference Standard Stock (DOC)
Preparation Record

Target is 6.05526 g/L NH_2CONH_2 to obtain 0.1 M C

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (L)	Conc (M)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 12D. Carbon Calibration Curve Preparation Record

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Weekly Dissolved Organic Carbon Calibration Standards
Preparation Record

Vol 0.1 M C cal stock (µL)	Final vol (mL)	Final conc (µM)
0	250	0.0
250	250	100
750	250	300
1250	250	500
5000	250	1000
3750	250	1500
5000	250	2000

Stable for one week

Use a new page when the lot number changes.
When the lot number is finished stroke out the remaining unused lines.

	Week of	Expiry date	Analyst initials
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			
For DOC analysis on			

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 12E. 1,000 µM Carbon Reference Standard

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1000 µM Carbon Reference Standard
Preparation Record

Stable for one year

Preparation date	Stock lab lot number	Stock expiry date	Stock conc (M)	Vol stock added (mL)	Final vol (mL)	Final conc (µM)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 12F. DOC Worksheet Shimadzu Full Run

DOC Worksheet Shimadzu Full Run

DATE: _____
ANALYSIS: _____
ANALYST: _____

Vial Num	Sample Name	DF
1	MQ	1
2	MQ	1
3	MQ	1
4	0 CAL	1
5	100 CAL	1
6	300 CAL	1
7	500 CAL	1
8	1000 CAL	1
9	1500 CAL	1
10	2000 CAL	1
11	1000 REF	1
12	MQ	1
13	Reference sample	1
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29	DUP	
30	1000 CHECK	1
31	MQ	1
32		
33		
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		
46		
47		

Vial Num	Sample Name	DF
48	DUP	
49	1000 CHECK	1
50		
51		
52		
53		
54		
55		
56		
57		
58		
59		
60		
61		
62		
63		
64		
65		
66		
67	DUP	
68	1000 CHECK	1
69		
70		
71		
72		
73		
74		
75		
76		
77		
78		
79		
80		
81		
82		
83		
84		
85	DUP	
86	MQ	1
87	0 CAL	1
88	100 CAL	1
89	300 CAL	1
90	500 CAL	1
91	1000 CAL	1
92	1500 CAL	1
93	2000 CAL	1

13.0 Absorbance Scan

13.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of dissolved organic carbon (DOC) optical characteristics in fresh water and precipitation.

13.2 Method principle

DOC in natural waters can be comprised of many different species, all with unique optical properties as a result of their differing molecular structures, molecular weights, and sources. Certain optical properties can be identified in an ultra-violet visible (UV-VIS) absorbance spectrum (200–800 nm). The absorbance ratios at certain wavelengths and the spectral slopes of certain sections have been shown to be indicative of the molecular properties of the DOC species. There are several reportable values with differing levels of significance and relation to the physical properties of carbon. The reportable values for the natural waters of boreal lakes are:

- Log-transformed spectral slopes of 275–295 nm ($S_{275-295}$) and 350–400 nm ($S_{350-400}$), and the slope ratio (S_r) of these slopes, respectively;
- Absorbance ratios of a_{250}/a_{365} and a_{255}/a_{436} (E_2:E_3 and E_2:E_4);
- Spectral slopes in the UV-A, UV-B, and UV-C range (250–280 nm, 280–315 nm, and 315–400 nm); and
- Specific UV-absorbance at 254 nm ($SUVA_{254}$).

S_r has been found by Helms et al. (2007) to be inversely related to the molecular weight of DOC.

Lei et al. (2019) split the UV portion of absorbance (250nm–400nm) into its three components—UV-A, UV-B, and UV-C—for better resolution on the behaviour of DOC in that range. Lei et al. (2019) also found that E_2:E_3 and E_2:E_4 correlate with $S_{uv\text{-}b}$ and $S_{uv\text{-}a}$, respectively.

$SUVA_{254}$ has been shown by Weishaar et al. (2003) to be strongly correlated to % aromaticity of DOC species in aquatic waters ($R^2 = 0.97$).

13.3 Occupational health and safety

There are no occupational health and safety concerns associated with this analysis.

13.4 Validation

13.4.1 Operating range

200 nm–800 nm

13.5 Sample conditions

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must be

- filtered through a 1.2 µm GF/C filter within 24 hours of sample collection,
- stored in pre-washed amber glass vials (refer to Section 2, *Labware Cleaning* for details),
- stored in the dark at 4 ± 2 °C, and
- stored for no more than 14 days.

pH has occasionally had a significant effect on absorbance scans, which Weishaar et al. (2003) found to be negligible outside of a range of 2.0–8.6. For IISD-ELA waters, which range primarily from ~ 4.5 to 8.5, a pH adjustment is unnecessary.

Both Fe^{2+} and Fe^{3+} are known to display characteristics in the UV-C absorption range. Weishaar et al. (2003) tested this and found that for most natural waters (< 0.5 mg/L Fe), interference was negligible. NO_3 can also interfere: Weishaar et al. (2003) found that the tail of the peak for NO_3 was relevant only in concentrations above 40 mg/L. For these reasons, Fe and NO_3 concentrations in samples should be monitored and noted if they are above these concentrations.

13.6 Equipment

- Shimadzu UV-1800 scanning spectrophotometer
- a computer running UV probe software
- two 10 mm pathlength quartz cuvettes
- two 100 mm pathlength quartz cuvettes

13.7 Prepared reagents

- Preparation information for reference samples is recorded in the *Reagent Preparation* logbook.
- Reference sample containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reference samples before pouring in the bulk of new reference samples.

13.7.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

13.7.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

13.7.3 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference Sample Preparation*. Briefly, the reference sample consists of water from Lake 239 that has been aged for at least one year at 4 °C, filtered through a 1.2 µm GF/C filter, and stored in the dark.

13.8 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective action is taken before testing is resumed.

13.8.1 Blanks

An instrument blank containing solely Milli-Q water will be run at the beginning of each run, ensuring that at no point during the spectrum the blank registers more than 0.005 absorbance units.

13.8.2 Reference sample

A reference sample of aged Lake 239 water is included in every analytical run. The values obtained must be within 5.0% of the expected values. Results for check sample performance are charted and assessed for drift and bias.

13.8.3 Instrument duplicate

Instrument duplicates are run every 16 samples to ensure replicability of the instrument. Duplicates must agree to within 5.0% of each other. If duplication is inadequate, samples bracketed by the duplicates are reanalyzed.

13.8.4 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verification*. All glassware is cleaned according to Section 2, *Labware Cleaning*.

13.9 Quality assurance

Proficiency testing is not currently conducted for the absorbance scans.

While solid-state standards are not currently used to confirm the absorbance wavelength on the spectrophotometer, this practice will be implemented in future studies.

13.10 Sample preparation

1. A subsample of filtered sample is transferred to a pre-labelled, 20 mL polyethylene vial and stored in the dark at 4 ± 2 °C.
2. Remove samples from the refrigerator ~30 minutes prior to analysis to avoid condensation buildup on quartz cuvettes; keep samples in the dark.

13.11 Analysis

1. Turn on the power to the Shimadzu UV-1800 Spectrophotometer and allow it to warm up for at least 45 minutes. Once warm, on the spectrophotometer, click *Enter* followed by *F4* to allow for PC control.
2. On the desktop PC, double-click the *UV Probe Absorbance* scan method shortcut.
3. Click the *Connect* button at the bottom of the UV Probe window.
4. Create a new folder in the \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Absorbance scan\yyyy_Absorbancescan directory with the yyyymmdd_Absorbancescan format with yyymmdd reflecting the analysis date (e.g. 20200601_Absorbancescan). This folder will function as the direct saving location for spectrum and text files.
5. Once the lamp is warm, rinse two 10-mm quartz cuvettes with Milli-Q water. Use a Kimwipe™ to ensure there is no smudging or dust particles.
6. Fill both quartz cuvettes with Milli-Q water and visually inspect them to confirm the cleanliness of the light path of the cell. Place both cuvettes filled with Milli-Q in the two holders in the spectrophotometer.
7. Select the *Baseline* option at the bottom of the screen. Confirm baseline from 800 nm to 200 nm and click *OK*.

8. Confirm that the front cuvette is still free from contaminants, and then click *Auto Zero* at the bottom of the screen.
9. Click *Start* at the bottom of the PC screen. A filepath will be presented to you. Select the filepath by clicking … , and direct it to the folder created for this analysis. Change your filename from File_xxxxx_xxxxx to yyyymmdd_MQ_abscan, click *Open*, then *OK*.
10. When the spectrum for the Milli-Q has finished, ensure that the absorbance has not gone above 0.005 at any point during the spectrum. If successful, continue to step 11. If not, rinse both cuvettes well and repeat steps 6–10.
11. Go to the *Operation* tab at the top of the window. Select *Data Print*, and the wavelengths and absorbances should appear in the centre of the screen.
12. Select all the wavelengths by clicking *RawData* at the top of the table and then open the "File" tab and click *Save As*. Save two copies, one as "Spectrum Files (*.spc)" and one as "Data Print Table (.txt)." If the filepath was set up correctly in step 9, you should be able to click *Save*. If not, find the correct folder and create the correct name for the sample and then proceed to save.
13. Rinse the front cuvette at least three times with Milli-Q water, and then rinse and fill with the reference sample. Repeat step 9, but save the file as yyyymmdd_L239_abscan.
14. While the sample is running, begin to calculate your reportable values. Minimize the UV Probe window, go to \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Absorbance scan, and open "Absorbance Scan Calculation Template.xls."
15. Open the yyyymmdd_MQ_abscan.txt file in Notepad. Select *All* (*Ctrl-A* on the keyboard or select from the *Edit* tab), and *Copy* (*Ctrl C* on the keyboard or select from the *Edit* tab).
16. Navigate to the "Raw Data" tab in the "Absorbance Scan Calculation Template.xls" and select the square in the spreadsheet with "Paste Special Here."
17. Paste the .txt file by clicking on the arrow below the *Paste* button on the top left of the screen and select "Use Text Import Wizard." A window will open. Ensure that "Delimited" is selected, and click *Next*. In the next window, select "comma" in the "Delimiters" section and click *Finish*.
18. Ensure that the data has properly pasted and then save the file as yyyymmdd_MQ_abscan.xlsx in the folder created in step 4.

    Note: Each sample should now have three files associated with it: .spc, .txt., and .xlsx.
19. Copy the yellow squares (S275–S295 to a254) in the "Reportable values" tab and open the Absorbance Scan Benchsheet in the \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Absorbance scan directory. Select the cell to the right of the appropriate "Sample ID" (e.g. the S275-295 cell in the MQ row) and right-click Paste Special with "Values" and "Transpose" selected.
20. Once the scan of the reference sample has finished, if satisfactory results are obtained, repeat step 12 to save the .txt and .spc files for the reference sample.

21. Rinse the front cuvette at least three times with Milli-Q water, and then rinse and fill with sample. Repeat step 9 but save the file as SampleID_abscan.
22. While the sample is running, repeat steps 14–19, but for the reference sample (e.g. yyyymmdd_L239_abscan).
23. Once the scan of the sample has finished, repeat step 12 to save the .txt and .spc files for the sample.
24. Repeat step 21.
25. While the sample is running, repeat steps 14–19, but for the sample (e.g. SampleID_ abscan).
26. Repeat steps 23–25 until all of the samples have been analyzed, including a duplicate every 16 samples, and reported values are copied to the Absorbance Scan Benchsheet.
27. Enter the analyst and analysis date in Absorbance Scan Benchsheet and save it as yyyymmdd_absorbancescans in the folder created in step 4.

13.12 Clean up

The equipment is cleaned according to Section 2, Labware Cleaning. Briefly, the cuvettes and vials are cleaned by rinsing three times with hot water, followed by three times with DRO water.

13.13 Calculations

All calculations of the reportable values from the absorbance scans are performed by using the "Absorbance Scan Calculation Template.xls" worksheet located in the \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Absorbance scan directory and following steps 14–19 in Section 13.11.

Calculations are done using the Napierian absorption coefficient, which returns values in m^{-1}. This is calculated using equation 13.1.

Equation 13.1.

$$a_\lambda = \frac{2.303 A_\lambda}{l}$$

where λ = the wavelength in nm, a = the absorption coefficient, A = absorbance, and l = path length of the cell (m).

Two absorption ratios are reported – a_2:a_3 and a_2:a_4. These are calculated by equations 13.2 and 13.3, respectively:

Equation 13.2.

$$a_3 = \frac{a_{255}}{a_{355}}$$

Equation 13.3.

$$a_2{:}a_4 = \frac{a_{255}}{a_{456}}$$

where the subscripts denote the wavelength of the absorption coefficients in nm.

Absorbance spectrums function similarly to exponential decay functions, thus they can be log transformed, and spectral slopes can be reported in this manner. The entire spectrum can be log transformed in Microsoft Excel by reporting value ln*a* for all values *a*. The slopes are determined for all parameters, $S_{275\text{-}295}$, $S_{350\text{-}400}$, $S_{uv\text{-}a}$, $S_{uv\text{-}b}$, and $S_{uv\text{-}c}$ by using the line of best fit calculated by Microsoft Excel over those wavelengths. S_r is then calculated by equation 13.4:

Equation 13.4.

$$S_R = \frac{S_{275\text{-}295}}{S_{350\text{-}400}}$$

$SUVA_{254}$ is calculated by normalizing the Napierian absorption coefficient at λ =254 nm to the DOC concentration (eq. 13.5):

Equation 13.5.

$$SUVA_{254} = \frac{a_{254}}{[DOC]}$$

13.14 Data entry

The reportable values for each sample, which are calculated using the "Absorbance Scan Calculation Template.xls," as well as the QC data, are entered into the Sample Master LIMS. The completed Absorbance Scan Benchsheet (saved as yyyymmdd_absorbancescans) is printed and filed into the "Absorbance Scan" folder.

13.15 References

Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., & Mopper, K. (2007). Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography, 53,* 955–969.

Lei, X., Pan, J., & Devlin, A. T. (2019). Characteristics of absorption spectra of chromophoric dissolved organic matter in the Pearl River estuary in spring. *Remote Sensing*, *11,* 1533–1548.

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., & Mopper, K. (2003). Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science and Technology, 37,* 4702–4708.

14.0 Nitrite, Nitrate, and Ammonia: Colourimetric method

14.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of nitrite, nitrate, and ammonia in fresh water and precipitation.

14.2 Method principle

This automated procedure is based on methods designed for the analysis of nitrite, nitrate, and ammonia using the SEAL Analytical AutoAnalyzer 3 HR Continuous Segmented Flow Analyzer (SEAL Analytical, 2015; SEAL Analytical, 2016). The nitrate analysis uses the method whereby nitrate is reduced to nitrite by a copper-cadmium reduction column (Bendschneider, 1952; Brewer & Riley, 1965; Morris and Riley, 1963; Stainton, 1974; Stainton et al., 1977; Woods et al., 1967). Under appropriate conditions, nitrite complexes with an N-(1-Naphthyl)ethylenediamine dihydrochloride (NNED), sulfanilamide and a phosphoric acid-based reagent. Extreme care is taken while handling the phosphoric acid to avoid phosphorus contamination in labware. When the nitrite reacts with this colour reagent under acidic conditions, it forms a diazo compound, which couples with the N-1-NNED to form a purple azo dye. The colour intensity is proportional to the amount of nitrite in the sample. The absorption maximum is 543 nm. Under appropriate conditions, ammonia complexes with an ethylenediamine tetra-acetic acid (EDTA) and tri-sodium citrate dehydrate-based reagent. When the ammonia and complexing reagent react with the phenol and hypochlorite, an indophenol blue is formed. The colour intensity is proportional to the amount of ammonia in the sample. The absorption maximum is 660 nm.

14.3 Occupational health and safety

The phenol reagent used is corrosive, toxic and a health hazard. Avoid contact with skin. Handle in a fume hood and wear a face shield and gloves when handling and diluting.

Cadmium is toxic and should be handled carefully. Avoid contact with skin or inhaling. Wear gloves and use a handle in the fume hood.

The concentrated hydrochloric acid (HCl) used for reagent preparation is corrosive and reacts violently with water. Handle in a fume hood to avoid HCl vapours. Avoid contact with skin. Wear goggles and gloves when handling and diluting. Always pour acid into water slowly while stirring. **Never pour water into acid.**

14.4 Validation

14.4.1 Operating range

0.50–50 µg/L NO_2-N

1.4–100 µg/L NO_3-N

10.6–500 µg/L NH_3-N

14.4.2 Detection limit and limit of quantitation

The detection limit, which is based on the residuals along the standard curve, is calculated for each run using equations 14.1 through 14.3:

Equation 14.1.

$$C_{dl} = \frac{y_d - b}{m}$$

where b is the y-intercept, m is the slope, and y_d is the instrument response detection limit, which is calculated using equation 14.2:

Equation 14.2.

$$y_d = 3s_y + b$$

where s_y is based on the residuals between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration. It is calculated using equation 14.3:

Equation 14.3.

$$s_y = \sqrt{\frac{\sum di^2}{n-2}}$$

where n is the number of standards in the calibration curve, and di is the difference between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration.

The limit of quantitation is calculated in the same manner as the detection limit, except equation 14.2 is replaced with equation 14.4:

Equation 14.4.

$$y_q = 10s_y + b$$

The averages of the detection limits and limits of quantitation for samples run between 2019 and 2020 were as follows:

Table 14.1. Average (± standard deviation) detection limit and limit of quantitation for nitrite, nitrate, and ammonia analyses conducted in 2019 and 2020

	Detection limit (µg/L)	Limit of quantitation (µg/L)
NO_2-N	0.5 ± 0.2	1.6 ± 0.6
NO_3-N	1.4 ± 1.1	4.6 ± 3.7
NH_3-N	10.6 ± 6.3	35 ± 21

14.5 Sample condition

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must be

- field sampled in a Nalgene polyethylene bottle,
- filtered within 24 hours,
- stored in a sealed Nalgene polyethylene bottle,
- stored and analyzed within two days, and
- stored at 4 ± 2 °C.

14.6 Equipment

- SEAL AutoAnalyzer 3 HR
  - three digital colorimeters
  - high-precision pump outfitted with the pump tubes outlined in the system maps (SEAL Analytical, 2015, 2016).
  - three multitest manifolds
  - auto sampler
- computer running AACE 7.09 software
- 11 mL plastic tubes for samples (sample cups)
- 14.5 mL plastic tubes for standards

14.7 Purchased reagents

14.7.1 NO_2 calibration standard stock

Commercial certified (traceable to NIST) NO_2-N standard

1.0 mL = 0.1 ± 0.01 mg NO_2-N

The standard is stable until the expiry date on the manufacturer's label.

14.7.2 NO_2 reference standard stock

Commercial certified (traceable to NIST) NO_2-N standard

1.0 mL = 1 ± 0.01 mg NO_2-N

The standard is stable until the expiry date on the manufacturer's label. It must be from a different manufacturer than the standard used for calibration standards.

14.7.3 NO_3 calibration standard stock

Commercial certified (traceable to NIST) NO_3-N standard

1.0 mL = 0.1 ± 0.01 mg NO_3-N

The standard is stable until the expiry date on the manufacturer's label.

14.7.4 NO_3 reference standard stock

Commercial certified (traceable to NIST) NO_3-N standard

1.0 mL = 1 ± 0.01 mg NO_3-N

The standard is stable until the expiry date on the manufacturer's label. It must be from a different manufacturer than the standard used for calibration standards.

14.7.5 NH_3 calibration standard stock

Commercial certified (traceable to NIST) NH_3-N standard

1.0 mL = 0.1 ± 0.01 mg NH_3-N

The standard is stable until the expiry date on the manufacturer's label.

14.7.6 NH_3 reference standard stock

Commercial certified (traceable to NIST) NH_3-N standard

1.0 mL = 1 ± 0.01 mg NH_3-N

The standard is stable until the expiry date on the manufacturer's label. It must be from a different manufacturer than the standard used for calibration standards.

14.7.7 Cadmium – 20–60 mesh size

Cadmium is stable indefinitely at room temperature.

14.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.

14.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

14.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

14.8.3 Mixed calibration standard stock – 0.5 µg/mL NO_2-N, 0.5 µg/mL NO_3-N, and 5 µg/mL NH_3-N

Dilute 0.5 mL of the 0.1 mg/mL NO_2-N, 0.5 mL of the 0.1 mg/mL NO_3-N, and 5.0 mL of the 0.1 mg/mL NH_3-N to 100 mL with Milli-Q water in a volumetric flask. Make fresh weekly.

Table 14.2. Mixed calibration standard stock preparation volumes

Analyte	Stock conc. (mg/mL)	Vol. added (µL)	Flask vol (mL)	Final conc. (µg/mL)
NO_2	0.1	500	100	0.5
NO_3	0.1	500	100	0.5
NH_3	0.1	500	100	5.0

14.8.4 Mixed reference standard stock – 10 µg/mL NO_2-N, 10 µg/mL NO_3-N, and 100 µg/mL NH_3-N

Dilute 1.0 mL of the 1.0 mg/mL NO_2-N, 1.0 mL of the 1.0 mg/mL NO_3-N, and 10.0 mL of the 1.0 mg/mL NH_3-N reference stocks to 100 mL with Milli-Q water in a volumetric flask. Make fresh monthly.

Table 14.3. Mixed reference standard stock preparation volumes

Analyte	Stock conc. (mg/mL)	Vol. added (µL)	Flask vol (mL)	Final conc. (µg/mL)
NO_2	1000	1000	100	10
NO_3	1000	1000	100	10
NH_3	1000	10 000	100	100

14.8.5 Mixed reference standard – 25 µg/L NO_2-N, 50 µg/L NO_3-N, and 250 µg/L NH_3-N

Dilute 0.25 mL of mixed reference standard stock to 100 mL with Milli-Q water in a volumetric flask. Make fresh weekly.

14.8.6 NO_2 standard – 50 µg/L NO_2-N

Dilute 0.05 mL of the 0.1 mg/mL NO_2-N to 100 mL with Milli-Q water in a volumetric flask. Make fresh weekly.

14.8.7 NO_3 standard – 50 µg/L NO_3-N

Dilute 0.05 mL of the 0.1 mg/mL NO_3-N to 100 mL with Milli-Q water in a volumetric flask. Make fresh weekly.

14.8.8 NO_2 standard – 2000 µg/L NO_2-N

Dilute 0.2 mL of the 0.1 mg/mL NO_2-N to 100 mL with Milli-Q water in a volumetric flask. Make fresh monthly.

14.8.9 NO_3 standard – 2000 µg/L NO_3-N

Dilute 0.2 mL of the 0.1 mg/mL NO_3-N to 100 mL with Milli-Q water in a volumetric flask. Make fresh monthly.

14.8.10 Colour reagent

- 10 g sulfanilamide
- 0.5 g NNED
- 100 mL concentrated phosphoric acid

Dissolve sulfanilamide, NNED, and concentrated phosphoric acid into 1 L of Milli-Q water. Heat if necessary. Stored in an amber bottle; it is stable for one month.

14.8.11 Ammonium chloride

40 g ammonium chloride

710 µL ammonium hydroxide

Dissolve ammonium chloride and ammonium hydroxide into 1 L of Milli-Q water. The pH of this reagent should be 7.5 ± 0.1. Adjust the ammonium hydroxide as necessary using high-purity hydrochloric acid or ammonium hydroxide to achieve this pH. It is stable for one month.

14.8.12 Complexing reagent

- 15 g EDTA
- 120 g *tri*-sodium citrate dihydrate
- 0.5 g sodium nitroprusside
- 3 mL Brij-35, 22–30% solution

Dissolve EDTA, *tri*-sodium citrate dihydrate, and sodium nitroprusside into 1 L of Milli-Q water and then add Brij-35, 22–30% solution. Store in an amber bottle; it is stable for two weeks.

> **Note:** If the calcium or magnesium concentrations of samples are likely to exceed 0.005 m/L and 0.04 mol/L, respectively, the EDTA should be increased to 30 g/L.

14.8.13 Hypochlorite

Dilute commercial unscented bleach (preferably Chlorox) with 10 parts Milli-Q water to 1 part bleach. It is stable for 24 hours.

14.8.14 Phenol

- 50 g phenol
- 30 g sodium hydroxide

Dissolve phenol and sodium hydroxide into 1 L of Mill-Q water. Store in an amber bottle. It is stable until the solution turns brown.

> **Note:** Final pH should be 11.5–11.9. If the final pH is too high, reduce the sodium hydroxide concentrations. If the pH is too low, increase the sodium hydroxide concentration.

14.8.15 Brij-35 – 1mL/L

Dilute 1 mL of Brij-35, 22–30% solution to 1 L with Milli-Q water. It is stable indefinitely.

14.8.16 Brij-35 – 2 mL/L

Dilute 2 mL of Brij-35, 22–30% solution to 1 L with Milli-Q water. It is stable indefinitely.

14.8.17 Brij-35 – 6 mL/L

Dilute 6 mL of Brij-35, 22–30% solution to 1 L with Milli-Q water. It is stable indefinitely.

14.8.18 Copper sulfate solution – 2%

Dissolve 2 g of copper sulfate in 1 L of Milli-Q water. It is stable indefinitely.

14.8.19 Hydrochloric acid – 6 N

Cautiously, while swirling, add 495 mL of concentrated hydrochloric acid to ~400 mL of Milli-Q water. Cool to room temperature. Dilute to 1 L with Milli-Q water. It is stable indefinitely.

14.8.20 Cadmium column

Place ~10 g of cadmium mesh in a 250-mL beaker. Add 50 mL of 6 N HCl to the beaker and swirl to clean the cadmium for at least one minute. Decant the HCl into waste, add another 50 mL of 6 N HCl, and swirl to clean for another minute. Decant the HCl and rinse the cadmium with at least three successive rinses of Milli-Q water. Decant the Milli-Q and add 50 mL of

copper sulfate solution and swirl until the blue colour is gone from the supernatant. Decant the copper sulfate and rinse the cadmium with at least three successive rinses of Milli-Q water. Add another 50 mL of copper sulfate and swirl until the blue colour is gone from the supernatant. Decant the copper sulfate and gently rinse with Milli-Q until all of the floating precipitate is gone.

Close one end of an 18-cm piece of 0.110" I.D. Tygon tubing with glass wool. Connect the other end of the tubing to a small funnel using a small piece of 1/8" I.D. tubing. Elevate the funnel using a ring stand and attach a 50-mL syringe to the end with wool. Fill the funnel with ammonium chloride and use the syringe to pull the ammonium chloride into the tubing. Ensure there are no air bubbles in the tubing and transfer the prepared cadmium into the tubing using the funnel. Be careful not to expose the cadmium to air, do not allow air bubbles to be trapped in the column, and ensure that the cadmium is packed tightly with no dead space. Remove the funnel and close the tubing with glass wool and an N6 nipple. Remove the syringe and close the tubing with a N6 nipple.

Attach the column to the manifold and condition the column with ~2000 µg/L NO_3-N for five minutes, followed by ~2000 µg/L NO_2-N for five minutes.

14.8.21 NO_x and NH_3 calibration standard curves

The standard curve is created using the "Build Standards" option in the AACE software. See step 8 in Section 14.12, *Analysis* for how the standard curve is created using the AACE software.

14.8.22 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference sample preparation*. Briefly, the reference sample consists of water from Lake 239 that has been aged for at least one year, diluted by a factor of 2, and spiked with 25 µg/L of NO_2 and 250 µg/L of NH_3. The reference sample is stored at 4 ± 2 °C.

14.9 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective action is taken before testing is resumed.

14.9.1 Cadmium column conversion efficiency

The conversion efficiency of the cadmium column is checked prior to conducting an analytical run by measuring the peak areas of 100 µg/L NO_3-N (A_{NO3}) and 100 µg/L NO_2-N (A_{NO2}) standards. The cadmium column efficiency, which is calculated using equation 14.5, must be

greater than 0.95. If the cadmium column conversion efficiency (X_{Cd}) is less than 0.95, the column needs to be either reconditioned or remade and conditioned.

Equation 14.5.

$$X_{Cd} = \frac{A_{NO3}}{A_{NO2}}$$

14.9.2 Blanks

A reagent blank, consisting of Milli-Q water and the reaction reagents, is incorporated as a baseline, which is run every 5–10 samples.

14.9.3 Reference standards

A mixed reference standard is run twice every run, once before samples are run and once afterward. The NO_x and NH_3 concentrations must be within 5.0% of the calibration standard, representing the same concentration.

14.9.4 Calibration curve

The linear correlation coefficients obtained from the calibration standards must have an $R^2 \geq 0.995$ with a first order fit. If necessary, new calibration standards should be prepared.

14.9.5 Reference sample

A reference sample is included at the beginning of every run. The NO_x and NH_3 values obtained must agree to within 5.0% of the expected value. Results for check sample performance are charted and assessed for drift and bias.

14.9.6 Duplicates

Random duplicates are run every 15 samples. Samples for duplicate analysis are chosen at the beginning of the analysis run (before the first analysis is done). Duplicates' relative percent difference (RPD) must agree to within 5.0%. If duplication is inadequate, samples bracketed by the duplicates are re-tested.

14.9.7 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verifications*. All glassware is cleaned according to Section 2, *Labware Cleaning*.

14.10 Quality assurance

Proficiency testing is conducted for the NO_x and NH_3 analyses via the Environment and Climate Change Canada Proficiency Testing (ECCC-PT) program.

14.11 Sample preparation

No additional sample preparation is required prior to analysis.

14.12 Analysis

1. Turn on power on the SEAL AutoAnalyzer 3 HR by turning on the power bar.
2. On the desktop of the PC, double-click the *AACE 7.09* shortcut.
3. Click the Charting button displayed in the *Sys. 1 [AA3 HR]* system window (Figure 14.1.). Select the appropriate analysis (.anl) file.

   Note: When charting is started, the Base, Gain, and Light Power values that were used the last time this .anl file was run are downloaded to the colorimeters.
4. Replace Milli-Q water in the wash bottles, washpot bottle, and syringe diluter receptacle.
5. Pump system wash solutions (6 mL/L Brij-35 for NO_x and 2 mL/L Brij-35 for NH_3) through the reagent lines for 10–15 minutes to "wet" the tubes with surfactant.
6. Pump Milli-Q water through the lines and allow the baseline to stabilize, checking the bubble pattern for the proper bubble shape. Set the base to 5% (right-click in the channel charting window and click *Set Base*).

Figure 14.1. The Sys. 1 [AA3 HR] system window

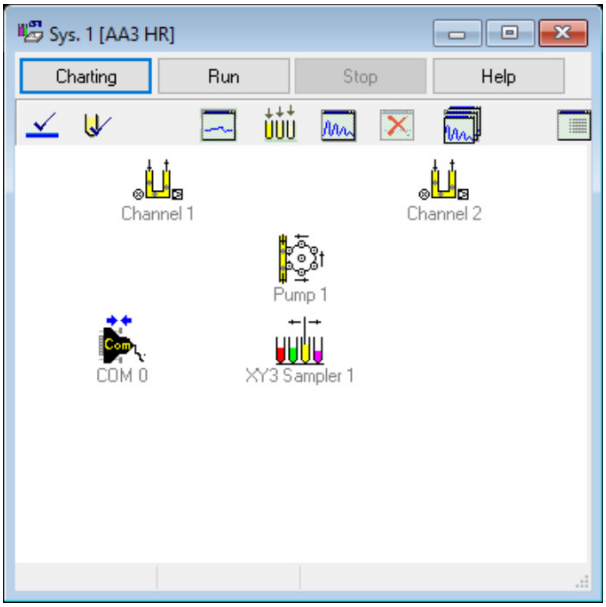
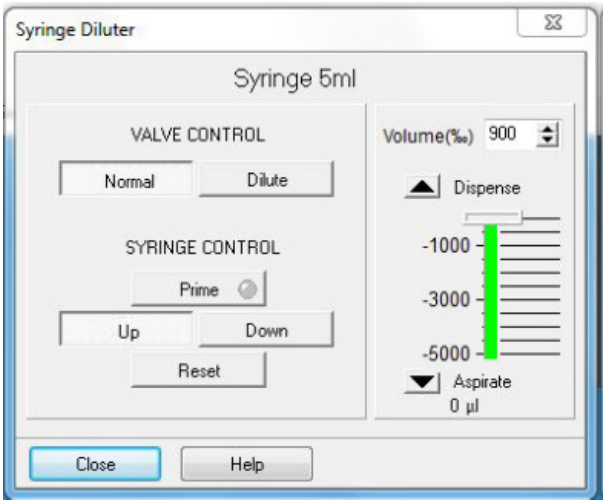


Figure 14.2. The Syringe Diluter control window



7. Check reagents' absorbance of each channel by setting the Gain (double click on channel icon in the system window) to 10 and setting the base to 5%. Then pump reagents, open the cadmium column, and let the NH_4Cl run through to prepare the column for the conversion of NO_3 to NO_2. Once the baseline on each channel stabilizes, record the reagent absorbance as the percent difference between baseline absorbance and reagent absorbance.
8. Standards are created by diluting the mixed calibration standard stock using the built-in syringe diluter. Ensure that there is fresh Milli-Q water in the syringe diluter receptacle and empty vials in slots 901 through 908 of the autosampler standard tray. Place a 14-mL tube of mixed calibration standard in slot 910.

   Click on the *Syringe Diluter* icon in the system window, click the *Reset* button and then click the *Prime* button to prime the syringe diluter with fresh Milli-Q water.

   Select the *Build Standards* icon from the system window and select the *190503A.run* file in the "Nitrogen stds" directory. Ensure that the concentration field is set to 500 and that the calibrants are set as shown in the *Build Standards* image (Figure 14.3).
9. Verify the Gain on each channel by sampling the highest calibrant for 120–180 seconds. Click the *Sampler* icon in the system window, type the cup position of the highest calibrant, and click *Sample*. After 120–180 seconds have elapsed, click *Wash* to move the

Figure 14.3. The Build Standards set up window

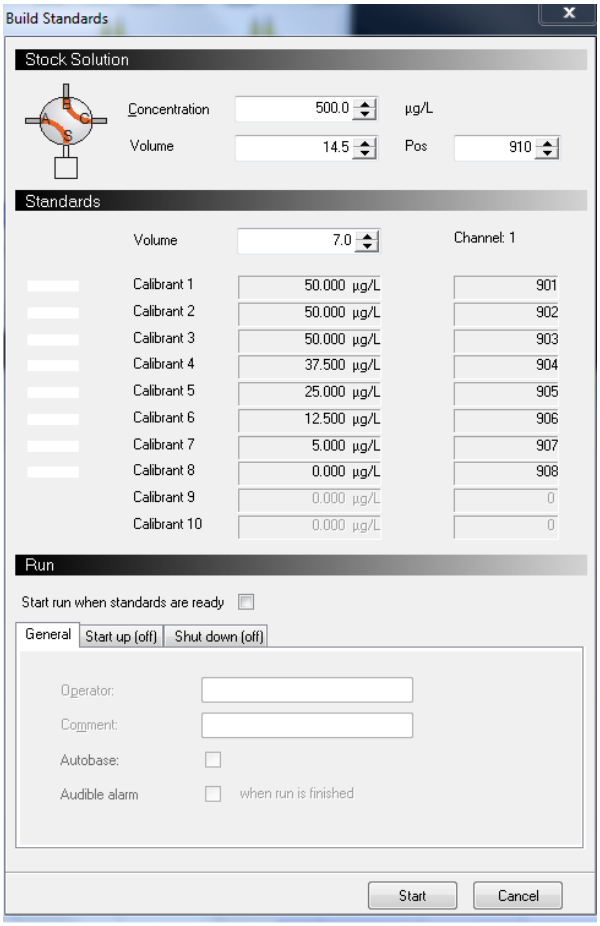
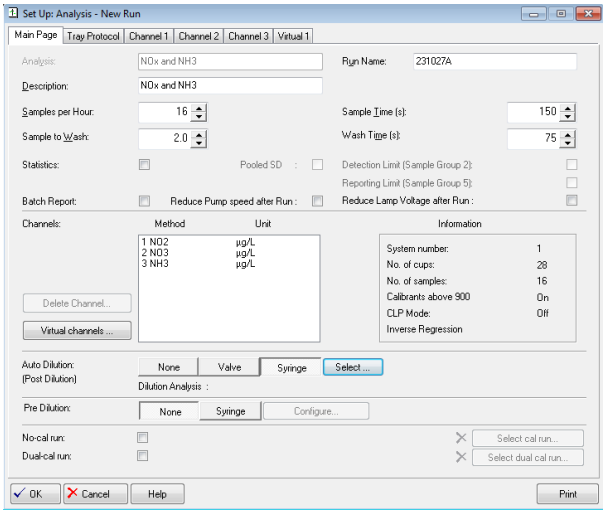


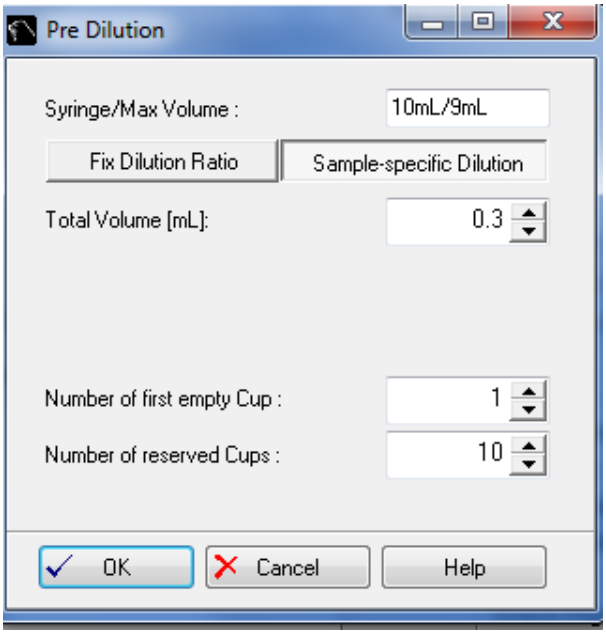
Figure 14.4. The Set Up: Analysis – New Run window



sampler back to the wash receptacle. Once the plateau of the standard peak has been reached and stabilized set the Gain (right-click on the channel charting window and click *Set Gain*). Make sure enough standard remains for the run. If not, it may be necessary to make more standard.

10. Double-check the channel QC parameters, such as light power, reference energy, sensitivity, and smoothing, if necessary.
11. Click *Set Up* and *Analysis/Run* on the top bar of the window and navigate to the NO_xNH_3 directory, select the "NOxNH3.anl" file and click *New Run*.
12. The *Set Up: Analysis – New Run* window will open wherein the analytical run is configured. The following settings are stipulated on the Main page tab.
    - Sample Time: 150
    - Wash Time: 75
    - Samples per Hour: 16
    - Sample to Wash: 2.0
    - Channels
      - Channel 1: NO_2
      - Channel 2: NO_2 + NO_3
      - Channel 3: NH_3
      - Virtual 1: (NO_3)
    - Data fields:
      - (Weight)
      - Dilution
      - **Note:** A field in parentheses indicates that the field is set as inactive.
    - Auto Dilution (Post Dilution): Syringe
    - Pre-Dilution: Syringe

Figure 14.5. Pre-Dilution configuration window



13. If any of the samples need to be pre-diluted, the run can be set up to automatically dilute samples. Click the *Syringe* button on the Main Page and click *Configure* to open the Pre-Dilution configuration window. Select the *Sample-specific Dilution* button, ensure that the "Syringe/Max Volume" is set to 10mL/9mL (i.e. the volume of the syringe diluter), enter 9.0 for the "Total Volume (mL)," and enter the autosampler tray location of the first empty cup and how many empty cups are placed on the autosampler tray. (**Note:** Make sure to put the empty cups in order and that they are different than the empty cup setup in the NO_x and NH_3 Autodilution.anl file). Next, click *Virtual channels* and open the data

fields by clicking *Change* button. Activate the dilution data field by selecting *Yes* in the active column in the dilution row and click *OK*. Now, the dilution data can be stipulated in the tray protocol.

14. Click the "Tray Protocol" tab. The table should already be populated with the calibration standards and the recovery standards (Figure 14.6). Click the *Samples* button and stipulate the number of samples you would like to add to the table and the start cup. Stipulate which sample will be in each sample cup. The run should include a primer (highest calibrant), the calibration curve, at least two drift standards (highest calibrant), recovery standards, a reference standard, a reference sample, and at least one instrument duplicate for every 15–20 samples.

    Put an X in the "PD" column of all samples that need to be diluted (Figure 14.7) and stipulate the dilution factor in the "Dilution" column (only available if Pre-Dilution was set up).

    Click *Print Tray* to print the Tray Protocol and use it to fill each of the sample cups with the appropriate standard/sample.

Figure 14.6. Example Tray Protocol window

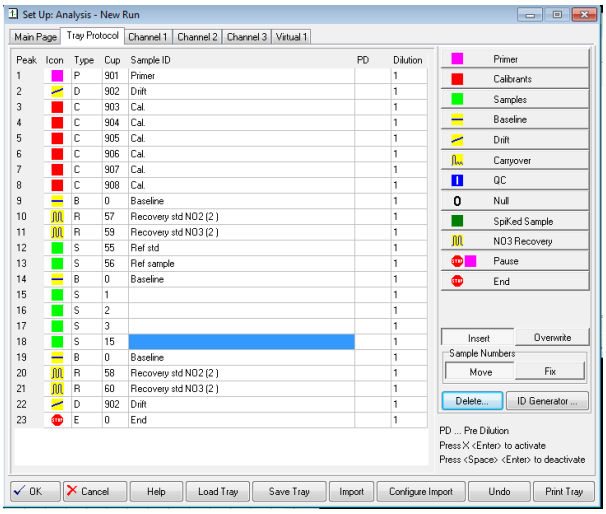


Figure 14.7. Example of Pre-Dilution configuration in the Tray Protocol window

Peak	Icon	Type	Cup	Sample ID	PD	Dilution
1		P	1	Primer		1
2		D	2	Drift		1
3		C	1	Cal.		1
4		C	2	Cal.		1
5		S	10		X	1
6		S	11			1
7		S	12		X	1
8		S	13			1

PD ... Pre Dilution
Press X <Enter> to activate
Press <Space> <Enter> to deactivate

15. Once the tray protocol is set up, any necessary Pre-Dilution settings are set, and the sample cups are filled and placed in the correct positions, click *Run* and select the created run from the directory to start the analytical run.

    Note: If the run was set up with Pre-Dilution, a [run name] PREDIL.run file is created and the samples are diluted prior to the run. The tray protocol of the original run file (i.e. yymmddX.run) is updated with the sample cup locations of the diluted samples.

16. Once the run is complete, click *Retrieve* on the top bar of the window and select *View chart* to view the peaks of the appropriate run. Any modifications that need to be made to the marking of peaks should be done before printing out a report. Peak markers are moved by clicking the move marker icon and moving the marker as needed.

17. Click *Display Report* on the top bar of the window to retrieve the results of the run of interest and save a copy of the run report at \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\AA3\AA3 Runs\NOx and NH3 in the yymmddX_NOxNH3 (X = A, B, C, etc.) format.
18. Turn off the cadmium column and pump Milli-Q water through the reagent lines for 15–20 minutes, followed by pumping air through the lines until dry.
19. Shut down the system by turning off the power bar and removing the peristaltic pump plaque and the system lines from tension.

14.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning*. Briefly, the vials are cleaned by rinsing three times with hot water and three times with DRO water.

14.14 Calculations

The calculation of NO_2-N and NH_3-N in samples is performed by the AACE 7.09 software using equation 14.6:

Equation 14.6

$$A = lg\left(\frac{I_0}{I}\right) = C \times \varepsilon \times d \quad \text{or} \quad C = \frac{A}{\varepsilon \times d}$$

where C is the concentration of the sample, A is the sample absorbance, lg is the logarithm function base 10, I_0 is the original measured light intensity, I is the reduced measured light intensity, ε is the molecular extinction coefficient, which is a constant, and d, which is the path length of the flow cell, which is also constant. The sample concentration is reported to the nearest integer.

The calculation of NO_3-N in the samples is performed by the AACE 7.09 software using equation 14.7, which accounts for the use of a mixed calibrant:

Equation 14.7

$$NO_3 = \frac{TON \times (A \times Recovery + B)/(A+B) - NO_2}{Recovery}$$

where *TON* is the total oxidized nitrogen (i.e. NO_2 + NO_3), A is the NO_3 concentration of the TON calibrant, B is the NO_2 concentration of the TON calibrant, NO_2 is the concentration of NO_2 in channel 1 (i.e. NO_2 concentration of the sample), and Recovery is the percentage of NO_3 that was converted to NO_2.

If a dilution has been made, the concentration of the sample needs to be corrected for the dilution factor, which is calculated using equation 14.8:

Equation 14.8

$$D = \frac{V_1}{V_0}$$

where V_1 is the volume of the diluted sample and V_0 is the volume of the undiluted sample.

14.15 Data entry

The NO_x and NH_3 concentrations for each sample, which are calculated using the AACE 7.09 software, as well as the QC data, are entered into the Sample Master® LIMS.

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Appendix 14A. Mixed Calibration Standard Stock Preparation Record

NOx/NH3 Mixed Calibration Standard Stock

Preparation Record

Stable for one week

Preparation Date	Standard	Manufacturer	Manufacturer lot number	Expiry date	Conc (µg/mL)	Vol added (µL)	Final vol (mL)	Final conc (µg/mL)	Expiry date	Analyst initials
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Ammonia (NH_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Ammonia (NH_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Ammonia (NH_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Ammonia (NH_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Ammonia (NH_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Ammonia (NH_3)									

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14B. Mixed Reference Standard Stock Preparation Record

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NOx/NH3 Mixed Reference Standard Stock
Preparation Record

Stable for one month

Preparation date	Standard	Manufacturer	Manufacturer lot number	Expiry date	Conc (µg/mL)	Vol added (µL)	Final vol (mL)	Final conc (µg/mL)	Assigned lab lot number	Expiry date	Analyst initials
	Nitrite (NO_2)										
	Nitrate (NO_3)										
	Ammonia (NH_3)										
	Nitrite (NO_2)										
	Nitrate (NO_3)										
	Ammonia (NH_3)										
	Nitrite (NO_2)										
	Nitrate (NO_3)										
	Ammonia (NH_3)										
	Nitrite (NO_2)										
	Nitrate (NO_3)										
	Ammonia (NH_3)										
	Nitrite (NO_2)										
	Nitrate (NO_3)										
	Ammonia (NH_3)										
	Nitrite (NO_2)										
	Nitrate (NO_3)										
	Ammonia (NH_3)										
	Nitrite (NO_2)										
	Nitrate (NO_3)										
	Ammonia (NH_3)										

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14C. Mixed Reference Standard Preparation Record

iisd ela
experimental lakes area

NOx/NH3 Mixed Reference Standard
Preparation Record

Stable for one week

Preparation date	Stock lab lot number	Stock vol (mL	Final vol (mL)	Final vol conc (µg/L)	Standard	Expiry date	Analyst initials
					Nitrite (NO_2)		
					Nitrate (NO_3)		
					Ammonia (NH_3)		
					Nitrite (NO_2)		
					Nitrate (NO_3)		
					Ammonia (NH_3)		
					Nitrite (NO_2)		
					Nitrate (NO_3)		
					Ammonia (NH_3)		
					Nitrite (NO_2)		
					Nitrate (NO_3)		
					Ammonia (NH_3)		
					Nitrite (NO_2)		
					Nitrate (NO_3)		
					Ammonia (NH_3)		
					Nitrite (NO_2)		
					Nitrate (NO_3)		
					Ammonia (NH_3)		
					Nitrite (NO_2)		
					Nitrate (NO_3)		
					Ammonia (NH_3)		
					Nitrite (NO_2)		
					Nitrate (NO_3)		
					Ammonia (NH_3)		

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 14D. 50 µg/L NO_2 and NO_3 Standard Preparation Record

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experimental lakes area

50 µg/L NO_2 & NO_3 Standards
Preparation Record

Stable for one week

Preparation date	Standard	Manufacturer	Manufacturer lot number	Expiry date	Conc (µg/mL)	Vol added (µL)	Final vol (mL)	Final conc (µg/L)	Expiry date	Analyst initials
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14E. 2000 µg/L NO_2 and NO_3 Standard Preparation Record

2000 µg/L NO_2 & NO_3 Standards
Preparation Record

Stable for one month

Preparation date	Standard	Manufacturer	Manufacturer lot number	Expiry date	Conc (µg/mL)	Vol added (µL)	Final vol (mL)	Final conc (µg/L)	Expiry date	Analyst initials
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									
	Nitrite (NO_2)									
	Nitrate (NO_3)									

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14F. Colour Reagent Preparation Record

Colour Reagent
Preparation Record

Store in amber bottle

Stable for one month

Preparation Date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Vol (mL)	Final vol (L)	Expiry date	Analyst initials
	Sulphanilamide								
	NNED								
	Conc. H_3PO_4								
	Sulphanilamide								
	NNED								
	Conc. H_3PO_4								
	Sulphanilamide								
	NNED								
	Conc. H_3PO_4								
	Sulphanilamide								
	NNED								
	Conc. H_3PO_4								
	Sulphanilamide								
	NNED								
	Conc. H_3PO_4								
	Sulphanilamide								
	NNED								
	Conc. H_3PO_4								
	Sulphanilamide								
	NNED								
	Conc. H_3PO_4								
	Sulphanilamide								
	NNED								
	Conc. H_3PO_4								

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14G. Ammonium Chloride Preparation Record

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Ammonium Chloride
(NO_3 and NO_2)
Preparation Record

Stable for one week

Preparation date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Vol (µL)	Final vol (L)	Expiry date	Analyst initials
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								
	NH_4Cl								
	NH_4OH								

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14H. Complexing Reagent Preparation Record

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Complexing Reagent
Preparation Record

Stable for three months

Preparation Date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Vol (mL)	Final vol (L)	Expiry date	Analyst initials
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14I. Hypochlorite Preparation Record

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Hypochlorite
Preparation Record

10:1 hypochlorite to water

Prepare daily

Preparation date	Manufacturer	Manufacturer lot number	Grade	NaOCl vol (mL)	Final vol (mL)	Expiry date	Analyst initials
			Commercial bleach				

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Authorization date: May 2019

Appendix 14J. Phenol Preparation Record

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Phenol
Preparation Record

Stable for three months

Preparation Date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Expiry date	Analyst initials
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14K. 1 mL/L Brij-35 Preparation Record

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1 mL/L Brij-35
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot Number	Brij-35 vol (mL)	Final vol (L)	Expiry date	Analyst initials

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Authorization date: May 2019

Appendix 14L. 2 mL/L Brij-35 Preparation Record

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2 mL/L Brij-35 (NH_3 System Wash)
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot Number	Brij-35 vol (mL)	Final vol (L)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14M. 6 mL/L Brij-35 Preparation Record

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6 mL/L Brij-35 (NH_3 System Wash)
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot Number	Brij-35 vol (mL)	Final vol (L)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14N. Copper Sulfate Preparation Record

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Copper Sulfate
Preparation Record

Stable indefinitely

PreparationDate	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 14O. 6 N Hydrochloric Acid Preparation Record

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6 N HCl
(NO_3 and NO_2)
Preparation Record

Stable indefinitely

Preparation Date	Manufacturer	Manufacturer lot number	Grade	HCl vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2019

15.0 Total Dissolved Nitrogen: UV oxidation and colourimetry method

15.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of total dissolved nitrogen (TDN) in fresh water and precipitation.

15.2 Method principle

This automated procedure is based on the method designed for the analysis of ammonia using the SEAL Analytical AutoAnalyzer 3 HR Continuous Segmented Flow Analyzer (SEAL Analytical, 2016) that was modified for the analysis of TDN by the inclusion of a zinc column. Under ideal conditions, organic nitrogen compounds can be oxidized into inorganic constituents after a four-hour exposure to high-intensity short-wave UV radiation (Armstrong & Tibbitts, 1968; Hensricksen, 1970; Stainton et al., 1977). The products of oxidation are nitrite, nitrate, and ammonia. These products can be measured after the photo oxidized sample is passed through a zinc column that has been conditioned with sulfuric acid, which reduces nitrite and nitrate to ammonia (Stainton et al., 1977). If only total inorganic nitrogen species are to be measured, simply skip the UV oxidation. Under appropriate conditions (i.e. pH 11.5–12.3), ammonia complexes with an ethylenediamine tetra-acetic acid (EDTA) and tri-sodium citrate dehydrate-based reagent. When the ammonia and complexing reagent react with the phenol and hypochlorite, an indophenol blue is formed (Harwood & Huyser, 1970). The colour intensity of the indophenol blue, which is proportional to the amount of ammonia in the sample, is measured on a colorimeter measuring absorbance at a wavelength of 660 nm.

15.3 Occupational health and safety

The short-wave UV irradiator, used for UV oxidation of the samples, can cause serious and potentially permanent damage to the eyes. Do not look at or expose skin to the UV light and ensure that there is a sign posted in a conspicuous location warning others of this hazard.

Hydrogen peroxide (30% w/w), also used for UV oxidation of the samples, is a strong oxidant that can burn skin, eyes, or the respiratory tract if inhaled. Avoid contact with skin. Wear a face shield, lab coat, and gloves when handling.

The phenol reagent used for reagent preparation is corrosive, toxic, and a health hazard. Avoid contact with skin. Wear a face shield, lab coat, and gloves when handling.

The concentrated sulfuric acid (H_2SO_4), used for UV oxidation of the samples, is corrosive and reacts violently with water. Avoid contact with skin. Wear a face shield, lab coat, and gloves when handling. Always pour acid into water slowly while stirring. **Never pour water into acid.**

15.4 Validation

15.4.1 Operating range

6.89–1,000 µg/L NH_3

15.4.2 Detection limit and limit of quantitation

The detection limit, which is based on the residuals along the standard curve, is calculated for each run using equations 15.1 through 15.3:

Equation 15.1

$$C_{dl} = \frac{y_d - b}{m}$$

where b is the y-intercept, m is the slope, and y_d is the instrument response detection limit, which is calculated using equation 15.2:

Equation 15.2

$$y_d = 3s_y + b$$

where s_y is based on the residuals between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration. It is calculated using equation 15.3:

Equation 15.3

$$s_y = \sqrt{\frac{\sum di^2}{n-2}}$$

where n is the number of standards in the calibration curve, and di is the difference between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration.

The limit of quantitation is calculated in the same manner as the detection limit, except equation 15.2 is replaced with equation 15.4:

Equation 15.4

$$y_q = 10s_y + b$$

The averages of the detection limits and limits of quantitation for samples run between 2019 and 2020 were as follows:

Detection limit	19 ± 10 µg/L NH_3-N
Limit of quantitation	64 ± 34 µg/L NH_3-N

15.5 Sample conditions

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must be

- filtered within 24 hours of sample collection,
- stored and analyzed within seven days,
- kept away from NH_3 environments, and
- stored at 4 ± 2 °C.

15.6 Equipment

- Short-wave UV irradiator
  - 550-watt medium-pressure mercury vapour lamp
  - cooling fan
  - quartz jacket tube to shield the mercury lamp from the cooling effect of the fan
  - a transformer to supply power at the correct voltage
  - an hour meter for monitoring lamp life
  - on-off automatic timer
  - support assembly
  - snorkel to vent free radicals and ozone
- SEAL AutoAnalyzer 3 HR
  - digital colorimeters
  - high-precision pump outfitted with the pump tubes outlined in the System Map (SEAL Analytical, 2016) that have been modified to include:
    - a 4 way valve connected to the sample pump tube (red/red instead of yel/yel)
    - a 0.04N H_2SO_4 pump tube (blk/blk)
    - a zinc column (see section 15.8.15) so that the sample and H_2SO_4 is pumped through the zinc column prior to reaching the manifold
  - multitest manifold
  - auto sampler
- computer running AACE 7.09 software
- quartz sample tubes: 16 mm I.D. × 18 mm O.D. × 20 cm length, 35 mL capacity, calibrated and etched to 25 mL
- carousel for quartz sample tubes

- 11-mL plastic sample tubes
- 14.5-mL plastic tubes for standards

15.7 Purchased reagents

15.7.1 NH_3 calibration standard stock

Commercial certified (traceable to NIST) NH_3-N standard

1.0 mL = 0.1 ± 0.01 mg NH_3-N

The standard is stable until the expiry date on the manufacturer's label.

15.7.2 NH_3 reference standard stock

Commercial certified (traceable to NIST) NH_3-N standard

1.0 mL = 1 ± 0.01 mg NH_3-N

The standard is stable until the expiry date on the manufacturer's label. It must be from a different manufacturer than the standard used for calibration standards.

15.7.3 Sulfuric acid, high purity

OPTIMA (Fisher Scientific), TraceMetal™ Grade (Fisher Chemical™), *Ultrex* (J.T. Baker), and *Aristar* (B.D.H.) brands have been found to have acceptable nitrogen levels. Store in an acid cabinet at room temperature within a sealed plastic bag. It is stable as per the manufacturer's specifications or until contaminated with phosphorus or nitrogen.

15.7.4 Hydrogen peroxide

Reagent grade, 30% w/w. **Caution: This is a dangerous oxidant.** Avoid contact with skin. Wear gloves and wash hands and arms after handling.

This reagent deteriorates if left open at room temperature. Store at 4 ± 2 °C. It is stable as per the manufacturer's specifications or until contaminated with phosphorus or nitrogen.

15.7.5 Zinc – 40 mesh

Zinc is stable indefinitely at room temperature.

15.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.

15.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

15.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q® Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

15.8.3 Sulfuric acid – 4 N

Dilute 106 mL of high-purity concentrated sulfuric acid into ~800 mL of Milli-Q water. Cool and make up to 1 L with Milli-Q water. It is stable indefinitely, or until contaminated, at room temperature.

15.8.4 Sulfuric acid – 1 N

Dilute 26.5 mL of high-purity concentrated sulfuric acid into 1 L of Milli-Q water. It is stable indefinitely, or until contaminated, at room temperature.

15.8.5 Sulfuric acid – 0.04 N

Dilute 1.06 mL of high-purity concentrated sulfuric acid into 1 L of Milli-Q water. It is stable indefinitely, or until contaminated, at room temperature.

15.8.6 Complexing reagent

- 15 g EDTA
- 120 g *tri*-sodium citrate dihydrate
- 0.5 g sodium nitroprusside
- 3 mL Brij-35, 22–30% solution

Dissolve EDTA, *tri*-sodium citrate dihydrate, and sodium nitroprusside into 1 L with Milli-Q water and then add Brij-35, 22–30% solution. Store in an amber bottle. It is stable for two weeks.

> **Note:** If the calcium or magnesium concentrations of samples are likely to exceed 0.005 m/L and 0.04 mol/L, respectively, the EDTA should be increased to 30 g/L.

15.8.7 Hypochlorite

Dilute commercial unscented bleach (preferably Chlorox) with 10 parts Milli-Q water to 1 part bleach. It is stable for 24 hours.

15.8.8 Phenol

- 50 g phenol
- 30 g sodium hydroxide

Dissolve phenol and sodium hydroxide into 1 L of Mill-Q water. Store in an amber bottle. It is stable until the solution turns brown.

> **Note:** Final pH should be 11.5–11.9. If the final pH is too high, reduce the sodium hydroxide concentrations. If the pH is too low, increase the sodium hydroxide concentration.

15.8.9 NH_3 calibration standard – 10 µg/mL

Dilute 10 mL of the 0.1 mg/mL NH_3-N to 100 mL with Milli-Q water in a 100 mL volumetric flask. Make fresh weekly.

15.8.10 NH_3 reference standard – 500 µg/L

Dilute 0.05 mL of the 1 mg/mL NH_3-N to 100 mL with Milli-Q water in a 100 mL volumetric flask. Make fresh weekly.

15.8.11 NO_3 standard – 500 µg/L

Dilute 50 µL of the 1000 µg/L NO_3 standard stock to 100 mL in a 100 mL volumetric flask. Make fresh weekly.

15.8.12 Urea check standard stock – 1000 µg/mL (as N)

Dissolve 2.1429 g of Ultrex (J.T. Baker) Urea (CH_4N_2O) into 1 L of Milli-Q water. Store at 4 ± 2 °C. It is stable for six months.

15.8.13 Urea check standard – 500 µg/L (as N)

Dilute 0.5 mL of the urea check standard stock to 1 L with Milli-Q water. It is stable for six months.

15.8.14 TDN calibration standard curve

The standard curve is created using the "Build Standards" option in the AACE software. See step 8 in Section 15.12 *Analysis* for how the standard curve is created using the AACE software.

15.8.15 Zinc column

Close one end of a 30-cm piece of 0.110" I.D. Tygon tubing with glass wool. Connect the other end of the tubing to a small funnel using a small piece of 1/8" I.D. tubing. Elevate the funnel using a ring stand and attach a 50-mL syringe to the end with wool. Fill the funnel with Milli-Q water and use the syringe to pull the Milli-Q water into the tubing ensuring there are no air bubbles in the tubing. Place zinc mesh in a small beaker with Mill-Q water and use a rod to stir the zinc mesh and dislodge air bubbles that attach to the zinc particles. Transfer the wetted zinc into the tubing using the funnel. Do not allow air bubbles to be trapped in the column and ensure that the zinc is packed with no dead space. Remove the funnel and close the tubing with glass wool and an N6 nipple. Syringe 15 mL of 1 N H_2SO_4 through the column, followed by 15 mL of Milli-Q water. Remove the syringe and close the tubing with a N6 nipple.

Attach the column to the manifold and condition the column with 0.04 N H_2SO_4 for 15–20 minutes.

15.8.16 Reference sample

Reference samples are prepared according to Section 4, *Reference Sample Preparation*. The reference sample consists of water from Lake 239 that has been aged for at least one year and filtered. The reference sample is stored at 4 ± 2 °C.

15.9 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated, reported in the *Non-Conformance* log and appropriate corrective action is taken before testing is resumed.

15.9.1 Zinc column conversion efficiency

The conversion efficiency of the zinc column is checked prior to conducting an analytical run by measuring the peak areas of 500 µg/L NO_3-N (A_{NO3}) and 100 µg/L NH_3-N (A_{NH3}) standards. The zinc column efficiency, which is calculated using equation 15.5, must be greater than 0.95. If the zinc column conversion efficiency (X_{Zn}) is less than 0.95, the column needs to either be reconditioned or remade and conditioned.

Equation 15.5

$$X_{Zn} = \frac{A_{NO3}}{A_{NH3}}$$

15.9.2 Blanks

A reagent blank, consisting of Milli-Q water and the reaction reagents, is incorporated as a baseline, which is run every 5–10 samples.

15.9.3 Calibration curve

The linear correlation coefficients obtained from the calibration standards must have an $R^2 \geq 0.995$ with a first order fit. If necessary, new calibration standards should be prepared.

15.9.4 Urea standard

A urea standard is included on every rack of UV oxidation samples to ensure that UV lamp energy is sufficient to oxidize organic nitrogen. The carbon nitrogen bonds found in urea are particularly resistant to UV decomposition, making it a good indicator of lamp performance. If the urea standard is not within 5.0% of the expected value (i.e. 475-525 µg/L), the UV oxidation is non-compliant.

15.9.5 Reference sample

A reference sample is included at the beginning of every run. The TDN value obtained must agree to within 5.0% of the expected value. Results for check sample performance are charted and assessed for drift and bias.

15.9.6 Duplicates

Random instrument duplicates are run every 15 samples. Samples for duplicate analysis are chosen at the beginning of the analysis run (before the first analysis is done). Duplicates' relative percent difference (RPD) must agree to within 5.0%. If duplication is inadequate, samples bracketed by the duplicates are re-tested. One method duplicate is included on each rack of UV oxidation samples and is included in the analytical run.

15.9.7 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verifications*. All glassware is cleaned according to Section 2, *Labware Cleaning*.

15.10 Quality assurance

Quality assurance for TDN is maintained through annual participation in the Environment and Climate Change Canada Proficiency Testing Program (ECCC-PT). Non-compliance is defined by ECCC-PT and is reported directly to the Research Chemist. Samples analyzed during the period of non-compliance are flagged in the database.

15.11 Sample preparation

15.11.1 UV oxidation

Note: Wear gloves when handling the quartz tubes, quartz surface of the lamp, or quartz jacket. Salt deposits ($NaHCO_3$ in particular) will permanently etch quartz surfaces, reducing their transparency, and hence their photo-digestion efficiency. Quartz surfaces should be inspected at least every six months for cleanliness and wiped with soft tissue, moistened first with Milli-Q water, followed by ethanol.

1. Fill out the UV Oxidation BenchSheet, which includes the rack number, the UV oxidation instrument used, sample order on the numbered rack, date and time of UV oxidation, any sample dilutions and the initials of the analyst. These worksheets are stored in a 3-ring binder located next to the UV oxidation instruments. See Figure 15.1. for an example of how standards/samples are typically loaded onto the UV oxidation rack.

Figure 15.1. Example UV Oxidation BenchSheet, which outlines the typical sequence of urea, blanks, reference sample, samples, and method duplicate

UV Oxidation Bench Sheet		
Date:	Date:	Date:
Time on:	Time on:	Time on:
Lamp #:	Lamp #:	Lamp #:
Rack #:	Rack #:	Rack #:
1 urea check standard	1	1
2 blank (Milli-Q water)	2	2
3 reference sample	3	3
4 sample 1	4	4
5 sample 2	5	5
6 sample 3	6	6
7 sample 4	7	7
8 sample 5	8	8
9 sample 6	9	9
10 sample 7	10	10
11 sample 8	11	11
12 sample 9	12	12
13 sample 10	13	13
14 sample 11	14	14
15 sample 12	15	15
16 sample 13	16	16
17 sample 14	17	17
18 sample 15	18	18
19 sample 16	19	19
20 method duplicate	20	20
Time off:	Time off:	Time off:
Analyst initials:	Analyst initials:	Analyst initials:

2. Add 25 mL of each QC sample/standard and filtered samples to quartz tubes and place them into the UV oxidation rack in the order as written on the UV Oxidation BenchSheet.
3. Use a repeater Eppendorf™ pipette to add 100 µL of 4N H_2SO_4 (high purity) and 100 µl of 30% H_2O_2 to each 25 mL tube (including urea standard, method blank and reference sample). There are two labelled, 50-µL, repeater pipette tips. One is for H_2O_2, and the other is for H_2SO_4. These repeater tips should be rinsed out with DRO water after each use and rinsed with reagent before being used to dispense.
4. Stopper the tubes with plastic caps and place the rack into the UV oxidization instrument and irradiate for four hours. Turn on the snorkel to vent out free radicals and ozone.
5. Once UV oxidation is complete, allow the samples to cool and check for evaporation. If evaporation has occurred, top up to the 25 mL line with Milli-Q water.

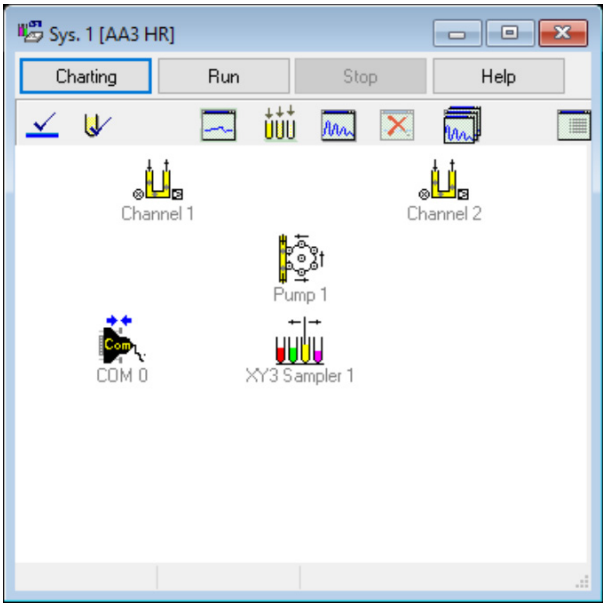
Caution: Do not look at or expose skin to the UV light and ensure that there is a sign posted in a conspicuous location warning others of this hazard.

15.12 Analysis

1. Turn on power on the SEAL AutoAnalyzer 3 HR by turning on the power bar.
2. On the PC, double-click the *AACE 7.09* shortcut.
3. Click the *Charting* button displayed in the *Sys. 1 [AA3 HR]* system window (Figure 15.2.). Select the appropriate analysis (.anl) file.

   Note: When charting is started, the Base, Gain, and Light Power values that were used the last time this .anl file was run are downloaded to the colorimeters.
4. Replace Milli-Q water in the wash bottles, washpot bottle, and syringe diluter receptacle.

Figure 15.2. The Sys. 1 [AA3 HR] system window



5. Pump system wash solution (2 mL/L Brij-35) through all of the reagent lines, except for 0.04 N H_2SO_4, for 10–15 minutes to "wet" the tubes with surfactant. The surfactant ensures proper flow and bubble pattern.
   - Bubbles must completely fill the tubing. Their length should be approximately 1.5 times the inner diameter of the tubing.

- Bubbles must be the same size, shape, and distance apart.
- Bubble shape in all plastic tubing must be round at the front and back. If the bubble looks straight or square at the back, there is insufficient wetting agent.

6. Pump Milli-Q water through the lines and allow the baseline to stabilize, checking the bubble pattern for proper bubble shape. Set the base to 5% (right-click on the channel charting window and click *Set Base*).
7. Check the reagent absorbance of each channel by setting the Gain (double click on the *Channel* icon in the system window) to 10 and setting the base to 5%. Then pump reagents until a stable baseline is reached. Once the baseline is reached, open the zinc column and let the H_2SO_4 run through to prepare the column for the conversion of NO_x to NH_3. Record the reagent absorbance.
8. Standards are created by diluting the 10 µg/mL NH_3 stock standard using the built-in syringe diluter. Ensure that there is fresh Milli-Q water in the syringe diluter receptacle, empty vials in slots 901 through 908 of the autosamplers standard tray, and ensure that a 14 mL tube of 10 µg/mL NH_3 stock standard is placed in slot 910. Click on the *Syringe Diluter* icon in the system window, click the *Reset* button and then the *Prime* button to prime the syringe diluter with the fresh Milli-Q water.
   Select the *Build Standards* icon from the system window and select the "TDN standards.run" file in the

Figure 15.3. The Syringe Diluter control window

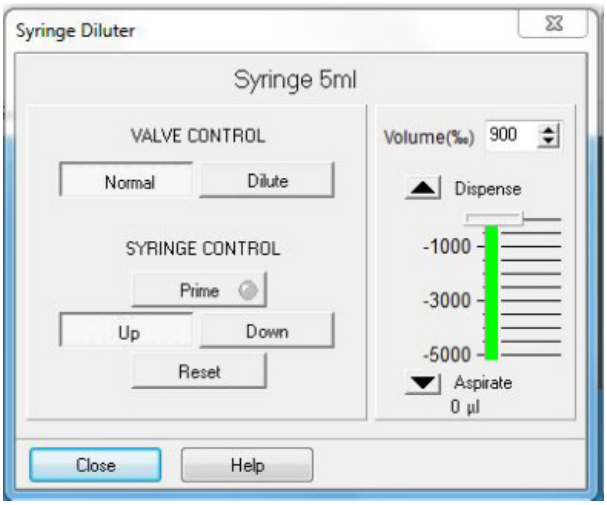
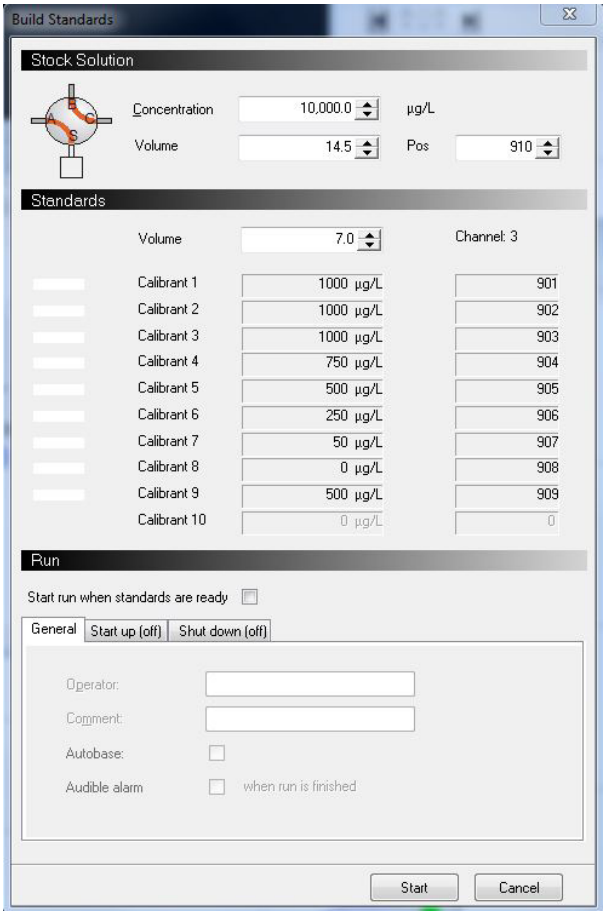


Figure 15.4. The Build Standards set up window



"TDN stds" directory. Ensure that the concentration field is set to 10 000 and that the calibrants are set as shown in the *Build Standards* image (Figure 15.4).

9. Verify the Gain on each channel by sampling the highest calibrant for 120 to 180 seconds. Click the *Sampler* icon, type the cup position of the highest calibrant (901, 902, or 903), and click *Sample*. After 120 to 180 seconds have elapsed, click *Wash* to move the sampler back to the wash receptacle. Once the plateau of the standard peak has been reached and stabilized, set the Gain (right click in the channel charting window and click *Set Gain*). Make sure enough standard remains for the run. If not, it may be necessary to make more standard.
10. Double-check the channel QC parameters such as light power, reference energy, sample:reference, and smoothing if necessary.
11. The efficiency of the Zn column to convert NO_x to NH_3 is checked prior to conducting the run. To check the Zn efficiency:

    a. Create a new run. Click *Set Up* > *Analysis*/*Run*, navigate to the Zn conversion directory, highlight the .anl file at the top and click *New Run*. The Set Up: Analysis – New Run window will open, wherein the analytical run is already configured for the Zn conversion test. Click OK.

    b. Place a 500-µg/L NO3 standard into slot 60 of the autosampler tray and ensure that there is sufficient 500 µg/L NH3 in slot 905 (created in the standard build).

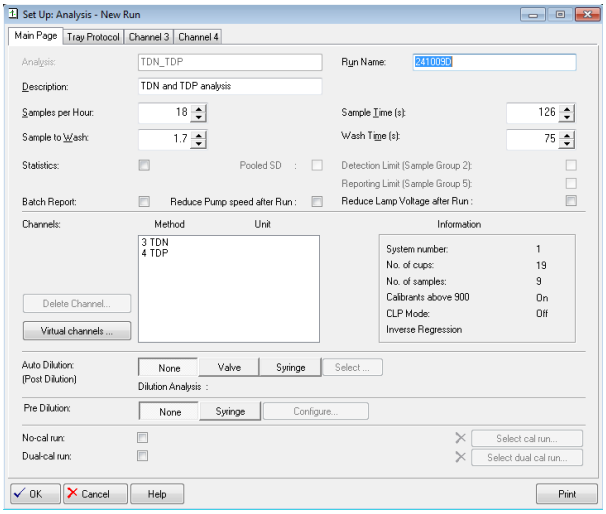
    c. Click *Run* and select the newly created Zn conversion file.

    d. Once the run is complete, retrieve the run Chart by clicking *Retrieve* > *View Chart*. Any modifications that need to be made to the marking of peaks should be done before printing out a report. Peak markers are moved by clicking the move marker icon and moving the marker as needed. Click *Display Report* on the top bar of the window to retrieve the results of the run.

    e. Change the units to Raw Data and calculate the Zn efficiency by dividing the 500 NO_3 raw data by the 500 NH_3 Raw Data.

    f. If the efficiency is > 0.95 (equation 15.5), proceed with the analytical run. If not, the Zn column either needs additional conditioning or a new Zn column needs to be made and conditioned.

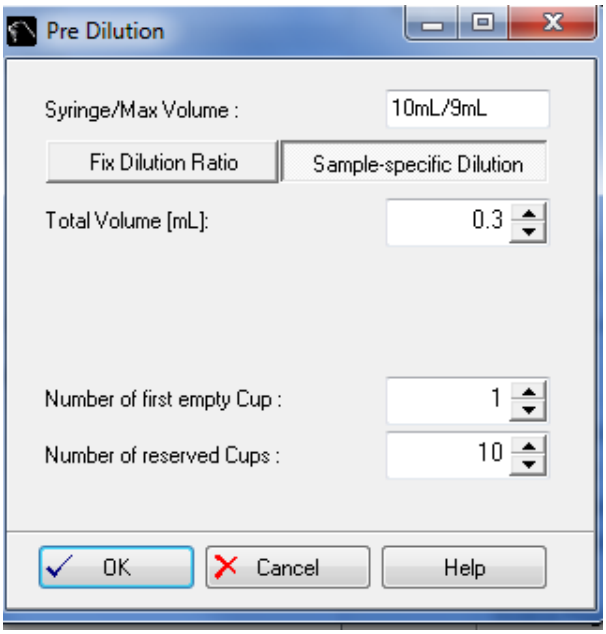
Figure 15.5. The Setup Up: Analysis – New Run window



12. To set up the analytical run, click Set Up and Analysis/Run on the top bar of the window, navigate to the TDN directory, select the "TDN.anl" file and click *New Run*.
13. The Set Up: Analysis – New Run window (Figure 15.5) will open, wherein the analytical run is configured. The following settings are stipulated on the Main tab.
    - Sample Time: 126
    - Wash Time: 75
    - Samples per Hour: 18
    - Sample to Wash: 1.7
    - Channels
    - Channel 3: TDN
        - Virtual Channels: none
    - Data fields:
        - (Weight)
        - Dilution
        - **Note:** A field in parentheses indicates that the field is set as inactive.
    - Auto Dilution (Post Dilution): Syringe
    - Pre-Dilution: Syringe
14. If any of the samples need to be pre-diluted, the run can be set up to automatically dilute these samples by clicking the *Syringe* button on the Main Page.
15. Select *Configure* to open the Pre-Dilution configuration window. Select the *Sample-specific Dilution* button, ensure that the "Syringe/Max Volume" is set to 10mL/9mL (i.e. the volume of the syringe diluter), enter 9.0 for the "Total Volume (mL)," and enter the autosampler tray location of the first empty cup and how many empty cups are placed on the autosampler tray (Figure 15.6).

    Note: Make sure to put the empty cups in order and that they are different than the empty cup setup in

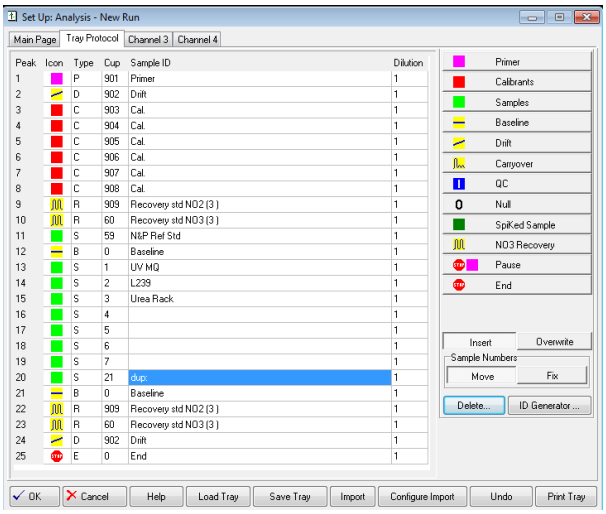
Figure 15.6. *Pre-Dilution* configuration window



the TDN Auto-Dilutions.anl. Next, click *Virtual channels* and open the data fields by clicking the *Change* button. Activate the dilution data field by selecting *Yes* in the active column in the dilution row and clicking *OK*. Now, the dilution data can be stipulated in the tray protocol.

16. Click the "Tray Protocol" tab. The table should already be populated with the calibration standards and the recovery standards (Recovery std NO_2 is actually NH_3). Click the *Samples* button and stipulate the number of samples you would like to add to the table and the start cup. Stipulate which sample will be in each sample cup. The run should include a primer (highest calibrant), the calibration curve, at least two drift standards (highest calibrant), recovery standards, a reference standard, a reference sample, and at least one instrument duplicate for every 15–20 samples (Figure 15.7).

Figure 15.7. Example Tray Protocol window



17. Put an X in the "PD" column of all samples that need to be diluted and stipulate the dilution factor in the "Dilution" column (Figure 15.8, only available if Pre-Dilution was set up).

Figure 15.8. Example of Pre-Dilution configuration in the Tray Protocol window

Peak	Icon	Type	Cup	Sample ID	PD	Dilution
1		P	1	Primer		1
2		D	2	Drift		1
3		C	1	Cal.		1
4		C	2	Cal.		1
5		S	10		X	1
6		S	11			1
7		S	12		X	1
8		S	13			1

PD ... Pre Dilution
Press X <Enter> to activate
Press <Space> <Enter> to deactivate

18. Click *Print Tray* to print the Tray Protocol and use it to fill each of the sample cups with the appropriate standard/sample.

19. Once the tray protocol is set up, any necessary Pre-Dilution settings are set and sample cups are filled and placed in the correct positions, click *Run* and select the created run from the directory to start the analytical run.

    Note: If the run was set up with Pre-Dilution, a [run name] PREDIL.run file is created and the samples are diluted prior to the run. The tray protocol of the original run file (i.e. yymmddX.run) is updated with the sample cup locations of the diluted samples.

20. Once the run is complete, click *Retrieve* on the top bar of the window and select *View chart* to view the peaks of the appropriate run. Any modifications that need to be made to the marking of peaks should be done before printing out a report. Peak markers are moved by clicking the move marker icon and moving the marker as needed.

21. Click *Display Report* on the top bar of the window to retrieve the results of the run of interest and save a copy of the run report at \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\AA3\AA3 Runs\TDN in the yymmddX_TDN (X = A, B, C etc.) format.
22. Turn off the zinc column and pump Milli-Q water through the reagent lines for 15–20 minutes, followed by pumping air through the lines until dry.
23. Shut down the system by turning off the power bar and removing the peristaltic pump plaque and the system lines from tension.

15.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning.* Briefly, the vials are cleaned by rinsing three times with hot water and three times with DRO water.

15.14 Calculations

The calculation of NH_3-N in samples is performed by the AACE 7.09 software using the following equation:

Equation 15.6

$$A = lg\left(\frac{I_0}{I}\right) = C \times \varepsilon \times d \quad \text{or} \quad C = \frac{A}{\varepsilon \times d}$$

where C is the concentration of the sample, A is the sample absorbance, lg is the logarithm function base 10, l_0 is the original measured light intensity, I is the reduced measured light intensity, ε is the molecular extinction coefficient, which is a constant, and d is the path length of the flow cell, which is also constant. The sample concentration is reported to the nearest integer.

If a dilution has been made, the concentration of the sample needs to be corrected for the dilution factor (equation 15.7):

Equation 15.7

$$D = \frac{V_1}{V_0}$$

where V_1 is the volume of the diluted sample and V_0 is the volume of the undiluted sample.

15.15 Data entry

The TDN concentrations for each sample, which are calculated using the AACE 7.09 software, as well as the QC data, are entered into the Sample Master LIMS.

15.16 References

Armstrong, F. A. J., & Tibbitts, S. (1968). Photochemical combustion of organic matter in sea water for nitrogen, phosphorus and carbon determination. *Journal of the Marine Biological Association of the United Kingdom, 48,* 143–152. https://doi.org/10.1017/S0025315400032483

Harwood, J. E., & Huyser, D. J. (1970). Automated analysis of ammonia in water. *Water Research, 5,* 695–704. https://doi.org/10.1016/0043-1354(70)90031-X

Henriksen, A. (1970). Determination of total nitrogen, phosphorus and iron in freshwater by photo-oxidation with ultraviolet radiation. *Analyst, 95*, 601-605. (U of M Science Library Sci/Per/543/A532/Ch/v95. https://doi.org/10.1039/AN9709500601

SEAL Analytical. (2016). *Method for the AutoAnalyzer 3 High Resolution Continuous Flow Analytical Instrument* (SEAL Analytical Method G-171-96 Rev. 16). SEAL Analytical Corporation.

Stainton, M. P., Capel, M. J. &. Armstrong, F. A. J. (1977). *The chemical analysis of fresh water* (2nd ed). (Miscellaneous Special Publication 25). Fisheries Marine Service. https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/110147.pdf

Appendix 15A. 4 N Sulfuric Acid Preparation Record

iisd|ela experimental lakes area

4 N H_2SO_4
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	H_2SO_4 vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 15B. 1 N Sulfuric Acid Preparation Record

iisd|ela experimental lakes area

1 N H_2SO_4
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	H_2SO_4 vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 15C. 0.04 N Sulfuric Acid Preparation Record

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0.04 N H_2SO_4
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	H_2SO_4 vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 15D. Complexing Reagent Preparation Record

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experimental lakes area

Complexing Reagent
Preparation Record

Stable for three months

Preparation Date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Vol (mL)	Final vol (L)	Expiry date	Analyst initials
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								
	EDTA								
	Na3 citrate								
	Na nitroprusside								
	Brij-35								

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 15E. Hypochlorite Preparation Record

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Hypochlorite
Preparation Record

10:1 hypochlorite to water

Prepare daily

Preparation date	Manufacturer	Manufacturer lot number	Grade	NaOCl vol (mL)	Final vol (mL)	Expiry date	Analyst initials
			Commercial bleach				

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 15F. Phenol Preparation Record

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experimental lakes area

Phenol
Preparation Record

Stable for three months

Preparation Date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Expiry date	Analyst initials
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							
	Phenol							
	NaOH							

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 15G. 10 µg/mL NH_3 Calibration Standard Stock Preparation Record

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10 µg/mL NH_3 Calibration Standard Stock
Preparation Record

Stable for one week

Preparation Date	Manufacturer	Manufacturer lot number	Expiry date	Conc (µg/mL)	Vol added (mL)	Final vol (mL)	Final conc (µg/mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 15H. 500 µg/L NH_3 Reference Standard Preparation Record

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500 µg/L NH_3 Reference Standard
Preparation Record

Stable for one week

Preparation date	Manufacturer	Manufacturer lot number	Expiry date	Conc (µg/mL)	Vol added (mL)	Final vol (mL)	Final conc (µg/mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 15I. 500 µg/L NO_3 Standard Preparation Record

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500 µg/L NO_3 Standard
Preparation Record

Stable for one week

Preparation date	Manufacturer	Manufacturer lot number	Expiry date	Conc (µg/mL)	Vol added (mL)	Final vol (mL)	Final conc (µg/mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2019

Appendix 15J. 1000 µg/mL-N Urea Standard Stock Preparation Record

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1000 µg/mL-N Urea Standard Stock
Preparation Record

Target is 2.14379g/L Urea

Stable for six months

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight	Final vol (ml)	Conc (1mg/mL)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 15K. 500 µg/L-N Urea Standard Preparation Record

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500 µg/L-N Urea Standard Stock
Preparation Record

Stable for six months

	Urea stock						
Preparation date	Lab lot number	Vol (mL)	Conc (µg/mL)	Final vol (mL)	Final conc	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

16.0 Total Dissolved Phosphorus

16.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of total dissolved phosphorous (TDP), as PO_4^{-3}-P, in fresh water and precipitation.

16.2 Method principle

Organic phosphorus compounds under acidic conditions and, with adequate oxygen supply, can be photo-oxidized to orthophosphate (PO_4^{-3}) with short-wave UV radiation (Armstrong & Tibbits, 1968; Henriksen et al., 1970; Stainton et al.; 1977). Breakdown is quick (one to four hours). This method does not, however, appear to hydrolyze polyphosphate. If significant amounts of polyphosphates are suspected to be present, the UV oxidized sample should be boiled with HCl prior to the orthophosphate analysis (Strickland & Parsons, 1972).

The resulting orthophosphate is measured by the soluble reactive phosphorus method (Stainton et al., 1977). The UV oxidation procedure is identical to that for total dissolved nitrogen.

Phosphate, silicate, arsenate, and germanate ions react under acidic conditions with molybdate to form heteropoly acids that can be converted by suitable reducing agents to blue compounds of uncertain composition (Nagul et al., 2015; Stainton et al., 1977).

16.3 Occupational health and safety

The short-wave UV irradiator, used for UV oxidation of the samples, can cause serious and potentially permanent damage to the eyes. Do not look at or expose skin to the UV light and ensure that there is a sign posted in a conspicuous location warning others of this hazard.

Hydrogen peroxide (30% w/w), also used for UV oxidation of the samples, is a strong oxidant that can burn skin, eyes, or the respiratory tract if inhaled. Avoid contact with skin. Wear a face shield, lab coat, and gloves when handling.

The phenol reagent used for reagent preparation is corrosive, toxic, and a health hazard. Avoid contact with skin. Wear a face-shield, lab coat, and gloves when handling.

The concentrated sulfuric acid (H_2SO_4), used for UV oxidation of the samples, is corrosive and reacts violently with water. Avoid contact with skin. Wear a face shield, lab coat, and gloves when handling. Always pour acid into water slowly while stirring. **Never pour water into acid.**

16.4 Validation

This method is suitable for determining the TDP in water.

16.4.1 Operating range

3 – 50 µg/L PO_4^{-3}-P

16.4.2 Detection limit and limit of quantitation

The detection limit, which is based on the residuals along the standard curve, is calculated for each run using equations 16.1 through 16.3:

Equation 16.1

$$C_{dl} = \frac{y_d - b}{m}$$

where b is the y-intercept, m is the slope, and y_d is the instrument response detection limit, which is calculated using equation 16.2:

Equation 15.2

$$y_d = 3s_y + b$$

where s_y is based on the residuals between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration. It is calculated using equation 16.3:

Equation 16.3

$$s_y = \sqrt{\frac{\sum di^2}{n-2}}$$

where n is the number of standards in the calibration curve, and di is the difference between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration.

The limit of quantitation is calculated in the same manner as the detection limit, except equation 16.2 is replaced with equation 16.4:

Equation 16.4

$$y_q = 10s_y + b$$

The averages of the detection limits and limits of quantitation for samples run between 2019 and 2021 were as follows:

Detection limit	3.0 ± 1.5 µg/L
Limit of quantitation	10.0 ± 5.0 µg/L

16.5 Sample condition

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparations. The sample must be

- stored at 4 ± 2 °C,
- UV oxidized within seven days of sample collection,
- analyzed within seven days of UV oxidation, and
- analyzed at room temperatures < 28 °C.

16.6 Equipment

- short-wave UV irradiator
    a. 550-watt medium-pressure mercury vapour lamp
    b. cooling fan
    c. quartz jacket tube to shield the mercury lamp from the cooling effect of the fan
    d. transformer to supply power at the correct voltage
    e. hour meter for lamp life monitoring
    f. on-off automatic timer
    g. support assembly
    h. snorkel to vent out free radicals
- quartz sample tubes: 16 mm I.D. × 18 mm O.D. × 20 cm length, 35 mL capacity, calibrated and etched to 25 mL
- carousel for quartz sample tubes
- Shimadzu UV-1800 scanning spectrophotometer set at 885 nm
- UV Probe software
- 10-cm pathlength cuvettes
- 50-mL graduated polyethylene conical tubes and rack to hold them
- Eppendorf™ repeater pipette and pipette tip with 5-mL dispensing capacity

16.7 Purchased reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.

- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.

16.7.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

16.7.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

16.7.3 Sulfuric acid, high purity

OPTIMA (Fisher Scientific), TraceMetal™ Grade (Fisher Chemical™), Ultrex (J.T. Baker), and Aristar (B.D.H.) brands have been found to have acceptable nitrogen levels. Store in an acid cabinet at room temperature within a sealed plastic bag. It is stable as per the manufacturer's specifications or until contaminated with phosphorus or nitrogen.

16.7.4 Sulfuric acid, ACS grade

Store in an acid cabinet at room temperature. It is stable as per the manufacturer's specifications or until contaminated with phosphorus or nitrogen.

16.7.5 Hydrogen peroxide

Reagent grade, 30% w/w. **Caution: This is a dangerous oxidant.** Avoid contact with skin. Wear gloves and wash hands and arms after handling.

This reagent deteriorates if left open at room temperature. Store at 4 ± 2 °C. It is stable as per the manufacturer's specifications or until contaminated with phosphorus or nitrogen.

16.7.6 Calibration standard stock

Commercial certified (traceable to NISCT) PO_4^{-3}-P standard:

1.0 mL = 50 ± 1 µg PO_4^{-3}-P

Store at 2–6 °C until the expiry date on the manufacturer's label.

16.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.

16.8.1 4 N sulfuric acid

Caution: ALWAYS pour acid into water slowly while stirring. NEVER pour water into acid.

Slowly pour 26.5 mL of high-purity concentrated sulfuric acid into ~200 mL Milli-Q water. Cool and dilute to 250 mL with Milli-Q water. Store at room temperature. Stable until contaminated with phosphorus or nitrogen.

16.8.2 Ammonium molybdate

Caution: ALWAYS pour acid into water slowly while stirring. NEVER pour water into acid.

Dissolve into ~1500 mL of ultrapure water. Add in the order given.

- 15 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}$ $4H_2O$)
- 0.28 g of antimony potassium tartrate ($KSbOC_4H_4O_6$)
- 176 mL concentrated sulfuric acid, ACS grade

Cool and dilute to 2 L with Milli-Q water. Store at room temperature in a brown polyethylene bottle. It is stable for one year.

16.8.3 Ascorbic acid

Dissolve 1.25 g of L-ascorbic acid into 50 mL of Milli-Q water. Prepare fresh.

16.8.4 Mixed molybdate

Mix 50 mL ascorbic acid for every 200 mL ammonium molybdate. Prepare fresh.

16.8.5 Reference standard stock – 50 µg/mL PO_4

Dissolve 0.2197 g potassium dihydrogen phosphate (KH_2PO_4) in 100 mL of Milli-Q water.

1.0 mL = 50 µg PO_4^{-3}-P

Store at room temperature in a labelled glass bottle. It is stable for one year.

16.8.6 Working calibration standard stock – 25 µg/mL PO_4

Dilute 1250 µL of the 50 µg/mL calibration standard stock to 25 mL with Milli-Q water.

1.0 mL = 2.5 µg PO_4^{-3}-P

Prepare weekly.

16.8.7 Calibration curve

Dilute volumes of the 2.5 µg/mL working calibration standard stock according to Table 16.1 with Milli-Q water to make each of the following calibration standard concentrations. Prepare weekly.

Table 16.1. Total dissolved phosphorus calibration standards

Std conc. (µg/L)	Working stock vol. (µL)	Final vol. (mL)
0	0	100
2.5	100	100
5	200	100
10	400	100
25	1000	100
50	2000	100

16.8.8 Reference standard – 25 µg/L PO_4

Dilute 125 µL of the 50 µg/mL reference standard stock to 250 mL with Milli-Q water. Prepare weekly.

16.8.9 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference Sample Preparation*. The TDP reference sample consists of water from Lake 239 that has been aged for at least one year, filtered, and spiked with 25 µg/L PO_4^{-3}-P. The reference sample is stored at 4 ± 2 °C.

16.9 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective action is taken before testing is resumed. If reanalysis is not possible, the samples are flagged in the database as non-compliant.

16.9.1 Calibration curve

The linear correlation coefficients obtained from the calibration standards must have an $R^2 \geq 0.995$ with a first order fit. If necessary, new calibration standards should be prepared.

16.9.2 Blanks

An instrument blank, consisting solely of ultrapure water, and a reagent blank, consisting of ultrapure water and reaction reagents, are included at the beginning of a run. A method blank, consisting of ultrapure water, reaction reagents, and photo-digestion reagents, is run for each photo-digestion rack of samples. The absorbance of the blanks should be no higher than 0.003.

16.9.3 Urea standard

A urea standard is included on every photo-digestion rack of samples to ensure that UV lamp energy is sufficient to break down organic nitrogen. The carbon carbon-nitrogen bonds found in urea are particularly resistant to UV decomposition, making it a good indicator of lamp performance. See Method ELA-TDN for details of this urea standard. If the urea standard is not within 5.0% of the expected value (i.e. 475–525 µg/L), the photo-digestion is non-compliant.

16.9.4 Reference standard

A reference standard is run at the beginning and end of an analytical run and every 16 samples within. The absorbance of the 25-µg/L reference standard should be within 5.0% of the 25-µg/L calibration standard.

16.9.5 Reference sample

A reference sample is included on every photo-digestion rack of samples. The TDP value obtained must be within 5.0% of the expected value. Results for reference sample performance are charted and assessed for drift and bias.

16.9.6 Duplicates

A random method duplicate is included on every photo-digestion rack of samples (16 samples or less). Instrument duplicates are run every 16 samples. Duplicates must agree to within 5.0% of each other. If duplication is inadequate, samples bracketed by the duplicates are reanalyzed.

16.9.7 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verifications*. All glassware is cleaned according to Section 2, *Labware Cleaning*.

16.10 Quality assurance

Quality assurance for TDP is maintained through annual participation in the Environment and Climate Change Canada Proficiency Testing Program (ECCC-PT). Non-compliance is defined by ECCC-PT and is reported directly to the Research Chemist. Samples analyzed during the period of non-compliance are flagged in the database.

16.11 Sample preparation

16.11.1 UV oxidation

> **Note:** Wear gloves when handling the quartz tubes, quartz surface of the lamp, or quartz jacket. Salt deposits ($NaHCO_3$ in particular) will permanently etch quartz surfaces, reducing their transparency and hence their photo-digestion efficiency. Quartz surfaces should be inspected at least every six months for cleanliness and wiped with soft tissue, moistened first with ultrapure water, followed by ethanol.
>
> **Note:** If significant amounts of polyphosphates are suspected to be present, the UV oxidized sample should be boiled with HCl prior to the orthophosphate analysis (Strickland & Parsons, 1968).

This UV oxidation procedure is identical to that used to measure total dissolved nitrogen (TDN). The UV oxidation efficiency of TDP samples is evaluated by analyzing the urea standard on each rack for TDN using the Section 15, *Total Dissolved Nitrogen – UV Oxidation and Colourimetry Method.*

1. Fill out the UV Oxidation BenchSheet, which includes the rack number, the UV oxidation instrument used, sample order on the numbered rack, date and time of UV oxidation, any sample dilutions, and the analyst's initials. These worksheets are stored in a 3-ring binder located next to the UV oxidation instruments. See Figure 16.1 for an example of how standards/samples are typically loaded onto the UV oxidation rack.

2. Add 25 mL of each QC sample/standard and filtered samples to quartz tubes and place them in the UV oxidation rack in the order as written on the UV Oxidation BenchSheet. If TDP is expected to exceed 50 µg/L, the sample should be diluted with Milli-Q water at this stage (i.e. prior to UV oxidation).
3. Use a repeater Eppendorf™ pipette to add 100 µL of 4N H_2SO_4 (high purity) and 100 µl of 30% H_2O_2 to each 25-mL tube (including urea standard, method blank, and reference sample). There are two labelled 50-µL, repeater pipette tips. One is for H_2O_2, and the other is for H_2SO_4. These repeater tips should be rinsed out with distilled water after each use and rinsed with reagent before being used to dispense.
4. Stopper the tubes with plastic caps, place the rack into the UV oxidization instrument, and irradiate for four hours. Turn on the snorkel to vent out free radicals and ozone.
5. Once UV oxidation is complete, allow the samples to cool and check for evaporation. If evaporation has occurred, top up to the 25-mL line with Milli-Q water.

Figure 16.1. Example UV Oxidation BenchSheet, which outlines the typical sequence of urea, blanks, reference sample, samples, and method duplicate

UV Oxidation Bench Sheet		
Date:	Date:	Date:
Time on:	Time on:	Time on:
Lamp #:	Lamp #:	Lamp #:
Rack #:	Rack #:	Rack #:
1 urea check standard	1	1
2 blank (Milli-Q water)	2	2
3 reference sample	3	3
4 sample 1	4	4
5 sample 2	5	5
6 sample 3	6	6
7 sample 4	7	7
8 sample 5	8	8
9 sample 6	9	9
10 sample 7	10	10
11 sample 8	11	11
12 sample 9	12	12
13 sample 10	13	13
14 sample 11	14	14
15 sample 12	15	15
16 sample 13	16	16
17 sample 14	17	17
18 sample 15	18	18
19 sample 16	19	19
20 method duplicate	20	20
Time off:	Time off:	Time off:
Analyst initials:	Analyst initials:	Analyst initials:

16.11.2 Colorimetric reaction

1. Add 25 mL of each of the following into 50-mL conical tubes:
   - reagent blank, consisting of Milli-Q water
   - calibration standards (i.e. 0, 2.5, 5, 10, 25, and 50 µg/L PO_4^{-3}-P)
   - 25-µg/L reference standards (prepare enough to run at the beginning and end of an analytical run and every 16 samples within).
2. Add 5 mL of mixed molybdate to each calibration standard, reference standard, reagent blank, method blank, reference sample, sample, and duplicate. **Do not add mixed molybdate to the urea sample.** This is to be analyzed for TDN.

3. Wait 30 minutes for the colorimetric reaction, but no more than three hours prior to analysis on the spectrophotometer.

16.12 Analysis

1. Turn on the power on the Shimadzu UV-1800 scanning spectrophotometer and let it warm up for at least two hours.
2. On the PC desktop, double-click the UV Probe shortcut.
3. Click OK.
4. Ensure that the *Photometric* icon is selected (Figure 16.2).

Figure 16.2. The Photometric icon

5. Open the UV Probe software from the desktop and open the TDP method (\\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\TDP\TDP template.pmd).
6. Click the *Connect* button at the bottom. If not available, click *View* > *Photometric buttons.* The UV detector will now go through its startup sequence.
7. After the startup sequence has finished, if the instrument has passed, click *OK.*
8. Rinse two 10-cm pathlength cuvettes at least three times with Milli-Q water and fill them with Milli-Q water.
9. Wipe the cuvettes with lint-free tissue and place them in the appropriate holders in the spectrophotometer (the cuvette farthest away from you is the reference position). Ensure that the wavelength is set to 885 nm and click *Auto Zero* to set the photometric value at 885 nm of ultrapure water to an absorbance of 0. Leave the cuvette in the reference position for the duration of the analytical run.
10. Remove the front cuvette, rinse at least three times with Milli-Q water then rinse and fill with the first standard (0 µg/L PO_4^{-3}-P). Enter the Sample ID and standard concentration (e.g. 0 std and 0.0) and click *Read Std.*
11. Repeat step 10 for the remaining standards.
12. Once the calibration curve is complete, remove the front cuvette and rinse at least three times with ultrapure water, then rinse and fill with Milli-Q water. Enter the Sample ID (e.g. instrument blank) and click *Read Unk*.

13. Repeat step 12 for the remaining blanks, QC standard/samples, and samples. Run a random duplicate, a reference standard, and a reagent blank every 16 samples. See Table 16.2 for a typical run sequence of standards/samples.

Table 16.2. A typical sequence of blanks, standards, reference standards, reference samples, and samples for one UV oxidation rack. A reference standard, duplicate sample, and reagent blank are analyzed at least every 16 samples.

Scan #	Sample description
1	0 µg/L calibration standard
2	2.5 µg/L calibration standard
3	5.0 µg/L calibration standard
4	10 µg/L calibration standard
5	25 µg/L calibration standard
6	50 µg/L calibration standard
7	25 µg/L reference standard
8	instrument blank (Milli-Q water)
9	reagent blank (Milli-Q water + mixed molybdate)
10	method blank (UV Milli-Q water)
11	reference sample
12	sample 1
13	sample 2
14	sample 3
15	sample 4
16	sample 5
17	sample 6
18	sample 7
19	sample 8
20	sample 9
21	sample 10
22	sample 11
23	sample 12
24	sample 13
25	sample 14
26	sample 15
27	sample 16
28	method duplicate
29	instrument duplicate
30	25 µg/L reference standard

14. Once the analytical run is complete, copy and paste the raw data into the TDP Calculation Template located at \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\TDP\TDP Calculation Template.xls and save the file as yyyymmdd_TDP.xls in the \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\TDP\yyyy TDP directory
15. Save the photometric file as yyyymmdd_TDP.pho under \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\TDP\yyyy TDP.
16. Click *Disconnect* and close the UV Probe software.

16.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning*. Briefly, while wearing gloves, clean the quartz tubes, cuvettes, conical tubes, and repeater pipet tips by rinsing three times with hot water and three times with DRO water.

16.14 Calculations

The calculation of TDP in samples is performed by using the "TDP Calculation Template.xls" worksheet located at \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\TDP\TDP Calculation Template.xls.

Concentrations of TDP are calculated using equation 16.5:

Equation 16.5

$$C = \frac{A_{samp} - A_{blk} - b}{m}$$

where C is the concentration of the sample, A_{samp} is the sample absorbance, A_{blk} is the reagent blank absorbance, m is the slope, and b is the intercept of the calibration curve after correcting for the reagent blank absorbance. The sample concentration is reported to three decimals.

If a dilution has been made, the concentration of the sample needs to be corrected for the concentration of TDP in the dilution water using equation 16.6:

Equation 16.6

$$C = (C_0 \times D) - ((C_{dil} \times (D - 1))$$

where C_0 is the concentration of TDP in the diluted sample, C_{dil} is the concentration of TDP in the dilution water, and D is the dilution factor, which is calculated using equation 16.7:

Equation 16.7

$$D = \frac{V_1}{V_0}$$

where V_1 is the final volume of the diluted sample, and V_0 is the volume of the undiluted sample.

This spreadsheet template should be saved to a new file name to record the data. The file name should be in the format of yyyymmdd_TDP.xls (e.g. 20190207_TDP.xls) and saved in the directory \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\TDP\yyyy TDP.

To calculate the TDP concentrations in the samples, fill in each of the yellow fields with the Sample ID, dilution factor, and absorbance of each of the blanks, calibration standards, reference standards, reference samples, and samples.

Information on the date, analyst's initials, standard lot numbers, expiry date and stock concentrations, number of calibration standards (n), reference standard preparation date, and reference sample check batch number are also recorded in this spreadsheet in the yellow fields.

16.15 Data entry

The TDP concentrations of each sample, which are calculated using the "TDP Calculation Template," as well as the QC data, are entered into the Sample Master® LIMS. The completed "TDP Calculation Template.xls" is printed and filed in the TDP folder.

16.16 References

Armstrong, F. A. J., & Tibbitts, S. (1968). Photochemical combustion of organic matter in sea water for nitrogen, phosphorus and carbon determination. *Journal of the Marine Biological Association of the United Kingdom, 48,* 143–152. https://doi.org/10.1017/S0025315400032483

Henriksen, A. (1970). Determination of total nitrogen, phosphorus and iron in freshwater by photo-oxidation with ultraviolet radiation. *Analyst, 95,* 601–605. (U of M Science Library Sci/Per/543/A532/Ch/v95.) https://doi.org/10.1039/AN9709500601

Nagul, E.A., McKelvie, I.D., Worsfold, P., Kolev, S.D. (2015). The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Analytica Chimica Acta, 890,* 60–82. https://doi.org/10.1016/j.aca.2015.07.030

Stainton, M. P., Capel, M. J. &. Armstrong, F. A. J. (1977). *The chemical analysis of fresh water.* (2nd ed). (Miscellaneous Special Publication 25). Fisheries Marine Service. https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/110147.pdf

Strickland, J. D. H., & Parsons, T. R. (1972). *A practical handbook of seawater analysis.* (Bulletin no. 167). Fisheries Research Board of Canada.

Appendix 16A. 4 N Sulfuric Acid Preparation Record

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4 N H_2SO_4
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	H_2SO_4 vol (mL)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 16B. Ammonium Molybdate Preparation Record

Ammonium Molybdate
(TDP and Part P)
Preparation Record

Stable for one year

Preparation date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Vol (mL)	Final vol (L)	Assigned lab lot number	Expiry date	Analyst initials
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 16C. Ascorbic Acid Preparation Record

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Ascorbic Acid
(TDP & Part P)
Preparation Record

Prepare daily

Preparation date	TDP or Part P	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 16D. Mixed Molybdate Preparation Record

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experimental lakes area

Mixed Molybdate
(TDP & Part P)
Preparation Record

4:1 molybdate to ascorbic acid

Prepare daily

	Constituents					
Preparation date	Chemical	Lab lot number	Vol (mL)	Expiry date	Final vol (mL)	Analyst initials
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 16E. 50 µg/mL Reference Standard Stock Preparation Record

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Reference Standard Stock
(TDP and Part P)
Preparation Record

Target is 0.2197 g/L KH_2PO_4 to obtain 50 µg/mL PO_4-P

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Conc (µg/mL)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 16F. Calibration and Reference Standards Preparation Record

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Weekly Total Dissolved Phosphorus Calibration and Reference Standards

Preparation Record

Calibration Standards			
Std #	Vol 0.05 mg/mL P cal stock* (µL)	Final vol (mL)	Final conc (µg/mL)
1	1250	25	2.5

	Vol #1 std (µL)	Final vol (mL)	Final conc (µg/L)
2	200	100	5
3	1000	100	25
4	2000	100	50
5	3000	100	75
6	4000	100	100

Reference Standard		
Vol 50 µg/mL P ref stock* (µL)	Final vol (mL)	Final conc (µg/L)
125	250	25

Calibration standard manufacturer _____

Manufacturer lot # _____

Expiry date _____

Reference standard manufacturer _____

Assigned TDP ref stock lot # _____

* Reference standard stock and calibration standard stock must be from different manufacturers.

Stable for one week

Use a new page when the lot number changes.
When the lot number is finished stroke out the remaining unused lines.

	Week of	Expiry date	Analyst initials
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			
For TDP analysis on			

Authorized by: Sonya Havens

Authorization date: September 2016

17.0 Particulate Phosphorus

17.1 Introduction and scope

This method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of particulate phosphorous in fresh water and atmospheric precipitation. Particulate is operationally defined as that which is retained by a GF/C filter, which has a nominal pore size of 1.2 µm.

17.2 Method principle

Particulate matter that has been collected on a glass fibre filter is combusted to destroy organic matter and convert particulate phosphorus into phosphorus oxides by oxidation. After combustion, the phosphorus oxides are extracted from the filter and hydrolyzed to orthophosphate by heating with dilute hydrochloric acid. The resulting orthophosphate is measured using the molybdenum blue method (Nagul et al., 2015; Stainton et al., 1977).

Phosphate, silicate, arsenate, and germanate ions react under acidic conditions with molybdate to form heteropoly acids that can be converted by suitable reducing agents to blue compounds of uncertain composition, which are then measured spectrophotometrically (Nagul et al., 2015; Stainton et al., 1977).

17.3 Occupational health and safety

The muffle furnace is set to 500 °C, which presents a risk for burns when handling the furnace and items baked in the furnace. To reduce the risk of burns, vials and trays should be placed into the muffle furnace prior to turning it on. Allow the vials and trays to cool down and use protective gloves and/or tongs when removing them. Do not leave the muffle furnace door open to cool. Post a **HOT** warning to caution other lab staff about the risk of burns from the muffle furnace or items baked in the muffle furnace.

The concentrated hydrochloric acid (HCl) and sulfuric acid (H2SO4) used for reagent preparation are corrosive. Handle in a fume hood to avoid HCl vapours. Avoid contact with skin. Wear goggles and gloves when handling and diluting. Always pour acid into water slowly while stirring. **Never pour water into acid.**

17.4 Validation

This method is fit to measure particulate phosphorus in water.

17.4.1 Operating range

0.05–20 µg PO_4^{-3}-P per filter disc

The operating range is in terms of the mass of the sample collected per filter and depends on the volume of sample available for filtration or able to be passed through the filter.

17.4.2 Detection limits and limits of quantitation

The detection limit is calculated for each run using equations 17.1 through 17.3:

Equation 17.1

$$C_{dl} = \frac{y_d - b}{m}$$

where b is the y-intercept, m is the slope, and y_d is the instrument response detection limit, which is calculated using equation 17.2:

Equation 17.2

$$y_d = 3s_y + b$$

where s_y is based on the residuals between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration. It is calculated using equation 17.3:

Equation 17.3

$$s_y = \sqrt{\frac{\sum di^2}{n-2}}$$

where n is the number of standards in the calibration curve, and di is the difference between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration.

The limit of quantitation is calculated in the same manner as the detection limit, except equation 17.2 is replaced with equation 17.4:

Equation 17.4

$$y_q = 10s_y + b$$

The averages of the detection limits and limits of quantitation for samples ran between 2017 and 2019 were:

Detection limit	1.4 µg/L PO_4^{-3}-P
Limit of quantitation	4.7 µg/L PO_4^{-3}-P

17.5 Sample condition

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must

- consist of particulates collected (filtered) onto a pre-baked Whatman 4.25 cm GF/C glass fibre filter disc,
- be filtered within 24 hours of sample collection,
- be stored damp in an acid-washed glass vial with a polytetrafluoroethylene (PTFE)-lined screw-cap, and
- stored for up to one year at room temperature.

17.6 Equipment

- Cole-Parmer 2100 spectrophotometer
- muffle furnace set at 500 °C
- oven with timer set at 100 °C
- glass screw-cap vials; 16 mL capacity, 70 mm × 21 mm (OD), PTFE-lined screw caps, and caps with septa inserts
- numbered metal racks, with rows and columns numbered for identification purposes, to hold the vials and capable of withstanding 500 °C
- filtration apparatus to hold 42.5 mm filter discs
- pre-baked Whatman 42.5 mm GF/C glass fibre filter discs (see Section 6, *Sample Preparation and Filtration* for GF/C procurement and preparation)
- bottle-top dispenser set to deliver 12 mL
- 1-cm pathlength glass cuvettes
- Particulate phosphorus BenchSheet

17.7 Purchased reagents

17.7.1 PO_4^{-3} Calibration Standard Stock

Commercial certified (traceable to NIST) PO_4^{-3}-P standard

1.0 mL = 50 ± 1 µg PO_4^{-3}-P

Store at 2–6 °C until the expiry date on the manufacturer's label.

17.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.

17.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

17.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

17.8.3 Hydrochloric acid – 0.16 N

Caution: ALWAYS pour acid into slowly while stirring. NEVER pour water into acid.

Slowly pour 26 mL of concentrated HCl into ~1500 mL of Milli-Q water and make it to 2 L with Milli-Q water. Store in a bottle-top dispenser at room temperature for one year.

17.8.4 Ammonium molybdate

Dissolve into ~1500 mL of ultrapure water. Add in the order given.

- 15 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}\ 4H_2O$)
- 0.28 g of antimony potassium tartrate ($KSbOC_4H_4O_6$)
- 176 mL conc. sulfuric acid

Cool and dilute to 2 L. Store at room temperature in a brown polyethylene bottle. Stable for one year.

17.8.5 Ascorbic acid

Dissolve 1.25 g of L-ascorbic acid into 50 mL of Milli-Q water. Prepare fresh.

17.8.6 Mixed molybdate

Mix 50 mL ascorbic acid for every 200 mL ammonium molybdate. Prepare fresh.

17.8.7 Reference standard stock – 50 µg/mL PO_4

Dissolve 0.2197 g potassium dihydrogen phosphate (KH_2PO_4) in 100 mL of Milli-Q water.

1.0 mL = 50 µg PO_4^{-3}-P

Store at room temperature in a labelled glass bottle. Stable for one year.

17.8.8 Calibration curve

Dilute the volumes listed in Table 17.1 of the 50 µg/mL calibration standard stock with 0.16 N HCl in 100 mL volumetric flasks to make each of the calibration standard concentrations. Prepare weekly.

Table 17.1. Dilution volumes of calibration standards

Std conc (µg/L)	Calibration stock vol (µL)	Final vol (mL)
0	0	100
25	50	100
50	100	100
125	250	100
250	500	100
375	750	100
500	1000	100
1000	2000	100

17.8.9 Reference standard – 250 µg/L PO_4

Dilute 500 µL of the reference standard stock into 100 mL with 0.16 N HCl. Prepare weekly.

17.8.10 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference Sample Preparation*. Briefly, the reference sample consists of a GF/C filter that has been used to filter 50 mL of Lake 227 water collected during the summer bloom. The reference samples are vacuum desiccated and stored in the dark at ≤ -20 °C.

17.9 Quality control

This method has several conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data

recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective action is taken before the analysis is resumed. If reanalysis is not possible, the test is cancelled for each affected sample and the non-compliant issue is recorded in Sample Master (See Section 5, *Laboratory Information Management System – Sample Master* for how to cancel tests).

17.9.1 Calibration curve

The linear correlation coefficients obtained from the calibration standards must have an $R^2 \geq 0.995$ with a first order fit. If necessary, new calibration standards should be prepared.

17.9.2 Blanks

In each run, an instrument blank consisting solely of 0.16 N HCl, a reagent blank consisting of 0.16 N HCl and reaction reagents, and two method blanks consisting of an unused, ignited GF/C filter disc, 0.16N HCl, and reaction reagents are run. The absorbance of the blanks should be no higher than 0.003.

17.9.3 Internal standard

A vial containing 12 mL of 50 µg/mL PO_4^{-3}-P calibration standard stock is used as an internal standard to correct for evaporation should any vial cap seal failures occur.

17.9.4 Reference standard

A reference standard is run at the beginning and end of an analytical run and every 16 samples within. The absorbance of the 375 µg/L reference standard should be within 5.0% of the 375 µg/L calibration standard.

17.9.5 Reference sample

A reference sample is included on every rack of samples. The value obtained must be within 5.0% of the expected value. Results for reference sample performance are charted and assessed for drift and bias.

17.9.6 Duplicates

A random method duplicate is included on every rack of samples. A field duplicate, wherein a sample is collected in duplicate, is included in every run. Instrument duplicates are run every 16 samples. Duplicates must agree to within 5.0% of each other for duplicates with a concentration of > 20 µg/L; if the average concentration is ≤ 20 µg/L, the difference must be ≤ 1 µg/L. If duplication is inadequate, samples bracketed by the duplicates are reanalyzed.

17.9.7 Labware

Volumetric flasks are calibrated and maintained according to Section 3, Labware Calibration and Verification. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verifications*. All glassware is cleaned according to Section 2, *Labware Cleaning*.

17.10 Quality assurance

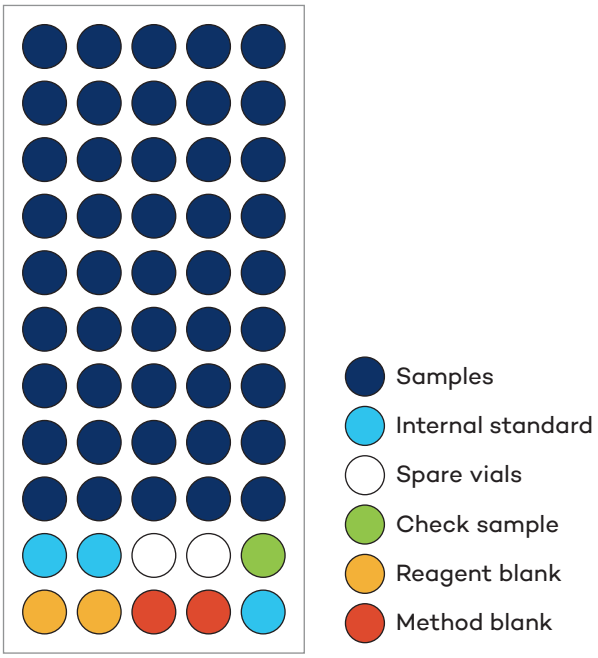
Quality assurance for particulate phosphorus is maintained through inter-laboratory calibrations using plant tissue (forest material). The certified reference material, Orchard Leaves (CRM 1571) from the National Bureau of Standards, has been used as the organic phosphorous standard to check the sample procedure periodically.

17.11 Sample preparation

17.11.1 Filter ignition

1. Organize samples in an aluminum ignition tray. The ignition tray must have labelled rows and columns etched into the metal. Multiple trays must be numbered. Place two empty vials, five vials with an unused ignited GF/C filter in each (two method blanks, three internal standards), two empty spare vials, and a vial with reference sample into the first two rows.
2. Add vials with sample filters to the remaining rows. Record Sample IDs and filtration volumes on the Particulate Phosphorus BenchSheet.
3. Remove the caps from the vials and store the caps so that they maintain the same order as the vials in the rack.
4. When ready, place the tray in the muffle furnace and heat to 500 °C and record the time in the test log. Take care not to scrape the sides of the furnace.
5. After 1.25 hours (1 h 15 m), turn off the muffle furnace and remove the tray using crucible tongs and protective gloves. Avoid touching any vials with the tongs because they

Figure 17.1. Arrangement of blank, standards, and sample vials in labelled aluminum tray



may crack upon cooling. Again, avoid scraping the sides of the muffle furnace. Record time in the test log.

6. Set the tray aside to cool. Post a sign near the tray to alert others that the tray is hot.

17.11.2 Digestion and extraction

1. When the vials have cooled, add 12 mL of 0.16 N HCl to each vial using the calibrated bottle-top dispenser.
2. For the internal standard vials,
   a. remove 100 µl of 0.16 N HCl from the vials,
   b. add 100 µl of 50 µg/mL PO_4^{-3}-P calibration standard stock.
3. With forceps, place a PTFE-coated septum into each cap, with the white (PTFE) side of the septum to the inside of the vial, and put the cap with septum onto the vial. Be sure the cap is not cross-threaded on the vials and make it finger-tight.

   Caution. Avoid over-tightening the caps to prevent crushing the vials.
4. Digest in an oven at 100 ± 4 °C for 2.2 hours (2 h 10 m). Record start and finish time.

   Caution. Do not extract longer than specified to prevent HCl from boiling off and possibly extracting Si from the filter into the liquid phase.
5. With crucible tongs or, if desired, temperature-resistant (Zetex) gloves, remove the tray from the oven and set aside to cool for at least 15 minutes.

   Note: Put up a **HOT** warning to other laboratory staff.

17.11.3 Colorimetric reaction

1. Using a 5 mL pipette, transfer blanks, standards, and samples into 15 mL Falcon™ tubes and place them in a numbered rack. Record the location of each blank, standard, and sample on the Particulate Phosphorus BenchSheet.
2. Add 1 mL of mixed molybdate to each blank, standard, and sample tube and vortex each tube.
3. Wait 30 minutes for the colorimetric reaction, but no more than three hours, prior to analysis on the spectrophotometer.

17.12 Analysis

1. Turn on the Cole-Parmer spectrophotometer to warm up. Click the *Mode* button to enter "Abso rbance" mode. Set wavelength to 885 nm.
2. Rinse and fill cuvettes with Milli-Q water and place them into the spectrophotometer. Click *Zero/100% Transmission* to zero the instrument. The absorbance should read -0.000.

Pull the knob toward you to read the absorbance in the sample cuvette. This should also show an absorbance of -0.000.

3. Leave the rear cuvette with Milli-Q water in the rear slot to serve as the reference absorbance. Analyze the blanks, standards, and samples according to Table 17.2. Rinse the sample cuvette three times with Milli-Q water and a small amount of sample in between absorbance readings. Record the absorbance value in the Particulate Phosphorus BenchSheet.

Table 17.2. Analysis sequence order

Scan #	Sample description	Scan #	Sample description
1	instrument blank	14	250 µg/L calibration standard
2	instrument blank	15	375 µg/L calibration standard
3	reagent blank	16	500 µg/L calibration standard
4	reagent blank	17	1000 µg/L calibration standard
5	method blank	18	250 µg/L reference standard
6	method blank	19	reference sample
7	internal standard	20–35	samples 1 - 15
8	internal standard	36	duplicate
9	internal standard	37	250 µg/L reference standard
10	0 µg/L calibration standard	38–53	samples 16 – 30
11	25 µg/L calibration standard	54	duplicate
12	50 µg/L calibration standard	55	250 µg/L reference standard
13	125 µg/L calibration standard	…	etc.

17.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning.* Briefly, the cuvettes, conical tubes, and repeater pipet tips are cleaned by rinsing three times with hot water and three times with DRO. The sample vials are rinsed three times with hot water, soaked in 10% HCl for at least four hours, and rinsed three times with DRO.

17.14 Calculations

The calculation of particulate phosphorus in samples is performed by using the "PartP Calculation Template.xls" worksheet.

Concentrations of particulate phosphorus are calculated using equation 17.5:

Equation 17.5

$$C = \frac{A_{samp} - A_{blk} - b}{m}$$

where C is the concentration of the sample, A_{samp} is the sample absorbance, A_{blk} is the reagent blank absorbance, m is the slope, and b is the intercept of the calibration curve after correcting for the reagent blank absorbance. The sample concentration is reported to one decimal.

If a dilution has been made, the concentration of the sample needs to be corrected for the concentration of phosphorus in the dilution solution (0.16 N HCl) using equation 17.6:

Equation 17.6

$$C = (C_0 \times D) - ((C_{dil} \times (D - 1))$$

where C_0 is the concentration of phosphorus in the diluted sample, C_{dil} is the concentration of phosphorus in the dilution solution, and D is the dilution factor, which is calculated using equation 17.7:

Equation 17.7

$$D = \frac{V_1}{V_0}$$

where V_1 is the final volume of the diluted sample and V_0 is the volume of the undiluted sample.

This worksheet template should be saved to a new file name to record the data. The file name should be in the format of "yyyymmdd_PartP.xls" (e.g. 20200821_PartP.xls) and saved in the directory \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\PartP\yyyy_PartP.

To calculate the particulate phosphorus concentrations in the samples, fill in each of the yellow fields with the Sample ID, dilution factor, filtration volume, and absorbance of each of the blanks, calibration standards, reference standard, reference sample, and samples.

Information on the date, analyst initials, standard lot numbers, expiry date, stock concentrations, number of calibration standards (n), reference standard preparation date, and reference sample check batch number are also recorded in this spreadsheet in the yellow fields.

17.15 Data entry

The particulate phosphorus concentrations of each sample, which are calculated using the "PartP Calculation Template.xls," as well as the QC data, are entered into the Sample Master LIMS. The completed "PartP Calculation Template.xls" is printed and filed into the PartP folder.

17.16 References

Nagul, E.A., McKelvie, I.D., Worsfold, P., Kolev, S.D. (2015). The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Analytica Chimica Acta, 890,* 60–82. https://doi.org/10.1016/j.aca.2015.07.030

Stainton, M. P., Capel, M. J. &. Armstrong, F. A. J. (1977). *The chemical analysis of fresh water.* (2nd ed). (Miscellaneous Special Publication 25). Fisheries Marine Service. https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/110147.pdf

Appendix 17A. Hydrochloric Acid Preparation Record

0.16 N HCl
(Part P)
Preparation Record

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Grade	HCl vol (mL)	Final vol (mL)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 17B. Ammonium Molybdate Preparation Record

Ammonium Molybdate
(TDP and Part P)
Preparation Record

Stable for one year

Preparation date	Constituent	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Vol (mL)	Final vol (L)	Assigned lab lot number	Expiry date	Analyst initials
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									
	Am. molyb.									
	Ant. pot. tart.									
	H_2SO_4									

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 17C. Ascorbic Acid Preparation Record

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Ascorbic Acid
(TDP & Part P)
Preparation Record

Prepare daily

Preparation date	TDP or Part P	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 17D. Mixed Molybdate Preparation Record

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experimental lakes area

Mixed Molybdate
(TDP & Part P)
Preparation Record

4:1 molybdate to ascorbic acid

Prepare daily

	Constituents					
Preparation date	Chemical	Lab lot number	Vol (mL)	Expiry date	Final vol (mL)	Analyst initials
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					
	NH_4 molybdate					
	Ascorbic acid					

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 17E. 50 µg/mL Reference Standard Stock Preparation Record

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Reference Standard Stock (TDP and Part P)
Preparation Record

Target is 0.2197 g/L KH_2PO_4 to obtain 50 µg/mL PO_4-P

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Conc (µg/mL)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 17F. Calibration and Reference Standard Preparation Record

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Weekly Particulate Phosphorus Calibration and Reference Standards

Preparation Record

Calibration Standards		
Vol 0.05 mg/mL P cal stock* (µL)	Final vol (mL)	Final conc (µg/L)
0	100	0.0
50	100	25
100	100	50
250	100	125
500	100	250
750	100	375
1000	100	500
2000	100	1000

Reference Standard		
Vol 50 µg/mL P ref stock* (µL)	Final vol (mL)	Final conc (µg/L)
1250	250	250

Calibration standard manufacturer _____

Manufacturer lot # _____

Expiry date _____

Reference standard manufacturer _____

Assigned Part P ref stock lot # _____

* Reference standard stock and calibration standard stock must be from different manufacturers.

Stable for one week

Use a new page when the lot number changes.
When the lot number is finished stroke out the remaining unused lines.

	Week of	Expiry date	Analyst initials
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			
For Part P analysis on			

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 17G. Particulate Phosphorus BenchSheet

Particulate Phosphorus Bench Sheet

Date:
Analyst:
QC Batch:

Position on: Tray	Position on: Rack	Sample Number	Dilution Factor	Filtration Volume (mL)	Absorbance at 885nm	Note
	1-A1	instrument blank (MQ only)	1			
	1-A2	instrument blank (MQ only)	1			
Row 1	1-A3	reagent blank	1			
Row 1	1-A4	reagent blank	1			
Row 1	1-A5	method blank	1			
Row 1	1-A6	method blank	1			
Row 1	1-A7	internal standard	1			
R2	1-A8	internal standard	1			
R2	1-A9	internal standard	1			
	1-A10	0 µg/L calibration standard	1			
	1-B1	25 µg/L calibration standard	1			
	1-B2	50 µg/L calibration standard	1			
	1-B3	125 µg/L calibration standard	1			
	1-B4	250 µg/L calibration standard	1			
	1-B5	375 µg/L calibration standard	1			
	1-B6	500 µg/L calibration standard	1			
	1-B7	1000 µg/L calibration standard	1			
	1-B8	250 µg/L reference standard 1	1			
R2	1-B9	reference sample				
Row 3	1-B10					
Row 3	1-C1					
Row 3	1-C2					
Row 3	1-C3					
Row 3	1-C4					
Row 4	1-C5					
Row 4	1-C6					
Row 4	1-C7					
Row 4	1-C8					
Row 4	1-C9					
Row 5	1-C10					
Row 5	1-D1					
Row 5	1-D2					
Row 5	1-D3					
Row 5	1-D4					
	1-D5	250 µg/L reference standard 2	1			
	1-D6	instrument duplicate				
	1-D7	reagent duplicate				

18.0 Soluble Reactive Silicon: Spectrophotometric method

18.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of soluble reactive silicon in fresh water and precipitation.

18.2 Method principle

Orthosilicate and possibly the dimer ($Si_2O_7^{-2}$) react with molybdate in acid solution to form heteropoly molybdates, which can be reduced to silicomolybdenum blues (Armstrong, 1951; Stainton et al., 1977; Strickland, 1952). The absorption spectrum, intensity and stability of the colour depend greatly on the acidity and other experimental conditions (Strickland, 1952). In this method, stannous chloride is used as a reductant. Phosphate interference is prevented by the addition of tartrate which complexes excess molybdate, preventing its reduction. The absorption maximum is in the near infra-red (800–820 mm). This colourimetric procedure is conducted using a spectrophotometer set to a wavelength of 820 nm.

18.3 Occupational health and safety

The concentrated hydrochloric acid (HCl) used for reagent preparation is corrosive and volatile. Handle in a fume hood to avoid HCl vapours. Avoid contact with skin. Wear goggles and gloves when handling and diluting. Always pour acid into water slowly while stirring. **Never pour water into acid.**

18.4 Validation

18.4.1 Operating range

0.003–2.50 mg/L Si

18.4.2 Detection limit and limits of quantitation

The detection limit, which is based on the residuals along the standard curve, is calculated for each run using equations 18.1 through 18.3:

Equation 18.1

$$C_{dl} = \frac{y_d - b}{m}$$

where b is the y-intercept, m is the slope, and y_d is the instrument response detection limit, which is calculated using equation 18.2:

Equation 18.2 $$y_d = 3s_y + b$$

where s_y is based on the residuals between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration. It is calculated using equation 18.3:

Equation 18.3 $$s_y = \sqrt{\frac{\sum di^2}{n-2}}$$

where n is the number of standards in the calibration curve, and di is the difference between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration.

The limit of quantitation is calculated in the same manner as the detection limit, except equation 18.2 is replaced with equation 18.4:

Equation 18.4 $$y_q = 10s_y + b$$

The averages of the detection limits and limits of quantitation for samples ran between 2017 and 2019 were:

Detection limit	0.05 ± 0.01 mg/L
Limit of quantitation	0.18 ± 0.04 mg/L

18.5 Sample conditions

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. The sample analyzed must be

- stored in a polyethylene container (do not store in glass),
- unfiltered,
- stored at room temperature, and
- stored for less than six months.

18.6 Equipment

- Shimadzu UV-1800 scanning spectrophotometer set to 820 nm
- a computer running UV probe software

- 1 cm plastic cuvettes
- 15 mL polyethylene conical tubes and racks to hold them
- 5 mL transfer pipette and tips
- repeater pipette and three Eppendorf™ Combitips to dispense 0.3 mL, 0.2 mL, and 0.1 mL of each of the three reagents
- vortex mixer

18.7 Purchased reagents

18.7.1 Calibration standard stock

Commercial certified (traceable to NIST) SiO_2-Si standard

1.0 mL = 1 ± 0.01 mg Si

The standard is stable until the expiry date on the manufacturer's label.

18.7.2 Reference standard stock

Commercial certified (traceable to NIST) SiO_2-Si standard

1.0 mL = 1 ± 0.01 mg Si

The standard is stable until the expiry date on the manufacturer's label. It must be from a different manufacturer than the silicone standard used for calibration standards.

18.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.

18.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

18.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

18.8.3 Ammonium molybdate

Dissolve 5 g of ammonium paramolybdate into 100 mL of Milli-Q water. Stable for one month.

18.8.4 Hydrochloric acid – 1 N

Dilute 8.3 mL of concentrated HCl into 100 mL of Milli-Q water. Stable indefinitely.

18.8.5 Mixed molybdate

Add 15 mL of 1N HCl to every 10 mL of molybdate stock as required. Prepare fresh.

18.8.6 Tartaric acid – 10% w/v

Dissolve 10 g of tartaric acid into 100 mL of Milli-Q water. Stable for one month.

Table 18.1. Dilution volumes of calibration standards

Std #	Cal std conc (µg/L)	Final vol (mL)	Conc Si (mg/L)
1	2500	1000	2.50
2	2000	1000	2.00
3	1500	1000	1.50
4	1000	1000	1.00
5	500	1000	0.50
6	250	1000	0.25
7	100	1000	0.10
8	10 mL Std #2	1000	0.02

18.8.7 Ascorbic acid

Dissolve 1.1 g ascorbic acid to 10 mL of ultrapure water. Stable for two weeks

18.8.8 Si calibration standard curve

Eight Si standards are prepared according to Table 18.1 in 1-L quantities from the 1 mg/mL calibration standard stock. Stable for six months.

18.8.9 Reference standard – 1.00 mg/L Si

Dilute 1.0 mL of the 1.0 mg/mL Si reference standard stock into 1 L of Milli-Q water in a 1-L plastic volumetric flask. Stable for six months

18.8.10 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference Sample Preparation*. The reference sample consists of water from Lake 239 that has been aged for at least one year and is diluted by a factor of 2. The reference sample is stored at room temperature.

18.9 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated, it is reported in the *Non-Conformance* log, and appropriate corrective action is taken before testing is resumed.

18.9.1 Blanks

An instrument blank, consistently solely of Milli-Q water, is included at the beginning of a run. A reagent blank, consisting of ultrapure water and reaction reagents, is run every 15 samples.

18.9.2 Reference standard

A reference standard is run every 15 samples. These must be within 5.0% of the calibration standard of the same concentration.

18.9.3 Calibration curve

The linear correlation coefficients obtained from the calibration standards must have an $R^2 \geq 0.995$ with a first order fit. If necessary, new calibration standards should be prepared.

18.9.4 Reference sample

A reference sample is included at the beginning of every run. The Si value must be within 5.0% of the expected value. Results for check sample performance are charted and assessed for drift and bias.

18.9.5 Duplicates

Random duplicates are run every 15 samples. Samples for duplicate analysis are chosen at the beginning of the analysis run and must agree to within 5.0% of each other for concentrations above 0.1 mg/L. For concentrations ≤ 0.1 mg/L, accept differences of 0.005 mg/L. If duplication is inadequate, samples bracketed by the duplicates are reanalyzed.

18.9.6 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verifications*. All glassware is cleaned according to Section 2, *Labware Cleaning*.

18.10 Quality assurance

Quality assurance for SRSi is maintained through annual participation in the Environment and Climate Change Canada Proficiency Testing Program (ECCC-PT). Non-compliance is defined by ECCC-PT and is reported directly to the Research Chemist. Samples analyzed during the period of non-compliance are flagged in the database.

18.11 Sample preparation

1. Add 5 mL of each of the following into a 15 mL conical tube using a 5 mL transfer pipette:
   - blanks of Milli-Q water (every 15 samples),
   - calibration standards (i.e. 0.00, 0.02, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L),
   - reference standard (every 15 samples),
   - reference (check) samples,
   - samples, and
   - duplicates (every 15 samples).

Table 18.2. Typical sequence of blanks, standards, reference standards, reference samples, and samples. Reference standards, duplicate samples, and reagent blanks are analyzed at least every 15 samples.

Scan #	Sample description
1	0.00 mg/L calibration standard
2	0.02 mg/L calibration standard
3	0.10 mg/L calibration standard
4	0.25 mg/L calibration standard
5	0.50 mg/L calibration standard
6	1.00 mg/L calibration standard
7	1.50 mg/L calibration standard
8	2.00 mg/L calibration standard
9	2.50 mg/L calibration standard
10	instrument blank
11	reagent blank
12	1.00 mg/L reference standard
13	reference sample
14	sample 1
15	sample 2
16	sample 3
17	sample 4
18	sample 5
19	sample 6
20	sample 7
21	sample 8
22	sample 9
23	sample 10
24	sample 11
25	sample 12
26	sample 13
27	sample 14
28	random duplicate from samples 1-14
29	1.00 mg/L reference standard
30	reagent blank 2
31	Sample 15
...	etc.

2. Add 0.3 mL of mixed molybdate to each of the conical tubes; mix with a vortex and wait 15 minutes.
3. Add 0.2 mL of tartaric acid to each of the conical tubes; mix with a vortex and wait two minutes.
4. Add 0.1 mL of ascorbic acid to each of the conical tubes; mix with a vortex and wait five minutes.

18.12 Analysis

1. Turn on the power on the Shimadzu UV-2401 PC spectrophotometer and let it warm up for at least two hours.
2. On the PC desktop, double-click the *UV Probe* shortcut.
3. Click *OK*.
4. Ensure that the *Photometric* icon is selected (Figure 18.1).

Figure 18.1. The Photometric icon

5. Open the UV Probe software from the desktop and open the SRSi method (\\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\SRSi\SRSi template.pho).
6. Click the *Connect* button at the bottom. If not available, click *View* > *Photometric* buttons. The UV detector will now go through its startup sequence.
7. After the startup sequence has finished, if the instrument has passed, click *OK*.
8. Rinse two 1-cm plastic cuvettes at least three times with Milli-Q water and fill them with Milli-Q water. Wipe the cuvettes with lint-free tissue and place them in the appropriate holders in the spectrophotometer (the cuvette farthest away from you is the reference position). Ensure that the wavelength is set to 820 nm and click *Auto Zero* to set the photometric value at 820 nm of Milli-Q water to an absorbance of 0. Leave the cuvette in the reference position for the duration of the analytical run.
9. Remove the front cuvette, rinse at least three times with Milli-Q water, and then rinse and fill it with the first standard (0.00 mg/L Si). Enter the Sample ID and standard concentration (e.g. 0.00 mg/L and 0.0) and click *Read Std*.
10. Repeat step 9 for remaining standards.
11. Once the calibration curve is complete, remove the front cuvette and rinse it at least three times with Milli-Q water, then rinse and fill it with Milli-Q water. Enter the Sample ID (e.g. instrument blank) and click *Read Unk*.
12. Repeat step 11, replacing the Milli-Q with each of the remaining blanks, QC standard/samples and samples. Run a random duplicate, a reference standard, and a reagent blank every 15 samples.
13. Once the analytical run is complete, copy and paste the raw data into the "SRSi Calculation Template.xls" located at \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\

SRSi\SRSi Calculation Template.xls and save the file as "yyyymmdd_SRSi.xls" under \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\SRSi\yyyy SRSi.

14. Save the photometric file as "yyyymmdd_SRSi.pho" under \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\SRSi\yyyy SRSi.
15. Click *Disconnect* and close the UV Probe software.

18.13 Clean up

The equipment is cleaned according to Section 2, *Labware Cleaning.* Briefly, the cuvettes, conical tubes, and repeater pipet tips are cleaned by rinsing three times with hot water and three times with DRO water.

18.14 Calculations

The calculation of Si in samples is performed by using the "SRSi Calculation Template.xls" worksheet located at \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\SRSi\SRSi Calculation Template.xls.

Concentrations of SRSi are calculated using equation 18.5:

Equation 18.5.

$$C = \frac{A_{samp} - A_{blk} - b}{m}$$

where C is the concentration of the sample, A_{samp} is the sample absorbance, A_{blk} is the reagent blank absorbance, m is the slope, and b is the intercept of the calibration curve after correcting for the reagent blank absorbance. The sample concentration is reported to three decimals.

If a dilution has been made, the concentration of the sample needs to be corrected for the concentration of SRSi in the dilution water using equation 18.6:

Equation 18.6.

$$C = (C_0 \times D) - ((C_{dil} \times (D - 1))$$

where C_0 is the concentration of DOC in the diluted sample, C_{dil} is the concentration of SRSi in the dilution water, and D is the dilution factor, which is calculated using equation 18.7:

Equation 18.7

$$D = \frac{V_1}{V_0}$$

where V_1 is the volume of the diluted sample, and V_0 is the volume of the undiluted sample.

This spreadsheet template should be saved to a new file name to record the data. The file name should be in the format of "yyyymmdd_SRSi.xls" (e.g. 20190204_SRSi.xls) and saved in the directory \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\SRSi\yyyy SRSi.

To calculate the SRSi concentrations in the samples, fill in each of the yellow fields with the Sample ID, dilution factor, and absorbance of each of the reagent blanks, calibration standards, reference standards, reference (check) samples, and samples.

Information on the date, the analyst's initials, standard lot numbers, expiry date and stock concentrations, number of calibration standards (*n*), reference standard preparation date, and reference sample check batch number are also recorded in this spreadsheet in the yellow fields.

18.15 Data entry

The SRSi concentrations of each sample, which are calculated using the "SRSi Calculation Template.xls," as well as the QC data, are entered into the Sample Master LIMS. The completed "SRSi Calculation Template.xls" is printed and filed in the SRSi folder.

18.16 References

Armstrong, F. A. J. (1951). The determination of silicate in sea water. *Journal of the Marine Biological Association of the United Kingdom, 30,* 149–160. https://doi.org/10.1017/S0025315400012649

Stainton, M. P., Capel, M. J. &. Armstrong, F. A. J. (1977). *The chemical analysis of fresh water.* (2nd ed). (Miscellaneous Special Publication 25). Fisheries Marine Service. https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/110147.pdf

Strickland, J. D. H. (1952). The preparation and properties of silicomolybdic acid. Part III: The combination of silicate and molybdate. *Journal of the American Chemical Society, 74,* 872–876. https://doi.org/10.1021/ja01124a004

Appendix 18A. Ammonium Molybdate Preparation Record

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Ammonium Molybdate
(SRSi)
Preparation Record

Stable for one month

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (L)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 18B. 1 N Hydrochloric Acid Preparation Record

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1 N HCl
Preparation Record

Stable indefinitely

Preparation date	Manufacturer	Manufacturer lot number	Grade	HCl vol (mL)	Final vol (mL)	Assigned lab lot number	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: May 2018

Appendix 18C. Mixed Molybdate Preparation Record

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Mixed Molybdate
(SRSi)
Preparation Record

15 mL HCl : 10 mL ammonium molybdate

Prepare daily

	Constituents					
Preparation date	Chemical	Lab lot number	Vol (mL)	Expiry date	Final vol (mL)	Analyst initials
	NH_4 molybdate					
	1 N HCl					
	NH_4 molybdate					
	1 N HCl					
	NH_4 molybdate					
	1 N HCl					
	NH_4 molybdate					
	1 N HCl					
	NH_4 molybdate					
	1 N HCl					
	NH_4 molybdate					
	1 N HCl					
	NH_4 molybdate					
	1 N HCl					
	NH_4 molybdate					
	1 N HCl					
	NH_4 molybdate					
	1 N HCl					

Authorized by: Sonya Havens
Authorization date: September 2016

Appendix 18D. Tartaric Acid Preparation Record

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Tartaric Acid
Preparation Record

Stable for one month

Preparation date	Manufacturer	Manufacturer lot number	Grade	Net weight (g)	Final vol (mL)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 18E. Ascorbic Acid Preparation Record

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Ascorbic Acid
(SRSi)
Preparation Record

Prepare daily

Preparation Date	Manufacturer	Manufacturer Lot Number	Grade	Net Weight (g)	Final Vol (mL)	Expiry Date	Analyst Initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 18F. Calibration and Reference Standards Preparation Record

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SRSi Calibration and Reference Standards
Preparation Record

Calibration Standards			
Std #	Vol 1 mg/mL Si cal std* (µL)	Final vol (ml)	Final conc (mg/L)
1	50	500	0.10
2	125	500	0.25
3	250	500	0.50
4	500	500	1.00
5	750	500	1.50
6	1000	500	2.00
7	1250	500	2.50
8	5 ml of #6 std	500	0.02

Reference Standard		
Vol 1 mg/mL Si ref std* (µL)	Final vol (ml)	Final conc (mg/L)
500	500	1.00

Calibration standard manufacturer _____

Manufacturer lot # _____

Expiry date _____

Reference standard manufacturer _____

Manufacturer lot # _____

* Reference standard stock and calibration standard stock must be from independent sources.

Standards stable for six months

Use a new page when the lot number changes.
When the lot number is finished stroke out the remaining unused lines.

	Week of	Expiry date	Analyst initials
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			
For SRSi analysis on			

Authorized by: Sonya Havens
Authorization date: September 2016

19.0 Particulate Iron

19.1 Introduction and scope

This method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of particulate iron (Part Fe) in fresh water and atmospheric precipitation. Particulate is operationally defined as that which is retained by a GF/C filter, which has a nominal pore size of 1.2 µm.

19.2 Method principle

Particulate matter that has been collected on a glass fibre filter is digested with nitric acid to liberate the iron from the filter and transfer it into solution. The concentrated acid is then diluted with ultrapure water and analyzed for iron with an atomic absorption spectrometer. The instrument atomizes iron in the sample using an acetylene flame, meaning that all iron will be measured regardless of its oxidation state in solution.

19.3 Occupational health and safety

Concentrated nitric acid is corrosive, a strong oxidizer, can be fatal if inhaled, and can cause severe eye, skin, respiratory, and digestive tract burns. Handle in a fume hood, avoid contact with skin, and wear goggles and gloves when handling and diluting. Always pour acid into water slowly while stirring. **Never pour water into acid.**

The acetylene flame of the instrument can generate temperatures up to 2800 °C. The acetylene flame can emit ultraviolet light. Do not look directly at the flame without UV protective eyewear, and do not leave the room while the flame is lit.

19.4 Validation

This method is fit to measure Part Fe in water.

19.4.1 Operating range

0.4–80 µg Fe per filter disc, given a post-digestion dilution of 20 mL.

The operating range is in terms of the mass of sample collected per filter and depends on the volume of sample filtered. A sample filtration volume of 100 mL corresponds to a concentration range of 4–800 µg/L in the filtered sample.

19.4.2 Detection limits

The detection limit is calculated for each run using equations 19.1 through 19.3:

Equation 19.1

$$C_{dl} = \frac{y_d - b}{m}$$

where b is the y-intercept, m is the slope, and y_d is the instrument response detection limit, which is calculated using equation 19.2:

Equation 19.2

$$y_d = 3s_y + b$$

where s_y is based on the residuals between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration. It is calculated using equation 19.3:

Equation 19.3

$$s_y = \sqrt{\frac{\sum di^2}{n-2}}$$

where n is the number of standards in the calibration curve, and di is the difference between the measured instrument response for each standard concentration and the calibration curve-predicted response for each standard concentration.

The limit of quantitation is calculated in the same manner as the detection limit, except equation 19.2 is replaced with equation 19.4:

Equation 19.4

$$y_q = 10s_y + b$$

The averages of the detection limits and limits of quantitation for samples run between 2017 and 2019 were:

Detection limit	2.6 µg/L Fe
Limit of quantitation	8.7 µg/L Fe

19.5 Sample condition

Refer to Section 6, *Sample Preparation and Filtration* for details on sample preparation. Briefly the sample:

- Consists of particulates collected (filtered) onto a pre-baked Whatman 4.25 cm GF/C glass fibre filter disc.
- Must be filtered within 24 hours of sampling.

- TIs stored in an acid-washed high-density polyethylene screw-cap vial at room temperature.
- Can be stored for up to one year at room temperature.

19.6 Equipment

- PerkinElmer Aanalyst 400 Atomic Absorption Spectrometer and WinLab 32™ software
- acid-washed high-density polyethylene screw-cap vials, 20 mL capacity
- filtration apparatus with a 47-mm diameter magnetic filter funnel
- 42.5 mm GF/C glass fibre filters, baked for 14 hours at 500 °C
- repeater pipette
- 25 mL capacity Eppendorf™ Combitip
- Eppendorf™ micropipettes and tips.
- volumetric flasks (100 mL, acid washed prior to use)
- spatula
- flat-ended filter forceps

19.7 Purchased reagents

19.7.1 High-purity concentrated nitric acid

Stable at room temperature until the expiry date on the manufacturer's label.

19.7.2 Calibration standard stock

Commercially-certified iron standard.

1.0 mL = 1000 ± 10 µg Fe

The standard is stable at room temperature until the expiry date on the manufacturer's label.

19.7.3 Reference standard stock

Commercially-certified iron standard

1.0 mL = 1000 ± 10 µg Fe

The standard is stable at room temperature and may be used past the expiry date on the label. It must be from a different manufacturer than the iron standard used for calibration standards.

19.8 Prepared reagents

- Preparation information for all standards is recorded in the *Reagent Preparation* logbook.
- All standards are labelled with an expiry date.
- Standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.
- All standards are prepared with Type 1 ultrapure water (Milli-Q).
- All standards are prepared in volumetric flasks.

19.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

19.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

19.8.3 Calibration curve

Dilute the volumes of the 1000 µg/mL Fe calibration standard stock according to Table 19.1 with 10 mL of concentrated nitric acid and Milli-Q water in 100 mL volumetric flasks to make each of the following calibration standard concentrations. **Add ~80 mL of Milli-Q water to your volumetric flasks before you add the acid.** Prepare weekly.

Table 19.1. Dilution volumes of calibration standards

Std conc (µg/L)	Calibration stock vol (µL)	Final vol (mL)
0	0	100
125	12.5	100
250	25	100
500	50	100
1000	100	100
2000	200	100

19.8.4 Reference standard – 2000 µg/L Fe

Dilute 200 µl of the 1000 µg/mL Fe reference standard stock with 10 mL of concentrated nitric acid and Milli-Q water in 100-mL volumetric flasks. **Add ~80 mL of ultrapure water to your volumetric flask before you add the acid.** Prepare weekly.

19.8.5 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference Sample Preparation*. Briefly, 50 mL aliquots of Lake 227 epilimnion water, collected during peak phytoplankton biomass, are filtered through GF/C filters. Each filter is placed in a 50-mm Petri dish, desiccated in the dark in a vacuum desiccator, and stored in the dark at -20 °C.

19.9 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the non-conformance log, and appropriate corrective action is taken before testing is resumed.

19.9.1 Blanks

Reagent blanks consisting of 3 mL of high-purity nitric acid and 18 mL of Mill-Q water and method blanks consisting of an unused, pre-baked GF/C filter disc, 2 mL of high-purity nitric acid, and 18 mL of Milli-Q water are included in each run.

19.9.2 Reference standard

A reference standard is run every 15 samples. These must be within 5.0% of the calibration standard of the same concentration (i.e. 2000 µg/L).

19.9.3 Calibration curve

The linear correlation coefficients obtained from the calibration curve must have an $R^2 \geq 0.995$ with a first order fit. If this criterion is not met, new calibration standards may need to be prepared.

19.9.4 Reference sample

A reference sample is included at the beginning of every run for Fe. The Fe value obtained must agree to within 5.0% of the expected value. Results for reference sample performance are charted and assessed for drift and bias.

19.9.5 Duplicates

Random duplicates are run every 15 samples. Samples for duplicate analysis are chosen at the beginning of the analysis run (before the first analysis is done). Duplicates must agree to within 5.0% of each other. If duplication is inadequate, samples bracketed by the duplicates are re-tested.

19.9.6 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibration and Verification*. The calibrations of the Eppendorf™ pipettes are verified on a weekly basis or prior to making standards using methods outlined in Section 3, *Labware Calibrations and Verifications*. All glassware is cleaned according to Section 2, *Labware Cleaning*.

19.10 Quality assurance

Proficiency testing is not currently conducted for the Part Fe analysis.

19.11 Sample preparation

19.11.1 Digestion and extraction

1. Compress the filter to the bottom of the vial so that as much of it as possible can be covered by the nitric acid.
2. In a fume hood, use a repeater pipette to add 2 mL of high-purity concentrated nitric acid to each vial.
3. Place the vials in a cardboard flat (or whatever else may be convenient) and put them in a fume hood. Allow the samples to digest at room temperature for at least two days.
4. After a 72-hour digestion period has passed, use a repeater pipette to add 18 mL of Milli-Q water to each vial. Be careful to avoid cross-contamination of vials from splash-back, wiping the repeater pipette with a Kimwipe™ as necessary. The samples are now ready for analysis.
5. Prior to analysis, the sample vials are inverted to mix.

19.12 Analysis

19.12.1 Atomic absorption spectrometer

1. Open the regulator to begin the flow of acetylene gas to the instrument. Ensure the tank has a pressure of at least 50 psi.
2. Turn on the atomic absorption spectrometer.
3. On the computer, open "WinLab32 for AA." The software will connect to the instrument as it starts up.
4. Click on the *Lamps* icon on the toolbar. Turn on the iron hollow cathode lamp (HCL) by selecting *Lamp X* under the "Set Up" column, with X being whichever port number the iron HCL is being housed in. The spectrometer can have up to four HCLs at a given time, so ensure you have selected the correct port. The "Energy" intensity of the lamp should be

around 75 once it is turned on, and the icon under the "On/Off" column will turn green. Close the *Lamp Setup* window (if it is open, the software will not let you run samples). Let the lamp warm up for at least 15 minutes before starting measurements.

5. Place the sample line in a beaker of fresh DRO water. Once the flame is turned on, it will begin aspirating the sample.
6. Click on the *Flame* icon on the toolbar. Click on the *On/Off* switch icon to turn on the flame. The acetylene flow should be at 2.50 L/min, while the airflow should be 10.00 L/min. Once the flame is turned on, adjust the acetylene pressure from the tank to 14 psi. Close the *Flame Control* window. Let the flame burn for at least five minutes before starting measurements.
7. Click on the *MethEd* icon on the toolbar. Select the "ELA Fe" method and click *OK*.
8. Click on the *Manual* icon on the toolbar. First, under "Info. File," click *Open* and open the "ELA Fe.sif" sample info file. Next, under "Results Data Set Name," click *Open*. Enter the name as "MMM DD ELA," and put the description as the range of sample numbers you will be analyzing. Click *OK*—do not worry about what is highlighted; as long as you have put in a new name and description, no old data will be over-written. Ensure the "Save Data" box under the "During Analyses:" heading is checked.
9. Click the *Results* icon on the toolbar and position the window on the screen such that both it and the Manual window are visible. Samples are now ready to be run.
10. In the Manual window, change the ID to a description of the sample (i.e. 500 std or 2017082801-01) about to be analyzed and write the sample information onto the BenchSheet. Take the sampling tube and hold it in the sample or standard to be measured. Filter particles can clog the sampling line, so try to avoid touching the filter or placing the sample line at the bottom of the vial where the filter particles tend to reside. When the sampling tube is in place, click the *Analyze Sample* button. Once the *Sample Progress* bar indicates the analysis is completed, remove the sampling tube from the sample and place it back into the beaker of DRO water. The sample readings should now be displayed in the Results window. Move on to the next sample once you are happy with them. Any samples with a reading higher than the largest calibration standard will need to be diluted. Refill the DRO beaker as needed. It is ok if the sample line aspirates air, though try to maintain liquid aspiration as much as possible.
11. Once the sample run is complete, let the instrument suck DRO water for five minutes.
12. After the rinsing is complete, click the *Flame* icon on the toolbar. Click on the *On/Off* switch icon to turn off the flame.
13. Click on the *Lamps* icon on the toolbar. Click the *green icon* next to the Fe lamp under the "On/Off" column to turn the HCL off. The power bar should drop to zero, and the icon will turn grey.
14. Close "WinLab32 for AA" and turn off the spectrometer. The program will ask, "Do you want to quit using WinLab?" Click *OK*. It will then tell you "Sample information has

changed" and ask you if you want to save the changes on disk before continuing. Click *No*. The window will then close after a couple of seconds.

15. Close the regulator to cease the flow of acetylene gas.

Table 19.2. A typical sequence of a particulate iron analytical run

Scan #	Sample description
1	DRO Water
2	DRO Water
3	2000 µg/L reference standard
4	0 µg/L calibration standard
5	125 µg/L calibration standard
6	250 µg/L calibration standard
7	500 µg/L calibration standard
8	1000 µg/L calibration standard
9	2000 µg/L calibration standard
10	2000 µg/L reference standard
11	filter blank
12	filter blank
13	L227 reference sample
14	L227 reference sample
15–27	samples 1-13
28	random duplicate from samples 1–13
29	2000 µg/L reference standard
30–44	samples 14–28
45	random duplicate from samples 14–28
46	2000 µg/L reference standard
34–48	samples 29–43
49	random duplicate from samples 29–43
50	2000 µg/L reference standard
51	filter blank (remeasure)
52	filter blank (remeasure)
53	0 µg/L calibration standard
54	125 µg/L calibration standard
55	125 µg/L calibration standard
56	500 µg/L calibration standard
57	1000 µg/L calibration standard
58	2000 µg/L calibration standard
59	2000 µg/L reference standard

19.12.2 Data retrieval

1. On the computer, open *Data Manager*.
2. Click on the *Result Name* matching the Results Data Set Name you gave at the start of the sample run. Click on the *Export* icon on the top toolbar. The *Data Export Wizard* will open.
3. Click on *Use Existing Design*. Click the *Browse* button and select "ELA Fe.xpt." Click *Next*. All samples should be highlighted. Click *Next* until you reach the page with the

heading "4. Select Export Options." Here, under "Name:" select the text box and name it "yyyymmdd PartFe" with the analysis date. Click *Next* until you reach the page with the heading "8. Export Data Set" and click the *Export Data* button. Click *Finish*.

4. The data set has now been exported to C:\Users\Public\PerkinElmer\AA\Data\Reports where it will be a .csv file. Copy the file to a flash drive. When you have access to a computer equipped with Microsoft Office, open it with Excel and save it as an Excel file.

19.13 Cleaning

The equipment is cleaned according to Section 2, *Labware Cleaning.* Briefly, filters are removed with forceps and discarded. Excess sample is discarded into the sink under running water. Vials and lids are rinsed three times with hot water, placed in a 10% HCl acid bath for at least four hours, and rinsed three times with DRO water.

19.14 Calculations

The calculation of Part Fe in samples is performed by using the "PartFe Calculation Template.xls" worksheet.

The corrected absorbance ($A_{corrected}$) is calculated using equation 19.5:

Equation 19.5.

$$A_{corrected} = A_{measured} - A_{blank}$$

where $A_{measured}$ is the absorbance of the sample, and A_{blank} is the average absorbance of all the method blanks. The concentration of iron in the extract ($C_{extract}$) is calculated using equation 19.6:

Equation 19.6.

$$C_{extract} = \left(\frac{A_{corrected} - b}{m}\right) \times D$$

where m is the slope, b is the intercept of the calibration curve after correcting for the reagent blank absorbance, and D is the dilution factor, which is calculated using equation 19.7:

Equation 19.7.

$$D = \frac{V_1}{V_0}$$

The concentration of iron in the sample (C_{sample}) is then calculated using equation 19.8:

Equation 19.8.

$$C_{sample} = \frac{C_{extract} \times V_{extract}}{V_{sample}}$$

where $V_{extract}$ is the extract volume (typically 20 mL), and V_{sample} is the volume of the sample filtered through the filter disc.

The worksheet template should be saved to a new file name to record the data. The file name should be in the format of "yyyymmdd_PartFe.xls" (e.g. 20200821_PartFe.xls) and saved in the directory \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\PartFe\yyyy_PartFe.

To calculate the particulate iron concentrations in the samples, fill in each of the yellow fields with the Sample ID, dilution factor, extract volume, filtration volume, and absorbance of each of the blanks, calibration standards, reference standards, reference samples, and samples.

Information on the date, analyst's initials, standard lot numbers, expiry date, stock concentrations, number of calibration standards (n), reference standard preparation date, and reference sample check batch number are also recorded in this spreadsheet in the yellow fields.

19.15 Data entry

The particulate iron concentrations of each sample, which are calculated using the "PartFe Calculation Template. xls," as well as the QC data, are entered into the Sample Master LIMS. The completed "PartFe Calculation Template. xls" is printed and filed in the PartFe folder.

Appendix 19A. Calibration and Reference Standards Preparation Record

iisd|ela experimental lakes area

Weekly Particulate Iron Calibration and Reference Standards

Preparation Record

Calibration Standards		
Vol 1000 µg/mL Fe cal stock* (µL)	Final vol (mL)	Final conc (µg/L)
0	100	0
12.5	100	125
25	100	250
50	100	500
100	100	1000
200	100	2000

Reference Standard		
Fe ref stock* (µL)	Final vol (mL)	Final conc (µg/L)
200	100	2000

Calibration standard manufacturer _____

Manufacturer lot # _____

Expiry date _____

Reference standard manufacturer _____

Manufacturer lot # _____

* Reference standard stock and calibration standard stock must be from different manufacturers.

Stable for one week

Use a new page when the lot number changes.

When the lot number is finished stroke out the remaining unused lines.

	Week of	Expiry date	Analyst initials
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			
For Part Fe analysis on			

Authorized by: Sonya Havens

Authorization date: September 2016

20.0 Chlorophyll-*a*: Gross fluorescence method

20.1 Introduction and scope

The following method is used in the IISD Experimental Lakes Area Analytical Service Laboratory for the determination of chlorophyll-a (Chl-*a*) in fresh water.

20.2 Method principle

Particulate matter from samples of lake water is collected on glass fibre paper, dried using vacuum desiccation and stored at ≤ -20 °C until analyzed. Photosynthetic pigments within the filter-retained particulate matter are quantitatively extracted in a mixture of methanol, acetone, and water. The extract is analyzed fluorometrically using the Environmental Protection Agency Method 445.0 (Arar & Collins, 1997).

For Chl-*a*, excitation between 430–450 nm gives an emission maximum between 650−675 nm. In the method described, a fluorometer equipped to provide excitation at 440 nm and detection emission at 660 nm is used. The output of the instrument is in arbitrary units, and it must be calibrated using a ch-*a* solution of known concentration.

Chl-*a* (from *Anacystis nidulans*) from Sigma Aldrich is a convenient source of pure Chl-*a* that is free of chlorophyll-b and can be used to calibrate the fluorometer.

Using the recommended fluorometer settings, the instrument responds almost exclusively to Chl-*a*. However, chlorophyll degradation products will also produce a response when present (as may be the case with samples from aphotic waters or sediments). Estimates using this "gross fluorescence" method will over-estimate Chl-*a* where degradation products are present. A high-performance liquid chromatography procedure should be used to specifically measure Chl-*a* where this distinction is required.

20.3 Occupational health and safety

This method requires the use of volatile solvents (methanol and acetone). Hand and eye protection must be used when working with solvents and should be performed in a fume hood.

20.4 Validation

20.4.1 Operating range

This depends on the mass of cells capable of being collected on a GF/C filter and, hence on the volume of lake water available or capable of being filtered. The lower limit of chlorophyll mass that can be detected is 0.5 ng. For the default volume of water sample filtered (200 ml) and 2 ml solvent used for pigment extraction, the method detection limit is 0.05 µg/L Chl-*a* in lake water. The upper limit of 100 µg/L can be attained by adjusting the filtration volume and extraction volume.

20.4.2 Detection limits

The method detection limit (MDL) goal for the Chl-*a* test is 0.5 ng or less.

The test performs as well, or better, than these established goals and hence this method is fit for determining Chl-*a* in water.

20.5 Sample conditions

See Section 6, *Sample Preparation and Filtration* for details on sample preparation.

The sample filter discs analyzed must be

- collected (filtered) on Whatman GF/C 4.25 cm glass fibre filters,
- vacuum desiccated for 16 hours,
- stored dry in individual Petri dishes,
- stored at ≤ -20 °C, and
- stored in the dark, preferably not longer than 12 months.

20.6 Equipment

- Shimadzu UV-1800 scanning spectrophotometer
- UV Probe software
- Turner Designs Trilogy Fluorometer equipped with a Chl-*a* Non-Acidification Fluorescence Module
- 1 cm low volume cuvettes (quartz windows)
- 13 mm (O.D.) glass cuvettes
- low volume glass transfer pipettes
- 25 mL glass volumetric flasks

- 9 mL test tubes with threaded neck and polytetrafluoroethylene (PTFE)-lined caps for extraction
- flat-ended filter forceps
- stainless steel rod
- repeating dispenser calibrated at 2.0 mL for dispensing the extracting solvent
- glass graduated syringes
- rack to support vials in an upright position
- Chlorophyll-a BenchSheet (Appendix 20D)

20.7 Purchased reagents

20.7.1 Methanol (HPLC or certified ACS grade)

Store at room temperature in the manufacturer's bottle. Stable as per the manufacturer's specifications.

20.7.2 Acetone (HPLC or certified ACS grade)

Store at room temperature in the manufacturer's bottle. Stable as per the manufacturer's specifications.

20.7.3 Chl-*a* calibration crystals

Chl-*a* from *Anacystis nidulans* algae is a crystalline solid obtained in nominal 1 mg quantities from Sigma Aldrich and is free of chlorophyll-b, unlike chlorophyll extracted from spinach. Stored at ≤ -20 °C (< 0 °C manufacturer's instructions) in the dark.

20.7.4 Chl-*a* reference crystals

Chl-*a* from spinach is a crystalline solid obtained in nominal 1 mg quantities from Sigma Aldrich and may contain chlorophyll-b. Stored at ≤ -20 °C (< 0 °C manufacturer's instructions) in the dark.

20.8 Prepared reagents

- Preparation information for all reagents and standards is recorded in the *Reagent Preparation* logbook.
- All reagents and standards are labelled with an expiry date.
- Reagent and standard containers are washed according to Section 2, *Labware Cleaning* and rinsed with new reagent before pouring in the bulk of new reagent.

- Unless otherwise indicated, all reagent chemicals are ACS grade.
- Unless otherwise indicated, all reagents and standards are prepared with Type 1 ultrapure water (Milli-Q).
- Unless otherwise indicated, all reagents and standards are prepared in volumetric flasks.

20.8.1 Type 2 ultrapure deionized reverse osmosis (DRO) water

Effluent from an Elix 20 Water Purification System (> 5 MΩ·cm purity). The feedwater for the Elix 20 system is water from Lake 239 that is filtered through a 20", 5-µm pleated paper filter, UV treated, and then filtered through an Extra Fine Pore Sediment Cartridge (Rainfresh), an Ion-Ex Cartridge (MilliporeSigma), and a Super-C Carbon Cartridge (MilliporeSigma).

20.8.2 Type 1 ultrapure water (Milli-Q)

Effluent from a Milli-Q Advantage A10 Water Purification System (> 18 MΩ·cm purity) with Type 2 DRO feedwater.

20.8.3 Extraction solvent

Using a graduated cylinder, mix the following proportions of solvent:

- 68% methanol
- 27% acetone
- 5% Milli-Q water

This is stable for six months at room temperature in a brown glass bottle.

Do not allow this solvent mix to sit open to atmosphere to evaporate, or proportions will change.

20.8.4 Chl-*a* calibration standard stock

Under low light conditions, ~1 mg of crystalline Chl-*a* from *A. nidulans* is dissolved in 100 ± 2 mL acetone to give a stock standard of ~10 mg/L (since the exact weight of the crystalline Chl-a is uncertain). The exact concentration is not essential since it is determined spectrophotometrically each time it is used to prepare the intermediate calibration standard.

This solution should be handled with care at all times. Exposure to acid vapours must be avoided as this will cause degradation of Chl-*a* to phaeophyton-*a*. Regular handling of the Chl-*a* calibration standard stock can lead to both photo-degradation of pigment and evaporation of solvent. While the concentration is determined spectrophotometrically (see Section 20.8.11) each time it is used, it is best to attempt to minimize the changes in the Chl-*a* calibration standard stock concentration as much as possible. Store the Chl-*a* calibration standard stock in an amber glass bottle, sealed with a PTFE-lined screw-cap, at ≤ -20 °C in the dark for up to one year.

20.8.5 Chl-*a* reference standard stock

The Chl-*a* reference stock is prepared and handled with the same precautions as the Chl-*a* calibration standard stock, as outlined above in section 20.8.4.

20.8.6 Intermediate Chl-*a* calibration standard

The standard should be prepared fresh and stored in the dark at room temperature while not in use.

The intermediate Chl-*a* calibration standard is prepared by diluting the Chl-*a* calibration standard stock with the extraction solvent to obtain a concentration of 500 ± 50 µg/L. The concentration of the intermediate Chl-*a* calibration standard is verified spectrophotometrically (see Section 20.8.11).

1. Remove ~1 ml of the Chl-*a* calibration standard stock that was just measured from the spectrophotometer cuvette using a glass transfer pipette with an attached bulb.
2. Place the ~1 ml of the Chl-*a* calibration standard stock into a 25 ml graduated cylinder with a glass stopper.
3. Dilute the Chl-*a* calibration standard stock with ~20 mL of extraction solvent from the 2 mL calibrated dispenser to obtain a concentration of 500 ± 50 µg/L.
4. Measure as an unknown on the spectrophotometer as per Section 20.8.11 below. If the intermediate Chl-*a* calibration standard is greater than 550 µg/L, add more extraction solvent and remeasure. If the daily Chl-*a* calibration standard stock is less than 450 µg/L, add more Chl-*a* calibration standard stock and remeasure. Continue adjustment until the 500 ± 50 µg/L concentration is achieved.

20.8.7 Intermediate Chl-*a* reference standard

The standard should be prepared fresh and stored in the dark at room temperature while not in use.

The intermediate Chl-*a* reference standard is prepared in the same manner as the intermediate Chl-*a* calibration standard, as outlined above in Section 20.8.6.

20.8.8 Chl-*a* calibration standard curve

The standard should be prepared fresh and stored in the dark at room temperature while not in use.

Five Chl-*a* standard concentrations are prepared by diluting the intermediate Chl-*a* calibration standard with extraction solvent in 25-mL volumetric flasks according to Table 20.1. Use glass graduated syringes to measure and deliver intermediate Chl-*a* standard. **Do not use plastic pipette tips as they are "wetted" with solvent and do not deliver accurate volumes.**

Table 20.1. Dilution volumes of calibration standards

Target conc.	DF	Intermediate Std Vol. (µL)	Final Vol. (mL)
0	extract solvent only	0	25
5	100	250	25
10	50	500	25
25	20	1250	25
50	10	2500	25
100	5	5000	25

Note: The actual standard concentrations will differ slightly from the target concentrations, depending on the concentration of the intermediate Chl-*a* standard. For example, if the intermediate Chl-*a* standard measured 520 µg/L, the 100, 50, 20, and 10 dilution factors will result in a calibration curve with concentrations of 5.2, 10.4, 26, 52, and 104 µg/L, respectively.

20.8.9 Chl-*a* reference standard – 10 µg/L

The standard should be prepared fresh and stored in the dark at room temperature while not in use.

A 10 µg/L Chl-*a* reference standard is prepared by diluting 500 µL of the intermediate Chl-*a* reference standard with 24.5 mL of the extraction solvent in a 25-mL volumetric flask.

20.8.10 Reference sample

Reference samples are prepared in bulk according to Section 4, *Reference Sample Preparation*. Briefly, the reference sample consists of a GF/C filter that has been used to filter 50 mL of Lake 227 water collected during the summer bloom. The reference samples are vacuum desiccated and stored in the dark at ≤ -20 °C.

20.8.11 Spectrophotometric determination of stock and intermediate standards

1. Turn on the power on the Shimadzu UV-1800 scanning spectrophotometer and let it warm up for at least two hours.
2. On the PC desktop, double-click the *UV Probe* shortcut.
3. Click *OK*.
4. Ensure that the *Photometric* icon is selected (Figure 20.1).

Figure 20.1. The Photometric icon

5. Open the method file under \\ela-camp.iisd.ca\shared\Chem Lab\Analysis Results\Chla\chlorophyll.pmd

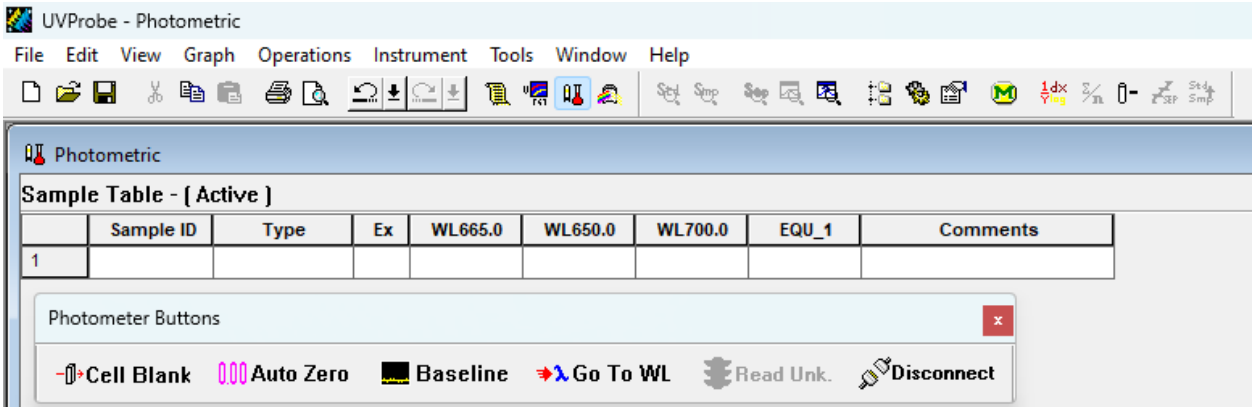
   Note: You must change the file type to *methods* in order to find the correct file.

6. The file will open with the correct wavelengths (665, 650, and 700 nm) selected, plus the calculation equation 20.1:

Equation 20.1.	Conc (µg/l) = 16.5(A665−A700) − 8.3(A650−A700)

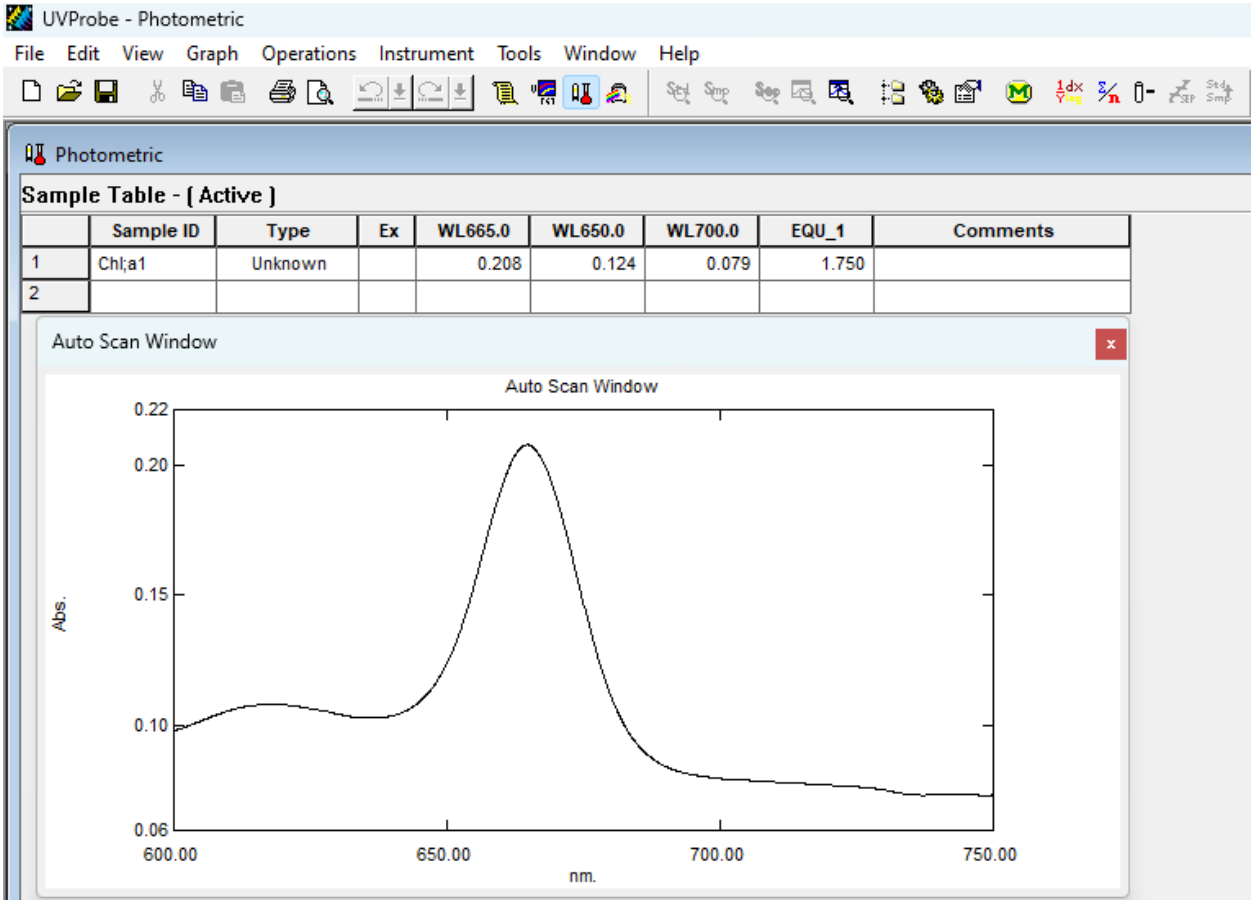
7. Click the *Connect* button at the bottom (Figure 20.2). If not available, click *View* > *Photometric buttons*. The UV detector will now go through its startup sequence.
8. After the startup sequence has finished, if the instrument has passed, click *OK*.
9. Rinse two 1-cm cuvettes with acetone and fill them with acetone.
10. Wipe the cuvettes with Kimwipes™ and place them in the appropriate holders in the spectrophotometer (the cuvette farthest away from you is the reference position).

Figure 20.2. Chl-*a* UV Probe photometric method window prior to connecting the UV Probe software to the Shimadzu UV-1800 scanning spectrophotometer



11. Click the *Baseline* (Figure 20.3) button, which will now be selectable. The baseline window options should be 400 to 800 nm. Click *OK*. The spectrophotometer will run through the two acetone samples to establish a standard baseline. After it has finished, all the buttons will be selectable (bold) again.

Figure 20.3. Chl-*a* UV Probe photometric method window



12. Remove the front cuvette, empty it, rinse it with stock solution, fill it with calibration stock, and return the cuvette to the front. Fill in the Sample ID of the standard table with "Chl stock_date" (format as mmdd, where the date is the current date of analysis). Click the *Read Unknown* button (Figure 20.3). Record the Chl-*a* concentration on the Chlorophyll-a BenchSheet (BenchSheet) and in the *Chlorophyll Standard* logbook. If this value is more than 5.0% different than the previous recorded value, repeat the scan. If repeat scan is still > 5.0% different, repeat again and record the mean of the three new readings.
13. Remove the front cuvette and transfer ~1 ml of the calibration stock to a 25-mL TC graduated cylinder with a tapered stopper. Dilute the calibration stock with enough

extraction solvent to yield a concentration of 500 ± 50 µg/L. Rinse the cuvette with methanol and then repeat step 12 using the diluted calibration stock (i.e. intermediate calibration standard) and labelling the Sample ID with "Cal_std_date" to measure and record the intermediate calibration standard.

14. Repeat steps 12 and 13 for the reference stock and intermediate reference standard, labelling the Sample IDs with "Ref_stock_date" and "Ref_std_date," respectively.
15. Save the results in \\ela-camp.iisd.ca\shared\Chem Lab\Analysis Results\Chla\yyyy_Chla in the format "yyyymmdd_Chla.unk" (e.g. 20140501_Chla.unk).
16. Once complete, click *Disconnect* and close the UV Probe software.
17. Ensure the spectrophotometer is turned off (if no longer needed) and rinse the two cuvettes with methanol.

20.8.12 Calibration of repeating dispenser

1. The dispenser used to distribute the extraction solvent must be checked prior to use to ensure it is delivering the proper volume.
2. Pump the dispenser twice to ensure there are no bubbles in the line.
3. Ensure the adjustable pointer is pointed at the 2-ml mark.
4. Take a 10-mL graduated cylinder and dispense five pumps of extraction solvent into it.
5. Volume should be 10 ± 0.1 mL.
6. If the volume is not acceptable, adjust the volume pointer. Loosen the knob, adjust the volume, and repeat the calibration.

20.9 Quality control

This method has a number of conditions for calibration standards, reference standards, reference samples, and blanks that must be met before samples can be analyzed and the resulting data is recorded. If any of the quality control (QC) expectations are not met, testing is stopped, the non-conformance is investigated and reported in the *Non-Conformance* log, and appropriate corrective action is taken before testing is resumed. If any QC failure persists, testing of samples is suspended until QC has been returned to within expectations.

20.9.1 Solid secondary standard

A solid secondary standard is used prior to fluorometric analysis to check the drift of the instrument over time. The raw fluorescence units (RFU) of the solid secondary standard at the time of analysis must be within 2.5% of the RFU of the solid secondary standard at the time of calibration. If not, the fluorometer must be recalibrated.

20.9.2 Reference standard

A reference standard is run every time a new calibration curve is run. The RFU of the reference standard must be within 5.0% of the RFU of the calibration standard of the same concentration (i.e. 10 µg/L).

20.9.3 Calibration curve

The linear correlation coefficients obtained from the calibration curve must have an $R^2 \geq 0.995$ with a first order fit. If this criterion is not met, new calibration standards may need to be prepared.

20.9.4 Reference sample

A reference sample is included at the beginning of every run for Chl-*a*. The Chl-*a* value obtained must agree to within 5.0% of the expected value. Results for check sample performance are charted and assessed for drift and bias.

20.9.5 Duplicates

Random duplicates of sample extracts are run every 15 samples. The duplicates must agree within 0.5 µg/L below concentrations of 10 µg/L or within 5.0% of the mean for concentrations greater than 10 µg/L.

20.9.6 Instrument blanks

Instrument blanks, consisting solely of extraction solvent, are analyzed at the beginning of each run. The instrument blanks must be below 0.10 µg/L; otherwise the problem must be investigated and corrected.

20.9.7 Labware

Volumetric flasks are calibrated and maintained according to Section 3, *Labware Calibrations and Verification*. All other glassware is cleaned according to Section 2, *Labware Cleaning*.

20.10 Quality assurance

Unlike most analytical methods, there are few known inter-laboratory calibration or certification programs available for the measurement of Chl-a, and these can be prohibitively expensive. We do not currently participate in proficiency testing. However, this method follows guidelines set forth by the United States Environmental Protection Agency Method 445.0, In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence.

20.11 Sample preparation

There is a 16-hour lag time between the extraction step and the analytical step.

20.11.1 Pigment extraction

A sample is collected by filtering a known volume of water through a Whatman GF/C 4.25-cm diameter GF/C filter. The default volume is 200 mL (see Section 6, *Sample Preparation and Filtration*).

Under low light conditions, the sample filter is desiccated under vacuum for 16 hours and processed immediately or stored at ≤ -20 °C in the dark in a small plastic Petri dish (see Section 6, *Sample Preparation and Filtration*). The filter is transferred to a glass extraction test tube with forceps by folding the filter in half twice, particulates facing in, placing the filter in the bottom of the extraction tube and using the aluminum rod to move the filter to the bottom of the tube. The filter must be placed far enough down in the tube to be covered by the 2.0 mL of extraction solvent that will be added, but it should not be packed tightly, as this will affect the extraction.

The extraction tube, with filter, is placed in a numbered rack, and the BenchSheet is filled out with the sample number, rack position, sample description (e.g. 239 epi), and filtration volume.

Using the calibrated dispenser, add 2.0 mL of extraction solvent to the extraction test tube containing the filter. Make sure the PTFE tip of the dispenser is free of bubbles and that the plunger is raised and lowered fully for each dispensing cycle. While the default extraction solvent volume added is 2.0 mL, filters with high levels of Chl-*a* (visual inspection of the biomass on the filter) are diluted at this stage with 4.0–8.0 mL of solvent. Record the extraction volume for each sample on the BenchSheet.

The cap is screwed on the test tube tightly and placed back into the rack, making certain that the filter is still at the bottom of the test tube and covered with solvent. After all samples are set up, the rack is covered by the bottom end of another rack so test tubes can be inverted three times to mix contents completely.

The sample is extracted while stored in the dark at 4 °C for a minimum of approximately 16 hours (overnight).

At the end of the extraction period, the sample vials are again inverted to mix the sample and solvent. Vials are kept in the dark at room temperature for one hour prior to analysis. This allows the solvent to warm and particles to settle.

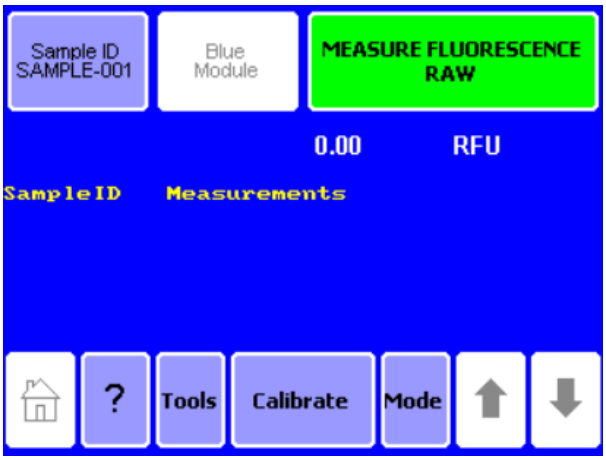
20.12 Analysis

20.12.1 Calibration curve

A calibration curve is conducted at the beginning of the field season and any time the RFU of the solid secondary standard at the time of analysis is more than 2.5% different than the RFU of the solid secondary standard at the time of calibration. The fluorometer is calibrated with the calibration standards created in Section 20.8.8 using the following steps:

1. Turn on the fluorometer and let it warm up for at least 30 minutes. The instrument goes to sleep after a period of inactivity; ensure that this does not occur.
2. Select the CHL-NA module. Close the door and dim the lights as dark as they can be without impairing your ability to read and write.
3. Put the instrument in raw fluorescence mode by clicking *Mode*. Remove the cuvette holder and measure the RFU of the solid secondary standard by clicking *Measure Fluorescence Raw* (Figure 20.4). Replace the cuvette holder and measure the RFU of the reference standard. Record these values on the BenchSheet.
4. Click *Calibrate* to enter the instrument's calibration mode and select µg/L as the unit of measure.
5. Fill a cuvette with at least 2 mL of extraction solvent, place in the cuvette holder and click *OK*.
6. Fill a cuvette with standard and enter the actual concentration of the standard (as calculated from the stock concentration, NOT the target concentration) and click *OK*. Record RFU values on the BenchSheet as they appear.
7. Click *Enter More Standards* and repeat step 6 for the remaining four standards.
8. Name the calibration with the date in the "yyyymmdd format" and click Save.
9. Enter the calibration curve and reference standard data (again use calculation, not target concentrations) into the Excel worksheet (\\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Chla\yyyymmdd_Chla.xls) and save the file as "yyyymmdd_Chla.xls" in \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Chla\yyyy_Chla directory. Ensure that the calibration curve has an $R^2 \geq 0.995$ and the reference standard is within 5.0% of the 10

Figure 20.4. Display screen of Turner Designs Trilogy Fluorometer in raw fluorescence mode



µg/L calibration standard. If they are not within acceptable values, the reference standard and/or the calibration curve may need to be remade and/or reanalyzed.

20.12.2 Fluorometric analysis

If samples are processed on the same date as the calibration curve was conducted, proceed to step 4.

1. Turn on the fluorometer and let it warm up for at least 30 minutes. The instrument goes to sleep after a period of inactivity; ensure that this does not occur.
2. Select the CHL-NA module. Close the door and dim the lights as dark as they can be without impairing your ability to read and write.
3. Put the instrument in raw fluorescence mode by clicking *Mode*. Remove the cuvette holder and measure and RFU of the solid secondary standard by clicking *Measure Fluorescence Raw* (Figure 20.4). Compare this value to the RFU of the solid secondary standard obtained on the date of calibration. If the RFUs are within 2.5%, proceed with an analysis of the samples. If not, a new calibration curve needs to be conducted.
4. Run an instrument blank by filling a cuvette with at least 2 mL of extraction solvent, placing it in the cuvette holder and clicking *OK*. Record the concentration and fluorescence value on the BenchSheet. Make sure to capture the fluorescence value before it disappears.
5. Run each sample in the same manner as the instrument blank.
   - If there is doubt about having enough sample to measure, remove the cuvette holder, place the cuvette with sample extract in it, and ensure the meniscus is above the window. If there is not enough sample, use a spatula to squeeze extra solvent out of the filter. If this still does not yield enough sample, use graduated glass syringes to dilute 1 mL of sample with 1 mL of extraction solvent and measure this, making note of the dilution.
   - If a sample is off-scale, dilute it as necessary with extraction solvent. Try to avoid this scenario by choosing the right amount of extraction solvent during the extraction step.
6. Run a random duplicate every 15 samples.
7. Once analysis is complete, turn off the fluorometer.

20.13 Cleaning

1. Dump any remaining solvent into the solvent waste container.
2. Remove filters from test tubes using the aluminum rod and discard them in the trash.

3. Rinse the cuvettes, test tubes, and caps with hot tap water and scrub the test tubes with a brush to remove any remaining filter debris. Rinse the cuvettes, test tubes, and caps three times with hot water, followed by three times with DRO water.
4. Dry the cuvettes, test tubes, and caps in an acid vapour-free environment.

All other labware is cleaned according to Section 2, *Labware Cleaning*. Briefly, the labware is rinsed three times with hot water, three times with DRO water, and air dried.

20.14 Calculations

A calibration curve, first order instrument response, R, is generated within the Trilogy fluorometer (equation 20.2):

Equation 20.2. $$C_{std} = (b + mR_{std})$$

where R_{std} is the instrument response to the calibration standard of concentration C_{std} in µg/L and b and m are the intercept and slope, respectively. This curve is then modified to calculate samples as:

Equation 20.3. $$C_{smpl} = (b + mR_{smpl}) \times \frac{v_{solv}}{v_{filt}}$$

where R_{smpl} is the instrument response to the sample of concentration C_{smpl}, and v_{solv} and v_{filt} are the volume of extraction solvent (mL) and the volume of sample filtered (mL), respectively.

20.15 Data entry

The Chl-*a* concentrations for each sample, which are calculated by the Trilogy fluorometer using the calibration curve, as well as the QC data, are entered into the Sample Master. The calibration curve and reference standard data (again, use calculation, not target concentrations) are entered into the Excel worksheet (\\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Chla\yyyymmdd_Chla.xls) and saved as "yyyymmdd_Chla.xls" in the \\ela-lab.iisd.ca\shared\Chem Lab\Analysis Results\Chla\yyyy_Chla directory. The *Chlorophyll-a BenchSheet* is filed in the Chl-a archived BenchSheet folder.

20.16 References

Arar, E. J., & Collins, G. B. (1997). *Method 445.0: In vitro determination of chlorophyll a and phaeophytin a in marine and freshwater algae by fluorescence.* U.S. Environmental Protection Agency.

Appendix 20A. Extraction Solvent Preparation Record

iisd ela experimental lakes area

Extraction Solvent
Preparation Record

Stable for six months

Preparation date	Constituents by volume	Manufacturer	Manufacturer lot number	Grade	Vol (mL)	Expiry date	Analyst initials
	Methanol 68%						
	Acetone 27%						
	Water 5%						
	Methanol 68%						
	Acetone 27%						
	Water 5%						
	Methanol 68%						
	Acetone 27%						
	Water 5%						
	Methanol 68%						
	Acetone 27%						
	Water 5%						
	Methanol 68%						
	Acetone 27%						
	Water 5%						

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 20B. Chlorophyll-*a* Calibration Standard Stock Preparation Record

iisd|ela experimental lakes area

Chlorophyll-*a* Calibration Standard Stock
Anacystis nidulans
Preparation Record

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Manufacturer expiry date	Nominal mass (g)	Acetone vol (mL)	Approx final conc (µg/L)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 20C. Chlorophyll-*a* Reference Standard Stock Preparation Record

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experimental lakes area

Chlorophyll-*a* Reference Standard Stock
Spinach
Preparation Record

Stable for one year

Preparation date	Manufacturer	Manufacturer lot number	Manufacturer expiry date	Nominal mass (g)	Acetone vol (mL)	Approx final conc (µg/L)	Expiry date	Analyst initials

Authorized by: Sonya Havens
Authorization date: October 2016

Appendix 20D. Chlorophyll-*a* BenchSheet

Chlorophyll-a Bench Sheet

Date _____ Analyst: _____

Cal. stock conc. = _____ Cal. conc. = _____ Sec. Solid Std. = _____

Ref. stock conc. = _____ Ref. conc. = _____ CalCurve = _____

Standard Curve					
Target Concentration	**DF**	**Vol. Cal/Ref Conc. (µL)**	**Make to volume (mL)**	**Actual Conc.**	**RFU**
0	extract solvent only	0	25		
5	100	250	25		
10	50	500	25		
25	20	1250	25		
50	10	2500	25		
100	5	5000	25		
Ref. 10	50	500	25		

Rack pos.	Sample Number	Sample Description	Filtration Volume (mL)	Extraction Volume (mL)	Concentration (ug/L)	F
A1						
A2						
A3						
A4						
A5						
A6						
A7						
A8						
A9						
A10						
A11						
A12						
B1						
B2						
B3						
DUP 1						
B4						
B5						
B6						
B7						
B8						
B9						
B10						
B11						
B12						
C1						
C2						
C3						
C4						
C5						

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Head Office

111 Lombard Avenue, Suite 325
Winnipeg, Manitoba
Canada R3B 0T4

Tel: +1 (204) 958-7700
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