NTL Research Protocols

NTL-LTER

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Welcome

This is the website for NTL-LTER research protocols.

Acknowledgements

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Part I

Water Chemistry Protocols

The following sections contains water chemistry laboratory protocols.

1 Alkalinity

Revised: Grace Wilkinson, March 2023

1.0.1 Purpose:

This procedure describes the steps to potentiometrically titrate water samples with standardized hydrochloric acid to calculate alkalinity according to Andersen, 2002 and USGS National Field Manual for the Collection of Water-Quality Data. The units of alkalinity for this analysis are microequivalents of carbonate per liter.

1.0.2 Sample Holding Time:

14 days @ 4° C unpreserved

1.0.3 Materials Required for Titration

(see materials for regents in Section 1.5):

- MilliQ water
- 0.05N Hydrochloric Acid
- 1000 ueq/L Sodium Carbonate standard
- pH meter and probe
- pH buffers
- Analytical balance
- P200 manual, adjustable pipette
- P100 electronic, adjustable pipette
- Pipet tips
- Small, graduated cups for titration
- Micro-stirbar
- MilliQ squirt bottle
- Kimwipes

1.0.4 Glassware Preparation:

The glass jar to hold waste rinses and the graduated cups used for titration should be rinsed with DI water from the tap at the sink and set upside down to dry.

1.0.5 Personal Protective Equipment / Waste Disposal:

Nitrile gloves and eye protection should be worn while titrating. Always use chemical resistant gloves (not latex), safety glasses, lab coat, and a fume hood while using concentrated acids to prepare the 0.05N HCl. This is not only for your protection, but also to prevent contamination of samples. Proper personal protective equipment is always required for safety and contamination prevention.

1.0.6 Quality Assurance/Quality Control:

- Blind samples for analysis (i.e., field duplicates)
- Triplicate analysis of standard solution

1.0.7 Waste Disposal:

Most of the reagent solutions used in this procedure can go down the drain; however the pH should be near neutral (pH 5 – 8). Flush during and after disposal by running tap water. Excess dry reagents from preparing the stock can go in the trash.

Consumables Ordering: Item Catalog # Item Catalog # Na2CO3 salt Fisher AA3648522 Buffer 3.557 (Ricca) Fisher 149816 Optima HCl (500 mL) Fisher A466500 Buffer 6.87 (Ricca) Fisher 154016

1.0.8 Consumables Ordering:

Item	Catalog #	Item	Catalog #
Na_2CO_3 salt	Fisher AA3648522	Buffer 3.557 (Ricca)	Fisher 149816
Optima HCl (500 mL)	Fisher A466500	Buffer 6.87 (Ricca)	Fisher 154016

1.1 Preparing for Analysis

1.1.1 Remove samples for analysis from the fridge to allow them to warm up to room temperature prior to analysis.

1.1.2 Turn on the pH probe by pressing any key. Make sure it is reading in mV; if not, press 'MODE' until mV is being read. NOTE: put the meter on "Standby" after analysis

1.1.3 Prepare the pH probe for analysis. NOTE: follow these directions in reverse to store the pH probe after analysis

1.1.3.1 Remove the storage solution and parafilm from the probe

1.1.3.2 Check that there is enough liquid in the probe

1.1.3.3 Rinse the probe with MilliQ water from the squirt bottle and dab with a Kimwipe. NOTE: Do not rub the probe with a Kimwipe as this creates static.

1.1.4 Using the pH probe, measure the standard buffers (pH = 3.557 and 6.87) to create a calibration curve.

1.1.4.1 Place the pH probe in the buffer solution and allow the reading to stabilize. Record the millivolts (mV) on the sample data sheet.

1.1.4.2 Rinse off the pH probe with MilliQ water and dab dry with a Kimwipe

1.1.4.3 If the millivolts are not close to the values recorded below, the pH probe may be faulty or need recalibration. Consult with the lab manager before proceeding.

Buffer pH	millivolts
3.557	168
6.87	-19

1.1.5 Make sure that all the sample cups and stir bars are clean and dry.

1.1.6 Turn on the electronic micropipette.

1.2 Analysis of Standard

1.2.1 Place a sample cup with a microstir bar on the analytical balance. Tare the balance.

1.2.2 Pour and pipette 16 ± 0.1 mL of the 1000 µe/L standard into the sample cup on the balance. Remember, 16 mL = 16 g.

1.2.3 Record the mass of the standard sample on the standards data sheet.

1.2.4 Pour an aliquot of 0.5 N HCl into one of the plastic cups and cover with parafilm for storage. This will be the working acid solution you use for titrations and pre-dosing.

1.2.5 Pre-dose the standard sample with 0.2-0.4 mL of 0.5 N HCl acid. Remember, 100 µL = 0.1 mL

1.2.6 Place the standard sample on the stir plate and carefully position the pH probe so that is submerged in the sample without touching the sides or the microstir bar. Turn on the stir plate.

1.2.7 Using the electronic micropipette, add 0.01 mL aliquots of HCl until the meter reads 120 mV. Keep track of the volume added!

1.2.7.1 Record the pre-dosing volume and volume added in the following step along with the mV reading on line 1.

1.2.7.2 Do not record any readings or volumes until the mV reading is greater than 120 mV.

1.2.8 Add an additional 0.01 mL aliquot of acid to the standard sample and let the pH reading stabilize. Record the new volume and mV.

1.2.9 Repeat this process for 10 total aliquots of 0.01 mL of acid. Record the volume and mV reading each time.

1.2.10 Cleaning up the standard sample:

1.2.10.1 Dump the sample down the sink.

1.2.10.2 Rinse off the pH probe with MilliQ water and dab dry with a Kimwipe

1.2.11 Repeat steps from Section 1.2.1 - Section 1.2.10 twice more for a total of three standard sample readings.

1.2.12 Compare the amount of acid added to reach 120 mV and the mV readings thereafter. If the standard samples are wildly different from each

Table 1.3: Northern Lakes Pre-Dosing

Abbreviation	Lake Name	Pre-Dosing Volume
AL	Allequash Lake	0.2 mL
SP	Sparkling	0.2 mL
TR	Trout Lake	0.2 mL
BM	Big Muskellunge	0.1 mL
CR	Crystal lake	None
CB (Bog 27.2)	Crystal Bog	None
TB (Bog 12.15)	Trout Bog	None

Table 1.4: Southern Lakes Pre-Dosing

Abbreviation	Lake Name	Pre-Dosing Volume
FI	Fish Lake	0.7–0.9 mL
WI	Wingra	0.9–1.1 mL
ME	Mendota	0.9–1.1 mL
MO	Monona	0.9–1.1 mL
WA	Waubesa	0.9 mL
KE	Kegonsa	0.9 mL

1.4 Calculations

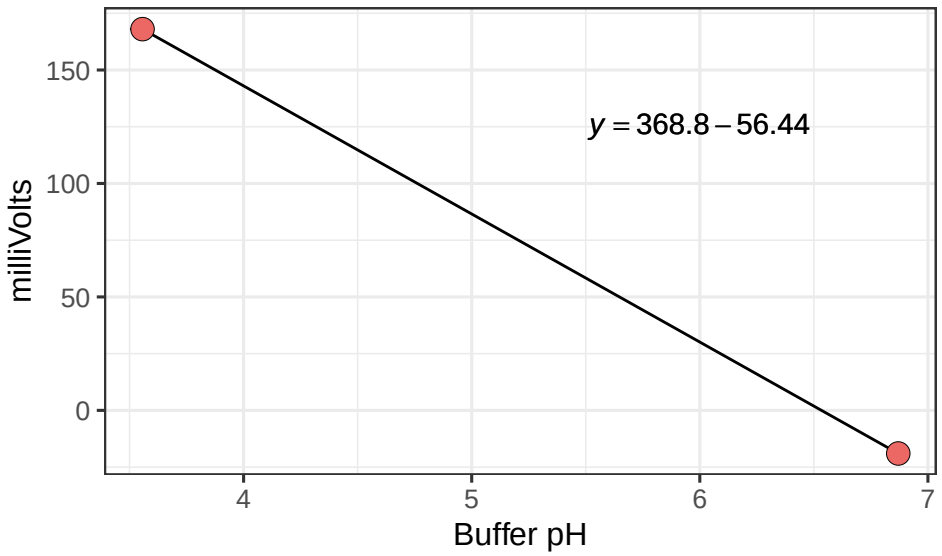
There is a calculation spreadsheet available on the shared drive. However, if the spreadsheet is not available or becomes corrupted, the steps below can be used to calculate alkalinity.

1.4.1 Use the calibration buffers to calculate a standard curve relating mV (millivolts) to pH. Calculate the slope (mstd) and intercept (bstd).

In this example, the slope (mstd) is -56.44 and the intercept (bstd) is 368.8. The slope should always be negative for the standard curve and the coefficients should be similar to the values in this example. If not, the pH probe may be faulty. Try calibrating.

1.4.2 Using the standard curve coefficients, calculate the pH for each point in the sample titration.

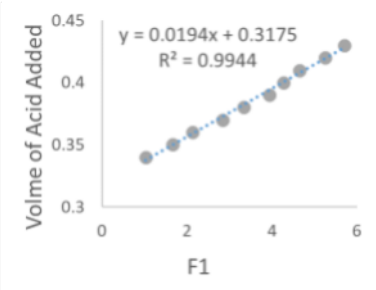
$$pH = \frac{(mV - \ b_{std})}{m_{std}}$$



1.4.3 The pH from the sample titration, the initial sample volume (mL), and volume of acid added (mL) are used to calculate F1 for each point in the sample titration.

$$F1 = 1000 \times \frac{VolumeAcidAdded + SampleVolume}{SampleVolume} \times 10^{-pH}$$

1.4.4 Regress the volume of acid added (mL) versus F1. The intercept (bF1) of this regression (mL) will be used to calculate alkalinity.



In this example, the intercept (bF1) is 0.3177. The slope should always be positive for this relationship and the R2 value should be >0.98. If not, the pH probe may be faulty, the pipette calibration is incorrect, or the analyst was not careful with pipetting. Try calibrating or reanalysis of the sample.

1.4.5 Calculate alkalinity (µEg/L) using molarity of the titration acid (nominally 0.05 N, but needs to be determined for each batch), initial sample volume (mL), and b_{F1}.

$$Alkalinity(\frac{Eq}{L}) = \frac{b_{F1} \times TitrantMolarity}{SampleVolume} \times 1e6$$

1.5 Preparation of Standards and Reagents

1.5.1 Preparation of 1000 µEq L^{-1} standard for alkalinity titration

Requires sodium carbonate (Na_2CO_3), 2 L of milliQ water, 2L volumetric flask, and standard solution storage bottles. The mathematical justification for the recipe below (note, there are 2 equivalents of Na+ per mol of Na_2CO_3):

$$0.1061g\ Na_2CO_3 \times \frac{1mol\ Na_2CO_3}{105.99g\ Na_2CO_3} \times \frac{1e6\ Eq}{Eq} \times \frac{2Eq}{mol} \times \frac{1}{2L} = \frac{1001\ Eq}{L}$$

1.5.1.1 Dry approximately 0.2 g of sodium carbonate (Na_2CO_3) salt in the drying oven for at least 3 hours prior making the standard

1.5.1.2 Label a 2 L volumetric flask and fill approximately half way with milliQ

1.5.1.3 Weigh out 0.1061 g of Na_2CO_3 salt using a weigh boat and the analytical balance. Add the salt to the volumetric flask with milliQ water (previous step)

1.5.1.4 Cap the volumetric flask with parafilm or the glass stopper, and swirl to dissolve and combine.

1.5.1.5 Fill the volumetric flask to the 2L mark with milliQ water and invert to mix.

1.5.1.6 Dispense the standard solution into the clean and labeled standard storage solution bottles. NOTE: the label should include the chemical composition, date of preparation, and preparer's initials.

1.5.2 Preparing 0.05 M HCl titration solution or alkalinity titration

Requires Fisher Brand Optima HCl, milliQ water, and a 1 L volumetric flask. Note, that for this solution, molarity is equivalent to normality because there is 1 H+ per Cl-. The mathematical justification for the recipe below:

For 100 g of solution, there will be 34 g of HCl, therefore:

$$34g\ HCl \times \frac{1mol\ HCl}{36.4611g\ HCl} = 0.9325mol\ HCl$$

The specific gravity (density) of HCl is 1.18 g/mL:

$$100g\ solution \times \frac{1mL\ solution}{1.18g\ HCl} \times \frac{1L\ solution}{1000mL} = 0.0847L\ solution$$

$$\frac{0.9325mol\ HCl}{0.0847L\ solution} = 11.01M\ (mols/L)$$

Based upon M1V1 = M2V2:

$$V1 = \frac{0.05\ HCl \times 1000mL}{11.01M} = 4.54mL$$

1.5.2.1 Fill the volumetric flask with ~800 mL of milliQ water.

1.5.2.2 Using a pipette, dispense 4.54 mL of HCl into the volumetric flask

1.5.2.3 Fill the volumetric flask to the 1 L mark with milliQ water. Cap and invert to mix.

1.5.3 Standardizing the HCl titration solution

Requires titrating the 1000 µEq L^{-1} standard multiple times and the intercept of F1 ~ volume of acid added (Section 1.4.4) is averaged to determine molarity.

1.5.3.1 Following the methods in Part 2 of this protocol, titrate at least 10 standard solution samples of 16 mL.

1.5.3.2 Enter the data into the calculation spreadsheet (based on calculations in Section 1.4 of this protocol) and record the intercept.

1.5.3.3 Average the intercepts from the 10 titrations of standard solution and use in the following equation to calculate molarity (equivalent in this case to normality):

$$\frac{1000}{1e6} \times \frac{16mL}{avg.intercept} = molarity\ of\ HCl\ solution$$

1.6 References

National Field Manual for the Collection of Water-Quality Data: U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9, Chaps. A1-A9, Chap. A6.6 "Alkalinity and Acid Neutralizing Capacity"

Andersen, C. B., 2002, Understanding Carbonate Equilibria by Measuring Alkalinity in Experimental and Natural Systems, Journal of Geoscience Education, v. 50, p. 389 – 403.

Part II

Old Protcols

The following section contains protocols taken from https://lter.limnology.wisc.edu/research/protocols

2 Ammonia and Nitrate/Nitrite

2.0.1 NITRATE/NITRITE

This automated procedure for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-napthylethylenediamine dihydrochloride to form a reddish-purple azo dye.

2.0.2 AMMONIA

This automated procedure for the determination of ammonia utilizes the Berthelot Reaction, in which the formation of a blue colored compound believed to be closely related to indophenol occurs when the solution of an ammonium salt is added to sodium phenoxide, followed by the addition of sodium hypochlorite. A solution of potassium sodium tartrate and sodium citrate is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium.

2.0.3 AUTOANALYZER REAGENTS

2.0.3.1 Nitrate/Nitrite

Ammonium Chloride-EDTA Buffer Reagent Ammonium Chloride (NH4Cl) 85 g MQ Water 1000 mL Disodium Ethylenediamine Tetraacetate (disodium EDTA) (C10H14N2Na2O8 $\cdot$ 2H2O) 0.5 mL Ammonium hydroxide(NH4OH) 6.5 mL (found in hood in acid washing room) TX10 0.05 mL
–Dissolve Ammonium chloride and EDTA in 900 mL Milli-Q water. Add Ammonium hydroxide and dilute to 1 L. Add TX10 to working reagent

3 Autoanalyzer Operating Instructions

Start up:

NOTE: To save time, start a warm water bath to bring the reagents up to room temp. Takes about 15 minutes to get hot water and about 20 minutes to warm the reagents.

1) Hook up the appropriate analysis module to the colorimeter and the autosampler lines. Hook up bubble lines to air pumping lines on the peristaltic pump. Check the pump tubing diagrams in the Lab Methods Manual if you have any questions.
2) Place the appropriate pair of filters in the colorimeter. Only the colorimeter in the back will ever need to have the filters changed. The Nitrite/Nitrate module uses the 550 nm filter for both Nitrite/Nitrate and Total Nitrogen analyses. The Silica module uses the 660nm filters; the ammonia module uses the 630 nm filters; the phosphorus module uses the 880 nm filters.
3) Fill the Milli-Q reservoir 1/2 full with fresh Milli-Q water.
4) Check the waste carboy. Empty if it is 2/3 full.
5) Place the pump tubing reagent lines into MQ while the reagents are warming up. Place the sampler(probe) into the autosampler and let it run on MQ. Once the reagents have warmed up, move the lines from the MQ tothe appropriate reagent bottles (lines and bottles are labeled).
6) Turn on power switches at the outlet board. Be sure the autosampler's power button is not engaged.
7) Install the platen plate on the peristaltic pump assembly and run pump on high speed once to get reagents flowing through lines.
8) Check to see if all reagents are flowing smoothly through the pump lines. If not, change lines position on the pump rollers or replace worn pump tubing.
9) After the bubbles in the lines are flowing evenly and are uniform in size, turn on the colorimeter and plug in the heating element(when needed). Set STD. CAL. knob to value used on previous analyses.
10) After 15 minutes, turn on the strip chart recorder(s), and install the pen(s).
11) Use the baseline knob on the colorimeter to establish a zero baseline.
12) Once a stable baseline has been established(usually 45-60 minutes), place the prepared tray on the autosampler (a standard 1 and a Milli-Q sample should be run first for calibration purposes). Press down the autosampler power button.
13) When the calibration standard's peak comes through on the chart paper, adjust the STD. CAL. knob on the colorimeter to attain a peak height that is almost the full width of the chart paper at the peak's maxima. When the Milli-Q sample is being recorded,

adjust the baseline knob of the colorimeter to place the recorder pen at its original zero position.

14) You now have the system calibrated and should not need to adjust the STD. CAL. knob the remainder of the analysis. If the baseline drifts up or down significantly over the course of the analysis, you may adjust it with the baseline knob when a Milli-Q sample is being detected. Record the adjustment with an arrow on the strip chart and label it with a message.
15) A full tray takes one hour and twenty minutes to be sampled completely. To assure that the autosampler stops at the end of the tray, place the red peg in the hole by the second-to-last sample cup. Continuous analysis may be maintained by taking the peg out and being sure to replace the sampled tray with an unsampled tray after the last sample has been aspirated.
16) Label the strip chart(s) with the name of the analysis, date, and analyst.

Shut down:

(Takes 30-40 minutes at end of analysis.) 0) Remove the cadmium column from the nitrate module without introducing any air into the column. 1) Remove reagent lines to a beaker filled with clean Milli-Q water. Run the pump on high speed one time. Place reagent bottles back in the walk-in cooler. 2) Remove the reagent lines to the small bottle half-filled with 0.1 N NaOH solution. Run the pump on high speed one time. 3) Remove the reagent lines to the beaker filled with Milli-Q water and run the pump one time on high speed. 4) Remove the reagent lines to a small bottle half-full of 0.1 N HCl. Run the pump on high speed one time. 5) Remove the reagent lines to the beaker of Milli-Q water and run the pump two or more times on high speed. 6) Remove the reagent lines to a plastic storage bag. Lift the autosampler "probe" out of the wash receptacle. Run the pump on high speed until liquid is no longer visible in the glass coils or the colorimeter tubing. 7) Remove the platen plate assembly and use a Kimwipe to clean the gray underside with isopropyl alcohol. 8) Turn off the power button on the autosampler and shut off all outlet power switches. 9) Turn off the power on the chart recorder and cap the recorder pen. 10) Check the waste carboy, empty if 2/3 full.

Additional Notes: If you are having problems with an unsteady or unusual baseline, the pump tubing and/or the pumping action are suspect. Most problems encountered are due to irregularities in the pumping action from worn pump tubing or blockages/air leaks in the lines. A level but noisy baseline may be caused by a small air bubble caught in the colorimeter's detection pathway. This may be "pulled through" by squeezing for a few seconds on the tube labelled TO PUMP and then releasing. A noisy baseline may also indicate worn pump tubing or a reagent which is precipitating. If you notice that liquid is dripping from the connecting lines of the mixing module and the colorimeter, you have probably forgotten to add a detergent (Aerosol 22 or Brij 35) to your reagents. See the Lab Methods Manual for details.

Tray Preparation for NO2&NO3/NH4 and N/ P Analyses: 1) Fill tray with conical-bottomed cups 2) Fill each cup completely with 1 N HCl. (83 mls conc. HCl/L) 3) Aspirate the acid

from each cup. 4) Fill each cup completely with fresh Milli-Q water. 5) Aspirate the Milli-Q water from each cup. 6) Repeat steps 4 and 5. 7) Aspirate thoroughly any water droplets in the bottom or clinging to the sides of the cups. This step is very important! 8) Fill sample tray immediately with the samples.

Tray Preparation for BRSi and DRSi analysis: 1) Fill tray with flat-bottomed cups. 2) Fill the sample tray with samples. (No washes or rinses are needed!)

last revision: 8/24/95 by James Thoyre