**Persulfate Digestion for Analysis of Total Phosphorus in Lake Water**

This analysis uses persulfate digestion to decompose organic phosphorus into phosphate, which is then measured using colorimetric analysis of phosphate (SRP analysis). Filtered samples are normally analyzed in this way to measure total dissolved P. Particulate N and P may be measured by simultaneous analysis of filtered and unfiltered subsamples, or by digesting material collected on glass=-fiber filters. However, other methods are usually superior for particulate forms, such as Anderson’s (1976) method for P.

**Supplies**:

* 60mL polypropylene bottles for digestion (soak in 10% HCl before use)
* Autoclave
* Spectrophotometer with 10 cm cuvette pathlength (in common lab 296)
* Supplies for SRP colorimetric analysis (see below)

**Reagents**:

Oxidizing solution: Add the following to about 350 mL of DI water, stir until dissolved (warm if necessary – over or extended heating can reduce effectiveness of the solution), then dilute to 500 mL. Prepare fresh for each use, adjusting reagent amounts to make only as much as you need. You will need 4mL for each sample and standard you plan to run. Store in the dark at room temperature in a polypropylene bottle.

1. 8.4 g NaOH (low N and P)
2. 18 g Boric Acid
3. 30 g potassium persulfate (K2S2O8; should be a special grade that is low in N).

Reagents for phosphate (SRP) analysis: See below.

**Standards**:

Working standard solutions (prepared before each analysis): Prepare P standards that bracket the sample concentrations. For surface waters, concentrations of 5-100 ppm (µg/L) TP are likely.

Use SPEX 1000ppm standard to make a 1ppm stock, from which you will make your standars

1. Make 10 mg/L (10ppm) working stock

Add 1000mL into 100mL of MilliQ in a volumetric flask

1. Made standard curve (in duplicate or triplicate) from working stock
   * If you will be running both TP and TN, you can combine the P and N standards. Just add both P and N standard to flask before filling with MilliQ

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Phosphorus**:  Std µg/L | **µL** of 10mg/L stock to   |  |  | | --- | --- | | **Nitrogen**:  Std ug/L | µL of 100mg/L stock to  add into 100mL MilliQ | | 0 | 0 | | 200 | 200 | | 400 | 400 | | 600 | 600 | | 800 | 800 | | 1000 | 1000 | | 2000 | 2000 |   add into 100mL MilliQ |
| 0 | 0 |
| 5 | 50 |
| 10 | 100 |
| 25 | 250 |
| 50 | 500 |
| 100 | 1000 |
| 200 | 2000 |

**Sample Storage:**

Unfiltered samples for TP should be frozen immediately if an analysis is not scheduled with a day or two. For TDP, filter samples immediately after collection. Although we often store filtered samples in the refrigerator for up to a few weeks before analysis, the best procedure is to add oxidizing solution as soon as possible after filtration. Oxidized samples can be stored at room temperature indefinitely.

**Procedure:**

1. Prepare the oxidizing reagent
2. Using a 30 mL volumetric pipet, measure 30 mL of samples and standards into the 60 mL **polypropylene** digestion bottles. Replicates are recommenced.
3. Add 4.0 mL of oxidizing solution using the repeater pipet with a 50 mL tip set to #4. Put caps on bottles, but **leave caps loose** **approximately ¼ turn.**
4. Place samples in autoclave and run as liquid for 45 minutes.
5. Allow samples to cool, then follow the protocol for the SRP analysis below (begin with step E).

**References:**Valderrama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10: 100-122.  
Langer, C.L. and P.F. Hendrix. 1982. Evaluation of a persulfate digestion method for particulate nitrogen and phosphorus. Water Research 16: 1451-1454.  
Ebina, J.T. and t. Shirai. 1982. Simultaneous determination of total nitrogen and total phosphorus in water using peroxydisulfate oxidation. Water Research 17: 1721-1726.

**Colorimetric analysis of SRP (phosphate)**

This method for the determination of soluble reactive phosphate (SRP) is taken from Wetzel and Likens (1991). In this method, the water sample reacts with a composite reagent containing molybdic acid, ascorbic acid, and potassium antimonyl-tartrate. The resulting heteropoly acid is reduced in situ to give a highly colored blue solution (Strickland and Parsons 1968). This analysis can also be used to measure the PO4-3 produced after persulfate digestion, as in the total dissolved P and particulate P analyses.

**Preparations:**

Samples must be filtered before analysis, and analysis should be performed relatively soon after filtration. Samples can be frozen if analysis cannot be completed within 3-4 days after sampling occurs. Samples should be analyzed at room temperature. Labware cleaning between routine uses does not require acid washing; thorough rinsing (at least 4 times) with DI water is sufficient. Labware should be acid washed after 4-5 uses. Never allow labware to dry out until thoroughly rinsed with DI water. Ascorbic acid reagent must be completely thawed prior to making the mixed reagent. Before handling labware, hands should be cleansed using a phosphate-free detergent, or clean gloves should be worn.

**Precautions:**

The capabilities of this test are approximately 3.0 – 100 µg P/L. This range corresponds to an absorbance range of approximately 0.015 – 0.600, as measured on a 10.0 cm pathlength. These estimates assume an absorbance blank of <0.006. Local waters are often around 3 µg/L or below, while wetland samples can be several hundred µg/L, especially if rich in DOC.

Mixed reagent may not be stored longer than 6 hours. Ascorbic acid should not remain thawed for longer than necessary.

Gloves should be worn to avoid contact with reagents, and safety glasses are recommended for washing labware and handling the reagents. See safety data sheets for ascorbic acid, potassium antimony-tartrate, soluble molybdenum compounds, and sulfuric acid.

**Reagents:**

1. Ammonium Molybdate solution: using a 250 mL volumetric flask, dissolve 7.5 g (NH4)6Mo7O24 4H20 in DI water and dilute to 250mL.
2. 15% H2SO4: in the hood, carefully add 75 mL of concentrated H2SO4 to 400 mL of DI water in an Erlenmeyer flask, use another 25 mL DI water to rinse the graduated cylinder (total volume ~500 mL). Store in glass
3. Ascorbic acid solution: using 200 mL volumetric flask, dissolve 10.8 g of L-ascorbic acid in DI water and dilute to 200 mL. Store in freezer.
4. Antimony potassium tartrate solution: dissolve 0.14 g of K(SbO)C4H4O6 0.5H2O in 100 mL of ID water. Store in glass.
5. Mixed reagent: Just before use, add the above reagents **in the order listed** in the proportions 2:5:2:1 to an appropriately sized beaker. Use a graduated cylinder to measure the volumes. Make enough to add 3.0 mL to each 30 mL standard and sample. The following table gives the proportions for several reagent batch volumes. The mixture should turn yellow after adding ascorbic acid.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Total Volume Desired (mL) | Ammonium Molybdate | Sulfuric Acid | Ascorbic Acid | Potassium Antimony Tartrate |
| 10 | 2 | 5 | 2 | 1 |
| 25 | 5 | 12.5 | 5 | 2.5 |
| 35 | 7 | 17.5 | 7 | 3.5 |
| 50 | 10 | 25 | 10 | 5 |
| 75 | 15 | 37 | 15 | 7.5 |
| 100 | 20 | 50 | 20 | 10 |
| 130 | 26 | 65 | 26 | 13 |
| 150 | 30 | 75 | 30 | 15 |
| 200 | 40 | 100 | 40 | 20 |
| 250 | 50 | 125 | 50 | 25 |
| 300 | 60 | 150 | 60 | 30 |

**Standards:**

Prepare standards that bracket the sample concentration. For surface waters, concentrations of 3-50 ppb P are likely. Total phosphate and total dissolved phosphate will yield higher values 5 – 100 ppb P are likely – adjust the standards used to accommodate this, if necessary. See above in the Persulfate Digestion protocol for how to make standards.

**Procedure**

1. Pour 30 mL samples, blanks, and standards in triplicate into 60 mL polypropylene bottles. If you digested samples, then you can skip this step.
2. Prepare the mixed reagent (see above).
3. Add 3 mL of mixed reagent to each standard using the repeater pipet with a 50 mL tip set at #3. Mix the bottles. Wait 10 minutes and then run them on the spec.
4. Read absorbance on a 10 cm pathlength at 885 nm. Borrow the cuvettes from Tank Lab. Use the Shimadzu spectrophotometer in the common lab (296). Fill one cuvette with MilliQ water and place in the back slot. This is the reference channel. Pour a small amount of your first standard into the second cuvette, rinse the cuvette, then dump into the waste. Then fill the cuvette with the rest of your standard and place it into the front slot of the spec. Record the absorbance when it is stable. Repeat for all of your standards.
5. Plot the standard curves and check to make sure that they are linear (the should have R2 around 0.95-1). If the standard curves look good, then proceed with step 6. If not, you’ll have to remake the standards and try again. If you are running TP then you’ll have to digest them again as well.
6. Add 3 mL of mixed reagent to each sample using the repeater pipet with a 50 mL tip set at #3. Mix the sample.
7. Run the samples on the spec between 10 minutes and 2 hours after adding the reagent. Follow the instructions for running the standards above (step 4).

**Waste disposal:**

All waste from this test should be stored in the designated hazardous waste container.

**Calculations:**

Average your standard curves and then calculate the slope and intercept of the regression line. This can be done in Excel using the =slope(y,x) and =intercept(y,x) commands where y is the concentrations and x is the abs values. Use the slope and intercept to calculate the concentrations from the abs values. Concentration = slope \* abs + intercept.

**References:**

Strickland, J.D.H. and T.R. Parsons. 1968. A practical handbook of seawater analysis. Fisheries Research Board of Canada, Ottawa.

Wetzel, R.G. and G.E. Likens. 1991. Limnological analyses. Second edition. Springer-Verlag.

**Method for Measuring Nitrate or TN with a UV Spectrophotometer  
(Olsen 2008)**

**Equipment and reagents:**

UV-Vis spectrophotometer  
1cm quartz cuvettes  
0.2 µm hydrophilic PTFE syringe filter  
10 mL syringe   
Potassium Nitrate

For TN also need:   
 Sodium Hydroxide (low N and P)  
 Boric Acid  
 Potassium Persulfate (Low N and P)  
 60mL acid washed polypropylene bottles

**Standards:**

Prepare standards with potassium nitrate. Create a stock of 100mg/L (180.5mg KNO3 in 250mL MilliQ). From the stock, make a standard curve that encompasses the range of your samples. Include a zero in the standard curve.

Potassium Nitrate (KNO3)

* KNO3 molec weight=101.1
* 250mL of 100 ppm stock N
* 100 mg N /L= (14mg N/101.1 mg KNO3) \* (x mg KNO3/0.25L)
* x= **180.5 mg KNO3 in 250mL MilliQ for 100 mg/L stock TN**

Make standard curve from stock

|  |  |
| --- | --- |
| Std ug/L | mL of 100mg/L stock to  add into 100mL MilliQ |
| 0 | 0 |
| 200 | 0.2 |
| 400 | 0.4 |
| 600 | 0.6 |
| 800 | 0.8 |
| 1000 | 1 |
| 2000 | 2 |

\*For TN, you need to digest the standards along with the samples.

**Sample Prep:**

TN: digest the samples with a persulfate oxidizing reagent

Oxidizing solution: Add the following to about 350 mL of DI water, stir until dissolved (warm if necessary – over or extended heating can reduce effectiveness of the solution), then dilute to 500 mL. Prepare fresh for each use, adjusting reagent amounts to make only as much as you need. Store in the dark at room temperature in a polypropylene bottle if necessary.

1. 8.4 g NaOH (low N and P)
2. 18 g Boric Acid
3. 30 g potassium persulfate (K2S2O8; should be a special grade that is low in N).

Add 4mL of oxidizing solution to each sample and standard. Can use the repeating pipette with 50mL tips set to #4.

Autoclave samples and standards for 45 minutes on liquid setting. Be sure to loosen caps ¼ turn prior to autoclaving. Allow samples to cool before proceeding with analysis.

**Analysis:**

Second Derivative method – this method works best:

Scan standards and samples on a UV-Vis spectrophotometer with 1 cm quartz cuvettes. For each standard/sample Scan from 200 to 250 nm and measure absorbance at 5 nm intervals. Fit a cubic polynomial to the data (y-axis is absorbance, x-axis is wavelength). The maximum value of the polynomial’s second derivative is used to calculate the nitrate concentration.

To run scans on Spectronic Genesys2 spec in common lab:

1. go to Advanced scanning from main menu
2. Push the test types button, then select Nitrate
3. Collect baseline (first time only) on a MilliQ blank
4. Scan (test settings: 200-250nm at 1nm invertals (medium speed))
5. Go to post process menu
6. Go to scale functions
7. Choose Tabular
8. Write down the values for 200-250nm at 5 nm intervals (i.e. 200, 205, 210…250)
9. Push exit until you are back at the scan screen. Repeat steps 4 through 8 for each sample. Rinse the cuvette 3x with the sample before filling. Only need to fill the cuvette about 2/3 full. If you accidently exit past the scan screen, you will need to collect a new baseline before continuing to scan. Make sure you do this on the MilliQ blank and not on a sample or empty chamber.

**Data analysis:**

You can analyze the data using the R script Nitrate.r or with an Excel file called NitrateScanCalculations.xlsx

**References:**

Olsen, KK. 2008. Multiple wavelength ultraviolet determinations of nitrate concentration, method comparisons from the Preakness Brook Monitoring Project, October 2006 to October 2006. Water Air Soil Pollut. 187: 195-202.