LAB 3: DETERMINATION OF PHOSPHATE

1. Background

In this experiment, we will analyze Hudson River water samples for their phosphate content. Phosphate-containing compounds are used in detergent formulation as water softeners. Thus, detergents can be the primary contributor to phosphate loads in rivers and lakes. Phosphate is not toxic to animals or plants. In fact, it is a plant nutrient, which stimulates the growth of aquatic weeds and algae. This may cause lakes and rivers to become clogged and overrun with plants (eutrophication). In the oceans, phosphate is limiting over geological time scales since the only source is weathering of continental rocks.

2. Scope and field of application

This procedure describes a method for the determination of reactive phosphorus in water samples, suitable for the assay of concentrations of 0.01- 2.5 μM. This method is a modification of Strickland and Parsons (1968).

3. Definition

The reactive phosphate concentration is given in units of μM.

4. Principle of Analysis

The determination of reactive phosphorus in seawater is based on the method proposed by Strickland and Parsons (1968). The seawater sample is allowed to react with a composite reagent containing ammonium molybdate, ascorbic acid and potassium antimonyl-tartrate. In the ascorbic acid-molybdate method orthophosphate reacts with molybdate to form phosphomolybdic acid. Phosphomolybdic acid is reduced by ascorbic acid to form a blue complex:

Phosphate + Molybdate →Phosphomolybdic Acid

Phosphopmolybdic Acid + Ascorbic Acid → Reduced Phosphomolybdate complex

The intensity of the blue color at 885 nm is proportional to the phosphate concentration in the solution. It has been demonstrated that in dilute, acidic solutions with an excess of the molybdate species present, Beer's Law is obeyed with respect to phosphate.

Beer's Law is frequently written as: $A = \varepsilon\, l\, C$
where: A = the absorbance; ε = the molar absorptivity; l = the path length in cm (in our case, this is 1 cm) and C = the concentration of absorbing species in moles per liter. If Beer's Law is obeyed, a linear plot of A vs. C should result. If one prepares a Beer's Law plot with standard solutions, an unknown can be determined by

comparison to the standard curve. The figure below was made using the following data:

Concentration μM	Absorbance
3.0	0.17
1.5	0.092
0.6	0.035
0.3	0.015
Blank	0

Phosphate Standard Curve

$y = 0.0574x + 0.0005$

Absorbance vs. Phosphate concentration (uM)
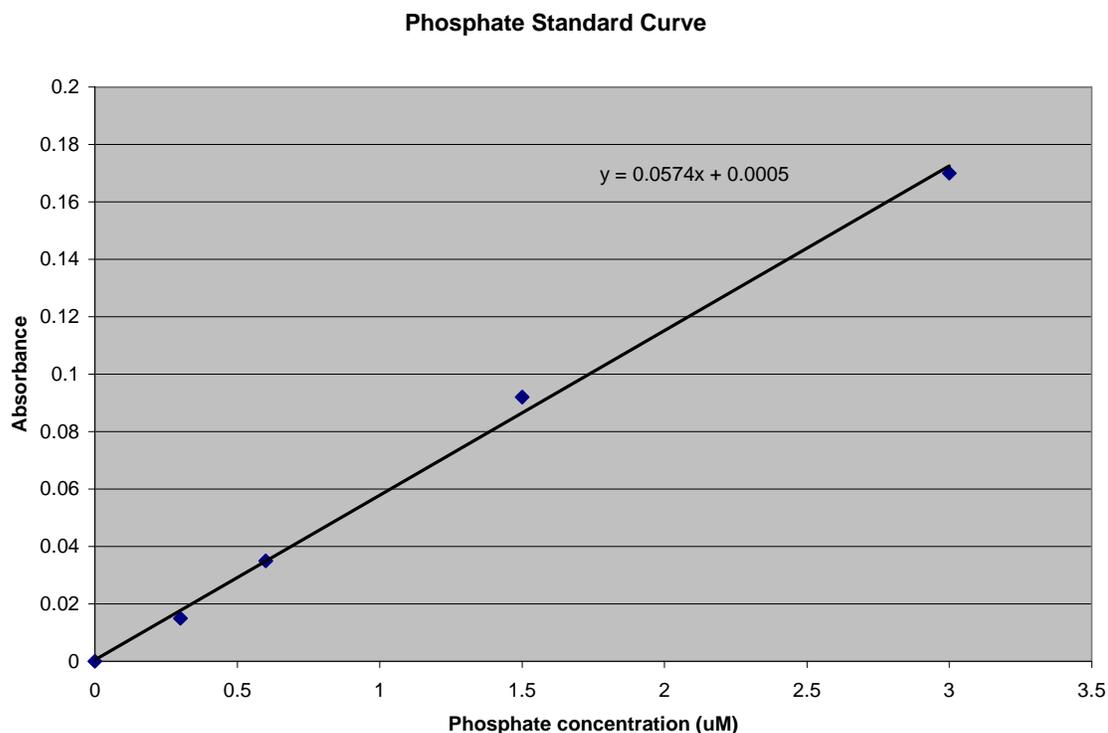
5. Apparatus

Contamination is a major problem with nutrient samples; all glassware and sample containers must be rigorously cleaned and dried. The cleaning begins by a wash with a phosphate-free detergent (Aquet) followed by a rinse with 10% HCl and three rinses with deionized water.

  a. Spectrophotometer and a set of test tubes

  b. Volumetric Flasks

    i. One 500 mL volumetric flask for preparing the ammonium molybdate solution

      ii. One 200 mL volumetric flask for preparing the potassium antimonyl-tartrate solution

      iii. Two 100 mL volumetric flasks for preparing dihydrogen phosphate standard and ascorbic acid.

      iv. One 1000 mL volumetric flask for preparing the potassium dihydrogen phosphate solution.

c. Reagent storage bottles

      i. Two 500 mL plastic bottle for storing the ammonium molybdate and potassium antimonyl-tartrate solutions.

      ii. Two 500 mL glass bottles for storing the sulfuric acid solution.

      iii. Two 500 mL amber glass bottles for storing the dihydrogen phosphate solution.

d. Graduated cylinders

      i. Two 250 mL graduated cylinders

      ii. One 1000 mL graduated cylinder

      iii. One 500 mL graduated cylinder

      iv. One 125 mL graduated cylinder

e. 150mL beakers.

f. Funnels for delivering dry chemicals into vol. flasks

g. Volumetric pipets and 200-1000μL pipettor for making standards

h. Acid cleaned squirt bottle for deionized water dispensing

i. Nutrient bottles: 125 plastic screw-cap bottles

j. Whatman disposable filter-cup (one per water sample).

k. Hand vacuum pump for filtering samples.

6. Reagents

Dry chemicals needed:

15 g. Ammonium molybdate
5.4 g. Ascorbic acid
0.272 g. Potassium antimonyl-tartrate
0.816 g. Potassium dihydrogen phosphate (KH_2PO_4)

Liquid chemicals needed:
   *Use distilled water or Milli-Q water if available for all phosphate reagents, particularly the standard. If distilled is not available, use deionized water although your blank may be higher.
   140 mL Sulfuric acid (concentrated)
   1 mL Chloroform

The ammonium molybdate, potassium anitmonyl-tartrate, sulfuric acid and potassium dihydrogen phosphate should all be made ahead of time. The ascorbic acid should be mixed at the beginning of analysis and the mixed reagent should be mixed only immediately before it is to be added to unknown samples.

   a. Ammonium molybdate

      i. Fill a clean 500 mL volumetric flask approximately 2/3 full of deionized water.

      ii. Using a clean small funnel, gradually pour 15 g ammonium molybdate into the flask.

      iii. Fill the flask to 500 mL, stopper and invert the flask several times to mix thoroughly.

      iv. Pour the ammonium molybdate solution into a plastic 500 mL stock bottle and mark with the name of the reagent, the date and the initials of the mixer.

      v. This solution is stable, but should not receive direct sunlight. If absorption values appear too high, this would be the first reagent to re-mix.

   b. Potassium antimonyl-tartrate

      i. Fill a clean 200 mL volumetric flask approximately 2/3 full of Milli-Q water.

      ii. Add 0.272 g potassium antimonyl-tartrate into the flask. Bring to volume with Milli-Q water.

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    iii. Pour this solution into a 500 mL glass or plastic stock bottle, mark the bottle with the reagent name, date and initials.

    iv. This solution is very stable.

c. Sulfuric acid (~2.4M)

    i. THIS OPERATION SHOULD TAKE PLACE INSIDE A FUME HOOD! GLOVES AND EYE PROTECTION MUST BE WORN AT ALL TIMES. THIS OPERATION REQUIRES QUALIFIED PERSONEL.

    ii. Pour 140 mL of concentrated sulfuric acid into a clean, dry 250 mL graduated cylinder and keep secure.

    iii. Pour 900 mL of deionized water into a 1000 mL graduated cylinder.

    iv. SLOWLY pour the sulfuric acid into the 1000 mL graduated cylinder using a clean funnel. ALWAYS ADD ACID TO WATER AND NEVER, NEVER ADD WATER TO ACID!!! This solution will be quite hot.

    v. Immediately pour the solution into two clean glass 500 mL stock bottles, stopper and place into a cool water bath. Once cool, label the bottles as described above and store.

    vi. This solution is stable.

d. Potassium di-hydrogen phosphate standard (0.01M)

    i. This is your standard, and the dry chemical should be very dry, that is dried in a drying oven, or at least stored with fresh dessicant overnight. All the phosphate values depend on the accurate weighing of this standard.

    ii. Fill a clean 1000 mL volumetric flask approximately 2/3 full of deionized water.

    iii. Add 0.816 g potassium dihydrogen phosphate (KH_2PO_4), fill to 1000 mL with deionized water and thoroughly mix.

    iv. Pour into two <u>amber</u> 500 mL glass stock bottles and label. Add 1 mL chloroform to each bottle and seal; it will not mix with the aqueous solution but will sit on the bottom as a little "bubble". The chloroform is a toxin that keeps mold and bacteria from

growing in your standard, and it will slowly evaporate over time. Check occasionally and replenish the chloroform if you can no longer see a small "bubble" of chloroform in the bottom, stopper and invert the flask several times to mix the solution.

v. This solution is stable for approximately four months as long as it remains clear.

e. Ascorbic acid

i. The ascorbic acid solution should be prepared on the day of analysis.

ii. Fill a clean 100 mL volumetric flask 2/3 full of deionized water, add 5.4 g ascorbic acid stir and fill to 100 mL with deionized water. Seal the top and label.

iii. This solution is not stable for more than 12 hrs.

7. Sampling

a. Collection of water, from the Niskin bottle or bucket sample is done after the dissolved oxygen sample has been collected.

b. The spigot is opened and water from the Niskin bottle tube is directed into a clean Filter cup, which is attached to the hand vacuum pump. The funnel stem and rubber stopper are placed snugly into the mouth of the sample bottle and vacuum is applied. Three sets of samples are collected from the water that passes through the filter. If the sample is being collected from a bucket, carefully pour the water into the Filter-cup. Rinse the sample bottle twice with a small amount of filtered sample water.

c. After rinsing, fill the bottle to just below the shoulder. Care must be taken to avoid overfilling of samples to be frozen. These bottles are transferred to a freezer (-20°C) and kept frozen until analysis. Record all sample information on data sheet, and make sure sample bottle is labeled accordingly. Water samples to be tested for phosphate (PO_4) concentration are not stable at room temperature and must be frozen if not analyzed immediately.

8. Phosphate Analysis

a. Prepare the phosphate standard solutions

i. All four standards and the reagent blank should be run each time a new batch of mixed reagent is made.

      ii. Using the volumetric pipet provided, transfer 2 mL of KH_2PO_4 primary standard solution (0.01M) into a clean 100 mL volumetric flask. Bring to 100 mL with Q water.

      iii. From this working solution ($2.0 \times 10^{-4}M$) prepare the following dilutions. Use the adjustable pipettor or volumetric pipets to deliver the standard to the labeled volumetric flasks, then bring to 100 mL with Q water:

4.0 µM: 2 mL of the working solution; bring to 100 mL with Milli-Q water.

2.0 µM: 1 mL of the working solution; bring to 100 mL with Milli-Q water.

1.0 µM: 500 µL of the working solution; bring to 100 mL with Milli-Q water.

0.5 µM: 250 µL of the working solution; bring to 100 mL with Milli-Q water.

   b. Prepare the unknown samples

      i. Remove the samples to be measured from the freezer well enough in advance of analysis so that the sample water may be brought to room temperature. (Note: this will be done for you).

      ii. Using the beakers provided, arrange and label enough beakers for each of your standards, a blank (Milli-Q water) and three replicates of each of the water samples at your station.

      iii. Using the 25mL graduated pipet, deliver 50 mL Milli-Q water into the beaker labeled as your blank.

      iv. Next, deliver 50 mL of sample to each pre labeled beaker. Rinse the pipet between samples (not replicates) with 25mL of the new sample.

      v. Finally, deliver 50 mL of each standard to the proper pre-labeled beaker.
         1. Do this from lowest to highest concentration.
         2. Rinse the pipet between each standard first with 25 mL of deionized water.
         3. Then rinse with 25mL of the next standard. Discard this as waste.
         4. Deliver 50 mL of each standard into its beaker.

   c. Prepare the reagents

    i. The majority of the reagents used in this lab will be made for you. The mixed reagent, however, must be made just prior to measurement.

    ii. Using the clean graduated cylinders provided, add the specified volumes of the reagents to the 300mL Erlenmeyer flask **in the following order**.

Reagent	Volume
Ammonium molybdate	50 mL
Sulfuric acid	125 mL
Ascorbic acid	50 mL
Potassium antimonyl-tartrate	25 mL
Total Volume	250 mL

  d. Treat the samples

    i. Using the 5 mL graduated pipet, add 5.0 mL of mixed reagent to each beaker or flask (including all standards, the blank, and the unknowns). Swirl each sample gently after adding the mixed reagent.

    ii. The samples must be allowed to develop color for at least 20 minutes, and should be run within 2 hours, though the loss over the first 24 hours is relatively minor.

  e. Measure samples on the spectrophotometer (885nm):

9. **Determining PO$_4$ concentration from absorbance**

  a. Using Microsoft Excel, plot the concentration of your standards in μM versus absorbance. This means concentration is your x-value and absorbance is your y-value. Be sure to include your blank value as a zero concentration.

  b. Find the regression line for your standard curve.

    i. On the chart itself (Go to "Add Trendline" under Chart; choose a linear fit with the equation and R^2-value displayed on the chart) display the trendline and hand in a copy with your data.

    ii. We want you to use the *worksheet* alternative to calculate the errors on your slope and intercept. In the worksheet, use the function LINEST. The syntax of this function is =LINEST(y-range, x-range, TRUE, TRUE). Hit Ctrl-Shift-Enter after typing this function because it is an "array" function in Excel. You will

get back a set of numbers 2 columns by 3 or 4 rows and the cells correspond to the following parameters:

Slope value	Intercept value
Error on slope	Error on intercept
R^2	Standard error on y values

c. Find the average and standard error of the triplicate absorbance values for each sample.

d. For each sample, find the concentration of phosphate using the inverse of the regression line you determined. Propagate the error on your sample, slope and intercept to estimate the error on your final concentration.

e. Find the average of your three concentration values for each sample.

f. Identify aspects of the procedure (from collection to measurement) that may have introduced some error into your final results. Suggest ways to minimize these errors.

10. References

Strickland, J.D.H., and Parsons, T.R. (1968). Determination of reactive phosphorus. In: A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Bulletin 167, 49–56.