Experiment 5: Analysis of Nutrients in Natural Waters

CH3600: Environmental Chemistry, Plymouth State University


Introduction:

Four of the most important biological nutrients found in varying amounts in aquatic systems are carbon (various organic and inorganic forms), nitrogen (typically as nitrate, NO$_3^-$), phosphorous (typically as phosphate, PO$_4^{3-}$), and oxygen (dissolved O$_2$ gas). In healthy natural waters, these nutrients are present in reasonable proportions that allow aquatic life to flourish. However, if any one of them is out of proportion, the water may not be suitable for aquatic life. In this lab, you will analyze samples of natural water for all nutrients.

**Carbon** exists in solution in both inorganic (CO$_2$ and CO$_3^{2-}$ carbonates) and organic forms. As far as nutrients and pollutants, the organic form is generally the more important, as an excess of biologically available organic carbon increases Biological Oxygen Demand and leads to hypoxia. Total Organic Carbon is typically measured a TOC instrument, which converts the organic carbon to CO$_2$ via combustion and then quantifies the CO$_2$ using infrared spectroscopy. Unfortunately, our TOC instrument is currently under repair.

**Phosphorous** is a primary nutrient usually found in limiting quantities, and an excess of it leads to eutrophication. It exists in solution as H$_3$PO$_4$, H$_2$PO$_4^-$, HPO$_4^{2-}$, or PO$_4^{3-}$ (collectively, "phosphates") depending on the pH of the water. In this lab, you will quantify the amount of phosphates by reaction with an ammonium-molybdate complex, which simultaneously converts all the species to PO$_4^{3-}$ and produces a deep blue color ("Molybdenum blue"), which can be quantified by UV spectroscopy.

**Nitrogen** exists in aqueous systems in either the oxidized form (nitrate [NO$_3^-$] / nitrite [NO$_2^-$]) or the reduced form (ammonia [NH$_3$]). Both nitrite and ammonia are toxic at relatively low levels, and although nitrate itself is not nearly as toxic, it is converted to nitrite in the gastrointestinal tract. In addition, excess available nitrogen in aquatic systems leads to eutrophication. In oxygenated natural waters, nitrate is the major form of nitrogen. Nitrate is analyzed by reaction with N-(1-Naphthyl)ethylenediamine dihydrochloride, which produces a deep red color that can be quantified by UV spectroscopy. In this lab, you will use a Hach kit to run this reaction and quantify the result. Ammonia may be measured using an ion-selective probe that measures a voltage in response to the concentration. Unknown solutions are compared to prepared standards via a standard curve. Unfortunately, our ammonia probe is notoriously finicky, especially at the low concentrations expected for this lab. Instead, we will use a nitrate-selective probe that will allow comparison of the values determined with the Hach kit.
Dissolved oxygen (DO) is present in water that is in contact with air, according to the equilibrium:

$$O_2 (g) \rightleftharpoons O_2 (aq)$$

Dissolved oxygen is only a problem when it is not present in large enough concentrations—a condition known as hypoxia. In this condition, fish and other aerobic species die, and anaerobic bacteria take over. Stagnant waters, such as swamps, are frequently naturally hypoxic, but hypoxia in lakes and streams is a sign of pollution. In this lab, you will measure dissolved oxygen using a DO probe that relies on the following electrochemical reaction:

$$2Ag (s) + 2Cl^- (aq) + \frac{1}{2} O_2 (aq) + H_2O \rightarrow 2AgCl (s) + 2OH^- (aq)$$

Safety Considerations: Read through the procedures and note any safety considerations.

Procedure

Note: Turn on the Spec 20's at the beginning of class to warm them up.

Total Organic Carbon

1. Sadly, our instrument is under repair, so this analysis will not be done.

Determination of Dissolved Oxygen

1. Pour about 50 mL of water into a beaker. Pour the water gently so as to not aerate it, which will cause more $O_2$ to be absorbed from the air and give you an artificially high DO reading (note: typically DO measurements should be done in the field at the time of sampling, as the amount of dissolved oxygen will change over time).
2. Insert the DO probe into the water. Stir it gently, then wait briefly until the reading stabilizes (about 1 min).

Determination of Nitrate (via Hach kit)

1. Rinse two color viewing tube several times with the water to be tested. Fill both the 5 mL mark with the water to be tested.
2. Place one tube in the left hand side of the color comparator. This is the Blank.
3. Using scissors, open one of the NitraVer5 Nitrate Reagent Powder Pillow and add the contents to the second color viewing tube. This is the Sample.
4. Stopper the Sample tube. Shake vigorously for exactly one minute. Note any color change.
5. Place the Sample tube in the right hand side of the color comparator.
6. Allow the Sample to sit undisturbed for one minute.
7. Rotate the disk in the color comparator until the color on the disk matches the color of the water sample.
8. Record the concentration (mg/L of nitrogen) shown on the scale window.
**Determination of Nitrate (via ion selective electrode)**

1. Make the Standards:
   a) A Nitrate Stock Solution containing ~100 mg of nitrogen per liter (mg N/L) will be provided. Make note of the exact concentration of this solution.
   b) Each group will be assigned one or two standards to be made. Make the standard by transferring the given volumes of Stock Solution and Ionic Strength Solution into the proper volumetric flask and diluting to the mark. Transfer 0.5-1.0 mL using an autopipette and for 5-10 mL using a volumetric pipette.

<table>
<thead>
<tr>
<th>Conc of Standard (mg N/L)</th>
<th>Dilution Factor</th>
<th>Vol. of Stock Solution (mL)</th>
<th>Volumetric Flask (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>5.0</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>5.0</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10.0</td>
<td>100</td>
</tr>
</tbody>
</table>

2. Measure concentrations
   a) If you are the first group to do this measurement: For each standard, use a graduated cylinder to measure and pour 50.0 mL into a clean, dry, labeled 100 mL beaker. Add 1.0 mL of Ionic Strength Solution. Immerse the nitrate ion-selective electrode and a reference electrode in the solution. Stir gently for a few seconds, then stop and wait 10-20 seconds for the instrument to stabilize. Record the voltage to the nearest millivolt. *Dilute solutions may never completely stabilize. Take the reading when it stays on roughly the same number for a few seconds.* Leave the solutions in clearly labeled beakers for other groups to use.
   b) For your water sample, follow the same procedure outlined in 2a for the Standards.

**Determination of Phosphate (via spectroscopy)**

1. Place 25 mL of the water sample to be analyzed in an Erlenmeyer flask.
2. Add one drop of phenolphthalein indicator. If the solution turns pink, add 6M HCl one drop at a time until the solution is clear.
3. Add 1.0 mL of ammonium molybdate solution to the flask. Swirl to mix thoroughly.
4. Add 2 drops of the tin chloride solution to the flask. Swirl to mix thoroughly. Note the color and set aside.
5. Prepare the spectrophotometer (*it should have been warmed up for at least 10 minutes*):
   a) With no sample or test tube in the machine, zero the reading with the left dial
   b) Set the wavelength on the spectrophotometer to 650 nm.
   c) Fill the test tube with distilled water. This will serve as your “blank”. Wipe the outside of the test tube with a Kimwipe to remove excess dust and fingerprints and place the tube in the spectrophotometer.
   d) Using the right dial, set the absorbance to zero for the blank.
6. Prepare a standard curve using the phosphate solutions provided:
   a) Four cuvettes containing 1, 2, 5, and 10 ppm of KH$_2$PO$_4$ standard solution will be provided (note: more exact values of standards will be provided at the time of lab. Be sure to record these and use all significant figures given in your analysis).
   b) For each Standard: Wipe the cuvette, insert into the spectrophotometer, and record the absorbance value in your laboratory notebook. Return the cuvette to the rack in the appropriate spot.

7. Analyze your prepared water sample(s). (Note: the water sample must be allowed to sit for 5-10 minutes after the addition of the ammonium molybdate to allow the color to fully develop.)
   a) Fill the cuvette with the treated water sample to be analyzed. Wipe the cuvette with a Kimwipe, insert into the spectrophotometer, and record the absorbance value in your laboratory notebook.
   b) Remove the cuvette from the spectrophotometer. Discard the solution IN THE APPROPRIATE WASTE CONTAINER. Rinse with distilled water several times.

Analysis: Lab notebook

The following should be done in your notebook. You may choose to do this after the lab, but your lab will not be signed off, and your duplicate pages will not be accepted, until it is completed. This analysis should also appear in your formal lab report.

1. Nitrate via Ion-selective Electrode: Calculate the exact concentration of your standards by dividing the given concentration of the Stock Solution by the Dilution Factor from the table in Step 2. Using a spreadsheet or a graphing calculator, prepare a graph of Potential (mV) versus Log [N] (where [N] is the concentration of nitrogen in mg/L of the standards you just calculated). Find the slope, intercept, and R-squared for the standard curve. Record these values in your notebook. Calculate the concentration of nitrate nitrogen in your sample.

2. Phosphate: Using the values your recorded, prepare a standard curve. Find the slope, intercept, and R-squared for the standard curve. Record these values in your notebook. Calculate the concentration of phosphate in your water sample.

3. Prepare a table with the values for Dissolved Oxygen, Nitrate Nitrogen via Hach kit, Nitrate Nitrogen via Ion-selective Electrode, and Phosphate

Analysis and Conclusions: Report

Answers to these questions must be included as part of your Conclusions in your written lab report. Include them in your Conclusions narrative, not as numbered list of questions and answers.

1. Include a copy of the standard curves for Nitrate via Ion-selective Electrode, including the formulas of the lines and the R$^2$ values. Comment on these curves. By visual inspection and by reference to the R$^2$ values, determine whether they are linear over the range of standards.

2. Compare the two methods used to determine nitrate nitrogen. Do you trust one more than the other? What are the relative merits of each method.

3. Do a little research via the Internet to find typical ranges in natural waters for the nutrients you measured. Present these values in table form in your report.

4. Comment on the overall quality of your water sample. Were any of your measurements particularly notable for being outside of the normal ranges for natural waters?