Lab 2: NITRATE ANALYSIS

Introduction:

Today we're going to measure nitrate concentrations in your Boston Harbor / Mass Bay samples. The two most common forms of inorganic nitrogen in aquatic systems are ammonium (NH_4^+) and nitrate (NO_3^-). Nitrate is the most oxidized form of nitrogen in aquatic systems and it cannot be used by organisms unless it is first reduced to ammonium. This reduction often occurs within the organism itself and the nitrogen is assimilated into organic nitrogen or excreted as ammonium. In riverine and coastal systems, nitrate is derived from terrestrial debris and agricultural fertilizers. In seawater, nitrate is usually higher than ammonium, but is still often low due to biological uptake by phytoplankton.

Method:

Nitrate cannot be detected in the water column in its natural state, but instead needs to be reduced to nitrite (NO_2^-) and then chemically altered to a photo-active complex. There are a number of methods for nitrate analysis, ranging from manual methods such as the method we will perform today to automatic methods such as a nitrate electrode. The choice of method depends on the anticipated nitrate concentrations and the funds available for analysis. Nitrate electrodes are commonly used for high concentrations, while manual methods are more sensitive and thus used for samples with low nitrate.

Chemistry: First, the nitrate (NO_3^-) in our samples will be quantitatively reduced over Cd granules (treated with $CuSO_4$) and converted to nitrite (NO_2^-). The nitrite will then be converted to an azo compound (N=N) with sulfanilamide and then complexed to N-(1-naphthyl)-ethylenediamine dihydrochloride. The resulting azo dye is a brilliant reddish-purple color and its concentration is measured spectrophotometrically. In this method, you assume that all nitrate is converted quantitatively to the azo-complex and that the concentration of complex is directly proportional to the original nitrate concentration.

Sample acquisition:

Water samples must be acquired and filtered through a 0.2µm filter (or a 0.45µm filter) to remove all biological organisms. Samples must then be frozen at –4°C as soon as possible for storage until analysis.

Sample analysis:

Reduction reagent: You will be given Cd granules that need to be treated with 2% $CuSO_4$. Put approximately 1-2 cm of Cd granules into the 250mL beaker provided. Add $CuSO_4$ solution and swirl until you see some red precipitates forming. Put the $CuSO_4$ solution into the waste container and repeat. Rinse the granules once with Milli-Q water; place the waste rinse into the waste container. Add enough Milli-Q water to your beaker to cover the Cd/Cu granules. Once you have activated your granules, please be careful not to let these granules dry out. Always keep a layer of water above the granules in your beaker and your column.

Reagents:

(A)   Color reagent: 800mL water + 100mL 85% phosphoric acid + 10g sulfanilamide; when sulfanilamide is dissolved completely, add 1g *N*-(1-naphthyl)-ethylenediamine dihydrochloride; bring to 1L with Milli-Q water - *stable for 1 month in amber bottle in refrigerator*

(B)   Stock NH_4Cl-EDTA solution: 39g NH_4Cl + 5.1g Na_2EDTA in 900mL Milli-Q water; adjust pH to 8.5 with concentrated NH_4OH; bring to 1L with Milli-Q water – *stable indefinitely*

(C)   Dilute NH_4Cl-EDTA solution: 40mL Reagent B to 200mL with Milli-Q water – *stable indefinitely*

Stock nitrate solution:

   1mM KNO_3

1. You must make 4 standard solutions with the following concentrations: 10µM, 20µM, 40µM, and 60µM. Using the formula, $M_1V_1 = M_2V_2$, calculate the volume of stock solution that you will need to make 100mL of each standard. Fill in the third line of the table below before coming to class:

Standard NH_4^+ solution	1	2	3	4
Target concentration (µM)	10	20	40	60
Volume stock needed (mL)				
Actual volume stock added (mL)				
Standard concentration (µM)				

2. Using the pipets provided, add the appropriate volume of stock solution to a 100mL volumetric flask for each standard. Bring to volume with Milli-Q water.
3. 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.
4. Make the dilute NH_4-EDTA solution (Reagent C) in the 500mL volumetric flask. You will need to make a number of batches of this reagent over the course of the experiment.
5. Make the Cd-reduction column at your station.
   a. First, put a plug of glass wool in the base of the column.
   b. Add the Cd-Cu granules to the column. Use your squirt bottles and spatulas to coax the granules into the column. Be sure to keep water above the Cd-Cu granules at all times so that the column doesn't dry out. Drain excess water into a waste beaker whenever needed.
   c. Make 200mL of a "wash" solution – 150mL Reagent C + 40mL Milli-Q water + 10mL stock NO_3^-. Add to the column. Drain until the water level is approximately 1 cm above the granules.
   d. Wash the column a second time but with 200mL dilute NH_4-EDTA solution (Reagent C).

    e. During the second rinse, adjust the flow rate of your column so that you are collecting between 10 and 15 mL / min. Ideally, it should take you 8 min to collect 100mL (the flow rate here is about 13mL/min).

NOTE #1: The reduction of NO_3^- to NO_2^- is time-sensitive. After you reduce each sample, quickly add the color reagent so that you capture as much nitrite as you can. You will reduce all your samples, blanks and standards. Do this in the following order: blank, samples, standards.

6. For the blank:
    a. Using a 100mL graduated cylinder, combine 25 mL Milli-Q water and 75 mL Reagent C. Reserve this graduated cylinder for use with your standards in step 7.
    b. Transfer into the appropriately marked beaker.
    c. Pour the mixture onto the column. You should have ample room for the entire sample within the bulb on top.
    d. Drain your sample through the column. Discard the first 30mL. Collect the remaining sample in the original beaker.
    e. Pour 50mL of sample into a mixing cylinder.
    f. Add 2mL color reagent (A). Mix well.
    g. Let the complex develop for 10min before spectrophotometric analysis.

NOTE #2: To maximize your efficiency, you should add the next sample for NO_3^- analysis as soon as the previous sample has drained through the column. Add the next sample when the level of the previous sample reaches 0.5 cm above the Cd-Cu granules. Do not worry about mixing the new sample with the previous one – this is why you discard the first 30mL.

7. For your samples:
    a. Using the other 100mL graduated cylinder, combine 25mL sample and 75mL Reagent C. You will use the same graduated cylinder for all your samples, so rinse with a little sample when you start a new one. You only have approx. 15mL of sample to use as a rinse so please be thrifty!
    b. Transfer the sample mixture into the appropriately marked beaker.
    c. Reduce the NO_3^- in the same manner as the blank (steps 5c and onward).
8. For your standards:
    a. Using the 100mL graduated cylinder, combine 25mL of your standard with 75mL Reagent C.
    b. Transfer to the appropriate beaker.
    c. Reduce the NO_3^- in the same manner as the blank (steps 5c and onward).

NOTE #3: We may not have enough mixing cylinders for each standard and sample replicate. You will have enough to do a large fraction of your samples. However, when you run out, rinse the cylinder three times with Milli-Q water and shake to remove any excess water. You can then use the mixing cylinder for your next sample(s).

9. Wait 10 min and transfer your sample to the tubes provided for the spectrophotometer. We will be analyzing our samples in Karen Casciotti's lab (basement of McLean building). Each sample will be loaded into the instrument by an auto-sampler so label your tubes in a way that you know what is contained in each tube.

Data analysis:
1. Using the spectrophotometer, you measured the absorbance of light (at 543nm) by the complex in your samples.
2. Using Microsoft Excel, plot concentration 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. Hand in a copy of your standard curve. Include the regression line and R^2 value.
3. Find the average and standard error of the triplicate absorbance values for each sample.
4. For each sample, find the concentration of nitrate 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.
5. 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.

Reference
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.