Determination of Nitrate (NO_3^-)

Background

The nitrate ion absorbs ultraviolet radiation at 220 nm but not at 275 nm. The nitrate absorption at 220 nm follows Beer's law with linear behavior to 11 mg nitrogen/L solution. Because dissolved organic matter may also absorb at 220nm, one must be able to distinguish between the absorbance of nitrate and the other organic matter in water. This is accomplished by making a measurement of the water sample's absorbance at 275 nm, a wavelength that the organic matter can absorb electromagnetic radiation but in which the nitrate cannot. Once known, an empirical correction factor can be applied to the 220 nm measurement to distinguish the nitrate from the organic matter. Because this is an empirical correction, this method is not recommended for waters requiring a significant correction for organic matter. It is main use is as a screening tool for water samples in which the organic matter is relatively constant. If it is desired to create a more accurate method, another approach to the calibration process can be performed. This approach, known as the method of standard additions, is beyond the scope of the intent of this lab, though your instructor can steer you to reading information to more fully describe this approach.

Sample preparation consists of two steps. First, the sample will be filtered, to prevent scattering of UV light by suspended particles in the water sample, which will change the measured absorbance. The samples will next be acidified using 1 N HCl to prevent interferences due to the absorption of either OH^- or CO_3^{2-}, both of which can absorb at 220 nm. The acidification should prevent interferences from these ions up to 1000 mg $CaCO_3$/L. Hydrochloric acid is used because Cl^- does not absorb light in 250 – 290 nm region of the spectrum. Some other ions, such as Cr^{6+} (as either chromate or dichromate ion), nitrite, chlorate and chlorite, may also interfere, but these are expected to be low or nearly non-existent in the water samples used in our analysis.

Procedure

All solution must be prepared using the nitrate-free water available in the laboratory. Do <u>not</u> use tap water or regular distilled water. Exercise care when using the nitrate-free water and do not waste it.

In a volumetric flask prepare 1.00 L of a 100 µg N/mL stock solution from solid KNO_3 which has been dried in an oven at 105 ºC for 24 hours. Note that the dried KNO_3 will be provided to you. You will need to determine the mass of KNO_3 that you actually used to the nearest milligram and calculate your exact concentration from this.

From the nitrate stock solution prepare 500.0 mL of a nitrate solution that contains 10 µg N/mL. Note that your actual value may be slightly different depending on the actual amount of KNO_3 you used to prepare the first solution.

Prepare NO_3^- calibration standards in the range of 0 to 7 µg N/mL (this corresponds to the addition of between 0 and 350 µg N per 50 mL of solution). Use 50.0 mL volumetric flasks and pipets to create these solutions. Volumes of diluted standard nitrate solution ranging between 0 and 35 mL will be sufficient to cover this range of concentrations. To be useful, the calibration curve should be created at least five different standard solutions (plus a "blank") that "bracket" the expected concentration of nitrate. Be sure to dilute carefully and mix thoroughly before using.

The water sample is prepared by first gravity filtering through acid-washed, ashless hard finish, filter paper to filter fine precipitates to remove large particles and debris. The filtered solution will then be filtered through a 0.45 μm pore diameter membrane filter using a syringe to force the water through the filter. Filter at least 75 mL of sample through the filter paper and then filter only as much sample as needed through the membrane filter. To each 50 mL of clear sample, add 1 mL of the 1 M HCl solution and mix thoroughly.

Use the Ocean Optics spectrometer and cells provided to measure the absorbance of each solution (the blank, the calibration standards and the water sample) at 220 nm and at 275 nm. You will be using a special sample cell for this experiment (either quartz or a special plastic), because ordinary glass and plastic do not transmit light in the ultraviolet region of the spectrum. Handle these cells very carefully, as they are extremely expensive and fragile. Also, be sure to keep fingerprints off of the surfaces that light passes through, as the acids in your fingerprints can be permanently etched into the surface of the expensive cuvette when exposed to UV light. If this happens, the cuvettes are ruined. Please see ChemLab.truman.edu to review proper spectrophotometer operation. Note if your absorbance readings are much larger than 1, or if your water sample's absorbance is much higher than your highest standard solution, you will need to dilute your samples accordingly.

Results

Subtract 2 times the absorbance reading of the water sample at 275 nm from the value of absorbance for that sample at 220 nm. This operation will give a value for the experimental absorbance due to NO_3^-.

From the corrected absorbances of the standard solutions create a calibration curve of absorbance as a function of the concentration of NO_3^- (in mg N/L) that includes best fit line though the data. Include the correlation coefficient for this fit.

Using the measured absorbances of your water samples, determine the concentration of nitrogen in each your water samples in μg N/mL solution. Convert this value to the concentration of NO_3^- in μg/mL solution. Don't forget that in the determination of any real samples, you should also report the standard deviation and confidence limits for your results.

What are the predominant sources of nitrate in natural waters? How do your results compare with what is expected of natural water sources in the Midwest? If elevated, what ways might the nitrate be remediated?

Determination of Dissolved Oxygen in Water

Background

Oxygen is poorly soluble in water. Where creatures that live in the atmosphere have about 19% oxygen available for consumption, organisms living in water have a maximum of about 0.15 % oxygen. Moreover, as temperature or as salt content in the water increases, the dissolved oxygen (DO) concentration decreases. DO in pure water (no salt content) ranges from 7.6 mg/L at 30°C to 14.6 mg/L at 0°C. Many organisms, such as the mountain trout, have specific oxygen needs, so that they can only live in a small range of these variable O_2 concentrations. The mountain trout is only found in icy cold water with the highest concentration of DO.

DO concentrations can be determined by the Winkler, or iodometric, method. This method involves a series of oxidation-reduction reactions starting with the oxidation of Mn^{2+} (added to the sample in the form of $MnSO_4$) by O_2 in basic solution (by addition of an alkaline potassium iodide solution, KI in NaOH) to give solid MnO_2, as shown in reaction (**1**).

$$2Mn^{2+}\,(aq) + 4\,OH^{-}\,(aq) + O_2\,(aq) \rightarrow 2MnO_2(s) + 2\,H_2O\,(l) \qquad (\mathbf{1})$$

The formation of MnO_2 stabilizes essentially locks up the dissolved O_2 in a form that allows for short term storage of the sample before it is analyzed.

To start the actual analysis of oxygen, H_2SO_4 and more KI are added to the solution containing the MnO_2 precipitate. At low pH, the MnO_2 oxidizes I^- completely into I_2, as shown in reaction (**2**).

$$MnO_2\,(s) + 2\,I^{-}\,(aq) + 4\,H^{+}\,(aq) \rightarrow Mn^{2+}\,(aq) + I_2\,(aq) + 2\,H_2O\,(l) \qquad (\mathbf{2})$$

Because both reactions (**1**) and (**2**) go to completion, the I_2 formed is directly related to the amount of DO in the sample.

The I_2 produced in reaction (**2**) is determined by titration with standardized sodium thiosulfate ($Na_2S_2O_3$), via another oxidation-reduction reaction in which thiosulfate reduces the I_2 back into I^-. This reaction is illustrated in reaction (**3**) below.

The endpoint of the reaction is detected using a starch indicator. When I_2 is present, the indicator has a deep blue color (due to a complexation of iodine by the starch), but when no I_2 is present in solution, the solution is colorless. So, once the equivalence point is reached, there will be no I_2 remaining in solution and the dark blue color that was initially present in the solution will disappear.

$$2\,S_2O_3^{2-}\,(aq) + I_2\,(aq) \rightarrow S_4O_6^{2-}\,(aq) + 2\,I^{-}\,(aq) \qquad (\mathbf{3})$$

From the known $S_2O_3^{2-}$ concentration and the volume of the $S_2O_3^{2-}$ solution used to reach the equivalence point, we can ultimately determine the number of moles of O_2 that were originally present in your sample of water. Since the volume of the original sample is known, we can calculate the concentration of O_2 in mg/L, which is what will be reported along with the temperature of the water when it was sampled.

To prevent nitrite interference (a real possibility in fresh water samples) sodium azide (NaN_3) is added with the alkali-KI reagent to the water sample as it is taken from the source. The addition of sulfuric acid then results in the removal of NO_2^- through the following series of reactions, (**4**) and (**5**) below.

$$NaN_3\,(aq) + H^+\,(aq) \rightarrow HN_3\,(aq) + Na^+\,(aq) \qquad (\mathbf{4})$$

$$HN_3\,(aq) + NO_2^-\,(aq) + H^+\,(aq) \rightarrow N_2\,(g) + N_2O\,(g) + H_2O\,(l) \qquad (\mathbf{5})$$

Sample Collection

The following steps are for collecting the samples. As you will not be collecting the samples yourself, these are included here for your information only.

Prepare a manganese(II) sulfate solution by dissolving 480 g of $MnSO_4 \cdot 4H_2O$ (or 400 g of $MnSO_4 \cdot 2H_2O$, or 364 g of $MnSO_4 \cdot H_2O$) in about 800 mL of deionized water. Filter the solution and dilute to 1.0 L. Prepare the alkali-iodide-azide reagent by dissolve 500 g of NaOH (or 700 g KOH) and 135 g of NaI (or 150 g KI) in deionized water and dilute to 1.0 L. Add 10 g of NaN_3 dissolved in 40 mL of deionized water.

Samples should be collected as close to analysis as possible. Collected samples should be stored with little or no head space, on ice and in the dark and care should be taken to avoid shaking samples.

To a 250-300 mL plastic sample bottle almost filled with the water sample, add 1 mL of $MnSO_4$ solution, followed by 1 mL of alkali-iodide-azide reagent (this is best done with graduated two graduated cylinders). If the solution turns white, no DO is present.

Gently squeeze the sample bottle to raise the sample's level in the bottle to its lip. Seal the sample bottle so that no air bubbles are present and mix by inverting the bottle rapidly a few times. When the precipitate has settled to half the bottle volume, repeat the mixing and allow the precipitate to resettle.

Procedure

All solutions can be disposed of down the drain.

Prepare the sodium thiosulfate titrant by dissolving 6.205 g of $Na_2S_2O_3 \cdot 5H_2O$ in deionized water. To this solution add 0.4 g of solid NaOH. Once the NaOH has dissolved dilute to 1.0 L. This solution is approximately 0.0250 M, but because the solid can be oxidized by oxygen in air during storage, we will need to standardize it with biiodate.

Dissolve 0.81 g of $KH(IO_3)_2$ in deionized water (measured to the nearest milligram) and dilute to 1.000 L. This solution should be 0.00210 M in $KH(IO_3)_2$ and will be our standard biiodate solution.

Approximately 2 g of KI (measure to the nearest 0.001 g) is dissolved in 100 – 150 mL deionized water in an Erlenmeyer flask and 1 mL of 6 M H_2SO_4 is added to the resulting solution. Pipet 20.00 mL of the standard biiodate solution into the flask; I_2 will be formed from the reaction.

Titrate the liberated I_2 with the thiosulfate titrant until a pale straw (yellow) color is reached. Add a few drops of starch indicator (already prepared for you by dissolving 2 g of lab-grade soluble starch and 0.2 g of salicylic acid as a preservative in 100 mL of hot distilled water and cooled before use). The solution should turn blue. Continue the adding titrant until the blue color is gone, which is the endpoint. If the solutions were made correctly with pure reagents, 2 g of KI and 20.00 mL of $KH(IO_3)_2$ should require 20.00 mL of the $Na_2S_2O_3$ titrant to reach the endpoint. Determine the concentration of thiosulfate and repeat the titration until three concentrations agree within about 3% of each other.

Once the $Na_2S_2O_3$ has been standardized, open the sealed sample bottle and add 1 mL of concentrated H_2SO_4. Reseal the bottle and mix by inverting the bottle rapidly and dissolve the precipitate. You may open the bottle and pour the sample at this point since the DO and reagents have been "fixed" and will not react further.

Titrate 200 mL of the water sample with your standard thiosulfate solution as in the standardization (first titrate to a pale straw color, add starch indicator, and titrate to the clear endpoint). Take care with these titrations! Only enough samples will be collected for each group to do four titrations. Once your allotment of samples is gone, there will be no more available!

Determine the DO concentration in the water samples and repeat the titrations at least twice more or until three concentrations agree within about 3%.

Results

Calculate the mean, standard deviation and confidence limit of the results of your titration. The concentration of O_2 should be reported in mg O_2 per L solution. Compare your results with that expected for standard fresh water at the temperature in which your sample was taken (this can be calculated from Henry's Law or looked up in reference books).

Determination of Ions Using an Ion Selective Electrode

Introduction

An ion-selective electrode (ISE) incorporates a special, ion-sensitive membrane, which may be glass, a crystalline inorganic material or an organic ion-exchanger. The membrane interacts specifically with the ion of choice, allowing the electrical potential of the half cell to be controlled predominantly by the ion's concentration. ISEs are available for measuring more than 20 different cations (e.g., Ag^+, Na^+, K^+, Ca^{2+}, Cu^{2+}) and anions (e.g., F^-, Cl^-, S^{2-}, CN^-).

The potential of the ISE is measured against a suitable reference electrode (another half cell which gives a complete electrochemical cell) using an electrometer or pH meter. The electrode potential is related to the logarithm of the concentration of the measured ion by the form of the Nernst equation given as Eqn. 1. In Eqn. 1 *n* is the ion charge (negative for anions), [*X*] is the concentration of the species of interest, and the factor 2.303 RT/F has a theoretical value of 59 mV at 25 °C.

$$E = E^0 + 2.303\frac{RT}{nF}\log[X] \qquad (\mathbf{1})$$

The Nernst equation is only valid for very dilute solutions or for solutions where the ionic strength, *I*, is constant. Ionic strength is defined by Eqn. 2, where Z_i is the charge on an ion and C_i is its concentration. In this analysis the ionic strength will be maintained by adding another salt to the solution in excess. This supporting electrolyte will be NaCl for a fluoride analysis and $NaNO_3$ for a chloride analysis.

$$I = \frac{1}{2}\sum Z_i^2 C_i \qquad (\mathbf{2})$$

Procedure

The following procedure assumes that you are doing a fluoride analysis. If you are performing a chloride analysis, substitute NaCl for NaF and use $NaNO_3$, instead of NaCl, as the supporting electrolyte.

Dry the NaF solid for 1 hour at 100 °C (already done for you) to remove any water that has adhered to the solid's surface.

Accurately prepare 100 mL of a 0.1 NaF solution by weighing out about 0.42 g of NaF into a 100-mL volumetric flask. Dissolve the salt in deionized water, dilute to the mark and mix well. This is your fluoride stock solution.

Prepare 100 mL of a 0.01 M NaF solution by dilution of the fluoride stock solution using volumetric glassware.

Weigh out 7.55 g of KCl on a top-loading balance and dissolve in 100 mL of deionized water. This solution is 1 M in KCl.

Prepare standard solutions in four 100-mL volumetric flasks as follows:

Flask Number	Volume of 0.01 M NaF (mL)	Volume of 1 M KCl (mL)
I	1.00	10.00
II	2.00	10.00
III	5.00	10.00
IV	10.00	10.00

Dilute each flask to volume with deionized water and mix well.

Add 1.00 mL of your unknown, then 10.00 mL of KCl to a 100 mL volumetric flask. Dilute to the 100 mL mark with deionized water.

Pour about 30 mL of either a standard or unknown solution into a clean, dry 100 mL beaker and immerse the electrodes in the solution to a depth of not more than 2 cm. Measure the electrode potential (in mV) with the voltmeter. **CAUTION!** Do not touch the ISE membrane. Rinse it with deionized water between measurements and then with a small volume of the new solution. Do not wipe it dry.

When you finish, rinse the electrodes with deionized water. Leave the reference electrode in the appropriate storage solution. The F^- ISE should be stored dry and loosely capped. DO NOT force the cap onto the electrode tip!

Results

Prepare a calibration curve in Excel relating log[NaF] in the standard solutions to the measured potential of each solution in mV. Determine the best line through the four standard solution experimental points. The slope of the calibration curve should be close to -59 mV.

Utilizing the calibration curve, determine the concentration of NaF in your original unknown solution.