Lab 1:

DETERMINATION OF DISSOLVED OXYGEN BY WINKLER TITRATION

1. Background

Knowledge of the dissolved oxygen (O_2) concentration in seawater is often necessary in environmental and marine science. It may be used by physical oceanographers to study water masses in the ocean. It provides the marine biologist with a means of measuring primary production - particularly in laboratory cultures. For the marine chemist, it provides a measure of the redox potential of the water column.

The concentration of dissolved oxygen can be readily, and accurately, measured by the method originally developed by Winkler in 1888 (*Ber. Deutsch Chem. Gos.*, **21**, 2843). Dissolved oxygen can also be determined with precision using oxygen sensitive electrodes; such electrodes require frequent standardization with waters containing known concentrations of oxygen. They are particularly useful in polluted waters where oxygen concentrations may be quite high. In addition, their sensitivity can be exploited in environments with rapidly-changing oxygen concentrations. However, electrodes are less reliable when oxygen concentrations are very low. For these reasons, the Winkler titration is often employed for accurate determination of oxygen concentrations in aqueous samples.

2. Scope and field of application

This procedure describes a method for the determination of dissolved oxygen in aqueous samples, expressed as mL O_2 (L water) $^{-1}$. The method is suitable for the assay of oceanic levels of oxygen in uncontaminated seawater and is based on the Carpenter (1965) modification of the traditional Winkler titration.
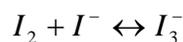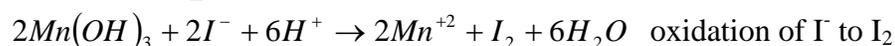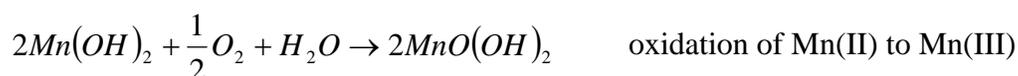
3. Definition

The dissolved oxygen concentration of seawater is defined as the number of milliliters of dioxygen gas (O_2) per liter of seawater (mL L $^{-1}$).

4. Principle of Analysis

The chemical determination of oxygen concentrations in seawater is based on the method first proposed by Winkler (1888) and modified by Strickland and Parsons (1968). Oxygen in the water sample oxidizes iodide ion (I^-) to iodine (I_2) quantitatively. The amount of iodine generated is then determined by titration with a standard thiosulfate ($S_2O_3^{-2}$) solution. The endpoint is determined by using starch as a visual indicator. The amount of oxygen can then be computed from the titer: one mole of O_2 reacts with four moles of thiosulfate.

At the time of sampling, dissolved oxygen is fixed by the addition of Mn(II) under basic conditions, resulting in a brown precipitate, manganic hydroxide ($MnO(OH)_2$). Prior to analysis, the sample is acidified to pH 1.0-2.5. This causes the precipitated hydroxides to dissolve, liberating Mn(III) ions. Mn(III) ions oxidize previously added iodide ions to iodine. Iodine forms a complex (I_3^-) with surplus iodide ions. Iodine and the complex exist in equilibrium; thus, I_3^- serves as a reservoir of I_2. The iodine is then titrated with thiosulfate; iodine is reduced to iodide and the thiosulfate is oxidized to tetrathionate. The stoichiometric equations for the reactions described above are:

$$Mn^{+2} + 2OH^- \rightarrow Mn(OH)_2$$

$$2Mn(OH)_2 + \frac{1}{2}O_2 + H_2O \rightarrow 2MnO(OH)_2 \qquad \text{oxidation of Mn(II) to Mn(III)}$$

$$2Mn(OH)_3 + 2I^- + 6H^+ \rightarrow 2Mn^{+2} + I_2 + 6H_2O \quad \text{oxidation of I}^- \text{ to I}_2$$

$$I_2 + I^- \leftrightarrow I_3^-$$

$$I_3^- + 2S_2O_3^{-2} \rightarrow 3I^- + S_4O_6^{-2} \qquad \text{oxidation of S}_2\text{O}_3{}^{-2} \text{ to S}_4\text{O}_6{}^{-2}; \text{ reduction of I}_3^- \text{ to I}^-$$

The thiosulfate solution is not stable and therefore must be standardized with a primary standard, typically potassium iodate (KIO_3). Standardization is based on the co-proportionation reaction of iodide with iodate, thereby forming iodine. As described above, the iodine binds with excess iodide, and the complex is titrated with thiosulfate. One mole of iodate produces three moles iodine, which are consumed by six moles of thiosulfate.

$$IO_3^- + 8I^- + 6H^+ \rightarrow 3I_3^- + 3H_2O$$

$$I_3^- + 2S_2O_3{}^{2-} \rightarrow 3I^- + S_4O_6{}^{2-}$$
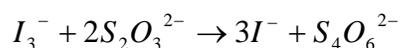
A note to the student:
The entire method is presented below. It is important that you understand the complete method from sample collection to analysis. In addition, you might need/ want to repeat this measurement in the future. All reagents will be made before the lab period. Additionally, the sodium thiosulfate solution will be standardized for you. If time allows, you may get a chance to do this. You will be responsible for measuring your own blank and samples. You are responsible for the procedures described in section 9c and onward.

5. **Sampling apparatus**

   a. Sample flasks: Glass stoppered dissolved oxygen bottles (115 mL nominal capacity – also called "BOD bottles"). 1 clean and dry bottle per sample, blanks and standard.

   b. Volumetric dispensers (or manual volumetric pipettes (disposable, glass)).

   c. Four dispensers capable of accurately dispensing 1 mL aliquots. These should be labeled "Reagent #1", "Reagent #2" and "Reagent #3", and "KIO_3 Blank".

      i. One dispenser capable of accurately dispensing a 10 mL aliquot. This should be labeled "KIO_3 Standard".

      ii. One dispenser capable of accurately dispensing a 50 mL aliquot. This should be labeled "Sample"

      iii. Tygon® tubing: long enough to reach from spigot to the bottom of the sample bottle.

6. **Titration apparatus**

   a. Titration box: a three-sided box containing the titration apparatus. The inside walls should be covered with white lab paper to aid in end point detection.

   b. Magnetic stirrer and stir bars.

   c. 10 mL reservoir-fill buret for thiosulfate titrations.

   d. Clean, dry 125 mL Erlenmeyer flasks (one per titration – 125mL beakers can also be used)

   e. Glass eye dropper bottle for starch indicator.

7. **Reagents**

   a. Reagent #1: Manganese (II) chloride (3M: reagent grade): Dissolve 600 g of $MnCl_2*4H_2O$ in 600 mL distilled water. After complete dissolution, make the solution up to a final volume of 1 liter with distilled water and then filtered into an amber plastic bottle for storage.

   b. Reagent #2: Sodium iodide (4M: reagent grade) and sodium hydroxide (8M: reagent grade): Dissolve 600g NaI in 600 ml of distilled water. If the color of solution becomes yellowish brown, discard and repeat preparation with fresh reagent. While cooling the mixture, add 320g NaOH to the solution, and make up the volume to 1 liter with distilled water. The solution is then filtered and stored in an amber glass bottle.

   c. Reagent #3: Sulfuric Acid (50% v/v): Slowly add 500 mL of reagent grade concentrated H_2SO_4 to 500 mL distilled water. Cool the mixture during addition of acid.

d. Starch indicator solution (manual titration only): Place 1.0g of soluble starch in a 100mL beaker, and add a little distilled water to make a thick paste. Pour this paste into 1000 mL of boiling distilled water and stir for 1 minute. The indicator should be stored in a refrigerator.

e. Sodium Thiosulfate Stock solution (0.18 M: reagent grade): Dissolve 45 g $Na_2S_2O_3*5H_2O$ and 2.5g sodium borate, $Na_2B_4O_7$ (reagent grade) for a preservative, in 1 liter of distilled water. This solution is stored in a refrigerator and used to make the working thiosulfate solution. Some variation on the method call for a 0.1N (Normal) solution, which can be purchased ready-made.

f. Sodium Thiosulfate working solution (0.018 M: reagent grade): Bring 100 mL of the sodium thiosulfate stock solution to 1000 mL with distilled water in a 1 liter volumetric flask. This solution is stored in a refrigerator and used for titrations. If a ready-made 0.1 N solution was used for the stock, a working solution of 0.01 N will be fine.

g. Potassium Iodate Standard (0.00167 M = 0.01 N: analytical grade): Dry the reagent in a desiccator under vacuum. Weigh out exactly 0.3567 g KIO_3 and make up to 1.0 liter with distilled water. It is important to note the temperature of the solution so that a precise molarity can be calculated.

8. Sampling

a. Collection of water, from the Niskin bottle or bucket sample, must be done soon after the sample has been collected, preferably before any other samples have been drawn. This is necessary to minimize exchange of oxygen, which typically results in contamination by atmospheric oxygen.

b. Before each water sample is collected, open the spigot on the Niskin bottle without opening the bleeder valve. If water flows out of the spigot then air must be entering the sampling bottle through a leak and the seawater within the bottle has probably been contaminated with water from shallower depths. Record this onto the data sheet.

c. If no water flows out of the spigot, attach a rubber tube to the spigot and stick the tube all the way into the sample bottle. With the spigot open, slowly open the bleeder valve to allow water to stream out of the spigot. If the sample is being collected from a bucket, carefully immerse the BOD bottle into the water so that water gently fills the bottle without creating bubbles. Rinse the sample bottle twice with the sample water.

d. After rinsing, fill the bottle slowly - always trying to minimize the amount of air bubbles introduced into the water. Adjust the bleeder valve to control the flow. Allow the water to overflow the neck of the bottle. Place the stopper into the bottle. Record all sample information on data sheet, and make sure BOD bottle is labeled accordingly.

e. Immediately after obtaining the water sample, the following reagents are introduced into the filled BOD bottles by submerging the tip of a pipette or automatic dispenser well into the sample: 1 mL of manganous chloride (Reagent

#1), followed by 1 mL of sodium iodide-sodium hydroxide solution (Reagent #2).

9. Titration Procedures

a. First, the precise concentration of thiosulfate in the titrating solution must be determined. Next, a "blank" is analyzed. Impurities in the reagents may participate in the oxidation-reduction reactions involved in the dissolved oxygen analysis and thus must be accounted for. Once the standard titer and blank have been determined, the samples can be titrated with thiosulfate delivered via the buret; the endpoint is determined visually using the starch indicator solution. Below, the procedures for standardization, blank determination, and sample analysis are described. As mentioned above, the sodium thiosulfate solution will be standardized for you. You will make your own blank and sample determinations. If time allows, you may try performing the standardization yourself.

b. Standardization: Label one clean, empty BOD bottle "KIO3 Standard"; add approximately 15 mL of Milli-Q water and a stir bar.

   i. Carefully add 10 mL of standard potassium iodate (0.00167 M = 0.01N) from an "A" grade pipette or equivalent. Swirl to mix. Immediately add 1 mL of the 50% sulfuric acid solution (Reagent #3). Rinse down sides of flask, swirling to mix, thus ensuring an acidic solution before the addition of reagents.

   ii. Add 1 mL of sodium iodide-sodium hydroxide reagent, swirl, then add 1 mL of manganese chloride reagent. Mix thoroughly after each addition. Once solution has been mixed, fill to the neck with deionized water.

   iii. From the "Standard" bottle, fill the 50 mL volumetric pipet with KIO_3 solution.

   iv. Empty the first 50 mL of solution into a waste container as a rinse.

   v. Re-fill the 50 mL pipet with KIO_3 solution and empty into a clean 125-mL Erlenmeyer flask. Add a magnetic stir bar.

   vi. Check the 10 mL buret to ensure that it is full of thiosulfate working solution (0.01N). Place the erlenmeyer flask under the buret and turn on the magnetic stirrer. Keep the speed of the stir bar moderate - do not create a vortex in the solution.

   vii. Slowly add thiosulfate to the solution until the solution turns a pale yellow color. Stop titrating.

   viii. Add three drops starch solution to the flask. Continue to titrate by adding thiosulfate drop by drop just until the solution becomes colorless.

   ix. Record the volume of thiosulfate added. Repeat steps 6 through 9 until you have three readings within 0.05 mL of each other.

c. Blank determination:

  i. Place approximately 15 mL Milli-Q water in a BOD bottle with a stir bar. Add 1 mL of the potassium iodate standard, mix thoroughly, then add 1 mL 50% sulfuric acid, again mixing the solution thoroughly.

  ii. Before beginning the titration, add the following reagents: 1 mL sodium iodide-sodium hydroxide reagent (#1), rinse, mix, then add 1 mL manganese chloride reagent (#2). Fill the BOD bottle to just below the neck with Milli-Q water. Titrate to the endpoint as described for the sample analysis procedure (below).

  iii. Pipet 1 mL of the standard solution into the same flask and again titrate to the end point.

  iv. The difference between the first and second titration is the reagent blank. Either positive or negative blanks are possible.

d. Sample analysis:

  i. Identify the sample you are working on by the number on the bottle and the corresponding data sheet; copy this information into your notebook.

  ii. Immediately prior to analysis, add 1 ml 50% H_2SO_4 (Reagent #3) below the water line in the sample bottle.

  iii. Cover and invert the sample bottle several times to mix the solution. The precipitate should dissolve completely and the solution should turn a deep yellow color. If some precipitate remains add a few more drops of Reagent #3 (this reagent may be added in excess of 1 ml).

  iv. DO NOT ALLOW THE SAMPLE TO SIT FOR ANY LENGTH OF TIME AFTER REAGENT #3 HAS BEEN ADDED - THE SAMPLE MUST BE RUN IMMEDIATELY.

  v. Fill the 50-mL volumetric pipet with sample solution and empty into the waste container (this is your pipet rinse).

  vi. Re-fill the 50-mL pipet with sample and empty into a clean 125-ml Erlenmeyer flask (or beaker) and add a magnetic stir bar.

  vii. Check the 10 mL buret to ensure that it is full of thiosulfate solution. Place the erlenmeyer flask (or beaker) under the buret and turn on the magnetic stirrer. Keep the speed of the stir bar moderate - do not create a vortex in the solution.

  viii. Slowly add thiosulfate to the sample until the solution turns a pale yellow color. Stop titrating.

ix. Add three drops starch solution to the flask. Continue to titrate by adding thiosulfate drop by drop just until the solution turns clear and colorless.

x. Record the volume of thiosulfate added. Repeat steps vi - ix until there are three readings (of thiosulfate added) within 0.05 mL of each other.

10. Calculation and expression of results

The calculation of oxygen concentration (mL O_2 L^{-1}) from this analysis follows in principle the procedure outlined by Carpenter (1965). Watch your units!

$$O_2\left(mL/L\right) = \frac{\left(\left(R - R_{blank}\right) * V_{IO_3} * N_{IO_3} * E\right)}{\left(R_{std} - R_{blank}\right) * \left(V_{bottle} - V_{rgts}\right)} - DO_{rgts}$$

where:

R = Volume of thiosulfate used to titrate the sample (mL)
R_{std} = Volume of thiosulfate used to titrate the KIO_3 standard (mL)
R_{blank} = Volume of thiosulfate used to titrate the blank as measured above (mL)
N_{IO3} = Normality of standard KIO_3 (equiv/L) – *use 0.01 N*
V_{IO3} = Volume of KIO_3 standard (mL) – *use 10 mL*
E = 5598 mL O_2 / equiv
V_{bottle} = Volume of sample bottle (mL) – *use 250 mL*
DO_{rgts} = Oxygen added in reagents – *use 0.0017 mL O_2 /L*
V_{rgts} = Volume of reagents – *use 2 mL*

The additional correction for DO_{rgnts} of 0.0017 mL oxygen added in 1 mL manganese chloride and 1 mL of alkaline iodide has been suggested by Murray, Riley and Wilson (1968).

Homework:

Calculate the average O_2 concentration and the uncertainty of this value for each sample you measured. You should have at least three values for each sample. Computing the average value is straightforward, but how do you estimate the uncertainty (i.e. how well you know that value)? For this exercise, you are concerned only with the precision of your measurements. Later, we will ask you to compare the measurement precision with the uncertainty generated from measurements made on replicate samples (i.e., separate samples collected from the same Niskin bottle).

To calculate the uncertainty of your average value, you will first calculate the standard deviation (σ) of your measurements of the volume of thiosulfate needed (R), using the following formula:

$$\sigma = \sqrt{\frac{1}{N-1}\sum(x_1 - \bar{x})^2}$$

Where :

N = the number of measurements

x_1 = the individual measurement

$\bar{x}$ = the average of your measurements

Calculation of the standard deviation can be performed as follows (you can do this in Microsoft Excel):

After calculating the average value of your three measurements, subtract it from each of the three measurements. Then, square each of these and add them together. Multiply this value by $1/(N-1)$ (in this case N=3), and take the square root. You can check your long hand calculations by using Excel's STDEV function. Please show all your work. ***You only need to show your work for one long-hand calculation of σ. All other calculations can be done in Microsoft Excel using the STDEV function.***

Now, estimate the uncertainty in the determination of the mean (sometimes called the standard error). Divide the standard deviation by the square root of the number of measurements.

Now calculate the dissolved oxygen concentration for each sample, using the average thiosulfate volume for your sample, R, and your blank, R_{blk}. Propagate the error of all three R values to estimate the error on your O_2 concentration. (A sample calculation is provided below for your convenience.)

Sample calculation:

A student titrated three aliquots of a water sample with thiosulfate. The three volumes of thiosulfate were 7.17mL, 7.36mL and 7.22mL. The average value is 7.25mL and the standard deviation is 0.10mL. The standard error is the standard deviation divided by the square root of the number of volume measurements (3) and is equal to 0.06mL.

The blank value measured by this student was 0.52mL. The error on this measurement is the error inherent in the buret (0.05mL) because there was only one measurement.

The dissolved oxygen concentration can be calculated by substituting the values into the equation given above:

$$O_2 = \frac{\left[(7.25\pm0.10mL - 0.52\pm0.05mL)*10mL*0.01\frac{equiv}{L}*5598\frac{mLO_2}{equiv}\right]}{(3.41\pm0.05mL - 0.52\pm0.05mL)*(250mL - 2mL)} - 0.0017mLO_2/L$$

I know you all know how to do the basic arithmetic in this equation, but you may not know how to propagate errors. The following rules apply. For addition and subtraction, the error on the result is the sum of the errors on each component. Thus:

$$(A\pm a)+(B\pm b)=(A+B)\pm(a+b)$$

and

$$(A\pm a)-(B\pm b)=(A-B)\pm(a+b)$$

For multiplication and division, the error on the result is the sum of the *relative* errors on each component. Thus:

$$(A\pm a)*(B\pm b)=(A*B)\pm\left(\frac{a}{A}+\frac{b}{B}\right)$$

and

$$\frac{(A\pm a)}{(B\pm b)}=\left(\frac{A}{B}\right)\pm\left(\frac{a}{A}+\frac{b}{B}\right)$$

So, the O₂ calculation started above can be continued:

$$O_2 = \frac{\left[(6.73\pm0.15mL)*10mL*0.01\frac{equiv}{L}*5598\frac{mLO_2}{equiv}\right]}{(2.89\pm0.10mL)*(248mL)} - 0.0017mLO_2/L$$

Now, multiply the values through, multiplying all the constants by the error as well.

$$O_2 = \frac{(3770\pm84mL^2)}{(717\pm2.5mL^2)}mLO_2/L - 0.0017mLO_2/L = \left[\left(\frac{3770}{717}\right)\pm\left(\frac{84}{3770}+\frac{2.5}{717}\right)mLO_2/L\right] - 0.0017mLO_2/L$$

$$O_2 = 5.26\pm0.03mLO_2/L$$

The appropriate range of O₂ values for freshwater and seawater is 0-10mL O₂ / L.

References

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