

# FISH 503 Advanced Limnology (University of Idaho, Moscow Idaho Campus)

## Oxygen Module Winkler titration lab

**Goal:** To familiarize the participants with the Winkler titration of oxygen determination in water; understand principles of the underlying chemistry; to understand standardization procedures of chemical solutions; perform sodium thiosulfate standardization; perform Winkler titrations; examine experimenter error and intra-experimenter error. Examine “kits”.

**Outcomes:** Ability to determine under what conditions Winkler chemistry is appropriate for determination of DO in water; ability to relate details of chemical reactions to and knowledge of indirect determinations; perform titrations and calculate titration standards, as well as mass of DO in samples; assess individual and among individual errors associated with method.

**General description:** You have received a new O<sub>2</sub> meter that the person giving to you ensures is ‘perfectly calibrated’ and ‘ready-to-go’. You are heading out on a one-shot chance to sample in the arctic, are you going to trust this person to have gotten it right. How do you check that your probe is actually giving you a believable number (yes does the light in the fridge really turn off when you close the door?) You will find out in this lab how to check your sensor.

### Your tasks:

- 1) Familiarize yourself with the Winkler titration chemistry so you know what you are doing once you get your hands on the bottles and chemicals.
- 2) Standardize the Sodium thiosulfate, so you know how much to mix for making a 0.025 N solution
- 3) Titrate up to five (5) individual Winkler samples (BOD) bottles - with up to three replicates in each.
- 4) Calculate the mass of Oxygen in the common water sample
- 5) Determine your error/variation in replicates from the same bottle
- 6) Determine your error/variation between bottles.
- 7) Determine the class error/variation
- 8) Plot cumulative variance versus cumulative number of samples. Does your curve level off?
- 9) Plot class cumulative variance versus cumulative number of samples. How many samples do you need to take versus that of the entire class considering the class is the number of chemists working in the lab.
- 10) Calculate % saturation for all of the samples using the current barometric pressure.

Run a couple of kit titrations to get the feel for them and report the values along with your thoughts on precision.

Prepare a very brief write-up of your results from today’s lab. Include your plots to examine how many samples you should be running.

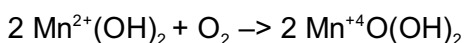
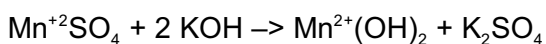
## Winkler titration - azide modification

### *Principle behind the method:*

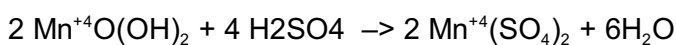
The Winkler titration method for the determination is based on the method developed by Winkler in 1888 (Winkler 1888) during his PhD. The method has seen several modifications to encompass interferences - (see APHA Standard methods for the examination of water and wastewater). It is an iodometric titration, in which the amount of oxygen in the sample is determined indirectly via iodine. It is the most precise and reliable titrimetric procedure for DO analysis. In summary, a divalent manganese solution is added to the sample, followed by strong alkali in glass stoppered bottles. Any DO present in the sample rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with sodium thiosulfate and starch as an indicator. The method is a typical standardization and calibration check for other equipment such as membrane sensors and LDO probes. For the analysis of field samples, it is generally recommended that DO analysis is best done in the field, as there is less chance for the sample to be altered by atmospheric equilibration, changes in temperature and chance of escape of floc or gasses. The method has also been adapted for very small samples (ml), including oxygen samples extracted from animal burrows by divers.

*The equations are as follows:*

i) Manganous sulfate reacts with potassium or sodium hydroxide to give a white precipitate of manganous hydroxide. In the presence of oxygen, brown manganic basic oxide is formed.

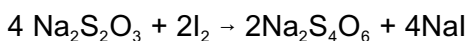


ii) Addition of sulfuric acid dissolves the brown manganic sulfate which reacts instantly with iodide to yield iodine.



iii) In effect, oxygen oxidizes  $\text{Mn}^{2+}$  to  $\text{Mn}^{4+}$  and the  $\text{Mn}^{4+}$  oxidizes  $\text{I}^-$  to  $\text{I}_2$ . Iodine is then determined titrimetrically via titration with Sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) with starch as an end point indicator (deep blue).

iv) Thiosulfate solution (made up as  $\text{Na}_2\text{S}_2\text{O}_3$ ) is used, with a starch indicator, to titrate the iodine.



From the above stoichiometric equations we can find that four moles of thiosulfate are titrated for each mole of molecular oxygen ( $\text{O}_2$ ).

Thus 1 ml of 0.025 M sodium thiosulfate is equivalent to 0.025 meq of oxygen. This

value is commonly multiplied by 8 mg/meq to convert to mg O<sub>2</sub>. When 200 ml of the original sample is titrated, then 1 ml of 0.025M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> = 1 mg dissolved oxygen/L. (200ml /1000ml = 0.2 mg O<sub>2</sub> and 8mg/meq\*0.025 = 0.2 mg O<sub>2</sub>)

### **Interferences:**

Some oxidizing agents liberate iodine from iodide (+ interference) while some reducing agents reduce iodine to iodide (-interference). Organic matter is oxidized partially when the manganese precipitate is acidified, causing negative errors. The azide modification removes interference caused by nitrate, common in biologically treated effluents. The permanganate modification is used to remove iron interference of >5 mg ferric iron salts/L. Ferrous interference can be removed by adding H<sub>3</sub>PO<sub>4</sub> for acidification (only if Fe<sup>3+</sup> is < 20mg/L). With suspended solids the alum flocculation modification works well, while for activated-sludge mixed liquor the copper sulfate-sulfamic acid flocculation modification can be used. In many cases, these interferences can be overcome by using the dissolved oxygen probe method.

### **General methods:**

A sample bottle (typical 300 ml glass BOD bottle with pointed ground glass stopper) is filled completely with water. Special precautions are required to prevent the entrainment of or solution of atmospheric oxygen or loss of DO. This is particularly important for samples at either end of the spectrum - anoxic samples tend to be highly sensitive to oxygen dissolution into the sample during handling, while those supersaturated may degas. To fill the bottle, it can be lowered into the water at the surface - ensuring that sample water enters the bottle without splashing, to avoid oxygen entrainment. Samples from depth should be collected with a remote sample such as a Kemmerer sampler, which has a tube on the outlet. The tube should be placed at the bottom of the BOD while it is filled, and the bottle allowed to overflow for 10s to ensure that representative sample water is obtained. For running water, two to three times bottle volume replacement is suggested to get a representative sample. Samples from turbulent streams may be collected via a funnel connected to a rubber tube which is placed in the bottom of the BOD bottle. This will ensure non-turbulent sample collection and avoid splashing in the bottle that could entrain oxygen. Temperature of the sample should be measured as accurately as possible.

### **Specific procedures:**

Reagents:

#### 1) Manganese sulfate

Dissolve 480 g of MnSO<sub>4</sub>·4H<sub>2</sub>O; 400 g of MnSO<sub>4</sub>·2H<sub>2</sub>O; or 364 g MnSO<sub>4</sub>·H<sub>2</sub>O in distilled water. Filter and dilute to 1 liter. The MnSO<sub>4</sub> solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

#### 2) Alkali-iodide-azide

i) for saturated or less-than-saturated samples

Dissolve 500 g NaOH (or 700 g KOH), and 135 g NaI (or 150 g KI), in distilled water and dilute to 1 liter. To this add 10 g sodium azide  $\text{NaN}_3$  dissolved in 40 ml of distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

ii) for super saturated samples - to be added

### 3) Sulfuric acid

Concentrated  $\text{H}_2\text{SO}_4$  (S.G. 1.84): one milliliter is equivalent to about 3 ml alkali-iodide-azide reagent.

### 4) Starch indicator

Bring 100 ml of distilled water to boil. Prepare a paste of 1 g potato starch in a few ml of distilled water. Add boiling water to the paste. Cool and store at  $5^\circ\text{C}$ . When a violet or grey tinge is noted, discard.

Alternatively

Use either an aqueous solution or soluble starch powder mixtures. To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as preservative, in 100 ml hot distilled water.

### 5) Standard sodium thiosulfate (Stock 0.1 M - 0.1N)

Dissolve 24.82 g  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  per liter, preserved with ammonium carbonate and chloroform 5 ml per liter after making up to mark. Deteriorates about 1% in 6 months.

Standard 0.025M (0.025 N) made by diluting 250 ml of stock to one liter. Add 5 ml chloroform after making up to mark. Deteriorates rapidly and should be made up fresh every month.

Alternatively: Dissolve 6.205 g  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  in distilled water. Add 1.5 ml 6N NaOH or 0.4 g solid NaOH and dilute to 1000 ml. Standardize with potassium bi-iodate or potassium dichromate before use. (Makes 0.025N  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ )

### 6) Potassium dichromate

Dissolve 4.903 g  $\text{K}_2\text{Cr}_2\text{O}_7$  in distilled water and bring up to 1 liter. This will make a 0.10 N solution. Use this to standardize the 0.1 N sodium thiosulfate (see standardization below).

Alternatively: To make a 0.025 N  $\text{K}_2\text{Cr}_2\text{O}_7$  solution, dissolve 1.225 g  $\text{K}_2\text{Cr}_2\text{O}_7$  in distilled water and make up to 1 liter.

### 7) Dilute acid for standardization

3.6 N  $\text{H}_2\text{SO}_4$  - 1 part concentrated  $\text{H}_2\text{SO}_4$  to 9 parts water

**Cautions:**

A variety of manuals is available that describe the method. Be sure you read and understand each before you launch into the analysis to avoid confusion. As occurs often, to save space, or because of things that are obvious to analytical chemists, not all methods offer all of the details you may want, or leave things out leading to questions between methods. I've tried to cobble all of the reasoning together here for a complete package.

Sources consulted included:

APHA standard methods for the examination of water and wastewater.

EPA standard method 360.2, a variety of books and appendices explaining Winkler titrations.

Carpenter, J. H. 1965. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. *Limnol. Oceanogr.* 10:141-143.

Stainton, M.P., M.J. Capel and F.A. Armstrong. 1977. The chemical analysis of freshwater. 2nd ed. *Fish. Envir. Can. Miscellaneous Special Publ.* 25 (available from the Freshwater Inst., Winnipeg, Manitoba)

ASTM E200-86 Standard practice for preparation, standardization, and storage of standard solutions for chemical analysis (59-63).

Most methods use a titration standard of 0.025 N sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) with an adjusted sample volume of between 200-203 ml depending if 1 or 2 ml of each reagent were added (see below) because at those proportions 1 ml of sodium thiosulfate is equal to 1 mg of DO which makes things simple. However, 200 ml out of a 300 ml bottle only gives you one shot at getting it right. You can do any number of titrations between 50 and 200 ml (using an adjusting formula - see below) so you can get replicates out of a single bottle.

**Standardize the 0.10 N sodium thiosulfate solution.**

- 1) Potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) primary standard solution (used to standardize the sodium thiosulfate - see below for details on primary standard solutions)

For our purposes - we will make a 0.1 N  $\text{K}_2\text{Cr}_2\text{O}_7$  solution. Take 4.903 g of  $\text{K}_2\text{Cr}_2\text{O}_7$  and dissolve in distilled water, make up to 1L.

$$\text{Normality of } \text{K}_2\text{Cr}_2\text{O}_7 = \frac{\text{(g dissolved in 1 L)}}{49.03}$$

- 2) Dissolve approximately 2 g of KI, free of iodate, in a 500-ml Erlenmeyer flask with 100-150 ml of distilled water;

add 10 ml of 10%  $\text{H}_2\text{SO}_4$  solution, followed by exactly 20.00 ml of standard dichromate solution (0.10 N  $\text{K}_2\text{Cr}_2\text{O}_7$ )

Place in the dark for 5 min.

Dilute to 300 ml with distilled water, and titrate the liberated iodine with the 0.10 N thiosulfate titrant to pale straw color;

Add starch 1-2 ml and continue until blue is discharged

Calculate the volume of the 0.10 N stock sodium thiosulfate that must be diluted to 1 L to get exactly 0.025 N standard sodium thiosulfate solution:

$$\text{Vol of stock Na}_2\text{S}_2\text{O}_3 = \frac{\text{Vol. of titrant (ml)} * 250.}{\text{solution required} \quad 20.0 \text{ (ml)}}$$

A couple of things about the above - typically you determine the Normality of your standard by the following calculation:

$$1 - \text{Normality of reagent} = \frac{(\text{normality of standard} * \text{volume of Standard used (ml)})}{\text{Volume of reagent used in titration (ml)}}$$

thus if your 0.1 N Sodium thiosulfate is exactly 0.1 N, then it should have taken 20.0 ml of reagent to dissipate the blue of the 20 ml of potassium dichromate standard.

If it is not 0.1 N - then you need to re-calculate how much you will need to make 0.025 N

2 - remember that normality of sodium thiosulfate = normality of Potassium dichromate

3 - remember that in this case then  $N_1 * V_1 = N_2 * V_2$  (N - normality, V - volume)

if your standard is 0.1 N, then to make a 0.025N solution you need:

$$V_1 = \frac{N_2 * V_2}{N_1} \quad N_1 - 0.10, N_2 - 0.025, V_2 - 1.0L$$

= 0.25 L or 250 ml of 0.1 N  $\text{Na}_2\text{S}_2\text{O}_3$  (this is where the 250 multiplication comes from in the above equation)

If the stock is not 0.1 N - then the above equation uses a ratio by which to adjust the typically required 250 ml. (Just make sure you use the same units - if you use L, then re-adjust the 250 by dividing by 1000ml/L to 0.25).

Potassium dichromate is an ideal primary standard solution because it can be made by direct weighing of a chemical.

Standard solutions are the ones whose exact concentration/normality/molarity is known and if it has to be made by direct weighing. Some of the desirable properties for making a primary solution are:

1. It must be solid. It is difficult to weigh exact quantity of gases/liquids.
2. Must be available in high purity (100 % +/- 0.02%) and available commercially.
3. Must be very stable and do not change its composition on storage/keeping or exposure to air/atmosphere.
4. Must have uniform composition.
5. Non-hygroscopic
6. Must readily dissolve and must be stable in solution form as well.
7. High equivalent weight (to minimize balance errors)
7. Preferably nontoxic

Barwick, V., Burke, S., Lawn, R., Roper, P., and Walker, R. 2001. Applications of Reference

Potassium dichromate: Chemical Formula  $K_2Cr_2O_7$  (CAS No. 7778-50-9)

Formula Weight 294.18

Equivalent Weight 49.03 (Molar = 6 Normal)

### Equivalent Weight

In chemistry, the quantity of a substance that exactly reacts with, or is equal to the combining value of, an arbitrarily fixed quantity of another substance in a particular reaction.

Substances react with each other in stoichiometric, or chemically equivalent, proportions, and a common standard has been adopted. For an element the equivalent weight is the quantity that combines with or replaces 1.00797 grams (g) of hydrogen or 7.9997 g of oxygen; or, the weight of an element that is liberated in an electrolysis (chemical reaction caused by an electric current) by the passage of 96,500 coulombs of electricity.

The equivalent weight of an element is its gram atomic weight divided by its valence (combining power). Some equivalent weights are: silver (Ag), 107.868 g; magnesium (Mg), 24.312/2 g; aluminum (Al), 26.9815/3 g; sulfur (S, in forming a sulfide), 32.064/2 g.

For compounds that function as oxidizing or reducing agents (compounds that act as acceptors or donors of electrons), the equivalent weight is the gram molecular weight divided by the number of electrons lost or gained by each molecule;

e.g., potassium permanganate ( $KMnO_4$ ) in acid solution, 158.038/5 g;

**potassium dichromate ( $K_2Cr_2O_7$ ), 294.192/6 g = (49.032)** - constant used in thiosulfate standardization

sodium thiosulfate ( $Na_2S_2O_3 \cdot 5H_2O$ ), 248.1828/1 g.

For all oxidizing and reducing agents (elements or compounds) the equivalent weight is the weight of the substance that is associated with the loss or gain of  $6.023 \times 10^{23}$  electrons.

The equivalent weight of an acid or base for neutralization reactions or of any other compound that acts by double decomposition is the quantity of the compound that will furnish or react with or be equivalent to 1.00797 g of hydrogen ion or 17.0074 g of hydroxide ion;

e.g., hydrochloric acid (HCl), 36.461 g;  
sulfuric acid ( $H_2SO_4$ ), 98.078/2 g;  
sodium hydroxide (NaOH), 74.09/2 g;  
sodium carbonate ( $Na_2CO_3$ ), 105.9892/ 2 g.

Equivalent weight. 2009. In Encyclopædia Britannica. Retrieved February 17, 2009, from Encyclopædia Britannica Online:

<http://www.britannica.com/EBchecked/topic/190933/equivalent-weight>

## Procedure:

1. Carefully fill a 300-mL glass Biological Oxygen Demand (BOD) stoppered bottle brim-full with sample water.
2. Immediately add 2mL of manganese sulfate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.
3. Add 2 mL of alkali-iodide-azide reagent in the same manner.
4. Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.
5. Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the floc. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper, and cap the bottle with aluminum foil and a rubber band during the storage period.
6. In a glass flask, titrate between 201 ml of the sample with sodium thiosulfate to a pale straw color. Titrate by slowly dropping titrant solution from a calibrated pipette into the flask and continually stirring or swirling the sample water.

Standard methods - suggests calculating a titration volume before you start titrating by calculating  $(200 \times 300) / (300 - 4) = 203$  ml.

Alternatively - you can use any volume between 50 and 200 ml - see calculations below for adjusting.

7. Add 2 mL of starch solution so a blue color forms.
8. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the blue color.
9. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each ml of sodium thiosulfate added in steps 6 and 8 equals 1 mg/L dissolved oxygen.

## 10. Calculations

$O_2$  in ppm (mg/L) =  $K \times 200 \times \underline{\text{Volume of 0.025 N thiosulfate used in titration}}$



## Volume of sample titrated

K is a constant to correct for displacement of oxygen in the sample when manganous sulfate and alkaline iodide azide were added. (No correction needed for acid unless floc is displaced)

$$\text{For a 300 ml bottle } K = \frac{\text{Volume of bottle}}{\text{Vol. Of bottle} - \text{Vol. of reagents}} = \frac{300}{(300-4)}$$

**Note 1** You should check to make sure you know the volume of each BOD bottle before you start. Determine this volumetrically or gravimetrically and write on bottle - or next to notes and number of bottle.

**Note 2** 1.0 ml of 0.025 N thiosulfate is equivalent to  $25 \times 10^{-6}$  equivalents of iodine which is in turn equivalent to  $25 \times 10^{-6}$  equivalents of oxygen

$$25 \times 10^{-6} \text{ equivalents of O}_2 = 200 * 10^{-6} \text{ g O}_2$$

If volume titrated is 200 ml then 1.0 ml of 0.025 N thiosulfate is equivalent to 1.0 mg O<sub>2</sub>/liter in the original sample. (This is where the 200 factor in the above calculation comes from)

If oxygen concentrations to 0.1 mg/L are acceptable, the above equality is sufficient to determine dissolved oxygen.

If greater accuracy is required, one must take into account the dilution effect of the first 3 reagent additions. This dilution effect is  $(V-4)/V$  where V is the volume in ml of the oxygen bottle used. Oxygen values obtained by assuming 1.00 ml of 0.025 N thiosulfate equivalent to 1.00mg/L dissolved oxygen should then be multiplied by  $V/(V-4)$  to correct for dilution.

Note 3 If neither 0.025 N titrant is used, other volumes are used and bottles are not 300 ml, then a convenient method to figure out mg O<sub>2</sub>/L is:

$$\text{mgO}_2/\text{L} = \frac{(\text{ml of titrant}) (\text{Molarity of Thiosulfate})(8000)}{(\text{ml of sample titrated}) \frac{(\text{ml of Bottle}-2)}{(\text{ml of bottle})}}$$

Adjust 2 in this equation to however many ml of reagents were added. 8000 is factor related to 8mgO<sub>2</sub>/meq (see above).