One Man's Advice on the Determination of Dissolved Oxygen in Seawater

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Introduction

With the exception of salinity determinations, dissolved oxygen is probably the most commonly measured chemical oceanographic variable. Usually, dissolved oxygen is determined by some modification of the classic Winkler (1888) method.

## Range of Oceanic Dissolved Oxygen Concentrations

Dissolved oxygen concentrations are generally expressed in millimolar, µMoles/kg, mg-atoms/liter or ml/liter (mg-atoms of O_2/liter = 0.08931 x ml/liter). Values from 0 to >13 ml/liter are encountered in the ocean. The equilibrium solubility values for dissolved oxygen increase with decreasing temperature, and salinity, and they range from about 4 to 9 ml/liter throughout most of the ocean. Generally, biological processes are responsible for the wide range of values which are encountered. Phytoplankton growth, for example, can cause oxygen supersaturations while respiratory processes can remove all of the oxygen originally present in some water masses.
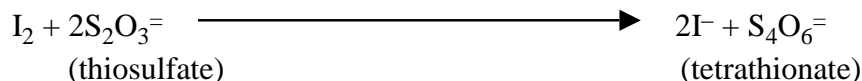
Chemistry of the Winkler Method

Manganous sulfate (or chloride) solution is added to a known quantity of seawater and is immediately followed by the addition of an alkaline sodium hydroxide-sodium iodide solution. Manganous hydroxide precipitates and reacts with the dissolved oxygen in the water with the formation of a hydrated tetravalent oxide of manganese.

$$Mn^{++} + 2OH^- \longrightarrow Mn(OH)_2 \quad (S)$$

$$2MN(OH)_2 + O_2 \longrightarrow 2MnO(OH)_2 \quad (S)$$
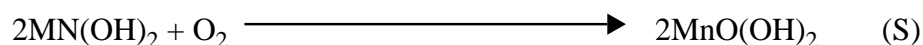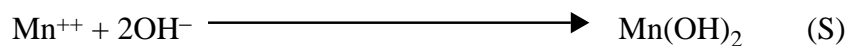
Upon acidification, the manganese hydroxides dissolve. In the acid solution, the tetravalent manganese in $MnO(OH)_2$ acts as an oxidizing agent and liberates free iodine from the iodide ions.

$$Mn(OH)_2 + 2H^+ \longrightarrow Mn^{++} + 2H_2O$$

$$MnO(OH)_2 + 4H^+ + 2I^- \longrightarrow Mn^{++} + I_2 + 3H_2O$$

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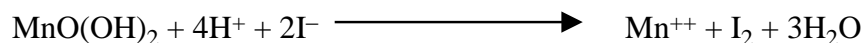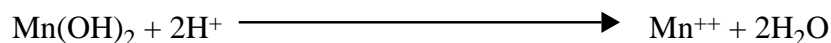
The liberated iodine, equivalent to the dissolved oxygen present in the water, is then titrated with a standardized sodium thiosulfate solution and the dissolved oxygen present in the sample is calculated. The reaction involved is as follows:

$$I_2 + 2S_2O_3^= \longrightarrow 2I^- + S_4O_6^=$$
$$\text{(thiosulfate)} \qquad\qquad\qquad \text{(tetrathionate)}$$

Accuracy of the Winkler Method

Under ideal conditions, the Winkler method is quite accurate. Carpenter (1965a), for example, has described one modification which has an accuracy of 0.1%, and using the Winkler method he (Carpenter, 1966) has produced a set of oxygen solubility tables which appears to be accurate within ~0.01 ml/liter (Murray and Riley, 1969). The field adaptation of Carpenter's technique (1965b) probably should have an accuracy of better than 0.05 ml/liter when carefully performed. (This estimate includes standardization, and "pickling" errors. Precision should be significantly better than 0.05 ml/l.)

Unfortunately, much of the historical oxygen data has been obtained using unsuitable variations of the Winkler method and/or by unskilled analysts. A study conducted several years ago indicated that errors approaching 0.5 ml/liter may be common in the historical data (Carritt and Carpenter, 1966). Such errors sometimes make it impossible for oceanographers to use existing oxygen data. Wust (1964), for example, could not compare oxygen data from different cruises in his study of the Caribbean Sea.

**The Carpenter Modification of the Winkler Method**

Carpenter's (1965b) modification of the Winkler method was designed to reduce the following errors:

1. Iodine Volatilization. Loss of the iodine produced after the sample has been acidified has been shown to be one of the most significant errors in some previous techniques. One way in which Carpenter's method reduces this error is by eliminating sample transfers which could contribute to iodine losses. This is done by using the apparatus shown in Fig. 1. Because the entire sample is titrated in the original collection flask, no transfers are necessary. Another advantage of this approach is that the collection flasks can be calibrated for volume with much greater accuracy than can usually be obtained when taking aliquots from the sample flask.

    In Carpenter's method, loss of iodine during exposure to the atmosphere is also minimized by encouraging the formation of the less volatile triiodide complex (I_3^-). This is done by using a high concentration of sodium iodide solution encouraging the formation of the triiodide complex according to the following formula:

$$I_2 + I^- \rightleftharpoons I_3^-$$
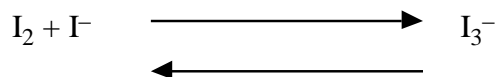
2. Air Oxidation of Iodide. Iodide reacts with oxygen in acidic solution:

$$4I^- + 4H^+ + O_2 \longrightarrow 2I_2 + 2H_2O$$
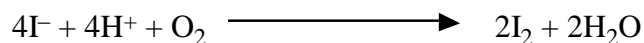
To minimize this source of error, the optimum pH to permit the proper reaction between thiosulfate and iodine and prevent the above reaction was determined. The optimum pH range was found to be 2.0-2.5 and the reagent concentrations were adjusted to attain a final pH in the appropriate range.

3. Improper Blank Determinations. In most previous methods, allowance was made only for positive blanks, but negative blanks caused by reducing impurities are also possible. Consequently, a method for determining positive and negative blanks was devised. This procedure will be described below.

4. Improper Standards. It was found that potassium dichromate, which had been used in some methods, was not a suitable standard. The best standard is potassium iodate, but potassium biiodate can also be used if it is dried by vacuum dessication. Potassium iodate can be dried in an oven at ~110°C.

    Comparison experiments indicate that the error introduced by not using the glassware necessary to eliminate transfers is less than 0.05 ml/liter if aliquots are drawn with care and the Carpenter reagents are employed.

Preparation of Reagents

Reagent Bottles. The reagent bottles used for the manganous sulfate (or chloride), potassium hydroxide-iodide, and sulfuric acid should be such that automatic filling of the pipets is possible. In order to prevent photochemical reactions, the alkaline iodide reagent bottles should be of brown glass or else the exterior painted black.

Manganous Chloride. Dissolve 600 g of reagent grade $MnCl_2 \cdot 4H_2O$ in distilled water. Adjust final volume to 1 liter. Manganous sulfate may be substituted for the chloride, but the chloride is suggested because of its solubility and its freedom from higher valence manganese compounds. When substituting manganous sulfate on a form of MnCl with more or fewer waters of hydration than in $MnCl_2 \cdot 4H_2O$, adjust for differences in formula weight.

Alkaline Sodium Iodide. Dissolve 320 g of reagent grade NaOH and 600 g of reagent grade NaI in distilled water. Dilute to 1 liter.

Sulfuric Acid. Make up 280 ml of concentrated reagent grade H_2SO_4 to 1 liter with distilled water. *Use caution, as a great deal of heat is liberated!*

Starch Solution. Dissolve 1-2 g of starch indicator in 100 ml of distilled water, or boil a similar amount of potato starch in distilled water for about 5 minutes. The indicator should be made up fresh every day if possible since bacterial action degrades it. One sign of a degraded indicator is a reddish tinge to the normally blue color. It is permissible to "play around" with the starch concentration a bit to produce an indicator color intensity that meets your personal preferences!

Sodium Thiosulfate Solution. Dissolve 1.6 g for the Aliquot method (see below) or 35 g (for the Carpenter method) of reagent grade $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter of distilled water. Add 1 pellet of NaOH and 2 drops of chloroform. Let stand (preferably for a few days) before using.

NOTES:

a.   The sodium hydroxide is added to negate the influence of any dissolved CO_2 as the presence of carbonic acid causes the following reaction:

$$S_2O_3^= + 2H^+ \longrightarrow S + SO_2 + H_2O$$
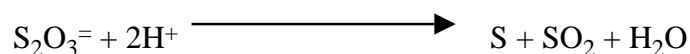
b.   Sodium thiosulfate is degraded by the action of bacteria. Chloroform is added to prevent bacterial growth.

Primary Standard. Dissolve 0.3567 g of oven dried (~105-110˚C) KIO_3 or 0.3250 g of $KH(IO_3)$ dried in a vacuum dessicator in distilled water. Make up to exactly 1 liter. This solution is 0.01000 N.
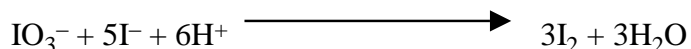
NOTE:

KIO_3 is preferred because of its stability during drying. $KH(IO_3)_2$ (BI-IODATE) decomposes at temperatures above ~100˚C.

Standardization of the Thiosulfate

The thiosulfate solution should be standardized under the same conditions as the actual procedure for the determination of dissolved oxygen. By so doing, possible errors are compensated. The best practice is to run standards and blanks before and after every sample run until you are _sure_ that the interval between standard runs can be extended.

To about 40 ml of distilled water add 1 ml of the H_2SO_4 reagent. Mix thoroughly. Add 1 ml of the NaOH•NaI solution. Mix and wash down the sides of the flask thoroughly. The solution should be distinctly acid, clear and colorless. If any basic microenvironments exist at this point due to insufficient washing of the reagents into the bottle and insufficient stirring, you will obtain poor results. Make good use of your wash bottle and stirring motion before going on! Add 1 ml $MnCl_2$ reagent and, after mixing, pipet into the flask 10 ml of the standard (BI) iodate solution. Stir for ~5 sec. Then titrate with thiosulfate in the same manner described below for samples.

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

$$\frac{10ml \ KIO_3 \ x \ 0.0100 \ N}{[ml \ Na_2S_2O_3 - B]} = \text{Normality of } Na_2S_2O_3$$

where 0.01000 is the normality of the iodate solution, and B = Blank. Often, it is preferable to weigh out standards that are not exactly 0.01000 N because striving for an exact weight takes extra time and tends to make the weighing process less exact. It is, of course, a simple matter to substitute a different normality when performing your calculations.

Blank Determination. This procedure is the same as the standardization procedure except

that exactly 1 ml of the standard KIO_3 is added instead of 10 ml, and, after the first titration, another 1 ml of standard is added and the solution is titrated again. The blank is equal to the ml of thiosulfate needed for the first titration minus the ml of thiosulfate needed for the second titration. The blank may be either positive or negative.

Sample Collection and Treatment

Samples for the determination of dissolved oxygen should be taken from the water sampling bottle immediately upon its arrival on deck. The sample should be collected in a calibrated, ground glass-stoppered container. Two types may be used—bottles approximating 250 ml and bottles approximating 125 ml. In the case of the latter, the entire contents of the flask is eventually used for titration while with the former, an aliquot is taken. We generally use 125 ml (approx.) clear glass bottles that are calibrated to contain with an accuracy of ±0.04 ml and titrate the entire bottle as suggested by Carpenter (1965).
To fill the oxygen bottle from the oceanographic sampling bottle (e.g., a Niskin bottle), you will need a length of amber surgical tubing (or similar material) with an inside diameter of approximately 1/4". The length of this tubing should be approximately 12", but keep the length as short as you can without sacrificing ease of movement while filling the oxygen bottle. To one end of this tubing attach a length of stiff plastic tubing or glass tubing that is a bit longer than the oxygen bottle. You are now ready to begin the challenging task of drawing a high quality oxygen sample without contamination while bathed in an atmosphere that is about 21% oxygen!! Believe it or not, it can be done, and with a bit of practice _you_ can do it!
A method that I have used will be described next, but remember the principle, which is to minimize contact of the seawater sample with the atmosphere. With this basic principle in mind, you can modify my technique to fit your particular blend of motor skills. When I draw an oxygen sample, I proceed as follows:
   1)   Attach the soft tubing to the spigot of the Niskin (or other type of bottle), and open the air vent on the Niskin bottle.
   2)   Open the spigot valve and flush the sampling tubing so that all air bubbles are removed. I find that the easiest way to do this is to hold the tubing in a straight line (more or less) and point the tubing downward while letting water flow through the tubing at maximum velocity for a brief period. At this point, you control the velocity of flow by pinching the soft tubing with your fingers. If the tubing is not completely free of bubbles after the first brief period of maximum velocity, repeat the process one or more times by turning the flow on and off by pinching and unpinching the rubber tubing. Some investigators prefer to try to remove the bubbles from the tubing by holding it in a U-shape with the open end up and letting the water flow gently through the tubing. Any method or combination of methods that removes all of the bubbles from the tubing is OK.

3)   The next step is to rinse your oxygen bottle while minimizing contact with the atmosphere. I do it by allowing the water to flow at a moderate pace out of the bubble-free tubing, inserting the tubing so that the stiff portion touches the bottom of the bottle, and then inverting the bottle. While in the inverted position, I move the stiff part of the tubing around so that a moderate "sheet flow" rinses the entire bottle with minimal turbulence.

4)   Next, I momentarily stop the flow by pinching the tubing and invert the bottle quickly. Then I start a moderate flow again and let the bottle fill as quickly as possible without forming a lot of turbulence while keeping the stiff part of the tubing near the bottom of the bottle. As the bottle begins to overflow, I let the overflow water rinse the ground glass stopper. After about half a bottle volume has overflowed, I begin to withdraw the tubing from the bottle by steadily raising while allowing another bottle volume (approx.) to overflow and continue to rinse the stopper.

5)   Now I immediately add 1 ml of the manganous reagent by placing the tip of the delivery pipet just below the surface of the sample in the oxygen bottle. This step is immediately followed by the addition (in like manner) of the alkaline-iodide reagent. Both reagents are very much denser than seawater, and they sink to the bottom and displace the upper 2 ml of seawater in the bottle, which is helpful since the upper water has been in contact with the atmosphere. It is best not to immerse more than about 1/8" of the tips of the delivery pipets since this should help to cut down on contamination. Not immersing the tips at all increases the possibility of contamination from the atmosphere.

6)   Now place the stopper in the bottle without trapping bubbles. I have found that the easiest way is also the way that works the best. Just drop the stopper into the bottle from a height of about one inch! If you place the stopper in the bottle slowly and carefully, you are more likely to trap a bubble. The stopper displaces the upper few ml of liquid in the neck of the bottle, thereby removing most of the atmospheric contamination that may have accumulated.

7)   Now push down on the stopper to make sure that it is tight, and closely inspect the bottle to make sure that it is free of bubbles. A bubble the size of a pencil eraser will completely invalidate any sample, and much smaller bubbles can also totally ruin a sample depending on ambient concentrations and the accuracy that you desire. The best rule of thumb is to start over again if you can see a bubble in your sample even though there exist exceptional situations in which heating or the addition of the first two reagents can cause bubbles to form within the sample.

8)   Now invert the bottle several times to mix the first two reagents with the sample. A precipitate will form. Allow the precipitate to settle, and repeat the mixing process one time. Generally, you should wait at least 15 minutes before repeating this mixing process. If you are using the Carpenter method (which we prefer), your sampling bottle will be a calibrated (for volume) bottle made out of clear glass. Because of the possibility of undesirable   photochemical reactions, it is important that you keep the bottle in the dark as much as possible. We accomplish this by placing the bottles in a carrying case (which is painted black on the inside) in between manipulations. Also try to keep the samples cool (i.e., in the shade).

9)   The next step is to add the acidic reagent. Some prefer to do this immediately after the precipitate in the oxygen bottle has settled for the second time, and others prefer to do it just before beginning the oxygen titration. I have uncovered no convincing evidence that favors one technique over the other, but I prefer to add the acid fairly soon after the second settling and have had good success with this approach. In the Carpenter version of the Winkler method, the reagents are adjusted so that the final pH is between 2 and 2.5.

This range of pH minimizes unwanted side reactions but is just barely acid enough to dissolve the precipitate. If the precipitate is not completely dissolved when you are about to start your titration, just add another drop of acid.

NOTES:

a.   Occasionally after the addition of sulfuric acid, a gas bubble will appear. This bubble is composed largely of carbon dioxide and a little nitrogen which may have been liberated from the solution. The former results from the chemical reaction of the acid and carbonates. The presence of the nitrogen is accounted for by the reduced solubility of this gas upon the addition in electrolytes of the reagents and the possible increased temperature of the sample.

b.   In running determinations of dissolved oxygen on fresh or slightly brackish waters, considerable difficulty may be experienced in obtaining complete solution of precipitate. However, solution may readily be affected by the addition of a few crystals of sodium chloride to the acid solution.

c.   In limnological studies, the use of hydrochloric acid may be substituted for the sulfuric acid. (Concentrated HCL is 12N and concentrated H_2SO_4 is 36N.)

Titration of Samples

If an aliquot of sample is drawn from a ~250 ml brown glass bottle, the buret used for measuring the quantity of standard sodium thiosulfate solution should deliver 25 ml and be graduated to 0.05 ml. Readings of the buret should be made to 0.01 ml. If a large number of samples are to be titrated, it is a marked convenience to have an arrangement so that the buret may be filled by gravity with an automatic adjustment to the zero point.

If an aliquot of the sample is taken for titration, a measured volume of 100 ml is titrated in an Erlenmeyer flask with a standardized thiosulfate solution introduced from the buret. About 1 ml of the starch indicator solution is added and the solution titrated until the blue starch-iodine color disappears. When the blue color just disappears, read the buret. The starch is generally not introduced until the solution is a fairly pale yellow which means about 1/2 to 2/3 of the way through the titration for most samples. When samples are taken from low oxygen zones they will, however, be a pale yellow color at the beginning of the titration.   We   have   seen   no compelling evidence to suggest any differences due to the point at which the starch is introduced *as long as it is before the end point*!

It is difficult to describe how to detect the end-point on the printed page, and my advice is to try to have an experienced analyst demonstrate this for you. In case you cannot find an experienced and competent analyst, here is a method that should work. 1) As the blue color begins to disappear, start to add the thiosulfate in small increments and begin to record the buret readings. 2) If your eyes are like mine, you will not see too much color change near the end-point. Instead, the solution will become progressively clearer and/or brighter. 3) Keep on adding thiosulfate until you no longer see changes in clarity and/or brightness upon the addition of more thiosulfate. 4) The reading that corresponds to the last thiosulfate addition that caused a visible change is the end-point! 5) Do not allow your desire to obtain the best end-point cause the titration to take too much time! See note (b) below.

With the Aliquot method, the dissolved oxygen concentration is calculated from the following equation:

$$DO = \frac{[R - R_{blk}] \; V_{IO_3} \cdot N_{IO_3} \cdot E}{[R_{std} - R_{blk}] \left[V_s - V_{reg} \cdot \dfrac{V_s}{V_b} \right]} - DO_{reg} \cdot \frac{125 \; ml}{V_b}$$

where

DO	= dissolved oxygen (ml/liter)
R	= sample titration buret reading
R_{blk}	= difference between blank titration buret readings (i.e., the blank value)
R_{std}	= standardization buret reading
V_{IO_3}	= volume of primary standard (ml)
V_b	= volume of sample bottle (ml)
V_s	= volume of titration aliquot (ml)
V_{reg}	= volume of sample displaced by reagents (2 ml for this method)
N_{IO_3}	= normality of primary standard
E	= 5,598 ml O_2/equivalent

DO_{reg} = 0.018 ml/liter, the amount of oxygen added with the reagents if you were using the 125 ml flasks recommended by Carpenter (1965b).

NOTES:

a.   Since the precipitate containing the dissolved oxygen has settled before the addition of the H_2SO_4, only 2 ml of sample are displaced by reagent additions.

b.   After completing the titration, if the solution is permitted to stand for a short period of time, the blue starch-iodine color may again become evident. *This should be ignored*, but, in general, titrations should be carried out as quickly as possible consistent with accuracy.

If the entire sample is to be titrated, the amount of dissolved oxygen may be calculated from the following equation which applies to the "no-transfer" Carpenter method that we prefer.

$$DO = \frac{[R - R_{blk}] \; V_{IO_3} \cdot N_{IO_3} \cdot E}{[R_{std} - R_{blk}] \; [V_b - V_{reg}]} - DO_{reg}$$

where

|	
DO	= dissolved oxygen (ml/liter)
R	= sample titration buret reading
R_{blk}	= blank correction factor
R_{std}	= standardization buret reading
V_{IO_3}	= volume of KIO_3 standard
V_b	= volume of sample bottle (ml)
V_{reg}	= volume of sample displaced by reagents (ml)
N_{IO_3}	= normality of KIO_3 standard (equivalents/liter)
E	= 5,598 ml O_2/equivalent, and
DO_{reg}	= oxygen added with reagents

For the procedure described,

$$DO = \frac{[R - R_{blk}] \; 559.8}{[R_{std} - R_{blk}] \; V_b - 2} - 0.018$$

NOTE:

Another advantage of the no-transfer method is that the bottles can be calibrated for volume with high accuracy (±0.03%). This is a much higher accuracy than is likely to be obtained when drawing aliquots in the field.

Sources of Error in the Winkler Determination Caused by
Substances Found Only Occasionally in Seawater

a. If nitrites are present in large quantities, high values for oxygen may be obtained because of the following chemical reaction:

$$2NO_2^- + 2I^- + 4H^+ \longrightarrow 2NO + I_2 + 2H_2O$$
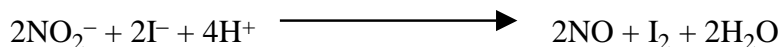
With the exception of oxygen deficient regions where O_2 is less than ~0.1 ml/l, the concentrations of nitrites in seawater are usually so small that any error introduced by their presence is negligible.

b. If hydrogen sulfide is present, the Winkler method for determining oxygen is not applicable. Hydrogen sulfide will react with the dissolved oxygen and with iodine. For example, one possible reaction is:

$$2H_2S + O_2 \longrightarrow 2H_2O + 2S°$$
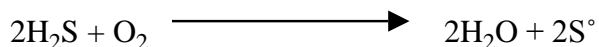
The sulfur formed is almost colloidal in nature and such waters will be devoid of oxygen, except for the possibility of an interface zone where $O_2 + H_2S$ may be present.

The hydrogen sulfide reacts with iodine and if present in sufficient quantities may be determined iodiometrically.

c. In waters polluted by industrial waste or containing relatively large concentrations of reducing material, Winkler's method is not applicable because any iodine liberated may react with the reducing substances.

Low Concentration Dissolved Oxygen Method

Broenkow and Cline (1969) have described a colorimetric dissolved oxygen method which is more suited to low concentrations (<0.4 ml/liter) than the more normal methods described above. The normal Winkler methods described above cannot resolve the 0 to ~0.15 ml/l dissolved oxygen range, and high nitrite levels often occur in low oxygen waters.

Automated Methods

A number of investigators (Williams and Jenkinson, 1982; Friederich, Sherman and Codispoti, 1984; Culberson and Huang, 1987) have recently automated the dissolved oxygen titration. These methods reduce eye-strain, improve precision, and allow the data to be directly acquired by computer. They do not require the addition of starch, since the yellow iodine color that is poorly perceived by the human eye can be detected with great precision electronically.

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