DETERMINATION OF DISSOLVED OXYGEN IN SEAWATER BY WINKLER TITRATION USING THE AMPEROMETRIC TECHNIQUE

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1. SCOPE AND FIELD OF APPLICATION

This paper describes procedures to be used for the determination of dissolved oxygen in discrete samples of seawater. These procedures are based on the modified Winkler titration method (Carpenter 1965). These modifications reduced the loss of I_2 during the titration due to volatilization by optimizing the concentrations of the "pickling" reagents to encourage the formation of the more stable triiodide complex I_3^- and by adopting the whole-bottle titration method which eliminates the loss of I_2 during transfer of sample aliquots. This procedure is suitable for the measurement of the full range of oceanic oxygen concentrations (0-400 μmol kg^{-1}) in uncontaminated seawater. The typical precision that can be achieved using automated amperometric endpoint detection systems is ±0.15 μmol kg^{-1}. Carpenter (1965) established the accuracy of the method as <0.1% or ±0.3 μmol kg^{-1}. This procedure is unsuitable for seawater containing hydrogen sulfide (H_2S). In oxygen-deficient regions (<5 μmol kg^{-1}) a high concentration of nitrite (NO_2^-) may cause a positive oxygen bias. Polluted waters may contain reducing substances that will react with the liberated I_2 resulting in a negative oxygen bias.

2. INTRODUCTION

Dissolved oxygen measurement is commonly a standard part of most hydrographic studies. The data is of interest to physical, chemical and biological oceanographers. Physical oceanographers use it to characterize water masses. Chemical oceanographers use it study to study the production and destruction of organic matter. Biological oceanographers use it determine rates of photosynthesis and respiration. Climate scientists are finding it to be a sensitive indicator of climate-related changes in the ocean circulation and ventilation of intermediate and deep water. It is also used in the quantification of the uptake of anthropogenic CO_2 by the ocean. The performance of oxygen sensors have dramatically improved in recent years; however, for the most precise work they still need to be calibrated frequently to correct for drift and temperature and pressure influences. The chemical titration method first described by Winkler (1888) remains the method of choice for the analysis of discrete water samples. The method is fast and inexpensive. The development of computer-controlled titrators has eliminated the tedium and operator variance of manual titration and has pushed the routine shipboard precision of the method to 0.06% or ± 0.15 μmol kg^{-1}.

In 1991, a report was published (Culberson,et al., 1991) describing an inter-comparison experiment between oxygen measuring groups at four institutions. The experiment compared three groups using automated endpoint detection methods (two amperometric, one photometric) with one group using the manual method with starch endpoint detection. The systematic differences between methods and groups was encouragingly small; i.e., worst case 0.6% and on average 0.3%. However, because the systematic differences were large relative to the precisions attained by the different groups (0.06-

0.15%), it was recognized that there was significant room for improvement. After considering possible causes of the systematic differences between groups the report came out with a set of recommendations for improving the accuracy of the dissolved oxygen method:

1) Calibrate the volume of all pipettes, volumetric flasks, burettes and oxygen flasks and include correction for buoyancy.
2) Correct for effect of thermal expansion on the masses of thiosulfate and iodate dispensed.
3) Measure the temperature of the seawater sample at the time of pickling in order that it's mass can be accurately determined.
4) Standardize the set of equations used to compute oxygen concentration and correct for the thermal expansion of the sample and solutions.
5) Determine the oxygen content of the pickling reagents as a function of temperature.
6) Perform intercomparison study at low oxygen concentration.
7) A study should be made of the seawater blank in coastal and open ocean waters at surface, oxygen minimum, nutrient maximum and bottom depths. Until more is known about the magnitude and variability of the seawater blank, only the pure water or reagent blank should be measured. While this will result in small errors in computed oxygen concentrations of -0.5 to + 0.8 μmol kg^{-1} they will be internally consistent between groups.

Implementation of the recommendations made by Culberson (1991) and Dickson (1995) with respect to the careful calibration of all glassware used in the method, the corrections for the effect of temperature in the lab on the mass of thiosulfate and iodate dispensed, and the effect of draw temperature on the mass of sample contained in the oxygen flask (i.e., recommendations 1-4) have contributed to a general improvement in the accuracy and precision of discrete dissolved oxygen measurements. It is important to note that recommendations 5-7 have still not been addressed. It would be wise in the not-too-distant future to conduct another intercomparison experiment both as an opportunity to judge how far systematic differences between groups have improved since 1991 and also to tackle the seawater blank and oxygen content of the reagents as a function of temperature.

In this paper I review the procedures that are followed to implement recommendations 1-4. Sample calculations are provided so that investigators coding the equations into their software can confirm that they are getting the correct values. New in this paper, I discuss the amperometric method of endpoint detection in detail and present the results of a side by side comparison of an amperometric and a photometric system and show that the oxygen concentrations obtained are identical to within the measurement uncertainties of 0.06% or ± 0.15 μmol kg^{-1}. Also new in the paper, I compare the "standard curve" method of thiosulfate standardization and reagent blank determination that some groups around the US are using with the methods described in Carpenter (1965) and in the 1991 and 1995 Standard Operating Procedures by Culberson et al. and Dickson, respectively.

3. PRINCIPLE OF THE WINKLER METHOD

Manganous chloride ($MnCl_2$) solution is added to a known quantity of seawater and is immediately followed by the addition of an alkaline sodium hydroxide-sodium iodide solution (NaOH/NaI). Manganous hydroxide ($Mn(OH)_2$) precipitates and reacts with the dissolved oxygen in the water with the formation of a hydrated tetravalent oxide of manganese ($MnO(OH)_2$).

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

$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2$

Upon acidification, the manganese hydroxides dissolve. In the acid solution, the tetravalent manganese in $MnO(OH)_2$ acts as an oxidizing agent and liberates iodine (I_2) from the iodide ions (I^-).

$2MnO(OH)_2 + 8H^+ + 4I^- \rightarrow 2Mn^{2+} + 2I_2 + 6H_2O$

Two moles of I_2 are formed for each mole of O_2 present in the sample. The amount of I_2 in the solution is determined by titration with a standardized sodium thiosulfate ($Na_2S_2O_3$) solution.

$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$

Two moles of thiosulfate are required to titrate each mole of I_2. Since two moles of I_2 were formed for each mole of O_2 the final stoichiometry is four moles of thiosulfate equals one mole of O_2. By knowing the concentration of the thiosulfate solution and the volume required to titrate the liberated I_2 the amount of the oxygen dissolved in the seawater sample can be easily computed.

4. AMPEROMETRIC ENDPOINT DETECTION

The method of titration in which the endpoint is detected by the sudden increase or decrease in current flow between two similar electrodes immersed in a solution due to polarization effects is known as the "dead-stop" endpoint amperometric method. Foulk and Bawden (1926) and Bradbury and Hambly (1952) investigated the advantages of using the amperometric method over the visual starch endpoint method in the titration of iodine with thiosulfate. The first report of the use of the amperometric method of endpoint detection applied to the Winkler titration is Truesdale and Knowles (1956). They described two methods, the first involved adding an excess of thiosulfate and then back-titrating with potassium iodate. The second method involved titrating directly with thiosulfate. It is the later method that is more widely used today (Culberson and Huang, 1987). The amperometric method involves applying a potential to an electrode placed in a solution so that an analyte is reduced and using the resulting current as an indicator of the concentration of that analyte. In the case of the Winkler titration the analyte is I_2. A potential of 100 mV is applied between two platinum electrodes. In an acidified solution the iodine is reduced at the cathode to iodide ($I_2 + 2e^- \rightarrow 2I^-$) and the reverse reaction occurs at the anode ($2I^- \rightarrow I_2 + 2e^-$). The result is a stable current proportional to the concentration of I_2 in solution. During the titration, thiosulfate ($Na_2S_2O_3$) is added and it reacts with the I_2 to form $2I^-$. As the I_2 concentration decreases so does the current measured at the electrodes. The endpoint is detected as the point at which the current ceases to decrease following the addition of a small aliquot (0.25 μL) of thiosulfate.

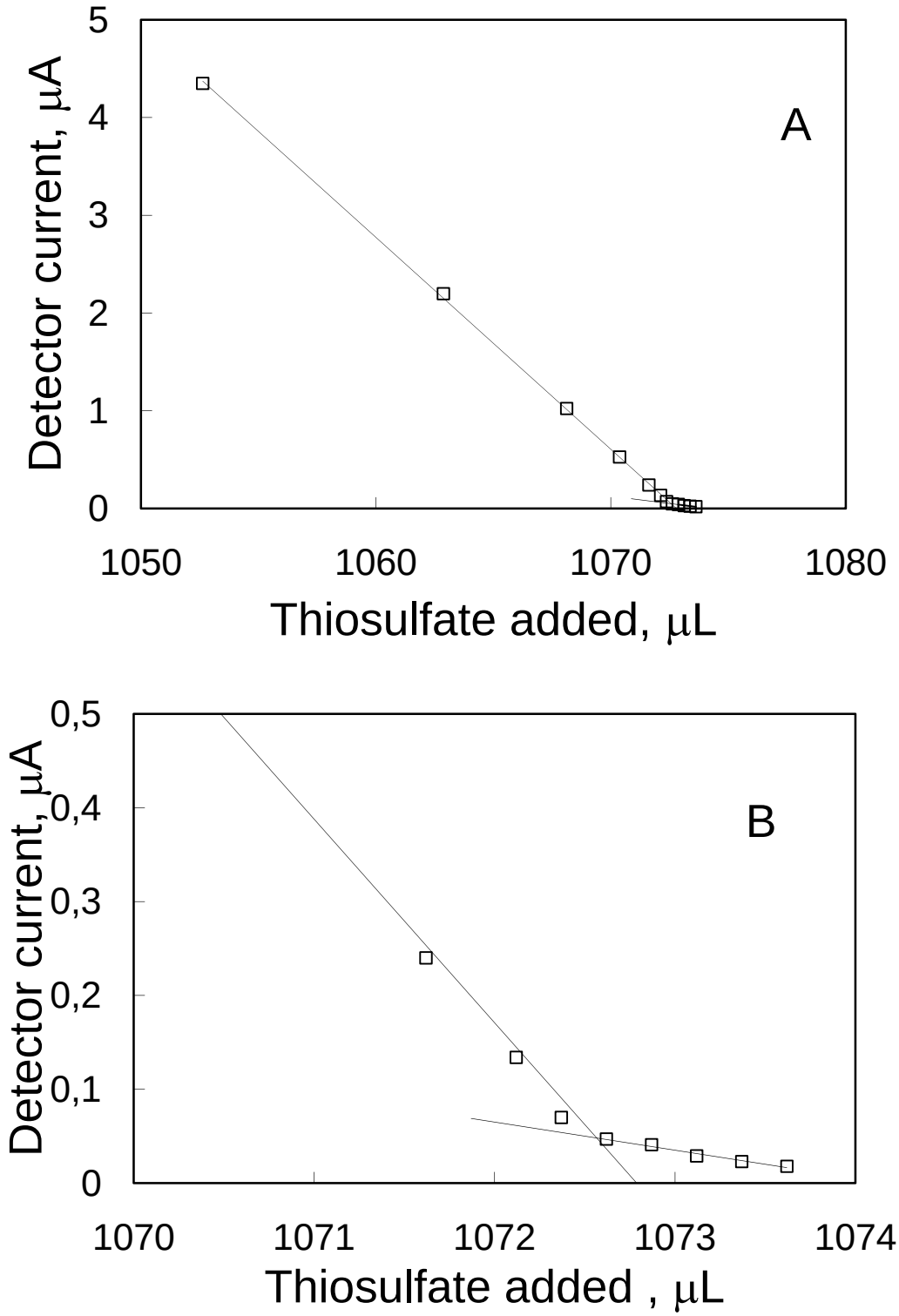

Figure 1. A) Typical titration curve showing linear decline in detector current as the endpoint is approached and sharp change in slope as the endpoint is passed. The endpoint is the intersection of the two line segments. B) Detail of the data and regression lines shown in panel A very near the endpoint where the titrator is adding just 0.25 μL of thiosultate with each addition.

The chief advantage of the amperometric method of endpoint detection over the photometric method is that the endpoint can be accurately detected in turbid or highly colored samples. Other advantages include that the glass oxygen flasks do not need to have uniform optical properties (they can even be opaque), the magnetic stir bars do not interfere with the light path and there is no light supply, no water bath that gathers interfering particles, nor detector to maintain in a very careful and regular manner.

5. APPARATUS

5.1 Sampling

a. Sample flasks: Pyrex "Iodine titration" flasks 125 ml nominal volume with flared necks and ground glass stoppers. Each flask must be numbered and a rubber leash should be used to keep the flask/stopper pair together. The flask/stopper pairs must be calibrated by weighing each when empty and filled with distilled water (DIW) of a known temperature and then applying a buoyancy correction (DOE, 1994). A balance with a capacity of 300 g and a precision of 0.001 g is required. With care the volume of each flask can be known to ±0.003 ml.

b. Reagent dispensers: Three accurate plunger type dispensers are required; two dispensers for the Reagent #1 – $MnCl_2$ and Reagent #2 – NaI/NaOH capable of delivering a 1.0 ml aliquot repeatable and accurate to ±0.02 ml. The ThermoScientific Repipet II works well. The dispenser containing the NaI/NaOH will require regular attention (don't wait for feedback from the samplers) because the plunger will begin to stick/bind to the glass. It is convenient to buy or build (often of plywood or grey P.V.C.) a carrying caddy for the $MnCl_2$ and NaI/NaOH reagent bottles and dispensers because they are carried back and forth repeatedly between the analytical lab and the CTD/rosette room or deck. The third dispenser mentioned is required during analysis for dispensing Reagent #3 – Sulphuric Acid solution.

c. The NaI/NaOH dispenser should be thoroughly cleaned and disassembled for long-term storage between voyages because otherwise the plunger will become stuck.

d. It is recommended to use amber colored reagent bottles to prevent any effect light may have on the reagents during an extended voyage. A square-form is more convenient for handling.

e. Flexible plastic drawing tube or "noodle": A length of thin-walled Tygon tubing long enough to reach from the petcock of the Niskin water sampler to the bottom of the oxygen sample flask. The diameter of the tubing must be large enough to allow a good rate of overflow of water as the flask is being filled. It is convenient to fit a short piece of highly flexible silicone tubing over one end of the drawing tube to make it easier to slip the tubing over the nipple of the petcock. It can be useful to store the "noodle" in DIW and occasionally rub and massage it for a time prior to drawing a dissolved oxygen sample. This thoroughly "wets" the inside and greatly cuts down on air entrapment when sampling.

f. Digital thermometer: A digital thermometer, with a thermistor on a thin flexible cable capable of being inserted into the oxygen flask along with the drawing tube for recording the temperature of the seawater as a sample is being drawn. The meter should measure the temperature to 0.1°C. This temperature is needed for computing the density of the seawater at the time of sampling and is used during data processing to permit conversion of oxygen

concentration from units of μmol L^{-1} to μmol kg^{-1}. The draw temperature is also useful as an indicator of whether the Niskin water sampler closed at the correct depth. This same thermometer can be used to measure the thiosulfate temperature in the lab. If thermistors are firmly adhered to the thiosulphate amber glass container, then the two can be wrapped in an insulating material; the temperature can be readily and accurately measured and will have little or no drift.

5.2 Titration apparatus

a. Titration controller: For automated titration setups there is a controller that interfaces a computer with a motorized burette for dispensing the thiosulfate titrant and with the components used to detect the endpoint either photometrically, amperometrically or potentiometrically.

b. Burette: A manual or motorized piston burette with a capacity of 1, 2 or 5 ml capable of accurately dispensing the thiosulfate in small increments. The Brinkman Dosimat is frequently used. The Manostat 2 ml Micrometer burette is also suitable used either manually or coupled to a stepper motor.

c. Dispenser: A dispenser, identical to those used for Reagent #1 – $MnCl_2$ and Reagent #2 – NaI/NaOH reagents, capable of dispensing 1.0 ml aliquots of the Reagent #3 – H_2SO_4 . Again the reagent bottles to which the accurate dispensers attach are recommended to be of amber glass and of square-form.

d. Precision dispenser: A high precision dispenser capable of delivering 1.0 and 10.0 ml aliquots of the potassium iodate (KIO_3) standard for the preparation of blanks and standards. The SOCOREX Calibrex 520 bottle-top dispenser is highly recommended for this application. It is adjustable from 1 to 11 ml in 0.25 ml increments. It should be calibrated by the repeated weighing of aliquots at the volumes you will use frequently; i.e., the 1.0 and 10.0 ml settings. The reproducibility of the dispensed volume is ± 0.002-0.005 ml. Another very accurate and repeatable option is Metrohm™ piston burettes.

e. Magnetic stirrer and a number of 25 mm (1") Teflon-coated magnetic stir bars.

f. Another piece of equipment that needs to be frequently used during the titration process is a plastic "squeeze bottle" of about 250ml filled with reagent grade or distilled water.

5.3 Reagents and preparation

a. Manganous chloride (3 M): Slowly add 600 g of $MnCl_2 \cdot 4H_2O$ to a graduated beaker containing 500-700 ml of DIW, stir until all the crystals have dissolved. The solution becomes cool upon preparation; allow it to reach ambient temperature then make the volume up to the 1 L mark with DIW. Filter the solution through a course glass fiber filter to remove any particulate material. If time or staffing is short, a magnetic stirrer can assist enormously. Please "BE SAFE" during make-up (behind a fume hood preferably) and please wear gloves and goggles.

b. Sodium iodide (4 M)/Sodium hydroxide (8 M): Dissolve 320 g of NaOH in a beaker containing roughly 500 ml of DIW. Allow the solution to cool. You want to place the

beaker in a tub of cold water to hasten the cooling. Then slowly add 600 g of NaI and allow the solution to cool again. Then add DIW to make the solution up to 1 L. Again, please "BE SAFE" whilst doing so (behind a fume hood preferably) and please wear gloves and goggles. It is particularly important to filter this solution because often there is a great deal of material that does not dissolve and some of this material has been found to be a reducing substance that contributes to the reagent blank.

c. Sulfuric acid (5 M): Slowly add 280 ml of concentrated reagent grade H_2SO_4 to a beaker containing roughly 500 ml of DIW. Again, please "BE SAFE" whilst doing so (behind a fume hood preferably) and please wear gloves and goggles. It is a good idea to have the beaker sitting in a tub of cold water because a great deal of heat is given off as the acid and water mix – enough to boil and injure. Allow the solution to cool to ambient and then make up the volume to 1 L. A graduated beaker is sufficiently accurate for the preparation of this solution.

d. Thiosulfate: The concentration of the thiosulfate is dependent on the volume of the burette. The concentration has to be great enough to titrate the highest oxygen concentration you expect to encounter. If you take 400 μmol kg^{-1} as the highest oxygen concentration you will ever encounter and your burette can dispense a maximum of 2 ml of titrant then the concentration of your thiosulfate should be:

$[Na_2S_2O_3] = 400*10^{-6}*140*4/BuretteVol$

or 0.11 M. Given that the formula weight of $Na_2S_2O_3 \cdot 5H_2O$ is 248.17 g $mole^{-1}$ you would weigh out 27.4 g and make the volume up to 1.0 L in a volumetric flask. You need to be careful because you can also buy the anhydrous form of thiosulfate. This has a formula weight of 158.09 g $mole^{-1}$ and in this case you would weigh out only 17.4 g. Adjusting the thiosulfate concentration so that you use almost the full capacity of your burette at the highest oxygen concentration you expect to find in the waters you are working will allow your titration system to return the maximum possible precision. For some reason, it has been found that the thiosulfate solution is more stable and consistent if it is left to age (say 2 – 5 days). This leads to more consistent blanks and calibrations day to day, saving time and frustration. As such it may be advisable to make it up in quantities > 1 L.

e. Potassium iodate (0.00167 M or 0.0100 N): Weigh out roughly 0.5 g of KIO_3 and dry in an oven at 170°C for several hours. Weigh out 0.3567 g of the dried KIO_3, dissolve in DIW and make up to exactly 1 L in an "A –grade" volumetric flask. Measure the laboratory temperature and denote it as t_p. Use a dropper to add the DIW at the end to bring the level of the meniscus up exactly to the line. It cannot be overstated that the absolute accuracy of your oxygen analyses are dependent on the care you take with the preparation of KIO_3 standard solution.

6. SAMPLING

6.1 Introduction

The collection of a seawater sample for oxygen analysis from the Niskin or other water sampling device should be done as quickly as possible after the water sampler reaches the surface. The first

consideration must be given to sampling from the first triggered (deepest) Niskin due to the fact that the sample water has been in this bottle the longest, and undergone the greatest changes in Pressure and Temperature. Then consideration must be given to the warming of the water on deck in the sampler which can result in outgassing and loss of oxygen. Once water sampling from a Niskin begins a headspace develops inside the sampler and gas exchange between that headspace gas and the water in the sampler will begin immediately. For this reason a strict hierarchy has been established for the order in which water samples for gas analyses are drawn from the sampler. Those gases that are likely to experience the greatest contamination from the headspace, are drawn infrequently, or are very expensive or labor intensive to analyze are drawn first. The order that is observed on the CLIVAR/Repeat Hydrography cruises is CFCs, helium, noble gases (argon and xenon), O^{17}, oxygen and pCO_2. It is just as important that all the sampling for dissolved gases happen as quickly as possible once the water sampler arrives on deck.

6.2 Sampling procedure

a. Confirm that flask and stopper is a matched pair. This is most easily done by using a rubber leash to keep the paired flask and stopper joined at all times. If a flask or stopper is broken, do not replace one or the other. Record the I.D. of the now lost pair and completely remove from use.

b. Please "BE SAFE" whilst sampling as the reagents you add are quite dangerous, so please wear goggles and if possible, gloves.

c. Connect the drawing tube to the nipple of the petcock on the water sampler and push in to start the flow of water. Dislodge any air bubbles within the tubing by squeezing or tapping.

d. Rinse the sample bottle twice by filling half-way to remove any residual reagent from a previous analysis. Leave the water flowing.

e. Fill the sample flask by inserting the drawing tube all the way to the bottom of the flask. Fill the flask smoothly to minimize turbulence and aeration. Restricting the flow at the beginning by squeezing the drawing tube helps to minimize the bubbles that will otherwise form if the flow is too fast. This can also be assisted by commencing the "sample fill" with the bottle starting on roughly a 45^{o} angle and as the fill progresses, move the flask to the upright position. Pay attention to how long the flask takes to fill. You should allow the flask to overflow for 2-3 times the time it took the flask to fill. This period of overflow is critical to obtaining a high quality sample. Your colleagues will complain about the waste of sample but it is critical to the precision of the oxygen analysis. The flask should be filled all the way to the top. Do not stopper the flask at this stage.

f. Note the sampling or draw temperature: while the flask is filling and overflowing insert the thermistor of a digital temperature meter into the flask and take a reading. This is an important step in computing the density of the seawater in the sample at the time that the oxygen concentration in the sample was "fixed" by the addition of the pickling reagents. The drawing temperature can be many degrees higher than the *in-situ* temperature recorded by the CTD at the time water sampler was tripped.

g. As mentioned, when full do not yet stopper the flask but move as rapidly as is safe to where the reagent caddy (holding Reagents 1 & 2) is located. Add the pickling reagents: add 1 ml of the $MnCl_2$ and then 1 ml of the NaI/NaOH reagents, stopper and shake. This step sounds simple but is a frequent source of error in the analysis. The bottle-top dispensers that are generally used for this job will frequently discharge a small amount of reagent as the barrel of the dispenser is drawn up. Do not place the sample flask under the dispenser until the barrel has been pulled up and this reagent has dripped from the tip of the dispenser. They may also discharge a small quantity of air (as bubbles) so it can be appropriate to discharge a small amount to waste and draw up to refill the dispenser. Immerse the dispensing tip fully and dispense the reagent slowly and not too forcefully. The objective is to get the reagent to the bottom of the flask without too much mixing in the flared neck of the flask because this reagent will be lost when the flask is stoppered and could bias the sample if reaction takes place in the upper neck. The NaI/NaOH dispenser will often begin to stick as the reagent dries out in the barrel of the dispenser (refer section 5.1b above). It is essential that the barrel of the dispenser be pulled fully up to the stop so that the full amount of this reagent is dispensed. This can become tricky to sense as the barrel becomes harder to pull up. Routinely you should disassemble and rinse the parts of the dispenser several times during a long cruise.

h. Stopper the flask being careful not to trap any air under the stopper. 2 ml of sample equal to the volume of the two reagents will be lost as the stopper is inserted. This will be taken into account in the calculations.

i. Shake the flask vigorously for several seconds to thoroughly mix the reagents with the sample using your thumb to secure the stopper. If possible, carry this out over a sink; and the shaking motion must be of a vigorous "snapping of the wrist" involving bottle inversion. This is of the utmost importance. If you merely shake, even vigorously, in a backward and forward motion; the sample and reagents will not be mixed properly and thus of no use, with the analyst obtaining exceptionally erratic (and false) results unless aware of the wrong mixing technique.

j. Add water to the neck of the flask using a squeeze bottle. This creates a seal and prevents air from entering the flask. This step is especially important for 2 prime reasons; the first being dry environments such as well air-conditioned ships, and the second being that warmer samples will still be contracting as they cool to ambient. If you fail to do this, bubbles will form in the flask during the wait before they are analyzed and the oxygen concentration will be several μ moles higher than it should be, and this cannot be corrected for as it is not consistent.

k. A second shaking of the flasks is recommended after approximately 30 minutes to ensure that all the oxygen in the flask has fully reacted with the reagents. After the second shaking, it is strongly advised to again seal the flared necks with a quantity of distilled water as there are still changes in flask temperatures occurring and they may not, for whatever reason, be able to be analyzed within a short time.

l. Storage: Generally the flasks are left in a covered crate to warm up in the lab for 1 ½-2 hours before analyzing. However, if the water seal is maintained, the flasks can be stored for many days without any noticeable change in concentration.

7. TITRATION PROCEDURES

7.1 Reagent blank determination – Carpenter (1965) method.

a. As always, even during analyses, please "BE SAFE" and wear goggles and if possible, gloves.

b. Fill flask half full with DIW and add stir bar and swirl to get a mix started.

c. Add 1.0 ml KIO_3 solution using precision dispenser and mix.

d. Add 1 ml of H_2SO_4 solution and mix.

e. Add 1 ml of NaI/NaOH solution and mix.

f. Add 1 ml of $MnCl_2$ solution and mix.

g. Fill flask up to neck with DIW.

h. Titrate the flask with thiosulfate exactly to the endpoint. Do not overshoot the endpoint. Record the endpoint as V1. On some automated titration systems it is not possible to prevent the titrator from overshooting the endpoint. In this case record the endpoint as V1 and final volume added as V3.

i. Add a second precise 1.0 ml aliquot of the KIO_3 standard solution to the same flask and titrate a second time. Record the endpoint as V2.

7.2 Standardization of thiosulfate solution – Carpenter (1965) method

a. Standardization should be performed at a temperature as close as possible to the temperature at which the oxygen samples will later be analyzed.

b. Fill flask half full with DIW and add stir bar and swirl to get a mix started.

c. Add 10.0 ml KIO_3 solution using precision dispenser and mix.

d. Add 1 ml of H_2SO_4 solution and mix.

e. Add 1 ml of NaI/NaOH solution and mix.

f. Add 1 ml of $MnCl_2$ solution and mix.

g. Fill flask up to neck with DIW.

h. Titrate the flask with thiosulfate and record the endpoint.

i. Generally four standards are run and the endpoints are averaged. The endpoints should be within ±0.3% of each other. It is possible also to aim for 3 concurrent endpoints. Record the average standard titre in units of ml or μl of titrant as V_{std}.

7.3 Standardization of thiosulfate and determination of reagent blank by "standard-curve" method

a. Prepare five standards as above using 2, 4, 6, 8 and 10 ml of KIO_3.

b. Titrate each standard and record the endpoint.

7.4 Titration of samples

a. Pour out the water "sealing" the neck of the flask and use a tissue to remove any remaining water.

b. Remove the stopper and add stir bar.

c. Add 1 ml of H_2SO_4, mix and allow the precipitate to dissolve. If all the precipitate does not dissolve, up to a second 1 ml of H_2SO_4 can be added. Once the pH is lowered by the addition of the H_2SO_4 the reaction of oxygen with the reagents and the subsequent liberation of I_2 ceases. This means that air bubbles present in the flask during mixing and titration are not a problem.

d. Note the temperature of the thiosulfate, t_L.

e. Titrate the sample to the endpoint and record. Denote the titre in units of ml or μl of titrant as V_{sam}.

f. Cleaning the flasks: the flasks and stopper pairs should be rinsed in fresh water to remove any residual reagents before reuse. It is not necessary to use DIW.

8. CALCULATION AND EXPRESSION OF THE RESULTS

8.1 Reagent blank

The reagent blank results from oxidizing or reducing impurities in the reagents that result in the amount of I_2 liberated not being exactly proportional to the amount of oxygen in the sample. It is given by the expression:

$$V_{blk} = V1-V2 \quad (1)$$

Note that the 1995 Oxygen SOP incorrectly gives V_{blk} as V2-V1.

If the titrator overshoots the endpoint V_{blk} can be computed by:

$$V_{blk} = V1 – (V2-(V3-V1)) = 2V1-V2-V3 \quad (2)$$

Where V1 is the volume of titrant used to titrate the first aliquot of KIO_3, V3 is the volume of titrant dispensed when the titrator stopped titrating the first aliquot and V2 is the volume of titrant used to titrate the second aliquot of KIO_3 to its endpoint. The units of V_{blk}, V1, V2 and V3 are ml of titrant.

Some investigators have noted the existence of redox species in the seawater sample that can alter the computed oxygen concentration by a few tenths of a μmole per kg. They have recommended that a seawater blank should also be determined. While it would be impractical to run a seawater blank on each sample it may be desirable for investigators to start running seawater blanks on a more frequent basis and to start reporting their values as this will make it possible to judge if the correction is variable in time or space and of a magnitude that may be important.

Note that there was an error in the WOCE (1995) Hydrographic Program Manual in the expression for computing the reagent blank; i.e., it also had V_{blk}=V2-V1.

8.2 Concentration of the standard KIO_3 solution

The concentration of the standard KIO_3 solution should be corrected from the temperature at which it was made up to 20°C by the expression:

$$M(KIO_3,20°C) = \frac{m(KIO_3)/(213.995 g \cdot mol^{-1})}{V_s} \times \frac{0.998206}{\rho_w(t_P)} \quad (3)$$

Where $m(KIO_3)$ is the mass of KIO_3 that was added to the volumetric flask and V_s is the volume of solution in the volumetric flask at the preparation temperature, t_P, 213.995 g mol^{-1} is the molar mass of KIO_3 and $\rho_w(t_P)$ is the density of pure water at the laboratory temperature when the solution was prepared.

Laboratory glassware is calibrated at 20°C. The volume of solution contained in the volumetric flask at the laboratory temperature is given by:

$$V_s = V_s[1+\alpha_V(t_L-20)] \quad (4)$$

Where α_V is the volumetric coefficient of expansion of Pyrex glass which is 9.75×10^{-6} ° K^{-1}.

8.3 Molarity of the thiosulfate titrant

The molarity of the thiosulfate solution at the laboratory temperature, t_L, can be calculated from the expression:

$$M(Na_2S_2O_3,t_L) = \frac{6000 \cdot V(KIO_3,t_L) \cdot M(KIO_3,t_L)}{(V_{std} - V_{blk})} \quad (5)$$

where

$$V(KIO_3,t_L) = V(KIO_3,20°C)\cdot(1+9.75\times10^{-5}(t_L-20)) \quad (6)$$

$$M(KIO_3,t_L) = M(KIO_3,20°C) \cdot (\rho_W(t_L)/0.998206) \quad (7)$$

6000 is (6 mol $Na_2S_2O_3$/1 mol KIO_3)*(10^3 µl/1 ml), V_{std} is the average volume of titrant used to titrate the KIO_3 standard solution and V_{blk} is the reagent blank, both in units of ml or µl of titrant.

8.4 Oxygen concentration in the seawater sample

The total number of moles of oxygen (sample + O_2 dissolved in the reagents) is given by the expression:

$$n(O_2) = (V_{sam}-V_{blk}) \cdot M(Na_2S_2O_3,t_L)*(1\ L/10^6\ \mu L) \cdot (1\ mol\ O_2/4\ mol\ Na_2S_2O_3) \quad (8)$$

$$C(O_2) = [n(O_2)-7.6x10^{-8}]/m(sample) \quad (9)$$

Where $7.6x10^{-8}$ mol O_2 is the amount of O_2 dissolved in the 2 ml of reagents ($MnCl_2$ + NaI/NaOH) added to the seawater sample and m(sample) is the mass of the seawater sample in kg. m(sample) is given by:

$$m(sample) = \{V(O_2\text{-flask},20\ °C) \cdot [1+9.75x10^{-5}(t_S-20)]-2\} \cdot \rho_{SW}(t_S,S) \quad (10)$$

where t_S is the temperature of the sample at the time it was pickled, 2 is the volume of seawater displaced by the addition of the pickling reagents and ρ_{SW} is the density of the seawater at the time of pickling.

8.5 Sample calculations

8.5.1 Preparing the KIO_3 standard solution

t_P = 24.0 °C
$m(KIO_3)$ = 0.3567 g
$Vs(t_P)$ = 1.00004 L --- Equation 4
$\rho_w(t_P)$ = 0.997299 g cm^{-3}
$M(KIO_3, 20\ °C)$ = 0.00166831 mol L^{-1} --- Equation 3

8.5.2 Reagent blank (Carpenter method)

V_{blk} is found as the difference between the titres of two standards prepared with 1 ml of KIO_3 using equation 1.

V1, µl	V2, µl	V1-V2, µl
68.09	65.59	2.5
69.09	67.09	2.0
68.59	66.09	2.5
Vblk	2.3	
SD	0.2	

8.5.3 Thiosulfate standardization (Carpenter method)

Prepare four standards using 10 ml of KIO3 standard solution and titrate.

Std	V_{std}, μL	M(thio,t_L)
1	989.97	0.100929
2	989.88	0.100939
3	989.21	0.101007
4	990.12	0.100914
Avg	989.80	0.10095
SE	0.17	0.00002

t_L = 25.1 °C
V_{blk} = 2.3 μL
V_{std} = 989.8 μL
$V(KIO_3, 20\ °C)$ = 9.9700 ml --- Calibrated volume of KIO_3 dispenser at 10 ml setting
$V(KIO_3,t_L)$ = 9.9705 ml --- Equation 4
$M(KIO_3, 20°C)$ = 0.0016631 mol L^{-1} --- From section 8.5.1
$M(KIO_3,t_L)$ = 0.00166633 mol L^{-1} --- Equation 7
$\rho_w(t_L)$ = 0.997022 g cm^{-3}
$M(Na_2S_2O_3,t_L)$ = 0.10095±0.00002 (SE) mol L^{-1} --- Equation 5

8.5.4 Thiosulfate standardization and reagent blank (Standard curve method)

Equation 5 can be rearranged to take the form:

$$V_{std} = \left(\frac{6x10^3 \cdot M(KIO_3, t_L)}{M(Na_2S_2O_3, t_L)} \right) V(KIO_3, t_L) + V_{blk} \qquad (11)$$

If a series of standards are prepared using different volumes of the KIO_3 say 2, 4, 8, and 10 ml and the titre of thiosulfate required to titrate these standards the molarity of the thiosulfate can be determined from the slope of the line and the reagent blank for the y-intercept. To illustrate, consider these data which were collected at the same time and using the same solutions as the example given in Section 8.5d.

$V(KIO_3,t_L)$, ml	V_{std}, μL
1.9761	201.6
3.9812	397.8
5.9853	596.3
7.9724	790.0
9.9705	989.8

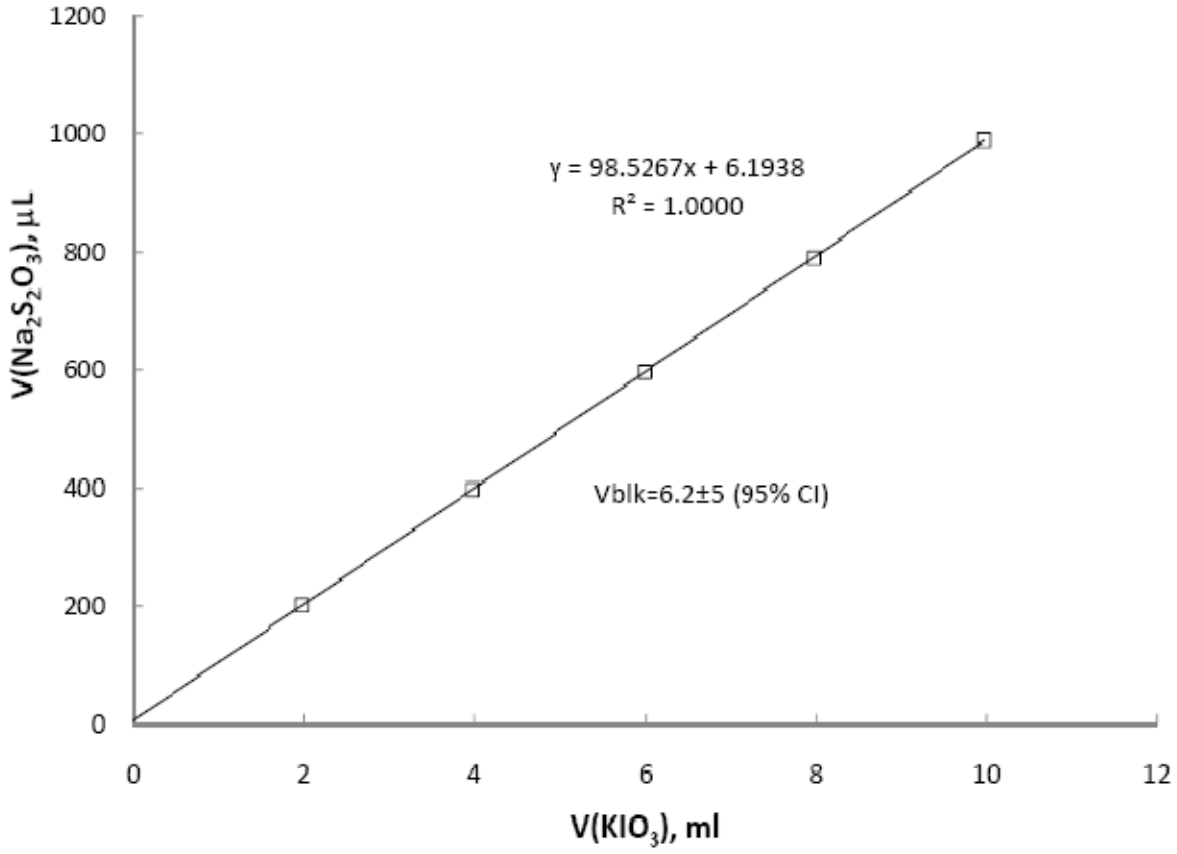

Figure 2. Standard curve method of estimating $M(Na_2S_2O_3)$ and V_{blk}.

The molarity of the thiosulfate can be computed from the slope of the regression line using the expression:

$$M(Na_2S_2O_3, t_L) = \left(\frac{6x10^3 \cdot M(KIO_3, t_L)}{m} \right) \quad (12)$$

where m is the slope of the standard curve.

$M(KIO_3,t_L)$ = 0.00166633 mol L^{-1}
m = 98.5267±0.21 (SE)
$M(Na_2S_2O_3,t_L)$ = 0.1015 ±0.0002 mol L^{-1}
V_{blk} = 6.2±1.4 (SE) µL

Estimates of the standard errors of the slope and intercept were obtained from the linear regression analysis. The error in the slope was propagated through Equation 12 to obtain the error in $M(Na_2S_2O_3,t_L)$.

Note that the errors in $M(Na_2S_2O_3,t_L)$ and V_{blk} obtained by the standard curve method are larger than those when the Carpenter (1965) methods are used.

For a typical oxygen sample the differences in $M(Na_2S_2O_3,t_1)$ and V_{blk} obtained by the two methods on the very same set of reagents and thiosulfate would amount to a difference of 0.6 μmol kg^{-1}. WOCE standards call for analyses to have accuracy better than 0.5% of the highest concentration that will be measured in the ocean. This is roughly 0.005*380 or 1.9 μmol kg^{-1}. So either method is adequate for meeting this standard but the Carpenter method for blank determination and thiosulfate standardization leads to more precise estimates of the parameters.

8.6 Comparison of blank determinations by the Carpenter and standard curve method

During Repeat Hydrography cruise A16S reagent blanks were determined by both the Carpenter and the standard curve methods. The results are shown in Figure 3.

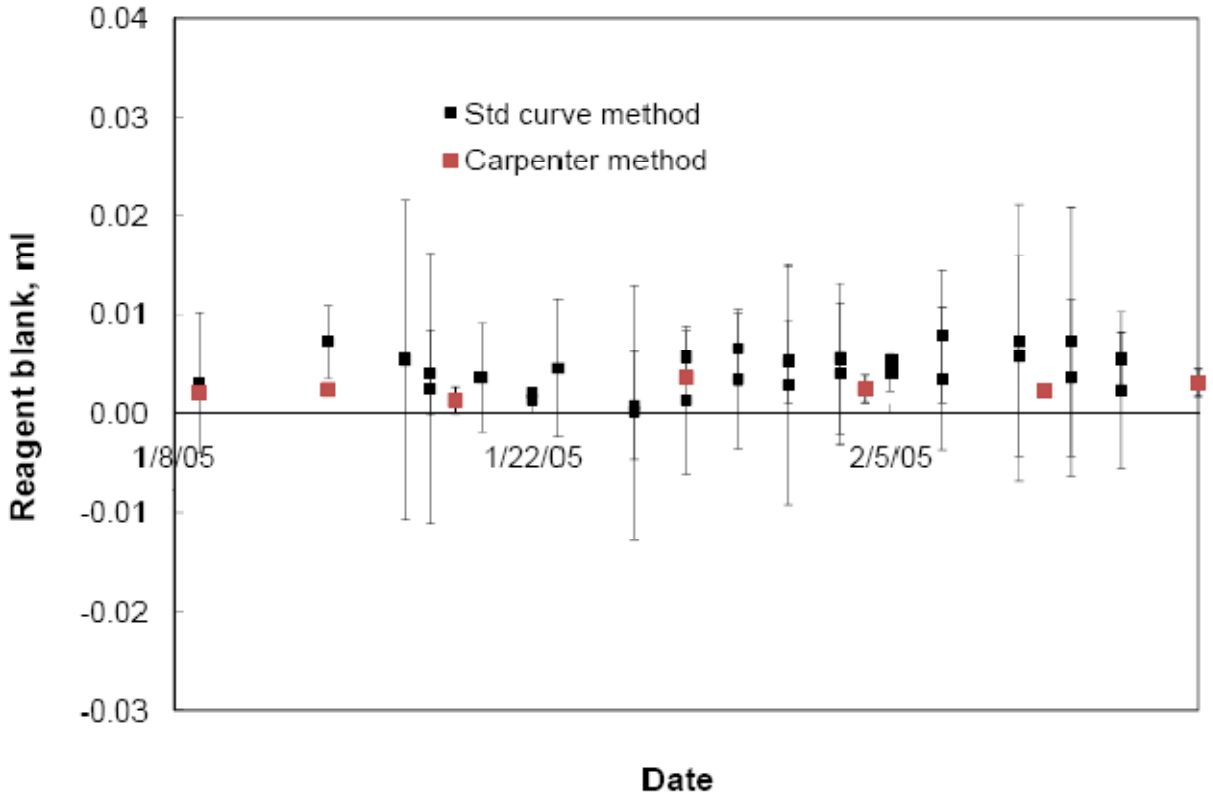

Figure 3. Reagent blanks determined by Carpenter method (red) and Standard Curve method (black). Error bars are the 95% CI.

The cruise-long average V_{blk} for the Carpenter method was 0.0025±0.0006 (SD) (range 0.001-0.004) and for the Standard Curve method it was 0.004±0.002 (SD) (range (0.0-0.008). Note the much larger range in V_{blk} by the Standard Curve method. The reagents were made from the same batch of chemicals so that V_{blk} would be expected to have constant value during the cruise. The wide confidence intervals on the V_{blk}'s determined by the 'standard curve" method are a reflection of the fact that they are determined by extrapolating the regression line to the y-axis.

Based on this comparison and the analysis of error in Section 8.5, it is recommended that the

original Carpenter (1965) methods for reagent blank determination and thiosulfate standardization be used for all CLIVAR/Repeat Hydrography work.

8.7 Comparison of amperometric and photometric endpoint methods

During the 2006 P16N cruise a comparison was conducted between a photometric end point titrator built by Gernot Friederich (1991) at MBARI and an amperometric end point titrator built by Chris Langdon at the University of Miami. Sets of duplicate flasks were drawn from the same rosette bottle and then analyzed by the two systems. The photometric system used a 5 ml Brinkman Dosimat to dispense the thiosulfate titrant and the amperometric system used a 2 ml Manistat burette driven by a stepper motor. Due to the different burette volumes the photometric system used a 0.040 M thiosulfate solution and amperometric system used a 0.14 M solution. Both burettes and the KIO_3 dispenser were calibrated gravimetrically before the cruise. Reagent blanks and standards for both systems were prepared using the same KIO_3 solution and the same dispenser. Data are summarized in Table 1. Each method was found to have a similar precision, i.e. ± 0.15 $\mu mol\ kg^{-1}$.

Table 1. Comparison of oxygen concentrations determined by photometric and amperometric endpoint detection methods.

			Photometric			Amperometric			Photo-Ampero
Cruise	CTD	Niskin	O2, µM	Mean	SD	O2, µM	Mean	SD	µM
A16S	106	8	259.8	259.75	0.05	259.70	259.65	0.05	0.10
			259.7			259.60			
A16S	106	20	175.6	175.55	0.05	175.80	175.80	0.00	-0.25
			175.5			175.80			
A16S	106	27	98.6	98.45	0.15	98.90	98.80	0.10	-0.35
			98.3			98.70			
A16S	106	33	218.6	218.80	0.20	218.70	218.75	0.05	0.05
			219.0			218.80			
P16N	58	8	124.9	125.10	0.20	125.50	125.60	0.10	-0.50
			125.3			125.70			
P16N	60	17	7.9	7.40	0.50	6.70	6.45	0.25	0.95
			6.9			6.20			
P16N	62	34	286.5	286.50	0.00	287.80	287.85	0.05	-1.35
			286.5			287.90			
P16N	66	33	307.4	307.53	0.09	307.52	307.47	0.05	0.05
			307.6			307.42			
P16N	68	33	313.8	313.92	0.08	313.96	313.95	0.01	-0.03
			314.0			313.94			
P16N	69	18	19.6	19.62	0.01	19.70	19.72	0.02	-0.10
			19.6			19.74			
P16N	70	1	145.9	145.96	0.06	146.14	146.09	0.05	-0.12
			146.0			146.03			
P16N	75	10	77.4	77.39	0.01	76.79	76.88	0.08	0.52
			77.4			76.96			
			Average		**0.12**			**0.07**	**-0.09**

9. REFERENCES

Bradbury, J. H. and A. N. Hambly. 1952. An investigation of errors in the amperometric and starch indicator methods for the titration of milli-normal solutions of iodine and thiosulfate. *Aust. J. Sci. Res. Ser. A* 5: 541-554.

Carpenter, J. H. 1965. The accuracy of the Winkler method for dissolved oxygen analysis. *Limnol. Oceanogr.* 10: 135-140.

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

Culberson, C. H. and S. Huang. 1987. Automated amperometric oxygen titration. *Deep-Sea Res.* 34: 875-880.

Culberson, C. H., G. Knapp, M. C. Stalcup, R. T. Williams and F. Zemlyak. 1991. *A comparison of methods for the determination of dissolved oxygen in seawater.* WHPO Publication 91-2.

Culberson, C. H. (1991). *Dissolved Oxygen*. WHPO Publication 91-1.

Dickson, A. D. 1995. *Determination of dissolved oxygen in sea water by Winkler titration.* WOCE Operations Manual, Part 3.1.3 Operations & Methods, WHP Office Report WHPO 91-1.

DOE. 1994. SOP 13 *Gravimetric calibration of volume contained using water.* Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water. A. G. Dickson and C. Goyet. ORNL/CDIAC74.

Foulk, C. W. and A. T. Bawden. 1926. *J. Amer. Chem. Soc.* 48: 2045-2049.

Friederich, G. E., Codispoti, L.A. and Sakamoto, C.M. 1991. *An easy-to-construct automated Winkler titration system.* MBARI Technical Report No. 91-6: Oxygen measurement.

Truesdale, G. A. and G. Knowles. 1956. Some recent work on dissolved oxygen in natural waters. *ICES J. du Conseil* 21(3): 263-267.

Winkler, L. W. 1888. Die Bestimmung des im Wasser gelosten Sauerstoffes. *Ber. Dtsch. Chem. Ges.* 21: 2843-2853.