Chapter 6. Determination of Dissolved Oxygen by the Winkler Procedure

Updated by: S. Bell and R. Johnson (April 1997), R. Little (April 1996); R. Kelly and R. Little (May 1994); R. Dow (July 1992); A. Michaels (March 1992); A. Michaels, R. Dow and N. Bates (May 1991)
Prepared by: A. Michaels and R. Dow (October 1989)
Modified from: Strickland and Parsons (1972)

1.0 Scope and field of application

This procedure describes a method for the determination of dissolved oxygen in seawater by means of an automated titration system which monitors changes in ultra-violet light transmission through the sample to determine the endpoint.[1] The method is suitable for the assay of oceanic levels, e.g. 0.5 to 350 μmol kg^{-1} of oxygen in uncontaminated seawater and is based on the Carpenter (1965) modification of the traditional Winkler titration. This method is unsuitable for seawater containing hydrogen sulfide.

2.0 Definition

The dissolved oxygen concentration of seawater is defined as the number of micromoles of oxygen gas (O_2) per kilogram of seawater (μmol kg^{-1}).

3.0 Principle of Analysis

The chemical determination of oxygen concentrations in seawater is based on the method first proposed by Winkler (1888) and modified by Strickland and Parsons (1968). The basis of the method is the quantitative oxidation of iodide ions to iodine by the oxygen in the seawater sample; the amount of iodine thus generated is determined by titration with a standard thiosulfate solution. The endpoint is determined by the absorption of ultraviolet light by the tri-iodide ion. The amount of oxygen originally contained in the sample can then be calculated from the titer: one mole of O_2 reacts with four moles of thiosulfate.

More specifically, dissolved oxygen is chemically bound to Mn(II)OH in a strongly alkaline medium which results in a brown floc/floculent, manganic hydroxide ($MnO(OH)_2$). After complete fixation of oxygen and formation of the mixed manganese (II) and (III) hydroxides floc, the sample is acidified to a pH between 2.5 and 1.0. This causes the precipitated hydroxides to dissolve, liberating the Mn(III) ions. The Mn(III)

[1] The automated titration system and the accompanying software used by BATS were designed by Robert Williams of Scripps Institute of Oceanography.

ions oxidize previously added iodide ions to iodine. Iodine forms a tri-iodide complex with surplus iodide ions. The complex formation is desirable because of its low vapor pressure and rapid decomposition when iodine is removed from the system. The iodine is then titrated with thiosulfate; iodine is reduced to iodide and the thiosulfate is oxidized to tetrathionate. The stoichiometric equations for the reaction described above are:

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

$$2Mn(OH)_2 + {}^1/_2O_2 + H_2O \rightarrow 2MnO(OH)_2$$

$$2Mn(OH)_3 + 2I^- + 6H^+ \rightarrow 2Mn^{2+} + I_2 + 6H_20$$

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

$$I_3^- + 2S_2O_3^{2-} \rightarrow 3I^- + S_4O_6^{2-}$$

The thiosulfate can change composition and therefore must be standardized with a primary standard, typically potassium iodate. Standardization is based on the co-proportionation reaction of iodide with iodate, thereby forming iodine. As described above, the iodine binds with excess iodide, and the complex is titrated with thiosulfate. One mole of iodate produces three moles of iodine, an amount that reacts with six moles of thiosulfate.

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

$$I_3^- + 2S_2O_3^{2-} \rightarrow 3I^- + S_4O_6^{2-}$$

4.0 Apparatus

4.1 *Sampling apparatus*

4.1.1 *Sample flasks*: Pyrex® iodine determination (BOD) flasks of 140 ml nominal capacity with ground glass barrel stoppers. The precise volume of each stopper/flask pair is determined gravimetrically by weighing with Milli-Q water. It is essential that each individual stopper/flask pair be marked to identify them and that they be kept together for subsequent use.

4.1.2 *Pickling reagent dispensers*: two dispensers capable of dispensing 1 ml aliquots of the pickling reagents. The accuracy of these dispensers should be 1% (i.e. 10 µl).

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

4.1.4 *Thermometers*: directly after sampling a thermometer is used to measure the water temperature to within 0.5°C.

4.2 *Automated titration apparatus*

4.2.1 *Metrohm 655 Dosimat burette*: a piston burette capable of dispensing 1 to 10 ml of KIO_3 for blank determination and standardization.

4.2.2 *Metrohm 665 Dosimat Oxygen Auto-titrator*: a piston burette capable of dispensing 2 µl aliquots of thiosulfate (the Dosimat 665), an ultra-violet light source UV and a detector to measures the change in UV transmission through the sample.

4.2.3 *PC computer*. The burette, endpoint detector and A/D convertor are controlled by an IBM compatible PC, in a system designed by R. Williams (SIO).

4.2.4 *Thermometers*. Platinum resistance temperature sensors are mounted to the bottles containing the potassium iodate standard and the thiosulfate solution, in order to correct the volumes dispensed to the corresponding volume at 20°C.

4.2.5 *Dispenser*: capable of delivering 1 ml aliquots of the sulfuric acid solution.

4.2.6 *Magnetic stirrer and stir bars*.

5.0 Reagents

5.1 *Manganese* (II) *chloride* (3M: reagent grade): 600 g of $MnCl_2 \cdot 4H_2O$ are dissolved in approximately 600 ml of Milli-Q water. After complete dissolution the solution is filtered into a Class A volumetric flask and then diluted with Milli-Q to a final volume of 1000 ml. The reagent is twice gravity filtered through 24µm particle retention paper filters, and stored in an amber plastic bottle.

5.2 *Sodium Iodide* (4M: reagent grade) and *sodium hydroxide* (8M: reagent grade): 600 g of NaI are dissolved in approximately 600 ml of Milli-Q water. 320 g of NaOH are added to the solution, usually in 80 g aliquots, while it is cooling in a water bath. The solution is then twice gravity filtered through 24µm particle retention paper filters into a Class A volumetric flask and diluted to a final volume of 1000 ml with Milli-Q. The reagent is stored in an amber glass bottle.

5.3 *Sulfuric Acid* (50% v/v): 500 ml of reagent grade concentrated H_2SO_4 are slowly added to 500 ml of Milli-Q water. The mixture is cooled in a water bath during the addition of acid and stored in a glass bottle.

5.4 *Sodium Thiosulfate* (0.18 M: reagent grade): Approximately 45 g of $Na_2S_2O_3 \cdot 5H_2O$ are dissolved in Milli-Q water to a final volume of 1000 ml. This solution is stored in an amber glass bottle in the refrigerator until use.

5.5 *Potassium Iodate Standard* (0.0100 N): A commercial standard (CSK standards solution) is used as the primary standard, purchased from Wako Chemicals (Wako Chemicals USA Inc. 1600 Bellwood Road, Richmond, VA 23237, USA. Tel: 804 271-7677).

6.0 Sampling

Collection of water from the OTE bottle must be done soon after opening, preferably before any other samples have been drawn. This is necessary to minimize exchange of oxygen with the head space in the OTE bottle which typically results in contamination by atmospheric oxygen.

6.1 Samples are collected at all depths in gravimetrically volume calibrated BOD flasks from each OTE bottle. The first sample is termed O2-1. For precision estimates and redundancy at extrema, a further two or more samples are drawn which are termed 02-2, 02-3, etc. Typically 30% of the depths are sampled in triplicate.

6.2 Before the oxygen sample is drawn the spigot on the OTE bottle is opened while keeping the breather valve closed. If no water flows from the spigot it is unlikely that the bottle has leaked. If water leaks, it is probable that the OTE bottle has been contaminated with water from shallower depths. The possibility of contamination is noted on the cast sheet for the appropriate bottle.

6.3 The oxygen samples are drawn into the individually iodine determination flasks. It is imperative that the flask and stopper are a matched pair. If replicates are to be taken from a particular OTE bottle, they are drawn immediately following the first sample (O2-1).

6.4 Great care is taken to avoid introducing air bubbles when drawing the sample. A 30–50 cm length of Tygon® tubing is connected to the OTE bottle spout. The end of the tube is elevated before the spout is opened to prevent the trapping of bubbles in the tube. With the water flowing, the tube is placed in the bottom of the horizontally held

sample flask in order to rinse the sides of the flask and the stopper. The flask is inverted upright for a few seconds so as to flush out any air bubbles that may be adhering to the flask walls. The flask is then returned to an upright position and four to five volumes of water are allowed to overflow. The tube is then slowly withdrawn from the flask while water is still flowing.

6.5 Immediately after obtaining the seawater sample, the tip of an automatic dispenser is submerged well into the sample to introduce 1 ml of manganous chloride into the flask. This is followed by addition of 1 ml of sodium iodide-sodium hydroxide solution from a second dispenser, also by submerging the tip in the sample.

6.6 The stopper is carefully placed in the bottle ensuring that no bubbles are trapped inside. The bottle is vigorously shaken, then reshaken approximately 20 minutes later after the precipitate has settled to the bottom of the bottle.

6.7 Once all oxygen samples from a particular OTE bottle have been drawn, the temperature of the water from the bottle is measured and recorded.

6.8 Sample bottles are stored upright in a cool, dark location and the necks are sealed with surface seawater. Samples are analysed after a period of at least 6-8 hours but within 36 hours. The samples are stable in this time period.

7.0 Titration Procedures

7.0.1 The basic steps in titrating oxygen samples differ little regardless of the system used. First the precise concentration of the thiosulfate must be determined. Next the blank - the impurities in the potassium iodate primary standards which participate in the series of oxidation-reduction reactions involved in the analysis - is calculated. Once the standard titer and blank have been determined, the samples can be titrated.

7.0.2 The auto-titrator used by BATS has a UV detector which measures the transmission of ultra-violet light through the sample (standard, blank or seawater-sample) as the thiosulfate is added. Initially the Metrohm 665 Dosimat rapidly dispenses 0.2 N thiosulfate. As the changes in UV absorption decrease the thiosulfate addition rate is slowed, and finally the continuous addition is stopped. The endpoint is approached by addition of ever-smaller increments of thiosulfate until no further change in absorption is detected, indicating that the endpoint has been passed. The actual end is determined by a least squares linear fit using a group of data points just prior to the endpoint, where the slope of the titration curve is steep, and a group of points after the endpoint, where the slope of the curve is close to zero. The intersec-

tion of the two lines of best fit is taken as the endpoint. Reproducibility should be better than 0.01 ml l^{-1}. Standardization, blank determination, and sample analysis are described below.

7.1 *Standardization*:

7.1.1 Using the Metrohm 655 Dosimat, 10 ml of standard potassium iodate (0.0100 N) are dispensed into a flask containing about 15 ml of Milli-Q water and a stir bar. The solution is swirled, and 1 ml of 50% sulfuric acid solution added. The sides of the flask are rinsed with additional Milli-Q and the flask swirled to ensure the acidic solution is well mixed before the addition of the pickling reagents.

7.1.2 1 ml of sodium iodide-sodium hydroxide reagent and then 1 ml of manganese chloride reagent are added to the acidified solution. The solution is mixed thoroughly after each addition. The solution is gently swirled and the sides of the flask rinsed with Milli-Q water and filled to the neck.

7.1.3 Titration of the liberated iodine with thiosulfate is started immediately, as described in Section 7.02. Reproducibility of standards should be better than 0.01 ml l^{-1}.

7.1.4 The mean value of at least four replicate standards is determined. The standard titer must have standard deviation of less than +/- 0.006 ml l^{-1} before samples can be run. Standards are run periodically throughout the time that samples are being titrated.

7.2 *Blank determination*:

7.2.1 1 ml of standard potassium iodate is added to a flask containing about 15 ml of Milli-Q water and a stir bar, using the Metrohm 655 Dosimat. The solution is swirled and then 1 ml of 50% sulfuric acid solution is added. The sides of flask are rinsed with additional Milli-Q and the flask swirled to ensure an acidic solution before the addition of the pickling reagents.

7.2.2 Before beginning the titration the pickling reagents are added. 1 ml of sodium iodide-sodium hydroxide reagent and 1 ml of manganese chloride reagent are added to the flask. The solution is gently swirled and the sides of the flask rinsed with Milli-Q water to ensure mixing. The flask is filled to just below the neck with Milli-Q water and then the blank is titrated to the endpoint, as described in Section 7.02.

7.2.3 A second 1 ml of the standard potassium iodate is added to the same solution which is again titrated to the end point. The difference between the first and second titration is the potassium iodate blank. Both positive or negative blanks are found.

7.3 *Sample analysis:*

7.3.1 Samples are analysed after at least 8 hours from when they were drawn. The water in the neck is carefully removed, taking care to minimize disturbance of the precipitate. The top of the flask is wiped with a KimWipe to remove any remaining moisture and the stopper carefully removed.

7.3.2 1 ml of 50% sulfuric acid and a stir bar are added to the flask. Care is taken to minimize disturbance of the precipitate.

7.3.3 Samples are titrated as described in Section 7.02.

8.0 Calculation and expression of results

The calculation of oxygen concentration (µmol l^{-1}) from this analysis follows in principle the procedure outlined by Carpenter (1965).

$$O_2\,(ml\,l^{-1}) = \frac{(R - R_{b/k})\,V_{IO_3} \cdot M_{IO_3} \cdot E}{(R_{Std} - R_{b/k})\,(V_b - V_{reg})} - \frac{DO_{reg}}{V_b}$$

Where:

R	=	Sample titration (ml)
$R_{b/k}$	=	Blank as measured above (ml)
V_{IO_3}	=	Volume of KIO_3 standard (ml)
M_{IO_3}	=	Molarity of standard KIO_3 (mol/l)
E	=	5,598 ml O_2/equivalent
R_{Std}	=	Volume used to titrate standard (ml)
V_b	=	Volume of sample bottle (ml)
V_{reg}	=	Volume of reagents (2 ml)
DO_{reg}	=	oxygen added in reagents

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

8.2 *Conversion to µmol kg*$^{-1}$: To make an accurate conversion to µmoles kg^{-1} two corrections are needed: (1) a correction for the actual amount of thiosulfate delivered by the burette (which is temperature dependent); and (2) a correction for the volume of the sample at the time it was drawn. Both calculations are undertaken automatically in many versions of software driven titration. Two pieces of information are required: (a) the temperature of the sample (and bottle) at the time of fixing; the reasonable assumption being that the two are the same; (b) the temperature of the thiosulfate at the time of dispensing. Some versions of the automatic titration may also call for *in situ* temperature, as well as salinity, which allow for the calculation of oxygen solubility and thus the percentage saturation and AOU.

9.0 Quality assurance

9.1 *Quality Control*: Thirty percent of the oxygen samples are replicated in at least triplicate. Replicates are taken at depths where the *in situ* CTD oxygen profile indicates extreme points. A mean squared difference (equivalent to a standard deviation of repeated sampling) is the measure of precision for these profiles. As this replication takes into account all sources of variability (e.g. sampling, storage, analysis) it gives a slightly larger imprecision than indicated by the analytical precision of the titration (e.g. repeated measures of standards in the lab). In addition, periodic precision tests are done by collection and analysis of 5–10 samples from the same OTE bottle. This precision should be better than 0.01 ml l^{-1}. Field precision can vary from 0.005 to 0.03 depending on the sea conditions and the performance of the auto-titrator. Samples are reduced to oxygen concentrations prior to the next cruise to identify any degradation of the precision before additional profiles are collected and analysed.

9.2 *Quality assessment*: No absolute standard exists for oxygen analysis. Standards are made by gravimetric and volumetric measurements of reagent grade chemicals. Standard solutions are relatively stable and provide an early warning of errors by changes in their titer. Profiles of oxygen are examined visually and numerically. At any depth where the replicates differ by 0.02 ml l^{-1} or greater, the samples are carefully scrutinized. The profile is compared with the historical profiles for consistency, particularly in the deep water. These profiles are also compared with the CTD oxygen sensor. Although CTD oxygen sensors are very imprecise and inaccurate, they provide a continuous record. Deviations from the general shape of the profile by a single oxygen sample is evidence of inaccuracy in the wet oxygen measurement.

10.0 Acknowledgments

We would like to thank Robert Williams (Scripps Institute of Oceanography) for his time and patience in getting our current automated titration system up and running. With his

assistance we have greatly improved the precision of oxygen measurements made at BATS.

11.0 References

Carpenter, J.H. (1965). The Chesapeake Bay Institute. Technique for the Winkler oxygen method. *Limnol. Oceanogr.*, **10**, 141–143.

Grasshoff, K. Ehrhardt, M, and K. Kremling (1983). Methods of Seawater Analysis. Grasshoff, Ehrhardt and Kremling, eds. Verlag Chemie GmbH. 419 pp.

Murray J.N., Riley, J.P. and Wilson, T.R.S. (1968). The solubility of oxygen in Winkler reagents used for the determination of dissolved oxygen. *Deep-Sea Res.*, **15**, 237–238.

Strickland, J.D.H., and Parsons, T.R. (1968). Determination of dissolved oxygen. in *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, Bulletin, **167**, 71–75.

Williams, P.J.leB., and Jenkinson, N.W. (1982). A transportable microprocessor-controlled precise Winkler titration suitable for field station and shipboard use. *Limnol. Oceanogr.*, **27** (3), 576–584.

Winkler, L.W. (1888). Die Bestimmung des in Wasser gelösten Sauerstoffen. *Berichte der Deutschen Chemischen Gesellschaft*, **21**: 2843–2855.