

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedure for Seawater Dissolved Oxygen Analysis

Version 1.1

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Signatures on File

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*Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.*

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
3/19/2007	1.0	Added "Seawater" to title; footer	all	Bill Kammin
8/22/2007	1.1	Minor changes to procedure	all	Adrienne Stutes

## Environmental Assessment Program

### Standard Operating Procedure for Dissolved Oxygen Analysis

#### **1.0 Scope and Application**

- 1.1 This Standard Operating Procedure (SOP) is for the analysis of dissolved oxygen samples collected during all seawater sampling events conducted by the Coastal and Estuarine Assessment Unit.
- 1.2 This SOP is not suitable for the analysis of dissolved oxygen in freshwater samples.

#### **2.0 Summary of Method**

- 2.1 Prepare and run standards.
- 2.2 Prepare and run blanks.
- 2.3 Prepare and run samples.

#### **3.0 Interferences**

- 3.1 After the fixed samples have been acidified, the iodide solution is sensitive to photochemical oxidation. Therefore, exposure of the iodide solution to sunlight or other UV light sources must be avoided and samples should be titrated as soon as possible after acidification.

#### **4.0 Sample Collection, Preservation, Storage and Holding Times**

- 4.1 Fixed samples must be kept in the cold and dark until acidification and analysis. Samples must be stored for no more than 5 days after collection.

#### **5.0 Apparatus and Materials**

- 5.1 De-ionized water (18 Megohm)
- 5.2 Safety apron
- 5.3 Safety goggles
- 5.4 Nitrile exam gloves
- 5.5 10 mL pipette and tips
- 5.6 1.0 mL pipette and tips
- 5.7 Kimwipes
- 5.8 Metrohm® 775 Dosimat titrator with magnetic stirrer and stir bar

## 6.0 Reagents

- 6.1 3 M Manganese chloride ( $\text{MnCl}_2$ ) (obtained from the University of Washington's Marine Chemistry Lab). This chemical is stable for 2 years when stored in sealed plastic bottles and kept in the dark. The MSDS may be found at <http://www.jtbaker.com/msds/englishhtml/m0767.htm>.
- 6.2 8 N Sodium hydroxide-sodium iodide sodium-azide ( $\text{NaOH-NaI-Azide}$ ) (obtained from the University of Washington's Marine Chemistry Lab). This chemical is stable for 2 years when stored in sealed plastic bottles and kept in the dark. Sodium azide is a suspected carcinogen and should be treated with care. The MSDS's for  $\text{NaOH}$ ,  $\text{NaI}$  and  $\text{NaN}_3$  can be found at <http://www.jtbaker.com/msds/englishhtml/S4034.htm>, <http://www.jtbaker.com/msds/englishhtml/S4202.htm> and <http://www.jtbaker.com/msds/englishhtml/S2906.htm>.
- 6.3 10 N Sulfuric Acid ( $\text{H}_2\text{SO}_4$ ) (obtained from the University of Washington's Marine Chemistry Lab). This chemical is stable for 2 years when stored in a sealed plastic or glass bottle and kept in an acid cabinet.  $\text{H}_2\text{SO}_4$  is extremely poisonous, corrosive and most likely carcinogenic. Extreme care must be used when handling this chemical. The MSDS for  $\text{H}_2\text{SO}_4$  may be found at <http://www.jtbaker.com/msds/englishhtml/S8234.htm>.
- 6.4 0.01 N Sodium Thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \bullet 5\text{H}_2\text{O}$ ) (obtained from the University of Washington's Marine Chemistry Lab). Sodium thiosulfate is made by dissolving 49.64g sodium thiosulfate + 0.1g  $\text{Na}_2\text{CO}_3$  to 1 liter volume with de-ionized water. This chemical is relatively inert and is stable for 2 years when stored in a sealed plastic bottle and kept in the dark. MSDS's for  $\text{Na}_2\text{S}_2\text{O}_3 \bullet 5\text{H}_2\text{O}$  and  $\text{Na}_2\text{CO}_3$  may be found at <http://www.jtbaker.com/msds/englishhtml/S5230.htm> and <http://www.jtbaker.com/msds/englishhtml/S3242.htm>.
- 6.5 Starch soluble, aqueous solution (obtained from the University of Washington's Marine Chemistry Lab). Starch aqueous solution is a super-saturated solution of starch soluble and de-ionized water. The starch solution does not pose any known health risks. The MSDS may be found at <http://www.jtbaker.com/msds/englishhtml/S6506.htm>.
- 6.6 0.01 N Potassium Iodate ( $\text{KIO}_3$ ) (obtained from the University of Washington's Marine Chemistry Lab). Potassium iodate is a strong oxidizer and should be handled with care. The MSDS may be found at <http://www.jtbaker.com/msds/englishhtml/P5895.htm>.

## 7.0 Procedure

### 7.1 Dissolved Oxygen Determination with Dosimat

7.1.1 *Notes:*

7.1.1.1 *\*This Standard Operating Procedure (SOP) does not attempt to describe the entire procedure for marine waters Dissolved Oxygen (D.O.) determination, but only the laboratory portion. It assumes that proper sampling protocols have been followed, that the sample was collected in a 130 mL D.O. flask, and that the sample has had 1 mL manganous chloride solution, followed by 1 ml of alkaline sodium hydroxide-sodium iodide reagent added soon after sampling. Care must have been taken to seal the sample bottle(s), excluding all air bubbles.*

7.1.1.2 *\*This is an analytical chemistry technique. The glassware and equipment -- standard and sample bottles, pipettes, stir bars, and buret tip must be kept **scrupulously clean**.*

7.1.1.3 *\*Thoroughly rinse the glassware with clean hot water before and after every analysis. Clean every three months using Liqui-Nox® and water. Clean the dosimat as needed.*

7.1.1.4 **\*\*Prior to titration, 1 mL of sulfuric acid must be added to samples and samples must be carefully shaken. If sample is still cloudy after addition of 1 mL of sulfuric acid, add another 1 mL of acid. This should result in a clear sample.\*\***

7.1.2 **Turn on** the Dosimat by pressing the **FILL** button at the same time you turn on the **POWER** button (the red button in back).

7.1.3 **Press GO.**

7.1.4 **Press CLEAR.** The display should read **DOS 0.000 ml.**

7.1.5 **Gently** lift the amber bottle of thiosulfate. Shake, then replace in the Dosimat.

7.1.6 **Turn** the dispense speed knob (labeled **dv/dt**) to 10. Dispense 15-20 ml of thiosulfate to flush out the buret (3-5 ml aliquots) by pressing the hand control button. Be sure there are no bubbles in the buret or moving bubbles in the line leading to the buret tip.

7.1.7 **Turn** the dispense speed knob to 1.

7.1.8 **Press** the **CLEAR** button.

7.1.9 **Rinse** off the buret tip with deionized water.

7.1.10 **Turn** the stirrer on.

- 7.1.11        **Pipetting Tips:**
- 7.1.12.1      **Always** shake the reagent before pipetting.
- 7.1.12.2      **Draw** reagent from a smaller vessel.
- 7.1.12.3      **Hold** the pipette straight up & down, never angled.
- 7.1.12        **IMPORTANT: NEVER DRAIN LIQUID BACK INTO THE REAGENT BOTTLE**
- 7.1.13        **Dispense** straight into the sample bottle. Do not put the tip of the pipette against the wall of the sample bottle.
- 7.1.14        **Rinse** the sides of the sample bottle w/ DI water after each chemical addition to rinse down any reagent that may have splashed onto the side.
- 7.2            Preparing and running O2 standards**
- 7.2.1        **Fill** clean standard sample bottle about  $\frac{3}{4}$  full of distilled water.
- 7.2.2        **Add** a clean stir bar.
- 7.2.3        Using a pipette, **add** 1 ml 10 N H<sub>2</sub>SO<sub>4</sub> and mix well.
- 7.2.4        Using a pipette, **slowly** add 1 ml of 8 N NaOH-NaI-azide solution. Mix well. If sample is not clear, discard and start again.
- 7.2.5        Using a pipette, **add** 10 ml of the 0.01 N KIO<sub>3</sub> standard.
- 7.2.6        Using a pipette, **add** 1 ml of starch aqueous solution.
- 7.2.7        **Rinse** inside of flask well with DI water to ensure no chemical residue has adhered to the sides of the flask.
- 7.2.8        **Position** the sample bottle on the stirrer; making sure the burette tip is under the surface of the sample.
- 7.2.9        **Make** sure that the Dosimat reads 0.000 ml (press **CLEAR** to zero).
- 7.2.10        **Titrate** sample to the endpoint by dispensing thiosulfate in the sample using the thumb button. The endpoint is achieved when all color is gone. Watch the vortex in the upper half of the bottle. The endpoint is a subtle difference between clear and sparkling clear.

- 7.2.11 **Record** endpoint.
- 7.2.12 **Remove** sample bottle and dispense a few drops of thiosulfate through the burette tip to flush out any sample residue.
- 7.2.13 **Rinse** down the burette tip with deionized water.
- 7.2.14 **Press** CLEAR to zero the Dosimat.
- 7.2.15 **Run** at least 3 standards; at least 2 out of 3 must agree to  $\pm 0.001$  ml.
- 7.3** **Blanks**
- 7.3.1 **Fill** a standard sample bottle  $\frac{3}{4}$  full of distilled water.
- 7.3.2 **Add** a clean stir bar.
- 7.3.3 Using a pipette, **add** 1 ml 10 N H<sub>2</sub>SO<sub>4</sub> and mix well.
- 7.3.4 Using a pipette, **slowly** add 1 ml of 8 N NaOH-NaI solution. Mix well. If sample is not clear, discard and start again.
- 7.3.5 Using a pipette, **add** 1 ml 3 M MnCl<sub>2</sub>. Mix well.
- 7.3.6 Using a pipette, **add** 1 ml of the 0.01 N KIO<sub>3</sub> standard.
- 7.3.7 Using a pipette, **add** 1 ml of starch aqueous solution.
- 7.3.8 **Rinse** inside of flask well with DI water to ensure no chemical residue has adhered to the sides of the flask.
- 7.3.9 **Position** the sample bottle on the stirrer; making sure the burette tip is under the surface of the sample.
- 7.3.10 **Make** sure that the Dosimat reads 0.000 ml (press **CLEAR** to zero).
- 7.3.11 **Titrate** sample to the endpoint. **Titrate slowly**. Remember this is only 1/10 as strong as the standard.
- 7.3.12 **Record** endpoint #1; this is Blank1.
- 7.3.13 **Add** 1 ml more of KIO<sub>3</sub> standard.

- 7.3.14 **Titrate** to endpoint #2.
- 7.3.15 **Blank 2** is obtained by subtracting (Endpoint #2) - (Endpoint #1)
- 7.3.16 **The Correction Blank** is obtained by subtracting (Blank1) - (Blank2). The **Correction Blank** must be  $\pm 0.001$ .
- 7.4 **Definitions:**
- 7.4.1 Blank 1 (in ml) = volume of thiosulfate needed to titrate the first 1 ml KIO<sub>3</sub> + reagents
- 7.4.2 Blank 2 (in ml) = volume of thiosulfate needed to titrate the second 1 ml KIO<sub>3</sub>
- 7.4.3 Therefore, Blank 1 – Blank 2 = correction factor to account for any impurities in reagents.
- 7.4.4 This value may be negative or positive or zero.
- 7.5 Samples**
- 7.5.1 **Carefully** remove the cap, and rinse the stopper into the sample bottle.
- 7.5.2 **Add** a clean stir bar.
- 7.5.3 Using a pipette, **add** 1 ml of starch aqueous solution.
- 7.5.4 **Position** the sample bottle on the stirrer; making sure the buret tip is under the surface of the sample.
- 7.5.5 **Rinse** inside of flask well with DI water to ensure no chemical residue has adhered to the sides of the flask.
- 7.5.6 **Make** sure that the Dosimat reads 0.000 ml (press **CLEAR** to zero).
- 7.5.7 **Titrate** sample to the endpoint by dispensing thiosulfate in the sample using the thumb button. The endpoint is achieved when all color is gone. Watch the vortex in the upper half of the bottle. The endpoint is a subtle difference between clear and sparkling clear.
- 7.5.8 **Record** endpoint.
- 7.5.9 **Remove** sample bottle; dispense a few drops of thiosulfate through the buret tip to flush out any sample residue.
- 7.5.10 **Rinse** down the buret tip with deionized water.
- 7.5.11 **Press CLEAR** to zero the Dosimat.

## 7.6 Back Titration

7.6.1 If you miss your endpoint (i.e. you titrate past the point where the sample turns clear), you must perform a 'back titration'. This is done as follows:

7.6.1.1 Using a pipette, **add** 1 ml of the 0.01 N KIO<sub>3</sub> standard.

7.6.2 **Titrate** to the endpoint.

7.6.3 **Take** this final reading and subtract the volume needed to titrate 1 ml of KIO<sub>3</sub> standard. This is your 'true' endpoint and can be recorded in the 'buret reading' column of the log sheet.

7.6.4 **Write** 'over-titrated - added 1 ml KIO<sub>3</sub>' in the "comments" column on the log sheet, followed by the final reading and volume subtracted used to calculate the "true" endpoint. For example: over-titrated – added 1 mL KIO<sub>3</sub>; (.955-.050)

## 7.7 Disposal

7.7.1 **The titrated** standards, blanks and samples are rinsed down the drain with copious amounts of tap water. The solution is acidic so it must be diluted as much as possible to reduce any impact on the wastewater treatment plant. Do not pour down the "live" sink.

7.7.2 **Rinse** all glassware, pipette tips and small plastic beakers with hot water (3 rinses), followed by 3 rinses with DI water

7.7.3 Bottle numbers and burette readings must be recorded on DO Analysis Data Sheets. Data should be entered into dissolved oxygen spreadsheets as soon as possible after samples are run.

## 8.0 **Calibration and Standardization**

8.1 Standards and blanks must be run before acidified samples are run (as per 'Procedure' above). At least 2 of the 3 standards must agree to  $\pm 0.001$  and the two blanks must agree to  $\pm 0.001$ . If standards and blanks are not agreeing, the reagents may be old or contaminated and new reagents should be used instead.

## 9.0 **Calculations**

9.1 The Correction Blank is obtained by subtracting (Blank 1) – (Blank 2). Blank 2 is obtained by subtracting (Endpoint #2) – (Endpoint #1). The correction blank must be  $\pm 0.001$ .

9.2 The equation for calculating O<sub>2</sub> (mg/L) is as follows:

9.2.1 
$$O_2 \text{ (mg/L)} = 16 * ([\text{Bottle factor} * (\text{sample reading} - \text{correction blank})] - 0.0016)$$
  
Bottle factor =  $50 / [(\text{bottle volume} - 2)(\text{Avg. of standards} - \text{correction blank})]$

## 10.0 Quality Control

10.1 This is an analytical chemistry technique. The glassware and equipment -- standard and sample bottles, pipettes, stir bars, and buret tip must be kept scrupulously clean.

10.2 Never pipette reagents straight out of the reagent bottle. Always decant a small amount into a clean vessel and pipette out of that. Never pour remaining reagents back into the reagent bottle – dispose of them as you would a titrated sample, standard or blank.

10.3 Thoroughly rinse the glassware with clean hot water before and after every analysis. Clean every three months using Liqui-Nox® and water. Clean the buret as needed.

10.4 Each analyzer must complete their own sets of standards and blanks. If someone cannot finish a set that they've acidified, the person finishing them MUST run their own set of standards and blanks.

10.5 Project-specific QA samples including replicates or duplicates may also be analyzed.

## 11.0 Precision and Accuracy

11.1 Standards must be within  $\pm 0.001$  of each other before they can be accepted.

11.2 The Correction Blank must be  $\pm 0.001$  before it can be accepted.

## 12.0 Safety

12.1 Follow general procedures for safety found in the *Environmental Assessment Program Safety Manual*.

12.2 The 8 N NaOH-NaI-azide solution and the 10 N H<sub>2</sub>SO<sub>4</sub> are suspected carcinogens and should be treated with care. Always wear safety glasses, gloves and a lab coat when handling these reagents. In addition, 10 N H<sub>2</sub>SO<sub>4</sub> is poisonous and corrosive. The 0.01 N KIO<sub>3</sub> solution is an oxidizer and should always be handled with care.

### **13.0 Hazardous Waste Disposal**

- 13.1 The titrated sample is washed down the drain with copious amounts of tap water. The solution is acidic so it must be diluted as much as possible to reduce any impact on the wastewater treatment plant.

### **14.0 References**

- 14.1 Codispoti, Lou. 1988. One Man's Advice on the Determination of Dissolved Oxygen in Seawater.
- 14.2 Environmental Assessment Program, 2006. Environmental Assessment Program Safety Manual. March 2006. Washington State Department of Ecology. Olympia, WA.
- 14.3 UNESCO. (1994). Protocols for the joint global ocean flux study (JGOFS) core measurements. pp. 104-118.