

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedure for Seawater Dissolved Oxygen Analysis

Version 3.0

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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
2/17/2012	2.1	Added picture, additional details for sample holding & treatment, Dosimat manual link and reference.	4.0, 7.0, 5.0 bibliography	Mya Keyzers, Julia Bos
3/19/2012	2.1	Recertified	All	Bill Kammin
1/9/2015	3.0	Added neutralization procedure Updated MSDS links Additions of apparatus and materials Minor edits and clarifications	5.0, 6.0, 7.0 (added new 7.7), 10.0, 13.0, 14.0	Laura Hermanson, Suzan Pool
1/9/2015	3.0	Recertified	All	Bill Kammin

## 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 Marine Monitoring 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 Deionized water (18 Megohm)
- 5.2 Safety apron or laboratory coat
- 5.3 Safety goggles or glasses
- 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 Small beakers, one for each reagent
- 5.9 Desk lamp to illuminate sample
- 5.10 White background (e.g., white paper or plastic) secured to clamp post
- 5.11 Metrohm® 775 Dosimat titrator with magnetic stirrer and stir bar  
<http://www.metrohmusa.com/search/Search.html?identifier=27750010&language=en>

- 5.12 pH strips
- 5.13 Plastic bucket

## **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.sciencelab.com/msds.php?msdsId=9924583>.
- 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.sciencelab.com/msds.php?msdsId=9924999>  
<http://www.sciencelab.com/msds.php?msdsId=9927270>  
<http://www.sciencelab.com/msds.php?msdsId=9927588>
- 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.sciencelab.com/msds.php?msdsId=9925146>.
- 6.4 0.01 N Sodium Thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) (obtained from the University of Washington's Marine Chemistry Lab). Sodium thiosulfate is made by dissolving 49.64 g sodium thiosulfate + 0.1g  $\text{Na}_2\text{CO}_3$  to 1 liter volume with deionized 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  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$  and  $\text{Na}_2\text{CO}_3$  may be found at <http://www.sciencelab.com/msds.php?msdsId=9927606>.
- 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 deionized water. The starch solution does not pose any known health risks. This solution should be kept refrigerated and has a shelf life of about 6-12 months. The MSDS may be found at <http://www.sciencelab.com/msds.php?msdsId=9925088>.
- 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.sciencelab.com/msds.php?msdsId=9927231>.

6.7 Sodium Bicarbonate ( $\text{NaHCO}_3$ ) (obtained from a store). Sodium bicarbonate, or baking soda, is a white powder that is commonly used to neutralize acid. The MSDS may be found at <http://www.sciencelab.com/msds.php?msdsId=9927258>

## 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 (DO) determination, only the laboratory portion. It assumes that proper sampling protocols have been followed, that the sample was collected in a 130 mL DO flask, and that the sample was fixed immediately with 1 mL manganese chloride solution followed by 1 ml of alkaline sodium hydroxide-sodium iodide reagent. 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 *\*\*Prior to titrations, samples **must** be at room temperature. If stored cold, remove from refrigerator at least 3 hours prior to titrations, keeping samples in dark conditions.*



Figure 1. Dosimat with DO flask on stir plate, beakers for each reagent, pipettes, deionized water in squeeze bottle, desk lamp, laboratory gloves, and Kimwipes.

7.1.2 Turn on the Dosimat using the red POWER switch in back.

- 7.1.3 Press CLEAR. The display should read DOS 0.000 ml.
- 7.1.4 Gently lift the amber bottle of thiosulfate. Shake and then replace in the Dosimat.
- 7.1.5 Turn the dispense speed knob (labeled dv/dt) to 10. Using the hand control button, dispense 15-20 ml of thiosulfate to flush out the buret to remove air bubbles from the Dosimat line and its buret. Be sure there are no bubbles in the buret or moving bubbles in the line leading to the buret tip.
- 7.1.6 Turn the dispense speed knob to 1.
- 7.1.7 Press the CLEAR button.
- 7.1.8 Rinse off the buret tip with deionized (DI) water.
- 7.1.9 Turn the stirrer on.
- 7.1.10 **Pipetting Tips:**
- 7.1.10.1 Always shake the reagent before pipetting.
- 7.1.10.2 Draw reagent from a smaller vessel (i.e., small beaker).
- 7.1.10.3 Hold the pipette straight up and down, never angled.
- 7.1.10 **IMPORTANT: NEVER POUR LIQUID BACK INTO THE REAGENT BOTTLE**
- 7.1.11 Dispense straight into the sample bottle. Do not put the tip of the pipette against the wall of the sample bottle.
- 7.1.12 Rinse the inside of the sample bottle with DI water after each chemical addition to rinse down any reagent that may have splashed onto the side.
- 7.2 Preparing and running O<sub>2</sub> standards
- 7.2.1 Fill clean standard sample bottle about  $\frac{3}{4}$  full of DI water.
- 7.2.2 Add a clean stir bar.
- 7.2.3 Turn stirrer on, setting speed as appropriate to size of stir bar. Leave stirrer running during entire titration of each sample to ensure complete mixing.
- 7.2.4 Using a pipette, add 1 ml 10 N H<sub>2</sub>SO<sub>4</sub> and mix well.
- 7.2.5 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.
- 7.2.6 Using a pipette, add 10 ml of the 0.01 N KIO<sub>3</sub> standard.
- 7.2.7 Using a pipette, add 1 ml of starch aqueous solution.
- 7.2.8 Rinse inside of flask well with DI water to ensure no chemical residue has adhered to the sides of the flask.
- 7.2.9 Position the sample bottle on the stirrer; making sure the burette tip is under the surface of the sample.

- 7.2.10 Make sure that the Dosimat reads 0.000 ml (press CLEAR to zero).
- 7.2.11 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.
- 7.2.12 Record endpoint.
- 7.2.13 Remove sample bottle and dispense a few drops of thiosulfate through the burette tip to flush out any sample residue.
- 7.2.14 Rinse down the burette tip with deionized water.
- 7.2.15 Press CLEAR to zero the Dosimat.
- 7.2.16 Run at least 3 standards; at least 2 out of 3 must agree to  $\pm 0.001$  ml. New technicians that have been running Winklers for less than two months should run at least 5 standards, with 3 out of 5 standards agreeing 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  $\text{H}_2\text{SO}_4$  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  $\text{MnCl}_2$ . Mix well.
- 7.3.6 Using a pipette, add 1 ml of the 0.01 N  $\text{KIO}_3$  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 inside 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 1/10th as strong as the standard.
- 7.3.12 Record endpoint #1; this is Blank1.
- 7.3.13 Add 1 ml more of  $\text{KIO}_3$  standard.
- 7.3.14 **Option 1:** Clear the first endpoint before titrating to the second endpoint.
- 7.3.15 **Option 2:** Do not clear the first endpoint and continue titrating to the next endpoint.
- 7.3.16 Titrate to the second endpoint.

7.3.17 **If Option 1 was used:** The Correction Blank is obtained by subtracting Blank 2 from Blank 1:

1. Blank 1 – Blank 2 = Correction Blank
2. The Correction Blank must be  $\pm 0.001$ .

7.3.18 **If Option 2 was used:** The Correction Blank is obtained by first subtracting Endpoint 2 from Endpoint 1 to get Blank 2 and then subtracting Blank 2 from Blank 1:

1. Endpoint 2 – Endpoint 1 = Blank 2
2. Blank 1 – Blank 2 = Correction Blank
3. 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> and 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 Check whether the sample bottle has any air bubbles in it. If there is any, record this on the log sheet.

7.5.2 Carefully remove the cap and rinse the stopper into the sample bottle.

7.5.3 Add a clean stir bar.

7.5.4 Using a pipette, add 1 ml of starch aqueous solution.

7.5.5 Position the sample bottle on the stirrer, making sure the buret tip is under the surface of the sample.

7.5.6 Rinse inside of flask well with DI water to ensure no chemical residue has adhered to the inside of the flask.

7.5.7 Make sure that the Dosimat reads 0.000 ml (press CLEAR to zero).

7.5.8 Titrate sample to the endpoint by dispensing thiosulfate in the sample using the thumb button. Watch the vortex in the upper half of the bottle. The endpoint is achieved when all color is gone. The endpoint is a subtle difference between clear and sparkling clear.

7.5.9 Record endpoint.

- 7.5.10 Raise buret tip and dispense a few drops of thiosulfate through the buret tip to flush out any sample residue into the sample bottle.
- 7.5.11 Remove sample bottle.
- 7.5.12 Rinse down the buret tip with deionized water.
- 7.5.13 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:
  - 7.6.2 Using a pipette, add 1 ml of the 0.01 N  $\text{KIO}_3$  standard.
  - 7.6.3 Titrate to the endpoint.
  - 7.6.4 Take this final reading and subtract the volume needed to titrate 1 ml of  $\text{KIO}_3$  standard. This is your ‘true’ endpoint and can be recorded in the “buret reading” column of the log sheet.
  - 7.6.5 Write “over-titrated - added 1 ml  $\text{KIO}_3$ ” 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  $\text{KIO}_3$ ; (0.955 – 0.050)

## 7.7 Neutralization and Disposal

- 7.7.1 The titrated standards, blanks, and samples are neutralized and rinsed down the drain with copious amounts of tap water. Do not pour down the “live” sink.
- 7.7.2 Neutralize the Winkler waste from all standards, blanks, and samples:
  - 7.7.2.1 Pour the waste into a plastic bucket with an excess amount (about 2 tablespoons) of baking soda.
  - 7.7.2.2 Use a pH strip to ensure that the final pH is near 7.
  - 7.7.2.3 Pour the contents of the bucket down the “dead” sink with copious amounts of water.
- 7.7.3 Rinse all glassware, pipette tips, and small plastic beakers with 3 rinses of hot water and then 3 rinses of DI water.
- 7.7.4 Bottle numbers and buret readings must be recorded on DO Analysis Data Sheets.

## 8.0 **Calibration and Standardization**

- 8.1 Standards and blanks must be run before acidified samples are run (as per ‘Procedure’ in section 7.0 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 subtraction: (Blank 1) – (Blank 2). Blank 2 is obtained by subtraction of endpoints: (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 
$$O_2 \text{ (mg/L)} = 16 * ([\text{Bottle factor} * (\text{sample reading} - \text{correction blank})] - 0.0016)$$
$$\text{Bottle factor} = 50 / [(\text{bottle volume} - 2)(\text{Avg. of standards} - \text{correction blank})]$$

## **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 Check whether a sample has any air bubbles in it before removing the bottle stopper as a single bubble can affect the final concentration of dissolved oxygen. Record any observations of air bubbles on log sheet.

10.3 Do not mix reagents of two different ages and ensure that there is sufficient reagent of one age before starting analysis.

10.4 Never pipette reagents straight out of the reagent bottle. Always decant a small amount into a clean vessel (i.e., small beaker) 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.

10.5 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.

10.6 Each analyzer must complete their own sets of standards and blanks because of bias between technicians. If someone cannot finish a set that they have acidified, the person finishing them MUST run their own set of standards and blanks.

10.7 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 neutralized with baking soda (i.e., sodium bicarbonate) and then washed down the drain with copious amounts of tap water. The non-neutralized solution is acidic, so it must be neutralized and diluted as much as possible to reduce any impact on the wastewater treatment plant.

### **14.0 References**

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