

## STANDARD OPERATING PROCEDURE

SOP 207  
Renumbered 5/12/2010

## DISSOLVED OXYGEN

**SCOPE AND APPLICATION:** This method is applicable to ground water and surface water, domestic and industrial wastes. The practical range of determination is 1 mg/L to 9 mg/L.

**APPLICABLE METHOD REFERENCES:** 18th edition, 1992, of Standard Methods for the Examination of Water and Wastewater, 4500-O, C, G.

**DISPOSAL OF SAMPLES:** Dispose of samples at an acid sink located in the general chemistry area.

\*\*\* DISSOLVED OXYGEN \*\*\*

1.0 GENERAL DISCUSSION

1.1 Principle:

**Iodometric method-** Probably the oldest standard method of analysis for dissolved oxygen is that first reported by Winkler in 1888. Although the method has been improved by variations it is still the basis for the majority of titrimetric analyses. The method is based on the addition of divalent manganese ( $Mn^{++}$ ) solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO (dissolved oxygen) rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states (i.e.  $Mn^{+++}$ ). In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate. The titration end point can be detected visually, with a starch indicator. Experienced analyst can maintain a precision of  $\pm 50 \mu g/L$ . The following equations for the various steps are:

- 1)  $Mn^{++} + 2OH^- \rightarrow Mn(OH)_2 \downarrow$  (alkali addition with subsequent manganous hydroxide precipitate)
- 2)  $2Mn(OH)_2 + \frac{1}{2}O_2 + H_2O \rightarrow 2Mn(OH)_3$  (dissolved  $O_2$  rapidly oxidizes  $Mn^{++} \rightarrow Mn^{+++}$ )
- 3)  $2Mn(OH)_3 + 6H^+ + 3I^- \rightarrow 2Mn^{++} + I_3^- + 6H_2O$  (oxidation of  $I^-$  by the  $Mn^{+++}$  in acid solution, liberates Iodine)
- 4)  $I_3^- \rightarrow I_2 + I^-$  (Iodine liberated, equivalent to original DO content)
- 5)  $I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$  (titration with sodium thiosulfate, determination of liberated iodine)

**Membrane Electrode-** The dissolved oxygen electrode is a polarographic device of the type first described by Clark in 1956. It consists of a pair of polarized silver electrodes and an electrolyte separated from the sample by a gas-permeable membrane. Oxygen diffuses across the electrode membrane and is reduced to hydroxyl ions at a silver cathode according to the reaction:  $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$ . The electrons necessary for this process are provided by a reaction at the silver anode. Because the electrolyte contains chloride ions, this reaction occurs as:  $Ag + Cl^- \rightarrow AgCl + e^-$ . At any given temperature, the current which flows between cathode and anode, is directly proportional to the level of oxygen outside of the membrane. The membrane passes oxygen at a rate proportional to the differences across it in partial pressure of oxygen. Since oxygen is rapidly consumed at the cathode, it can be assumed that the oxygen pressure under the membrane is zero. Hence, the force causing the oxygen to diffuse through the membrane is proportional to the partial pressure of oxygen outside the membrane. As the oxygen partial pressure varies, both the oxygen diffusion through the membrane and the electrode current will change proportionally. Oxygen electrodes do not directly measure the oxygen concentration, but rather they measure the partial pressure of oxygen. The conversion of oxygen concentration to partial pressure can be readily effected electronically, due to the constant and predictable relationship between oxygen solubility and, temperature and total atmospheric pressure.

**1.2 Sampling and Storage:** Collect samples very carefully. Methods of sampling are highly dependent on source to be sampled and, to a certain extent, on method of analysis. Do not let sample remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Collect surface water samples in narrow-mouth glass-stoppered BOD bottles of 300 mL capacity with tapered and pointed ground-glass stoppers and flared

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mouths. Avoid entraining or dissolving atmospheric oxygen. Fill bottle to overflowing and prevent turbulence and formation of bubbles while filling. Record sample temperature to nearest degree Celsius.

**1.3 Safety Considerations:** Wear gloves, laboratory coat and eye protection. Dispose of all samples in an acid sink. Reagents used are caustic, corrosive and toxic, avoid ingestion or inhalation and contact with the skin. For specific hazards consult the MSDS sheet, located in the laboratory office for the following compounds: Potassium Bi-iodate, Potassium Dichromate, Manganous Sulfate, Potassium Iodide, Sodium Azide, Sodium hydroxide, Sulfuric acid, Starch.

**1.4 Apparatus and Equipment:** Oxygen electrode (Orion model 97-08), pH meter, magnetic stirrer, 300 mL BOD bottles, 500 mL flask, reagent dispensers, 50 mL buret.

**1.5 Sample Preservation:** Determine DO immediately on all samples containing an appreciable oxygen or iodine demand. Samples with no iodine demand may be stored for a few hours without change after adding manganous sulfate ( $MnSO_4$ ) solution, alkali-iodide solution, and  $H_2SO_4$ , followed by shaking in the usual way. Protect stored samples from strong sunlight and titrate as soon as possible. For samples with an iodine demand, preserve for 4 to 8 hours by adding 0.7 mL concentrated sulfuric acid and 1 mL sodium azide solution (2g  $NaN_3/100mL$  reagent water) to the BOD bottle. This will arrest biological activity and maintain DO if the bottle is stored at the temperature of collection or water-sealed and kept at 10 to 20°C. As soon as possible, complete the procedure, using 2 mL  $MnSO_4$  solution, 3 mL alkali-iodide solution, and 2 mL concentrated  $H_2SO_4$ .

**1.6 Interference:**

**Modified azide method:** Certain oxidizing agents liberate iodine from iodides (positive interference) and some reducing agents reduce iodine to iodide (negative interference). Most organic matter is oxidized partially when the oxidized manganese precipitate is acidified, thus causing negative errors. Several modifications of the iodometric method are given to commonly used procedures. The azide modification (the method presented in this SOP) effectively removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples. When the sample contains 5 or more mg ferric iron salts per liter, add potassium fluoride as the first reagent in the azide modification. Alternately, eliminate Fe(III) interference by using 85 to 87% phosphoric acid instead of sulfuric acid for acidification.

**Membrane electrode method:** Plastic films used with membrane electrode systems are permeable to a variety of gases besides oxygen, although none is depolarized easily at the indicator electrode. Prolonged use of membrane electrodes in waters containing such gases as hydrogen sulfide tends to lower cell sensitivity. Eliminate this interference by frequently changing and calibrating the membrane electrode.

**1.7 Method Performance Criteria:** According to the reference method, Standard Methods 18ed method 4500-O C (azide modification). The reference method cites: DO can be determined with a precision, expressed as a standard deviation, of about 20  $\mu g/L$  in distilled water and about 60  $\mu g/L$  in wastewater and secondary effluents. In the presence of appreciable interference, even with proper modifications, the standard deviation may be as high as 100  $\mu g/L$ . Still greater errors may occur in testing waters having organic suspended solids or heavy pollution. Avoid errors due to carelessness in collecting samples, prolonging the completion of test, or selecting an unsuitable modification.

According to the reference method, Standard Methods 18ed method 4500-O D (membrane electrode). The reference method cites: With most commercially available membrane electrode systems an accuracy of  $\pm 0.1$  mg DO/L and a precision of  $\pm 0.05$  mg DO/L can be obtained.

**2.0 REAGENTS**

- 1) Standard Sodium Thiosulfate Titrant: Dissolve 6.205g  $Na_2S_2O_3 \cdot 5H_2O$  in distilled water. Add 1.5 mL 6N NaOH or 0.4g solid NaOH and dilute to

1000 mL. Standardize with 0.025N potassium bi-iodate or potassium dichromate.

- 2) Standard Potassium Bi-iodate 0.025N (0.0021M): Dissolve 812.4 mg  $\text{KH}(\text{IO}_3)_2$  and dilute to 1000 mL.
- 3) Standard Potassium Dichromate 0.025N: Dissolve 1.226g  $\text{K}_2\text{Cr}_2\text{O}_7$  (previously dried at  $103^\circ\text{C}$  for 2 hours) and dilute to 1000 mL.
- 4) Manganous Sulfate solution: Dissolve 480g  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  or 364g  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  in distilled water, filter, and dilute to one liter.
- 5) Alkali-iodide-azide reagent: Dissolve 500g NaOH and 150g KI in distilled water, and dilute to one liter. Add 10g  $\text{NaN}_3$  dissolved in 40 mL distilled water.
- 6) Sulfuric acid,  $\text{H}_2\text{SO}_4$  concentrated: One mL is equivalent to 3 mL, Alkali-iodide-azide reagent.
- 7) Starch indicator: Dissolve 2g laboratory grade potato or arrow root soluble powder starch and 0.2g salicylic acid (preservative) in 100 mL hot distilled water.
- 8) Potassium fluoride solution: Dissolve 40g  $\text{KF} \cdot 2\text{H}_2\text{O}$  in reagent water and dilute to 100 mL.

### 3.0 PROCEDURE

#### 3.1 Standardization of 0.025 N Sodium Thiosulfate, Titrant:

Add to a beaker in the following order:

- 1) Dissolve 2 grams KI and 150 mL reagent water
- 2) 1 mL 6N  $\text{H}_2\text{SO}_4$  or few drops of conc.  $\text{H}_2\text{SO}_4$
- 3) 20 mL 0.025N  $\text{K}_2\text{Cr}_2\text{O}_7$  or  $\text{KH}(\text{IO}_3)_2$
- 4) Cool 5 minutes in the dark if 0.025N  $\text{K}_2\text{Cr}_2\text{O}_7$  is used.  $\text{KH}(\text{IO}_3)_2$  if used need not be cooled in the dark.
- 5) Dilute to approximately 200 mL with reagent water
- 6) Titrate with Sodium thiosulfate titrant until a pale straw color is observed. **STOP TITRATING!**
- 7) Add starch indicator and titrate to a colorless endpoint.

#### CALCULATION:

$$\text{Normality of Titrant} = \frac{\text{mL } \text{KH}(\text{IO}_3)_2 \times 0.025}{\# \text{ mL titrant}}$$

$$\text{Mg/L DO/mL of titrant} = \frac{20}{\text{mL titrant (Na}_2\text{S}_2\text{O}_3)} \quad \text{OR} \quad \frac{\text{Normality of Titrant}}{0.025}$$

**3.2 Procedure for Azide Modification, sample analysis :** To the sample collected in a 300 mL bottle, add 1 mL MnSO<sub>4</sub> solution, followed by 1 mL alkali-iodide-azide reagent. If pipets are dipped into sample, rinse them before returning them to reagent bottles. Alternatively, hold pipet tips just above liquid surface when adding reagents. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times. When precipitate has settled sufficiently (to approximately half the bottle volume) to leave clear supernate above the manganese hydroxide floc, add 1 mL concentrated H<sub>2</sub>SO<sub>4</sub>. Re-stopper and mix by inverting several times until dissolution is complete. Titrate a volume corresponding to 200 mL original sample after correction for sample loss by displacement with reagents. Thus, for a total of 2 mL (1 mL each) of MnSO<sub>4</sub> and alkali-iodide-azide reagents in a 300 mL bottle, titrate,  $200 \times 300 / (300 - 2) = 201 \text{ mL}$ .

Titrate with 0.025M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution to a pale straw color. Add a few drops of starch solution and continue titration to first disappearance of blue color. *If end point is overrun, back-titrate with 0.0021M bi-iodate solution added dropwise, or by adding a measured volume of treated sample. Correct for amount of bi-iodate solution or sample. Disregard subsequent recolorations due to the catalytic effect of nitrite or to traces of ferric salts that have not been complexed with fluoride.*

### 3.3 Standardization of DO meter:

- 1) Fill to overflowing three BOD bottles with dilution water.
- 2) To two bottles add to each:
  - A) 2 mL manganous sulfate then cap and invert bottle to mix contents.
  - B) 2 mL alkali-iodine-azide reagent, mix by inverting bottle.
  - C) Let the floc settle 2-min. then add 2 mL conc. H<sub>2</sub>SO<sub>4</sub>, mix by inverting bottle.
- 3) Pour 203 mL of the contents of bottle into a beaker.
- 4) Titrate contents of beaker with standardized 0.025 N sodium thiosulfate titrant to a starch-iodine endpoint.
- 5) Calculate mg/L DO of the test bottles. Discard contents of the beaker.

**Calculation:** DO mg/L = (#mL titrant) X (mg/L DO /mLof titrant)\* Use the determined average of both bottles for the concentration of the third bottle to be used for the calibration of the DO meter.

\* previously determined in section 3.1.

- 6) The third bottle is used to calibrate the DO meter by immersing the electrode directly into the bottle. Place the bottle on a stirrer and turn on the meter by placing the power switch in the ON position. First check the zero by turning the mode switch on the DO electrode to the ZERO position, using the adjustment knob adjust display to read zero. Next check the DO by turning the mode switch on the DO electrode to the H<sub>2</sub>O position, after the displayed reading is stable for one minute, using the adjustment knob adjust display to read the determined dilution water DO, from the replicate bottles. Recheck the zero and calibration after every ten measurements.

**3.4 Procedure oxygen electrode, sample analysis:**

- 1) Insert the funnel into the sample bottle, making sure that the funnel is snugly seated. Slowly immerse the calibrated electrode into the funnel. Sample displaced by the electrode will collect in the funnel.
- 2) Place the bottle on a magnetic stirrer and stir gently.
- 3) Turn the mode switch of the electrode to the H<sub>2</sub>O position and set the meter to the pH mode.
- 4) Obtain and record stable reading. The result displayed is ppm O<sub>2</sub> dissolved oxygen.
- 5) Slowly remove the electrode from the funnel. Remove the funnel and re-stopper the bottle. Rinse electrode and funnel with reagent water and dry electrode. Gently blot membrane dry.
- 6) Place funnel and electrode in storage bottle.

**4.0 CALCULATION**

**4.1 Azide Modification:** For titration of 200mL sample, 1 mL 0.025M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> = 1 mg DO/L or use determined (mg/L DO/mL) x (# mL of titrant). The mg/L DO/mL determined in section 3.1

**4.2 Oxygen electrode:** No calculation, direct reading of results from meter display.

**5.0 REPORTING**

**5.1 Reporting:** Record the test results and all ancillary information, dates, times, analyst name, measurements and calculations in the appropriate DO book or BOD books and DO log and Titrant Standardization Log, the heading and footer information shall be filled out in total with no omissions. The BOD work sheets shall have the name of the analyst for both day on and day off and date and time of test, for both day on and day off. Enter results into the electronic system, LIMS.

**5.2 Control Charts-** All quality control data will be entered in the lab share drive by analysts performing this test. Quality assurance reviews are performed weekly, for complete details of control chart performance evaluations see QA SOP-005.

**6.0 TROUBLESHOOTING**

SYMPTOM	POSSIBLE CAUSES	REMEDY
Drift or Slow Response	Internal pressure in membrane/electrolyte module because the membrane/electrolyte module has been installed too quickly	Partially unscrew the module and retighten slowly
	New module not allowed to equilibrate long enough	Allow newly installed module to equilibrate for 15 to 30 minutes before calibrating
	Water droplets clinging to membrane during air standardization	Blot tip with tissue and recalibrate
	No stirring when electrode is in solution	Make sure that magnetic stirrer is turned on and that bar is in synchronization
	Batteries weak (reads <13.4 on battery check)	Check and replace if necessary
	Electrode not plugged in or meter not in pH mode	Check electrode plugs and meter mode switch

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Unable to Calibrate	Zero setting improperly adjusted	Check and reset if necessary
	Tip electrode wet	Blot dry
	Batteries weak (reads <13.4 on battery check)	Check and replace if necessary
	Membrane/electrolyte module damaged or dried out	Replace module

**7.0 MAINTENANCE** (membrane electrode) see Appendix I

**8.0 GENERAL INFORMATION** (membrane electrode) see Appendix II