

Standard Methods for the Examination of Water and Wastewater

4500-O OXYGEN (DISSOLVED)*#(1)

4500-O A. Introduction

1. Significance

Dissolved oxygen (DO) levels in natural and wastewaters depend on the physical, chemical, and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste treatment process control.

2. Selection of Method

Two methods for DO analysis are described: the Winkler or iodometric method and its modifications and the electrometric method using membrane electrodes. The iodometric method¹ is a titrimetric procedure based on the oxidizing property of DO while the membrane electrode procedure is based on the rate of diffusion of molecular oxygen across a membrane.² The choice of procedure depends on the interferences present, the accuracy desired, and, in some cases, convenience or expedience.

3. References

1. WINKLER, L.W. 1888. The determination of dissolved oxygen in water. *Berlin. Deut. Chem. Ges.* 21:2843.
2. MANCY, K.H. & T. JAFFE. 1966. Analysis of Dissolved Oxygen in Natural and Waste Waters. Publ. No. 999-WP-37, U.S. Public Health Serv., Washington, D.C.

4500-O B. Iodometric Methods

1. Principle

The iodometric test is the most precise and reliable titrimetric procedure for DO analysis. It is based on the addition of divalent manganese solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. 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, or electrometrically, with potentiometric or dead-stop techniques.¹ Experienced analysts can maintain a precision of $\pm 50 \mu\text{g/L}$ with visual end-point detection and a precision of $\pm 5 \mu\text{g/L}$ with electrometric end-point

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detection.^{1,2}

The liberated iodine also can be determined directly by simple absorption spectrophotometers.³ This method can be used on a routine basis to provide very accurate estimates for DO in the microgram-per-liter range provided that interfering particulate matter, color, and chemical interferences are absent.

2. Selection of Method

Before selecting a method consider the effect of interferences, particularly oxidizing or reducing materials that may be present in the sample. 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 minimize the effect of interfering materials.² Among the more commonly used procedures are the azide modification,⁴ the permanganate modification,⁵ the alum flocculation modification,⁶ and the copper sulfate-sulfamic acid flocculation modification.^{7,8} The azide modification (C) effectively removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples. Use the permanganate modification (D) in the presence of ferrous iron. When the sample contains 5 or more mg ferric iron salts/L, add potassium fluoride (KF) as the first reagent in the azide modification or after the permanganate treatment for ferrous iron. Alternately, eliminate Fe(III) interference by using 85 to 87% phosphoric acid (H_3PO_4) instead of sulfuric acid (H_2SO_4) for acidification. This procedure has not been tested for Fe(III) concentrations above 20 mg/L.

Use the alum flocculation modification (E) in the presence of suspended solids that cause interference and the copper sulfate-sulfamic acid flocculation modification (F) on activated-sludge mixed liquor.

3. Collection of Samples

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. Samples from any depth in streams, lakes, or reservoirs, and samples of boiler water, need special precautions to eliminate changes in pressure and temperature. Procedures and equipment have been developed for sampling waters under pressure and unconfined waters (e.g., streams, rivers, and reservoirs). Sampling procedures and equipment needed are described in American Society for Testing and Materials Special Technical Publication No. 148-1 and in U.S. Geological Survey Water Supply Paper No. 1454.

Collect surface water samples in narrow-mouth glass-stoppered BOD bottles of 300-mL capacity with tapered and pointed ground-glass stoppers and flared mouths. Avoid entraining or

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dissolving atmospheric oxygen. In sampling from a line under pressure, attach a glass or rubber tube to the tap and extend to bottom of bottle. Let bottle overflow two or three times its volume and replace stopper so that no air bubbles are entrained.

Suitable samplers for streams, ponds, or tanks of moderate depth are of the APHA type shown in Figure 4500-O:1. Use a Kemmerer-type sampler for samples collected from depths greater than 2 m. Bleed sample from bottom of sampler through a tube extending to bottom of a 250- to 300-mL BOD bottle. Fill bottle to overflowing (overflow for approximately 10 s), and prevent turbulence and formation of bubbles while filling. Record sample temperature to nearest degree Celsius or more precisely.

4. Preservation of Samples

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 h by adding 0.7 mL conc H_2SO_4 and 1 mL sodium azide solution (2 g NaN_3 /100 mL distilled 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 conc H_2SO_4 .

5. References

1. POTTER, E.C. & G.E. EVERITT. 1957. Advances in dissolved oxygen microanalysis. *J. Appl. Chem.* 9:642.
2. MANCY, K.H. & T. JAFFE. 1966. Analysis of Dissolved Oxygen in Natural and Waste Waters. Publ. No. 99-WP-37, U.S. Public Health Serv., Washington, D.C.
3. OULMAN, C.S. & E.R. BAUMANN. 1956. A colorimetric method for determining dissolved oxygen. *Sewage Ind. Wastes* 28:1461.
4. ALSTERBERG, G. 1925. Methods for the determination of elementary oxygen dissolved in water in the presence of nitrite. *Biochem. Z.* 159: 36.
5. RIDEAL, S. & G.G. STEWART. 1901. The determination of dissolved oxygen in waters in the presence of nitrites and of organic matter. *Analyst* 26:141.
6. RUCHHOFT, C.C. & W.A. MOORE. 1940. The determination of biochemical oxygen demand and dissolved oxygen of river mud suspensions. *Ind. Eng. Chem., Anal. Ed.* 12:711.
7. PLACAK, O.R. & C.C. RUCHHOFT. 1941. Comparative study of the azide and Rideal-Stewart modifications of the Winkler method in the determination of biochemical oxygen demand. *Ind. Eng. Chem., Anal. Ed.* 13:12.

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8. RUCHHOFT, C.C. & O.R. PLACAK. 1942. Determination of dissolved oxygen in activated-sludge sewage mixtures. *Sewage Works J.* 14:638.

4500-O C. Azide Modification

1. General Discussion

Use the azide modification for most wastewater, effluent, and stream samples, especially if samples contain more than 50 $\mu\text{g NO}_2^-$ -N/L and not more than 1 mg ferrous iron/L. Other reducing or oxidizing materials should be absent. If 1 mL KF solution is added before the sample is acidified and there is no delay in titration, the method is applicable in the presence of 100 to 200 mg ferric iron/L.

2. Reagents

a. *Manganous sulfate solution:* Dissolve 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 400 g $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, or 364 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ in distilled water, filter, and dilute to 1 L. The MnSO_4 solution should not give a color with starch when added to an acidified potassium iodide (KI) solution.

b. *Alkali-iodide-azide reagent:*

1) For saturated or less-than-saturated samples—Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L. Add 10 g NaN_3 dissolved in 40 mL distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

2) For supersaturated samples—Dissolve 10 g NaN_3 in 500 mL distilled water. Add 480 g sodium hydroxide (NaOH) and 750 g sodium iodide (NaI), and stir until dissolved. There will be a white turbidity due to sodium carbonate (Na_2CO_3), but this will do no harm. CAUTION—*Do not acidify this solution because toxic hydrazoic acid fumes may be produced.*

c. *Sulfuric acid, H_2SO_4 , conc:* One milliliter is equivalent to about 3 mL alkali-iodide-azide reagent.

d. *Starch:* Use either an aqueous solution or soluble starch powder mixtures.

To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

e. *Standard sodium thiosulfate titrant:* Dissolve 6.205 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in distilled water. Add 1.5 mL 6N NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with bi-iodate solution.

f. *Standard potassium bi-iodate solution, 0.0021M:* Dissolve 812.4 mg $\text{KH}(\text{IO}_3)_2$ in distilled water and dilute to 1000 mL.

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Standardization—Dissolve approximately 2 g KI, free from iodate, in an erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6*N* H₂SO₄ or a few drops of conc H₂SO₄ and 20.00 mL standard bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solutions are of equal strength, 20.00 mL 0.025*M* Na₂S₂O₃ should be required. If not, adjust the Na₂S₂O₃ solution to 0.025*M*.

3. Procedure

a. To the sample collected in a 250- to 300-mL bottle, add 1 mL MnSO₄ 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 1.0 mL conc H₂SO₄. Restopper 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 MnSO₄ and alkali-iodide-azide reagents in a 300-mL bottle, titrate $200 \times 300 / (300 - 2) = 201$ mL.

b. Titrate with 0.025*M* Na₂S₂O₃ 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 0.0021*M* 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.

4. Calculation

a. For titration of 200 mL sample, 1 mL 0.025*M* Na₂S₂O₃ = 1 mg DO/L.

b. To express results as percent saturation at 101.3 kPa, use the solubility data in Table 4500-O:I. Equations for correcting solubilities to barometric pressures other than mean sea level and for various chlorinities are given below the table.

5. Precision and Bias

DO can be determined with a precision, expressed as a standard deviation, of about 20 µg/L in distilled water and about 60 µg/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 µg/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.

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6. References

1. BENSON, B.B. & D. KRAUSE, JR. 1984. The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. *Limnol. Oceanogr.* 29:620.
2. BENSON, B.B. & D. KRAUSE, JR. 1980. The concentration and isotopic fractionation of gases dissolved in fresh water in equilibrium with the atmosphere: I. Oxygen. *Limnol. Oceanogr.* 25:662.
3. MORTIMER, C.H. 1981. The oxygen content of air-saturated fresh waters over ranges of temperature and atmospheric pressure of limnological interest. *Int. Assoc. Theoret. Appl. Limnol.*, Communication No. 22, Stuttgart, West Germany.
4. SULZER, F. & W.M. WESTGARTH. 1962. Continuous D. O. recording in activated sludge. *Water Sewage Works* 109: 376.
5. UNITED NATIONS EDUCATIONAL, SCIENTIFIC & CULTURAL ORGANIZATION. 1981. Background Papers and Supporting Data on the Practical Salinity Scale 1978. Tech. Paper Mar. Sci. No. 37.

4500-O D. Permanganate Modification

1. General Discussion

Use the permanganate modification only on samples containing ferrous iron. Interference from high concentrations of ferric iron (up to several hundred milligrams per liter), as in acid mine water, may be overcome by the addition of 1 mL potassium fluoride (KF) and azide, provided that the final titration is made immediately after acidification.

This procedure is ineffective for oxidation of sulfite, thiosulfate, polythionate, or the organic matter in wastewater. The error with samples containing 0.25% by volume of digester waste from the manufacture of sulfite pulp may amount to 7 to 8 mg DO/L. With such samples, use the alkali-hypochlorite modification.¹ At best, however, the latter procedure gives low results, the deviation amounting to 1 mg/L for samples containing 0.25% digester wastes.

2. Reagents

All the reagents required for Method C, and in addition:

- a. *Potassium permanganate solution*: Dissolve 6.3 g KMnO_4 in distilled water and dilute to 1 L.
- b. *Potassium oxalate solution*: Dissolve 2 g $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$ in 100 mL distilled water; 1 mL will reduce about 1.1 mL permanganate solution.
- c. *Potassium fluoride solution*: Dissolve 40 g $\text{KF} \cdot 2\text{H}_2\text{O}$ in distilled water and dilute to 100 mL.

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mL.

3. Procedure

a. To a sample collected in a 250- to 300-mL bottle add, below the surface, 0.70 mL conc H_2SO_4 , 1 mL KMnO_4 solution, and 1 mL KF solution. Stopper and mix by inverting. Never add more than 0.7 mL conc H_2SO_4 as the first step of pretreatment. Add acid with a 1-mL pipet graduated to 0.1 mL. Add sufficient KMnO_4 solution to obtain a violet tinge that persists for 5 min. If the permanganate color is destroyed in a shorter time, add additional KMnO_4 solution, but avoid large excesses.

b. Remove permanganate color completely by adding 0.5 to 1.0 mL $\text{K}_2\text{C}_2\text{O}_4$ solution. Mix well and let stand in the dark to facilitate the reaction. Excess oxalate causes low results; add only enough $\text{K}_2\text{C}_2\text{O}_4$ to decolorize the KMnO_4 completely without an excess of more than 0.5 mL. Complete decolorization in 2 to 10 min. If it is impossible to decolorize the sample without adding a large excess of oxalate, the DO result will be inaccurate.

c. From this point the procedure closely parallels that in Section 4500-O.C.3. Add 1 mL MnSO_4 solution and 3 mL alkali-iodide-azide reagent. Stopper, mix, and let precipitate settle a short time; acidify with 2 mL conc H_2SO_4 . When 0.7 mL acid, 1 mL KF solution, 1 mL KMnO_4 solution, 1 mL $\text{K}_2\text{C}_2\text{O}_4$ solution, 1 mL MnSO_4 solution, and 3 mL alkali-iodide-azide (or a total of 7.7 mL reagents) are used in a 300-mL bottle, take $200 \times 300 / (300 - 7.7) = 205$ mL for titration.

This correction is slightly in error because the KMnO_4 solution is nearly saturated with DO and 1 mL would add about 0.008 mg oxygen to the DO bottle. However, because precision of the method (standard deviation, 0.06 mL thiosulfate titration, or 0.012 mg DO) is 50% greater than this error, a correction is unnecessary. When substantially more KMnO_4 solution is used routinely, use a solution several times more concentrated so that 1 mL will satisfy the permanganate demand.

4. Reference

1. THERIAULT, E.J. & P.D. MCNAMEE. 1932. Dissolved oxygen in the presence of organic matter, hypochlorites, and sulfite wastes. *Ind. Eng. Chem., Anal. Ed.* 4:59.

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Endnotes

1 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1993.