DISSOLVED OXYGEN

(D.O.)

DISSOLVED OXYGEN

Background

Oxygen is present in solution as both the free, dissolved form and as a bound form such as the oxygen contained in compounds, including sulfates, SO_4^{-2}, nitrates, NO_3^-, etc. When we measure dissolved oxygen in a solution, we are measuring the free oxygen gas dissolved in the solution. The free, dissolved oxygen is the oxygen that is available for use in respiration, or breathing, by fish, aquatic insects, plants, and aerobic bacteria. Without dissolved oxygen, the waters go septic and the result can be unsightly and foul-smelling. Oxygen enters water from the air through the surface by diffusion and happens much more rapidly in waterfalls, rapids, and riffle areas than in quiet pools. Oxygen also enters the water from the daytime photosynthetic activities of aquatic plants. The levels of dissolved oxygen or DO in streams varies from a low of 0-3 mg/l for polluted waters to a high of 12-15 mg/l in clear, very cold streams. Seven to nine (7-9) mg/l is the average in natural waters. The levels are dependent on: 1) physical conditions such as rapids, dams, pools and temperature of the water; 2) chemical action such as rusting, etc.; and 3) biological activity going on in the water such as that of fish, insects bacteria, and plants.

We test for DO in treatment plant effluents to determine the amount there, to aid in the stream's natural waste stabilization process; in secondary plant processes to determine if the amount of DO present is sufficient to maintain good aerobic biological treatment; and in influents to avoid septic conditions which may result in the formation of hydrogen sulfide and its corrosive results.

Methods of Measurement

The two methods of dissolved oxygen measurement we will describe are the Winkler titration method with azide modification and the membrane electrode method.

Winkler Method. The Winkler procedure is based on the oxidizing property of DO or the tendency of free oxygen to attach to certain ions. The azide modification is used to eliminate the interference of nitrite which is found in many biologically treated effluents, some streams at certain times of the year, and in the BOD test.

In the presence of dissolved oxygen, Mn^{+2} (the manganous ion) from DO solution #1 (manganous sulfate) reacts with the dissolved oxygen under alkaline conditions supplied by the addition of DO solution #2 (the alkali-azide-iodide solution) to form a brown (manganic hydroxide) floc.

$$2\ Mn^{+2} + O_2 + 4\ OH \ \text{---}> \ 2\ MnO(OH)_2$$

With the addition of DO solution #3 (sulfuric acid), the oxidized manganese is reduced back to the divalent form, and iodine is released (from the iodide ions supplied in DO solution #2.)

$$MnO(OH)_2 + 6\ I^- + 6\ H^+ \ \text{---}> \ Mn^{+2} + 2\ I_3^- + 3\ H_2O$$

The amount of iodine formed is proportional to the amount of dissolved oxygen originally present in the solution.

By titrating with a standard solution of Sodium Thiosulfate we can measure the amount of iodine present which as stated is directly proportional to the DO concentration.

$$H^+ + I_3^- + 2\ Na_2S_2O_3 \ \text{---}> \ 2\ NaI + Na_2S_4O_6 + HI$$

The addition of starch indicator to the solution before titration makes endpoint determination easier by producing a dark blue color which contrasts the colorless endpoint.

Membrane Electrode Method. The membrane electrode method is based on the fact that the diffusion of dissolved oxygen across the electrode membrane produces a change in the potential of the electrode. This voltage change is measured by the DO meter. These electrodes, like pH electrodes, are sensitive to changes in temperature but most modern DO meters are equipped with automatic temperature compensation circuits. This method is especially useful for sludges, fast measurements in the plant, respiration rates and, when equipped with a stirring bottle probe, for BOD measurements.

Sampling Handling and Preservation

Samples for DO should be taken with a minimum of turbulence, to avoid air entrapment, into a 300 ml DO bottle. The bottle should be filled into the neck and stoppered tightly. Samples should be doped (the DO solutions added) and titrated immediately, although they can be held for up to eight (8) hours if fixed (DO #1 and #2 added) and stored in the dark (true for Winkler Method only). After the addition of DO #3 (sulfuric acid) the titration must be completed within 45 minutes.

When a Kemmerer sampler is used, the BOD sample bottle should be filled to overflowing. (Overflow for approximately 10 seconds.) The outlet tube of Kemmerer should be inserted to bottom of BOD bottle. Care must be taken to prevent turbulence and the formation of bubbles when filling bottle.

At the time of sampling, the sample temperature should be recorded as precisely as required.

Do not delay the determination of dissolved oxygen in samples having an appreciable iodine demand or containing ferrous iron.

WINKLER METHOD (w/ azide modification)

OPTION #1 - using 203 mls of sample

NOTE: The 300 ml (full bottle method) is described later in this section

Equipment

3 2 ml automatic or graduated pipets
200 ml volumetric flask with the neck cut off at 203 mls and a rubber stopper bored out to fit. (The best means of doing this is to order a plastic 200 ml volumetric flask. Fill it to the 200 ml mark and, with a 5 ml graduated pipet, carefully add 3 mls. Mark this level and cut off with a hack saw as level as possible. Bore out a #2 rubber stopper to fit the neck and slide down flush with top cut.)
2 500 ml Erlenmeyer flasks
buret, 50 ml or 10 or 25 ml automatic filling buret
2 dropping bottles, for starch and back titrant solution
10 ml volumetric pipet
100 ml graduated cylinder
magnetic stirrer (optional)

Reagents (Winkler 203 ml Method)

MANGANOUS SULFATE SOLUTION $MnSO_4$ D.O. Solution #1

Dissolve 480 grams of Manganous Sulfate ($MnSO_4 * 4H_2O$) OR 400 grams of ($MnSO_4 * 2H_2O$) OR 364 grams of ($MnSO_4 * H_2O$) in 500 mls distilled water. Filter if necessary and dilute to one-liter with distilled water.

ALKALI-IODIDE-AZIDE SOLUTION OH-,I-,NaN_3 D.O. Solution #2

CAUTION: Use **extreme caution** when preparing this solution! It should definitely be prepared only under a hood as the fumes are extremely dangerous. Also wear gloves and glasses. This solution can produce a great deal of heat when being mixed. Avoid contact with skin or eyes.

NOTE: The instructions call for the use of beakers when combining solutions, NOT volumetric flasks or other narrow mouthed glassware.

Gradually add and dissolve 500 grams of reagent grade Sodium Hydroxide (NaOH) OR 700 grams Potassium Hydroxide (KOH) in 600 mls distilled water in a 2 liter beaker. In another beaker add 135 grams Sodium Iodide (NaI) OR (NaN_3) in 40 mls of distilled water. Carefully add this solution to the Sodium Hydroxide solution, cool to room temperature, pour into one-liter volumetric flask, and dilute to one-liter with distilled water.

CONCENTRATED SULFURIC ACID, 36N (H_2SO_4) D.O. Solution #3

Concentrated reagent grade Sulfuric Acid should be purchased.

(STOCK) SODIUM THIOSULFATE SOLUTION 0.1N ($Na_2S_2O_3$)

The stock solution of Sodium Thiosulfate, if stored in an amber glass bottle with screw top and refrigerated, has a shelf life of approximately six months and can be used to make up the working solution (0.025 N) every two weeks to a month.

Dissolve 24.82 grams of $Na_2S_2O_3 * 5H_2O$ in freshly boiled and cooled distilled water. Dilute to 500 mls with distilled water. Preserve this solution by adding 5 mls Chloroform or 0.4 grams per liter of 0.025N Sodium Hydroxide.

STANDARD TITRANT, 0.025 SODIUM THIOSULFATE SOLUTION ($Na_2S_2O_3$)

Dilute 250 mls of stock Sodium Thiosulfate solution to 1 liter with distilled water. Preserve by adding 5 mls of Chloroform or 0.4 grams per liter of 0.025N Sodium Hydroxide. This solution should be stored in a glass amber bottle with screw top. This solution must be standardized with 0.25N Potassium Bi-iodate solution each time it is prepared. SEE STANDARDIZATION PROCESS IN APPENDIX I at the end of the Dissolved Oxygen Section.

NOTE: 0.025 N Phenylarsine Oxide can be used in place of the 0.025 N Sodium Thiosulfate solution as a titrant. It is more stable than sodium thiosulfate and has a longer shelf life. To prepare this solution, dissolve 4.20 grams of Phenylarsine Oxide crystals in 700 mls of freshly boiled and cooled distilled water. Dilute to 1 liter with distilled water. Preserve with 2.5 mls Chloroform.

(STOCK) POTASSIUM BI-IODATE SOLUTION 0.10 N ($KH(IO_3)_2$)

This stock solution of Potassium Bi-iodate, if stored in an amber glass bottle with screw cap and refrigerated, has a shelf life of approximately six months and can be used to make up the working solution of Potassium Bi-iodate every two weeks to one month.

After drying 5 grams of reagent grade Potassium Bi-iodate for two hours at 103°C, cool to room temperature in desiccator, weigh out exactly 3.249 grams and dissolve this in 600 mls distilled water. Dilute to 1 liter with distilled water.

POTASSIUM BI-IODATE PRIMARY STANDARD 0.025N ($KH(IO_3)_2$

Dilute exactly 250 mls of the 0.10N Potassium Bi-iodate solution to 1 liter with distilled water. Store in an amber glass bottle with screw top. Discard after one month.

STARCH INDICATOR SOLUTION

Prepare an emulsion of 10 grams soluble (potato) starch in a beaker with a small amount of distilled water. Pour this emulsion into 1 liter of boiling distilled water and allow to boil for a few minutes. Let the solution settle overnight and pour off the semi-clear portion. Discard the thick substance left in the bottom of the container. Preserve the starch solution by adding 1 gram salicylic acid, or 5 mls Chloroform or a couple drops of Toluene. This solution should be kept refrigerated. The starch indicator solution can be purchased from most of the chemical suppliers.

Procedure - 203 ml Method

Hold the pipet just above the liquid surface when adding reagents.

1) Remove the stopper and add 2 mls solution #1 ($MnSO_4$) and add 2 mls solution #2 (OH,I,N_3) the same way, slowly and just above the sample surface, to avoid adding any air bubbles.

2) Re-stopper and mix by inverting the bottle 10 to 15 times.

3) Allow the bottle to settle so that the brown floc occupies ½ the bottle volume or less.

4) Invert the bottle again 7 to 10 times.

5) Allow the floc layer to settle again to ½ the bottle volume or less.

6) Add 2 mls concentrated H_2SO_4 (Solution #3).

7) Stopper and shake until the solution is uniformly mixed and the floc is completely dissolved.

8) Using the cut-off volumetric flask, pour off 203 mls and put into a 500 ml Erlenmeyer flask. Reserve the remaining solution.

9) While swirling the flask, titrate with 0.025N sodium thiosulfate solution from the starting orange-yellow until it becomes a pale yellow color.

10) Add 1 ml starch solution, the sample will turn blue.

11) Continue titration until the blue goes to clear.

12) Check the endpoint by adding one (1) drop of the back titrant, 0.025N $KH(IO_3)_2$. If the solution does not turn blue, add another drop of back titrant, mix and check for blue. Continue adding back titrant, drop-by-drop, and mixing until the blue comes back. To figure how much you have overshot the endpoint, count the drops of back titrant added to reach the blue endpoint and subtract 1 (the first drop is free). Multiple this number by 0.05 and subtract this from the mls of thiosulfate used.

EXAMPLE: Suppose we titrated 8.50 mls of thiosulfate and overshot the endpoint. We add 5 drops of back titrant to reach the blue color. Four drops times 0.05 mls per drop equals 0.20 mls to be subtracted, giving us a thiosulfate volume of 8.5 mls - 0.20 mls = 8.30 mls. If you overshot by more than 10 drops, it is best not to use the titration. Instead, measure out the solution we reserved in step 8 above, and record the amount. Put this into a 500 ml Erlenmeyer flask and titrate as before, being very careful not to overshoot the endpoint. To figure the amount of thiosulfate which would have been used for a full sample, using the following formula:

$$\text{Volume of thiosulfate} = \frac{\text{mls thiosulfate used from reserved volume}}{\text{volume reserved}} \times 203 \text{ mls}$$

For example: Suppose we titrate a reserved volume of 87 mls and use 3.50 mls of thiosulfate doing so. The volume of thiosulfate used for a full sample would be:

$$\text{Volume of thiosulfate} = \frac{3.50 \text{ mls} \times 203 \text{ mls}}{87 \text{ mls}}$$

$$= 8.15 \text{ mls or } \underline{8.15 \text{ mls}}$$

If the sample goes clear and then slowly comes back to blue, don't continue to titrate; when the sample goes clear, the endpoint has been reached.

Calculation

When using a 203 ml sample and 0.025N sodium thiosulfate, then 1 ml thiosulfate used = 1 mg/L DO present in the sample.

WINKLER-IODOMETRIC METHOD (w/ azide modification)

OPTION #2 - 300 ml (Full Bottle)

Equipment

3 2 ml automatic or graduated pipets
2 500 ml Erlenmeyer flasks
buret, 50 ml or 10 or 25 ml automatic filling buret
2 dropping bottles, for starch and back titrant solution
10 ml volumetric pipet
100 ml graduated cylinder
magnetic stirrer (optional)
sample bottles - 300 ml (± 3 ml) capacity BOD incubation bottles with tapered around glass pointed stoppers and flared mouths

Reagents

MANGANOUS SULFATE SOLUTION $MnSO_4$ DO Solution #1

Dissolve 480 g $MnSO_4$ - $4H_2O$ in distilled water and dilute to 1 liter.

Alternatively, use 400 g of $MnSO_4$ $2H_2O$ or 364 g of $MnSO_4H_2O$ per liter. When uncertainty exists regarding the water of crystallization, a solution of equivalent strength may be obtained by adjusting the specific gravity of the solution to 1.270 at 20°C.

ALKALINE IODIDE-AZIDE SOLUTION OH-,I-,NaN_3 DO Solution #2

Gradually add and dissolve 500 grams of reagent grade Sodium Hydroxide (NaOH) OR 700 grams Potassium Hydroxide (KOH) in 600 mls distilled water in a 2 liter beaker. In another beaker add 135 grams Sodium Iodide (NaI) OR (NaN_3) in 40 mls of distilled water. Carefully add this solution to the Sodium Hydroxide solution, cool to room temperature, pour into one-liter volumetric flask, and dilute to one-liter with distilled water.

SULFURIC ACID CONCENTRATED 36N H_2SO_4 DO Solution #3

SODIUM THIOSULFATE, STOCK SOLUTION, 0.75N: Dissolve 186.15 g $Na_2S_2O_3$ $5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

SODIUM THIOSULFATE STANDARD TITRANT, 0.0375N: Prepare by diluting 50.0 ml of stock solution to 1 liter. Preserve by adding 5 ml of chloroform. Standard sodium thiosulfate, exactly 0.0375N is equivalent to 1.0 mg of DO per 1.00 ml. Standardize with 0.0375N potassium bi-iodate. SEE STANDARDIZATION PROCESS IN APPENDIX II AT THE END OF THE DISSOLVED OXYGEN SECTION.

*NOTE: 0.0375 N Phenylarsine Oxide can be used in place of the 0.0375 N Sodium Thiosulfate solution as a titrant. To prepare this solution, dissolve 6.30 grams of Phenylarsine Oxide crystals in 700 mls of freshly boiled and cooled distilled water. Dilute to 1 Liter with distilled water. Preserve with 2.5 mls Chloroform.

POTASSIUM BI-IODATE SOLUTION (STOCK) 0.15N $KH(IO_3)_2$

This stock solution of Potassium Bi-iodate if stored in an amber glass bottle with screw cap, and refrigerated, has a shelf life of approximately 6 months and can be used to make up the working solution of Potassium Bi-iodate every 2 weeks to 1 month.

After drying 5 grams of reagent grade Potassium Bi-iodate for 2 hours at 103°C, weigh out exactly 4.873 grams and dissolve this in 600 mls distilled water. Dilute to 1 liter with distilled water.

POTASSIUM BI-IODATE PRIMARY STANDARD 0.0375N ($KH(IO_3)_2$

Dilute exactly 250 mls of the 0.15N Potassium Bi-iodate solution to 1 liter with distilled water. Store in an amber glass bottle with screw top. Discard after 1 month.

*NOTE: The primary standard potassium bi-iodate $KH(IO_3)_2$ should be used in place of potassium dichromate, for standardization of the thiosulfate solution. Potassium bi-iodate seems to eliminate the return of the blue color that is titrated out.

STARCH INDICATOR SOLUTION

Prepare an emulsion of 10 grams soluble (potato) starch in a beaker with a small amount of distilled water. Pour this emulsion into 1 liter of boiling distilled water and allow to boil for a few minutes. Let the solution settle overnight and pour off the semi-clear portion. Discard the thick substance left in the bottom of the container. Preserve the starch solution by adding 1 gram salicylic acid, or 5 mls Chloroform or a couple of drops of Toluene. This solution should be kept refrigerated.

Procedure - 300 ml Method

1) Remove the stopper and add 2 mls solution #1 ($MnSO_4$). Hold the pipet just above the liquid surface and add 2 mls solution #2 (OH^-, I^-, N_3^-) the same way, slowly and just above the sample surface, to avoid adding any air bubbles.

2) Re-stopper and mix by inverting the bottle 10 to 15 times.

3) Allow the bottle to settle so that the brown floc occupies ½ the bottle volume or less.

4) Invert the bottle again 10 to 15 times.

5) Allow the floc layer to settle again to ½ the bottle volume or less.

6) Carefully, add 2 mls concentrated H_2SO_4, solution #3.

7) Stopper and shake until the solution is uniformly mixed.

8) Pour the entire contents into a 500 ml Erlenmeyer flask.

9) While swirling the flask, titrate with 0.0375N sodium thiosulfate solution from the starting orange-yellow until it becomes a pale yellow color. (0.0375N phenylarsine oxide (PAO) may be substituted as a titrant.)

10) Add 1 ml starch solution, the sample will turn blue.

11) Continue titration until the blue goes to clear.

12) Check the endpoint by back-titration (as described on page 6 of this section).

NOTE: Occasionally, a dark brown or black precipitate persists in the bottle after acidication. This precipitate will dissolve if the solution is kept for a few minutes longer than usual or, if particularly persistent, a few drops of H_2SO_4 will effect dissolution.

Calculation

When using a 300 ml sample (full DO bottle) and 0.0375N sodium thiosulfate, then 1 ml thiosulfate used = 1 mg/l DO present in sample.

The Winkler method with azide modification is not applicable under the following conditions: a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; b) samples high in suspended solids; c) samples containing organic substances which are readily oxidized in a highly alkaline solution or which are oxidized by free iodine in an acid solution; d) untreated domestic sewage; e) biological flocs; and f) where sample color interferes with endpoint detection. In instances where the azide modification is not applicable, the DO probe should be used.

DISSOLVED OXYGEN
TROUBLESHOOTING GUIDE
(Winkler Titration Method with Azide Modification)

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Cannot get floc to dissolve completely after addition of sulfuric acid (DO #3)	High DO in sample. Insufficient quantity of acid added to dissolve the iodine formed.	Add more sulfuric acid until the entire floc is dissolved. Mix by inversion.
	Insufficient mixing.	Continue to mix by inversion.
A white floc forms after the addition of manganous sulfate (DO #1) and the alkaline iodide - azide (DO #2) solutions. * Referred to as a "snowball" or "white-out".	Very little or no dissolve oxygen present in sample.	Add the sulfuric acid solution after allowing the floc to settle. If the sample turns clear (or milky) there is no dissolved oxygen in the sample.
Difficult to determine trace endpoint - "Fading" endpoint.	Background color interfering with reading.	Use a white buret stand or place a white piece of paper under the flask to help differentiate clear from light blue sample appearance.
	Starch solution weak.	Prepare fresh solution - Re-run analysis.
	The starch indicator solution was added without first titrating the sample to a pale straw yellow color.	Re-run the analysis. Before adding the starch indicator solution titrate the sample to a light - straw yellow color. Then add starch indicator and continue the titration to the clear endpoint.

DISSOLVED OXYGEN
TROUBLESHOOTING GUIDE (continued)
(Winkler Titration Method with Azide Modification)

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Result is unreasonably high.	Improper concentration of Sodium Thiosulfate titrant (too weak).	Restandardize the titrant to 0.025N for the 203 ml version of the test or 0.0375N for the 300 ml version. Re-run analysis.
	Sample has a chlorine residual.	Neutralize any chlorine residual before performing the analysis.
Result is unreasonably low.	Improper concentration of sodium thiosulfate titrant (too strong).	Restandardize the titrant. Re-run analysis.
	The sample contains >5 mg/l ferric iron salts.	Add potassium fluoride (approximately 1 milliliter) to the sample before adding the manganous sulfate (DO #1) solution **OR** Add an 80% to 85% solution of phosphoric acid in place of the sulfuric acid for acidification of the sample (approximately 1 milliliter).

Quality Control for
DISSOLVED OXYGEN
(Winkler Titration w/ azide modification)

Document

Sampling

Grab - Exact time and Date sampled
Exact time and Date analyzed
Volume
Location
Temperature

Glassware

203 or 300 ml version
Glassware properly washed and rinsed

Reagents

N I S T traceable
Preparation and Expiration dates

Duplication Schedule

REPORTING DISSOLVED OXYGEN
(Winkler Titrant)

Dissolved Oxygen (Winkler Method) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE:
SAMPLE VOLUME (method):
SAMPLE TEMPERATURE:
ANY MODIFICATION TO NEGATE INTERFERENCES:
BURET READING: Start _____ Endpoint _____
AMOUNT OF BACK TITRANT USED TO PRODUCE BLUE COLOR:
CALCULATIONS (Back Titration):
RESULTS IN mg/L:

MEMBRANE ELECTRODE METHOD for DISSOLVED OXYGEN ANALYSIS

Equipment and Reagent

DO meter, YSI model 51, 54, 57, etc. or equivalent
DO probe
spare membranes
thermometer
probe filling solution
razor blade (if using older style probe)

Calibration of DO Meter

DO meters can be calibrated by any of three methods. These are: atmospheric calibration, barometric calibration and calibration against the Winkler method. The atmospheric calibration method is fast and simple and is fine for in-plant process control testing but it is not generally a recommended method when calibrating for BOD readings. For BOD analysis the DO meter should be calibrated using either the barometric or Winkler method. Each of these methods will be discussed below.

Before going through the entire process of calibration it is always a good idea to check the membrane on the DO probe. Membranes must be replaced quite often. Some indication of the need for membrane replacement are: any sign of bubbles under the membrane, drifting of the meter and the inability to reach calibration. To replace the membrane, follow the manufacturer's instructions closely. Look for bubbles!

Once the membrane has been replaced:

1) Attach probe to meter.

2) Set master control to "red line" and adjust needle so that it lines up with the red line on the face with the "red line" knob.

3) Set the master control to "zero" and adjust the needle to zero with the zero knob.

4) Switch the master control to "calibrate" and allow the probe 15 minutes to polarize. Then proceed with one of the following methods of calibration.

   NOTE: The probe should be stored in a wet environment; the sponge in the plastic cap provided, should be wetted.

Atmospheric Calibration

With the probe polarized, read the room temperature and consult the saturation value either in Standard Methods or printed on the back of the meter. This value should be corrected by the atmospheric correction factor given on the back of the meter and this corrected value set on the meter with the "calibration" knob. For example, if the thermometer reads 19°C and the plant is located at about 500 feet above sea level:

19°C saturation value is 9.3 mg/l
500 feet correction factor is 0.98
9.3 mg/l x .98 - 9.1 mg/l

The value of 9.1 mg/l should be set on the meter. The meter is now calibrated and ready to use.

Winkler Calibration

(Use with DO bottle probes with stirrer for BODs). With the probe polarized, fill a graduated cylinder with distilled water. Siphon this out carefully into 3 DO bottles. Put the probe into one bottle and do a Winkler titration on the other two bottles as described previously. Calibrate the meter to the average of the two Winklers.

Most DO probes need a minimum flow rate past the probe in order to read accurately. In moving water, this is not so much a problem but when taking DOs on clarifiers, lakes or BOD bottles, etc., some means of agitation must be provided. In BOD bottles, magnetic stirrers can be used if your bottle probe is not equipped with its own stirrer. In large, quiet bodies of water, the lead to the probe can be moved up and down to provide an artificial flow.

Barometric Calibration

The third method for calibrating a DO meter is called barometric calibration. It is quicker to calibrate the meter by this method than by the Winkler calibration and it is permissible to utilize this method when using the meter for BOD analysis.

The meter should be warmed and polarized. The DO probe is put in a DO bottle partially filled (50 mls or so) with water, and is allowed 15 minutes to equilibrate. The temperature of this water vapor is then taken and by using the chart below the solubility of oxygen at this temperature is determined. For example, assume the temperature is 21°C. The chart below tells you that the solubility of saturated water vapor(s) at 21°C is 8.9 mg/l.

Solubility of Oxygen in Water Exposed to Water-Saturated Air

Temperature °C	O Chloride Concentration in Water, mg/l
10	11.27
11	11.01
12	10.76
13	10.52
14	10.29
15	10.07
16	9.85
17	9.64
18	9.45
19	9.26
20	9.07
21	8.90
22	8.72
23	8.56
24	8.40
25	8.24
26	8.09
27	7.95
28	7.81
29	7.67
30	7.54

If the barometer being used gives the barometric pressure reading in inches, use the following equation to calibrate the solubility at which the DO meter should be set:

$S^1 = \frac{S \times P}{29.92}$ = the solubility that you will set the meter at

S = the solubility of saturated water vapor at 101.3 KPa (from the chart in Column "O")

P = Barometric pressure, in inches

29.92 = a constant

In the example, the calculation works out:

$S^1 = \frac{8.90 \times 29.75"}{29.92}$

$S^1 = 8.849 - 8.85$

A different equation is used when the barometric pressure is measured in mm HG. In this case the following equation should be used.

$S^1 = \frac{S \times P}{760}$ = the solubility that you will set the meter at

S = the solubility of saturated water vapor at 101.3 KPa (from the chart under Column "O")

P = Barometric pressure, in mm Hg

760 = a constant

At the same temperature (21°C) and same barometric pressure (29.75" = 745 mm Hg), the example calculation becomes:

$S^1 = \frac{8.90 \times 756}{760}$

$S^1 = 8.853 - 8.85$

These directions hold true for elevations less than 1,000 meters (3,281') and for temperatures below 25°C.

Quality Control

For accurate data, it is important that all reagents are standardized correctly in the frequency required. The DO meter is standardized daily (each use) by the means outlined above and properly maintained so as to insure optimum performance.

DISSOLVED OXYGEN
TROUBLESHOOTING GUIDE
(Membrane Electrode Method)

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Air bubbles appear at the top of the BOD bottle after probe is inserted.	Membrane not cut around 'o' ring - (older type probes). Air is captured under membrane	Trim excess membrane just above 'o' ring.
Drifting, erratic reading **OR** Cannot get meter properly standardized.	Crease in membrane **OR** filling solution low.	Replace membrane; add fresh potassium chloride filling solution - Restandardize meter and re-run analysis.
	Flow rate of sample past the probe membrane is insufficient.	Increase meter speed - If probe stirrer is malfunctioning use a magnetic stirrer.
	Probe is not allowed to polarize before analysis.	Turn meter on 15 minutes before standardization or analysis.

Quality Control for
DISSOLVED OXYGEN ANALYSIS
(Membrane Electrode Method)

Document

Sampling

Grab - Exact Time and Date Sampled
Exact Time and Date Analyzed
Location
Temperature

Glassware

Properly washed and rinsed

Instrument

Calibration and Maintenance
Method of Standardization
Date
Analyst's initials

Duplication/Replication Schedule

REPORTING DISSOLVED OXYGEN RESULTS
(Membrane Electrode Method)

Dissolved Oxygen (Membrane Electrode Method) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE:
SAMPLE VOLUME (method):
SAMPLE TEMPERATURE:
METER STANDARDIZATION METHOD: (atmospheric, Winkler, etc.)
RESULT in mg/L:

DISSOLVED OXYGEN ON ACTIVATED SLUDGE USING WINKLER METHOD

Since activated sludge is a biological floc that will continue to use oxygen after taking a sample, it is necessary to stop the oxygen uptake as soon as possible to get a valid result. This is done by adding copper sulfate-sulfamic acid to the flocculate and settling out the activated sludge providing a clear supernatant on which to perform the Winkler method.

Equipment

In addition to the equipment and reagents required for the Winkler Method (Section 11. Page 3), you will need:

1 liter glass stoppered bottle

Reagents

COPPER SULFATE-SULFAMIC ACID INHIBITOR SOLUTION, $CuSO_4$-NH_2SO_2OH.

Dissolve 32 g of technical-grade NH_2SO_2OH in 475 ml distilled water. (DO NOT use heat to help dissolve). Dissolve 50 g $CuSO_4$ - $5H_2O$ in 500 ml distilled water. Mix these two solutions and carefully add 25 ml concentrated acetic acid.

Procedure

1) Add 10 ml $CuSO_4$-NH_2SO_2OH inhibitor to the 1 liter glass stoppered bottle.

2) Take a sample of the activated sludge by placing the 1 liter bottle in a special sampler that fills the bottle via a tube near the bottom.

3) Fill the bottle to the very top and stopper the bottle immediately and mix by inverting several times..

4) After suspended solids have settled, siphon off the clear layer (supernatant) into a 300 ml DO bottle being careful not to introduce any air by turbulence. Stopper the bottle tightly.

5) Continue Winkler method as described in Section 11 on Page 3 or Page 7 of this manual.

It is important to be aware of the difficulty of avoiding air entrapment into the bottle. It is recommended that a DO probe be used in the actual tank when DOs less than 1 mg/l are present. The Operations and Management Section may be able to lend a DO meter to those who cannot borrow one from a nearby plant.

Troubleshooting, quality control and reporting are the same as described for in the Winkler Titration method with azide modification (Section 11, Pages 11 through 13).

References

Winkler Method (with Azide Modification): Standard Methods 18th Edition 4500-OC, Page 4-100 (General Reference)

The solubility chart and barometric pressure calculations can be found on pages 4-101 through 4-105 of the 18th Edition Standard Methods

The Membrane Electrode Method for the analysis of dissolved oxygen can be found in pages 4-103 through 4-105 in the 18th Edition of Standard Methods for the Examination of Water and Wastewater.

The Copper Sulfate-Sulfamic Acid method used for analysis of DO in activated sludge is described on page 4-103 of Standard Methods for the Examination of Water and Wastewater - 18th Edition.

APPENDIX I

STANDARDIZATION OF 0.025N SODIUM THIOSULFATE

Run in duplicate:

1. Dissolve 2 g potassium iodide, KI, in 100 mls distilled water in a 500 ml Erlenmeyer flask.

2. Slowly, while stirring, add 10 ml 10% sulfuric acid, H_2SO_4. (10% H_2SO_4 = 10 mls concentrated H_2SO_4 diluted to 100 mls with distilled water)

3. Add 20.0 ml (use a volumetric pipet) of 0.025N potassium bi-iodate ($KH(IO_3)_2$) and mix.

4. Place flasks in dark for 5 minutes.

5. Dilute to approximately 300 mls with distilled water.

6. Titrate with approximately 0.025N sodium thiosulfate, to a pale straw color, add starch (1-2 ml) and titrate until blue color disappears.

7. Should use exactly 20.0 mls of thiosulfate if the normality is exactly 0.025N. Also true if using 0.0375N sodium thiosulfate with 0.0375N potassium bi-iodate.

If you use more than 20 mls, the thiosulfate is weaker than 0.025N; if you use less than 20 mls, it is stronger. If the thiosulfate is too weak, adjust normality to 0.025N by adding thiosulfate crystals; if it is too strong, adjust by diluting with distilled water. Two examples follow:

Example #1 - For 0.025N Solution - 21.40 mls thiosulfate used in the standardization titration. Using the normality, volume equation, the normality of the thiosulfate is calculated.

Bi-iodate		Thiosulfate
N_1V_1	=	N_2V_2
0.025N x 20.0 mls	=	N_2 x 21.40 mls
0.50	=	N_2 x 21.40
N_2	=	0.50 / 21.40

N_2 = 0.0234, thiosulfate is too weak and the normality must be adjusted upward by adding thiosulfate crystals. For every increase of 0.0001 N you wish to make in a liter of thiosulfate, add 0.0248g of crystals. In this example, 0.0250N - 0.0234N is an increase of 0.0016N desired. Therefore, we want to add 16 x 0.0248g of crystals or 0.397g to a liter.

Example #2 - For 0.025N Solution - 19.35 mls thiosulfate used to titrate the standards. Again using the equation $N_1V_1 = N_2V_2$, the normality of the thiosulfate is calculated:

$N_1V_1 = N_2V_2$
$0.025N \times 20.0 \text{ mls} = N_2 \times 19.35 \text{ mls}$
$N_2 = \frac{0.50}{19.35}$

$N_2 = 0.0258N$, thiosulfate is too strong and the solution must be diluted to the proper strength. To find out how much distilled water to add, again use the formula $N_1V_1 = N_2V_2$.

Remember, we used two 19.35 ml volumes (39 mls) to do the first titration so we are left with 961 mls.

$N_1V_1 = N_2V_2$
$0.0258N \times 961 \text{ mls} = 0.025N \times V_2$
$V_2 = \frac{24.79}{0.025}$
$V_2 = 992 \text{ mls}$

We need to add 992 - 961 = 31 mls of distilled water.

In both examples, the normality of the adjusted thiosulfate should be re-checked.

APPENDIX II

STANDARDIZATION OF 0.0375 SODIUM THIOSULFATE

Run in duplicate:

1. Dissolve 2 g potassium iodide, KI, in 100 mls distilled water in a 500 ml Erlenmeyer flask.

2. Slowly, while stirring, add 10 ml 10% sulfuric acid, H_2SO_4.

3. Add 20.0 ml (use a volumetric pipet) of 0.0375N potassium bi-iodate ($KH(IO_3)_2$ and mix.

4. Place flasks in dark for 5 minutes.

5. Dilute to approximately 400 mls with distilled water.

6. Titrate with approximately 0.0375N sodium thiosulfate, to a pale straw color, add starch and titrate until blue color disappears.

7. Should use exactly 20 mls of thiosulfate if the normality is exactly 0.0375.

   If you use more than 20 mls, the thiosulfate is weaker than 0.0375N, if you use less than 20 mls, it is stronger. If the thiosulfate is too weak, adjust the normality to 0.0375N by adding thiosulfate crystals; if it is too strong, adjust by diluting with distilled water. Two examples follow:

   Example #1: For 0.0375N Sodium Thiosulfate

N_1V_1	=	N_2V_2
0.0375N x 20 mls	=	N_2 x 21.40 mls
.75	=	N_2 x 21.40 mls
N_2	=	.75
	=	21.40
N_2	=	.0350 TOO WEAK!

The thiosulfate solution is too weak and must be adjusted upward by adding thiosulfate crystals. For every increase of 0.0001N you wish to make in a liter of thiosulfate, add 0.0248g of crystals. In this example, 0.0375N - 0.035N is an increase of .0025N desired. Therefore we want to add 25 x .0288g of crystals or 0.62g to a liter.

Example #2 For 0.0375N Sodium Thiosulfate - 19.35 mls thiosulfate used in the standard titration. Again using the equation $N_1 \times V_1 = N_2 \times V_2$, the normality of the thiosulfate is calculated:

$N_1 \times V_1$	=	$N_2 \times V_2$
0.00375N x 20 mls	=	N_2 x 19.35 mls
N_2	=	.75
	=	19.35

N_2 = .0388, thiosulfate is too strong and the solution must be diluted to the proper strength. To find out show much distilled water to add, again use the formula $N_1V_1 - N_2V_2$

$N_1 \times V_1$	=	$N_2 \times V_2$
.0388 x 961 mls	=	.0375N x V_2
V_2	=	37.29
	=	.0375
	=	994.4

We need to add 994.4 - 961 mls of distilled water = 33.4 mls.

That is, to the 961 mls of sodium thiosulfate remaining we add 33.4 mls of distilled water.