

USEPA¹ Azide Modification of Winkler Method²

Method 8229

1 to more than 10 mg/L DO

Buret Titration

Scope and Application: For water, wastewater and seawater.¹ USEPA approved.² Adapted from *Standard Methods for the Examination of Water and Wastewater*, (Standard Method 4500 O C)

Test preparation

Before starting the test:

Dissolved oxygen can be lost from the sample during sample collection. Review the precautions in [Sample collection, preservation and storage](#) before the test is started.

Standard APHA solutions for dissolved oxygen can be used in place of the powder pillow reagents by substituting 1 mL of Manganous Sulfate Solution, 1 mL of Alkaline Iodide-Azide Reagent and 1 mL of Sulfuric Acid (concentrated) in place of the powder pillows. These solutions must be dispensed below the surface of the liquid.

Collect the following items:

| Description | Quantity |
|---|----------|
| BOD bottle, 300-mL | 1 |
| Alkaline Iodide-Azide Reagent Powder Pillow | 1 pillow |
| Manganous Sulfate Powder Pillow | 1 pillow |
| Sulfamic Acid Powder Pillow | 1 pillow |
| Sodium Thiosulfate Standard Solution (titrant), 0.025 N | 1 bottle |
| Starch Indicator Solution | 1 bottle |
| Buret, Class A, 25-mL, with support stand | 1 |
| Graduated cylinder, 250-mL | 1 |
| Erlenmeyer flask, 250-mL | 1 |

See [Consumables and replacement items](#) for reorder information.

Buret titration

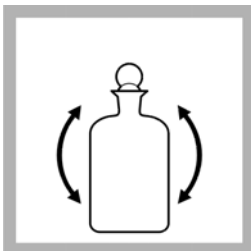


1. Collect a water sample in a clean, 300-mL, glass stoppered BOD bottle.

Overflow the bottle for two or three minutes to remove any trapped air bubbles to make sure that a representative sample is available.



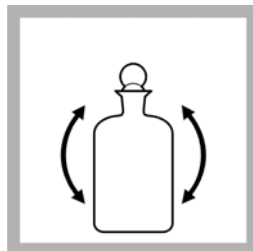
2. Add the contents of one Manganous Sulfate Powder Pillow and one Alkaline Iodide-Azide Reagent Powder Pillow.



3. Immediately insert the stopper so that no air is trapped in the bottle. Invert several times to mix.

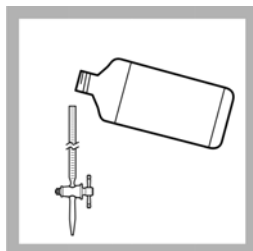
A flocculent precipitate will form. It will be orange-brown if oxygen is present or white if oxygen is absent.

The floc will settle very slowly in salt water. Wait five more minutes before proceeding to step 4.



4. Again invert the bottle several times and wait until the floc has settled and the top half of the solution is clear again.

Waiting until floc has settled twice makes sure that the reaction is complete. Results will not be affected if the floc does not completely settle.

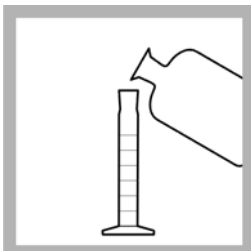


5. Fill a 25-mL buret to the zero mark with 0.025 N Sodium Thiosulfate Solution.



6. Remove the stopper and add the contents of one Sulfamic Acid Powder Pillow. Replace the stopper without trapping air in the bottle and invert several times to mix. This is the prepared sample.

The floc will dissolve and leave a yellow color if oxygen is present.



7. Pour the prepared sample into a 250-mL graduated cylinder to the 200-mL mark.

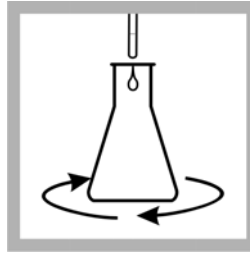


8. Pour the contents of the graduated cylinder into a 250-mL Erlenmeyer flask.

Buret titration (continued)



9. Titrate the sample while gently swirling the flask until it turns a very pale yellow color.



10. Add two 1-mL droppers of Starch Indicator Solution. Swirl to mix.

The solution will turn dark blue.



11. Continue the titration until the solution changes from dark blue to colorless.

The amount of titrant used to reach the end point is equal to the concentration of dissolved oxygen in the sample.

mL titrant used = mg/L DO

Interferences

Nitrite interference is eliminated by the azide in the reagents. Other reducing or oxidizing substances may interfere. If these are present, use an alternate method, such as the High Range Dissolved Oxygen Method, (Hach method 8166 - colorimetric) or a dissolved oxygen electrode (Standard Method 4500 O G).

Pretreatment procedure for activated sludge samples

A sample pretreatment is necessary for activated sludge samples.

1. Add 10 mL of Copper Sulfate-Sulfamic Acid Inhibitor Solution to a clean 1000-mL graduated cylinder.
2. Fill the cylinder with the sample using a tube that empties near the bottom of the cylinder and allow the sample to overflow by about 200 mL.
3. Swirl the cylinder to mix the contents. Allow the suspended solids to settle.
4. Siphon the relatively clear top layer into a BOD bottle through a siphon tube extended to the bottom of the bottle. Withdraw the siphon tube while the water is flowing. Make sure that no air bubbles are trapped in the bottle. Continue with step 2—step 11 of the test procedure.

Sample collection, preservation and storage

Sampling and sample handling are important considerations in obtaining meaningful results. The dissolved oxygen content of the water being tested varies with depth, turbulence, temperature, sludge deposits, light, microbial action, mixing, travel time and other factors. A single dissolved oxygen test rarely reflects the overall condition of a body of water. Several samples taken at different times, locations and depths are recommended for most reliable results.

Collect samples in a clean BOD bottle as described in step 1. If storage is necessary, complete steps 1–4 of the test procedure and store in the dark at the temperature of the water source or water seal at 10–20 °C. (Sealing with water is done by pouring a small amount of water into the

flared lip area of a stoppered bottle. Snap a BOD bottle cap over the flared lip). Samples preserved in this manner can be held four to eight hours. Start the test at step 6.

Accuracy check

The standard solution method can be used to confirm analytical technique and reagent performance.

Standard solution method

Complete the following test to make sure the concentration of the titrant is accurate.

Required for accuracy check:

- Iodate-Iodide Standard Solution, 0.00125 N (equivalent to 10 mg/L as O₂)
1. Add 200.0 mL of Iodate-Iodide Standard Solution, 0.00125 N, to an Erlenmeyer flask.
 2. Add one Sulfamic Acid Powder Pillow and swirl to mix.
 3. Follow steps 9–11 of the test procedure to titrate the standard to the end point. The titration should use 10.0 mL of the titrant solution. If more than 10.5 mL is used, discard the titrant and replace it with a fresh supply.

Summary of method

The Azide Modification of the Winkler Method is the standard test for dissolved oxygen. In the analysis, manganous ion reacts with the dissolved oxygen present in the alkaline solution to form a manganese (IV) oxide hydroxide flocculent. Azide is then added to suppress interference from any nitrite, which would react with the iodide. The solution is then acidified and the manganese (IV) floc is reduced by iodide to produce free iodine as I₃⁻ in proportion to the oxygen concentration. The liberated iodine is then titrated to the starch-iodide end point.

Consumables and replacement items

Required reagents

| Description | Quantity/Test | Unit | Catalog number |
|---|---------------|------------|----------------|
| Alkaline Iodide-Azide Reagent Powder Pillows | 1 pillow | 50/pkg | 107266 |
| Manganous Sulfate Powder Pillows | 1 pillow | 50/pkg | 107166 |
| Sodium Thiosulfate Standard Solution (titrant), 0.025 N | varies | 1 L | 2409353 |
| Starch Indicator Solution | 2 mL | 100 mL MDB | 34932 |
| Sulfamic Acid Powder Pillows | 1 pillow | 100/pkg | 107399 |

Required apparatus

| Description | Quantity/Test | Unit | Catalog number |
|--------------------------------------|---------------|------|----------------|
| Bottle, glass-stoppered, BOD, 300-mL | 1 | each | 62100 |
| Buret, Class A, 25-mL | 1 | each | 2636540 |
| Buret Clamp, double | 1 | each | 32800 |
| Clippers, for opening powder pillows | 1 | each | 96800 |
| Cylinder, graduated, 250-mL | 1 | each | 50846 |
| Flask, Erlenmeyer, graduated, 250-mL | 1 | each | 50546 |
| Support Stand | 1 | each | 56300 |

Recommended standards

| Description | Unit | Catalog number |
|--|--------|----------------|
| Iodate-Iodide Standard Solution, 0.00125 N | 500 mL | 40149 |

Optional reagents and apparatus

| Description | Unit | Catalog number |
|---|--------|----------------|
| APHA reagents: | | |
| Alkaline Iodide-Azide Reagent Solution | 500 mL | 27749 |
| Manganous Sulfate Solution | 500 mL | 27549 |
| Sodium Thiosulfate Standard Solution, 0.025 N | 1 L | 35253 |
| Sulfuric Acid, ACS | 500 mL | 97949 |
| Thermometer, Non-Mercury, -10 to 225 °C | each | 2635700 |
| Graduated cylinder, 1000 mL | each | 50853 |
| BOD bottle caps | 6/pkg | 241906 |
| BOD bottles, serialized | 24/pkg | 2898700 |
| Copper Sulfate-Sulfamic acid inhibitor | 100 mL | 35732 |
| Copper Sulfate-Sulfamic acid inhibitor | 500 mL | 35749 |



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