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**Environmental Science Education Title:**

Dissolved Oxygen in Surface Water: The Azide-Winkler Titration Method

**Overview:** Dissolved oxygen (DO) measurements calculate the amount of gaseous oxygen dissolved in surface water, which is important to all oxygen-breathing life in river ecosystems, including fish species preferred for human consumption (e.g. bluegill and bass), as well as decomposer species critical to the recycling of biogeochemical materials in the system.

The oxygen dissolved in lakes, rivers, and oceans is crucial for the organisms and creatures living in it. As the amount of dissolved oxygen drops below normal levels in water bodies, the water quality is harmed and creatures begin to die. In a process called eutrophication, a body of water can become hypoxic and will no longer be able to support living organisms, essentially becoming a “dead zone.”

Eutrophication occurs when excess nutrients cause algae populations to grow rapidly in an algal bloom. The algal bloom forms dense mats at the surface of the water blocking out two essential inputs of oxygen for water: gas exchange from the atmosphere and photosynthesis in the water due to the lack of light below the mats. As dissolved oxygen levels decline below the surface, oxygen-breathing organisms die-off in large amounts, creating an increase in organic matter. The excess organic matter causes an increase in the oxygen-breathing decomposer populations in the benthic zone, which further depletes the remaining dissolved oxygen levels during the metabolic decomposition activity. Once the oxygen levels become this low, mobile oxygen-breathing species (e.g. fish) will move away, leaving no aerobic life in the water and creating a dead zone.

The Azide-Winkler titration method uses titration to determine the concentration of an unknown in a sample. Specifically, sodium thiosulfate is used to titrate iodine, which can be stoichiometrically related to the amount of dissolved oxygen in a sample.

**Principles:** The Azide-Winkler method is used to measure DO on site, where surface water is collected. Manganese (II) sulfate and potassium hydroxide are added to the sample, and the dissolved oxygen in the sample oxidizes the manganese and forms a brown precipitate. Azide is added in the form of a purchased alkaline iodide-azide reagent to correct for the presence of nitrites, which are found in wastewater samples and can interfere with the Winkler oxidation procedure.

MnSO4 + 2 KOH Mn(OH)2 + K2SO4

2 Mn(OH)2 + O2 2 MnO(OH)2 (precipitate)

Sulfuric acid is then added to acidify the solution, and the precipitate dissolves. Under these conditions, the iodide from the alkaline iodide-azide reagent in the solution is converted into iodine.

2 MnO(OH)2 + 4 H2SO4 2 Mn(SO4)2 + 6 H2O

2 Mn(SO4)2 + 4 KI 2 MnSO4 + 2 I2 + 2 K2SO4

Thiosulfate is then used to titrate the iodine in the presence of an added starch indicator.

4 Na2S2O3 + 2 I2 2 Na2S4O6 + 4 NaI

4 moles of S2O32-: 1 mole of O2

At the endpoint of this titration, the blue solution will turn clear. The amount of dissolved oxygen in the sample is quantified in direct proportion to the amount of thiosulfate required to reach the endpoint.

1ml S2Os 🡪 1mg/l O:

1 ml S2O3 1L 0.025 moles 1 moles 32 g O2 1000mg 1000ml

-------------- x ----------- x ----------------- x --------------- x ----------- x ---------------- x --------------- = 1mg/l O2

200ml 1000 mL L 4 moles 1 mole 1g 1L

**Procedure:**

1. **Measure Dissolved Oxygen in sample.**
   1. At water collection site, use a calibrated pipette to add 2 mL manganous sulfate to a clear 300 mL BOD bottle filled with the sample water. Be careful not to introduce oxygen into the sample by inserting the pipette tip under the sample surface and carefully dispensing manganous sulfate. This will avoid creating bubbles until the sample is “fixed” and prevent changes to the dissolved oxygen concentration.
   2. Using the same technique, add 2 mL alkaline iodide-azide reagent.
   3. Immediately insert the stopper, tilting the bottle slightly and quickly pushing the stopper in place so no air bubbles are trapped in the bottle.
   4. Carefully invert several times (without creating air bubbles) to mix. A floccule (floc) will form from a precipitated aggregation of material with a cloudy appearance (Fig. 1).
   5. Wait until the floc in the solution has settled. Again, invert the bottle several times and wait until the floc has settled. The sample is now fixed to prevent change in dissolved oxygen content and can be transported back to the lab and stored for up to 8 hours, if needed, in a cool and dark condition.
   6. If storing, samples should be sealed using a small amount of deionized water squirted around the stopper, and the stopper should be wrapped in aluminum foil, secured with a rubber band.
   7. Pipette 2 mL of concentrated sulfuric acid into the sample by holding the pipette tip just above the sample surface. Invert carefully several times to dissolve the floc (Fig. 2).
   8. In a glass flask, and using a calibrated pipette, titrate 201 mL of sample water with 0.025 N standardized sodium thiosulfate, swirling and mixing continuously until a pale straw color forms (Fig. 3).
   9. Add 2 mL droppers of starch indicator solution and swirl to mix. Once the Starch Indicator is added, the solution will turn blue (Fig. 4).
   10. Continue the titration, adding one drop at a time until one drop dissipates the blue, causing the colorless endpoint. Be sure to add each drop of titrant carefully and to evenly mix each drop before adding the next. Holding the sample against a white piece of paper can help enhance visualization of the endpoint.
   11. The concentration of DO is equivalent to the volume (mL) of titrant used. Each milliliter of sodium thiosulfate added to the water sample equals 1 mg/L dissolved oxygen.
2. Representative Results:

2.1 A dissolved oxygen level of 6 mg/L is sufficient for most aquatic species. Dissolved oxygen levels below 4 mg/L are stressful to most aquatic animals. Dissolved oxygen levels below 2 mg/L will not support aerobic aquatic life (Fig. 5).

2.2 The maximum amount of oxygen that can be dissolved in water varies by temperature (Table 1).

**Applications:** Slow-moving rivers are particularly vulnerable to low DO levels, and in extreme cases, these DO levels can lead to hypoxic conditions, creating “dead zones” where aerobic life is no longer supported by a body of water. Once plants and animals die-off, the build-up of sediment that occurs can also raise the riverbed, allowing plants to colonize over the water and could lead to the loss of the river all together. Surface waters at higher altitudes are also more vulnerable to low DO levels, as atmospheric pressure decreases with increasing altitude, and less oxygen gas is suspended in the water.

Low DO levels support life forms considered unappealing or unfit for human use, including leeches and aquatic worms (*Oligochaeta)*.

**Legend:**   
Fig. 1: A sample after the alkaline iodide-azide reagent has been added and mixed, showing floc formation at the top of the sample before settling.

Fig. 2: A sample with dissolved floc after addition of sulfuric acid.

Fig. 3: A sample after addition of sodium thiosulfate displaying a pale straw color.

Fig. 4: A sample showing the blue color after the starch indicator is added and mixed.

Fig. 5: A dissolved oxygen level of 6 mg/L is sufficient for most aquatic species. Dissolved oxygen levels below 4 mg/L are stressful to most aquatic animals. Dissolved oxygen levels below 2 mg/L will not support fish and below 1 mg/L will not support most species.

Fig. 6: Map of dissolved oxygen concentrations across the Louisiana shelf showing the dead zone region.

Fig. 7: Photograph of the Caspian Sea showing severe eutrophication in the north end.

Table 1: Maximum amounts of oxygen that can be dissolved in water by temperature.