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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, of import 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 Azide-Winkler titration method uses a 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.

MnSO4 + 2 KOH Mn(OH)2 + K2SO4

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

Sulfuric acid is then added to acidify the solution and the precipitate dissolves. Under these conditions, the iodide 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 a starch indicator.

4 Na2S2O3 + 2 I2 2 Na2S4O6 + 4 NaI

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

1 mole of O2 4 moles of S2O32-

**Procedure:**

1. **Measure Dissolved Oxygen in sample.**
   1. Using a calibrated pipette, add 2 mL manganous sulfate to a clear 300 mL BOD bottle filled with your sample water. Be careful not to introduce oxygen into the sample by inserting pipette tip under the sample surface and carefully dispensing manganous sulfate to avoid creating bubbles.
   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 and 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” and can be 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 stopper and 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 0.0025 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 you add the Starch Indicator, 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 5 - 6 mg/L is sufficient for most aquatic species. Dissolved oxygen levels below 3 mg/L are stressful to most aquatic animals. Dissolved oxygen levels below 2 or 1 mg/L will not support fish.

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

2.3 DO measurements in mg/L are converted to % saturation using water temperature and the conversion chart below.(fig 5)

**DISSOLVED OXYGEN LEVELS (% SATURATION)**

Excellent 91 – 110

Good 71 – 90

Fair 51 – 70

Poor < 50

**Applications:** Slow-moving rivers are particularly vulnerable to low DO levels, and in extreme 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 river bed allowing plants to colonize over the water and 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 also 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 plate straw color.

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

Fig 5: DO measurements are converted to % saturation using the water’s temperature. The water’s temperature (10 oC in this example) on the top horizontal axis and the measured DO value (4.5 mg/L in this example) on the bottom horizontal axis. Use a ruler to draw a line between the two values and record where the line meets the middle diagonal axis for % saturation (40% in this example).

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