6.2.2 SPECTROPHOTOMETRIC METHOD

The spectrophotometric method described here is recommended for accurate determination of DO concentrations in suboxic waters (less than 1.0 mg/L DO concentration). The method is based on a Rhodazine-D™ colorimetric technique adapted by White and others (1990), which minimizes atmospheric interaction with the water sampled.

- This technique has a sensitivity to 0.2 µmoles/L (0.006 mg/L)—an order of magnitude lower than the amperometric method.
- The technique was developed for ground water but it can be adapted for work in anoxic zones of lakes and reservoirs.

6.2.2.A EQUIPMENT AND SUPPLIES

Two sampling systems can be used: an in situ (downhole) sampler (see White and others, 1990) or a closed-system flowthrough cell through which sample water is pumped. Either sampling system uses partially evacuated oxygen-free glass ampoules containing Rhodazine-D™, that are broken along a prescored capillary tip while they are submerged in the water to be analyzed. Equipment and supplies needed for this method are listed on table 6.2–5.

Table 6.2–5. Equipment and supplies (Rhodazine-D™ technique)
[mL, milliliters; µS/cm, microsiemens per centimeter at 25 degrees Celsius]

✓ Portable spectrophotometer, Bausch and Lomb Minispect-10™ or equivalent
✓ Ampoules with reagents, 10-mL glass, CHEMetrics Inc., Model K7553™
✓ Downhole sampler, to meet criteria described in White and others (1990)
✓ Flowthrough cell, modified to a closed-system device (alternative to sampling tool)
✓ Safety gloves, glasses, and apron
✓ Waste disposal container
✓ White background sheet
✓ Deionized water (maximum conductivity of 1 µS/cm)
✓ Bottle, squeeze dispenser, for deionized water

Kits available from CHEMetrics Incorporated contain prepackaged glass ampoules filled with a Rhodazine-D™ dye solution for two concentration ranges of dissolved oxygen: 0 to 1 mg/L (0 to 310 µmoles/L) or 0 to 40 mg/L (0 to 13 µmoles/L).

White and others (1990) used a portable Milton Roy Minispect-10™ battery-powered spectrophotometer. Any spectrophotometer of equal or better quality can be used.

> TECHNICAL NOTE: The closed-system cell is not the same as the flowthrough-chamber system used in routine ground-water field measurements. The cell consists of a three-way tee to which inflow, outflow, and discharge tubing sections are fitted tightly; outflow is fitted with a short length of 3/8-in. tubing.

CALIBRATION AND INTERFERENCES 6.2.2.B

Dissolved oxygen is measured as percent absorbance by the spectrophotometer.

- A calibration chart is provided in the CHEMetrics kit, along with a regression formula to convert absorbance to micrograms per liter of DO for use with the Minispect-10™ spectrophotometer. No other standards are provided.
- The CHEMetrics kit contains a blank ampoule used to zero the spectrophotometer.
- Interferences from total salinity and major dissolved inorganic species are negligible.
- The method is affected significantly by the presence of reducible inorganic species such as ferric and cupric ions and hexavalent chromium, resulting in high-biased DO readings. The effect from reducible inorganic species can be corrected if the concentrations of the interfering species are known.
- Additional calibration is needed if the method will be used for heavily contaminated or acidic waters, by equilibrating a water sample with known partial pressures of atmospheric oxygen (White and others, 1990). Atmospheric oxygen standards are available from suppliers of gas chromatography equipment.

6.2.2.C MEASUREMENT

Rhodazine-D™ reagent reacts with DO to produce an oxidized complex characterized by a red-blue color. The color intensity is proportional to the concentration of the initial DO present.

Follow the 7 steps below to measure DO using the Rhodazine-D™ method:

1. Zero the spectrophotometer, using the blank provided in the kit (follow the manufacturer's instructions).

2. Set the spectrophotometer to the correct wavelength.
   - The Minispect-10™ spectrophotometer is set at a wavelength of 615 nm for calibrating and measuring.
   - Refer to the manufacturer's instructions for the correct wavelength when using a different spectrophotometer.

3. Collect the sample. Install either the downhole sampling tool (White and others, 1990) or use a closed-system flowthrough cell with a suitable pump.
   - **Downhole system**—
     a. Carefully lower a sampling tool attached to a wire line.
     b. At the collection point (in the well or surface water), break the scored tip of the ampoule using a sharp upward tug on the sampling tool. (This permits sample water to be drawn into the ampoule. During transit to the surface, progressively decreasing pressure in the ampoule prevents cross contamination from overlying water through the capillary tip.)
   - **Closed-system flowthrough cell**—
     a. Fit inflow, outflow, and discharge tubing tightly into the three-way tee. Fit the outflow with a short length of 3/8-in. tubing. All fittings must be airtight to prevent aerating the sample.
     b. Insert the glass ampoule, tip first, into the outflow tubing. The seal must be airtight.
     c. Pinch the tubing so that the scored tip of the ampoule will break in the flow of water.

4. Insert the ampoule directly into the 1.27-cm spectrophotometer cell holder immediately after retrieval.

5. Read absorbance to the nearest 0.2 µmoles/L on the analog meter.
   - Allow the readings to stabilize first (usually within 2 minutes).
   - Read each DO value three times and record the median value.

6. Calculate the DO concentrations using regression equations (White and others, 1990).
   - To correct for appreciable concentrations of oxidized species of transition metals, use the stoichiometric relationships as described by White and others (1990).

7. **Quality control**—
   - Repeat steps 4 through 6 twice to document precision.
   - To document the variability of DO concentrations within the water system, repeat steps 3 through 6 on three sequentially collected samples.

Analyze samples in the field immediately.