

Standard Operating Procedure (SOP) 3.3.2.1

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MEASURING NITROGEN

Nitrogen is a nutrient that occurs naturally in both fresh and salt water. It is essential for plant growth in an aquatic ecosystem. Problems occur when large amounts nitrogen are introduced into the stream ecosystem. As a result, there can be excessive algal growth depleting the available oxygen in the stream that fish and other aquatic organisms depend upon.

Nitrogen exists in numerous forms but most commonly is found in water as nitrate (NO_3^-), nitrite (NO_2^-) and or ammonia (NH_3). Of these forms, nitrate is usually the most stable and is the form most often found in aquatic environments. Nitrite can be found in suboxic waters. Nitrogen is an essential nutrient for growth of algae and other aquatic plants, and nitrates can be present at high levels due to inputs from a variety of sources. Nitrate is very difficult to measure directly, so it is reduced to nitrite and the resulting nitrite concentration is measured. The measurement gives the combined concentration of nitrite (if present) and nitrate concentrations. Because we are interested in the nitrate measurement, background levels of nitrite also have to be measured. Ammonia is toxic in high concentrations, and is preferentially utilized (taken up) by phytoplankton and green plants. Nitrate measurements are reported as nitrate nitrogen (mg/L). Nitrite measurements are reported as nitrite nitrogen (mg/L). Ammonium measurements are reported as ammonium nitrogen (mg/l).

Measuring nitrate levels in water.

Materials and Tools

- 1) 50 mL beaker or flask
- 2) Nitrate Test Kit (if you have salt or brackish water, be sure to use an appropriate test kit)
- 3) 100 mL graduated cylinder
- 4) 500 mL graduated cylinder
- 5) 3 500-mL bottles or jars
- 6) Distilled water

Preparation

Read all instructions carefully in the test kit before beginning. Make sure kit includes all the materials listed. Review proper levels of nitrate that are acceptable in water (10 mg/L nitrate-nitrogen for drinking water).

Calibration and Quality Control

Standards should be run at least every six months to verify your technique and the integrity of your chemicals. Fresh standard should be prepared each time unless the standard has been stabilized. Measuring the standards will help to clarify the instructions in test kits where wording may be unclear.

Nitrate Standards

Nitrate standards do not come with test kits and need to be either ordered separately or prepared as follows:

- **Stock Nitrate Solution:** Dry KNO_3 (potassium nitrate) in an oven for 24 hours at 105°C . Then dissolve 3.62 g of KNO_3 in distilled water. Dilute to 500 mL in your 500 mL graduated cylinder using distilled water. Carefully swirl the solution to mix (do not shake). Store in a 500 ml bottle or jar. Label with masking tape (include date). This makes a 7200 mg/L KNO_3 (or a 1000 mg/L nitrate nitrogen) solution.
Note: To calculate nitrate nitrogen ($\text{NO}_3^{-2}\text{-N}$), take into account the molecular composition of KNO_3 (the ratio of the molecular weight of N to KNO_3 is 0.138): $7200 \text{ mg/L } \text{KNO}_3 \times 0.138 @ 1000 \text{ mg/L nitrate nitrogen } (\text{NO}_3\text{-N})$.
- **Standard Nitrate Solution:** Measure 50 mL of the stock nitrate solution using the 100 mL graduated cylinder. Pour into the 500 mL graduated cylinder and dilute to 500 mL with distilled water. Carefully swirl the solution to mix. The result is a 100 mg/L nitrate nitrogen standard. Store in a 500 ml bottle or jar. Label with masking tape (include date).
- Make a new stock nitrate solution each time a calibration is conducted if the stock solution has not been preserved. Standard nitrate solutions should be made fresh each time regardless of whether the stock solution has been preserved or not. The stock nitrate solution can be preserved and stabilized for up to six months using chloroform (CHCl_3) if you have safe access to this chemical. To preserve a stock nitrate standard add 1 mL of CHCl_3 to 500 mL of stock solution.

Quality Control Procedure

1. Dilute the 100 mg/L standard to make a 2 mg/L standard. Use this standard to test the accuracy of the nitrate kit. Measure out 10 mL of the 100 mg/L standard nitrate solution using the 100 mL graduated cylinder. Pour this into the 500 mL flask or beaker. Measure out 490 mL of distilled water in the 500 mL graduated cylinder and add to 500 mL bottle or jar.

Label with masking tape (include date). Carefully swirl the solution to mix the standard.

2. Follow the directions in the *Protocol* section to measure the standard. Where it says "sample water" this is where you use the standard that you made.
3. Record the value of the standard after testing on the Hydrology Investigation Data Work Sheet.
4. If the nitrate standard is off by more than 1 mg/L, prepare new dilutions and repeat the measurement. If still off, make a new stock solution and repeat the procedure.

Tip: Standards should be run at least every six months to verify your technique and the integrity of your chemicals. Fresh standard should be prepared each time unless the standard has been stabilized. Measuring the standards will help to clarify the instructions in test kits where wording may be unclear.

How to Measure Nitrate Nitrogen

1. **Nitrate nitrogen plus nitrite nitrogen:** Follow the manufacturer's nitrate instructions in the kit. The kits are based on the technique of adding a reagent that reacts with nitrate to form nitrite. The nitrite reacts with a second reagent to form a color. The intensity of the color is proportional to the amount of nitrate in the sample. The concentration is determined by comparing the sample color, after addition of reagents, to a color comparator included in the kit. If the kit calls for shaking the sample, be sure to shake for the specified period of time. Failure to follow the times specified in the directions will result in inaccurate measurements.
2. Record the nitrate concentration on data work sheet. (**Note:** Hold the comparator up to a light source such as a window, the sky or a lamp. Do not hold it up directly to the sun.)
3. Take the average of the three readings. If the recorded values are all within 1 mg/L of the average, record the average on the data work sheet. If they reading are not within 1 mg/L of the average have the reader reread the color comparator, then record and average the new values. (**Note:** do not reread if more than 5 minutes has elapsed.) If your remaining values are now all within 1 mg/L of the new average, record this new average on the data work sheet. If there is still an outlier (a value far different from the rest) discard that value and calculate a new average of the other values. If there is a wide scatter (more than 1 mg/L) of data points within the results, discuss the procedure and the potential sources of error with the reader/monitors and then repeat the protocol to produce a reportable measurement.
4. **Nitrite nitrogen:** Follow the manufacturer's instructions for nitrite. It is the same procedure, except the reagent to reduce nitrate to nitrite is not used.
5. Repeat steps 3 and 4 to obtain nitrite values.

Note: Test results should be reported as mg/L nitrate nitrogen (NO_3^{-2} -N; the same units as your standards), and not as mg/L nitrate (NO_3^{-2}).

For general information: To convert mg/L nitrate to mg/L nitrate nitrogen divide by 4.4, the ratio of their molecular weights. For example: 44 mg/L NO_3^{-2} is equivalent to 10 mg/L NO_3^{-2} -N. To convert mg/L nitrite to mg/L nitrite nitrogen divide by 3.3, the ratio of their molecular weights.

Test Kits:

Measuring Nitrogen as Nitrate

Nitrate Nitrogen Tablet Kit (LaMotte)

- 1) Fill a vial with 5ml of sample water.
- 2) Add one Nitrate Tablet (2799).
- 3) Cap the vial and mix until the tablet disintegrates.
- 4) Add one Nitrate #2 tablet (NN-3703).
- 5) Cap the vial and mix until the tablet disintegrates.
- 6) Wait 5 minutes.
- 7) Insert Nitrate-Nitrogen Ocat-Slide Bar into the Octa-Slide Viewer (Color Comparitor).
- 8) Insert the vial into the Octa-Slide Viewer.
- 9) Match the sample color to a color standard. Record as ppm Nitrate-Nitrogen.

To convert to nitrate multiply results by 4.4 and record as Nitrate.

Measuring Nitrogen as Ammonia in Water

Ammonia-Nitrogen Test Kit - Salicylate Method (LaMotte)

- 1) Fill a test tube (0124) to the 5ml mark with sample water.
- 2) Add 10 drops of Salicylate Ammonia #1 (3978).
- 3) Cap the test tube and mix.
- 4) Add 7 drops of Salicylate Ammonia #2 (3982).
- 5) Cap the test tube and mix.
- 6) Wait 20 minutes.
- 7) Insert Ammonia Nitrogen Octa-Slide Bar into the Octa-Slide Viewer (Color Comparitor).
- 8) Insert the tube into the Octa-Slide Viewer.
- 9) Match sample color to a color standard. Record as ppm Ammonia Nitrogen.

Colorimeters:

The accuracy and precision of the nitrate, nitrite, and ammonia tests can be improved by using a colorimeter in place of the color comparator. The colorimeter is a simplified spectrophotometer, which measures the amount of color in the sample at a specific wavelength of light. By choosing the appropriate nutrient test preprogrammed in the colorimeter, the correct wavelength is utilized.

Although the chemistry is the same as for the color comparator kits, the reagents used may be slightly different. Thus, be sure to only use those chemicals specified for the colorimeter method.

The advantage of this method (using a colorimeter) is that it places the accuracy on an electronic measurement. Thus it removes individual subjective differences in readings and removes the problem of trying to decide which color value (from the comparator) your sample is most similar to.

References: LaMotte Instructions: Nitrate Nitrogen Tablet Kit,
G.L.O.B.E. Teachers Manual 1997