

# Determining the Amount of Nitrate in Water

## INTRODUCTION

The tests described here are used to measure the concentration of nitrate ions,  $NO_3^-$ , in a water sample. The concentration of nitrate will be expressed throughout this section in units of mg/L  $NO_3^-$ -N. The unit,  $NO_3^-$ -N, means simply “nitrogen that is in the form of nitrate.”

Nitrate ions found in freshwater samples result from a variety of natural and manmade sources. Nitrates are an important source of nitrogen necessary for plants and animals to synthesize amino acids and proteins. Most nitrogen on earth is found in the atmosphere in the form of nitrogen gas,  $N_2$ . Through a process called the *nitrogen cycle*,<sup>1</sup> nitrogen gas is changed into forms that are useable by plants and animals. These conversions include industrial production of fertilizers, as well as natural processes, such as legume-plant nitrogen fixation, plant and animal decomposition, and animal waste.

### Sources of Nitrate Ions

- Agriculture runoff
- Urban runoff
- Animal feedlots and barnyards
- Municipal and industrial wastewater
- Automobile and industrial emissions
- Decomposition of plants and animals

Although nitrate levels in freshwater are usually less than 1 mg/L, manmade sources of nitrate may elevate levels above 3 mg/L. These sources include animal feedlots, runoff from fertilized fields, or treated municipal wastewater being returned to streams. Levels above 10 mg/L in drinking water can cause a potentially fatal disease in infants called *methemoglobinemia*, or Blue-Baby Syndrome. In this disease, nitrate converts hemoglobin into a form that can no longer transport oxygen.

High nitrate concentrations also contribute to a condition in lakes and ponds called *eutrophication*, the excessive growth of aquatic plants and algae. Unpleasant odor and taste of water, as well as reduced clarity, often accompany this process. Eventually, dead biomass accumulates in the bottom of the lake, where it decays and compounds the problem by recycling nutrients. If other necessary nutrients are present, algal blooms can occur in a lake with as little as 0.50 mg/L  $NO_3^-$ -N.

Nitrate pollution of surface and groundwater has become a major ecological problem in some agricultural areas. Although fertilizer in runoff is most often blamed, there is evidence that concentration of livestock in feedlots is now the major source of agricultural nitrate pollution. Runoff from fertilized fields is still a significant source of nitrate, although fertilizer use peaked in 1981 and has remained fairly constant since.

## Expected Levels

The nitrate level in freshwater is usually found in the range of 0.1 to 4 mg/L  $NO_3^-$ -N. Unpolluted waters generally have nitrate levels below 1 mg/L. The effluent of some sewage treatment plants may have levels in excess of 20 mg/L.

In a study based on 344 USGS sites throughout the United States,<sup>2</sup> 80% of the sites reported nitrate levels less than 1 mg/L, 16% were in the range of 1–3 mg/L, and 4% were greater than

<sup>2</sup> U.S. Geological Survey, *National Water Summary 1990–91, Hydrologic Events and Stream Water Quality*, Water-Supply Paper 2400, United States Government Printing Office, 1993, 122–123.

3 mg/L. The percentage of various land types reporting greater than 1 mg/L of nitrate were range land <5%, forested land ~10%, urban areas ~30%, and agricultural land ~40%.

Table 1: Nitrate Concentration in Selected Sites		
Site	Nitrate spring level (mg/L NO <sub>3</sub> <sup>-</sup> -N)	Nitrate fall level (mg/L NO <sub>3</sub> <sup>-</sup> -N)
Mississippi River, Clinton, IA	0.55	1.20
Mississippi River, Memphis, TN	1.60	2.90
Rio Grande River, El Paso, TX	0.38	0.59
Ohio River, Benwood, WV	0.87	1.30
Willamette River, Portland, OR	0.28	0.98
Missouri River, Garrison Dam, ND	0.40	0.14
Hudson River, Poughkeepsie, NY	0.49	0.64
Platte River, Sharpes Station, MO	1.90	1.30

## Summary of Method

### Method 1: Nitrate Ion-Selective Electrode

A Vernier Nitrate Ion-Selective Electrode (ISE) is used to measure the nitrate-ion concentration in the water, in mg/L NO<sub>3</sub><sup>-</sup>-N, either on site or after returning to the lab.

## Method 1: NITRATE ION-SELECTIVE ELECTRODE

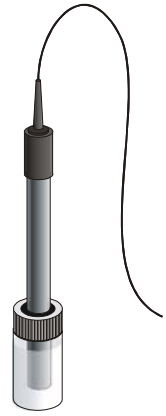
### Materials Checklist

- |  |   |
|--|---|
| <input type="checkbox"/> LabPro or CBL 2 interface             | <input type="checkbox"/> tissues or paper towels                                  |
| <input type="checkbox"/> TI Graphing Calculator                | <input type="checkbox"/> Low Standard (1 mg/L NO <sub>3</sub> <sup>-</sup> -N)    |
| <input type="checkbox"/> DataMate program                      | <input type="checkbox"/> High Standard (100 mg/L NO <sub>3</sub> <sup>-</sup> -N) |
| <input type="checkbox"/> Nitrate Ion-Selective Electrode       | <input type="checkbox"/> distilled water  |
| <input type="checkbox"/> small paper or plastic cup (optional) |   |

## Advanced Preparation

The Vernier Nitrate Ion-Selective Electrode (ISE) must be soaked in the Nitrate High Standard solution (included with the ISE) for approximately 30 minutes prior to use. **Important:** Make sure the ISE is not resting on the bottom of the container, and that the small white reference contacts are immersed. Make sure no air bubbles are trapped below the ISE.

If the ISE needs to be transported to the field during the soaking process, use the Short-Term ISE Soaking Bottle. Remove the cap from the bottle and fill it  $\frac{3}{4}$  full with High Standard. Slide the bottle's cap onto the ISE, insert it into the bottle, and tighten. **Important:** Do not leave the ISE soaking for more than 24 hours. Long-term storage should be in the Long-Term ISE Storage Bottle.



*ISE soaking  
for travel*

## Collection and Storage of Samples

1. This test can be conducted on site or in the lab. A 100-mL water sample is required.
2. It is important to obtain the water sample from below the surface of the water and as far away from shore as is safe. If suitable areas of the stream appear to be unreachable, samplers consisting of a rod and container can be constructed for collection. Refer to page Intro-4 of the Introduction of this book for more details.
3. If the testing cannot be conducted within a few hours, store samples in an ice chest or refrigerator.

## Testing Procedure

1. With the ISE still soaking in the High Standard solution, plug it into Channel 1 of the LabPro or CBL 2 interface. Use the link cable to connect the TI Graphing Calculator to the interface. Firmly press in the cable ends.
2. Turn on the calculator and start the DATAMATE program. Press **CLEAR** to reset the program.
3. Set up the calculator and interface for the ISE.
  - a. Select SETUP from the main screen.
  - b. If the calculator displays NO<sub>3</sub> ISE (MG/L) in CH 1, proceed directly to Step 4. If it does not, continue with this step to set up your sensor manually.
  - c. Press **ENTER** to select CH 1.
  - d. Select ION SELECTIVE from the SELECT SENSOR menu.
  - e. Select NO<sub>3</sub> ISE (MG/L) from the ION SELECTIVE menu.
4. Set up the calibration for the Nitrate ISE.
  - If your instructor directs you to manually enter the calibration values, select CALIBRATE, then MANUAL ENTRY. Enter the values, select OK, then proceed to Step 5.
  - If your instructor directs you to perform a new calibration, follow this procedure.

### First Calibration Point

- a. Select CALIBRATE, then CALIBRATE NOW.
- b. When the voltage reading is stable, press **ENTER**.
- c. Enter "100" as the concentration of the standard in mg/L NO<sub>3</sub><sup>-</sup>-N.

### Second Calibration Point

- d. Rinse the ISE thoroughly with distilled water and gently blot it dry with a tissue or paper towel. **Important:** Failure to carefully rinse and dry the ISE will contaminate the standard.
- e. Place the tip of the ISE into the Low Standard (1 mg/L NO<sub>3</sub><sup>-</sup>-N). Be sure that the ISE is not resting on the bottom of the bottle and that the small white reference contacts are immersed. Make sure no air bubbles are trapped below the ISE.
- f. After briefly swirling the solution, hold the ISE still and wait approximately 30 seconds for the voltage reading to stabilize. Press ENTER.
- g. Enter "1" as the concentration of the standard in mg/L NO<sub>3</sub><sup>-</sup>-N.
- h. Select OK to return to the setup screen.

5. Set up the data-collection mode.
  - a. To select MODE, press  once and press .
  - b. Select SINGLE POINT from the SELECT MODE menu.
  - c. Select OK to return to the main screen.
  
6. Collect nitrate concentration data.
  - a. Rinse the ISE with distilled water and gently blot it dry with a tissue. Place the tip of the ISE into the stream at Site 1, or into a cup with sample water from the stream. Make sure the small white reference contacts are immersed, and that the ISE is not resting on the bottom of the cup. Be sure no air bubbles are trapped below the ISE.
  - b. After briefly swirling the solution, hold the ISE still and wait approximately 30 seconds for it to stabilize.
  - c. Select START to begin sampling. **Important:** Hold the ISE still for the next 10 seconds.
  - d. After 10 seconds, the nitrate concentration will appear on the screen. Record this value on the Data & Calculations sheet (round to the nearest 0.01 mg/L NO<sub>3</sub><sup>-</sup>-N). **Note:** The sensor does not read values accurately below 0.1 mg/L. If the reading is less than 0.1, write <0.1 on the Data & Calculations sheet.
  - e. Press  to return to the main screen.
  - f. Select START to obtain a second reading. Record this value on the Data & Calculations sheet (round to the nearest 0.01 mg/L NO<sub>3</sub><sup>-</sup>-N).
  - g. Press  to return to the main screen.

# DATA & CALCULATIONS

## Method 1: Nitrate Ion-Selective Electrode

Stream or lake: \_\_\_\_\_ Time of day: \_\_\_\_\_

Site name: \_\_\_\_\_ Student name: \_\_\_\_\_

Site number: \_\_\_\_\_ Student name: \_\_\_\_\_

Date: \_\_\_\_\_ Student name: \_\_\_\_\_

Column	A
Reading	Nitrate (mg/L NO <sub>3</sub> <sup>-</sup> -N)
1	
2	
Average	

Column Procedure:

A. Record the nitrate concentration from the calculator, in mg/L NO<sub>3</sub><sup>-</sup>-N.

Field Observations (e.g., weather, geography, vegetation along stream) \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Test Completed: \_\_\_\_\_ Date: \_\_\_\_\_

## TEACHER INFO NITRATES

### Method 1: Nitrate Ion-Selective Electrode

1. The range of the Vernier Ion-Selective Electrode is 0.10 to 14,000 mg/L  $\text{NO}_3^-$ -N. As stated in the introduction, most values you measure from streams and lakes will be in the range of 0.1 to 4 mg/L-the lower end of the useful range of the sensor. If students obtain values lower than 0.1 mg/L, they should report these as  $< 0.1$  mg/L.
2. Two standard solutions are included with the Nitrate ISE-a High Standard that is 100 mg/L  $\text{NO}_3^-$ -N, and a Low Standard that is 1 mg/L  $\text{NO}_3^-$ -N.3 You can replace these standards using these directions:

High Standard (100 mg/L  $\text{NO}_3^-$ -N)

- a. Add 0.607 g of  $\text{NaNO}_3$  to enough distilled water to prepare one liter of solution.

Low Standard (1 mg/L  $\text{NO}_3^-$ -N)

- b. Dilute the High Standard from 100 mg/L to 10 mg/L by combining 100 mL of the High Standard with 900 mL of distilled water. Mix well.
- c. Combine 100 mL of the 10-mg/L solution with 900 mL of distilled water. Mix well. This standard is now 1 mg/L  $\text{NO}_3^-$ -N.

Prior to 1999, the Low Standard Solution shipped with each Nitrate ISE was 10 mg/L  $\text{NO}_3^-$ -N. This standard can be diluted to 1 mg/L by carefully measuring out 10 mL of the 10 mg/L standard, and adding enough distilled water (-90 mL) to make 100 mL of 1 mg/L  $\text{NO}_3^-$ -N standard

3. The Nitrate ISE can be used in a wide range of pH values, pH 2.5 to 11. The ions that are known to interfere with the Nitrate ISE ( $\text{ClO}_3^-$ ,  $\text{I}^-$ ,  $\text{ClO}_4^-$ ,  $\text{CN}^-$ ,  $\text{BF}_4^-$ ) will not generally be encountered in significant concentrations in freshwater samples.
4. Even better results can be obtained if you bring all samples to the same ionic strength. This is especially important when working with very low concentrations of ions. This can be accomplished by the addition of ionic strength adjuster, ISA. Add Nitrate ISA in the ratio of approximately 1 to 50; for example, if your water sample is 50 mL in volume, add about 1mL of ISA.

To prepare 100 mL of Nitrate ISA solution, 2.0 M  $(\text{NH}_4)_2\text{SO}_4$ , add 26.42 g of solid ammonium sulfate,  $(\text{NH}_4)_2\text{SO}_4$  to enough water to prepare 100 mL of solution.

5. Soaking your ISE prior to use is very important. The Advanced Preparation section recommends approximately 30 minutes of soaking in the High Standard solution. This is usually sufficient, but an hour-long soak is even better. If you are going out into the field, you can "soak as you go" using the Short-Term ISE Soaking Bottle. We began shipping Short-Term ISE Soaking Bottles with Vernier Ion-Selective Electrodes in January of 1999. If you purchased your ISEs prior to this and would like to use ISE Soaking Bottles, they can be purchased from Vernier Software (BTL-ISE, \$10 per package of 5 bottles). Important: Do not let the ISE soak longer than 24 hours. Long-Term storage should be in the Long-Term ISE Storage Bottle.
6. The Nitrate ISE has a PVC membrane with a limited life expectancy. It is warranted to be free from defects for a period of twelve (12) months from the date of purchase. It is possible, however, that you may get somewhat longer use than the warranty period. If you start to notice a reduced response (for example, distinctly different voltages or voltage ranges during calibration), it is probably time to replace the membrane module. Important: Do not order membrane modules far in advance of the time you will be using

them; the process of degradation takes place even when they are stored on the shelf.

7. The SINGLE POINT data-collection mode was designed to make measurements easier and more accurate. When SINGLE POINT mode is used, the interface takes readings for 10 seconds. These readings are averaged and this average value is displayed on the calculator. This method has several advantages over other data-collection modes: (1) It eliminates the need for students to choose one value over another if that value is fluctuating; (2) If the readings are fluctuating a little, an average of the values is desirable; (3) It requires the students to hold the sensor in the water longer than they might tend to otherwise.

### Methods 2 and 3: Nitrate Using a Colorimeter

8. The following reagents can be ordered directly from LaMotte Company (P.O. Box 329, Chestertown, MD 21620, Tel: 800-344-3100, www.lamotte.com):

- **Mixed** Acid Reagent, two 60-mL bottles ..... V -6278-H
- **Nitrate** Reducing Agent ..... V -6279-C
- **0.1g** Plastic Measuring Spoon.....0699

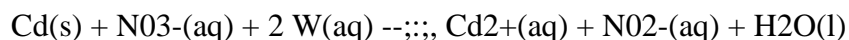
A Nitrate-N kit can also be ordered from LaMotte Company that includes both reagents:

- **Nitrate-N** Kit (spoon included).....3649-SC

9. We selected LaMotte Company reagents for the Nitrate colorimetric test because: (a) The recommended wavelength, 540 nm, is very close to the 565-nm wavelength setting on the Vernier Colorimeter,<sup>4</sup> and (b) We found the range of this test, 0.1 to 3.0 mg/L NO<sub>3</sub><sup>-</sup>-N to be very convenient for most freshwater samples. If samples are found to have nitrate levels higher than 3.0 mg/L, they may be diluted so they fall within this range. For example, a freshwater sample can be diluted to 1/2 of its concentration by adding 100 mL of distilled water to 100 mL of the water sample. If the concentration of the diluted sample was found to be 2.6 mg/L using either colorimetric method, then the final value would be multiplied by 2, for a reported value of 5.2 mg/L.

Using a wavelength that is somewhat different from that recommended by LaMotte does not degrade results. Absorbance values may be somewhat lower, but a valid Beer's law relationship still exists at 565 nm.

10. In this test, nitrate concentration is not measured directly. Upon addition of the Nitrate Reducing Agent, nitrate is reduced to nitrite, NO<sub>2</sub><sup>-</sup> by this reaction:



Note: The 0.1-g plastic spoon (included with the Nitrate-N kit) can be used to measure out 0.2 g of Nitrate Reducing Agent-this reagent is added in excess, so it is not necessary to use an electronic balance.

After adding the Mixed Acid Reagent, the nitrite ion forms a reddish-purple color. The color is then compared to one standard solution containing the same added reagent (Method 2), or to a series of standards using Beer's law (Method 3). A Vernier Colorimeter is used to measure the absorbance of the unknown and standard solution(s). Because of the conversion of nitrate ions to nitrite ions, this test actually measures the *sum* of the nitrate and nitrite ion concentrations.

The Mixed Acid Reagent contains sulfanilamide and N-(1-naphthyl)-ethylenediamene-dihydrochloride (NED).



11. After performing the nitrate test, all waste should be placed in a labeled waste container. The waste contains a small amount of cadmium metal. Dispose of the waste according to your state and local regulations.

**HAZARD ALERT:**

Cadmium metal (in LaMotte Nitrate Reducing Agent): A known carcinogen; dust or fume inhalation especially toxic. Hazard Code: B-Hazardous.

The hazard information reference is Flinn Scientific, Inc., *Chemical & Biological Catalog/Reference Manual*, 1999, P.O. Box 219, Batavia, IL 60510.

12. The directions in Methods 2 and 3 call for a nitrate standard solution with a concentration of 2.5 mg/L NO<sub>3</sub><sup>-</sup>-N. This solution can be prepared following these steps:
  - a. Prepare a 100-mg/L standard by adding 0.607 g of NaNO<sub>3</sub> to enough distilled water to prepare 1 liter of solution.
  - b. Dilute the 100-mg/L standard to 10 mg/L by combining 100 mL of 100 mg/L solution with 900 mL of distilled water.
  - c. Dilute the 10-mg/L solution to 2.5 mg/L by combining 250 mL of the 10-mg/L solution with 750 mL of distilled water. The resulting solution has a concentration of 2.5-mg/L NO<sub>3</sub><sup>-</sup>-N.

**General Information about Nitrate**

13. Samples that are brought back to the lab for analysis should be stored in an ice chest or refrigerator prior to testing. You should try to test all samples within 24 hours of collection, so that biochemical processes do not change the nitrate levels.
14. Nitrate concentration in this test is reported in units of mg/L NO<sub>3</sub><sup>-</sup>-N. The ending, NO<sub>3</sub><sup>-</sup>-N, means simply "nitrogen that is in the form of nitrate." Expressing nitrate concentration in terms of nitrogen is commonly done because other forms of nitrogen also exist in water samples (nitrite, NO<sub>2</sub><sup>-</sup>-N, and ammonium, NH<sub>4</sub><sup>+</sup>-N). If different forms of nitrogen are each expressed in terms of N, comparisons are easier to do.

Test results are sometimes published in units of mg/L NO<sub>3</sub><sup>-</sup> instead of NO<sub>3</sub><sup>-</sup>-N. To convert 100 ml/L NO<sub>3</sub><sup>-</sup>-N to mg/L NO<sub>3</sub><sup>-</sup>, you would perform this calculation:

$$\frac{100 \text{ mg N}}{1 \text{ L}} \times \frac{62 \text{ g NO}_3^-}{14 \text{ g N}} = 443 \text{ mg/L NO}_3^-$$

15. Because many fertilizers contain nitrate ions, monitoring nitrate levels in a stream that borders fertilized fields may show significant seasonal differences in NO<sub>3</sub><sup>-</sup> concentrations. Fertilizers are designated by numbers such as 6-12-8, indicating the percentages of N (6%), phosphorus as P<sub>2</sub>O<sub>5</sub> (12%), and potassium as K<sub>2</sub>O (8%). Farm manure corresponds to only 0.5-0.24-0.5 fertilizer. In the past, nitrate has been added to fertilizers in the form of ammonium nitrate, NH<sub>4</sub>NO<sub>3</sub>, and sodium nitrate, NaNO<sub>3</sub>. Due to the hazardous nature of these two substances, urea, H<sub>2</sub>NCONH<sub>2</sub>, is now the most-favored nitrogen-containing fertilizer. Intensive use of any of these fertilizing agents will result in increased levels of nitrate in streams adjacent to fields.
16. Formation of much of the nitrate in freshwater results from a series of processes in the *nitrogen cycle*. Ammonia (NH<sub>3</sub>) is converted to nitrite (NO<sub>2</sub><sup>-</sup>) by *nitrosomona* bacteria. Nitrite is then quickly converted to nitrate (NO<sub>3</sub><sup>-</sup>) by *nitrobacter* bacteria.

17. Significant flow rate in a stream can prevent the formation of algae blooms, even if essential nutrients, such as nitrate ions, are present. As a result, algae blooms may not occur in a stream until well into summer-when flow rates in the lower portions of a stream decrease. In lakes and ponds, where no appreciable current exists, if other nutrients are present, nitrate levels as low as 0.50 mg/L may result in significant growth of algae.

Essential plant nutrients include nitrogen, phosphorus, carbon (CO<sub>2</sub>), potassium, sulfur, Magnesium, calcium, and a number of other micronutrients (E, Cl, Co, Cu, Fe, Mo, Mn, Na, Si, V, Zn). In most cases, phosphorus is the plant nutrient most likely to be *limiting*; that is, the one depleted first.

18. Nitrate levels measured in an aquarium will typically be higher than levels found in streams, often as high as 20-40 mg/L.
19. During the wet season, particularly when plants are dormant, rain or snowmelt may leach large amounts of nitrate into the water table.

### How the Nitrate Ion-Selective Electrode Works

The Nitrate Ion-Selective Electrode is a membrane-based electrode that measures nitrate ions in an aqueous solution. The membrane is a porous plastic disk, permeable to the ion exchanger, but impermeable to water. When the membrane of the ISE is in contact with a solution containing the nitrate ion, a voltage, dependent on the level of nitrate in the solution, develops at the membrane. The interface reads the voltage and calculates the ion concentration.

### How the Vernier Colorimeter Works

The Vernier Colorimeter works by shining light of one wavelength (565 nm in this test) through a cuvette containing the sample solution. Some of the incoming light is absorbed by the solution. The light that does pass through the cuvette is detected by a photodiode and produces a voltage that is linear to percent transmittance. Absorbance is then calculated from percent transmittance according to the equation

$$A = \log(1/T)$$

The DataMate program will automatically make this calculation for you.

