Test
8

Nitrate

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*,[1] 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.

[1] See *Test 10: Ammonium Nitrogen*, p. 10-1, for further information on the nitrogen cycle.

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,[2] 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 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_3^--N)	Nitrate fall level (mg/L NO_3^--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 Methods

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_3^--N, either on site or after returning to the lab.

Method 2: Nitrate—Colorimeter with a Single Standard

A Vernier Colorimeter is used to create a 2-point standard curve of absorbance *vs*. nitrate concentration using a blank and one nitrate standard solution. This method is faster and easier than the multiple-standard method, but because your measurement depends upon one standard, the chances for error are somewhat higher.

Method 3: Nitrate—Colorimeter with Multiple Standards

A Vernier Colorimeter is used to create a 4-point standard curve of absorbance *vs*. nitrate concentration using a set of four nitrate standards. This method takes more time and effort than the single-standard method, but the standard curve will be based on four points, reducing the chance of error.

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[2] 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.

Method 1: NITRATE ION-SELECTIVE ELECTRODE

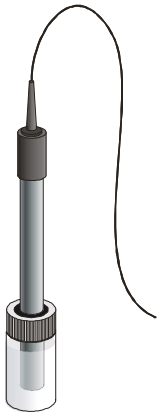
Materials Checklist

___ LabPro or CBL 2 interface
___ TI Graphing Calculator
___ DataMate program
___ Nitrate Ion-Selective Electrode
___ small paper or plastic cup (optional)
___ tissues or paper towels
___ Low Standard (1 mg/L NO_3^--N)
___ High Standard (100 mg/L NO_3^--N)
___ distilled water

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 ¾ 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 NO3 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 NO3 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_3^--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_3^--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_3^--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 ENTER.
   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_3^--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 ENTER 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_3^--N).
   g. Press ENTER 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_3^--N)
1	
2	
Average	

Column Procedure:

A. Record the nitrate concentration from the calculator, in mg/L NO_3^--N.

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

_____

_____

_____

Test Completed: _____ Date: _____

Method 2: NITRATE—COLORIMETER WITH A SINGLE STANDARD

Materials Checklist

___ LabPro or CBL 2 interface
___ TI Graphing Calculator
___ DataMate program
___ Vernier Colorimeter
___ 0.1 g plastic measuring spoon
___ tissues (preferably lint-free)
___ pipet pump or pipet bulb
___ one cuvette
___ one 125-mL Erlenmeyer flask *per test*
___ one rubber stopper per Erlenmeyer flask
___ two 10-mL pipets (or graduated cylinders)
___ nitrate standard (2.5 mg/L NO_3^--N)
___ Nitrate Reducing Reagent
___ Mixed Acid Reagent
___ distilled water

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, refrigerate the samples. Do not keep samples more than 24 hours.

Testing Procedure

1. Obtain collection site samples and standard for testing.
   a. Label one Erlenmeyer flask for each of the collection site samples you will be testing.
   b. Measure 5 mL of sample into each corresponding Erlenmeyer flask.
   c. Label one Erlenmeyer flask for the nitrate standard solution and measure 5 mL of standard into the flask.

2. Prepare the collection site samples for testing.
   a. Add 5 mL of Mixed Acid Reagent to each flask (also to the 2.5 mg/L nitrate standard). Stopper each flask and shake. Wait 2 minutes for a complete reaction to occur.
   b. Use the 0.1-g plastic spoon to add two spoonfuls (~0.2 g) of Nitrate Reducing Reagent to each of the flasks.
   c. Stopper the flasks and invert at a rate of 50–60 times per minute for 2 minutes. Wait 12 minutes for a complete reaction. During the 12-minute reaction period, proceed to Step 3. **Note:** Any undissolved portion of Nitrate Reducing Agent that remains in the bottom of the tube will not adversely affect results.

3. Plug the Colorimeter 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.

4. Prepare a *blank* by filling an empty cuvette ¾ full with distilled water. Seal the cuvette with a lid. To correctly use a colorimeter cuvette, remember:

   All cuvettes should be wiped clean and dry on the outside with a tissue.

   Handle cuvettes only by the top edge of the ribbed sides.

   All solutions should be free of bubbles.

   Always position the cuvette with its reference mark facing toward the white reference mark at the right of the cuvette slot on the colorimeter.

5. Turn on the calculator and start the DATAMATE program. Press [CLEAR] to reset the program.

6. Set up the calculator and interface for the Colorimeter.

   a. Place the blank in the cuvette slot of the Colorimeter and close the lid.
   b. Select SETUP from the main screen.
   c. If the calculator displays COLORIMETER in CH 1, set the wavelength on the Colorimeter to 565 nm (Green). Calibrate by pressing the AUTO CAL button on the Colorimeter and proceed directly to Step 7. If it does not, continue with this step to set up the Colorimeter manually.
   d. Press [ENTER] to select CH 1.
   e. Select COLORIMETER from the SELECT SENSOR menu.
   f. Select CALIBRATE from the SETUP menu.
   g. Select CALIBRATE NOW from the CALIBRATION menu.

   First Calibration Point

   h. Turn the wavelength knob of the Colorimeter to the 0% T position. When the voltage reading stabilizes, press [ENTER]. Enter "0" as the percent transmittance.

   Second Calibration Point

   i. Turn the wavelength knob of the Colorimeter to the Green LED position (565 nm). When the voltage reading stabilizes, press [ENTER]. Enter "100" as the percent transmittance.
   j. Select OK to return to the setup screen.

7. Set up the data-collection mode.

   a. To select MODE, press [▲] once and press [ENTER].
   b. Select EVENTS WITH ENTRY from the SELECT MODE menu.
   c. Select OK to return to the main screen.

8. Collect absorbance-concentration data for the blank and the nitrate standard (2.5 mg/L). This process will create a standard curve that will be used to determine the nitrate concentrations of the samples.

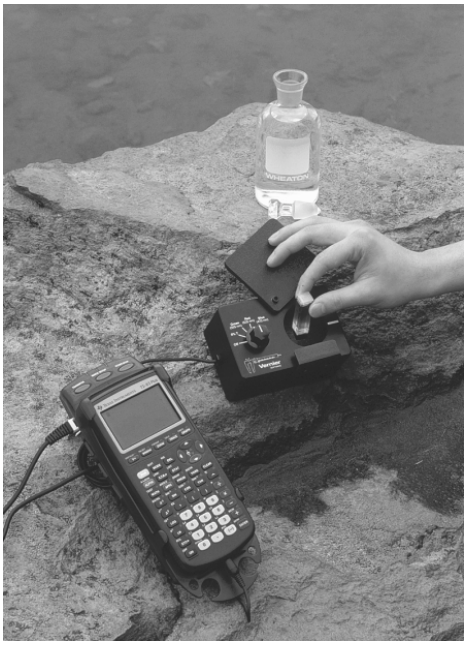
   a. Select START from the main screen.
   b. Press [ENTER] and enter "0" as the concentration in mg/L NO_3^--N.
   c. Discard the water in the cuvette. Using the 2.5 mg/L nitrate standard, rinse the cuvette twice with ~1-mL amounts and then fill it ¾ full. Seal the cuvette with a lid. Wipe the outside of the cuvette and place it in the colorimeter. After closing the lid, wait for the value displayed on the screen to stabilize.
   d. Press [ENTER] and enter "2.5" as the concentration in mg/L NO_3^--N.

e. Press STO▶ to stop data collection. The absorbance and concentration values have now been saved for the standard.

f. Press ENTER to return to the main screen.

g. Dispose of the remaining solution in the flask as directed by your instructor. **CAUTION:** *Any remaining solid particles in the flask are cadmium, a toxic metal.*

9. Find the absorbance of the sample.



a. Rinse the cuvette twice with solution from the first flask and fill it about ¾ full. Seal the cuvette with a lid. Wipe the outside of the cuvette and place it in the colorimeter. Close the lid.

b. Monitor the absorbance value displayed on the calculator. When this value has stabilized, record it on the Data & Calculations sheet (round to the nearest 0.01 mg/L NO_3^--N).

c. Dispose of the remaining solution in the flask as directed by your instructor. **CAUTION:** *Any remaining solid particles in the flask are cadmium, a toxic metal.*

10. Determine the nitrate concentration of the sample water by interpolating the absorbance value on the standard curve created in Step 8.

a. Select ANALYZE from the main screen.

b. Select CURVE FIT from the ANALYZE OPTIONS menu.

c. Select LINEAR (CH 1 VS ENTRY) from the CURVE FIT screen. The equation statistics will be given.

d. Press ENTER to display the graph of absorbance *vs.* concentration.

e. To interpolate along the curve, press ▲. A cursor is displayed on the regression line, along with its X and Y coordinates below the graph. Use ► or ◄ to move the cursor to an absorbance value (Y value) that is closest to the absorbance reading you obtained in Step 9 above. The nitrate concentration, in mg/L NO_3^--N, will be equal to the corresponding X value. Record this value on the Data & Calculations sheet (round to the nearest 0.01 mg/L).

f. Press ENTER to return to the ANALYZE OPTIONS menu.

g. Select RETURN TO MAIN SCREEN.

11. Repeat Steps 9-10 for each of the remaining flasks. When you are finished, discard the solutions, as directed by your instructor. **CAUTION:** *Any remaining solid particles in the flask are cadmium, a toxic metal.*

DATA & CALCULATIONS

Method 2: Nitrate—Colorimeter with a Single Standard

Stream or lake: _____ Time of day: _____

Site name: _____ Student name: _____

Site number: _____ Student name: _____

Date: _____ Student name: _____

Column	A	B
Reading	Absorbance	NO_3^--N (mg/L)
1		
2		
	Average Nitrate (mg/L NO_3^--N)	

Column Procedure:

A. Record the absorbance value from the calculator.
B. Record the NO_3^--N concentration as determined by interpolation of the standard curve.

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

_____

_____

_____

Test Completed: _____ Date: _____

Method 3: NITRATE—COLORIMETER WITH MULTIPLE STANDARDS

Materials Checklist

___ LabPro or CBL 2 interface
___ TI Graphing Calculator
___ DataMate program
___ Vernier Colorimeter
___ 0.1 g plastic measuring spoon
___ tissues (preferably lint-free)
___ pipet pump or pipet bulb
___ one cuvette
___ stirring rod
___ one 125-mL Erlenmeyer flask *per test*
___ one rubber stopper per Erlenmeyer flask
___ two 10-mL pipets
___ 10-mL graduated cylinder
___ Nitrate Reducing Reagent
___ Mixed Acid Reagent
___ nitrate standard (2.5 mg/L NO_3^--N)
___ distilled water

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, refrigerate the samples.

Testing Procedure

1. Add about 30 mL of 2.5 mg/L NO_3^--N standard solution to a 100-mL beaker. Obtain about 30 mL of distilled water in another 100-mL beaker.

2. Label four clean, dry, Erlenmeyer flasks 1–4. Pipet 4, 6, 8 and 10 mL of 2.5 mg/L NO_3^--N solution into Flasks 1–4, respectively. With a second pipet, deliver 6, 4, and 2 mL of distilled water into Flasks 1–3, respectively. (Flask 4 has no distilled water added to it.) *Thoroughly* mix each solution with a stirring rod. Clean and dry the stirring rod between stirrings. Volumes and concentrations for the trials are summarized below:

Flask number	2.5 mg/L NO_3^--N (mL)	Distilled H_2O (mL)	Concentration (mg/L NO_3^--N)
1	4	6	1.0
2	6	4	1.5
3	8	2	2.0
4	~10	0	2.5

3. Measure 5 mL of the standard from Flask 1 into a graduated cylinder. Discard the solution remaining in the flask as directed by your instructor.

4. Add 5 mL of Mixed Acid Reagent to the graduated cylinder containing the standard from Flask 1, to bring the volume to a total of 10 mL.

5. Pour the contents of the graduated cylinder back into the flask. Stopper each flask and shake.
6. Repeat Steps 3–5 for each of the remaining standards.
7. Use the 0.1-g plastic spoon to add two spoonfuls (~0.2 g) of Nitrate Reducing Reagent to each of the flasks.
8. Stopper the flasks and invert at a rate of 50–60 times per minute for 2 minutes. Wait 12 minutes for a complete reaction and best test results. During the 12-minute reaction period, proceed to Step 9 to continue with lab preparation. **Note:** Any undissolved portion of Nitrate Reducing Agent that remains in the bottom of the tube will not adversely affect results.
9. Plug the Colorimeter 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.
10. Prepare a *blank* by filling an empty cuvette ¾ full with distilled water. Seal the cuvette with a lid. To correctly use a colorimeter cuvette, remember:

    All cuvettes should be wiped clean and dry on the outside with a tissue.

    Handle cuvettes only by the top edge of the ribbed sides.

    All solutions should be free of bubbles.

    Always position the cuvette with its reference mark facing toward the white reference mark at the right of the cuvette slot on the colorimeter.

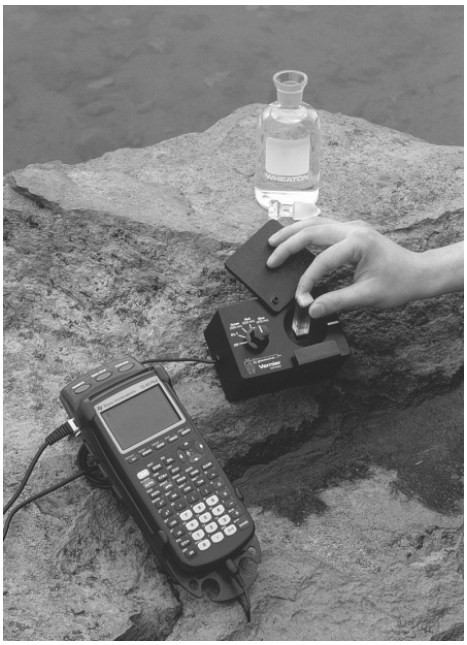
11. Turn on the calculator and start the DATAMATE program. Press [CLEAR] to reset the program.
12. Set up the calculator and interface for the Colorimeter.
    a. Place the blank in the cuvette slot of the Colorimeter and close the lid.
    b. Select SETUP from the main screen.
    c. If the calculator displays COLORIMETER in CH 1, set the wavelength on the Colorimeter to 565 nm (Green). Calibrate by pressing the AUTO CAL button and proceed to Step 13. If it does not, continue with this step to set up the Colorimeter manually.
    d. Press [ENTER] to select CH 1.
    e. Select COLORIMETER from the SELECT SENSOR menu.
    f. Select CALIBRATE from the SETUP menu.
    g. Select CALIBRATE NOW from the CALIBRATION menu.

    First Calibration Point

    h. Turn the wavelength knob of the Colorimeter to the 0% T position. When the voltage reading stabilizes, press [ENTER]. Enter "0" as the percent transmittance.

    Second Calibration Point

    i. Turn the wavelength knob of the Colorimeter to the Green LED position (565 nm). When the voltage reading stabilizes, press [ENTER]. Enter "100" as the percent transmittance.
    j. Select OK to return to the setup screen.
13. Set up the data-collection mode.
    a. To select MODE, press [▲] once and press [ENTER].
    b. Select EVENTS WITH ENTRY from the SELECT MODE menu.
    c. Select OK to return to the main screen.



14. After the 12-minute reaction period has ended, you are now ready to collect absorbance-concentration data for the four nitrate standard solutions. This process will create a standard curve that will be used to determine the nitrate concentrations of the samples.

    a. Select START from the main screen.

    b. Empty the water from the cuvette. Using the solution in Flask 1, rinse the cuvette twice with ~1-mL amounts and then fill it ¾ full. Seal the cuvette with a lid. Wipe the outside with a tissue and place it in the Colorimeter. After closing the lid, wait for the value displayed on the calculator screen to stabilize and press [ENTER].

    c. Enter "1.0" as the concentration in mg/L NO_3^--N. The absorbance and concentration values have now been saved for the first solution.

    d. Discard the cuvette contents as directed by your teacher. Using the solution in Flask 2, rinse the cuvette twice with ~1-mL amounts and then fill it ¾ full. Seal the cuvette with a lid. Wipe the outside with a tissue and place it in the Colorimeter. After closing the lid, wait for the value displayed on the calculator screen to stabilize and press [ENTER].

    e. Enter "1.5" as the concentration in mg/L NO_3^--N.

    f. Repeat the procedure for Flask 3 (2.0 mg/L) and Flask 4 (2.5 mg/L).

    g. Press [STO▶] to stop data collection. Examine the data points along the displayed graph of absorbance *vs*. concentration. As you move the cursor right or left, the concentration (X) and absorbance (Y) values of each data point are displayed below the graph.

    h. Press [ENTER] to return to the main screen.

    i. Dispose of the remaining solution in the flask as directed by your instructor. **CAUTION:** *Any remaining solid particles in the flask are cadmium, a toxic metal.*

15. Plot a graph of absorbance *vs*. concentration with a linear regression curve displayed.

    a. Select ANALYZE from the main screen.

    b. Select CURVE FIT from the ANALYZE OPTIONS menu.

    c. Select LINEAR (CH 1 VS ENTRY) from the CURVE FIT screen. The linear-regression statistics for these two lists are displayed for the equation in the form

    $$y = ax + b$$

    where *y* is absorbance, *x* is concentration, *a* is the slope, and *b* is the y-intercept.
    Note: One indicator of the quality of your data is the size of *b*. It is a very small value if the regression line passes through or near the origin. The correlation coefficient, *r*, indicates how closely the data points match up with (or *fit*) the regression line. A value of 1.00 indicates a nearly perfect fit.

    d. To display the linear-regression curve on the graph of absorbance *vs*. concentration, press [ENTER]. This graph should indicate a direct relationship between absorbance and concentration, a relationship known as Beer's law. The regression line should closely fit the four data points *and* pass through (or near) the origin of the graph.

    e. Press [ENTER] to return to the ANALYZE OPTIONS menu.

    f. Select RETURN TO MAIN SCREEN.

16. Prepare the water samples for testing. Repeat Steps 3–8 using samples from each of the collection sites you will be testing. If necessary, obtain more flasks and label them appropriately.

17. After the 12-minute reaction period has finished, find the absorbance of the water sample.

    a. Rinse the cuvette twice with solution from Flask 1 and fill it about ¾ full. Seal the cuvette with a lid. Wipe the outside with a tissue and place it in the Colorimeter. Close the lid.
    b. Monitor the absorbance value displayed on the calculator. When this value has stabilized, record it on the Data & Calculations sheet (round to the nearest 0.01 mg/L NO_3^--N).
    c. Dispose of the remaining solution in the flask as directed by your instructor. **CAUTION:** *Any remaining solid particles in the flask are cadmium, a toxic metal.*

18. Determine the nitrate concentration of the sample water by interpolating the absorbance value on the standard curve created in Step 15.

    a. Select ANALYZE from the main screen.
    b. Select CURVE FIT from the ANALYZE OPTIONS menu.
    c. Select LINEAR (CH 1 VS ENTRY) from the CURVE FIT screen. The equation statistics will be given.
    d. Press ENTER to display the graph of absorbance *vs.* concentration.
    e. To interpolate along the curve, press ▲. A cursor is displayed on the regression line, along with its X and Y coordinates below the graph. Use ► or ◄ to move the cursor to an absorbance value (Y value) that is closest to the absorbance reading you obtained in Step 17 above. The nitrate concentration, in mg/L NO_3^--N, will be equal to the corresponding X value. Record this value on the Data & Calculations sheet (round to the nearest 0.01 mg/L).
    f. Press ENTER to return to the ANALYZE OPTIONS menu.
    g. Select RETURN TO MAIN SCREEN.

19. Repeat Steps 17-18 for the remaining flasks. When you are finished, discard the solutions, as directed by your instructor. **CAUTION:** *Any remaining solid particles in the flask are cadmium, a toxic metal.*

DATA & CALCULATIONS

Method 3: Nitrate—Colorimeter with Multiple Standards

Stream or lake: _____ Time of day: _____

Site name: _____ Student name: _____

Site number: _____ Student name: _____

Date: _____ Student name: _____

Column	A	B
Reading	Absorbance	NO_3^--N (mg/L)
1		
2		

Average Nitrate (mg/L NO_3^--N)	

Column Procedure:

A. Record the absorbance value from the calculator.
B. Record the NO_3^--N concentration as determined by interpolation of the standard curve.

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

_____

_____

_____

Test Completed: _____ Date: _____

ADDITIONAL INFORMATION

Tips for Instructors

Method 1: Nitrate Ion-Selective Electrode

1. The range of the Vernier Ion-Selective Electrode is 0.10 to 14,000 mg/L 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 NO_3^--N, and a Low Standard that is 1 mg/L NO_3^--N.[3] You can replace these standards using these directions:

   High Standard (100 mg/L NO_3^--N)

   a. Add 0.607 g of $NaNO_3$ to enough distilled water to prepare one liter of solution.

   Low Standard (1 mg/L 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 NO_3^--N.

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 (ClO_3^-, I^-, ClO_4^-, CN^-, 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 1 mL of ISA.

   To prepare 100 mL of Nitrate ISA solution, 2.0 M $(NH_4)_2SO_4$, add 26.42 g of solid ammonium sulfate, $(NH_4)_2SO_4$, 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

[3] Prior to 1999, the Low Standard Solution shipped with each Nitrate ISE was 10 mg/L 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 NO_3^--N standard.

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 that 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
   0.1-g 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,[4] and (b) We found the range of this test, 0.1 to 3.0 mg/L NO_3^--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 ½ 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.

10. In this test, nitrate concentration is not measured directly. Upon addition of the Nitrate Reducing Agent, nitrate is first reduced to nitrite, NO_2^-, by this reaction:

$$Cd(s) + NO_3^-(aq) + 2\ H^+(aq) \longrightarrow Cd^{2+}(aq) + NO_2^-(aq) + H_2O(l)$$

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,[5] 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.

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[4] 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.

[5] 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. See *Appendix I* of this book, *Water Quality with Calculators*, for more information.

12. The directions in Methods 2 and 3 call for a nitrate standard solution with a concentration of 2.5 mg/L NO_3^--N. This solution can be prepared following these steps:

    a. Prepare a 100-mg/L standard by adding 0.607 g of $NaNO_3$ 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-mL/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_3^--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_3^--N. The ending, NO_3^--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_2^--N, and ammonium, NH_4^+-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_3^- instead of NO_3^--N. To convert 100 mg/L NO_3^--N to mg/L NO_3^-, 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_3^- concentrations. Fertilizers are designated by numbers such as 6–12–8, indicating the percentages of N (6%), phosphorus as P_2O_5 (12%), and potassium as K_2O (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_4NO_3, and sodium nitrate, $NaNO_3$. Due to the hazardous nature of these two substances, urea, H_2NCONH_2, 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_3) is converted to nitrite (NO_2^-) by *nitrosomona* bacteria. Nitrite is then quickly converted to nitrate (NO_3^-) 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. [6] 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.

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.

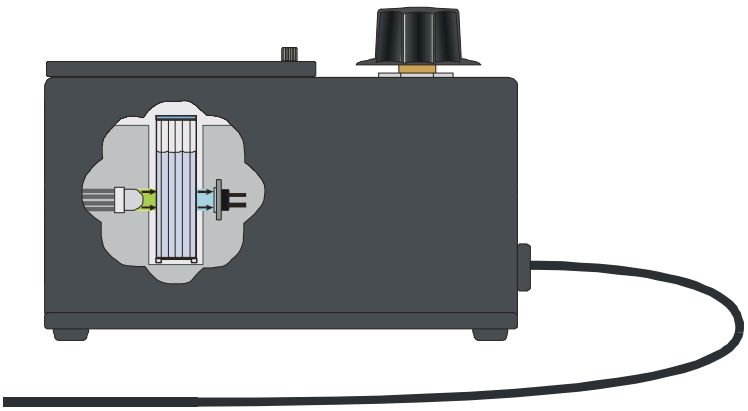
How the Nitrate Ion-Selective Electrode Works

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.

[6] Essential plant nutrients include nitrogen, phosphorus, carbon (CO_2), potassium, sulfur, magnesium, calcium, and a number of other micronutrients (B, 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.