To close the yellow note, click once to select it and then click the box in the upper left corner.
To open the note, double click (Mac OS) or right click (Windows) on the note icon.

#21 Measuring Nitrate by Cadmium Reduction

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INTRODUCTION

Description

In this experiment, each student is assigned the task of designing an experiment to evaluate the effects of various treatments on the nitrogen cycle in a freshwater aquarium. The students are required to maintain a laboratory notebook of all work, measure the key analytes of the bio-system at periodic intervals, analyze and interpret data through appropriate tables and graphs, and write a formal report at the end of the term.

The students set up the experiment using small goldfish bowls and incorporate some treatment, for example, a freshwater plant. This arrangement might have a bowl with fish only, one with a plant only, one with fish plus plant, and one with neither plant nor fish. A variety of techniques for analysis of nitrate and ammonia is included depending on the time available and level of sophistication desired. Since nitrite is typically not observed in this experiment, a technique for its determination is not included.

Student Audience

The experiments are written for freshman college chemistry, however, if aquarium test kits are used, this experiment could be modified for use even at the elementary school level.

Goals

The desired outcomes are that the students will:

- learn to do a rudimentary search of the literature.
- properly maintain a laboratory notebook.
- operate successfully in a research group.
- reinforce previously learned skills such as solution preparation, pipetting, and standards preparation.
- learn to interpret data based on existing theories.
- learn to use a spectrophotometer.
- prepare a formal written report following the format of a journal article.

Recommended Placement in the Curriculum

This experiment, as written, is intended as a capstone activity for freshman college chemistry and would be implemented at the end of the second quarter or near the beginning of the second semester.

Student Handouts

Research Project: Factors Affecting the Nitrogen Cycle in a Freshwater Aquarium

The purpose of the research project is to:

- introduce you to the fundamentals of doing rudimentary research of the literature and to provide background information for the project;
- bring together many of the concepts you have learned in chemistry to explain a real-world situation; and
- give you practice in putting all of the data and information together into a final report.

In this project you will:

- investigate what forms of nitrogen are produced as waste in a freshwater aquarium is acted upon by bacteria;
- follow the changes of concentration of these forms of nitrogen over a period of six to seven weeks; and
- attempt to show the effect of a water plant on this process.

Project Requirements

I. Cooperative Research Group:

Each group will consist of two individuals.

A. Responsibilities to the Group:

Every individual will be expected to actively participate in every aspect of the project.

B. Individual Responsibilities:

Everyone is ultimately responsible for the recording of data in his/her notebook and the conclusions written in the laboratory report.

C. Role Playing:

Although every individual is responsible for every aspect in the project, there are times when each member of your group must serve a different function. In a real-world research project, members of the team take on different functions. For example, when a decision needs to be made, someone should take the role of project manager to keep the research moving forward. In those instances when a question arises that no one can answer, one group member should serve as the project liaison. This individual will serve as the contact person to the instructor. In a real-world situation, the project liaison might communicate with a vice-president of the company. In any research group, some individual should take time to evaluate the project to see if the goals of the research group are being met and offer suggestions for future work. A person serving in this role would be called a project evaluator. It is not expected that any single individual would serve a particular role for the entire research project. It is more likely that each individual in a group should experience every role at least once.

II. Record Keeping:

You must keep a record of all work in a laboratory notebook. Typically a laboratory notebook is bound with consecutively numbered pages.

A. Notebook Organization:

1. Page One—Important Information:
The following information should be centered on the page:

Title of Project
Name
Address
Phone
Course
Instructor's Name
Group Members' Names

2. Pages Two and Three:
Table of Contents: Keep the information in the table current.

3. Consecutive Page Numbering:
Every page in your lab notebook must be numbered consecutively in the top, right-hand corner. There should be no writing on the reverse sides of pages.

4. Daily Requirements:
Never, never write data on a scrap piece of paper and then into the laboratory notebook. The purpose of a laboratory notebook is to serve as a record of all work done in the lab. Neatness is not as important as the recording of all information as the work is done. The current date should be recorded in the right-hand margin. Your signature should appear at the bottom of the page as well as the signature of one other person as a witness. At the end of each laboratory period, you must get your instructor to initial the last page of the day's work.

B. Carbon Copy Paper:
You will need to utilize carbon paper and make copies of pages in your laboratory notebook. Unless you are told otherwise, you will hand the copies in at the end of each laboratory period.

C. Ink:
All information must be recorded in ink. Errors should be crossed out with a single line. The correct entry should be written just above or next to the error. If a large section needs to be discarded, cross it out with a single X.

D. Reproducibility:
Neatly record your work so that anyone could repeat your work if necessary.

E. Miscellaneous Materials:
Your copies of miscellaneous materials should be kept in a separate folder.

III. Communication and Dissemination:

Communication is an important part of the collaborative process. You will need to submit the following documents:

A. Laboratory Report:

By the final exam, you should submit a typewritten report of your work. The report should be much like a journal report with the following sections:

Abstract—a summary of the experiment

Discussion—a discussion of the experiment that includes the three main types of bacteria involved in the nitrogen cycle,

Procedure—a description of the experimental procedure

Results—a data table and graphs

Conclusion—a plausible explanation of the results of the experiment

B. Laboratory Notebook:

Your laboratory notebook should be turned in at the same time as the laboratory report.

Measuring Nitrate by Cadmium Reduction

Purpose

Upon the completion of this experiment you should be able to:

- prepare a series of nitrate ion standards;
- successfully convert the nitrate in water to nitrite by cadmium reduction;
- develop the color of the nitrite ion by addition of appropriate reagents;
- measure the light absorbance of the color developed by the nitrite ions with a spectrophotom eter;
- prepare a calibration curve for the series of standards; and
- measure the nitrate in a sample of tap water.

Scenario

Because of the health risks associated with elevated levels of nitrates, the nitrate ion, NO_3^- is routinely measured in our drinking water by the local water treatment plant. Infants are especially sensitive to high levels of nitrates. Levels of nitrates which are toxic to fish can also accumulate in a freshwater aquarium if the water is not changed periodically.

Nitrates can be measured by a variety of techniques such as the nitrate electrode, ion chromatography, and the method used in this experiment, cadmium reduction. The cadmium reduction technique converts the nitrate ion to nitrite ion by allowing a solution of nitrate ions to pass slowly through a layer of copper-coated cadmium. (See Figure 1.) The reaction is believed to be the following (1):

$$2H^+ + NO_3^- + Cd_{(s)} \Leftrightarrow Cd^{+2} + NO_2^- + H_2O$$

The cadmium is treated with a solution of copper sulfate, which reacts with the cadmium and leaves a coating of copper on the surface of the cadmium. This copper is responsible for the reduction of the nitrate to nitrite. This reduction step is necessary because the nitrite ion forms a highly colored azo dye by coupling diazotized sulfanilamide with N-(1-naphthyl)-ethylenediaminedihydrochloride (NED dihydrochloride). By forming the colored azo dye, the concentration can be measured by light absorbance in a spectrophotometer. The color system obeys Beer's law up to 180 mg of nitrogen in the form of the nitrate ion, NO_3^--N per liter (written mg NO_3^--N/L) with a 1-cm light path at 543 nm. There are other chemicals which will react with nitrate to form a color, but they are not as sensitive as the NED dihydrochloride; i.e., they will not produce a measurable color at low nitrate concentrations. Some color-producing reagents work fairly well, but are extremely toxic.

Overview

In this experiment, the cadmium reduction column will be ready to use and the solutions needed will be prepared for you with the exception of the nitrate standards. You will work in groups of three or four. Your tasks will be:

1. Prepare 0.1, 0.5, 1.0, 2.0, and 5.0 ppm $N0_3^-$-N standards from the 100 ppm you had made previously.
2. Reduce the nitrate standards and a tap water sample with the cadmium reduction column.
3. Develop the color and read the light absorbance at 543 nm.

4. From the calibration curve constructed from the standards, calculate the NO_3^--N concentration in the tap water sample.

Safety, Handling, and Disposal

Wear eye protection and appropriate gloves. Cadmium is a highly toxic substance. Since the cadmium is immobilized in the buret, this drastically reduces the hazards associated with using it. If you break the buret and spill the cadmium, do not attempt to clean it up. See the lab instructor for instructions. Dispose of used reagents according to local ordinances.

Materials Needed

- 100 ppm nitrate standard (prepared previously)
- cadmium reduction column
- 250-mL Erlenmeyer flask
- 1-, 2-, 5-, 10-, 20-, 25-, and 50-mL pipets
- pipet bulb
- 6 100-mL volumetric flasks
- 3 250-mL beakers
- stopwatch or watch with second hand
- 10-mL graduated cylinder
- 6 50-mL volumetric flasks
- spectrophotometer
- 1-cm spectrophotometer cells
- 0.1 M hydrochloric acid
- 0.1 M sodium hydroxide
- pH meter

Procedure

A. Preparation of Standards

From the 100 ppm NO_3^--N standard you made previously, prepare 100 mL of the following standards: 0.1, 0.5, 1.0, 2.0, and 5.0 ppm NO_3^--N

B. Preparation of Sample and Standards for Reduction

1. Take a clean 250-mL Erlenmeyer flask to a faucet or drinking fountain and after letting the water run for at least three minutes, collect approximately 200 mL of tap water for analysis.

2. Check the pH of the tap water with the pH meter provided. If the pH is not between 7 and 9, increase the pH by adding 0.1 M sodium hydroxide or decrease the pH by adding 0.1 M hydrochloric acid. **Caution:** Sodium hydroxide and hydrochloric acid are caustic and should be handled with care. Wear goggles!

3. Pipet 25 mL of your 0.1 ppm standard into a 100-mL volumetric flask and bring to volume with the ammonium chloride-EDTA solution. Mix. Repeat this procedure for each standard and the tap water sample. You should now have six 100-mL volumetric flasks of solutions ready for reduction.

C. Reduction of Sample and Standards

1. Fill a 250-mL graduated beaker to the 100-mL mark with **column conditioning solution**. **Place the column conditioning solution and all of the diluted standards and sample on the bench next to the reduction column.** You will also need a 250-mL beaker with 50 mL of dilute ammonium chloride-EDTA, an empty graduated 50-mL beaker, and an empty 250-mL beaker.

2. Pour a conditioning solution through the column to improve the reduction capabilities of the copperized cadmium and allow you to adjust the flow rate of the solution through the column. The conditioning solution will be discarded after it has passed through the column. Remember that the nitrates are reacting with the copper on the cadmium granules and if the solution passes too quickly through the layer of cadmium granules, there may be insufficient time for that reaction to occur.

You will find the copperized reduction column with a small amount of the dilute ammonium chloride-EDTA solution covering the cadmium granules. (See Figure 1.) These granules should always be covered with a solution and kept moist. If allowed to dry, the column may not reduce the nitrate as efficiently. To prepare the column for use, start by filling the buret with the **column conditioning solution** and allow the buret to drain into the 250-mL beaker at a rate of 7 to 10 mL per minute. This solution will be discarded when it has all passed through the column. **Be ready to follow this solution with the standards and sample.** The 7 to 10 mL per minute flow rate will be a rapid drip. Using a 10-mL graduated cylinder, check the flow rate by measuring the volume of solution emitted from the tip of the buret in one minute. Continue to pour the **column conditioning solution** into the buret until all of it is in the buret.

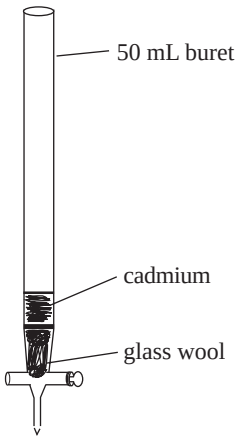

Figure 1: Cadmium reduction column

3. When the column conditioning solution has almost reached the top of the cadmium, pour the diluted 0.1 ppm standard into the buret. Place the empty 50-mL beaker under the buret and collect 25 mL and discard. Collect the rest of the standard in the original volumetric flask. Without stopping the flow, repeat this process with the remaining standards, going from 0.1 ppm to 5.0 ppm, then finish with the tap water sample. When the tap water sample has drained almost to the top of the cadmium, pour in enough of the dilute ammonium chloride-EDTA solution to fill the buret. Let the buret drain until the surface of the solution is several centimeters above the top of the cadmium. Turn off the stopcock and store the copperized cadmium in this solution.

D. Color Development and Measurement

1. As soon as possible, and not more than 15 minutes after reduction, your lab partner should pipet 2 mL of color reagent into a 50-mL volumetric flask and fill to the mark with the reduced standard or tap water sample and mix. Between 10 minutes and 2 hours after developing the color, measure the absorbance in the spectrophotometer at 543 nm using 1 cm cells. Use a distilled water blank.

Calculations

1. Using a spreadsheet program, prepare a graph of the five standards with concentration in ppm NO_3^--N on the x-axis and absorbance on the y-axis. In addition, do a linear regression analysis on the data and obtain the slope and intercept of this line. Although this graph is a *straight line,* it is generally referred to as a *standard curve.*
2. Use the slope and intercept of the standard curve to calculate the ppm NO_3^--N in the tap water sample from its absorbance.

Questions

1. If the tap water sample contained nitrite ions, how would this affect the nitrate results? How might this be corrected?

2. Why do you think it is necessary to maintain a certain flow rate through the reduction column?

Reference

1. "Hach Water Analysis Handbook, 2nd Ed." p783.

Report Sheet

Name_____ Date_____ Section No. _____

Data Table

Sample	Absorbance	
0.1 ppm	_____	
0.5 ppm	_____	
1.0 ppm	_____	
2.0 ppm	_____	
5.0 ppm	_____	
tap water	_____	_____ ppm NO_3^--N

Attach spreadsheet print-out here.

Instructor Notes

Measuring Nitrate in Water by Cadmium Reduction

Time Required

6 hours

Group Size

3-4 students per group

Materials Needed

Reagents:

a. *6 M hydrochloric acid*: Dilute concentrated HCl to make a 50:50 dilution.

b. a*mmonium chloride-EDTA solution*: Dissolve 13 g NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust the pH to 8.5 with concentrated NH_4OH and dilute to 1 L.

c. *dilute ammonium chloride-EDTA solution*: Dilute 300 mL NH_4Cl-EDTA solution to 500 mL with water.

d. *2% copper sulfate solution*: Dissolve 20 g $CuSO_4 \cdot 5H_2O$ in 500 mL water and dilute to 1 L.

e. c*olor reagent*: To 800 mL water, add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving the sulfanilamide completely, add 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride (NED). Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in a refrigerator.

f. *stock nitrate solution*: Dry potassium nitrate (KNO3) in an oven at 105°C for 24 hours. Dissolve 0.7218 g in water and dilute to 1000 mL; 1.00 mL = 100 mg NO3-N, ie., 100 ppm. Preserve with 2 mL 1 M boric acid/ liter. This solution is stable for at least 6 months.

g. *intermediate nitrate solution*: Dilute 100 mL stock nitrate solution to 1000 mL with water; 1.00 mL = 10 mg NO_3-N. Preserve with 2 mL 1 M boric acid/L. This solution is stable for 6 months.

h. *column conditioning solution* - Dilute 250 mL 1 ppm NO_3^--N to 1000 mL with ammonium chloride-EDTA solution.

i. 0.1 M hydrochloric acid - Dilute 4.2 mL concentrated HCl to 500 mL.

j. 0.1 M sodium hydroxide - Dissolve 2.0 g NaOH in enough water to make 500 mL of solution.

Equipment:

- cadmium reduction column
- 250-mL Erlenmeyer flask
- 1-, 2-, 5-, 10-, 20-, 25-, and 50-mL pipets
- pipet bulb
- 6 100-mL volumetric flasks
- 3 250-mL beakers
- 50-mL beaker
- stopwatch or watch with second hand
- 10-mL graduated cylinder
- 6 50-mL volumetric flasks
- spectrophotometer
- 1-cm spectrophotometer cells
- pH meter

Safety, Handling, and Disposal

Although not mentioned in any of the references, due to the toxicity of cadmium, avoid breathing any dust by handling the dry cadmium in the fume hood with gloves. Once prepared, the cadmium granules can be used repeatedly. Unless the students break the buret with the granules, they will not be exposed to cadmium. Dispose of used reagents according to local ordinances.

Points to Cover in Pre-Lab Discussions

Standards:

I generally have the students prepare a stock nitrate solution in advance. We go through the steps involved in calculating the amount of KNO_3 needed to prepare a 100 ppm NO_3^--N solution. We discuss the best method to prepare the 0.1, 0.5, 1.0, 2.0, and 5.0 ppm standards. I have them prepare a 10 ppm standard from the 100 ppm and then prepare the 0.1 to 5.0 ppm standards by dilutions of the 10 ppm. Use the following amounts:

- For 10 ppm use 10 mL 100 ppm diluted to 100 mL.
- For 0.1 ppm use 1 mL 10 ppm diluted to 100 mL.
- For 0.5 ppm use 5 mL 10 ppm diluted to 100 mL.
- For 1.0 ppm use 10 mL 10 ppm diluted to 100 mL.
- For 2.0 ppm use 20 mL 10 ppm diluted to 100 mL.
- For 5.0 ppm use 50 mL 10 ppm diluted to 100 mL.

Using the Reduction Column:

The procedure is time-consuming, and it is critical that once the students begin passing the standards through the column that they continue without interruption. Give the students an overview of what must be done in the reduction process and stress the importance of having everything ready before they begin.

Procedural Tips and Suggestions

I use this project in our Chemistry 103 class in addition to the student's regular lab schedule, and it is easily completed in one quarter. Hughes (2) utilized a marine aquarium in quantitative analysis and was able to build their entire lab schedule around it. This project utilizes goldfish in

a freshwater aquarium and concentrates on only the nitrogen cycle. The advantages are that it utilizes readily available materials and is much less expensive than a marine aquarium. To simplify the analysis, we used aquarium test kits which have a source of ready-made reagents. We used kits from Aquarium Pharmaceuticals, P. O. Box 218, Chalfont, PA 18914. The nitrate ion is the most difficult to measure, and a variety of techniques for its determination follows.(1) The ammonia is determined by developing the color with the test kit reagents and measuring the color intensity with a spectrophotometer.

The Nitrogen Cycle

A good biology text or aquarium book can be consulted for more information, but essentially the following occurs: when the aquarium or goldfish bowl is first established, heterotrophic bacteria act upon food or fecal matter and convert it to ammonia; the ammonia is acted upon by the Nitrosomonas bacteria and converted to nitrite; and finally the nitrite is acted upon by Nitrobacter bacteria which convert the nitrite to nitrate. My experience thus far with a non-aerated goldfish bowl was that the ammonia showed up first as expected, there was no measurable nitrite, and after a few weeks the nitrate developed in the bowl.

Initial Set-up

Several weeks before beginning the project, set up an aquarium with the goldfish. If the goldfish are sick when purchased (which happened to us) you can get the problem solved before the goldfish are needed. If you have an aquarium with a pump filter, several goldfish can be kept in one aquarium. The students will need at least three containers (we found goldfish bowls at a local discount store for $1.00 a piece). I asked them to furnish the containers. The experimental design should be left up to the students. It is important to remember that a treatment that has no effect on the nitrogen cycle can be as important as something that does affect it. Some ideas might be aerating vs. non-aerating, plant vs. no plant, moving water vs. still water, and even the effect of various medicines, such as ich medicine or algaecides. Many of the students purchased aquarium gravel for their bowls which looked nice and held the plants down, but does provide another variable in the experiment. Also, some students realized that one bowl with water only should be set up as a control. A fungus eventually developed in this bowl which did generate some nitrate! We used spring water for the bowls so no special treatment was necessary; however, if tap water is used it should be allowed to sit overnight to remove some of the chlorine.

Column Preparation

1. Wash 25 g of 40- to 60-mesh Cd granules (EM Laboratories, 500 Exec Blvd., Elmsford, NY, Catalog No. 2001) with 6M HCl in beaker with swirling, and rinse with deionized water.
2. Swirl Cd with 100 mL 2% $CuSO_4$ solution for 5 minutes or until blue color partially fades, decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate forms, and flush with water to remove precipitated Cu.
3. Place approximately 1.5 cm of glass wool in the bottom of a 50-mL buret. Add the Cd granules to the buret by scraping granules from beaker with a spatula into the buret. Wash column by passing 200 mL diluted NH_4Cl-EDTA solution and leave Cd granules covered with diluted NH_4Cl-EDTA solution to avoid air entrapment.
4. Activate column by passing through it, at 7 to 10 mL/min, at least 100 mL of a solution made by diluting 25 mL of 1 ppm NO_3 - N to 100 mL with NH_4Cl-EDTA solution.

Note: The column appears to work better after it has been used two or three times, cleaned with hydrochloric acid, and regenerated with copper.

The cadmium reduction technique has been in use for many years as a method for measuring nitrate in water samples. It has been the method of choice because it is not subject to many interferences and is sensitive to fairly low levels of nitrate. It is a labor-intensive technique, so most labs use an automated system which pumps the sample through a reduction column and then automatically mixes with the color-developing reagents and on to a detector.

Description

Principle: NO_3^- is reduced to NO_2^- in the presence of cadmium granules treated with $CuSO_4$.

NO_2^- is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediaminedihydrochloride (NED) to form a red color.

Must correct for NO_2^- that is present.

Limits of Detection = 0.07761 ppm

Limits of Quantitation = 0.25869 ppm

Interferences

- suspended matter
- high levels of metals, such as iron and copper, lower reduction efficiency and can be eliminated by EDTA
- residual chlorine can oxidize Cd column, which reduces efficiency
- colored sample that absorbs around 540 nm

Hints for using the cadmium reduction column

By using a 10- or 25-mL buret, the sample size could probably be reduced to 10-mL sample diluted to 50 mL. This would also reduce the time needed for reduction.

When using a 50-mL buret (instead of the column described in the standard procedure), the 25 mL wash doesn't completely wash the column. We found that in going from a 10 ppm NO_3 - N sample to a 3.8 ppm sample, there was a carry-over of approximately 0.5 ppm, i.e. the sample read 4.3 ppm.

The column seems to work best after it has been used two or three times, cleaned with HCl, and regenerated with copper.

Miscellaneous

Since water will evaporate from the fish bowls, we marked the initial water level in each bowl when they were set up and then refilled the bowl *after* each round of testing.

Since the ammonia/nitrate levels are somewhat dependent upon the amount of unused food, I fed all the fish so there would be some degree of consistency in the amount of food received by the fish. I did not measure the food provided.

The number of goldfish used per bowl can be determined by the size of the fish and the bowl. We put one fish into a small fishbowl. If larger containers are used, obviously more fish would be needed to generate reasonable levels of nitrate and ammonia.

The first time this project was attempted, the water was not changed for 50 days and the fish suffered no detrimental effects. However, the appearance of the fish tanks was poor, to say the least!

Answers to Questions

1. If nitrite is present in the tap water sample, a nitrite measurement would need to be made first and this value subtracted from the nitrate results.

2. If the flow rate is too slow, the nitrite is further reduced; if the flow rate is too fast, incomplete reduction can occur.

Extensions and Variations

One possible extension, where practical, would be to have the class monitor a pond or lake over a period of several months. One could also monitor other ions of interest.

References

1. Greenberg, A.E., "Standard Methods for the Examination of Water and Wastewater, 16th ed."; APHA, Wash., DC, 1980, p 394.

2. Hughes, Kenneth D. *Anal. Chem.* **1993**, *65*, 883A.

3. "Hach Water Analysis Handbook, 2nd Ed." p 783.

STUDENT HANDOUTS (INQUIRY BASED)

MEASURING NITRATES IN WATER

Part I

Because of the health risks associated with elevated levels of nitrates, the nitrate ion, NO_3^-, is routinely measured in our drinking water by the local water treatment plant. Infants are especially sensitive to high levels of nitrates. Levels of nitrates which are toxic to fish can also accumulate in a freshwater aquarium if the water is not changed periodically.

We will investigate a method for measuring nitrate. **Put on your goggles now. Wear appropriate gloves. Handle all solutions with care and wash immediately if spilled on the skin.** Prepare 100 mL 1 ppm NO_3^--N from the 100 ppm solution you made previously. You will also need a solution labeled "NED" and a 1 ppm nitrite solution, both prepared by the instructor. Pipet 2 mL NED solution into each of two test tubes. Fill the first test tube half full with the nitrate standard and mix. Fill the second test tube half full with the nitrite standard. Mix and record any observed changes.

Did you observe any changes that could be used to measure nitrate?

When you have finished, show your answers to the instructor to go on to the next part.

MEASURING NITRATES IN WATER
Part II

As you may know, any colored solution gets its color by selectively absorbing particular wavelengths (colors) of light from the visible spectrum. If an ion or molecule is colored, then as the concentration of that particular colored substance increases, the color gets darker and it will absorb more light.

Based upon your observations in Part I, could you use NED and the absorbance of light to measure the concentration of nitrate? nitrite?

MEASURING NITRATES IN WATER
Part III

Wear eye protection and gloves.

Safety Note: In this experiment the buret contains cadmium metal. Cadmium is a highly toxic substance. Since the cadmium is immobilized in the buret, this drastically reduces the hazards associated with using it. If you break the buret and spill the cadmium, do not attempt to clean it up. Ask the lab instructor for instructions. The solutions used should be handled with care and if spilled on the skin, washed off immediately. Dispose of used reagents according to local ordinances.

In this experiment, obtain the following from the materials supplied:

- column (a buret with metal beads in bottom)
- 300 mL ammonium chloride-EDTA solution (note that there are two different ammonium chloride solutions)
- 50 mL column conditioning solution
- 10 mL NED solution. Do not get the NED solution until you are ready to use it.
- 50 mL dilute ammonium chloride solution

Glassware needed that is not in your lab drawer will be provided.

A. Preparation of Standards

From the 100 ppm NO_3^--N standard you made previously, prepare 100 mL of the following standards:
10.0 ppm NO_3^--N and use this to prepare 100 mL of:
0.1, 0.5, and 1.0 ppm NO_3^--N

B. Dilution of Standards

Pipet 25 mL of your 0.1 ppm standard into a 100-mL volumetric flask, bring to volume with the ammonium chloride-EDTA solution, and mix. Repeat this procedure for each standard. You should now have three 100-mL volumetric flasks of solutions ready to pass through the column.

C. Treatment of Standards

1. Fill a 250-mL graduated beaker to the 100-mL mark with column conditioning solution. **Place the column conditioning solution and all of the diluted standards and sample on the bench next to the column.** You will also need a 250-mL beaker with 50 mL dilute ammonium chloride-EDTA, an empty graduated 10-mL beaker, and an empty 250-mL beaker.

2. In this step, you will pour a conditioning solution through the column. The conditioning solution will be discarded after it has passed through the column. You will find the column with a small amount of the dilute ammonium chloride-EDTA solution covering the cadmium granules. (See Figure 1). These granules should always be covered with a solution and kept moist. To prepare the column for use, start by filling the buret with the column conditioning solution and allow the buret to drain into the 250-mL beaker at a rate of 7 to 10 mL per minute. This solution will be discarded when it has all passed through the column. **Be ready to follow this solution with the standards.** The 7 to 10 mL per minute flow rate will be a rapid drip. Using a 10-mL graduated cylinder, check the flow rate by measuring the volume of solution emitted from the tip of the buret in one minute. Continue to pour the column conditioning solution into the buret until all of it is in the buret.

3. When the column conditioning solution has almost reached the top of the cadmium, pour the diluted 0.1 ppm standard into the buret. Place the empty 50-mL beaker under the buret and collect 25 mL and discard. Collect the rest of the standard in the original volumetric flask. Without stopping the flow, repeat this process with the remaining standards, going from 0.1 ppm to 1.0 ppm. When the 1.0 ppm standard has drained almost to the top of the cadmium, pour in enough of the dilute ammonium chloride-EDTA solution to fill the buret. Let the buret drain until the surface of the solution is several centimeters above the top of the cadmium. Turn off the stopcock and store the cadmium in this solution.

D. Color Development and Measurement

1. As soon as possible, and not more than 15 minutes after the standards have been passed through the column, one of the lab partners should pipet 2 mL of color reagent into a 50-mL volumetric flask and fill to the mark with the 0.1 standard which has passed through the column. Mix. Repeat this process with the other two standards. Between 10 minutes and 2 hours after developing the color, measure a single percent transmittance of each solution in the spectrophotometer at 543 nm using 1 cm cells. Use a distilled water blank.

Follow-up

1. You treated the nitrate standards by passing them through a layer of cadmium which had been coated with copper. When you added the NED to the resulting solution, what color did you observe? Did it look familiar?

2. What do you think was the purpose for passing the nitrate standards through the column?

3. Could this technique be used to measure the concentration of nitrate?

4. Make a graph with the concentration on the x-axis and percent transmittance on the y-axis. What is the shape of the graph?

5. Now divide all of the percent transmittances by 100, take the log of them, and change their sign to its opposite. Call these numbers absorbances. Make another graph with the concentration on the x-axis and absorbances on the y-axis. What shape is it?

MEASURING NITRATES IN WATER

Part IV

Light Absorbance

You hopefully observed that as the concentration of the nitrate standards increased, the color darkened. Based upon the previous experiment, which would be more useful for measuring the concentration of a colored substance; the graph using percent transmittances or the one using absorbances? This procedure gives us a method for measuring the concentration of nitrate by measuring its absorbance of light. The Beer-Lambert law is given by the equation:

$$A = abc$$

where A is the absorbance of the light, a is the absorptivity of the substance which is unique for each substance, b is the path length (the distance the light travels through the colored substance), and c is the concentration. As you can see, the absorbance of light of a colored solution is directly proportional to the concentration.

MEASURING NITRATES IN WATER

Part V

The Chemistry

You noticed that when NED was added to nitrates, there was no color change, but when NED is added to nitrites, you did see a color develop. Did you observe that same color develop when NED was added to the nitrate standards that had been passed through the column? What is the column doing to the nitrates?

INSTRUCTOR NOTES

Measuring Nitrate in Water by Cadmium Reduction (Inquiry-Based)

Note: This is an inquiry-based format for the previous experiment. The same solutions, equipment, etc. would be used as in the previous procedure with the following addition: To prepare the 1 ppm nitrite solution, 20 mL per student group: dissolve 0.4928 g of sodium nitrite in water and dilute to 1000 mL. This will be 100 ppm. Dilute 1.0 mL of this solution to 100 mL. Because nitrite is oxidized readily, this standard is only approximately 1 ppm but will serve the purpose for Part I.

Introduction

This is an inquiry-based experiment, designed to have the students investigate an oxidation-reduction reaction and the Beer-Lambert Law. At the end of the second quarter, our First-Year Chemistry students are introduced to techniques of measuring nitrates and ammonia in water, which are to be used in a research project required in the third quarter. The research project requires the measurement of nitrates and ammonia in a freshwater aquarium over a period of several weeks. The cadmium reduction method, although time consuming, is accurate at levels below 1 ppm NO_3^--N. Note that there are five parts to the experiment. The students receive each part only after they have completed the previous one. The lab could be split over two lab periods by having the students prepare the standards and do Parts I and II during the first lab and the rest during the second lab. Ideally, the lab should be performed by groups of two to three students.

The cadmium reduction technique has been in use for many years as a method for measuring nitrate in water samples. It has been the method of choice because it is not subject to many interferences and is sensitive to fairly low levels of nitrate. It is a labor-intensive technique, so most labs use an automated system which pumps the sample through a reduction column and then automatically mixes with the color-developing reagents and on to a detector.

Answers to Questions

Part I

The NED will form a reddish-purple color with nitrite, but has no effect upon the nitrate. The nitrate would need to be converted to nitrite in order to measure it with NED.

Part II

One could use the NED to measure the concentration of nitrate only if it were converted to nitrite. One could use NED to measure nitrite.

Part III

1. The color was reddish-purple. Yes, like the nitrite and NED.

2. To convert them to nitrites.

3. Yes.

4. A curved line.

5. A straight line.

Part IV

The graph of absorbances would be better because it is a straight line and the statistical treatment of linear least squares can be used to analyze the data.

Part V

Yes. The column is reducing the nitrate to nitrite.

DETERMINATION OF NITRATE BY NITRATE ELECTRODE

Introduction

The nitrate electrode offers an attractive alternative to measuring nitrates by cadmium reduction. It is a rapid, easy-to-use technique. The main deficiencies are that the electrode is not sensitive below about 1 ppm and is subject to interferences. The following experiment provides an introduction to the nitrate electrode.

Description

Specific instructions are provided on use of the reference and ISE electrode. The experiment allows the students to construct a calibration curve for nitrate levels in the range of 2 to 15 ppm.

Student Audience

This experiment could be used for freshman level college chemistry or in a quantitative analysis course.

Goals

- Become familiar with the basic operation of a pH meter for measuring voltages.
- Demonstrate the mathematical relationship between concentration and voltage produced by a nitrate electrode.
- Prepare a nitrate electrode and double junction reference electrode for use.

Recommended Placement in the Curriculum

This experiment could be used in freshman chemistry to illustrate a practical application of the Nernst equation. Typically near the end of the third quarter or second semester.

STUDENT HANDOUT
Determination of Nitrate by Nitrate Electrode

Theory

The pH meter

The pH meter is nothing more than a voltmeter designed for specific uses. It is most commonly used with a pH electrode, which is a glass tube with a special glass bulb at one end that senses hydrogen ions. The electrode has a wire leading from it to make an electrical connection to the meter. A voltage (electrical potential) must be measured relative to something, i.e., it is impossible to measure the absolute value of a voltage. To provide that reference point, a **reference electrode** is required. There are various types of reference electrodes, but the one used here is a double junction reference electrode. It has an outer chamber which isolates the inner reference element from the sample.

Ion-Selective Electrodes

An Ion-Selective Electrode (ISE) has a membrane that senses a particular ion and develops an electrical potential which is proportional to the concentration of that ion. ISEs have been developed to sense a variety of ions. When an ISE and a reference electrode are immersed in a solution of the particular ion of interest, an electrical potential is developed which can be measured on the pH meter. Since the potential developed is affected by the ionic strength of the sample solution, an **Ionic Strength Adjustor** (ISA) is added to provide a relatively constant concentration of ions. The potential produced is described by the **Nernst Equation**:

$$E = E^{\circ} - \frac{0.059}{n} log(C)$$

where E is the measured potential, E° is the standard potential for the system, n is the number of electrons involved in the reaction, and C is the concentration. For an ion with a -1 charge, such as nitrate, a plot of measured potential as a function of log(C) will give a straight line with a slope of -0.059 V/decade, or -59 millivolts/decade.

Safety, Handling, and Disposal

Wear eye protection during this experiment. Dispose of used reagents according to local ordinances.

Materials Needed

- pH meter capable of displaying millivolts
- Orion Model 90-02 double junction reference electrode or equivalent
- Orion Model 93-07 nitrate electrode or equivalent
- electric stirrer, stir bars
- nitrate standards—2, 5, 7, 10, 15 ppm NO_3-N which has had Ionic Strength Adjustor added
- outer filling solution for reference electrode - 2 mL of ISA diluted to 100 mL
- inner filling solution - Orion Part No. 900002
- Kim-Wipes or equivalent
- Ionic Strength Adjustor solution

Procedure

Wear Eye Protection

The meter should always be left with the electrodes soaking in 100 ppm NO_3-N. The meter should be set on Standby (Stby). To prepare the instrument for analysis one must always: (a) prepare the electrodes for measurement, (b) set the appropriate switches on the pH meter for measuring millivolts, and (c) measure the millivolt readings of the five standards provided.

Preparing the electrodes

- The Nitrate Ion-Selective Electrode

The nitrate ISE is solid black and the sensing module is screwed into the bottom. The sensing membrane is the small, circular indentation in the bottom of the electrode. **Do not touch this membrane.** The oil from your hands could contaminate the membrane and interfere with its ability to sense nitrate. Other than thoroughly rinsing the module and membrane with deionized water prior to measuring, no special treatment is necessary. It will not cause any problems to leave the electrode suspended in air for several minutes.

- The Double Junction Reference Electrode

The double junction reference electrode has a white top and a clear outer sleeve which allows you to see the green solution in the inner chamber. Both the inner and outer chamber filling solutions should be changed immediately before making a series of measurements. This is done as follows:

1. Draining outer filling solution: While holding the reference electrode over a beaker, drain the outer solution by grasping the white top of the electrode in one hand and push the outer sleeve up into the white top with the other hand. This should allow most of the clear outer solution to drain out. If it does not drain, try tilting the electrode at a different angle and repeat.

2. Disassemble the electrode: Unscrew the white top from the outer sleeve and slide the white top and spring up the cable. Gently push down on the electrode inner chamber until the inner cone appears at the bottom of the sleeve. Grasp the cone with a tissue and pull the inner electrode chamber from the outer sleeve.

3. Drain the inner filling solution: Near the top of the inner chamber you will see a white rubber sleeve. If this is covering the fill hole, slide it down until the fill hole is visible. Twist a tissue into a sharp point, turn the inner chamber upside down and insert the tissue into the fill hole. Notice that the green solution will wick out of the inner chamber into the tissue. If the solution tends to stay in the chamber, flick it with your finger to get the solution to drain down to the fill hole. It will not cause any problems if a few drops of solution remain in the inner chamber.

4. Filling the inner chamber: The inner filling solution bottle has a "flip-top" spout. Flip up the spout and squirt the green solution into the inner chamber until the solution level is just below the fill hole. It will be necessary to flick the inner chamber with your finger to get the air bubbles out when filling. Notice there is a tiny vent hole above the fill hole. If the fill solution simply goes into the fill hole and out the vent hole do the following: remove the solution from both holes with a tissue and holding the spout a few centimeters away from the electrode, direct a stream of the solution into the fill hole. Rinse any filling solution from the outside of the inner chamber. Reassemble the electrode by reversing the disassembly procedure. Do not over-tighten the white top when reassembling the electrode.

5. Fill the outer chamber: Having reassembled the electrode, fill the outer chamber with the clear, colorless, outer filling solution by squirting it into the fill hole on the outer chamber. When full, push down on the white top and drain the solution you just put in. Refill the outer chamber and the electrode is ready to use. Wipe any excess filling solution from the outside of the electrode. Return the electrode back to the holder in which you found it.

Making the Measurements

- Setting up the pH meter

Set the meter to read millivolts by turning the knob in the upper right-hand corner to the left. Set the on-off switch to ON. The meter is ready to take readings.

- Reading the standards

Into a 50-mL beaker pour approximately 20 mL of the 2 ppm standard. Add a small stir bar and place the beaker onto the stirrer. Set the stir rate to a moderate speed. Before lowering the electrodes into the standards always rinse thoroughly with deionized water and blot dry with a tissue. (Discard this rinse water.) Avoid touching the sensing portion of the nitrate electrode. Immerse the electrodes into the standard while stirring. When the meter has stabilized, record the millivolt reading. Lift the electrodes out of the standard and rinse and blot dry as before. Continue reading the 5, 7, 10, and 15 ppm standards. When finished, leave the electrodes in the same solution in which you found them and turn the meter back to Stby.

Calculations

Make a graph of millivolt readings on the y-axis and log concentration on the x-axis. Sample concentrations can be determined from this calibration curve within the range of concentrations of the standards. Any samples with concentrations outside the 2–15 ppm range should be either diluted or additional standards prepared to verify the range of linearity of the electrode.

INSTRUCTOR NOTES

Determination of Nitrate by Nitrate Electrode

Time Required

3 hours

Group Size

2-4 students

Materials Needed

- pH meter capable of displaying millivolts
- Orion Model 90-02 double junction reference electrode or equivalent
- Orion Model 93-07 nitrate electrode or equivalent
- electric stirrer, stir bars
- nitrate standards—2, 5, 7, 10, 15 ppm NO_3-N which has had Ionic Strength Adjustor added
- outer filling solution for reference electrode—2 mL of ISA diluted to 100 mL
- inner filling solution - Orion Part No. 900002
- Kim-Wipes or equivalent
- Ionic Strength Adjustor solution

I generally have the students prepare a stock nitrate solution in advance. We go through the steps involved in calculating the amount of KNO_3 needed to prepare a 100 ppm NO_3^--N solution. Dry potassium nitrate (KNO_3) in an oven at 105∝C for 24 hours. Dissolve 0.7218 g in water and dilute to 1000 mL; 1.00 mL =100 mg NO_3^--N, i.e., 100 ppm. Preserve with 2 mL 1 M boric acid/ liter. This solution is stable for at least 6 months.

We discuss the best method to prepare the 2, 5, 7, 10, and 15 ppm standards. I have them prepare the standards by dilutions of the 100 ppm to 100 mL. Add 1 mL Ionic Strength Adjustor for every 100 mL of standard or sample.

The Ionic Strength Adjustor is prepared by dissolving 264 g $(NH)_2SO_4$ per liter of solution.

An interference suppressor solution can be used if necessary. It is added to the sample or standard at the ratio of 1:1. It is prepared by dissolving the following in enough water to make a liter of solution:

3.43 g Ag_2SO_4
1.28 g boric acid
17.32 g $Al_2(SO_4)_3$
252 g sulfamic acid

Safety, Handling, and Disposal

General laboratory safety procedures are sufficient for this lab. Eye protection is required. Dispose of used reagents according to local ordinances.

Points to Cover in Pre-Lab Discussions

It is important to emphasize care in handling the nitrate electrode. It is an expensive piece of equipment and should be treated accordingly. The most important factor in the student experiment that is not found in the literature is the importance of changing the reference electrode solutions each time the electrode is used.

Procedural Tips

The portions describing the use of the electrodes are sufficient as written. To analyze an unknown simply add ISA at the 1:1 ratio and read on the instrument. If the nitrate electrode is used daily the sensing module would last about six months.

TESTING FOR AMMONIA UTILIZING AQUARIUM TEST KITS

Introduction

Description

One of the simplest means of testing for the ammonia nitrogen is to utilize freshwater test kits from an aquarium store. The standards and samples are treated with the ammonia reagent according to the instructions in the test kit and then read on a Spectronic 20 instead of using the color chart.

Student Audience

This experiment is written for freshman college chemistry and is a continuation of the capstone activity.

Goals

The desired outcomes are that the students will:

- learn to do a rudimentary search of the literature.
- properly maintain a laboratory notebook.
- operate successfully in a research group.
- reinforce previously learned skills such as solution preparation, pipetting, and standards preparation.
- learn to interpret data based on existing theories.
- learn to use a spectrophotometer.
- prepare a formal written report following the format of a journal article.

Recommended Placement in the Curriculum

This experiment is intended as a capstone activity for freshman college chemistry and would be implemented at the end of the second quarter or near the beginning of the second semester.

STUDENT HANDOUT
Ammonia Nitrogen Using Aquarium Test-kits

Purpose

The purpose of this experiment is to use aquarium test kits to simplify the color development when using a spectrophotometer to measure the ammonia concentration in a freshwater aquarium.

Scenario

Ammonia, being part of the nitrogen cycle, is formed by the bacterial activity in a freshwater aquarium. In this experiment, you will be able to follow the ammonia concentration after setting up a new fish tank by measuring the ammonia concentration using aquarium test kits. To improve the accuracy, instead of using the color charts provided, you will measure the color intensity using a spectrophotometer.

Safety, Handling, and Disposal

Follow the instructions provided with the test kit for safe handling of the chemicals. Wear goggles throughout the procedure. Dispose of used reagents according to local ordinances.

Materials Needed

- ammonia aquarium test kit
- ammonium sulfate
- spectrophotometer with 1 cm cells
- 5 100-mL volumetric flasks
- 1,000-mL volumetric flask
- 5-, 10-, and 20-mL pipets

Procedure

Prepare a 1,000 ppm N as NH_4^+ as follows:
Dissolve 4.7170 g $(NH_4)_2SO_4$ in enough water to make 1 liter of solution.
Prepare standards of 100, 5, 10, 15, and 20 ppm from the 1000 ppm standard. Discuss your plan for preparing these standards with your instructor before proceeding.

Following the instructions with the test kits, develop the color for a blank, 5, 10, 15, and 20 ppm standards. Read the percent transmittance at 600 nm on the spectrophotometer after the color has developed. On some older models it is difficult to get an accurate absorbance reading. In this case simply read the percent transmittance and convert to absorbance:

$$T = \frac{\%T}{100}$$

$$Abs. = log\left(\frac{1}{T}\right)$$

Calculations

Create a plot of concentration on the x-axis and absorbance on the y-axis using a spreadsheet program. This plot should be linear.

Question

How can this plot be used to calculate the ammonia concentration of an unknown solution?

INSTRUCTOR NOTES

Ammonia Nitrogen Using Aquarium Test-kits

If the cells from one time to the next are reasonably the same, a new standard curve will not be necessary each time measurements are made on the fish tanks.

Time Required

3 hours

Group Size

3-4 students

Materials Needed

- ammonia aquarium test kit
- ammonium sulfate
- spectrophotometer with 1 cm cells
- 5 100-mL volumetric flasks
- 1,000-mL volumetric flask
- 5-, 10-, and 20-mL pipets

Safety, Handling, and Disposal

Follow the instructions provided with the test kit for safe handling of the chemicals. Wear goggles throughout the procedure. Dispose of used reagents according to local ordinances.

Points to Cover in the Pre-Lab Discussion

This experiment makes the assumption that the students already have used the Spectronic 20 or similar spectrophotometer. If they have not, this would need to be addressed. The older aquarium test kits used the Nessler reagent for ammonia nitrogen. If you use one of these, it contains mercury compounds and the necessary precautions and disposal procedures need to be followed. If the kit causes the solutions to be cloudy, the students may need to centrifuge the samples.

Procedural Tips and Suggestions

The ammonia test is usually much easier to use than nitrate and generally causes very few problems.