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To open the note, double click (Mac OS) or right click (Windows) on the note icon.

ACTIVITIES FOR A HIGH SCHOOL INSTRUMENTATION COURSE

developed through:

National Science Foundation, Department of Undergraduate Education, Grant DUE-9454518. "Partnership for the Advancement of Chemical Technology"

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INTRODUCTION

ABOUT THIS BOOK

This guide describes activities for use in a special after-school enrichment course on chemical instrumentation for high school students. The course is designed to give high school students exposure to the field of chemical technology as a viable career choice and to actively engage these prospective chemical technology students in using chemical instrumentation to solve problems technicians regularly confront in the workplace.

The High School Instrumentation Course was originally developed by Martha Brosz at Cincinnati State Technical and Community College and subsequently expanded at Miami University Middletown by Mark Sabo, Art Contreras, and Julie Hust. Our goal in assembling this collection is to provide activities that others can adapt or use in similar recruiting programs for high school students in their area.

This introduction provides a brief discussion of how we organized an instrumentation course. The activities provide both student handouts and instructor notes, some including sample data, for the activities we have used in this enrichment course.

WHAT IS A HIGH SCHOOL INSTRUMENTATION COURSE?

This enrichment course exposes high school students to an array of chemical instrumentation used in industry labs through a series of scenario problems designed to engage the students. We have organized our courses into five three-hour sessions. Each week students are presented with a different problem to solve. In the first meeting time is spent largely on introductory material with a graphing exercise and introductory activity on paper chromatography. (We used components of the activities included in this guide). During subsequent meetings, students work with their partners to operate one instrument per week.

While the instrumentation course can be offered at any time during the school year, we have chosen to offer ours in the late winter/early spring. This allows students to participate in the course with the knowledge of at least one semester of high school chemistry.

THE COURSE

Attracting Students into Chemical Technology

As previously noted, our goal with this course is to inform high school students about careers in chemical technology and to recruit students into our school's chemical technology program. As such, we want to locate students who enjoy chemistry lab, even if they are not A students. We ask teachers at collaborating high schools to nominate students to participate and to encourage students to participate by pointing out the honor associated with the nomination and the fun they will have. As you might expect, the high school teachers are the key to involving the target students. We like to meet with these teachers in a small group or on a one-to-one basis to describe the course and their role in helping us fulfill our goals. Personal contact can be the key.

Offering course credit may be an additional way to attract students to enroll in the course. However, it does require advanced planning since many universities require a year to

approve a new course. Alternatively, high school teachers can offer their students some type of extra credit or substitute credit for participation.

How to Pay for the Course

Our institution offers mini-grants for faculty who want to conduct or develop outreach programs to the community since this type of activity helps facilitate our mission statement. We have also found local industry to be receptive to partial subsidy of this program. Costs are not high, except in faculty time (which is often donated as a professional expense); equipment and chemicals are donated by our department, and only the cost of administration and pizza (or other refreshments) remains.

Activities

Science content is typically presented to the students on an as-needed basis so that they can safely operate instrumentation and complete analyses. We focus on instrument uses rather than the intricate details of the instrument itself. We discuss theory as the labs are being done and in post-lab discussion.

Activities are typically carried out in small groups (2–3 students), with groups rotating weekly to different activities and instruments. We try to keep groups small enough so that each student gets a chance to inject a sample or operate the instrument.

Because our course is not given for credit or a grade, we do not assign homework or expect formal write-ups. We do however include lab questions and points of discussion within the activities to which students respond in writing. This encourages students to become active in their own learning and gives them a sense of accountability and accomplishment.

Safety and Disposal

Safety is an important issue to consider for any chemistry course. This course provides us an opportunity to review good safety and disposal protocol and to discuss how industry would handle similar issues.

Dispose of any leftover materials or waste at your institution according to institution policies and regulations. Be sure goggles or safety glasses are provided to every student.

Always practice activities yourself before using them in the classroom. This is the only way to become thoroughly familiar with an activity, and familiarity will help prevent potentially hazardous (or merely embarrassing) mishaps. In addition, you may find variations that will make the activity more meaningful to the students.

ACTIVITIES INCLUDED IN THIS BOOK

pH Titration
Students will titrate pickle juice with sodium hydroxide to determine the amount of acetic acid present.

Determining Unknowns Using Standards—A Graphing Exercise
Students will construct a calibration curve and determine the concentration of a set of unknowns from data provided to them.

Paper Chromatography
Students used paper chromatography to determine what brand of black ink was used to write a ransom note.

Gas Chromatography
Students use gas chromatography to identify the type and amount of alcohol in mouthwash.

High Performance Liquid Chromatography
Students will use High Performance Liquid Chromatography (HPLC) to determine the amount of caffeine in two brands of soft drink.

Ultraviolet-Visible Spectroscopy
Students will use UV-Vis spectroscopy to determine the concentration of Red #40 dye in Gatorade®.

Infrared Spectroscopy
Students will identify samples of plastic film using reference spectra and an infrared spectrometer.

pH TITRATION—Introduction

DESCRIPTION

Students will titrate pickle juice with sodium hydroxide to determine the amount of acetic acid present.

GOALS FOR THE EXPERIMENT

In this experiment students will learn to:

- titrate a sample
- properly use and read a pH meter
- conduct quantitative analyses
- perform titration calculations

STUDENT SKILLS

- calculations
- titrations

pH TITRATION—Student Handout

PURPOSE

To use a pH meter to monitor a titration for its endpoint to determine the acidity of pickle juice.

SCENARIO

Pickling is defined as the preservation of food by impregnation with acid. Dill pickles are produced by fermenting cucumbers in a brine solution. The brine solution encourages the growth of acid-producing bacteria, but is strong enough to prevent growth of undesirable bacteria. The brine solution contains vinegar, salt, dill, and other spices. Vinegar is present to prevent the growth of undesired bacteria, which may make the pickles soft or hollow or give an off-flavor.

You are a chemical technician in the quality control (QC) department of Dudley's Dill Pickles. The company feels that they can better control the quality of their pickles if they can monitor the final acidity of the brine solution after the pickles are fermented. Unfortunately, the company does not know what the acidity value is after the pickles are made. It is your job to determine the starting and final acidity of the brine solution using the standard method provided below. Once the final acidity value is determined, the company can set guidelines to control the quality of their pickles. The company feels that this QC is important to maintaining the good taste and customer satisfaction that make Dudley's Dill Pickles so famous.

SAFETY, HANDLING, AND DISPOSAL

Sodium hydroxide (NaOH) is caustic—handle all solutions with care. If you should spill NaOH on your hands, rinse them with a lot of water.

The pH probe is a delicate instrument. The end of the probe consists of a thin glass bulb. Because this glass is thin, it can break easily. Handle the probe with care and follow your instructor's directions for cleaning. The pH probe can also dry out easily. Be sure it is kept in a pH 7 buffer solution when not in use. Do not store the probe in water.

PROCEDURE

1. Your instructor will give you directions on how to calibrate and operate the pH meter.

2. Pre-rinse the buret and tip with a small amount of 0.25 M sodium hydroxide (NaOH) solution, and then fill it to the 0.0 mL mark with the 0.25 M NaOH solution.

3. Using a 50-mL graduated cylinder, carefully measure 25 mL of stock brine solution and transfer it into a clean, dry, 150-mL beaker.

4. If a magnetic stirrer is used, place the beaker on the magnetic stirrer. Position the pH probe so that it is deep into the brine solution and does not touch the rotating stirring bar or sides of the beaker. If the probe is not deep enough to completely wet the tip, distilled water can be added to the beaker.

5. Position the beaker, pH probe, and buret so that the NaOH solution can be easily added to the brine solution. Take and record the initial pH reading of the brine solution.

6. Add the NaOH solution and record the pH after each addition using the following parameters. Add NaOH solution in:
   - 5 mL increments until 30 mL of NaOH solution has been added;
   - 1 mL increments until pH 5 is reached;
   - 0.5 mL increments until pH 11.5 is reached; and
   - 2 mL increments for five more additions.

7. Discard the solution according to your instructor's directions. Be sure to rinse the pH probe and beaker with distilled water before each titration.

8. Repeat steps 2–7 for the brine solution. Record your data in the table below.

9. Repeat steps 2–7 twice more for the pickle juice. However, this time add NaOH in 1 mL increments until 8 mL have been added. Then add NaOH in 0.5 mL increments until you reach a pH of 11.5 or greater. Add NaOH in 2 mL increments for five more additions. Be sure to record your data in the table below.

Data Table For Titrations							
Brine Solution Titration #1		Brine Solution Titration #2		Pickle Juice Titration #1		Pickle Juice Titration #2	
mL NaOH	**pH**	**mL NaOH**	**pH**	**mL NaOH**	**pH**	**mL NaOH**	**pH**

CALCULATIONS

1. Using graph paper or a spreadsheet, graph pH vs. mL of NaOH added for the first titration. This is called a titration curve. The equivalence point (where moles acid = moles base) can be found at the inflection point. The inflection point on the curve represents the maximum rate of change of pH per unit volume of NaOH added. The mL of NaOH at this inflection point can be used to calculate the moles of acid titrated. Your instructor may need to help you determine the equivalence point of your titration. Record the amount of NaOH used at the equivalence point.

2. To determine the moles of acetic acid in the solution, use the fact that for this system, at the equivalence point, moles acid = moles base. To find the moles of base you can use the volume of NaOH added at the equivalence point and the concentration of the NaOH. So:

   moles NaOH = M_1V_1.
   M_1= concentration of NaOH (moles/L)
   V_1 = amount of NaOH added at equivalence point (in liters)
   moles NaOH = _____

   Since at the equivalence point moles acid = moles base, moles acetic acid = moles NaOH calculated above.

$$mass\ acetic\ acid = \left(\frac{60\ grams\ acetic\ acid}{1\ mole\ acetic\ acid}\right)(moles\ acetic\ acid)$$

3. Use the moles of acetic acid in the sample (found in step 2) to determine the grams of acetic acid in the sample.

4. Use the mass of acetic acid determined in step 3 to calculate percent concentration (m/v) using the following equation:

$$\%\ Concentration = \left(\frac{mass\ of\ acetic\ acid\ (g)}{25\ mL\ of\ solution\ tested}\right) \times 100$$

5. Repeat the calculations above for the other three titrations.

QUESTIONS

1. What was the % acid in the brine solution? In the pickle juice?

2. Did the % acid in solution increase or decrease after fermentation of the pickles was complete? What do you think caused the increase or decrease in acidity of the solution?

3. How close were the acid values for the duplicate runs?

4. What are the potential sources of experimental error?

pH TITRATION—Instructor Notes

Time Required for Preparation	30–45 minutes
Time Required for Procedure	2–3 hours
Group Size	2 students

MATERIALS NEEDED

Getting Ready

- sodium hydroxide
- distilled water
- phenolphthalein
- 95% ethanol

Procedure

Per group

- pH meter (or Vernier pH probe and CBL unit/TI graphics calculator or computer)
- 50-mL buret
- buret clamp
- ring stand
- distilled water in a wash bottle
- Kimwipes or other lint-free tissues
- 250-mL beaker (for waste)
- narrow-mouth 150-mL beaker
- 50 mL dill pickle juice
- 50 mL brine solution
- 250 mL 0.25 M sodium hydroxide
- magnetic stirrer and stirring bar (optional) or glass stirring rod

SAFETY, HANDLING, AND DISPOSAL

All chemicals may be disposed of in accordance with your local laws. Review the MSDS sheets of all chemicals used in this experiment.

GETTING READY

1. To make the stock brine solution, add 1.5 L of white vinegar to 1.5 L of water (can be tap water). Add 1-1/2 cups of kosher salt. Stir to dissolve. (For best results, use either kosher salt or chemistry lab salt in the brine solution rather than table salt. Table salt has an anti-caking agent (silica) that will not completely dissolve and as a result clouds the solution.)

2. To make the 0.25 M NaOH solution, weigh 2.5 g of sodium hydroxide into a 250-mL volumetric flask. Dilute to volume with distilled water.

3. You may be able to obtain pickle juice from a local store, deli, or sandwich shop.

4. You should perform a quick titration to check that the pickle juice can be titrated with less than 50 mL of titrant. If not, adjust the sample size accordingly.

PRE-LAB DISCUSSION

pH

Discuss with students the pH scale, its range (from 1—acidic to 14—basic) and what it means (pH is related to the hydrogen ion concentration by the equation pH = -log [H^+]). You can tell them the approximate pH of items they are familiar with such as:

- human blood, pH = 7.4
- lemon juice, pH = 2 (varies)
- carrots, pH = 5
- household ammonia, pH = 11
- bleach, pH = 12
- vinegar, pH = 3

Acetic Acid

To make the experiment more interesting to students, tell them about the substances they are analyzing. Some suggestions include:

- vinegar has been known for over 4,000 years. Ancient Mesopotamians referred to it as "sour beer."
- production of vinegar was mostly a matter of chance until Louis Pasteur discovered the secrets of fermentation. He discovered that bacteria convert alcohol to acetic acid. It is the acetic acid that makes vinegar sour.

PROCEDURAL TIPS AND SUGGESTIONS

After students plot their data, be sure to discuss the titration curve with them and show them how to determine the equivalence point.

SAMPLE RESULTS

The pickle juice was ~0.66% acetic acid (m/v). The brine solution was determined to be ~2.4% acetic acid (m/v).

PLAUSIBLE ANSWERS TO QUESTIONS

1. What was the % acid in the brine solution? In the pickle juice?
   Students found the average values for acetic acid to be 2.4% in the brine solution and 0.66% in the pickle juice.

2. Did the % acid in solution increase or decrease during fermentation of the pickles was complete? What do you think caused the increase or decrease in acidity of the solution?
   % acid decreased during fermentation of the pickles. Some of the acid is absorbed by the cucumbers, which turns them into pickles.

3. How close were the acid values for the duplicate runs?
   Answers will vary. As a variation, you may want to have the students average the class data and determine the standard deviation.

5. What are the potential sources of experimental error?

Sources of error are primarily in accurately determining the end point, reading the buret, measuring out 25 mL using the graduated cylinder, and not properly rinsing the buret with the 0.25 mL NaOH and pH probe and beaker with distilled water before each titration.

REFERENCES

Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis.* New York: Saunders College Publishing, 1992.

CBL System Experiment Workbook. Texas Instruments Incorporated, 1994, pp 65–67.

DETERMINING UNKNOWNS USING STANDARDS—A GRAPHING EXERCISE—Introduction

DESCRIPTION

Students will construct a calibration curve and determine the concentration of a set of unknowns from data provided to them.

GOALS FOR THE EXERCISE

Students will be able to:

- understand how/why standards are used
- draw a "best fit" line
- determine the concentration of unknown samples by extrapolation

STUDENT SKILLS

- algebra
- graphing
- equation of a line

DETERMINING UNKNOWNS USING STANDARDS—A GRAPHING EXERCISE—Student Handout

PURPOSE

To learn methods of determining the concentration of unknowns that cannot be measured directly.

SCENARIO

Lately, citizens in your community, Marysville, USA, have been concerned about the possibility of lead contaminating the water supply. Some of the lead pipes which carry the water supply throughout the community (including some pipes in old buildings) are old and it is feared that lead may leach into the water. The EPA regulatory action level of lead in drinking water is 15 parts per billion (ppb). This means that no more that 15 grams of lead can be present in one billion (10^9) grams of water! (This amounts to about 260,000 gallons! Another way to look at it is no more than 45 grams of lead can be present in enough water to fill an Olympic sized swimming pool.)

For a lab project in chemistry class, your instructor has collected water samples for analysis. Water samples have been taken from 36 spots throughout Marysville. Your instructor has already run the analyses; you will analyze the data she collected.

While there is not an instrument available that can directly measure the concentration of lead in water, an atomic absorption (AA) spectrometer can be used to provide the relative amount of lead particles present in that sample compared to some standard. A standard is a sample with a known concentration. To determine an unknown concentration, a series of standards are made and a reading is taken on the AA for each standard. A measurement is also made on an unknown (in this case, drinking water) and then compared with the data from the standards. In this case, the standards all contain known concentrations of lead. Table 1 provides the readings obtained for the lead standards used to calibrate the AA.

Table 1: Calibration Data

Standards with known concentration (ppb)	Absorbance read on the AA (au)
0.5	0.013
1.0	0.024
5.0	0.126
10.0	0.257
20.0	0.532

PROCEDURE

Part I: Best Fit Line—Graphing Method

1. Use the data for the standards to plot absorbance (y-axis) versus lead concentration (x-axis). You want the graph to fill the entire page. The larger the scale, the more accurately you can graph your data.

2. Using a ruler or other straight edge, draw a "best fit" line. A best-fit line does NOT connect all the points; it is a straight line and is draw so that all points are as close to the line as possible.

3. Choose three samples (provided by your instructor) for analysis. Using the best-fit line and the absorbance of your samples, determine the concentration of lead in your samples from the graph. The absorbance values for your three samples will be provided to you by your instructor. Fill in the concentrations in the chart below.

Graphing Method		
Site #	Absorbance	Lead Concentration (ppb)

Part II: Regression Analysis Using a Graphing Calculator or Computer

1. Using a computer or a calculator (such as a TI-82 or TI-85) that can perform regression analysis, enter the data provided above. Your instructor may need to show you how to do this.

2. Perform a regression analysis on the data and obtain the equation that describes the best-fit line. Write the equation here:_____.

3. Using your best-fit line above and the absorbance of your samples, determine the concentration of lead in your samples. The absorbance values for your three samples will be provided to you by your instructor. Fill in the concentrations in the chart below.

Regression Method		
Site #	Absorbance	Lead Concentration (ppb)

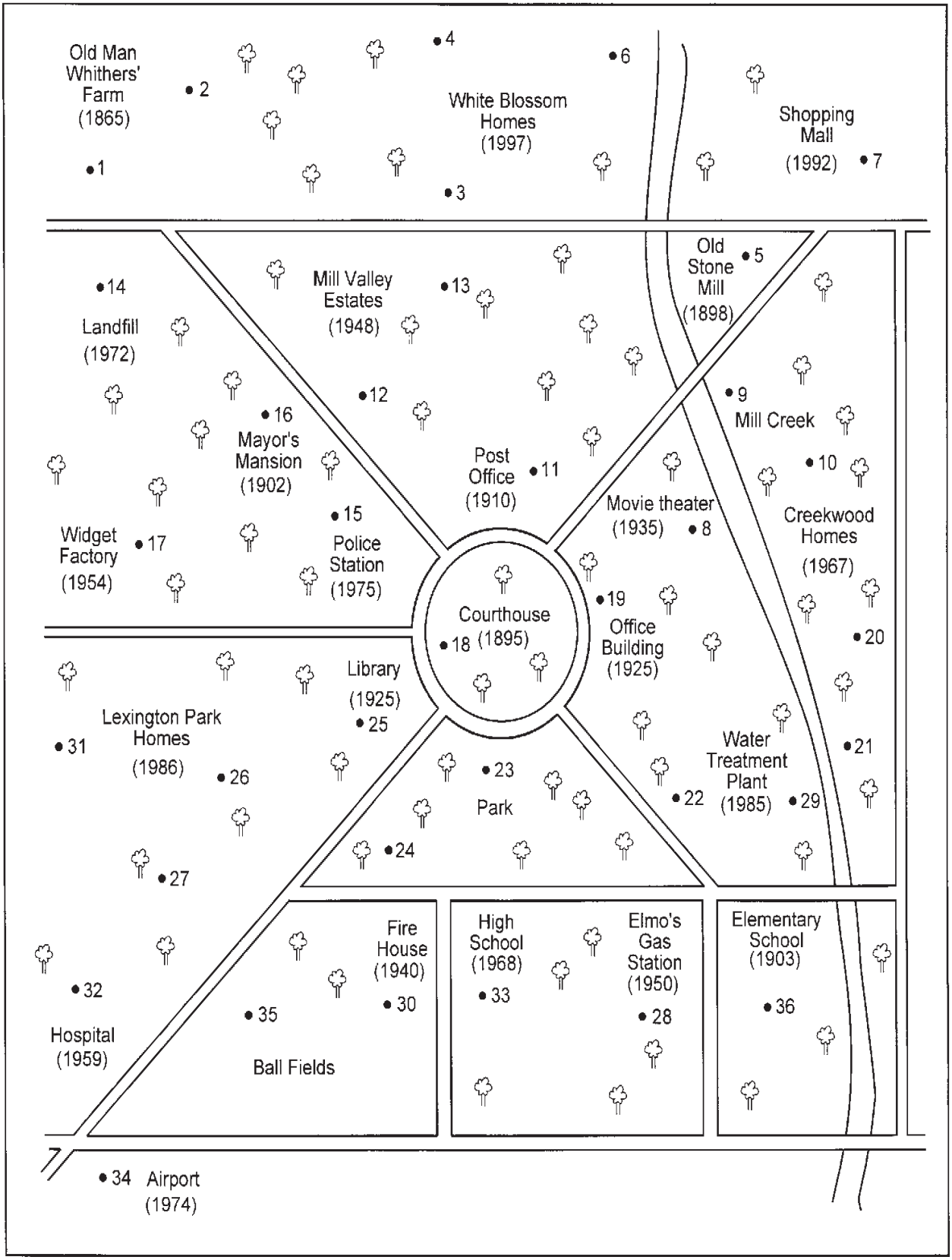
POINTS TO PONDER

1. How do your concentration values for your samples calculated in Part I and Part II compare? Discuss reasons for any variations.

2. Based on your determinations, is the water safe to drink in each of the three spots where your samples were taken? Why or why not?

3. Compile all the class data using the map provided. Can you determine the likely cause of contamination?

Map of Anytown, USA



DETERMINE UNKNOWNS USING STANDARDS—A GRAPHING EXERCISE—Instructor Notes

Time Required for Preparation	none
Time Required for Procedure	20–45 minutes
Group Size	1 student

MATERIALS NEEDED

Per student

- graph paper
- ruler or other straight edge
- 3 unknown "cards" (contain absorbance values; provided on page 7)

Per 1–6 students

- computer with graphing software (such as Excel, DeltaGraph, or Lotus) or graphing calculator

SAFETY, HANDLING, AND DISPOSAL

No special safety, handling, or disposal procedures are required.

PRE-LAB DISCUSSION

Before beginning the activity, review units of concentration such as mg/L and μg/L:

- 1 mg/L corresponds to 1 part per million (ppm) 1 μg/L corresponds to 1 part per billion (ppb), etc.
- Explain the units ppm and ppb are used when concentrations are extremely small, as is the case with many environmentally monitored toxins. Use examples students can relate to, such as 1 ppb compared to the whole is like 1.5 inches compared to the circumference of the earth.

Discuss standards and instrument response:

- absorbance readings have units of absorbance units, abbreviated au.
- how instruments measure response and provide examples. (A heartbeat is a good example of peak height because students are familiar with it.) The response helps one to graph a line which shows the relationship between concentration and the response. This line is what helps us to determine the concentration of an unknown.
- Standards are used to calibrate the response of an instrument. The concentration of the standards are known since a known amount of analyte (the species of interest whose concentration we determine—in this case lead) is added to a known amount of solvent (the liquid that dissolves the analyte—in this case water).

Discuss the issue of lead contamination in drinking water, including:

- the most frequent cause of contamination is the corrosion of indoor plumbing.
- until the early 1900's it was very common to use lead for indoor plumbing; all pipes installed before 1930 are made of lead.

- lead contamination of drinking water is most common in very old or new houses. The plumbing in new houses, while they are "lead-free" pipes (those that contain less than 0.2% lead), are susceptible to lead leaching into the water. After a few years, pipes build up a mineral deposits that keep the water from coming into direct contact with the pipes.
- Gasoline was also a cause of lead contamination before leaded gas was outlawed.
- Amendments were made to the Safe Drinking Water Act in 1986 that required the use of "lead-free" solder, pipes, and flux in the installation or repair of any public water system or any plumbing in residential or non-residential buildings that are connected to a public water system.
- initially, the federal guidelines set the action level of lead in drinking water at 50 ppb. It was later reduced to 15 ppb, where it stands today.

PROCEDURAL TIPS AND SUGGESTIONS

It is recommended that students hand graph the data to improve their graphing skills first in Part I. In Part II students can graph the data using a computer, calculate the regression line, and compare their results (and realize how much more accurate the regression method is). If a computer or graphing calculator is not available, you can give them the slope and intercept of the line and the formula for the line. The slope of the line is 0.0279 and the y-intercept 0.00167. Using the formula y = mx + b, the line can be expressed by the equation:

$$y = 0.0266x + 0.00407$$

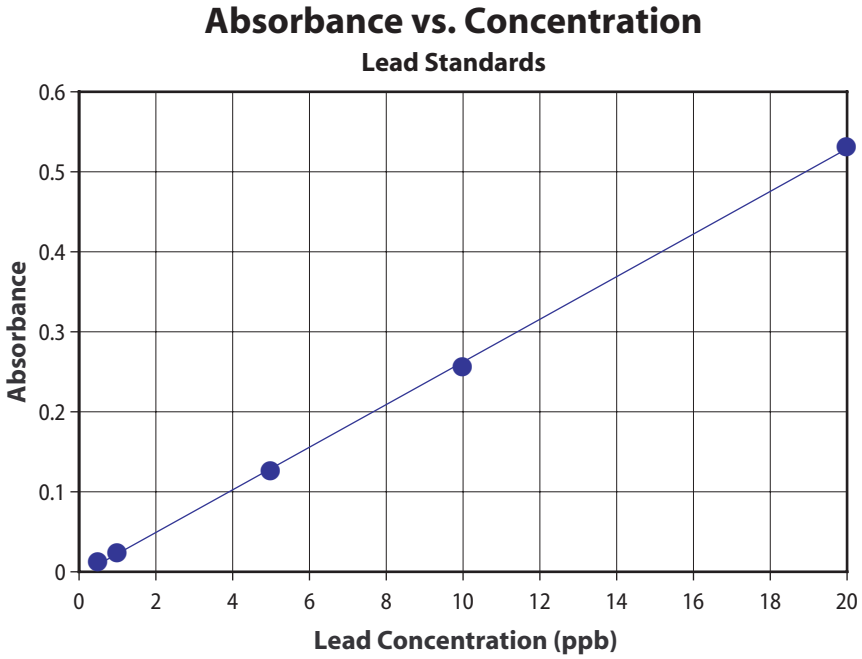
You may want to copy the "cards" that contain the absorbance readings of the unknowns on cardstock or other heavy paper, especially if these will be reused.

Absorbance Readings of Unknowns			
Site #1 Old Man Whithers' Farm (Farm House) Absorbance Reading: 0.134 au	**Site #2** Old Man Whithers' Farm (Barn) Absorbance Reading: 0.145 au	**Site #3** The Garlands' Home Absorbance Reading: 0.356 au	**Site #4** The Durlings' Home Absorbance Reading: 0.446 au
Site #5 Old Stone Mill (Restroom) Absorbance Reading: 0.427 au	**Site #6** The Collins' Home Absorbance Reading: 0.377 au	**Site #7** Shopping Mall (Water Fountain) Absorbance Reading: 0.158 au	**Site #8** Movie Theater (Water Fountain) Absorbance Reading: 0.348 au
Site #9 The Gallaghers' Home Absorbance Reading: 0.310 au	**Site #10** The Ross' Home Absorbance Reading: 0.193 au	**Site #11** Post Office (Restroom) Absorbance Reading: 0.571 au	**Site #12** The O' Daniels' Home Absorbance Reading: 0.204 au
Site #13 The Allisons' Home Absorbance Reading: 0.161 au	**Site #14** Landfill (Employee Restroom) Absorbance Reading: 0.079 au	**Site #15** Police Station (Water Fountain) Absorbance Reading: 0.222 au	**Site #16** The Mayor' s Mansion Absorbance Reading: 0.513 au
Site #17 Widget Factory (Employee Restroom) Absorbance Reading: 0.063 au	**Site #18** Courthouse (Water Fountain) Absorbance Reading: 0.590 au	**Site #19** Office Building (Water Fountain) Absorbance Reading: 0.475 au	**Site #20** The Sommers' Home Absorbance Reading: 0.116 au
Site #21 The Wades' Home Absorbance Reading: 0.108 au	**Site 22** Water Treatment plant (Waste Water) Absorbance Reading: 0.217 au	**Site #23** Park (Fountain) Absorbance Reading: 0.121 au	**Site #24** Park (Water Fountain) Absorbance Reading: 0.236 au
Site #25 Library Absorbance Reading: 0.462 au	**Site #26** The Wilsons' Home Absorbance Reading: 0.172 au	**Site #27** The Hughes' Home Absorbance Reading: 0.094 au	**Site #28** Elmo' s Gas Station (Restroom) Absorbance Reading: 0.113 au
Site #29 Water Treatment plant (Treated Water) Absorbance Reading: 0.039 au	**Site #30** Fire House (Water Fountain) Absorbance Reading: 0.121 au	**Site #31** The Woodwards' Home Absorbance Reading: 0.150 au	**Site #32** Hospital (Water Fountain) Absorbance Reading: 0.217 au
Site #33 High School Absorbance Reading: 0.097 au	**Site #34** Airport (Water Fountain) Absorbance Reading: 0.188 au	**Site #35** Ball Fields (Water Fountain) Absorbance Reading: 0.166 au	**Site #36** Elementary School (Water Fountain) Absorbance Reading: 0.129 au

Lead Concentrations of Unknowns			
Site #1 Old Man Whithers' Farm (Farm House) Lead Concentration: 5.2 ppb	**Site #2** Old Man Whithers' Farm (Barn) Lead Concentration: 5.6 ppb	**Site #3** The Garlands' Home Lead Concentration: 13.5 ppb	**Site #4** The Durlings' Home Lead Concentration: 16.9 ppb
Site #5 Old Stone Mill (Restroom) Lead Concentration: 16.2 ppb	**Site #6** The Collins' Home Lead Concentration: 14.3 ppb	**Site #7** Shopping Mall (Water Fountain) Lead Concentration: 6.1 ppb	**Site #8** Movie Theater (Water Fountain) Lead Concentration: 13.2 ppb
Site #9 The Gallaghers' Home Lead Concentration: 11.8 ppb	**Site #10** The Ross' Home Lead Concentration: 7.4 ppb	**Site #11** Post Office (Restroom) Lead Concentration: 21.6 ppb	**Site #12** The O' Daniels' Home Lead Concentration: 7.8 ppb
Site #13 The Allisons' Home Lead Concentration: 6.2 ppb	**Site #14** Landfill (Employee Restroom) Lead Concentration: 3.1 ppb	**Site #15** Police Station (Water Fountain) Lead Concentration: 8.5 ppb	**Site #16** The Mayor' s Mansion Lead Concentration: 19.4 ppb
Site #17 Widget Factory (Employee Restroom) Lead Concentration: 2.5 ppb	**Site #18** Courthouse (Water Fountain) Lead Concentration: 22.3 ppb	**Site #19** Office Building (Water Fountain) Lead Concentration: 18.0 ppb	**Site #20** The Sommers' Home Lead Concentration: 4.5 ppb
Site #21 The Wades' Home Lead Concentration: *4.2 ppb*	**Site #22** Water Treatment plant (Waste Water) Lead Concentration: *8.3 ppb*	**Site #23** Park (Fountain) Lead Concentration: *4.7 ppb*	**Site #24** Park (Water Fountain) Lead Concentration: *9.0 ppb*
Site #25 Library Lead Concentration: 17.5 ppb	**Site #26** The Wilsons' Home Lead Concentration: 6.6 ppb	**Site #27** The Hughes' Home Lead Concentration: 3.7 ppb	**Site #28** Elmo' s Gas Station (Restroom) Lead Concentration: 4.4 ppb
Site #29 Water Treatment plant (Treated Water) Lead Concentration: 1.6 ppb	**Site #30** Fire House (Water Fountain) Lead Concentration: 4.7 ppb	**Site #31** The Woodwards' Home Lead Concentration: 5.8 ppb	**Site #32** Hospital (Water Fountain) Lead Concentration: 8.3 ppb
Site #33 High School Lead Concentration: 3.8 ppb	**Site #34** Airport (Water Fountain) Lead Concentration: 7.2 ppb	**Site #35** Ball Fields (Water Fountain) Lead Concentration: 6.4 ppb	**Site #36** Elementary School (Water Fountain) Lead Concentration: 4.8 ppb

SAMPLE RESULTS

Below is a graph, including the regression line and its equation, for the lead standards.



To use the graph directly, the concentration of an unknown with an absorbance of 0.429 would show a concentration of 16 ppb from the graph. The equation of the line is known. For this case it was determined to be:

$$y = 0.0266x - 0.00407$$

If a sample gives an absorbance reading of 0.429 absorbance units, using the equation the concentration of the sample in ppb would be determined as follows:

First, the equation needs to be rearranged to solve for x.

$$x = \frac{y + 0.00407}{0.0266}$$

The absorbance value of 0.429 is substituted for y and solved for x

$$x = \frac{0.429 + 0.00407}{0.0266} = 16.3$$

So, the sample concentration of lead is 16.3 ppb. The advantage of this method is accuracy. Although the graphing method is easier, the lead concentration can be determined more accurately using the regression method. You will need to point this out to students.

PLAUSIBLE ANSWERS TO "POINTS TO PONDER"

1. How do your concentration values for your samples calculated in Part I and Part II compare?

Discuss reasons for any deviations.
Concentration values for each sample should be close (within approximately 1 ppb) but will vary depending on the student's graphing technique. The regression method results are more accurate since the "best-fit" line is calculated instead of estimated. The line the student draws will probably have a different slope than that calculated by the regression.

2. Based on your determinations, is the water safe to drink in each of the three spots where your samples were taken? Why or why not?
The actual lead concentrations are given on the page entitled "Lead Concentrations of Unknowns." If the concentration is greater than 15 ppb (the EPA determined action level of lead in drinking water) the water is not safe to drink.

3. Compile all the class data using the map provided. Can you determine the likely cause of contamination?
In buildings that were constructed before 1930, the pipes are most likely made of lead. Some of the lead is leaching into the water, resulting in the high concentration of lead. In the newer homes, mineral deposits have not yet formed and so the water is coming into direct contact with the pipes. Even though pipes used today are "lead-free" (meaning they contain less than 0.02% lead) lead can still leach out into the water. However, with time the amount of lead in water will decrease.

REFERENCES

Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis;* Saunders College Publishing: New York, 1992.

Chemical Education for Public Understanding Program (CEPUP). *Investigating Groundwater: The Fruitvale Story;* Addison-Wesley: New York, 1991.

United States Environmental Protection Agency web page: http://www.epa.gov

PAPER CHROMATOGRAPHY—Introduction

DESCRIPTION

Students use paper chromatography to determine what brand of black ink was used to write a ransom note.

GOALS FOR THE EXPERIMENT

In this experiment students will learn to:

- conduct qualitative analyses
- use standards to determine the identity of an unknown
- perform a chromatographic separation

STUDENT SKILLS

- identification of an unknown
- measuring

PAPER CHROMATOGRAPHY—Student Handout

PURPOSE

To use a type of chromatography to identify an unknown ink pigment based on standards.

SCENARIO

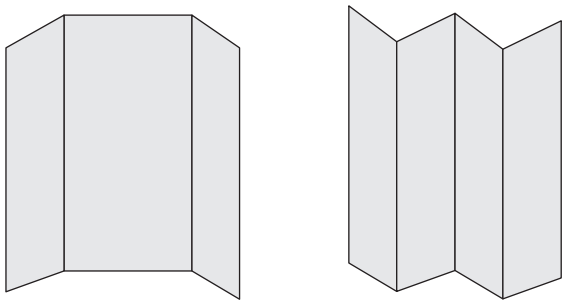
You are an analytical chemist in a crime lab. A police detective has a ransom note written in black. He found a black marker in the car of the suspect, so you will test that marker along with two or three other common brands. You will extract some of the ink off of the ransom note and analyze it by paper chromatography. You will also perform paper chromatography on all the black markers. If the brand of marker found in the suspect's car matches the ink from the ransom note, a search warrant may be issued to search the suspect's apartment for more clues.

PROCEDURE

Part 1: Preparing the Standards

1. Cut a piece of filter paper into a 10-cm x 10-cm square.

2. Divide the paper into three or four sections using a tri-fold or accordion fold.



tri-fold accordion fold

3. Orient the filter paper vertically and use a pencil and ruler to draw a line across the filter paper perpendicular to the fold lines 1.5–2 cm from an edge. Treat this edge as the bottom of the paper.

4. Using a pencil, label the bottom of each pleated segment (below the line drawn in Step 3) with the brand name of the felt-tipped marker to be used on that segment.
   Note: DO NOT use a marker for labeling the filter paper.

5. Use one brand of marker to make a small dot of ink (smaller than the diameter of a pencil eraser) in the middle of the pencil line in the appropriately labeled pleated segment of the paper.

6. Repeat Step 5 using each of the two remaining markers on a different segment of the paper.

7. Refold the spotted filter paper square along the original fold lines so that it will stand up with the ink spots near the bottom.

Part II: Developing the Chromatogram

1. Add 1% salt solution to the beaker to a depth of 1 cm.

2. Stand the paper outside the glass. Be sure that the ink spots will be above the solution; if too much solution was added, pour some out before continuing.

3. Carefully stand the folded filter paper in the glass containing the solvent. Cover the top of the glass with plastic wrap, aluminum foil, or invert a plastic sandwich bag over the glass.

   NOTE: Watch as the solution "creeps" up the paper; the components of the ink rise along with the solution, some moving faster than others. The development time depends on the kinds of paper and solvents used.

4. Remove the paper from the glass when the location of the solution level (solvent front) reaches about 2 cm from the top edge of the filter paper. Using a pencil, immediately mark the location of the solvent front.

5. Carefully lay the filter paper on a clean, dry paper towel and let it dry.

6. Complete the table below for each brand of felt-tipped marker, listing the colors found in the chromatogram in descending order (from the one that traveled the longest distance up the chromatogram listed first, down to the color that traveled the shortest distance).

Pen #1		**Pen #2**		**Pen #3**	
Brand:		Brand:		Brand:	
Color	R_f	Color	R_f	Color	R_f

7. Once the chromatogram is dry, measure the distance from the middle of the original spot to the edge of the solvent front marked in Step 4. This is the distance that the solvent traveled.

8. Measure the distance from the middle of the original colored spot to approximately the middle of each color. This gives an estimated value for the distance that each color or color component traveled.

9. Calculate the R_f (retention factor) for each color:

$$R_f = \frac{\text{distance solute moved}}{\text{distance solvent moved}}$$

10. Enter the R_f values in the chart above. Compare R_f values for the same color found in different brands of markers.

11. Compare the chromatograms of the different brands of markers to determine similarities and differences. (For example, is blue always the fastest-traveling color? How do the pigments used in the different brands of markers differ in color composition? Are there one or two colors that are present in all the different brands of markers?)

    Question 1: Why do you think some pigments traveled farther than others?

    Part III: Analyzing the Ransom Note Ink

1. Cut a 1.5-cm x 2.5-cm piece of the ransom note that contains a lot of writing (the more ink the better).

2. Put the piece of ransom note into a 50-mL beaker, ink side up. Add 40 drops (2 mL) of water. Swirl the water around so that it comes into contact with all of the ink on the note. Continue swirling the beaker until as much ink as possible has dissolved into the water. Remove the paper.

3. Place the beaker on a hot plate and boil off almost all of the water. (There should be approximately 0.5 mL of water left in the beaker.)

4. Swirl the beaker so the remaining water dissolves all of the ink. Draw some of this liquid up into a capillary tube.

5. Using the capillary tube, spot the ink onto a piece of filter paper. Let the ink dry and spot using the capillary tube again, touching the capillary tube to the center of the ink spot. Spot the ink a total of four times. Be sure to let the ink dry in between each spotting or the diameter of the spot will become too large.

6. Develop the chromatogram and calculate R_f values as instructed above (Part II, Steps 1–10).

Ransom Note Pen	
Color	R_f

7. Compare your ransom note ink chromatogram and the R_f values to the standards.

8. Record which brand of pen was use to write the ransom note here:_____.

Question 2: Is the brand of pen used to write the ransom note the same brand that was found in the suspect's car?

Part IV: Investigating a Different Solvent System

1. Repeat Parts I and II, but this time using acetone as your solvent in place of the 1% salt solution. Measure the R_f values and complete the chart below.

Pen #1		**Pen #2**		**Pen #3**	
Brand:		Brand:		Brand:	
Color	R_f	Color	R_f	Color	R_f

Question 3: What differences in the chromatogram (if any) did you observe between the two solvent systems (1% salt solution and acetone)?

PAPER CHROMATOGRAPHY—Instructor Notes

Time Required for Preparation	10–20 minutes
Time Required for Procedure	1–2 hours
Group Size	1–4 students

MATERIALS NEEDED

Getting Ready

- filter paper
- water-soluble markers
- scissors
- white paper

Procedure

Per group

- 10–30 mL of 1% Salt solution: dissolve 1 g of sodium chloride in 100 mL of water (can be tap water).
- 250-mL beaker
- 50-mL beaker
- acetone
- several pieces of filter paper at least 15 cm in diameter
- 3–4 different brands of black, water-soluble, felt-tipped pens and markers
  Note: Crayola® and Vis-a-Vis® brands work well. Erasable markers do not work well. 13-cm x 13-cm (5-in x 5-in) piece of aluminum foil, plastic wrap, or plastic sandwich bag
- metric ruler calibrated in millimeters
- scissors
- pencil
- 1.5-cm x 2.5-cm piece of ransom note

RESOURCES

Filter paper can be purchased from a chemical supply company such as Flinn Scientific, P.O. Box 219, Batavia, IL 60510-0219, 800/452-1261. filter paper—catalog # AP3105, 15-cm diameter, for 100 sheets

SAFETY, HANDLING, AND DISPOSAL

If acetone or rubbing alcohol is used, caution students about flammability.

PRE-LAB DISCUSSION

Before beginning the activity, introduce chromatography and related concepts including:

- The definition of chromatography, a technique to separate mixtures.
- Mixtures are separated based on the interaction of the samples with the mobile and stationary phases. In paper chromatography, the solvent is the mobile phase and the paper is the stationary phase. The solvent travels up the paper (or other media) via capillary action or "wicking." As the solvent moves up the filter paper, it carries with it the pigments in the ink. Each pigment travels at its own speed depending on its interaction

with the solvent (mobile phase) and the paper (stationary phase). Differences in these interactive forces among the various pigments cause some pigments to move up the paper at different rates. The pigments that are more soluble or are strongly attracted to the solvent move up the paper at a faster rate than those that are less soluble and have a smaller degree of attraction for the solvent. This separation of pigments becomes more apparent as the distance traveled by the solvent increases.

- A closer look at the chromatogram indicates that there is more going on than just pigments travelling at different rates. Some of the pigments end up in very concentrated zones; others are spread out in diffused bands. Some pigments tend to streak out in lines; others tend to form fan-like patterns. These effects are very likely a result of the intermolecular forces that exist between the molecules of the various pigments.
- There are other types of chromatography including high performance liquid chromatography (HPLC) and gas chromatography (GC). In both HPLC and GC the stationary phase is a solid. The mobile phase in HPLC is a liquid and in GC it is a gas. Included in this guide are experiments using both GC and HPLC.
- Point out the need for standards. In this experiment, standards are needed to compare the suspect's ink against to identify the ink from the ransom note. One of the standards is the ink from the pen found in the suspect's car.

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PROCEDURAL TIPS AND SUGGESTIONS

The level of the solvent must be below the ink dots or the ink will dissolve in the solvent.

The 1% sodium chloride solution provides a sharper separation than water alone.

When writing the ransom note, use block letters. This provides more ink for each sample.

To avoid each student analyzing the same unknown, you can set up the unknowns and experiment where each student group represents a different crime lab and kidnapping case.

PLAUSIBLE ANSWERS TO QUESTIONS

1. Why do you think some pigments traveled farther than others?
   The different pigments in the ink move at different speeds based on their differing attractions for the solvent. The pigment that travels the farthest is strongly attracted to the mobile phase. A pigment that has no attraction for the mobile phase would not move at all.

2. Is the brand of pen used to write the ransom note the same brand that was found in the suspect's car?
   This answer will depend on the brand of marker chosen by the instructor as the brand found in the suspect's car.

3. What difference in the chromatogram (if any) did you observe between the two solvent systems (1% salt solution and acetone)?
   The difference occurs in the order of separation of the pigments in the marker ink. Due to the differences in the polarity of the solvents, the order of the pigments developed in the salt solution will be reversed from the order of the pigments developed in the acetone.

VARIATIONS

1. Use other types of pigments and colorful mixtures:
   - Candy coatings from M&M's, Reese's Pieces, and Skittles—dissolve the coating from the candy with minimum amount of water (2–3 drops), dot the color coating on the paper, and use a 1% salt solution as the chromatographic solvent.
   - Food colors—Use a 1% salt solution as the chromatographic solvent.
   - Kool-Aid drinks—Make a concentrated slurry of the powder in water and use a rubbing alcohol/water mixture as the chromatographic solvent.
   - Grass or leaves—Extract the chlorophyll from the leaves using hot rubbing alcohol and use acetone as the chromatographic solvent.
   - Skins of fruits and vegetables—Extract the pigments by making a pureé in a blender. Spot the colored liquid and use a 0.1% salt solution as the chromatographic solvent.

2. Perform radial chromatography. Place a spot of ink in the center of a piece of circular filter paper. Add solvent and the ink will separate outward from the center. Use permanent markers in all colors on T-shirt material and rubbing alcohol as the chromatographic solvent.

3. Use media other than paper:
   - Cheap, uncoated chalk—Use chalk just like the paper. Place a spot of ink about 2 cm from the bottom of the stick. Place the chalk, with the ink spot down, in a glass filled with about 10 mL of solvent. Cover. It should develop like the paper did, with the color separating as the solvent moves up the chalk.
   - Column chromatography (disposable pipets packed with chalk or paper shavings)—Place ink or dye at the top of the column and pour the solvent (acetone or acetone/water) through until you see bands of color appear. This technique allows for the separation and collection of the components of the dye mixture.

REFERENCES

"Colorful Separations;" *Fun With Chemistry: A Guidebook of K–12 Activities;* Sarquis, M., Sarquis, J., Eds.; Institute for Chemical Education: Madison, WI, 1992; Vol. 2, pp 1–35.

"Paper Chromatography of Inks;" *Science in Our World: Strong Medicine—Chemistry at the Pharmacy,* Sarquis, M. Ed.; Center for Chemical Education, Terrific Science Press: Middletown, OH; pp 57–63.

GAS CHROMATOGRAPHY—Introduction

DESCRIPTION

Students use gas chromatography to identify the type and amount of alcohol in mouthwash.

GOALS FOR THE EXPERIMENT

In this experiment students will learn to:

- use a gas chromatograph
- conduct qualitative and quantitative analyses
- inject a sample into the gas chromatograph using a GC syringe
- use retention time of standards to determine unknown substances
- make and use a calibration curve to determine the concentration of alcohol in two brands of mouthwash

STUDENT SKILLS

- graphing
- making a calibration curve
- using a graph to interpolate

GAS CHROMATOGRAPHY—Student Handout

PURPOSE

Use gas chromatography to identify the type and amount of alcohol present in two popular brands of mouthwash.

SCENARIO

You are a chemical technician working for a company that wants to start production on a new brand of mouthwash. You have been assigned to investigate leading brands of mouthwashes. It is your job to identify and quantify the active ingredient in each mouthwash. Gas chromatography will be used to accomplish this task. You will start by analyzing two major brands of mouthwash, Scope® and Listerine®.

SAFETY, HANDLING, AND DISPOSAL

Goggles should be worn when performing this activity.

The alcohols used in this activity are a fire hazard; keep away from open flames or sources of heat. Wash your hands if alcohol spills on them.

Keep liquids away from the instrument—do not put beakers on the instruments and do not pour liquids while working over the instrument.

Handle syringes with care; avoid puncture wounds and breakage, gas chromatography syringes are very expensive. The instructor will provide details about the use, cleaning of the syringes, and keeping contamination out of samples. It is very important to follow these directions.

Do not adjust the dials on the instrument unless the instructor specifically instructs you to do so.. Also, when injecting your samples into the instrument, do not touch the metal fitting that holds the septum through which the syringe needle is inserted. It may be extremely hot!

PROCEDURE

Your instructor will demonstrate the proper use of the gas chromatograph (GC) and the GC syringe. Between trials, rinse the syringe at least three times with acetone and then rinse three times with your next sample, dispensing the waste into a waste beaker each time. Slowly draw your sample into the syringe and push the plunger so that 1 mL remains in the syringe. (Be sure there are no air bubbles in the syringe. Your instructor will show you the way to remove the air bubbles.) After you have 1 µL of liquid in the syringe, pull the plunger up so that you have at least 5-6 µL of air with your 1 µL of sample.

<u>Part A: Identifying the Alcohol (Qualitative Analysis)</u>

1. Inject a 1 µL mixture of alcohols provided by your instructor into the gas chromatograph. Look at the data from the recorder. This is called a chromatogram and is a plot of detector response versus time.

Question 1: What do you notice about the chromatogram? If you made only one injection, why does the chromatogram have more than one peak?

2. Inject 1 mL of ethanol into the GC. Print the chromatogram and write the name of this alcohol on the printout. Rinse the syringe. Repeat this step for propanol, butanol, and pentanol.

3. For each alcohol injected in step 1, you must determine the adjusted retention time. The adjusted retention time is the difference in time between the retention time of your analyte and the time of the air peak for that injection (usually the first peak in the chromatogram).

4. Record this data in the following table.

Table 1: Retention Times of Alcohols

Alcohol	Adjusted Retention Time (min)
Ethanol (C_2H_5OH)	
Propanol (C_3H_7OH)	
Butanol (C_4H_9OH)	
Pentanol ($C_5H_{11}OH$)	

5. Inject 1 mL of Listerine. Print the chromatogram and write the name of the sample on the printout. Rinse the syringe according to the outlined procedure.

6. Repeat step 5 using Scope.

7. Fill in the table below and determine the adjusted retention times for Listerine ® and Scope®.

Table 2: Retention Times of Alcohols in Mouthwash

Mouthwash	Adjusted Retention Time (min)
Listerine	
Scope	

8. Compare the adjusted retention times of the alcohol in the mouthwashes to the adjusted retention times of the alcohol samples. Which alcohol do you believe is in the:

Listerine _____

Scope _____

Question 2: How did you identify the type of alcohol present in the mouthwash?

Question 3: What information does the retention time give you?

Part B: Amount of Alcohol (Quantitative Analysis)

1. Ask your instructor for the alcohol standards. Inject 1 mL of the 5% standard for the alcohol identified in Step 8. Write the alcohol percentage on the printout. Rinse the syringe. Identify the area of the standard from the printout and record this information below. Then fill in the "Area x 10^{-3}" column. Repeat for the remaining standards (10%, 20%, and 30%).

Table 3: Standard Areas

% Alcohol	Area	Area x 10^{-3*}
5		
10		
20		
30		

Question 4: What do you notice about the relative areas for the standards listed above?

Question 5: What does the area of the peak represent?

Question 6: How could you use the area of these standards to determine the concentration of alcohol in each of the mouthwashes?

2. Using the chromatograms from the Scope and Listerine injections (Part A, step 4), find the area of the alcohol peak and write it in the table below.

Table 4: Mouthwash Areas

Mouthwash	Area	Area x 10^{-3}
Listerine		
Scope		

3. Looking at the area for the Listerine and Scope, compare the areas to the standards. How much alcohol would you estimate is in the mouthwashes?

Listerine _____

Scope _____

4. Using graph paper or a graphing software program, plot area on the y-axis and concentration on the x-axis for each of the standards listed in step 1. This is a calibration curve. Draw the best fit line through the data points. Ask your instructor if you need help.

5. Using your graph, determine the concentration of the alcohol in Listerine and Scope.

   Listerine _____

   Scope _____

GAS CHROMATOGRAPHY—Instructor Notes

Time Required for Preparation	30-45 minutes
Time Required for Procedure	2-3 hours
Group Size	2-4 students

MATERIALS NEEDED

Procedure

Per class

- Gas Chromatograph with integrator or computer

Per group

- 10 mL methanol
- 10 mL ethanol
- 10 mL propanol
- 10 mL butanol
- 10 mL pentanol
- 10 mL Listerine®
- 10 mL Scope®
- 5 vials (to store alcohols in)
- 50-mL beaker (for waste)
- 5-mL Mohr pipet
- 3 10-mL volumetric flasks
- gas chromatography syringe
- acetone
- distilled water
- ethanol
- graph paper or access to a computer with graphing software and printer

SAFETY, HANDLING, AND DISPOSAL

Goggles should be worn when performing this activity.

The alcohols use in this activity are a fire hazard; keep away from open flames or sources of heat. Wash your hands if alcohol spills on them.

Keep liquids away from the instrument—do not put beakers on the instruments and do not pour liquids while working over the instrument.

Handle syringes with care; avoid puncture wounds and breakage. Provide details about the use, cleaning of the syringes, and keeping contamination out of samples. It is very important that the students follow these directions.

Provide a container for waste solutions.

GETTING READY

1. To prepare for this activity, make a solution that contains an equal volume of ethanol, pro-

panol, butanol, and pentanol. This will be the alcohol mixture students inject in Part A , step 1.

2. Make 5%, 10%, 20%, and 30% ethanol in water standards in the 10-mL volumetric flasks. Inject the mouthwash samples into the GC to be sure that their concentrations fall within the range of the standards. Both Scope® and Listerine® should fall within the range of the standards as both brands contain less than 30% ethanol.

3. Warm up the instrument at least 1-2 hours before the start of the experiment. The following GC instrument parameters can serve as a guideline:

Gas Chromatograph:	GOWMAC Series 350
Column:	4 feet x 1/4 inch, 20% Carbowax 20M on ChromosabP, 80/100 mesh
Column temperature:	130°C
Gas flow rate:	50 mL/min
Bridge current:	150 mA

4. Cover the ingredient information on the back of the mouthwash bottles using tape or some other method. You may want to peal off the label before students perform this lab.

RESOURCES

A GC syringe can be purchased from a chemical supply company such as Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA, 15219-4785, 800/766-7000. Gas chromatography syringe—catalog #14-824-3, 10 mL, removable needle syringe.

PRE-LAB DISCUSSION

Describe the basics of GC, including the definitions of a mobile and stationary phase and how components of a mixture are separated. Remember to keep things simple—these are high school students. Other topics to discuss include:

- how to operate the GC.
- the difference between GC and HPLC (what is gas chromatography versus liquid chromatography).
- what a chromatogram looks like and the information it contains.
- how the peak area is related to concentration.
- the standards are used to develop the calibration curve; the calibration curve can then be used to determine the concentration of an unknown.
- the many uses of GC: to analyze gasoline additives, testing for alcohol in blood, purity of flavors in food, testing urine for steroids or other illegal drugs.
- the fact that such a small sample is required and how this makes the technique especially useful for forensics and medical tests.

NOTE: Some of these topics can be left for a post-lab discussion, as students will learn some of this information by performing the lab.

PROCEDURAL TIPS AND SUGGESTIONS

The actual percentage of ethanol in Listerine and Scope is printed on the sides of the containers. (Scope: ~19%; Listerine: ~26%).

An injection of 1 mL should give an adequate peak height for all alcohols, mouthwashes, and standards.

SAMPLE RESULTS

Table 1: Retention Times of Alcohols

Alcohol	Adjusted Retention Time (min)
Ethanol (C_2H_5OH)	0.46
Propanol (C_3H_7OH)	0.60
Butanol (C_4H_9OH)	1.02
Pentanol ($C_5H_{11}OH$)	1.75

Table 2: Retention Times of Alcohols in Mouthwash

Mouthwash	Adjusted Retention Time (min)
Listerine	0.45
Scope	0.45

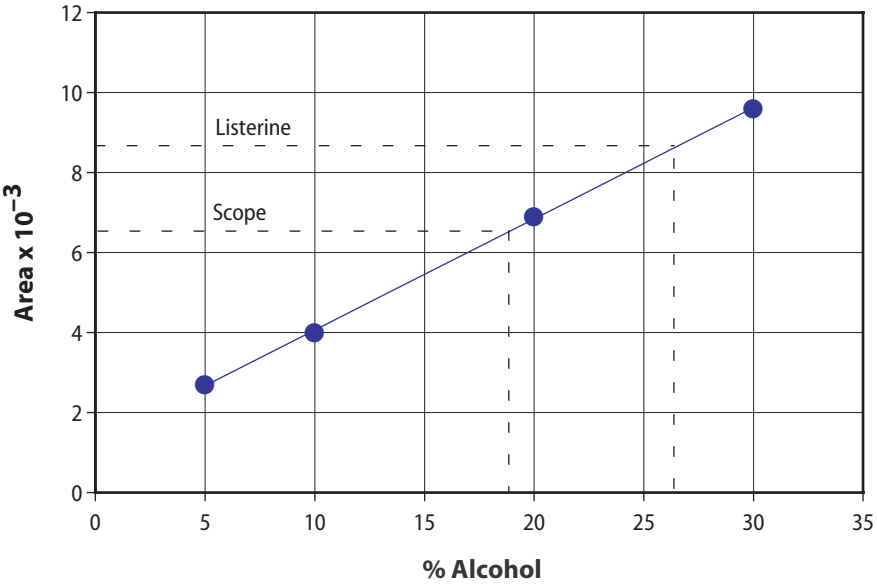
Table 3: Standard Areas

% Alcohol	Area	Area x 10^{-3*}
5	2735	2.7
10	4021	4.0
20	6897	6.9
30	9632	9.6

Table 4: Mouthwash Areas

Mouthwash	Area	Area x 10^{-3}
Listerine	8489	8.5
Scope	6501	6.5

Figure 1: Concentration vs. Area for Alcohol Standards



Regression Analysis	
Regression Equation	y = a + bx
Y intercept (a)	1.3
Slope (b)	0.3
Correlation coefficient	.99
Number of data points	4

PLAUSIBLE ANSWERS TO QUESTIONS

1. What did you notice about the chromatogram? If you made only one injection, why is there more than one peak in the chromatogram?
   There are four peaks in the chromatogram representing the four compounds in the mixture (ethanol, propanol, butanol, and pentanol). By interaction with the mobile and stationary phases, the mixture was separated into the individual components.

2. How did you identify the type of alcohol present in the mouthwash?
   The retention time of the peak due to the alcohol in the mouthwash was compared with the retention times of the peaks due to ethanol, propanol, butanol, and pentanol. Ethanol was identified as the alcohol present in the mouthwash since its retention time closely matched the retention time of the mouthwash alcohol.

3. What information does the retention time give you?
   Retention time only allows you to identify a compound by comparing the retention time of an unknown to the retention time of a known compound.

4. What do you notice about the relative areas for the standards listed above?
   As the concentration of alcohol increases, the area increases.

5. What does the area of the peak represent?
   The area of the peak is directly related to the relative amount of alcohol present in the sample.

6. How could you use the area of these standards to determine the concentration of alcohol in these mouthwashes?
   Alcohol standards of known concentrations are injected into the GC. The corresponding areas of the alcohol peaks are plotted versus their known concentration. Using this standard curve, the area of the mouthwash alcohol peak can be used to determine the concentration of the alcohol in the mouthwash.

EXTENSIONS AND VARIATIONS

- If time allows, students could make the standards.
- A mouthwash with no alcohol could be included.

REFERENCES

Sawyer, D. T.; Heineman, W. R.; Beebe, M. M.*Chemistry Experiments for Instrumental Methods.* New York: John Wiley & Sons, 1984.

Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis.* New York: Saunders College Publishing, 1992.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY—Introduction

DESCRIPTION

Students will use High Performance Liquid Chromatography (HPLC) to determine the amount of caffeine in two brands of soft drink.

GOALS FOR THE EXPERIMENT

In this experiment students will learn to:

- use an HPLC
- conduct quantitative analyses
- inject a sample into the HPLC using a syringe
- make and use a calibration curve to determine the concentration of two unknowns

STUDENT SKILLS

- graphing data
- making a calibration curve
- using a graph to interpolate

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY—Student Handout

PURPOSE

To use High Performance Liquid Chromatography (HPLC) to determine the amount of caffeine in soft drinks.

SCENARIO

Soft drinks and regular coffee contain caffeine, which acts as a stimulant. A 7 ounce cup of regular coffee can contain 90–150 mg of caffeine and tea contains 30–70 mg per 7 oz. cup. You wonder how this compares to the caffeine content of Mountain Dew® and Surge® (which are known for their high caffeine content), so you decide to analyze the concentration of caffeine in these soft drinks.

SAFETY, HANDLING, AND DISPOSAL

Make sure no carbonation remains in the soft drinks. The bubbles from the carbon dioxide can affect the separation in the HPLC.

Do not ingest any materials, soft drinks, etc. in the lab.

Handle syringes with care; avoid puncture wounds and breakage, HPLC syringes are very expensive. The instructor will provide details about the use and cleaning of the syringes, and keeping contamination out of samples. It is very important to follow these directions.

PROCEDURE

1. Before you start this experiment, your instructor should introduce the basic operation of the HPLC and injection techniques. Pay particular attention to the precautions outlined by your instructor.

2. Fill the syringe with 40 mL of the least concentrated caffeine standard (50 ppm). Inject the contents of the syringe into the HPLC. The instrument will have a sample loop of 20 µL. This loop must be filled completely without air bubbles in order to get good results.

3. The peak from the caffeine will be recorded by the detector. Repeat steps 1 and 2 twice more for the 50 ppm standard.

4. Repeat steps 2 and 3 for the 100, 150, and 200 ppm standards. Complete three injections for each standard. Make sure you inject the standards in order from least concentrated to most concentrated.

5. Fill in the table below. Average the peak areas of the three trials for each standard.

Table 1: Standard Injections			
Standard	Trial #	Peak Area	Averaged Peak Area
50 ppm	1		
	2		
	3		
100 ppm	1		
	2		
	3		
150 ppm	1		
	2		
	3		
200 ppm	1		
	2		
	3		

Question 1: What do you notice about the relative area for the caffeine standards listed above?

Question 2: What does the area of the peak represent?

Question 3: How could you use the area of these standards to determine the concentration of caffeine in soda pop?

6. Inject each soft drink sample three times filling in the table below. Average the caffeine peak areas of the three trials for each soft drink sample.

Table 2: Soft Drink Injections			
Soft Drink	Trial #	Peak Area	Averaged Peak Area
	1		
	2		
	3		
	1		
	2		
	3		

Looking at the average area for the Surge and Mountain Dew, compare the areas to the standards. How much caffeine would you estimate is in each soda pop?

Surge _____ ppm
Mountain Dew _____ ppm

7. On graph paper (or on a computer), plot the average peak area from the standards on the y-axis versus concentration on the x-axis. This will be your calibration curve.

   Question 4: If you had based the calibration curve on peak height rather than peak area, would your estimates of the amount of caffeine in the soft drinks have been as accurate? Explain.

8. Determine the concentration of caffeine (in ppm) in each soft drink using the calibration curve.

   Surge _____ ppm
   Mountain Dew _____ ppm

   Question 5: If 12 ounces (one can of soda) is equal to 0.355 L, how many mg of caffeine are in each can of soft drink? (Hint: 1 ppm = 1 mg/L)

   Question 6: How does the amount of caffeine found in the soft drinks compare to the amount of caffeine in coffee and tea?

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY—Instructor Notes

Time Required for Preparation	30-45 minutes
Time Required for Procedure	2-3 hours
Group Size	2-3 students

MATERIALS NEEDED

Getting Ready

- 4 100-mL volumetric flasks
- 1-L volumetric flask
- 2-mL, 4-mL, 8-mL pipets
- 1 L of solvent: 20% methanol, 80% water, adjusted to pH 3.5
- reagent grade caffeine
- HPLC grade acetonitrile

Procedure

Per class

- High Performance Liquid Chromatograph

Per group

- 50-mL HPLC syringe
- graph paper
- 2 cans of different caffeinated citrus or other clear soft drinks, such as Mountain Dew® or Surge®
- 50-mL beaker (for waste)
- acetone (to clean syringe after injection of soft drink)
- caffeine standards (50, 100, 150, and 200 ppm)
- Kimwipes or other type of lab tissue
- glacial acetic acid
- acetonitrile (HPLC grade)
- water (HPLC grade)
- saturated sodium acetate solution

SAFETY, HANDLING, AND DISPOSAL

Do not allow the students to drink the leftover soft drinks, as the soft drinks have been exposed in the lab they may be contaminated.

Pure caffeine is quite toxic if ingested. Have students review the MSDS.

All chemicals may be disposed of in accordance with your local laws. Review the MSDS sheets of all chemicals used in this experiment.

GETTING READY

1. Decarbonate the soft drinks by either letting them sit open overnight, pouring them into a larger bottle and shaking vigorously, or by pulling a vacuum over the liquid.

2. Prepare caffeine standards of 50 ppm, 100 ppm, 150 ppm, and 200 ppm in water.

3. The mobile phase (1M acetic acid in 10% acetonitrile) can be prepared by diluting 50 mL of acetonitrile and 28.5 mL glacial acetic acid with water to a final volume of 500 mL. This solution must then be adjusted to pH=3 by adding saturated sodium acetate.

4. All standards, samples, and mobile phase must be filtered through a 0.2-0.45 μm filter prior to entry into the HPLC. You should filter these into a vial and mark the vial for student use. Alternatively you could have your students filter these samples.

5. Turn on the pump and detector. Set the pump flow rate at 0.75 mL/min. Flush the column by allowing the mobile phase to pass through the column for at least 20 minutes. Record the detector responses to be sure no substances remain on the column.

6. Determine whether the caffeine in the soda samples falls within the range of the standards. If the samples do not fall within range, adjust the standard concentrations so that they do.

   Warm up the instrument at least 30 minutes before the start of the experiment. The following instrument conditions can serve as a guideline:

Column:	C8 or C18 packed column or similar
Injection size:	10–20 mL, depending on injection loop size
Flow rate:	1.5 mL/min
Detector:	UV detector, set at 254 nm
Mobile phase:	1 M acetic acid in 10% acetonitrile—adjusted to pH=3.0

PRE-LAB DISCUSSION

HPLC

Describe the basics of HPLC, including the definitions of a mobile and stationary phase and how components of a mixture are separated. Remember to keep things simple—these are high school students. Other topics to discuss include:

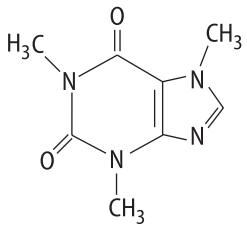
- how to operate the HPLC.
- the difference between HPLC and GC (what is liquid chromatography versus gas chromatography).
- how the peak area is related to the sample concentration.
- the standards are used to develop the calibration curve; the calibration curve can then be used to determine the concentration of an unknown.
- the many purposes of HPLC: to analyze air and water pollutants, to isolate natural products, to check the purity of pharmaceutical and food products (to name just a few).

NOTE: Some of these topics can be left for a post-lab discussion as students will learn some of the information by performing the lab.

Caffeine

To make the experiment more interesting to students, tell them about the substance they are detecting, caffeine. Some suggestions include:

- Caffeine is an alkaloid, found naturally in cola nuts, coffee, tea, cacao beans, and other plants and has the following structure:



- Over-the-counter caffeine preparations such as Nodoz® and Vivarin® are sometimes purchased by those wanting to stay alert. Excedrin® also contains 60 mg caffeine.
- Caffeine stimulates the central nervous system, cardiac muscle, and respiratory system.
- Caffeine also has the affect of making a person feel less drowsy and fatigued, more capable of rapid and clear thought. Caffeine can also make a person feel shaky and anxious.
- Caffeine is now being added to water. A product called Krank$_2$O contains 100 mg of caffeine per 500 mL.

PROCEDURAL TIPS AND SUGGESTIONS

Each injection will usually take less than 5 minutes. If you find yourself stretched for time, it is not necessary to have students do three injections of each standard. One should be sufficient since they are constructing a curve. You should, however, encourage the students to do three injections of the soda pop.

SAMPLE RESULTS

Students have typically determined that the concentration of caffeine in Mountain Dew is approximately 150 ppm. Surge has a caffeine concentration of approximately 110 ppm.

PLAUSIBLE ANSWERS TO QUESTIONS

1. What do you notice about the relative area for the caffeine standards listed above?
   As the concentration of caffeine increases, the area increases.

2. What does the area of the peak represent?
   The area of the peak is directly related to the amount of caffeine present in the sample.

3. How could you use the area of the standards to determine the concentration of caffeine in soda pop?
   The corresponding areas of the caffeine standard peaks are plotted against their known concentrations. Using this concentration curve, the area of the soda pop caffeine peak can be used to determine the concentration of caffeine in the soda pop.

4. If 12 ounces (one can of soda) is equal to 0.355 L, how many mg of caffeine are in each can of soft drink? (Hint: 1 ppm = 1 mg/L)
   According to the above student data, Mountain Dew contains approximately 53 mg per can and Surge contains approximately 39 mg per can. The companies that make these soft drinks have reported caffeine of 55 mg and 51 mg respectively.

5. How does the amount of caffeine found in the soft drinks compare to the amount of caffeine in coffee and tea?
   Coffee contains 12.9–21.4 mg of caffeine per ounce. A similar calculation for tea gives 4.3–10 mg/oz. Surge contains 3.25 mg caffeine/ounce and Mountain Dew contains 4.4 mg caffeine/ounce. Coffee and tea contain more caffeine per ounce when compared to Mountain Dew and Surge.

6. If you had based the calibration curve on peak height rather than peak area, would your estimates of the amount of caffeine in the soft drinks have been as accurate? Explain.
   If the amount of caffeine in the soft drinks had been determined by peak height, the concentration values would be close to the actual values, but not as accurate as when areas are used. The peak height doesn't take into account the shape of the peak. A shorter, but broader peak may have the same area as a tall thin peak, but the peak height may differ greatly.

REFERENCES

Kenkel, John. 1994. *Analytical Chemistry for Technicians.* Boca Raton: Lewis Publishers.

Sawyer, D.T.; Heineman; W.R.; and Beebe, M.M. 1984. *Chemistry Experiments for Instrumental Methods.* New York: John Wiley & Sons. p. 427.

Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis.* New York: Saunders College Publishing.

ULTRAVIOLET-VISIBLE SPECTROSCOPY—Introduction

DESCRIPTION

Students will use UV-Vis spectroscopy to determine the concentration of Red #40 dye in Gatorade®.

GOALS FOR THE EXPERIMENT

In this experiment, students will learn to:

- use a UV-Vis spectrophotometer or Spectronic-20
- conduct quantitative analyses
- make a calibration curve to determine the concentration of red dye in an unknown
- understand difference between transmittance and absorbance

STUDENT SKILLS

- graphing
- interpolate unknown concentration from a graph

ULTRAVIOLET-VISIBLE SPECTROSCOPY—Student Handout

PURPOSE

To determine the concentration of FD&C Red #40 in Gatorade® Tropical Burst sports drink using a double-beam UV-Vis Spectrophotometer or Spectronic-20.

SCENARIO

As a supermarket employee was stocking the shelves with Gatorade, she noticed that the new shipment of Gatorade Tropical Burst was a much darker red than the bottles remaining on the shelf from the previous shipment. She returned one bottle from the new shipment to the manufacturer for testing.

You are a lab technician at the company that makes Gatorade. Quality control guidelines mandate that Gatorade Tropical Burst should have a concentration of FD&C Red #40 dye in the range of 32–40 ppm. You will use a double-beam UV-Vis spectrophotometer to determine the actual concentration of Red #40 in this bottle of Gatorade Tropical Burst.

SAFETY, HANDLING, AND DISPOSAL

Goggles should be worn when performing this activity.

Do not drink the Gatorade or the standards. Since the Gatorade has been exposed in the laboratory, it may be contaminated.

Do not place beakers or other items on the instrument. Do not pour anything while working over the instrument.

PROCEDURE

The light strikes the lower portion of the cuvette, so only handle the upper portion of the cuvettes. Any liquid or smudges on the outside of the cuvette must be wiped with a Kimwipe, or other lint-free wipe, never with paper towels. There should be no lint or fingerprints on the outside of the cuvette and no air bubbles inside the cuvette. Cuvettes should be rinsed with distilled water, never with cleaning solution. Cuvettes for the UV-Vis come in matched sets and are very expensive; please be very careful with them.

<u>Preparing Your Samples</u>

1. Have your instructor show you the proper use of pipettes.

2. Using a 5-mL pipette, transfer 5.0 mL of the “good” Gatorade into a 25-mL volumetric flask and dilute to the mark.

3. Repeat step 2 for the “bad” Gatorade sample.

<u>Filling the Cuvette</u>

1. Rinse the cuvette with distilled water and then rinse it three times with your diluted “good” Gatorade sample.

2. Fill the cuvette half full with the sample.

3. Wipe the outside of the cuvette with a Kimwipe and place it in the sample compartment of the UV-Vis.

4. Rinse another cuvette, fill with distilled water, and place this in the reference compartment of the UV-Vis. This is your blank.

5. Make sure the lid is closed.

6. By scanning from 300-600 nanometers (nm), record a spectrum of the "good" Gatorade to determine the wavelength of maximum absorbance. (Note: your instructor may have to help you with this step.)

7. When the scan is finished, print the spectrum.

8. Choose the optimum wavelength for the Gatorade.

9. Enter your optimum wavelength in the table below.

Measuring Absorbance of Samples

1. With the help of your instructor, set the UV-Vis to measure absorbance at a fixed wavelength. Use the optimum wavelength to record the rest of your data.

2. Record the absorbance of the "good" and "bad" Gatorade samples as well as the standards listed in the table. Start with the most dilute sample and work to the most concentrated sample. (Be sure to rinse the cuvette three times with your sample.)

3. Enter absorbance readings on the chart provided.

4. Answer the questions below.

Data Table 1: Absorbances of Samples

Sample	Absorbance (Wavelength = _____)
1 ppm	
5 ppm	
10 ppm	
20 ppm	
Good Gatorade	
Bad Gatorade	

Question 1: Why do you think the reference cuvette contained distilled water for the blank? If your unknown used ethanol as the solvent, what would the blank consist of?

Question 2: Justify your choice of wavelength for the analysis of FD&C Red #40.
Question 3: Does the concentration of dye affect absorbance directly or indirectly?

Determining the Concentration of the "Good" and "Bad" Gatorade Samples
NOTE: If a computer is available in the lab, you may plot your data using the appropriate software. See your instructor for details.

1. Using data from the table above, plot the data points for the standards (1, 5, 10, 15, and 20 ppm) on a piece of graph paper. Be sure your graph has absorbance on the y-axis and concentration on the x-axis. Your graph should occupy the entire piece of paper.

2. Draw the best fit line through your data.

3. Use the graph to determine the concentration of the "good" Gatorade and the "bad" Gatorade.

Concentration of red dye in diluted "bad" Gatorade _____, diluted "good" Gatorade _____ according to the graph.

Question 4: What was the dilution factor you used to prepare your Gatorade samples at the beginning of this lab?

4. Using the dilution factor, determine the concentration of Red #40 dye in undiluted "bad" and "good" Gatorade.

Concentration of Red #40 dye in "bad" Gatorade _____, "good" Gatorade _____,

Question 5: Is the concentration of red dye in the "bad" Gatorade within the quality control specifications? How do you know?

ULTRAVIOLET-VISIBLE SPECTROSCOPY—Instructor Notes

Time Required for Preparation	60 minutes
Time Required for Procedure	2 hours
Group Size	2–4 students

MATERIALS NEEDED

Getting Ready

For the Procedure

- pipets, 2 mL, 4 mL, and 6 mL
- 4 100-mL volumetric flasks
- FD&C Red #40
- distilled water

Procedure

Per group

- ultraviolet-visible spectrophotometer (Spectronic 20 or single- or double-beam scanning instrument)
- 1, 5, 10, 15, and 20 ppm standard solutions of Red #40
- distilled water
- 5 disposable pipets
- 2 cuvettes
- Kimwipes or other laboratory tissues (lint-free)
- graph paper or graphing program and printer
- 10 mL Gatorade® Tropical Burst

NOTE: You may use another flavor of Gatorade if you can locate the appropriate dye. You would need to determine the concentration for the standards.

SAFETY, HANDLING, AND DISPOSAL

Goggles should be worn when performing this activity.

Do not place beakers or other items on the instrument. Do not pour anything while working over the instrument.

Do not allow the students to drink the leftover Gatorade, as the Gatorade has been exposed in the lab and may be contaminated.

Dispose of all waste in agreement with your local ordinances.

RESOURCES

FD&C Red #40 (also known as Allura® Red AC, molar mass 496.43) may be purchased from B.F. Goodrich, Hilton-Davis Division, 2235 Langdon Farm Road, Cincinnati, OH, 45237. 800/477/1022. Code no. 10-21-EA-6056—FD&C Red #40 dye.

Disposable pipets may be purchased from a chemical supply company such as Fisher Scientific, 711 Forbes Avenue, Pittsburgh, PA, 15219-4785, 800/766-7000.
catalog # 13-711-9A—graduated pipets, nonsterile

GETTING READY

1. Prepare 1, 5, 10, 15, and 20 ppm standard solutions of Red #40 dye.

2. To make the "bad" Gatorade sample, add approximately 0.030 grams of Red #40 to 1 quart of Gatorade Tropical Burst sports drink. (It contains approximately 30 ppm.) Do not dilute the Gatorade sample further.

3. Set the wavelength of the instrument to 500 nm. Obtain a calibration curve to determine whether the Gatorade absorbance falls within the range of the standards. If it doesn't fall within range, make the appropriate standard(s). Make sure the absorbance of the standards fall within 0.1 to 0.9 or whatever range satisfies Beer's Law for the instrument used.

4. Warm up the instrument at least 15 minutes prior to the start of the experiment.

PRE-LAB DISCUSSION

UV-Vis

You may want to discuss the electromagnetic spectrum, all types of radiation, and how ultraviolet radiation (190–400 nm) relates to visible radiation (400–700 nm) in terms of wavelength.

Describe the basics of UV-Vis spectroscopy, including:

- how to operate the instrument.
- light is energy and UV and visible light contain enough energy to promote outer electrons to higher energy levels.
- UV-Vis spectrophotometry is usually applied to molecules and inorganic ions or complexes in solution. This technique is very useful for quantitative measurements.
- the many purposes of UV-Vis spectrophotometry. It can be used to measure stabilizers in plastics and sugars in soft drinks. Biologists use it to measure bacteria. One of the most widely used purposes of UV-Vis is as a detector in HPLC.
- standards are used to develop a calibration curve; the calibration curve can then be used to determine the concentration of an unknown.

Color Additives

Include the following points:

- color is added to many consumer products to improve appearance. Industries add pigments to food, cosmetics, plastics, and other household products as a marketing strategy.
- you could ask students why color is added to food when it provides no flavor enhancement. Color is added to correct natural variations in foods such as butter, cheese, and orange rinds. It is also added to fruit-flavored drinks to replace more expensive, natural ingredients such as fruit juice.
- color is added to soft drinks to provide a clue to the flavor of beverage (which otherwise

may not be identified). You could remind students of Crystal Pepsi, a colorless cola soft drink introduced several years ago that was not well accepted by the public.

- In 1975, research on the health effect of red dyes led the U.S. Food and Drug Administration to ban the use of FD&C Red #4 in food. Red #4 was found to cause atrophy of adrenal glands and cancerous tumors in dogs. However, it is still used in externally applied drugs and cosmetics. In 1976, the use of FD&C Red #2 was also banned in food, drugs, and cosmetics due to research concluding that it causes cancer, birth defects, fetal deaths, and sterility in lab animals. Red #2 is currently used in color photography and the dyeing of some fabrics. FD&C Red #40 was produced to replace Red #4; it was approved for use in the United States and Canada but not in the United Kingdom, Sweden, Switzerland, and a few other countries. A recent survey by the National Academy of Sciences found that FD&C Red #40 is the most common colorant used today.

PROCEDURAL TIPS AND SUGGESTIONS

A group can complete activities for the UV-Vis spectrophotometer and infrared spectrometer during the same class meeting if the standards are made for the students.

Instructions were written for a double-beam instrument. If you use a single-beam instrument such as a Spectronic 20, you will need to adjust the directions accordingly.

SAMPLE RESULTS

Included below is a sample data table. Your students' data will vary since it will be acquired on a different instrument and possibly with different standard concentrations.

Data Table 1: Absorbances of Samples

Sample	Absorbance (Wavelength = _____)
1 ppm	0.047
5 ppm	0.235
10 ppm	0.476
15 ppm	0.699
20 ppm	0.929
Good Gatorade	0.353
Bad Gatorade	0.620

Regression Analysis	
Regression Equation	y = a + bx
y-intercept (a)	0.004
slope (b)	0.046
correlation coefficient	.9999
number of observations	5

The concentration of FD&C Red #40 in "good" Gatorade Tropical Burst was determined to be approximately 37 ppm, while the "bad" Gatorade had approximately 66 ppm red dye #40.

PLAUSIBLE ANSWERS TO QUESTIONS

1. Why do you think the reference cuvette contained distilled water as a blank? If your unknown used ethanol as the solvent, what would the blank consist of?
   Gatorade is a water-based sports drink. The majority of the remaining components or matrix (minus the red dye), consists of water. In a double-beam instrument, the reference beam takes into account all the other compounds that may be present in the sample (called the matrix). This has the effect of "subtracting" out all these compounds from the sample spectrum. The spectrum can then be considered to represent only the substance of interest. (The assumption here is that the background closely resembles the sample minus the substance of interest, so we assume that distilled water closely resembles Gatorade minus the red dye. This is not exactly true since Gatorade contains other additives such as sugar and flavorings, but for our purposes this is okay.) If ethanol were the solvent, you would put ethanol in the reference cuvette.

2. Justify your choice of wavelength for the analysis of FD&C Red #40.
   Students should choose a wavelength of 500 nm. The wavelength was chosen because it gave the highest absorbance reading; this ensures maximum sensitivity when measuring the absorbance of samples.

3. Does concentration affect absorbance directly or inversely?
   Concentration has a direct affect on the absorbance of a sample. An increase in concentration leads to an increase in absorbance of that wavelength.

4. What was the dilution factor you used to prepare your Gatorade samples at the beginning of this lab?
   1/5 (5.0 mL/25.0 mL)

5. Is the concentration of red dye in the "bad" Gatorade within quality control specifications? How do you know?
   The results of each individual group may vary. However, the results shown above indicate that the "bad" Gatorade had a red dye concentration of 66 ppm which is out of the quality control range of 32-40 ppm.

EXTENSIONS AND VARIATIONS

If time allows, students may make the standards.

REFERENCES

Sawyer, D. T.; Heineman, W. R.; Beebe, M. M. *Chemistry Experiments for Instrumental Methods.* New York: John Wiley & Sons,

Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis.* New York: Saunders College Publishing, 1992.

INFRARED SPECTROSCOPY—Introduction

DESCRIPTION

Students will identify samples of plastic film using reference spectra and an infrared spectrometer.

GOALS FOR THE EXPERIMENT

In this experiment, students will learn to:

- prepare samples for analysis
- compare and identify spectra
- use a polystyrene standard for comparison

STUDENT SKILLS

- collecting data
- interpreting data
- drawing conclusions

INFRARED SPECTROSCOPY—Student Handout

PURPOSE

To use infrared spectroscopy to determine the identity of plastic films.

SCENARIO

You are a chemical technician in a company than utilizes a robot and infrared spectroscopy to sort plastic samples. Periodically, you need to collect infrared spectra of the polymer samples to be sure the robot is sorting them correctly. This ensures that quality control is maintained.

Your supervisor has given you a box filled with different types of plastic film. These samples have already been sorted by the robot; you need to make sure they were identified correctly. You are to use the infrared spectrometer to determine what kind of plastic each sample contains by comparing your spectra to reference spectra in the Sadtler Atlas of Polymer Spectra.

SAFETY, HANDLING, AND DISPOSAL

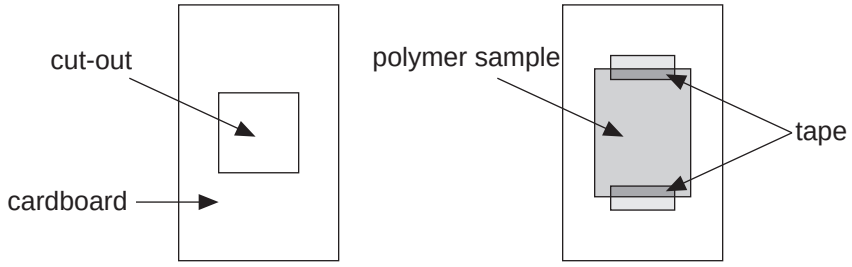
Students must wear goggles at all times when performing this activity.

Do not look at the helium-neon laser (used for alignment in an FT-IR spectrometer); it can damage the eyes.

PROCEDURE

1. Your instructor will provide details on proper use of the infrared spectrometer and how to print out your spectrum after it is recorded.

2. To mount the plastic samples, place the sample over the square cut-out of the paperboard and tape it into place. Make sure the film is stretched smoothly and tightly over the cut-out. Handle the films by the edges only or with forceps.



3. Record an infrared spectrum of each sample.
   Question 1: Look at the IR spectrum of each polymer sample. Do the spectra look the same? What is the difference in each spectrum?

4. Determine what plastic each sample is by comparing the spectrum from each unknown plastic sample to the reference spectra provided to you by your instructor. In the table below, list the code letter of the unknown, brand name and the chemical name for the plastic as listed on the reference spectrum.

Code letter of film	Brand name and specific plastic name

5. Be sure to check with your supervisor (instructor) to see if you correctly identified the plastic sample.
   Question 2: In this experiment, did you perform qualitative or quantitative analyses? Explain.

6. Determine the recycling symbol for each plastic sample and fill in the table below. Then fill in the common uses of this type of plastic from information provided by your instructor.

Code letter of film	Symbol	Type of polymer and use

   Question 3: Can you think of any other applications that a chemical company might use infrared spectroscopy for?

INFRARED SPECTROSCOPY—Instructor Notes

Time Required for Preparation	<30 minutes
Time Required for Procedure	45–60 minutes
Group Size	2–4 students

MATERIALS NEEDED

Getting Ready

- fine point permanent marker
- paperboard or cardstock
- scotch tape
- scissors
- ruler

Procedure

Per group

- infrared spectrometer (FT-IR or dispersive; FT-IR has the major advantage of quick spectral acquisition)
- reference spectra handbook such as the *Sadtler Atlas of Polymer Spectra, Hummel-Scholl Atlas of Polymer & Plastics Analysis,* or the *Coblentz Society's Desk Book of Infrared spectra* (These are just a few suggestions; there are other reference books available.)
- paperboard mount
- tape
- 3-cm x 5-cm pieces of transparent plastic film, including the following:
  - different types of plastic wrap
  - 2-L soda bottles (colorless)
  - plastic bags (freezer bags and/or sandwich bags)
  - transparent lids of yogurt containers (such as Dannon®)
  - roasting bags, if available

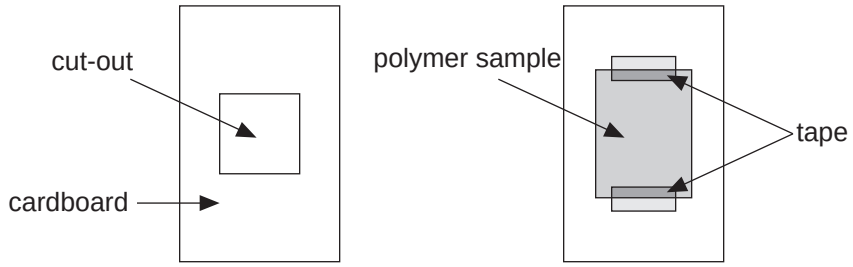
SAFETY, HANDLING, AND DISPOSAL

Goggles should be worn when performing this activity.

Do not touch or breathe on cell window surfaces or samples. Handle the polymer samples with forceps to minimize contamination.

GETTING READY

1. To prepare holder for the film, cut a 5 cm x 8 cm piece of cardboard or paperboard. Cut a window 2.5 cm x 2.5 cm in the center of the holder, as shown below. Be sure this fits into the sample holder of your IR. Alternatively, you could tape a polymer sample over the opening in the sample compartment of the instrument. Just be sure the IR beam goes through the sample and not the tape.



2. Cut the polymer samples into 3-cm x 5-cm squares. Using a permanent marker, label each sample with a unique letter. Be sure to place the label in the upper corner, out of the path of the infrared light beam.

3. Warm up the instrument for at least 5 minutes prior to the start of the experiment.

PRE-LAB DISCUSSION

Infrared Spectroscopy

Discuss with students the basics of infrared spectroscopy, including:

- how to operate the instrument.
- instrumentation (source, detector, grating or interferometer).
- absorption of infrared light, the vibration of bonds that result, and the fact that different types of bonds vibrate at specific and known frequencies (and can be used to identify functional groups).
- a spectrum plots intensity of an absorption versus the frequency of the absorption, reported in wavenumbers (cm^{-1}), which is inversely related to the frequency of the vibration.
- reference spectra and how to compare an unknown spectrum and a reference spectrum.
- the difference between absorbance (amount of light absorbed by the sample) and transmittance (the amount of light passed through a sample).
- although in this experiment infrared spectrometry is used for qualitative analysis, this technique can also be used for quantitative analysis if a standard curve is obtained.
- the many uses of infrared spectrometry: forensic analysis of paint chips at a vehicle accident site, real-time process control in the manufacture of chemicals, quality control (to name just a few).

Polymers

Discuss some basics of organic polymers including:

- the terms mono (one), and poly (many). Mer comes from the Greek word “meros” meaning parts or units. You can then asked students to define monomer and polymer.
- You can simulate polymerization by using pop beads or paper clips. Define one bead or paper clip as a monomer and hook several together to form a chain and represent a polymer.
- show students the structure of a monomer unit such as styrene and the polymer polystyrene.

- the term "polymer" is often used as a synonym for "plastic," but many biological and inorganic molecules are also polymeric. All plastics are polymers, but not all polymers are plastics.
- a homopolymer results from polymerizing only one kind of monomer. A copolymer results from using different kinds of monomers. Homopolymers have the same repeating unit, while copolymers (which can be random, block, or graft) can have different numbers of repeating units.
- One of the largest industries that use polymers is the food container industry. The packaging industry uses codes to identify the type of plastic used for a given package. The codes aid in recycling, since plastics that are similar to each other must be recycled together. There are six specific categories, and a seventh category for "other." See Table 1 for a description of each category.

PROCEDURAL TIPS AND SUGGESTIONS

Students may complete this activity on the same day they complete the UV-Vis activity. A discussion of ultraviolet light can be given to two groups, then each of the two groups can start on one instrument and switch when they have completed the activity.

You may want to encourage students to bring in polymer samples from home prior to the experiment.

SAMPLE RESULTS

Some possible uses of films are listed below in an example of a completed table from Step 6 in the procedure.

PLAUSIBLE ANSWERS TO QUESTIONS

1. Look at the IR spectrum of each polymer sample. Do the spectra look the same? What is the difference in each spectrum?
   No, they do not look alike. The spectra have different absorption bands and these bands occur at different wavenumbers. Since each IR is a fingerprint of a polymer sample, each sample would be expected to have a different IR spectrum, unless there are two identical samples.

2. In this experiment, did you perform quantitative or qualitative analyses? Explain.
   In this experiment, qualitative analysis was performed. Qualitative analysis is defined as the determination of the presence or absence of a particular substance. Quantitative analysis is defined as the determination of the amount of a given substance that is present in the sample.

3. Can you think of any other applications that a chemical company might use infrared spectroscopy for?
   See "Pre-Lab Discussion" section.

REFERENCES

Griffiths, P. R.; de Haseth, J. *A. Fourier Transform Infrared Spectrometry.* New York: John Wiley & Sons, 1986.

Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis.* New York: Saunders College Publishing, 1992.

An excellent reference on FT-IR spectrometry is a series of three articles written by W. D. Perkins:
J. Chem. Educ., 1986, 63(1), A5.
J. Chem. Educ., 1987, 64(11), A269.
J. Chem. Educ., 1987, 64(12), A299.

PROCEDURAL TIPS AND SUGGESTIONS

Students may complete this activity on the same day they complete the UV-Vis activity. A discussion of ultraviolet light can be given to two groups, then each of the two groups can start on one instrument and switch when they have completed the activity.

SAMPLE RESULTS

Some possible uses of films are listed below in an example of a completed table from Step 6 in the procedure.

Table 1

Recycling Symbol	Name of Polymer	Sample Uses
	polyethylene terephthalate	• soft drink bottles • carpets • fiberfill • rope • scouring pads • fabrics • Mylar tape (cassette and computer)
	high density polyethylene	• milk jugs • detergent bottles • bags • plastic lumber • garden furniture • flowerpots • trash cans • signs
	vinyl	• cooking oil bottles • drainage and sewer pipes • tile • bird feeders • institutional furniture • credit cards
	low density polyethylene	• bags • Elmer's® glue bottles and other squeeze bottles • wrapping films • container lids
	polypropylene	• yogurt containers • automobile batteries • bottles • lab equipment • carpets • rope • wrapping films
	polystyrene	• disposable cups and utensils • toys • lighting and signs • construction • foam containers and insulation
	all other polymers	• catsup, snack and other food containers • hand cream, toothpaste, and cosmetic containers