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

#18 Gas Chromatography: Introduction and Application

Patricia D. Sharp, Libertyville High School, Libertyville, IL 60048

INTRODUCTION

Description

This laboratory exercise is designed to introduce a student to gas chromatography using a discovery-based approach. The student is presented with a scenario/industrial application where their job is to verify that the company's mouthwash contains a specific percentage of ethanol. They must discover that ethanol can be detected by gas chromatography and that peak height can be related to percentage of ethanol. This requires preparation of standard ethanol solutions.

A batch of mouthwash is then suspected of isopropyl alcohol contamination and the student must develop a method to determine whether there is contamination. The technique of indirect determination by a standard addition method is introduced here.

Student Audience

Introductory (freshman) chemistry laboratory suitable for advanced placement chemistry and two-year technical college courses.

Goals for the Experiment

- Discover principles of gas chromatography.
- Eliminate the typical "cookbook" laboratory experience.
- Connect laboratory chemicals and "real-life" products.
- Convey some of the excitement of discovery in experimental science.
- Require some creative thinking from students.

Recommended Placement in the Curriculum

Introductory (freshman) college chemistry or advanced placement chemistry following discussion of mixture separation techniques.

Student Handout

Gas Chromatography: Introduction and Application

Introduction

Chromatography encompasses a diverse group of separation methods that are of great importance to the analytical chemist, for they enable a mixture to be separated, isolated, and identified that may otherwise have been difficult to resolve. This experiment is an introduction to gas chromatography (GC), which is the technique of choice for the separation of thermally stable and volatile organic and inorganic compounds. The GC consists of four components: a sample injector, a column, a mobile phase, and a detector. In gas-liquid chromatography (GLC) the separation is accomplished by partitioning the components of a chemical mixture between a moving (mobile) gas phase and a stationary liquid phase coated on a solid support. Gas-solid chromatography (GSC) uses a solid adsorbent as the stationary phase. The availability of versatile and specific detectors and the possibility of coupling the gas chromatograph to a mass spectrometer or an infrared spectrophotometer further enhance the usefulness of gas chromatography.

In GLC, separation occurs in a column packed with an inert solid that serves as a support for the liquid coating. Samples dissolved in a solution are introduced by injection through a self-sealing silicone rubber diaphragm called a septum by means of a hypodermic syringe. The injection port is heated to a temperature above the boiling point of the sample so that the sample vaporizes rapidly. This allows the sample to be introduced as a gas at the head of the column. Those components that have a finite solubility in the stationary liquid phase distribute themselves between this phase and the gas according to the law of chemical equilibrium. Elution is then accomplished by forcing an inert gas, such as nitrogen or helium, through the column. There are two types of columns employed in gas chromatography: capillary and packed. The capillary types are fabricated from capillary tubing, the inside of the tubing being coated with a very thin film of the liquid phase. Packed columns have larger capacities than capillary columns and consist of a glass or metal tube that can be folded or coiled to fit into an oven. A variety of solid supports are used, including finely ground fire brick and diatomaceous earth. The liquid coatings are nonvolatile, inert compounds such as polyglycols, Carbowax 1000, silicone oils, and phthalate esters. In this laboratory investigation, GLC will be used with a packed column.

The detector used in GC must sense the presence of a minute quantity of an eluted solute in a much larger amount of the carrier gas. A thermal conductivity detector (TCD) will be used here, where changes in the thermal conductivity of the gas stream are sensed by changes in the electrical conductivity of a heated filament or a suitably located thermistor. The thermal conductivity of the carrier gas is roughly six to ten times greater than that of most organic compounds. Thus the presence of even small amounts of organic materials causes a relatively large decrease in the thermal conductivity of the column effluent. The filament retains more heat, its temperature rises, and its electrical resistance goes up. The resulting conductivity change can be exhibited on a chart recorder or a computer screen as a function of time. Retention time data can be used for the identification of components of mixtures, but a number of variables need to be controlled in order to yield reproducible retention times. GC provides an excellent means of confirming the presence of a suspected compound in a mixture, provided a sample of the pure compound is available for comparison. No new peaks in the chromatogram of the mixture should appear upon addition of the pure compound, and enhancement of one of the existing peaks

should be observed. Quantitative analysis for a component in a mixture can be achieved by measurement of peak area or in the case of tailing, measuring peak height.

Purpose

- To learn the operation of a gas chromatograph
- To learn microliter syringe injection techniques
- To discover the use of GC in analysis of mixtures
- To discover that modern analysis can be carried out on very small samples
- To quantify ethanol in a mixture
- To discover quality control technician responsibilities in an industrial application

Equipment

Instrument: Gow-Mac Gas Chromatograph Series 150 equipped with Thermal Conductivity Detector
Column: $^1/_4$-inch x 4-inches DC 200
Column Temperature: 63°C
Carrier: Helium at 20 mL/min

Materials

200 proof ethanol, isopropyl alcohol, Equate Mint mouthwash, 10-uL syringe

Safety

Eye protection should be worn at all times. Do not set flammable liquids on top of the gas chromatograph. Exercise caution handling the syringe. Do not touch the heated injection and detection zones on the instrument. *They are hot.* All organic liquids used in this experiment are to be considered harmful. Review the MSDSs. Avoid ingestion of liquid or inhalation of vapors.

Background Information

The instructor will conduct an introduction to the instrument indicating temperature setting, injection procedure, carrier gas flow rate adjustment, and use of the detector.

Syringe Technique: In filling the syringe, it is desirable to exclude all the air initially by repeatedly drawing liquid into the syringe and rapidly expelling it into the liquid. Slowly draw up about twice as much liquid into the syringe as you plan to use.

The following should be done to adjust the volume of liquid in the syringe: Hold the syringe vertically with the needle pointing up. Put the needle through a tissue so that the liquid that is expelled will be absorbed by it. Any air in the syringe should go to the top of the barrel. Push the plunger until it reads the desired value, expelling the excess air at the same time. Wipe off the needle with the tissue. Draw some air into the syringe which will mark the adjusted retention time on the chromatogram and prevent any liquid from being expelled if the plunger is pushed accidentally. Hold the syringe with two hands. The left hand is to guide the needle and prevent bending while going into the septum. The other hand pushes the barrel while the right thumb prevents the plunger from being expelled by the gas pressure. To inject, insert the needle through the septum and as far into the injection port as possible, depress the plunger, hesitate a second or two, then withdraw the needle as rapidly and as smoothly as possible while keeping the plunger depressed. Always rinse the syringe three times and discard rinse liquid when changing to a new liquid.

Procedure

Scenario/Industrial Application:

You are employed by the Equate Mouthwash Company and your job as a quality control technician is to verify that each batch of Equate Mint Mouthwash matches product specifications for percent ethanol content, which is 18.9%. You have available a standard Equate Mint Mouthwash sample for comparison. You are to develop a protocol that will permit you to quickly test each batch for % ethanol using gas chromatography.

1. Inject 3 uL of 100% ethanol into the column.
   a. How many peaks do you see?
   b. Is the peak resolved on the chart paper or does it extend beyond it?
   c. What was the retention time, t_r?

2. Inject 3 uL of Equate Mouthwash.
   a. How many peaks are there?
   b. Is the shape of the peak the same as the 100% ethanol? Why or why not?
   c. What was the retention time, t_r?

3. Prepare an 80% ethanol solution and inject 3 uL into the column.
   a. Is the retention time and peak height the same as the 100% ethanol?
   b. How can you quantitatively determine the amount of ethanol in the mouthwash?

4. Prepare standards of 60%, 40%, 20% and 10% ethanol in water. Inject 3 uL of each. Wait until the recorder returns to baseline before injecting a new sample.

5. Prepare a graph of % ethanol versus peak height and draw a best fit straight line or use a spreadsheet and regression analysis to obtain an equation relating these two.

6. Determine the % ethanol in the sample of Equate mouthwash. Percent ethanol is expected to remain within 2% of company specifications.

MEMO July 26

To: Equate Mouthwash Company Purchasing Department
From: Liberty Chemical Company

We have just become aware that some of our current ethanol stock is found to contain contamination of isopropyl alcohol. The questionable shipments would have been shipped to Equate Mouthwash Company beginning on July 20.

MEMO:

To: Quality Control Technicians
From: Purchasing Department

Did we ship any Equate Mouthwash that was contaminated with isopropyl alcohol? Will a product recall be required? Develop a method that will check our batches prepared since July 20 for possible contamination with isopropyl alcohol.

7. You are a quality control technician. What will you do?

8. Inject 3 uL of isopropyl alcohol.
   a. What is its retention time, t_r?
   b. Can the ethanol and isopropyl alcohol peaks be resolved? How can you determine this?

9. Inject a mixture of ethanol and isopropyl alcohol.

10. Inject a sample of Equate Mint Mouthwash manufactured on July 21 and one made today.
    a. How can you tell whether the mouthwash was contaminated with isopropyl alcohol?
    b. Are you certain of the results?

11. Since there was not an obvious peak for today's batch, you need to do a standard addition method for an indirect determination of isopropyl alcohol. This involves adding a known amount of isopropyl alcohol to a known amount of ethanol and getting its chromatogram, then adding that **same** amount of isopropyl alcohol to the questionable mouthwash and getting its chromatogram. Determine the area of both isopropyl alcohol peaks by cutting them out and massing on an analytical balance. If the questionable mouthwash has a greater isopropyl alcohol peak mass than the isopropyl alcohol peak mass from the known sample then there was isopropyl alcohol contamination in the mouthwash.
    a. Did today's sample contain isopropyl alcohol contamination?
    b. Are you certain of the results?

Questions

1. Explain what is meant by retention time of a substance.

2. Why can chromatography be used to separate constituents of a chemical mixture?

3. Describe how gas chromatography separates mixtures.

4. What does volatile mean?

5. What other commercial products might you expect to be able to analyze using gas chromatography?

References

Grob, Robert L. *Modern Practice of Gas Chromatography*, 3rd Ed., John Wiley & Sons, New York, New York, 1995, p 33-34.

Willard, H. H., Merritt, L. L., Jr., Dean, J. A., and Settle, F. A. *Instrumental Methods of Analysis*, 7th Ed., Wadsworth Publishing Co., Belmont, CA, 1988, p 513-515.

Van Atta, R. E.; Van Atta, R. L. *J. Chem. Educ.* **1980**, *57*, 230-231.

Leary, J. J. *J. Chem. Educ.* **1983**, *60*, 675.

Burns, D. S.; Berka, L. H.; Kildahl, N. *J. Chem. Educ.* **1993,** *70,* A100-102.

Instructor Notes

Gas Chromatography: Introduction and Application

Time Required:

one 3-hour laboratory period

Group Size

With three gas chromatographs, a section of 30 students can be divided into six groups of five students. Two groups can work on one gas chromatograph. Each student in the group should perform all aspects of the procedure.

Materials Needed

200 proof ethanol, isopropyl alcohol, Equate Mint mouthwash (purchased from Wal-Mart) or any mouthwash containing ethanol, 10-uL syringe, sample of Equate labeled July 21 prepared to contain 1.0 mL isopropyl alcohol in 10.0 mL Equate, sample of Equate "made today" contains only Equate.

Equipment Needed

Instrument: Gow-Mac Gas Chromatograph Series 150 equipped with Thermal Conductivity Detector
Column: $^1/_4$-inch x 4-inches DC 200
Column Temperature: 63°C
Carrier: Helium at 20 mL/min

Safety, Handling, and Disposal

Eye protection should be worn at all times. Do not set flammable liquids on top of the gas chromatograph. Exercise caution handling the syringe. Do not touch the heated injection and detection zones on the instrument. They are hot. All organic liquids used in this experiment are to be considered harmful. Avoid ingestion of liquid or inhalation of vapors. Dispose of used reagents according to local ordinances.

Points to Cover in Pre-Lab

Introduce the operation of a gas chromatograph. Demonstrate proper syringe technique and injection. Discuss the concept of retention time. Tell students that, for a given column and set of run conditions, retention time is characteristic of a substance and can be used to identify it. Present the industrial application/scenario to the students by discussing the students' position at the Equate Mouthwash Company as quality control technicians. Their role is to monitor the percentage of ethanol in each batch of mouthwash produced and to trouble shoot for the company.

Procedural Tips and Suggestions

Encourage students to recognize the "real-life" application that laboratory experiments have to future workplace experience. The student handout can be passed out in segments. Once the student has given the instructor satisfactory answers to the proposed questions, another part of the laboratory can be issued. A convenient break for interacting with the instructor comes after procedure number 3, number 6, number 7, number 8, and number 10. Warn of the necessity to make accurate dilutions of standard solutions to obtain reliable results.

Plausible Answers to Procedure Questions

1. a. one peak for 100% ethanol

   b. adjust the attenuation or the size of sample injected if necessary

   c. determine from chart speed and adjust from air peak on chart

2. a. one peak for Equate mouthwash (Figure 1 is sample chromatogram)

b. the peak is not the same, it has considerable tailing caused from presence of water.

   c. same retention time as for 100% ethanol

3. a. same retention time as for 100% ethanol, but peak height is lower

   b. compare peak height to % ethanol; the tailing due to presence of water prevents easy determination of peak area and using it quantitatively.

5. See Figure 2 for graph of Peak Height vs. % Ethanol.

7. Inject isopropyl alcohol and get its retention time.

8. a. determine from chart speed and adjust from air peak on chart

   b. the ethanol and isopropyl alcohol peaks can be resolved because they have different retention times. Inject an ethanol isopropyl alcohol mixture.

10. Figure 3 shows chromatogram for ethanol-isopropyl alcohol mixture.

    a. look for an isopropyl alcohol peak in addition to the ethanol peak. The July 21 alcohol sample will contain isopropyl alcohol. The "made today" sample will not contain isopropyl

    b. the student cannot be sure that IPA is not present. They must do an indirect determination for IPA using the standard addition method. Note: There will be a point where IPA may be present but the concentration is so low that is not detectable by GC. (0.5 pph IPA cannot be detected)

11. Figure 4 shows chromatogram for Equate Mouthwash manufactured July 21.

    a. no obvious contamination of IPA

    b. cannot be certain that IPA is not present because very low concentrations of IPA will not be detectable by GC.

Plausible Answers to Laboratory Questions:

1. Retention time is the time required for the maximum concentration of solute to pass through the column and reach the detector. The retention behavior reflects the partitioning of a solute between the mobile phase and stationary phase.
2. Chromatography can be used to separate constituents of a chemical mixture because not all components of a mixture interact with the stationary phase and mobile phase in the same way.
3. In gas chromatography, a mixture of volatile substances is sent through a long column packed with a stationary material which attracts each substance to a different extent, depending upon the structure of the substance. The more strongly a substance is attracted to the column packing, the longer it takes to pass through the column.
4. The substances must vaporize quickly.

5. Other commercial products that could be analyzed using GC are alcoholic beverages, lighter fluid, household cleaning solvents, etc.

Extensions and Variations:

1. Other mixtures that could be used in this scenario are: analysis of % ethanol in whiskey, analysis of lighter fluid, analysis of a household cleaning solvent.
2. If time constraints are a problem, procedure #11 on indirect determination by standard addition method can be eliminated.

References:

Grob, Robert L. *Modern Practice of Gas Chromatography*, 3rd Ed., John Wiley & Sons, New York, New York, 1995, p.33-34.

Willard, H. H., Merritt, L. L., Jr., Dean, J. A., and Settle, F. A. *Instrumental Methods of Analysis*, 7th Ed., Wadsworth Publishing Co., Belmont, CA, 1988, p.513-515.

Van Atta, R. E.; Van Atta, R. L. *J. Chem. Educ.* **1980**, *57*, 230-231.

Leary, J. J.*J. Chem. Educ.* **1983**, *60*, 675.

Burns, D. S.; Berka, L. H.; Kildahl, N. *J. Chem. Educ.* **1993**, *70*, A100-102.

Sample Laboratory Results

Figure 1: Chromatogram for Equate Mouthwash

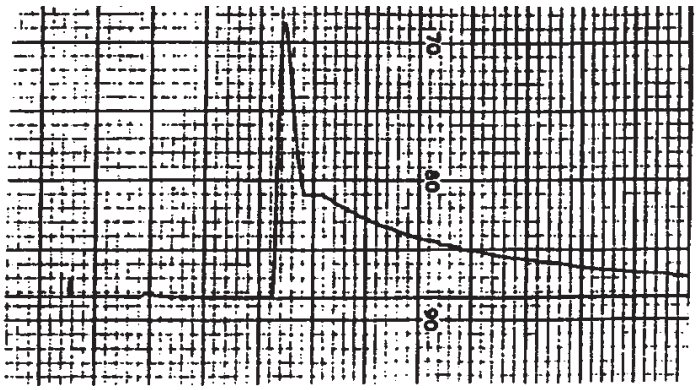

Figure 2: Graph of Peak Height vs. % Ethanol

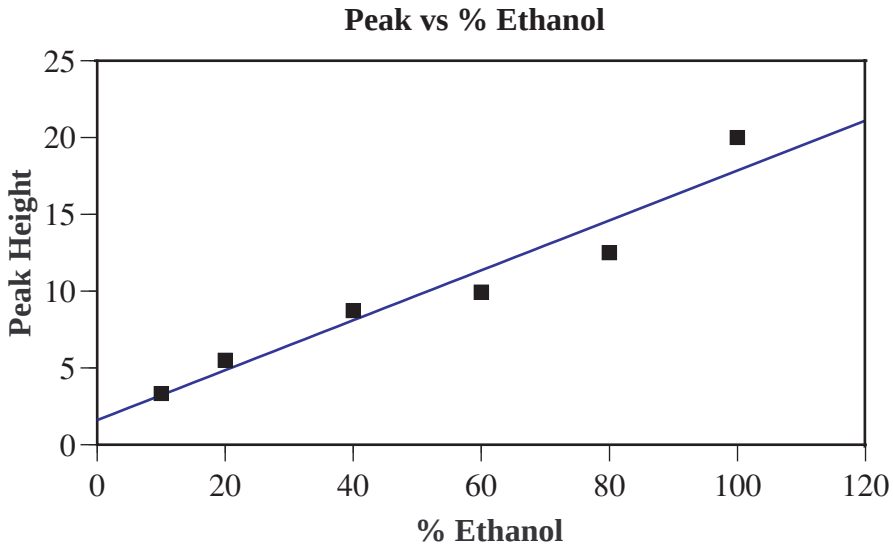

Figure 3 Chromatogram for Ethanol-Isopropyl Alcohol Mixture

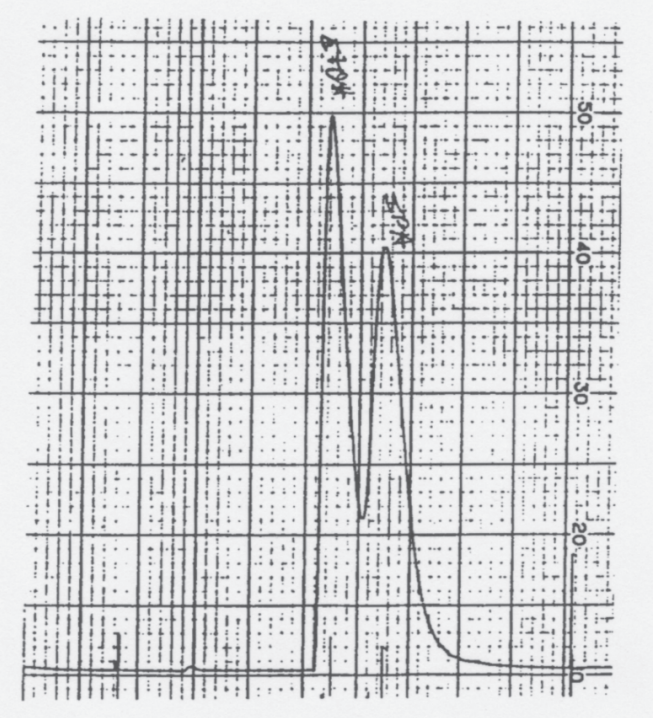

Figure 4 Chromatogram for Equate Mouthwash Manufactured July 21