Gas Chromatography: Identifying Unknown Compounds

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Last Update: May 12, 2016

Introduction

Most materials that we encounter are mixtures, and this includes food, gasoline, car tires, sea water, animals, and the list goes on and on. Often we want to determine the composition of a mixture, and a common approach is to separate the components of the mixture. We may want to know qualitatively what is present in a sample, or we may want to quantify the amount of salt or protein or residual pesticide in a food. Perhaps we want to obtain gasoline from petroleum.

How can we separate the components of a mixture? If we want to obtain millions of gallons of gasoline, we use huge distillation towers in a refinery where the components of petroleum are separated into fractions such as gasoline that have a narrower range of boiling points. We can also do distillation in a chemistry laboratory where we might take milliliters of a mixture and distill it, again separating components based on their boiling points. The boiling point and the vapor pressure of a substance depend on the strength of intermolecular forces within the liquid. When we distill a liquid, we must heat the sample until the random kinetic motion of the molecules can overcome the attractive intermolecular forces holding molecules together at the surface of the liquid.

Another technique that often depends on the strength of intermolecular forces is chromatography. Sample sizes for chromatography range from 0.1 μL up to mL or even L, but generally chromatography allows one to use much smaller samples than for distillation. There are many different types of chromatography, and it is applied in many fields. Biochemists use liquid chromatography to separate proteins. Chemists use gas chromatography (GC), thin layer chromatography (TLC), and high-pressure liquid chromatography (HPLC) to identify organic compounds. Forensic scientists and other specialties use gas chromatography for drug tests, toxin screens, and environmental analysis.

The effect of intermolecular forces can be different in distillation and chromatography. In chromatography there is a stationary phase which is a solid or a liquid which coats a solid. The mobile phase sweeps past the stationary phase, and the mobile phase is a gas or a liquid. When a component is introduced into the mobile phase over the stationary phase, the component experiences an attraction to stay in the mobile phase, but there is also an attraction to the stationary phase. The distribution of molecules of a component between the mobile and stationary phases depends on the relative strengths of intermolecular forces between a molecule and the mobile phase as compared to the intermolecular forces between a molecule and the stationary phase. As the mobile phase moves, it drags the sample components along as well. Those sample components that interact only weakly with the stationary phase will move more quickly to the end of the chromatography device, while components that interact more strongly with the stationary phase will take a longer time to reach the end. As a result, individual components of the mixture reach the end of the device one component at a time (ideally).

The Vernier Mini GC uses a 11-meter-long steel column (inner diameter 0.53 mm) which is coated with a nonpolar silicone polymer that serves as the stationary phase. A sample, consisting of one or more compounds, is injected into the column and is carried by atmospheric air, which is the mobile phase. Organic compounds flowing out of the chromatography column are indicated as a peak on a chromatograph, as shown in Figure 1. The unique time it takes for a specific compound to exit the column after it is injected is called the *retention time*. Using a GC, a compound may be identified from a mixture of compounds by its retention time, but it is easier to prove the absence of a compound than its presence, as discussed below.

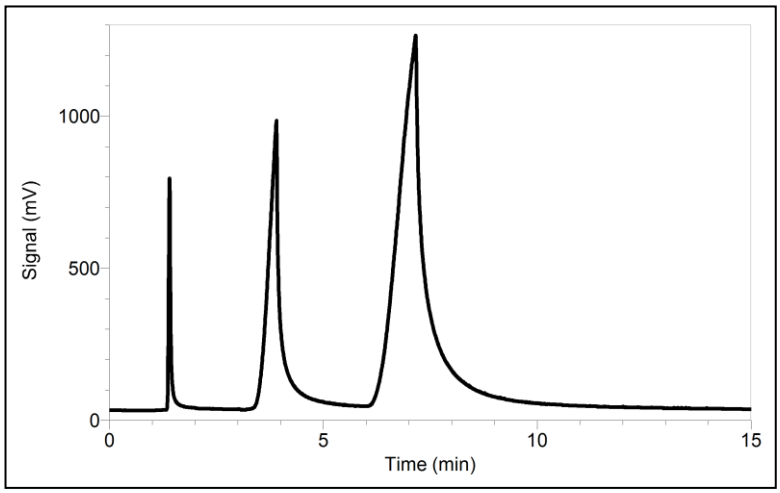

Figure 1 Sample gas chromatogram

An important intermolecular force between molecules of ethanol is hydrogen bonding, and the boiling point is 78 °C. But if ethanol is put into a gas chromatography column, we must consider intermolecular forces between ethanol and the gas of the mobile phase, and intermolecular forces between ethanol and the stationary phase material. There are probably negligible interactions between ethanol and gas phase molecules (N_2 or He or H_2) since the density of gas molecules is low. We can focus on the interaction of ethanol with a nonpolar stationary phase which occurs through London dispersion and dipole-induced dipole forces. Another liquid with a similar boiling point is ethyl acetate which boils at 77 °C, and it is difficult to separate ethanol from ethyl acetate by distillation. But these two substances can be separated easily by gas chromatography since the dominant interaction of both molecules with a nonpolar stationary phase is by London dispersion forces which are much larger for the larger, more polarizable ethyl acetate molecule. (See Petrucci *et al*., *General Chemistry: Principles and Modern Applications*, 10th ed., ©2011, §12-1 for more information.) Thus ethyl acetate molecules spend more time than ethanol molecules adsorbed on the stationary phase, and ethanol molecules spend more time in the mobile phase which sweeps them to the detector quicker. Unfortunately it is also possible that two substances with different boiling points such as ethanol and acetone can have similar retention times.

In this experiment you will explore the process of identifying one or more unknown species using gas chromatography. First, you will practice using a gas chromatograph by testing several known substances. You will then use this information to identify the substances present in an unknown mixture.

SAFETY

- Keep the chemicals away from open flames or sparks since they are flammable.
- Glacial acetic acid is a strong irritant to the nose, skin, and eyes. Wash off immediately!

MATERIALS

Computer with Logger Pro 3 (version 3.8 or newer) installed
Vernier Mini GC
1 μL glass syringe
Kimwipes® or lint-free tissue
Chemicals listed below and an unknown mixture

PRE-LAB EXERCISE

The information below is a common starting point for understanding retention times of substances.

Compound	Formula	Boiling point (°C)	Molar mass (g/mol)
Acetone	CH_3COCH_3	56	58.080
2-Butanone	$CH_3COCH_2CH_3$	80	72.107
2-Pentanone	$CH_3COCH_2CH_2CH_3$	102	86.134
4-Methyl-2-pentanone	$CH_3COCH_2CH(CH_3)_2$	117	100.161
2-Heptanone	$CH_3COCH_2CH_2CH_2CH_2CH_3$	151	114.188
Methanol	CH_3OH	65	32.042
Ethanol	CH_3CH_2OH	78	46.069
Ethyl acetate	$CH_3COOCH_2CH_3$	77	88.106
Acetic acid	CH_3COOH	118	60.052

1. Draw Lewis electron dot structures for the compounds above.

2. Predict the order in which these compounds will exit the GC column (known as *elution order*). Rate the compound you think will exit the column first with the number 1; the last compound to exit the column is given the highest number. Your predicted order might be guided by molar mass, a combination of boiling point and molar mass, or calculated polarizability.

3. Read the directions for care of the syringe by going to
http://www.vernier.com/files/manuals/gc-mini.pdf
and scroll down to **Appendix C: Syringe Usage Instructions**
or go to U:_SC Student File Area\Pultz\ and read the file called
GasChromatographyInvestigationsWithTheMiniGC_AppendixA-SyringeUsageAndCare.

a. Never pull the plunger on the GC syringe back more than ___ % of its total volume.

b. Once the plunger has been pulled out, what happens?

c. Depressing the plunger too rapidly can cause the plunger to _____.

d. Where should you hold the plunger and why?

e. In which direction should you wipe the needle?

PROCEDURE

1. Wear goggles. Work in a well-ventilated room.

2. Obtain a glass syringe and a set of vials containing the known substances and one unknown mixture containing three to five substances to be identified. You will not only test acetone but use it to clean the syringe needle. CAUTION: *Handle the glacial acetic acid with care. It can cause painful burns if it comes in contact with the skin.*

3. Prepare the Vernier Mini GC for data collection.
   a. Turn on the Mini GC. (*The order for steps a and b is unimportant.*)
   b. Connect the Mini GC to the USB port on your computer using a USB cable.
   c. Start Logger Pro and choose New from the File menu.
   d. Click Collect in Logger Pro to bring up the Temperature-Pressure profile.
   e. Set the Temperature-Pressure values to:

Start temperature	35°C
Hold time	1 min
Ramp rate	10°C/min
Final temperature	65°C
Hold time	6 min
Total length	10.0 min
Pressure	4.0 kPa

   f. Select Done to initiate the Mini GC warm up and continue to step 4. The computer will display "Do not inject until GC is ready", the LED on the GC will be red, and the GC display will read "GC Not Ready". The GC will take a few minutes to warm up and stabilize. When the GC is ready for injection in Step 6, the computer will show "Inject and click Collect simultaneously", the LED should turn green, and the GC display will cycle to show "Ready to Inject".

4. Follow the steps below to clean and flush the syringe with acetone. **Important**: The glass syringe is fragile. Be careful not to bend the needle or bend the plunger. Never pull the plunger back more than 50% of its total volume. Be careful not to bend the plunger as you press it down.
   a. Depress the plunger fully.
   b. Submerge the tip of the syringe needle into the vial of acetone.
   c. Pull back the plunger to fill the barrel about 1/3 full of acetone. Examine the barrel of the syringe and estimate the amount of acetone in the barrel.
   d. Expel the liquid onto a Kimwipe or a paper towel.
   e. Repeat Steps a–d at least two times, until you are comfortable pulling up a liquid into the syringe and measuring the volume in the syringe barrel. Use a Kimwipe® or a paper towel to carefully pat around the tip of the syringe needle.

5. Collect a volume of acetone for injection.
   a. Submerge the tip of the syringe needle into the vial of acetone one last time.
   b. Draw up 0.15 μL of liquid. Record your actual volume; you may need to decrease the volume in order to obtain the sharp peaks shown in Figure 1.
   c. After collecting your sample, gently wipe the needle, from barrel to tip, with a lint-free tissue such as a Kimwipe.

6. Prepare for injection and the start of data collection. It is important for you and your lab partner to divide the tasks in this step. One person will operate the syringe and the other person will operate the computer controls.
   a. The Mini GC should now have reached the correct start temperature and pressure and the LED should be green.
   b. To insert the needle of the syringe into the injection port of the Mini GC, hold the syringe with one hand and steady the needle with your other hand, as shown in Figure 2. Insert the needle into the injection port until the needle stop is fully seated. If the needle sticks, rotate it slightly while inserting. Do not move the plunger yet.
   c. Simultaneously, depress the syringe plunger and select Collect to begin data collection. Pull the needle out of the injection port immediately.

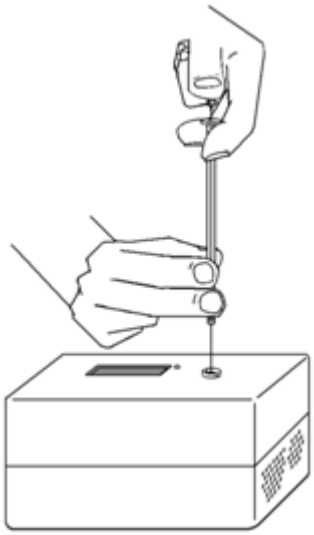

Figure 2

7. While the data collection proceeds, repeat Step 4 to thoroughly clean the syringe and needle with acetone. It may take more than three flushes to feel the syringe plunger move smoothly again, which is your indicator that the syringe and needle are both suitably clean. After rinsing with acetone, rinse with the next chemical to be injected into the GC.

8. The data collection will end after 10 minutes. You may stop the data collection early if you are certain that the entire injected sample has passed through the detector. And you may need to use a longer total length of time for one of the compounds.

9. Save the chromatogram to either a flash drive or to your Y: drive. If you save as a CMBL file type and then open the file on a campus computer or other computer with a recent version of Logger Pro, you will see the chromatogram. Choose Save or Save As from the File menu; if you have already saved a file, clicking the Save button will save over the pre-existing file.

10. Follow the steps below to test the next sample in your set of substances.
    a. Click Collect in Logger *Pro* and make a choice: If you click "Store Latest Run" all of your chromatograms that you have taken will be shown on the same graph by default. If you click "Erase and Continue" you must choose a new file name when you store the next chromatogram since otherwise it will write over your previous data. After making your choice, the Temperature-Pressure profile will appear and it will be the same as for your previous run. Select Done to initiate the Mini GC profile.
    b. While the Mini GC adjusts to its Temperature-Pressure profile, load the syringe with the next sample. Gradually decrease the volume that you use from 0.15 μL to about 0.10 μL as the molar mass of the compound increases. Record the volumes that you inject for each compound. For at least one sample, see what happens when you use different volumes.
    c. After the Mini GC is ready, repeat Steps 6-9 for the next sample.

11. Repeat Step 10 for the remaining pure substances and unknown mixture. For the unknown, you may need to inject 0.3 µL; record the volume that you use. After testing the unknown mixture, you may wish to clear the graph of data. In Logger *Pro*, choose Graph Options from the Options menu. Choose Axes Options. Scroll down the list in the y-axis box and remove the check marks in each of the runs for your known compounds. Leave only the Latest box checked. Click done.

12. After you have completed your final data collection, turn off the Mini GC. You may wish to copy the data into Excel and make graphs. If so, go to the spreadsheet portion of Logger Pro, click on a cell, press the Ctrl key and A key simultaneously to select all, press the Ctrl key and C key simultaneously to copy, and then paste into Excel. Remember that data for the last chromatogram will be in the two columns labelled Latest and data for the first chromatogram will be in the columns labelled Run 1.

DATA TABLE

Compound	Formula	Retention time (min)
Acetone	CH_3COCH_3	
2-Butanone	$CH_3COCH_2CH_3$	
2-Pentanone	$CH_3COCH_2CH_2CH_3$	
4-Methyl-2-pentanone	$CH_3COCH_2CH(CH_3)_2$	
2-Heptanone	$CH_3COCH_2CH_2CH_2CH_2CH_3$	
Methanol	CH_3OH	
Ethanol	CH_3CH_2OH	
Ethyl acetate	$CH_3COOCH_2CH_3$	
Acetic acid	CH_3COOH	

DATA ANALYSIS

Determine retention times for each compound. Decide whether you should use the time when the signal is largest or when the peak begins. Explain your decision.

Discuss the retention times of the substances with regard to molar masses, boiling points, and polarizability. Describe any patterns that emerge and explain both the patterns and deviations.

Report your unknown number and identify the substances that are present in your mixture. Are there more than one possible compound which might give a peak in your unknown? What compounds can you exclude from being in your unknown? Support your identification. In your analysis be guided by the Critical Thinking Rubric condensed from Portfolio version. "Communicates effectively" also includes presentation of data in tables and graphs.

Adapted from http://www.vernier.com/products/sensors/gc-mini/ accessed 16 June 2013
and http://www.vernier.com/files/manuals/gc-mini.pdf accessed 16 June 2013
and Mlsna, Deb and Randall, Jack, Gas Chromatography Investigations with the Mini GC, 1st ed., ©2009 by Seacoast Science, Inc. and Vernier Software & Technology, Experiment # 1
and unpublished experiment by Brian Lamp, "Characterizing Mixtures by Chromatography" June 2012

To install Logger Pro on a personal computer, go to chemistry.truman.edu which morphs into the web address http://www.truman.edu/majors-programs/majors-minors/chemistry-major/ and click on **For Chemistry Students, Faculty, & Staff**
Log in, click on **Installing Logger Pro**, and follow the directions. Thanks to Dr. Brian Lamp for setting up this easy installation method.