

Gas Chromatography and Refractive Index

In today's lab two different analysis methods will be used to determine the composition of a mixture of two esters. The results of the two methods will be compared.

Gas chromatography (GC) is a very fast, repeatable and accurate analysis method for volatile organic materials. GC is most often used for analysis of very small samples of liquid or gas mixtures in an analogous manner to Column Chromatography. In gas chromatography the mobile phase is an inert gas called the carrier gas. The carrier gas is usually nitrogen (N_2) or Helium (He). The stationary phase is a high-boiling (low volatility) liquid coated onto an inert support.

The GC technique is useful for quantitative analysis (identifying how much of a compound is present) and qualitative analysis (determining the identities of compounds present). GC is widely used in industrial, research and analytical laboratories. GC instruments are reasonably priced and flexible in their analysis methodologies. It is not uncommon for an analytical testing center to have 20 or more GC machines operating simultaneously.

GC can also be used on a small scale to separate mixtures into their components and recover them in a way analogous to column chromatography. This time intensive procedure is called preparative scale GC.

Refractive Index (RI) is a method that indirectly measures the change in the velocity of light as it passes through different materials. The refractive index of a material is a physical characteristic of that substance (as are melting points or boiling points). The determination of the RI of a substance can identify an unknown substance or determine the composition of a mixture of materials.

Today both GC and RI will be used to determine the composition of a mixture of ethyl acetate and butyl acetate.

PRE-EXPERIMENT ASSIGNMENT

For GC and RI, study this chapter, and the lecture notes on the Chemistry Department web site.

A student who has prepared for this experiment should be able to:

1. Define and explain: chromatography, gas chromatography (GC), stationary phase, mobile phase, carrier gas, retention time. Explain the

differences between GC and the chromatographic methods you used earlier.

2. Draw a diagram of a gas chromatography apparatus, and label the components of the apparatus in a drawing.
3. Identify and explain the reasons chemists use gas chromatography (to ID compounds in a mixture using retention times, to separate components of small samples, and to determine the amount of given compounds present, for mixtures of volatile compounds only).
4. Explain the general principles behind the RI method.
5. Draw the structure given the name, or give the name from the structure, of the possible unknowns used in the GC portion of the day's experiment.
6. Carry out the experiment in a safe manner.

Quizzes given after the experiment has been performed may also include:

8. Explain and predict the effects of experimental variables such as flow rate, column temperature, and column length have on retention time and resolution of peaks.
9. Read a refractometer scale accurately.
10. Be able to properly correct a sample RI for water offset.
11. If given the literature RI values for two pure compounds and the corrected RI value for a mixture of these two compounds, calculate the composition of the mixture.
12. If given the boiling points of pure materials and a GC of a mixture of these compounds be able to identify which peak belongs to each material.
13. Be able to correctly interpret a GC trace. Identify each peak according to retention time (RT) and be able to calculate or read the percent composition for each material.
14. If given a GC trace without integrated values, be able to calculate the area of each peak and the percent composition of each

Safety Precautions

GC syringes are sharp and can easily prick skin. If a syringe is left too long in the injection port without depressing plunger, the liquid will volatilize and expand, shooting the plunger out at an unsuspecting student. The injection port is hot and can easily burn skin. Be sure to wear goggles while operating GC.

The organic esters have a moderately low toxicity and a high flammability.

The experiment

Your instructor will describe how a Refractometer works, how to properly read the RI scale, how a GC works, how to operate a GC and

how to interpret GC results. A problem set will be distributed to work on in-class to hone your newly acquired interpretative skills.

You will be given a sample containing a mixture of ethyl acetate and butyl acetate.

The sample will be analyzed by RI and GC. The detailed procedure for each analysis is given below. A comparison of the composition as determined by the two methods will be made.

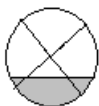
Be very careful not to bend or break the GC syringe. These are fragile and expensive. Record the GC number and instrument operating conditions (column temperature, injector temperature, detector temperature, column type, flow rate, and sample size) in your laboratory notebook.

Abbe Refractometer Operating procedure

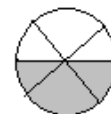
Measure the index of refraction for deionized water and for your distillate using an Abbe refractometer as described below. The procedure will be demonstrated in the lab.

1. Open the plate by moving the illuminating prism arm to the left.
2. Clean the prism plates with a few drops of ethanol and wipe with a Kim-wipe. Let air dry for a few moments.
3. Add two drops of your sample (water if obtaining your offset) and close the prism plates.
5. Turn on the light by moving the switch to the center position. The switch is located on the left rear side of the unit.
6. Swing lamp arm up to shine in prism.
7. Look through the eye piece and simultaneously turn the large dial on the right side rear until a "horizon line" appears (A light upper half over a

dark lower half.)



8. Center this horizon in the exact middle of the crosshairs.
9. If the "horizon line" is fuzzy or colored, turn the chromatic adjustment wheel on the front of the unit until the line is sharp and clear. Repeat step 7 to ensure horizon is centered.
10. Press and hold the switch (located on left rear) to the downward position. A scale will appear. Read and record value. Ignore the lower scale. The value should be read to **4** decimal places.
11. Pull the switch up to shut off the meter.
12. Clean the prism with ethanol and a Kim-wipe. Throw Kim-wipe in trash.



Determine a correction for the refractometer by subtracting the value that you measured for water from the literature value for water (1.3330).

Pay attention to the sign. Add this correction to the value that you measured for your distillate. This is the corrected index of refraction for your unknown.

Using the known RI values for ethyl acetate and butyl acetate, determine the composition of the unknown mixture.

GOW-MAC GC Operating procedure

1. Carefully rinse syringe 2-3 times with acetone by pulling up approximately 5 μl of acetone and dispensing on Kim wipe.
2. Carefully rise syringe 2-3 times with sample by pulling up approximately 5 μl of sample and dispensing on Kim wipe.
3. Carefully pull up 5 μl of sample into syringe.
4. Being careful to keep syringe level and perpendicular, insert needle into port A. Do not bend needle. Insert syringe until glass barrel almost touches metal port ring. Depress plunger then immediately pull syringe out and hit "START" on integrator.
5. Wait until all desired peaks have eluted. (The next person can clean and load syringe while previous sample is coming off column.)
6. When all peaks have eluted, press "STOP" on integrator. Write name by chromatograph. After everyone has gone, the chromatograms will be cut up and distributed.
(Do not pull or tear paper. This causes the integrator to jam).
7. The next person can inject their sample.
8. The last person to inject sample, be sure to rinse the syringe 2-3 times with acetone before returning to Styrofoam tray.

Vernier mini-GC Procedure

1. Ensure the lap top has an installed version of *Logger Pro 3*. If not, place CD containing *LoggerPro* in lap top and install. This takes less than 4 minutes. This works on both PC and Mac.

Important: The glass syringe is fragile and can be easily damaged. Be careful not to bend the needle or bend the plunger. If the plunger is accidentally pulled out of the glass barrel, reinserting it is extremely difficult, sometimes impossible.

2. Prepare the Vernier Mini GC for data collection.
 - a. Turn on the Mini GC. The switch is on left side of machine.
 - b. Connect the USB cable of the Mini GC to the USB port on the laptop.

- c. Start the data-collection program,
 - d. Choose New from the File menu.
3. Click Collect in *Logger Pro* (This is the **large green button** on the right side of the header) to bring up the Temperature-Pressure profile.
Set the Temperature-Pressure values to:

Start temperature	80°C
Hold time	0 min
Ramp rate	50°C/min
Final temperature	90°C
Hold time	0 min
Total length	2.0 min
Pressure	9.0 kPa

Select “Done” to initiate the Mini GC warm up. Note: A new message will appear, “Do not inject until GC is ready,” and the LED on the Mini GC is red. The Mini GC will take a few minutes to warm up and stabilize. When the Mini GC is ready for injection in Step 6, the message will read, “Inject and select Collect simultaneously,” and the LED will turn to green. Continue with Steps 4 and 5 during warm up.

4. Follow the steps below to clean and flush the syringe with sample.

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 sample vial.
 - c. Pull back the plunger to fill the barrel about 1/3 full of sample. 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 more 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. Fill syringe with 0.3 μL of the sample to be injected into Mini GC. Make this measurement as accurately as possible; it will make your

analysis much easier if the injection volumes are the same for each sample. Use a Kimwipe to gently wipe the needle from barrel to tip.

6. Prepare for injection and the start of data collection. The collect button needs to be depressed immediately after injection. It is a good idea to ask another student to assist. One person will operate the syringe and the other person will operate the computer controls.

- a. When the Mini GC has reached the correct start temperature and pressure, the message reads, "Do not inject until GC is ready" and the LED on the Mini GC is 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. Insert the needle into the injection port until the needle stop is fully seated, as shown in Figure 2. If the needle sticks, rotate it slightly while inserting. Do not force. Do not move the plunger yet.

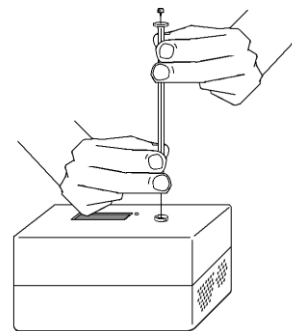


Figure 2

- c. Simultaneously, depress the syringe plunger and select Collect to begin data collection. Pull the needle out of the injection port immediately.

7. While the data collection proceeds, repeat Steps 4 and 5 to thoroughly flush the syringe and needle with the next sample. 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.

8. Data collection will end after 2 minutes. Observe the graphed data that describe the chromatogram for this mixture. Note the two peaks on the graph. At this point in your experiment, the identity of each peak may not be evident, but it can be helpful to speculate about the order in which the compounds elute through the Mini GC. (Remember, GC separation is based on boiling points)

9. Choose Peak Integration from the Analyze menu.

- a. Select and integrate your peaks. To do this, drag from a little before the left most peak, to just after the end of left most peak that includes all of the peak. Then choose Add.

- b. Analyze the other peak on the same graph, by repeating Steps a.

- c. When you are finished with all of the peaks, select OK.

- d Record the peak number, name, retention area, total area, and calculated peak area percent in a data table in your lab notebook. The data file may be printed or saved to a portable drive.

10. Either return to step 3 to proceed to next sample or proceed to step 11 to turn off Mini GC.

11. Rinse syringe three times with acetone. When finished using the mini-GC, simply turn the mini-GC off with the rocker switch on the left side. Disconnect the cords, wrap neatly and replace all pieces in carrying case.

POST-EXPERIMENT ASSIGNMENT

Complete data sheet. Turn in data sheet and white notebook pages. Prepare for the upcoming quiz on Gas Chromatography and Refractive Index.

Revised January 27, 2012, S. L. Weaver