GAS CHROMATOGRAPHY, RAMAN SPECTROSCOPY, & ESTERIFICATION

OBJECTIVE

A miniature gas chromatograph (GC) will be used to separate and analyze a mixture of ethanol, acetic acid, and ethyl acetate. Each compound in the mixture will be identified by comparing the chromatogram & Raman spectrum of the mixture to chromatograms and Raman spectra of individual compounds. Students will run the chromatographic separation 3 times to find the parameters for a good separation of the compounds. Once these optimal parameters are found, a calibration curve will be created by injecting standard solutions of ethanol, acetic acid, and ethyl acetate. Finally, the progress of the esterification reaction between ethanol and acetic acid to synthesize ethyl acetate will be monitored by GC and Raman.

INTRODUCTION

Raman spectroscopy was introduced in a previous lab. Please review that material.

Gas chromatography is a common analytical technique frequently used to separate complex mixtures and, just like liquid chromatography, consists of a **stationary phase** and a **mobile phase**. However, gas chromatography differs from liquid chromatography in that the stationary phase is chemically adhered to the walls of the column and the mobile phase is a gas instead of a liquid. Columns are made of fused silica and have a very small diameter, usually less than 1 mm. As compounds flow through the column they **adsorb** onto the surface of the stationary phase. (Compounds do not absorb into the interior of the stationary phase like a sponge, instead compounds just sit on the surface.) Once the compounds **desorb** from the stationary phase they are carried further down the column by the mobile phase.

Compounds are separated by intermolecular interactions between the compound and the stationary phase. If one compound is more attracted by the stationary phase than other compounds, then it stays adsorbed on the walls of the column for a longer period of time than the rest of the compounds. The other compounds then move down the column with the mobile phase; the adsorbed compound comes out of the column later. Compounds come out of the column and enter the chemical detector at the end of the column which will produce an electrical signal for each compound that is proportional to the concentration of the compound. Figure 1 shows a sample chromatogram with three peaks, one for each compound found in the sample.

To identify peaks on a chromatogram chemists refer to a peak's retention time. **Retention time** is the time, in minutes, at which the highest point of the peak appears. If a compound is not adsorbed onto the walls of the column at all it will exit the column with the very first part of the mobile phase immediately after injection. The compound is not held back or, retained, at all and so has a retention time of 0 minutes. If a compound comes out of the column 1 minute after injection, it will have a retention time of 1 minute and so on for each compound in the sample.

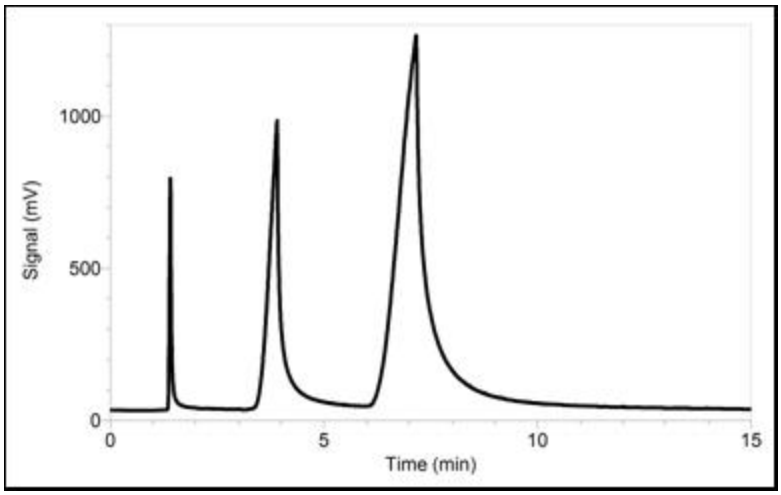


Figure 1: Sample Chromatogram of three compounds.

For example, in Figure 1, the first compound has a retention time of approximately 1.4 minutes, the second is at 3.9 minutes, and the third is at 7.2 minutes. If the sample in Figure 1 was ran again, each peak would be at about the same retention time ± 0.05 minutes and would be about the same size, assuming the same amount was injected and all of the GC parameters were kept the same.

Parameters

The adjustable parameters of a GC are:

- column temperature,
- ramp rate (how quickly the oven will change temperatures),
- injector temperature (varied to heat up a sample up before introduction to the column),
- hold time (how long the oven is kept at a temperature before the temperature is increased),
- flow rate of the mobile phase (how quickly the mobile phase is flowing through the column).

In this experiment you will only change the *column temperature* and the *flow rate*.

If parameters are kept the same from one run to another, runs should appear identical. However, if one of the parameters is changed, the chromatogram will look different. Figure 2 shows two chromatograms containing the same chemicals, but ran at different temperatures. The top chromatogram is at 70 °C and the peaks have retention times of 1.10 minutes and 1.81 minutes. The bottom chromatogram is at 110 ºC and the same peaks have new retention times of 1.07 and 1.40 minutes. Not only does the retention time change, so does area of the peaks. From Figure 2 we can surmise that as the temperature of the run is increased, the peaks will come out closer together.

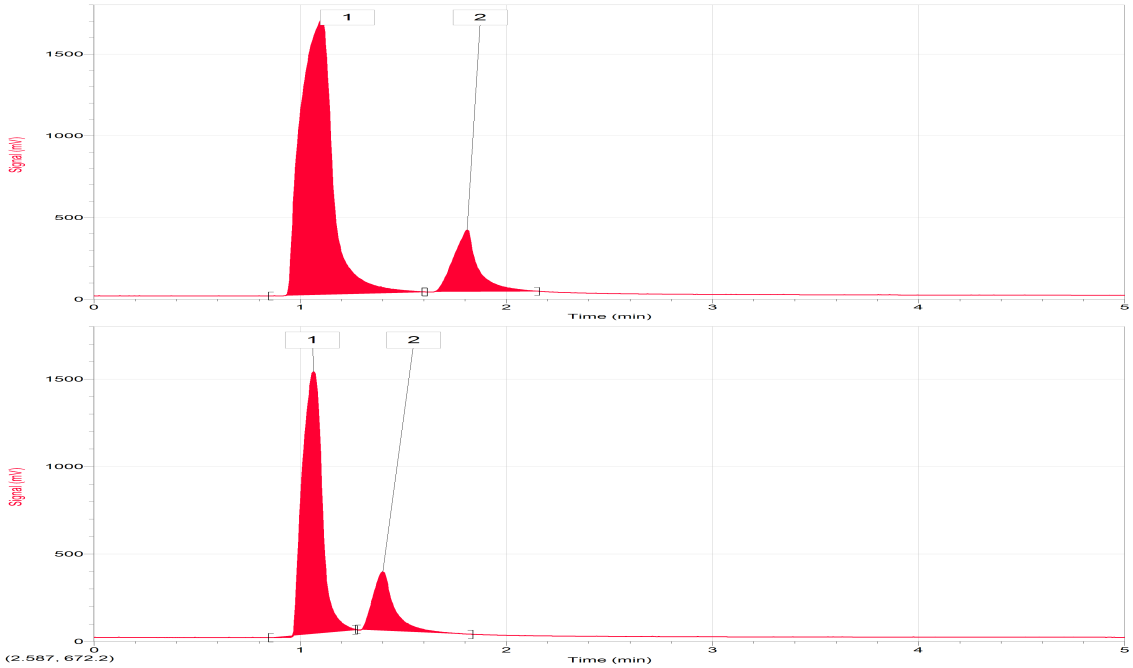


Figure 2: Sample chromatogram at 2 different temperatures.

Figure 3 shows two chromatograms of the same sample at the same temperature but different flow rates. Flow rate refers to the pressure of the carrier gas, which is the mobile phase.

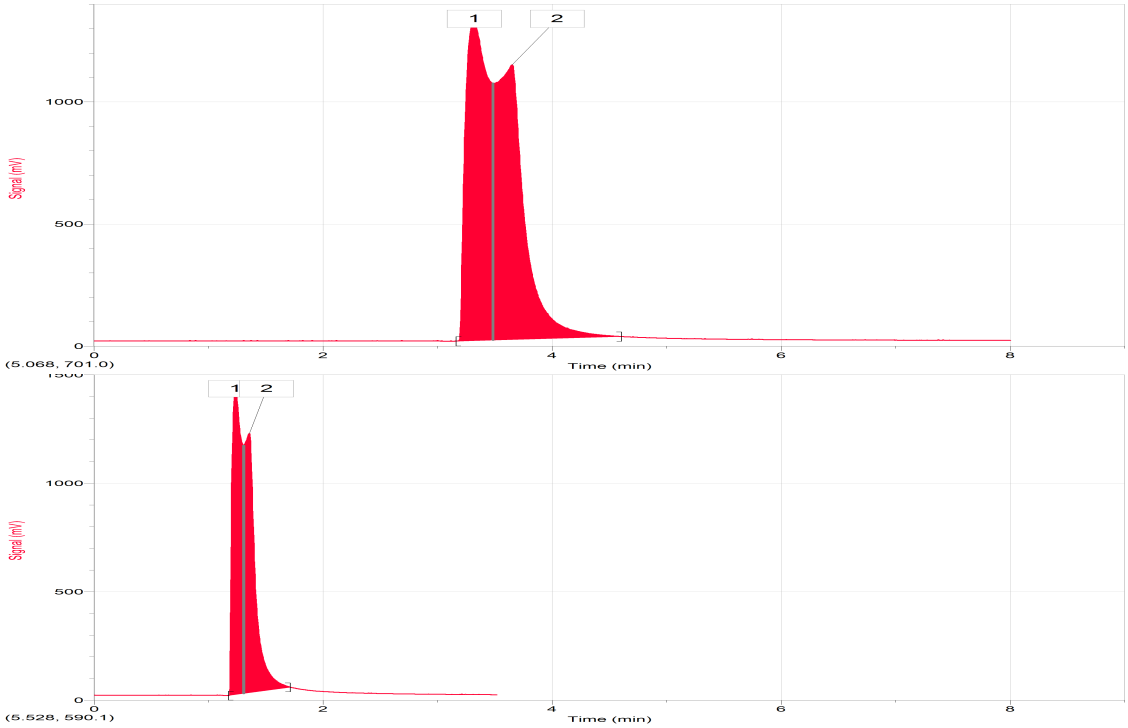


Figure 3: Sample chromatogram at 2 different pressures.

The mobile phase can be any type of gas that doesn't react or interact with the stationary phase In this experiment the carrier gas will be ordinary laboratory air, however, the traditional carrier gasses are either nitrogen or helium. The top chromatogram is at 1.5 kPa and the bottom chromatogram is at 4 kPa.

As you can see in Figure 3, increasing the pressure (or flow rate) of the carrier gas causes the sample to come out faster with narrower peaks. Notice that peaks for the two compounds come out together in each chromatogram. This is coelution and should be avoided if possible. **Coelution** occurs when two or more compounds have similar molecular properties. For example, if the column separates compounds based on boiling point, two compounds with similar boiling points would coelute.

The column used in this experiment separates compounds based on their intermolecular interactions with the stationary phase. The stationary phase of the column is polydimethylsiloxane (PDMS), and is shown in Figure 4. The brackets are enclosing the main group which can repeat up to 600 times to form the polymer.

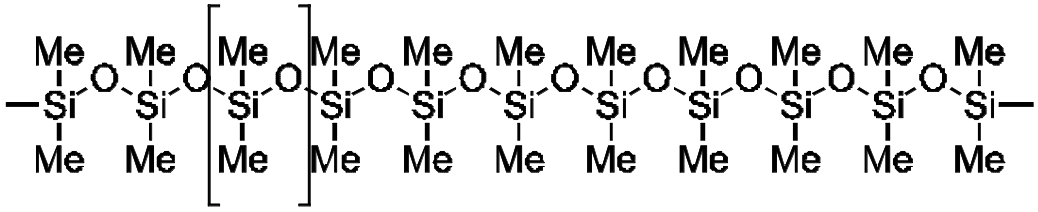


Figure 4: Polydimethylsiloxane with brackets denoting the repeating group

Usually, compounds will **elute** in order of vapor pressure, from highest to lowest. Elution order is usually highest to lowest vapor pressure because, compounds with a higher vapor pressure exist in the gas phase more readily while compounds with lower vapor pressure adsorb onto the stationary phase easier due to stronger intermolecular interactions with the stationary phase. However, this is only a general rule and is not always true. If a compound has very strong intermolecular interactions with the stationary phase it will frequently elute after another compound even though it may have a higher vapor pressure than that compound.

Within this context, it makes sense that heating a column up would cause the compounds to elute faster because they have more energy to overcome the intermolecular attraction to the stationary phase. The same holds true for raising the pressure of the carrier gas, as - more strain is put on the intermolecular interactions. Therefore, if a run is taking too long and compounds are very far apart on the chromatogram, raising the temperature of the oven or raising the pressure of the carrier gas will bring the compounds closer together and shorten the run time. The converse is also true, lowering the oven temperature or the carrier gas pressure will help to separate the compounds more.

In a polysiloxane column, the general elution order is from highest to lowest vapor pressure. However, if a compound becomes strongly adsorbed with the polysiloxane, it will provide an exception to this rule.

Quantitative Analysis

A **calibration curve** comes from plotting some **dependent variable** versus the **independent variable** (typically the **analyte** concentration). A great variety of variables can be correlated to other variables in calibration curves. For example, the density of a sugar solution is dependent on the mass of dissolved sugar. Another example is the relationship between the concentration of a colored solute in solution and its absorbance. A sample calibration curve is shown in Figure 5. The points used to make the calibration curve are **standards**, and are solutions containing known quantities of analyte. **Unknowns** are solutions whose concentration is not known.

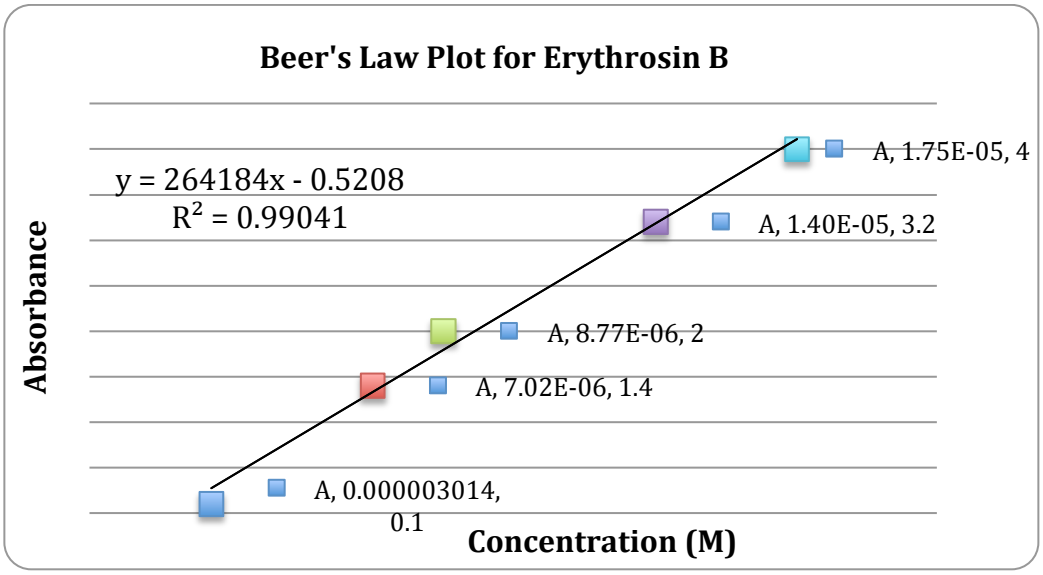


Figure 5: *A sample calibration curve.*

The black trend line in Figure 5 is calculated using a method known as the **method of least squares** or simply **least squares**. The method of least squares is somewhat complicated; however, Excel performs it easily, adding the trend line and its equation. Once created, a calibration curve can be used to determine the concentration an unknown. The concentration is determined by measuring the response of the analyte in the unknown and plugging that value into the least squares line equation. For example, in Figure 1, an absorbance of 2.5 would correspond to a concentration of 1.2×10^{-5} M.

In a **linear** calibration the instrument's signal is proportional to the quantity of analyte at all concentrations. Realistically, very few instruments have a linear calibration curve at all concentrations. Instead instruments have a **linear range** - the range over which the instrument will give a response proportional to the quantity of analyte present. Some instruments, however, do not have a linear range, or their linear range is very small. In these instances, try fitting a trend line from one of the many possibilities given in Excel: linear, polynomial, power, and logarithmic, etc. After selecting the correct trend line, the options tab allows the addition of the line equation on the chart as well as the **R-squared** value, which tells how accurately the data fits the line. An R^2 value of 1 fits the data perfectly and a value of 0 does not fit at all.

Gas chromatography detectors, like almost every other detector, produce an electronic response measured in millivolts (mV). GC detectors use the area of the peak, instead of the highest peak point (as in absorbance measurements). To use peak area, the area under the curve of the peak needs to be found with calculus. Fortunately, computer programs **integrate** peaks easily and automatically. The only source of error, with respect to area, comes from incorrectly integrated responses. Peaks must be integrated from "**valley to valley**", from just before the peak starts to rise until just after the peak ends. The end of the peak is a somewhat arbitrary position, therefore, Figure 6 gives a good example of where peaks "end".

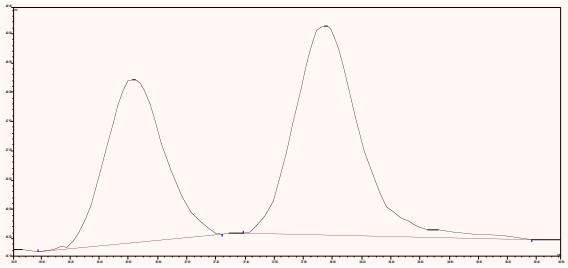


Figure 6: Correctly integrated peaks.

The red lines on the chromatograms in Figures 6, 7, and 8 are **baselines** and they are drawn in manually, indicating the area under the peak to be integrated. Notice how the integration on the left peak begins just before the peak starts to rise and how integration of the second peak ends just after the peak comes back down. Integrating the little "hump" on the tail end of the second peak is important because that little extra bit can dramatically change the area of some peaks. Occasionally the separation of two peaks is not complete and some peaks **coelute**; the compounds exit the column and enter the detector at almost the same time. When coelution occurs integration is still possible. Instead of integrating two separate peaks, the major peak is integrated first and then the second peak is split off of the first. Figures 7 and 8 show how to integrate two coeluting peaks.

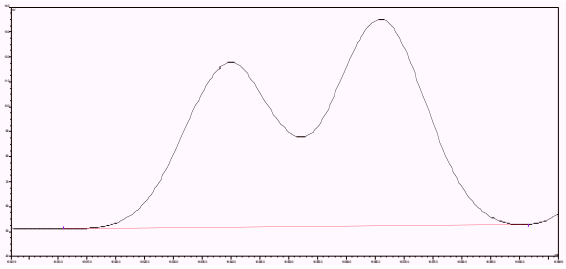


Figure 7: 1st step of integrating coeluting peaks: Integrate coeluted peaks as one peak.

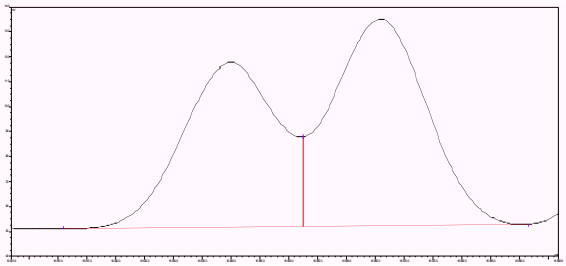


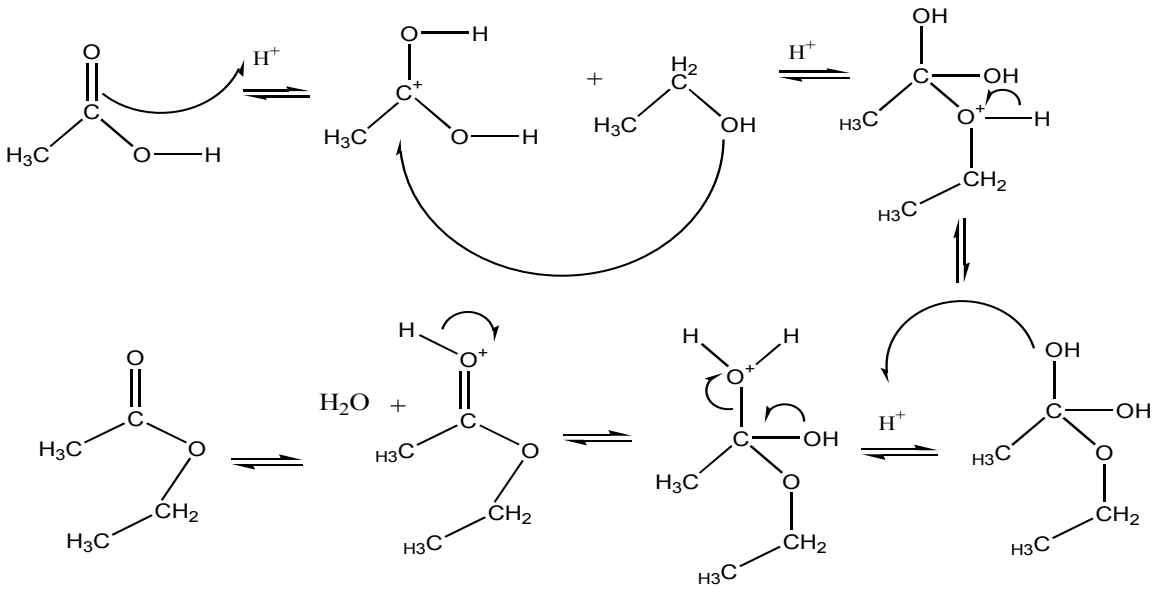
Figure 8: 2nd step of integrating coeluting peaks: Split coeluted peaks into two at the valley.

Reaction Progress

Chromatography is frequently used to follow the progress of a reaction. A chromatograph is taken periodically to measure the amount of products with respect to reactants. The reaction can be stopped when all the reactants disappear or when equilibrium appears to have been established between reactants and products.

In this experiment, ethanol (an alcohol) reacts with acetic acid (a carboxylic acid) to form ethyl acetate (an ester) and water. This particular esterification needs an acid catalyst or no ethyl acetate will form. Instead of a concentrated acid such as sulfuric acid, an ion exchange resin will be used in this experiment. An ion exchange resin is polymer beads containing a repeating characteristic functional group. The functional group in the ion exchange resin used in this experiment is a sulfonic acid. A proton (H^+) is temporarily borrowed from the resin to catalyze the reaction.

The following mechanism shows the reaction:



SAFETY

Safety goggles and aprons must be worn at all times. Glacial or 5M acetic acid should be handled with gloves because it can cause painful burns if it comes into contact with skin and securely capped when not in use because its vapors are irritating. Handle the syringe with care, it is very delicate and sharp and it will puncture skin easily. Wear gloves when using ion exchange resins.

PROCEDURE

Plan your time wisely. Parts can be done out of order. Part E & F must be done in one lab period. All chromatograms and Raman spectra should be saved to your ELN.

Part A: Optimal Parameters for Separation and Standard Retention Times

1. Your TA will make 100 mL of a fresh 1:1:1 mixture of ethanol, ethyl acetate, & acetic acid at the beginning of lab.

2. Obtain a microliter syringe and about a ~ 5mL of the solution your TA just made. The solution should be kept in a capped vial to prevent evaporation.

3. Turn on the LabQuest2 and allow the device to boot. Once ready, connect the GC and turn the device on. The LabQuest2 should automatically sense the device and display the appropriate plots.

4. Click on the button on the bottom left of the screen that looks like a play button. It will bring up the screen to edit the parameters (heading of screen says Temperature Pressure Profile). Press on the numbers to highlight them, then click OK to set the parameters. (Make sure all the values are correct before clicking OK - sometimes the second hold time or the duration changes on its own.)

Parameters	**Set #1**
Start Temperature	70 °C
Hold Time	5 min
Ramp Rate *(may be greyed out)*	0 °C/min
Final Temperature	70 °C
Hold Time	5 min
Duration	10 min
Pressure	16.0 kPa

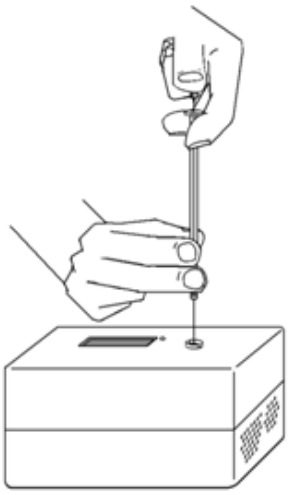
5. Be sure the time scale starts at zero and ends at 10 min. Use the arrows to adjust. Do not use the GC until the light on top is green.

6. While the GC warms up, clean the syringe by rinsing it 10 times with the liquid from the vial. **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.

   With the plunger fully depressed, place the needle into the sample. Slowly draw up the plunger to obtain a sample in the syringe. Remove the syringe from the sample. Discharge this sample into the sink or onto a Kimwipe. Depress the plunger, and put the syringe needle back into the sample. Draw up a second sample and discharge it. Repeat. **Do not push on the plunger when the needle is inside a sample.**

7. Place the syringe needle back into the sample and withdraw over 0.2 μL. Remove the needle from the sample bottle, then depress the plunger to the 0.2 μL line. Wipe the needle with a Kimwipe. Cap vial when not in use to avoid the escape of irritating vapors into the laboratory.

8. 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 (at least until half of the needle is inside the GC). Do not force the needle into the injection port. If the needle sticks, rotate it slightly while inserting. Do not move the plunger yet.



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

10. When the sample peak returns to baseline, the run may be stopped by pressing the end button on the LabQuest2. After pressing end, save the graph. Be sure to label the graphs. When beginning the next run, make sure you save the last chromatograph.

11. Repeat the above steps using the following parameters for the mixture:

Parameters	**Set #2**	**Set #3**
Start Temperature	50 °C	30 °C
Hold Time	5 min	5 min
Ramp Rate	0 °C/min	0°C/min
Final Temperature	50 °C	30 °C
Hold Time	5 min	5 min
Total Length	10 min	10 min
Pressure	8.0 kPa	5.0 kPa

12. Choose the parameter set that gives the best separation of the mixture. Obtain 3 vials that contain the pure chemicals (the standards: ethanol, ethyl acetate, and acetic acid). Repeat the

above steps with your chosen parameter set for each standard. Identify the chemicals responsible for the peaks on the chromatograph ran at the same (chosen) parameter set. Remember to keep vials tightly capped when not in use to prevent vapors from escaping into the laboratory.

Make sure to clear your email address and password of the LabQuest2 so others can't access your email account. Shutdown the LabQuest2 and not simply put it to sleep. To shutdown the LabQuest2: press the home key, select System → Shut Down → OK.

Part B: Contact Angles

1. A digital camera is set up to measure the contact angle. Obtain a PDMS plate from your TA. Use the jack stands to position the camera and the PDMS plate so that the camera is looking head on to the PDMS plate.

2. Using a piece of scotch tape, blot the surface of the PDMS plate to remove any dust. With a microliter pipette, place a 4 µL drop of ethanol close to the edge of the PDMS plate. Make sure that the camera is looking at the plate head on, not upwards or downwards, and focus the camera on the droplet.

3. Take a picture of the droplet, recording the file number. Once you have taken the picture, use a chemwipe and blot the surface of the PDMS plate dry. Take a piece of scotch tape use it to remove any surface dust.

4. Repeat steps 2 and 3 for ethyl acetate and acetic acid.

5. Your TA will upload all of the photos to the EEE Dropbox.

6. Measure the contact angle of each of the three compounds keeping in mind that the contact angle is the interior angle between the droplet and the PDMS plate.

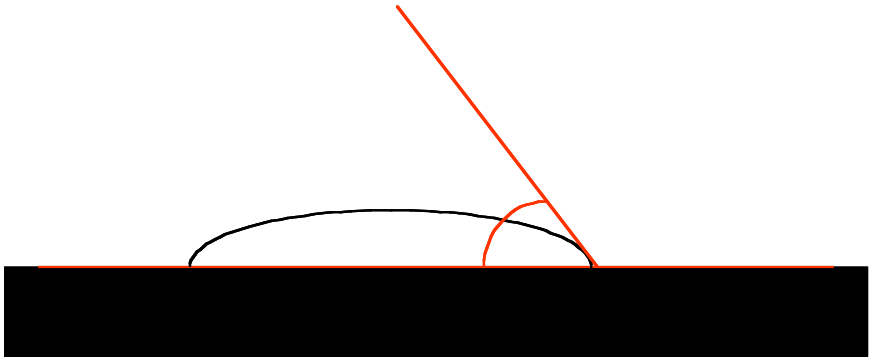


Figure 10: Contact Angle

Part C: Raman Analysis of Standards

Refer to the iRaman supplemental procedure page for operation of Raman spectrometer.

1. Turn the iRaman power on using the black switch on the back of the machine, followed by the laser by turning the key above the power switch to the on position (up). (If done already, proceed to the next step) and start the BWTEK software.
2. Switch the laser gun to open on the liquid analysis stage.
3. Ensure the integration time is 5000 ms, averaging is set to 1, and multiplier is set to 5 in the measurement settings boxes
4. Run a dark current measurement by pressing the dark current icon as indicated in the iRaman procedures guide, and select dark subtracted in the display options box once the measurement is complete.
5. Turn the laser power on in the settings, and make sure it is set to 30% at most.
6. Fill a cuvette with ~3 mL of ethanol and cap the solution. Place the cuvette in the sample holder with the transparent face towards the laser input. Place the cap on top of the sample holder to shield the cuvette from room light during the scan. Press **Acquire**.
7. Repeat this measurement for ethyl acetate, glacial acetic acid, and the 1:1:1 mixture from Part A. Collect all 4 separate spectra.

Part D. Quantitative Analysis

1. Make a ~5 M ethyl acetate solution by placing 50 mL into a 100 mL vol flask. Dilute to volume with ethanol. This will be your stock solution. Calculate its exact concentration.

2. Using 5 M ethyl acetate in ethanol stock solution, prepare 10 mL of five standard solutions of 2.5 to 4.5 M ethyl acetate in ethanol *(Ethanol is the solvent, not water)*. Cap solutions tightly to prevent evaporation and mix the solutions by inverting the container. Show one sample calculation for the dilution. The remaining data needs to be in a table. All of the calculations need to be completed before coming to lab. Remember to keep all liquids capped when not in use.

3. Enter the parameters from Part A that gave the best separation. Press "Collect" to open the input window. Once all of the parameters are input press "Done" to start the GC warm up. When the GC is ready for injection the LED on the GC will turn green and the message "Inject and select collect simultaneously" should be on the screen.

4. Clean the syringe with ethanol while the GC is warming up. Repeat the process to rinse the syringe with the first (lowest concentration) standard solution. Using the same technique as in Part A, inject 0.20 μL of liquid. It is important that the volume be very close to 0.20 μL and that you inject the same volume of each standard.

5. While the data collection proceeds, thoroughly clean the syringe and needle with ethanol and then prerinse it with the next standard. *Cap solution vials when not in use.*

6. If both peaks come out before the run ends and the signal returns to baseline, you may end the run early by selecting the "Stop" button. Once completed, analyze the chromatogram by going to the "Analyze" menu and selecting "Advanced" and then "Peak Integration", then "Signal". Zoom in as needed to correctly integrate the peaks. To integrate, highlight the peak from the correct starting point to the correct end point and then press "Add". Name the peak. Repeat this for all peaks present. If two peaks coelute and need to be split, correctly integrate them as one peak, then click on the integrated peak where you want to split then and press "Split". Print out the chromatogram.

7. Repeat this process for the rest of the standards including the 5 M solution. Do this in order of increasing concentration.

8. Create a calibration curve for ethyl acetate by plotting the % area of the ethyl acetate peak versus concentration.

Make sure to clear your email address and password of the LabQuest2 so others can't access your email account. Shutdown the LabQuest2 and not simply put it to sleep. To shutdown the LabQuest2: press the home key, select System → Shut Down → OK.

Part E: Esterification

1. Perform this part in the ***fume hood***. Fill a 400 mL beaker half-full with water and place it on a hotplate. Place a thermometer in the water to monitor the temperature (it Must stay between 70 – 80 °C).

2. Place a *small* amount of ion-exchange resin (about equal to two garden peas) into 3 scintillation vials. (Too much resin will result in strange background signals in the Raman spectra.)

3. Add 25 drops glacial acetic acid and 25 drops ethanol to each of the vials. *Determine the volume in milliliters*.

4. Cap the scintillation vials and place all 3 vials into the hot water.

5. **Monitor the water temperature**. If it drops below 70 °C, turn up the heat; if it rises above 80 °C, turn the heat down or off and add some cold water. At the end of ten minutes remove and label one of the vials carefully. *Why is temperature control crutial under these conditions? Consider both cooler and warmer temperatures. Discuss this issue with your TA.*

6. At the end of twenty minutes, remove and label another vial.

7. At the end of thirty minutes, remove and label the third vial.

8. Allow all three scintillation vials and contents to cool. When the vials are cool enough to handle, procede to Part F.

Part F: Verification of Esterification

1. Following the procedures and best parameters found in Part A, obtain a chromatograph of the contents in each of the 3 test tubes. Inject 0.**2** μL of each sample. **Do not stop the run until peaks for ethanol, glacial acetic acid, and ethyl acetate have all been obtained.**
2. After injecting the last sample, rinse the syringe well with ethanol.
3. Measure the Raman spectra for all 3 samples.

Make sure to clear your email address and password of the LabQuest2 so others can't access your email account. Shutdown the LabQuest2 and not simply put it to sleep. To shutdown the LabQuest2: press the home key, select System → Shut Down → OK.

Report Guidelines:

*The directions below are meant as guidelines – you are encouraged to be **thorough**, but **concise**. The section titles (Introduction, Data, etc.) should appear on your report. All work should be neatly formatted and grammatically correct. Part of being a scientist is communicating with the written word! The entire document should be written in MS Word (or a similar word processor) and then uploaded into this week's experiment folder in the ELN. You are also required to submit the Word document to turnitin.com.*

1. *Introduction.* Already written… it's the first few pages of this document. (You don't need to repeat it.)

2. *Data.* You must use some text to describe your data. (Do not just create a couple pages of plots.) All figures should have numbers and concise descriptive captions.
   - Display & annotate GCs of 3 standards & mixture with best parameters (clearly indicate the parameters).
   - Display & annotate Raman spectra of 3 standards & mixture. Clearly label the bond type responsible for the peaks on each standard's spectrum. On the mixture, label peaks with the bond and (if possible) the chemical (ethanol, acetic acid, or ethyl acetate) responsible for the peak.
   - Photos with contact angles.
   - Calibration Curve from Part D.

3. *Discussion/Results.*
   - What parameters worked for the separation of the 3 chemicals in this experiment and why? What is the effect of increasing the column temperature on a chromatogram's appearance? What is the effect of increasing the carrier gas pressure?
   - How does vapor pressure and the contact angle of ethanol, acetic acid, and ethyl acetate relate to the order of elution of the chemicals in the GC?
   - Discuss the use the GC, Raman, and IR (Spartan) of each of the standards (pure ethanol, pure acetic acid, and pure ethyl acetate to analyze the GC, Raman, and IR data for the mixtures in Part A & E/F. Provide an interpretation of the data.
   - Use the calibration curve created in Part D to quantify the amount of ethyl acetate formed at 10, 20, and 30 minutes.
   - If errors occurred, discuss them here.

4. *Conclusion.* What instruments did you use? What did you use them for? How much ester was formed?

5. *Experimental.* Provide the procedure with observations and chemical amounts incorporated within the text. Include instrumental parameters and results (GC retention times and Raman peak frequencies). You can assume the reader knows how to use a Vernier mini-GC and an iRaman, so do not include the procedural steps about pushing particular buttons, etc.