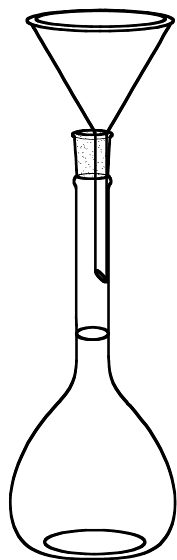
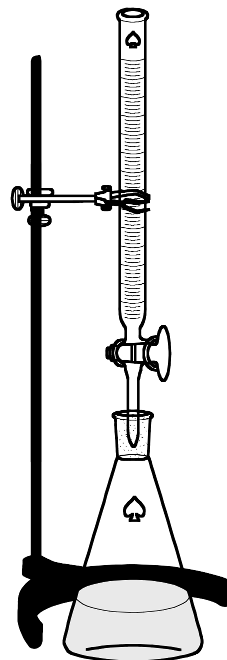
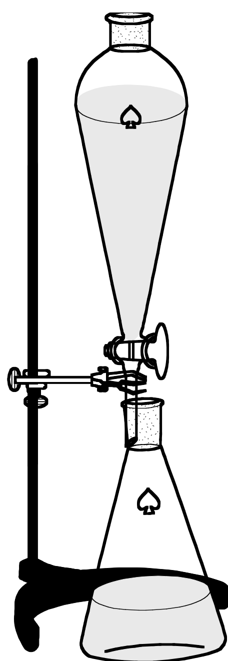
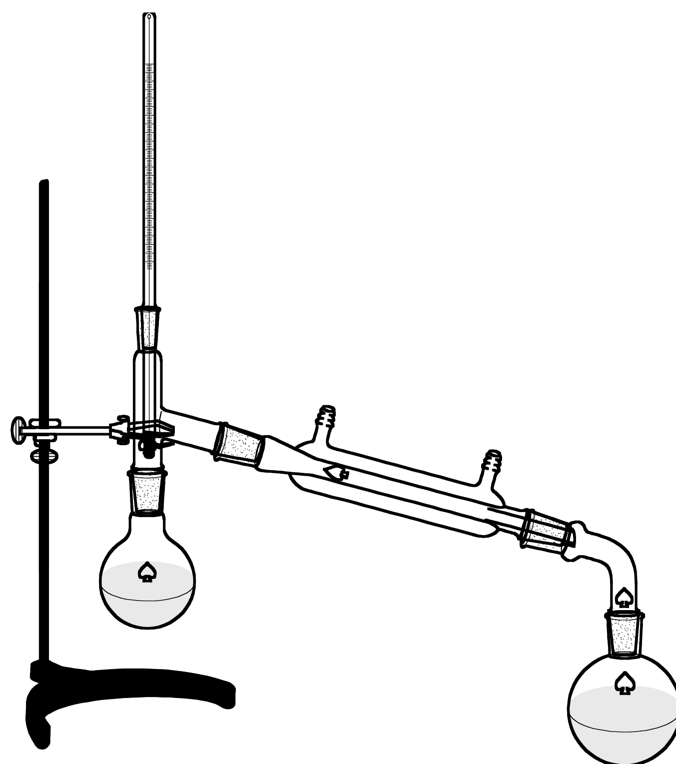
Standard Operating Procedures









Standard Operating Procedures

OVERVIEW

In the following laboratory exercises you will be introduced to some of the glassware and techniques used by chemists to isolate components from natural or synthetic mixtures and to purify the individual compounds and characterize them by determining some of their physical properties. While working collaboratively with your group members you will become acquainted with:

a) Volumetric glassware
b) Liquid-liquid extraction apparatus
c) Distillation apparatus

OBJECTIVES

After finishing these sessions and reporting your results to your mentor, you should be able to:

- Prepare solutions of exact concentrations
- Separate liquid-liquid mixtures
- Purify compounds by recrystallization
- Separate mixtures by simple and fractional distillation

EXPERIMENT 1

Glassware Calibration, Primary and Secondary Standards, and Manual Titrations

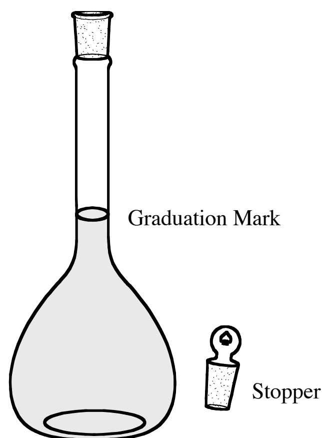
PART 1. Volumetric Glassware Calibration

Volumetric glassware is used to either contain or deliver liquids at a specified temperature. Glassware manufacturers indicate this by inscribing on the volumetric ware the initials TC (to contain) or TD (to deliver) along with the calibration temperature, which is usually 20°C[1]. Volumetric glassware must be scrupulously clean before use. The presence of streaks or droplets is an indication of the presence of a grease film.

To eliminate grease from glassware, scrub with detergent solution, rinse with tap water, and finally rinse with a small portion of distilled water.

Volumetric flasks (TC)

A volumetric flask has a large round bottom with only one graduation mark positioned on the long narrow neck.



The position of the mark facilitates the accurate and precise reading of the meniscus. If the flask is used to prepare a solution starting with a solid compound, add small amounts of solvent until the entire solid dissolves. Cap the flask with its stopper and invert it three times to insure good mixing. If the temperature changes during dissolution, wait until the flask reaches room temperature before proceeding to fill the flask to the mark.

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[1] Skoog D.A., West D.M., Analytical Chemistry, 2nd Ed, pp. 89-91, Holt, Rinehart and Winston (1965).

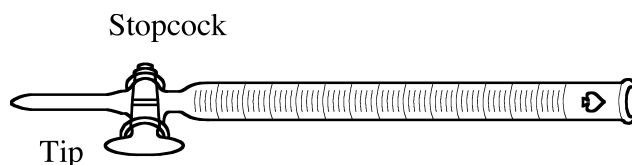
When filling the volumetric flask, stop the transfer of liquid when the liquid level is about an inch below the graduation mark and invert the flask as you did previously to homogenize the solution. Then use a Pasteur pipette to add liquid slowly to the mark. Once liquid is filled to the mark, cap the flask with a stopper and mix as before. Overfilling the flask above the graduation mark ruins the volume measurement. In this case, the content inside the volumetric flask should be discarded.

Pipettes (TD)

A pipette bulb should be used to withdraw and deliver liquids when using a pipette. In this course you will use graduated and volumetric pipettes according to the precision required by the experiment. Graduated pipettes deliver different amounts of liquid by making use of incremental markings inscribed along the pipette. Volumetric pipettes deliver fixed amounts of liquid indicated by a single graduation mark inscribed on them. The volumetric pipette possesses an enlarged portion below the graduation mark in order to reduce the speed of suction and allow a precise volume reading.

Burettes (TD)

A burette is a long glass tube with a stopcock near the tip, which precisely controls the rate of flow of liquid down the constricted tip of the burette.



Before use, burettes must be rinsed first with distilled water, then with a small portion of the liquid to be measured as follows: add approximately 2 mL of liquid to the burette, hold the burette horizontally and rotate it to allow the liquid to coat the entire inside. Drain the liquid down the tip. When filling the burette using a funnel, make sure the funnel is perfectly clean, the stopcock is closed, and the burette is over a waste beaker. If you see air bubbles trapped inside

the tip of the burette, eliminate them by draining a few mL of the liquid out the tip while holding the burette diagonally. Your mentor will demonstrate the correct way to rinse and fill the burette.

Depending on the type of glassware, the gravimetric calibration procedure at room temperature consists of:

a) Determining the mass of distilled water that fills the TC-ware until the bottom of the meniscus coincides with the graduation mark; or collecting the amount of water delivered by the TD-ware in a tared beaker. Repeat the procedure at least three times to assure reproducibility.
b) Measure the exact temperature of the water with a 0.1°C precision thermometer before each measurement.
c) Calculate the calibrated volume using the density of water at the calibration temperature (*Knovel Critical Tables* http://www.knovel.com/knovel2/crc_handbook.jsp)

Warning: The Mettler balance has a capacity of 510g.

Statistical Analysis of Data

Your group will be assigned 4 or 5 pieces of glassware to calibrate. Each group member should calibrate one piece of glassware. Your mentor will give you instructions about the volumes to measure. Input the data from your notebook into the spreadsheet that corresponds to each piece of glassware.

1. Record the name of the manufacturer. Fill up the information as in the example below.

VOLUMETRIC FLASK

Measured Volume (mL)	Measured mass (g)	Temperature of water (°C)	Density (g/mL)	Calculated volume (mL)

2. Using Excel compute the average, variance, and standard deviation for each piece of glassware. Compute the same statistics for the mass determined with the Mettler balance
3. Find the uncertainty given by the glassware manufacturer and by the Mettler Company

4. Compare the values of the computed statistic with the uncertainty provided by the manufacturers. Comment on the statistics given by the manufacturer.
5. Discuss the precision of the calibrated volume for each piece of glassware and for the Mettler balance
6. Report your conclusions

PART 2. Solution Preparation: Primary and Secondary Standards

1. Tare two watch glasses and weigh on each a small amount of sodium hydroxide, NaOH and potassium hydrogen phthalate, KHP, a monoprotic acid. Record the masses to the nearest 1 mg. Leave the systems exposed to air for 25 minutes.
2. While you are waiting for the watch glass systems, take a 400 mL beaker of distilled water and measure the pH of the water. Is this the value you expect? Boil the distilled water uncovered with a stir bar for 10 minutes and let it cool to room temperature covered with a watch glass. Measure the pH again. Is there a difference in the reading obtained? If so, discuss why.
3. After time has elapsed, weigh the watch glasses and record your observations. Based on the observations you made, decide which one of the two substances can be weighed directly and dissolved in a well-measured volume of water to prepare a solution of exact concentration.
4. Prepare a 10^{-1}M solution of the substance you decided on in step 3.
5. Calculate the volume needed from this concentrated solution to prepare 250 mL of a 10^{-2}M solution. Use a volumetric pipette to withdraw the aliquot calculated from the concentrated solution. Is it OK to use a graduated pipette to take the aliquot? Set this solution aside to determine its exact concentration later.

PART 3. Standardization of Sodium Hydroxide Solution

After preparing a solution with the substance that changed while exposed to air, it is necessary to perform a standardization (find the exact concentration of the solution) using a procedure called <u>TITRATION</u>. The solution should be kept minimally exposed to air after standardization. The TITRATION procedure entails reacting a volume of a solution whose concentration is exactly

known or a mass of solid with known molecular weight with a volume of a solution which concentration is unknown. The volume of sodium hydroxide solution used in a manual titration is the volume needed to reach the *endpoint* of the titration (where the indicator changes color), while the one obtained from the potentiometric titration is referred to as the *equivalence point* of the titration. At the equivalence point the number of moles of titrant used is equal to the number of moles of the titrated compound.

1. Add about 450 mL of distilled water to about 150 mL of NaOH (aq) solution provided (note the approximate concentration of the NaOH(aq) from the bottle). Cover the bottle and homogenize. Neither of these volumes needs to be exact since the solution will be standardized. **Keep the dilute solution covered as much as possible**.
2. Weigh two samples of about 0.2-0.3 g of KHP (Potassium Hydrogen Phthalate, record exactly the amount of KHP weighed) and **quantitatively** transfer them into two separate, clean 250 mL Erlenmeyer flasks. Dissolve the samples in about 100 mL distilled water (you may need to slightly heat for complete dissolution, but make sure the solutions cool to room temperature before titrating) and add 2-3 drops of phenolphthalein indicator.
3. Rinse a clean burette with distilled water and then with small portions of the dilute solution prepared in step 1. Discard the rinse solutions in the liquid waste container and clamp the burette to the burette clamp. Fill the burette with the dilute solution prepared in step 1.
4. Knowing the exact amount of KHP and its molar mass (204.22 g/mol), can you estimate the approximate volume of dilute sodium hydroxide solution needed to reach the endpoint of the titration?
5. Titrate the KHP solution with the diluted NaOH solution from the burette, swirling continuously, until the faint pink color lasts about 20 seconds. **Each group member should perform at least one manual titration.**
6. Calculate the concentration of the solution from the volume used in each titration. If the values of the volume used for each titration fall within the experimental error, use the average value as the concentration of the solution. If the values are scattered showing low precision, repeat the titration one more time.

RESULTS

Primary versus non-primary standard

Time(min)	Mass NaOH (g)	Mass KHP (g)
0		
25		

Observations:

Standardization of NaOH
Manual Titration

	Trial 1	Trial 2	Trial 3
Weight of KHP used (g)			
Initial burette reading (mL)			
Final burette reading (mL)			
Volume NaOH used (mL)			

DISCUSSION

Discuss with your group whether the endpoint volume of the equivalence point should be used to calculate the concentration of the titrated sodium hydroxide solution. What is the exact concentration of the sodium hydroxide solution?

Comment on the pH range at which phenolphthalein changes color. Is phenolphthalein a good indicator for standardizing NaOH solutions against KHP? Explain.

EXPERIMENT 2

Potentiometric Titration of Coca-Cola and MicroLab Analysis of Vinegar

PART 1. Potentiometric Titration of Coca-Cola

Use the sodium hydroxide solution you previously prepared to potentiometrically titrate a sample of Cola as follows:

1. You should degas your soda by stirring under vacuum. Pour approximately 200 mL of your soda into a clean vacuum filter flask, add a stir bar, and stopper. Your mentor will demonstrate how to correctly use a vacuum system. Make sure your system has a trap and attach it to your flask. Turn on the water full-blast to create a vacuum and stir your soda vigorously on a stir-plate until you no longer observe bubbles. Why is it necessary to de-gas your sample before beginning the titration?
2. Rinse and full a burette with your standardized NaOH solution, and calibrate the pH meter with the buffer solutions provided. Why should you calibrate the pH-meter with a buffer solution?
3. Set up the potentiometric titration system (pH-meter and burette) and turn on the stirring to assure that the titrated solution is thoroughly mixed, being careful not to break the glass electrode with the magnetic bar.
4. Measure the initial pH and perform the titration recording the pH value every 0.50 mL of NaOH added.

PART 2: Titration of Vinegar using a Microlab

Vinegar is an incredibly versatile liquid. A preservative and condiment to the Babylonians, they began flavoring it with herbs. In its pure form it was a beverage to the Roman legionnaires. Cleopatra demonstrated she could consume a fortune in a single meal by dissolving pearls in vinegar to win a bet. Biblical references mention the use of vinegar for soothing and healing purposes. And even Hannibal when he crossed the Alps heated boulders and doused them with vinegar to crack crumble them from his path. In World War I, vinegar was used to treat wounds. And today it is recommended for treatment of rashes, bites and other minor ailments when camp-

ing. The frugal homemaker even recommends vinegar for uses such as laundry, metal polishing and insect repulsion.

Vinegar can be made from any fruit, or from any material containing sugar: apple juice being most commonly used. Vinegar is made by two distinct biochemical processes, both the result of the action of microorganisms. The first process is fermentation during which yeast converts sugars to alcohol. The second process converts the alcohol to acid using bacteria (*"Acetobacter"*). This is the acetic, or acid fermentation that forms vinegar.

This experiment will combine titration techniques we have learned previously with instrumentation and software called MicroLab. You will determine the concentration (V/V) of acetic acid in a commercial sample of vinegar.

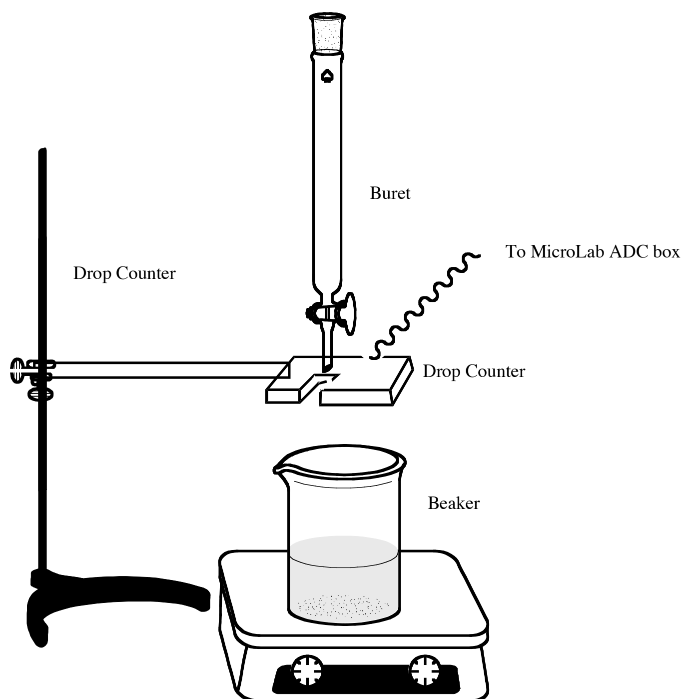
Calibration of Drop Counter using Distilled Water

The drop counter on the MicroLab will be calibrated in order to know the volume of one drop of solution. The NaOH(aq) solution (which is standardized using KHP and an indicator) will be used to titrate a sample of commercial vinegar using the MicroLab system.

Set up the burette/syringe above a test beaker with the MicroLab interface between so the drops pass through the path of MicroLab sensor, which will count the drops. Follow the procedure given below (or in the help menu of the MicroLab software) to calibrate the drops from the burette.

1. Connect the MicroLab interface to the USB port of the computer and turn it on.
2. Turn on the computer and open the MicroLab software. (If software doesn't open, make sure that the MicroLab interface is powered on and connected to the computer.)
3. When prompted to select an experiment type, select "Microlab Experiment" under the "New" tab. In the lower right corner, change the experiment name from "Untitled" to "Counter Calibration" and click OK. A new screen with multiple windows should open.
4. Connect the MicroLab drop counter to port A on the MicroLab interface. (The green light on the drop counter should light up.)

5. In the upper left window entitled “Data Sources/Variables,” click “Add Sensor.” A new window entitled “Choose sensor” should open. Where it says “<Choose sensor>,” select “Counter” and then select the input (port A) by clicking on the picture of the MicroLab interface; click “Next.”
6. Select “Continually increasing count” and click “Finish.” This should close the window and return you to the previous screen, and “Counter” should be added to the list of sensors in the upper left corner.
7. Click and drag “Counter” from the upper left window into the lower right window where it says “Digital Display.” This window should now read “Counter = 0.00.”
8. Set up a 10 mL syringe and the drop counter so that liquid will flow through from the syringe, through the sensor on the drop counter, and into a clean beaker as shown. Make sure that the stopcock on the syringe is in the closed position (horizontal). Fill the syringe with about 10 mL of the liquid to be distilled.



9. Slowly turn the stopcock toward the open position until drops of liquid begin to flow from the syringe. Adjust the stopcock until the drops flow at a rate of about 1 drop a second. You should notice the red light on the drop counter blink every time a drop passes

through the sensor. You may need to adjust the position of the drop counter in order for the sensor to detect each drop. You should put the counter as close to the syringe as possible without allowing the sensor to get wet.

10. Once you have the set up adjusted, close the stopcock and refill the syringe to exactly 10 mL. (You can use the liquid that you collected in the beaker during the previous step.) You are now ready to begin the calibration experiment.
11. First, go back to the MicroLab program and click the "Start" button on the left. The counter display on the right should now be in boldface. Now slowly turn the stopcock toward the open position until you can get the drops to flow at a rate of about 1 drop a second. The counter display should increase for each drop. (Don't worry if the counter is going up in multiples of 2 or more; the refractive power of different liquids can cause individual drops to be read as multiples of itself. Our calibration will compensate for this.)
12. Keep dispensing liquid until the volume in the stopcock reaches 5 mL. At this precise point, click the "Stop" button (located next to the "Start" button) to stop the counter. You should now have a value for the number of drops there are in 5 mL of solution.
13. To calculate the volume of each drop, divide the volume dispensed by the total number of drops counted (use the value given by the drop counter). For example, if the drop counter finished at 300, you would have the following equation:

    Volume of a drop = 5 mL / 300 drops = 0.0167 ml/drop

14. You can use this value to convert the drops counted by the drop counter during the titration. Click on "Add Formula" in the "Data Sources/Variables" window and input a formula that will convert a drop count to volume based on your calibration. Drag this formula from the "Data Sources/Variables" pane to the X-axis of the graph on the right.

Calibration of pH Meter

1. Click on "Add Sensor" and choose "pH meter." Clicking "Next" will bring you to a calibration screen. Select "Perform New Calibration."
2. Click "Add New Calibration Point" and immerse the electrode in the provided pH 4 buffer. A calibration window will appear with a "Rate of Change" and "Sensor His-

tory" graph. The "Rate of Change" graph will have a vertical bar whose position fluctuates about a central position. When its position becomes constant (or fluctuates only slightly within the green zone of the graph), enter the pH of the buffer in the "Actual Value" box and click "OK."

3. Add a calibration point for the pH 7 buffer as above. Then select "First Order (linear) fit" from the left panel of the calibration window. Click "Accept and Save this Calibration."
4. Drag the pH sensor from the "Data Sources/Variables" pane to the Y-axis of the graph on the right.

Titration of Vinegar

Use the standardized NaOH solution prepared above to titrate 1 mL of the commercial vinegar sample provided to the group using a MicroLab. Dilute the sample of vinegar using water. Titrate the solution drop by drop (using the drop counter) the titration is complete at about pH 12.

RESULTS

Potentiometric Titration

Volume of Coca-Cola titrated ______$\pm$_____g
Initial pH ______$\pm$_____pH units

During the course of the potentiometric titration, you should record the pH after every addition of 0.5 mL NaOH. Tabulate your data as follows:

Buret reading (mL)	pH	ΔpH	Vol. NaOH Used (mL)	ΔVol. NaOH Used (mL)	ΔpH/ΔVol. NaOH Used

Input these data into an Excel worksheet. Perform the following operations on the worksheet using ***formula***: calculate the values of volume of NaOH added (Δvol), change in pH (ΔpH), and change in pH per volume of base added (ΔpH/Δvol. NaOH added). You will use these values to

plot pH (y-axis) versus volume NaOH added (x-axis) and ΔpH/Δvol base added (y-axis) versus volume NaOH added (x-axis). Predict the shape of the latter graph based on your first graph and your knowledge of Calculus. How would you name these graphs?

Look at the ingredients of Coca-Cola. What acid do you suppose you're observing? Do your plots support your conclusion? From the ΔpH/Δvol vs. volume of base plot determine the equivalence point(s) and the amount of base added to reach them. Use these values to determine the concentration of acid in your soda. Next, find the half-equivalence point(s) and determine the dissociation constants, K_a. On this same graph label the equivalence point and the half-equivalence point(s) where the K_a value(s) were found.

Titration of Vinegar

Determine the concentration of your vinegar and find the half-equivalence point and K_a as you did for your soda. Assuming the density of commercial vinegar is close to that of water (1.00 g/mL), calculate the percent (by mass) of acetic acid in vinegar.

DISCUSSION

- In your own words, define primary and non-primary standards.
- What would be the effect of using a more concentrated solution of NaOH in the titrations? How would this affect the titration curves and the ΔpH/Δvol vs. volume base graphs?

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EXPERIMENT 3

Liquid-liquid Extraction and Recrystallization

BACKGROUND

Extractions

Extraction is one of the oldest chemical operations known; it involves transferring a solute from one phase to another. The preparation of a cup of tea or coffee represents a process of extraction of flavor and odor components from dried material into water. When a compound is extracted from a solid material into a liquid, the process is referred to as a **solid-liquid extraction;** if the transfer occurs from one liquid into another is called **liquid-liquid extraction.** Most organic synthetic procedures are followed by workups employing extraction to isolate the product of interest.

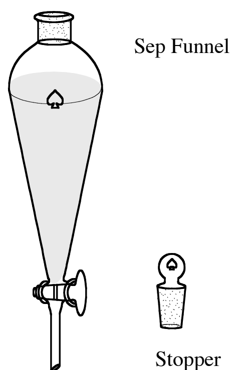
How would a mixture of saccharin and NaCl be separated? Both are soluble in water. However, saccharin is somewhat soluble in diethyl ether while salt is not. If you dissolve the mixture in water and then add ether, two layers will form because ether and water are *immiscible*. Most of the saccharin will be extracted into the *ether layer*. Notice that the separation is not absolute. The salt and saccharin (the *solutes*) are distributed between the two solvents, and a dynamic equilibrium is established. The ratio of the concentration of a solute in a second solvent (*e.g.* ether) to its concentration in water is constant, called the *partition coefficient K*:

$$K_{solute} = C_{ether} / C_{water}$$

C_{ether} and C_{water} represent the molar concentration of the solute in ether and water respectively.

This constant depends on the solvent used, the solute itself, and temperature. In this particular case $K_{saccharin}$ is a large number because saccharin is more soluble in ether than water while K_{salt} is a small number because salt is slightly soluble in ether. Using this constant, one can show that extracting a component from a mixture several times with small portions of solvent is more efficient than extracting it with one large portion.

The separatory funnel is the tool of trade for liquid-liquid extraction. In order to increase the surface area between the two layers, and speed the attainment of equilibrium, the separatory funnel is shaken and vented. The organic layer (ether) is then separated from the aqueous layer, and *dried.* Any water dissolved in the ether can be removed by utilizing a *drying agent* such as anhydrous magnesium sulfate ($MgSO_4$) and filtering off the hydrate ($MgSO_4 \cdot xH_2O$) that forms. Another way to dry an ether layer is to *wash* it with saturated NaCl solution (*brine*) before adding drying agent. The brine transfers the water from the ether layer to the aqueous layer. The dry ether solution is evaporated by a *rotary evaporator* (see the Instrumentation Guide) and the solute remains in the flask. If the ether is not properly dried, the remaining solute in the flask will be moist.



Other organic solvents that are used in extractions include ethyl acetate ($CH_3CO_2C_2H_5$), methylene chloride (CH_2Cl_2), chloroform ($CHCl_3$), hexane ($CH_3(CH_2)_4CH_3$), and benzene (C_6H_6). Benzene and chloroform are usually avoided as solvents due to their carcinogenic nature. Methanol and ethanol are not useful extraction solvents because they are miscible with water and will not form a separate layer. Chloroform and methylene chloride are denser than water, while most other organic solvents are not as dense as water. Therefore, the organic layer could be above or below the aqueous layer depending on the organic solvent used. If you are not sure which layer is the organic or the aqueous layer, perform the water drop test: add a drop of either layer on top of a watch glass filled with water. The aqueous layer will readily mix with water. You may need to add several drops, as some solvents have a small, but significant, solubility in water.

The formation of an ***emulsion*** is a common problem when performing extractions. An emulsion is a stable dispersion of one liquid in a second immiscible liquid. Emulsions delay the separation of two liquids, making it necessary to "break" the emulsion. This can be done mechanically (settlers, cyclones, centrifuges, filtration through Celite) or chemically (addition of salt or a saturated NaCl solution called brine). The addition of salt increases the surface tension of the droplets and increases the density of the aqueous layer, thereby forcing separation. If one of the solvents being used is water, the addition of a saturated aqueous sodium chloride solution will help destroy the emulsion. You should avoid shaking a solution that tends to form emulsions. Depending on the impurities being removed extractions can be classified as :

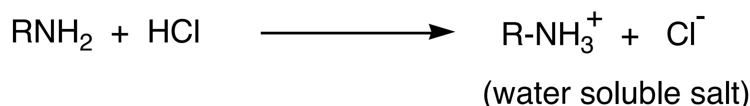
- Aqueous extraction
- Acidic extraction
- Basic extraction

Aqueous Extraction

An organic mixture is extracted with water to remove highly polar materials such as inorganic salts, strong acids or bases, and low molecular weight polar substances. Normally, water extractions are used immediately following extractions of a mixture with either an acid or base to ensure that all traces of the acid or base have been removed.

Acidic Extraction

Extracting an organic mixture with a dilute acid (5% HCl) removes any basic impurities such as amines. Bases are converted to their cationic salts by the acid.



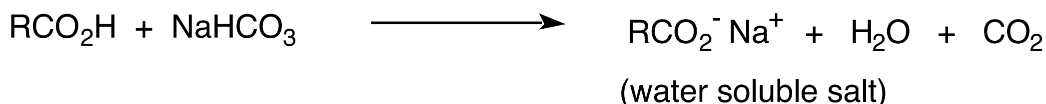
$$RNH_2 + HCl \longrightarrow R\text{-}NH_3^+ + Cl^-$$

(water soluble salt)

This cationic salt can be converted to its neutral form by adding base to the acid extract.

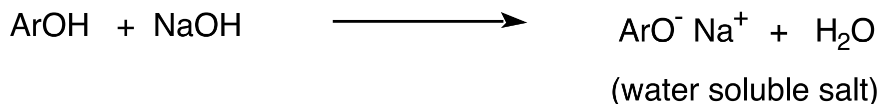
Basic Extraction

Extracting an organic mixture with a dilute base (5% sodium bicarbonate or NaOH) converts any **strongly** acidic impurities to their anionic salts.



This anionic salt then can be regenerated by acidifying the basic extract. If a weak acid is present,

extracting an organic mixture with a **stronger** dilute base (5% NaOH) will convert **weak** acids to their anionic salts:



This anionic salt then can be regenerated to its neutral form by acidifying the basic extract.

Purification by Recrystallization

The technique of recrystallization is used to purify inorganic and organic compounds in the solid state. Essentially, impurities are separated from the desired product by selecting a solvent, or a solvent mixture, that will keep the impurities in solution at all temperatures, thus prohibiting these impurities from precipitating along with the product crystals. The goal is to prepare a *saturated solution* of solute in the solvent at its boiling point and allow it to cool. If the solvent or solvent mixture is properly chosen, the compound will have decreased solubility at lower temperatures, and the solution will precipitate crystals as it cools.

Recrystallization Using a Single Solvent

Ideally, the solute is very soluble in the solvent at its boiling point, but virtually insoluble at 0°C. Usually, recrystallization is carried out by first dissolving the solid in a boiling hot solvent.

Next, the solution is cooled below room temperature, and the crystals that crash out are collected by vacuum filtration. To do this the substance to be recrystallized is placed in an Erlenmeyer flask and a **minimal** amount of hot solvent is added to dissolve the solid, as the solvent is heated to maintain it at its boiling point. The solution is then allowed to **slowly** cool to room temperature undisturbed. Allowing crystals to grow slowly is important because crystals consisting entirely of the same repeating unit will have the most uniform and strongest intermolecular interactions. For pure crystals to grow, however, there must exist a thermodynamic equilibrium between solid- and dissolved-phase solute. If the solution is shocked and cooled rapidly, crystals will grow haphazardly and are more likely to incorporate impurities.

Once recrystallization is complete, the crystals must be separated from the mother liquor via suction filtration, washed a few times with the appropriate **ice-cold** solvent (to discourage the now-recrystallized solid from dissolving) and dried either in air or in a desiccator.

Recrystallization Using Solvent Mixtures

In many cases, a single solvent does not fit the above requirement satisfactorily, and as a result, a mixture of solvents is used. By using a solvent mixture, we are attempting to create a solvent system that closely resembles an ideal solvent as shown in the Figure below.

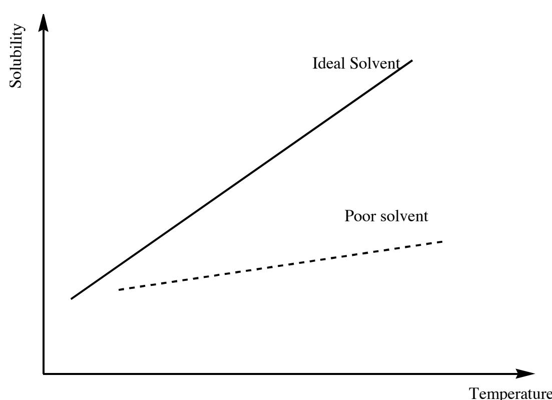


Figure 1. Ideal- and poor-recrystallization solvents.

In this situation, a solvent is chosen that will readily dissolve the solid. After dissolution, the system is filtered to remove any solid impurities (if necessary). A second solvent miscible with the first, but in which the solute has little solubility, is then added dropwise to the hot solu-

tion to achieve saturation. The first solvent in the dual solvent system readily dissolves the solid at high temperatures. However, at the boiling point of this first solvent, the solution is not saturated, and thus, this single solvent does not exhibit ideal behavior. Upon addition of the second solvent, the solution approaches saturation at the boiling point of this solvent system. This new dual solvent system best approaches ideal solvent behavior.

General Procedure

The material to be recrystallized is dissolved in the <u>minimum</u> amount of the solvent in which the compound is most soluble at its boiling point. While the solution boils, the solvent in which the compound is less soluble is added drop wise until it just turns the solution cloudy. If necessary, a little of the more-soluble solvent is added to clear the mixture; as the mixture cools, the desired compound will crystallize out. When using mixed solvents keep in mind that that they must be miscible so that separate layers do not form.

Table 1. Some Miscible Solvent Pairs For Recrystallization

Methanol - Water	Ether - Acetone
Ethanol - Water	Ether - Petroleum Ether
Ether - Methanol	Methylene Chloride - Methanol
Ethyl Acetate – Hexanes	Ethyl Acetate - Ether

In this list, the first solvent listed is the principal solvent used to dissolve the "impure" crystals. The second solvent, present in significantly lower quantity, enables saturation of the solution and thus initiates the gradual precipitation.

Melting Points

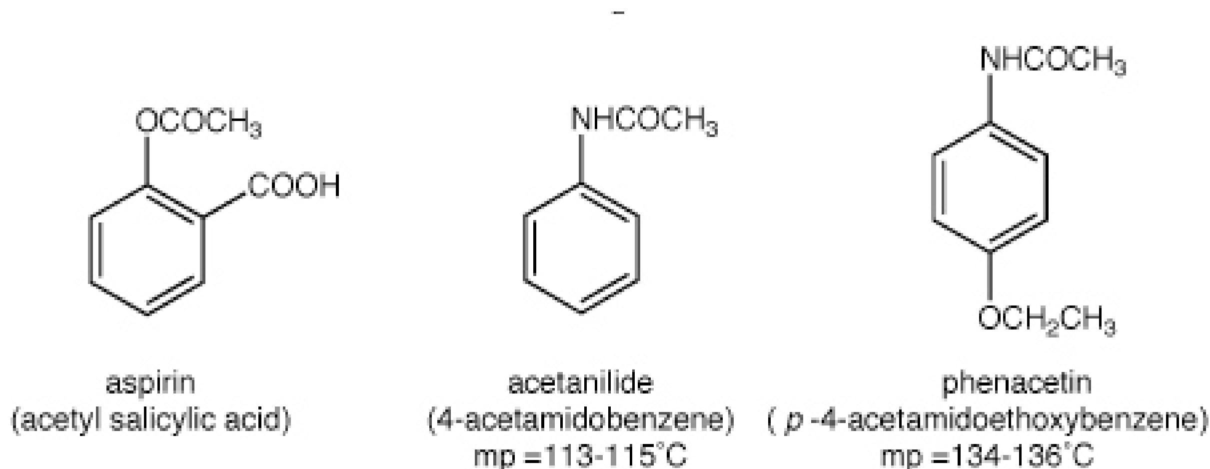
Once an organic solid has been isolated, the melting point range is measured to establish the compound's identity and purity. The melting point of a solid compound is the temperature at which a phase transition from solid to liquid occurs. This is a demonstration of *colligative properties,* which can be rationalized by the lowering of the vapor pressure of pure liquids due to the presence of impurities.

It should be apparent that the impurity must be soluble in the compound in order to cause a melting point depression; i.e., an insoluble impurity such as sand or charcoal will not depress the melting point. The impurity does not need to be a solid. It can be a liquid such as water or

an organic solvent. Melting points are generally measured and reported as a range rather than as a single discrete temperature.

EXPERIMENTAL OUTLINE

In this experiment you will separate a mixture of aspirin and an unknown that is either acetanilide or phenacetin by making use of their solubility and acid-base properties. You will purify the unknown by recrystallization and determine which of the two substances it is based on its melting point.



PROCEDURE

PART 1. Separation of Aspirin and Unknown

Before you begin, discuss how you might separate a mixture of aspirin and either acetanilide or phenacetin with you group. How can you take advantage of the acid-base properties of the compounds and their solubilities in aqueous and organic solvents?

1. Weigh about 3 g of the unknown mixture and transfer to a clean, dry 125 mL Erlenmeyer flask. Dissolve in approximately 50 mL dichloromethane. *Dichloromethane may be*

harmful if ingested, inhaled, or absorbed through the skin. Minimize contact with the liquid and handle it under the fume hood.

2. Prepare at least 100 mL of a saturated sodium bicarbonate solution (about 10% w/v). Add your solution from step 1 to a seperatory funnel and extract the aspirin in two 25 mL portions of $NaHCO_3$. Your mentor will demonstrate the proper use of a seperatory funnel. Collect the organic and aqueous layers in separate Erlenmeyer flasks. Why will aspirin be extracted into the aqueous layer?
3. Set your organic layer aside. **Slowly** add 6 M HCl to your aqueous layer while stirring until the pH is about 2. Cool the solution and filter off the solid by vacuum filtration. Wash your solid with cold distilled water and dry to constant mass. Take the melting point.
4. Dry your organic layer with sodium sulfate and gravity filter into a **pre-weighed** round-bottom flask. Evaporate the solvent using a rotovap and determine the mass of solid you obtain. Determine the melting point of your crude solid.

PART 2. Recrystallization of Unknown

5. Dissolve your solid in a minimal amount of boiling water in an Erlenmeyer flask. Why is an Erlenmeyer flask ideal for recrystallizations?
6. If the solution is colored, add a small amount of activated carbon and gravity filter the hot solution into a second flask. Add additional hot solvent to your solution before filtering and use a funnel pre-heated with vapors from your boiling solvent to prevent recrystallization and loss of product.
7. Heat your solution until the solute is completely dissolved and then allow it to cool to room temperature. Cool for 10-15 minutes on an ice bath to complete the recrystallization and collect the crystals by vacuum filtration.
8. While your solution cools, convince yourself that water is an appropriate solvent for recrystallization by performing three solubility tests. Take about 10 mg of your crude unknown (the tip of a spatula) and place in a test tube with about 0.3 mL of either distilled

water, hexanes, ethyl acetate, acetone, or ethanol. Observe the degree to which the solid dissolves at room temperature, at 0°C, and at the solvent's boiling point.

9. After your recrystallized unknown is sufficiently dry, determine its melting point and identify it as either acetanilide or phenacetin. Confirm your results by grinding a 50/50 mixture of your unknown and a pure sample of the compound you suspect, and determine the melting point. How does this technique work to confirm your result?

EXPERIMENT 4

Separation of Liquid Mixtures by Distillation

BACKGROUND

Distillation is one of the most useful methods for the separation and purification of liquids. It is perhaps the oldest separation technique known. It is most commonly used to purify a liquid from either liquid or solid contaminants by exploiting differences in their boiling points.

Types of Distillation

Simple distillation can separate compounds cleanly if the difference in boiling points between the compounds is greater than 70 °C. The process is useful to further purify relatively pure liquids, to get rid of volatile organic solvents, or to separate the liquid from polymerization products and mineral impurities.

Fractional distillation is a separation technique that is used when the boiling point differences of the compounds in a mixture to be separated are not large enough to employ the simple distillation technique. The components to be separated are collected in different fractions, whose identity are usually then confirmed via spectroscopy or TLC (thin layer chromatography). The first fraction, (or the first mixture between fractions) usually just a few drops, is called the *forerun*. It contains any highly volatile substances that were present in the sample, and is a kind of first rinse of the distillation glassware. It may be combined with the next fraction if analysis warrants.

Vacuum distillation is used to purify compounds that can decompose before reaching their boiling point at atmospheric pressure. By lowering the gas pressure above a liquid, that liquid can be encouraged to boil at a lower temperature. This is important if it would break down at a higher one.

Steam distillation is also used for high boiling point substances that decompose before the boiling point is reached. In this case, instead of using a vacuum, the liquid in question is mixed with another, immiscible liquid. The presence of the second liquid causes both to boil at a temperature lower than the regular boiling points of either liquid. For example, naphthalene, a

solid at room temperature that boils at 218°C, can be melted and mixed with water. Because the two are immiscible, they will steam distill at a temperature around 90°C, less than 100°C (the boiling point of water) and well below 218 °C. In all steam distillations, the distillate collected will be a mixture of both liquids, but since they are immiscible, they can generally be separated easily via extraction.

PROCEDURE

Fractional Distillation Using Microlab

In this experiment you will determine the composition of a mixture of ethanol and water. You will separate the components of the mixture by fractional distillation. You will use the MicroLab data acquisition software to monitor the progress of the distillation. A thermistor (an electronic temperature sensor) will be used to observe the boiling points of the components of the mixture, and the volume of each will be determined by collecting the fractions in a graduated cylinder.

PART 1. Calibration of Thermistor.

At the beginning of the experiment, the thermistor must be calibrated to ensure accurate temperature readings. You will calibrate the thermistor using samples of water at well-defined temperatures.

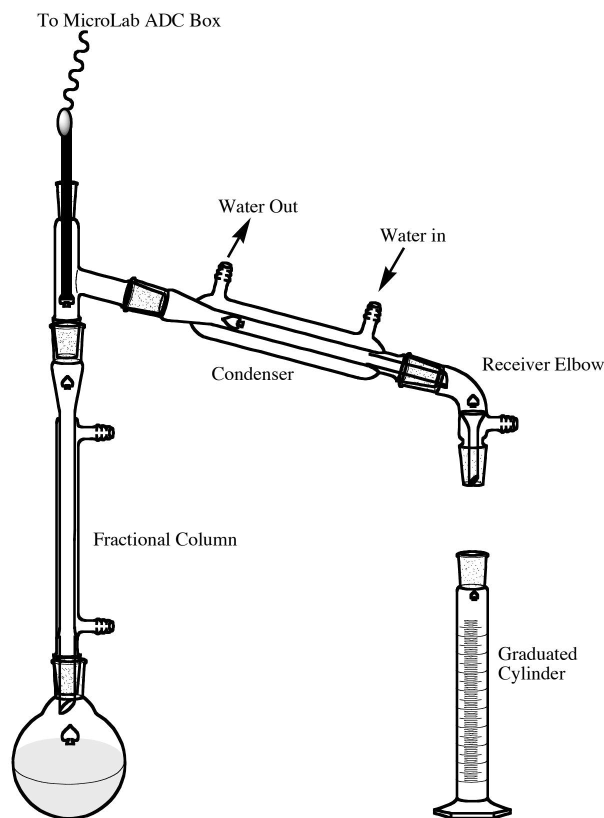
1. For the calibration, you will need about 50 mL of boiling water. Heat this water in a beaker on a hot plate. Remember to add a boiling stick!
2. Connect the MicroLab thermistor to a port on the MicroLab interface and open a new experiment.
3. Click "Add Sensor," select "Temperature (thermistor)" from the pull-down menu, and select the port where you inserted it. Click "Next" to bring you the thermistor calibration window.
4. Click "Perform New Calibration." This will open another window entitled "Calibrate a Sensor."

5. Click “Add Calibration Point.” Insert the thermistor and a thermocouple into the beaker of boiling water. You should notice the measured value of the thermistor increasing. Once the rate of change of the measured value becomes constant, read the temperature on the thermocouple (to the nearest 0.1°C) and type that in as the actual value. Then click “OK.”
6. Prepare an ice bath (with crushed ice and some water) in a recrystallizing dish. Insert the thermistor and the thermocouple and calibrate this point as you did for the boiling water. Do the same for a sample of lukewarm tap water.
7. In the “Calibrate a sensor” window, you should see a graph of the three calibration points you made. To obtain a best-fit curve, select “First Order (Linear)” from the choices on the left. Click “Accept and Save this Calibration.” A small window will open asking for units; enter “(C).” Save the calibration as “Thermistor Calibration.” (If this file already exists, replace it.) Click “Save” and then click “Finish.”

The thermistor is now calibrated to track the temperature of the vapor during the distillation.

PART 2. Distillation Setup.

1. Place about half of your sample (roughly 10 mL) in a 50 mL round bottom flask. Since you will be boiling this solution, add a magnetic stir bar. Why is it important to use a stir bar in a distillation?
2. Set up a distillation apparatus according to the diagram below. Clamp the 50 mL round-bottom flask (the pot) at least a foot above the floor of the hood and construct a fractionating column by packing a condenser with steel wire or glass wool. You should **lightly** grease all joints in your setup unless they are still greasy from previous usage.



Fractional Distillation setup

3. Insert the thermistor into a rubber thermometer adapter fitted onto a glass straight tube adapter by placing a drop of glycerol onto the bulb of the thermometer and gently pushing the thermometer through the thermometer adapter. Your TA will demonstrate the proper way to insert a thermometer. To avoid injury make sure that as you push you gently rotate the bulb of the thermometer. Proceed slowly.
4. Adjust the thermistor so that the bulb is completely below the sidearm (leading to the condenser) in the three-way adapter. This will result in an accurate temperature reading because the thermistor will be totally immersed in boiled vapor. Seal up the hole using teflon wrap to prevent vapors from escaping during the distillation.
5. Seal the condenser to the open joint of the three-way adapter and clamp it. Use a blue plastic clip to help hold it in place. Attach a vacuum adapter to the end of this condenser and clip it as before.

6. Place a 100 mL graduated cylinder at the bottom of the vacuum adapter. This last flask, the collection flask, should be surrounded with an ice bath to ensure that any liquid you collect does not evaporate out.
7. Place a stirring-hot plate on a jack under your pot. This plate will be used only to stir and not to heat.
8. Obtain a heating mantle and plug it into a variac. Plug the variac into the wall and switch it on. The variac is a device that modulates AC current. Wall current is 120 V; a variac can adjust that to anywhere between 0 and 120 V. Plugging the heating mantle into a variac allows you to control the temperature by controlling the voltage supplied to it. Test the mantle and variac by briefly turning the dial up to 100%. The mantle should get warm within ten seconds or so. Turn the variac down to 0 once you have confirmed that it and the mantle work. The variac should be left at 0 until you are ready to heat. Place the heating mantle on the hot plate under your pot.
9. The hot plate and mantle should be lifted up on a jack. Raising your heat source allows you to quickly remove it in case of violent boiling or fire. If you need to cool your pot quickly, you can simply lower the jack. Without the jack, you would have to slide the hot mantle or hot plate out or lift the hot pot and risk a burn.
10. The last step towards completing the distillation apparatus is running water through the condenser. What would be the best way?
11. Ask your TA to inspect your setup before beginning to heat. When ready, start stirring and turn on the variac. It should be set low at first, to 20-30%, to avoid boiling too quickly. If, after a few minutes, the solution does not boil, you may increase the variac to 50% or so. Ask your TA before turning it any higher. If increasing the variac setting doesn't work, you may want to insulate your pot with some cotton wrapped in aluminum foil. Avoid heating too quickly--boiling too fast can cause the vapor to travel past the thermometer too quickly, resulting in an artificially low measured boiling point. Furthermore, too much heat could cause your compound to decompose.

You should eventually see your liquid boil. Monitor the temperature on the MicroLab software. Typically the measured temperature will stay at or near room temperature until the boiled liquid (now a gas) rises up to the thermistor, at which point the temperature will jump up rapidly to the boiling point of that liquid. As your liquid boils, the temperature should stay more or less constant as newly formed gas continues to warm the thermistor. As you distill, you should also see drops of liquid roll down the inside of your condenser and fall into your collection flask. Continue distilling until the pot is nearly dry (less than 25% of its original volume). Again, you should never distill to dryness. Lower the hot plate and heating mantle and switch off the variac. Remove your collection flask (now full of your compound) from the ice.

EXPERIMENT 5

Visible Spectroscopy of Kool-Aid

BACKGROUND

This experiment will introduce you to spectroscopy. Spectroscopy is a field of study that deals with the interaction of matter and **electromagnetic** (EM) radiation. Matter can absorb, reflect or scatter EM radiation (although in most cases there is actually a combination of these interactions). Depending on the mode of interaction, we can distinguish between absorption spectroscopy, reflection spectroscopy and scattering spectroscopy. Different regions of the electromagnetic spectrum indicate different categories of spectroscopy such as visible (Vis), ultraviolet (UV), infrared (IR), and microwave.

Spectroscopy is used for the identification and quantitative measurement of organic and inorganic compounds in products such as foodstuffs, pharmaceuticals, paints, etc. The method is essential in both research and quality control in fields such as medicine and the life sciences.

Beer-Lambert's Law

The fundamental value considered in absorption spectroscopy is **Transmittance (*T*)**, defined as the ratio of the intensity (*I*, light power per unit area) of light after passing through the medium being studied to the intensity of the light before encountering the medium (I_O):

$$T = \frac{I}{I_o}$$

Transmittance can be expressed as a percentage:

$$\%T = \frac{I}{I_o} \text{ x } 100$$

Often the same spectroscopic information that is reported as percent transmittance is more conveniently expressed as **Absorbance (A)**; mathematically:

$$A = -log_{10}T = -log_{10}\frac{I}{I_o}$$

Beer-Lambert's Law is expressed as:

$$A = \epsilon c l$$

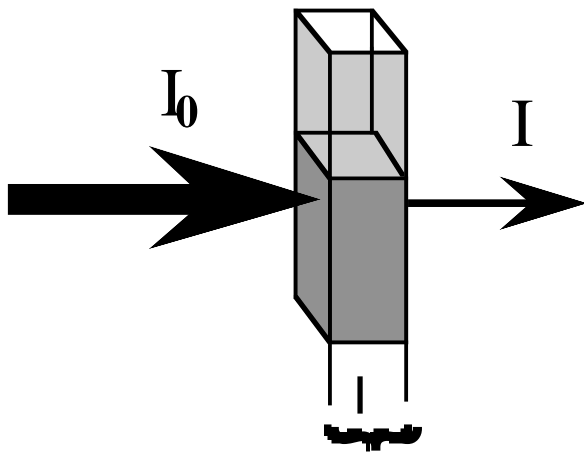
where: ε is the *extinction coefficient* (M^{-1} cm^{-1}).

c is the molar concentration of an absorbing substance (M or mol/L).

l is the path length through which the light travels (cm).

When most of the light striking a sample is absorbed, almost no light is transmitted; the medium is said to have a high absorbance and a low transmittance. The amount of light absorbed is directly proportional to the amount of sample present in the path of the light. This concept can be understood by examining the following diagram (figure 1):

Figure 1: Beer's-Lambert law



The intensity of the light beam diminishes as it travels through the absorbing medium. If the concentration (c) of the absorbing species is lowered (diluting the solution), then the absorbance decreases and the solution transmittance increases. The same effect is achieved if the path-length (l) is decreased by using narrower cuvettes.

The extinction coefficient, ε, is a proportionality constant which depends on the nature of the solute and solvent, and on the wavelength of the light passing through it.

Beer-Lambert's law is a limiting law: it holds true only for dilute solutions whose concentrations are less than 0.01 M for organic compounds. At higher concentrations, the average distance between the molecules of the absorbing species decreases such that each molecule affects the charge distribution of its neighbors or form aggregates, thereby causing deviations

from the linear relationship between absorbance (*A*) and concentration (*c*). Some organic molecules, like methylene blue, dimerize in solution even at concentrations below 10^{-6}M. In these cases the linear dependence of *A* vs. *c* is not observed. Finally, deviations from linearity can arise simply because of instrumental error.

Spectrophotometric Analysis

Different types of radiant energy affect matter in different ways. Radio waves cause molecules to rotate. Infrared light causes molecules to rotate, bend, and stretch (rotational and vibrational transitions); visible and ultraviolet light cause electrons to jump from one orbital of lower energy to another of higher energy (electronic transitions). In each case, the energy absorbed is specific to the end event (i.e., it takes *x* amount of IR energy to cause a molecule to rotate, but *y* amount of energy to cause a molecule to bend).

Organic molecules containing double bonds are often photoreactive. The double bonds in these molecules are formed by π electrons. Whenever double bonds alternate with single bonds (i.e., the double bonds are conjugated), the probability that an electronic transition will occur in the visible region of the EM spectrum is increased. Organic molecules that contain a conjugated system of π electrons are called chromophores. Good examples of such molecules are dyes, which are chemicals used to color fabrics, plastics, etc. They contain multiple bonds like C=C, C=O, etc., which may be conjugated to other groups to form complex chromophores. A typical example of a chromophore is β-carotene, which is the orange color in carrots (Figure 2).

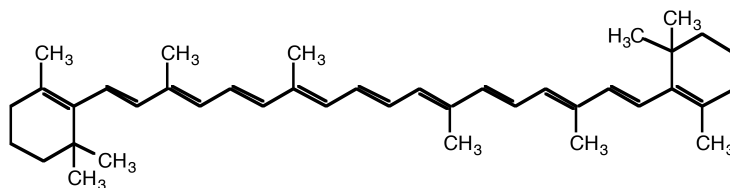
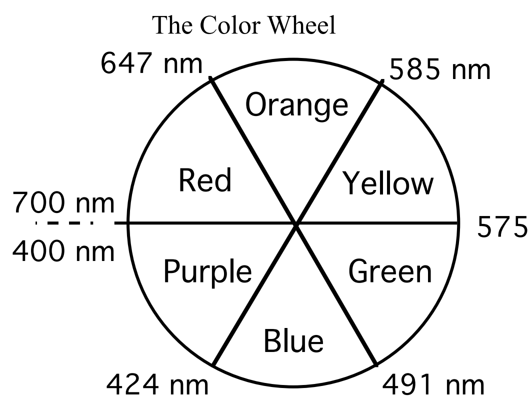


Figure 2: β-carotene, a chromophore

This molecule absorbs blue-green light (wavelength 460 nm), which is the complementary color of orange (620 nm).

In the case of transition metal salts and metallic complexes like copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), potassium permanganate ($KMnO_4$), iron (III) thiocyanate, ($[Fe(SCN)]^{2+}$), etc., the color observed depends on the oxidation state of the metal and involves different types of electronic transitions.

Visible Spectrum



Colors that are complementary are found opposite each other on the color wheel. Complementary colors combine to form white light. If only one color is filtered out from white light, such as yellow for example, light of the complementary color results, violet in this particular case. In color technology (paper, textiles, paints, and coatings) colored pigments are combined. Combinations of complementary colored pigments will form black.

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PROCEDURE

PART 1. The Spectrum of Kool-Aid

You will be provided with a stock solution of purple Kool-Aid (7.2 g/100 mL), the concentration of which is similar to that of a drinkable glass.

Use an LKB spectrometer to obtain a visible spectrum (400-700 nm) of a 1:5 dilution of your stock solution. Set the spectrometer's precision to "0-1A," and fill a cuvette about two-thirds with distilled water. This is your blank. Fill another cuvette with the diluted Kool-Aid solution and record the absorbance of this solution at wavelengths between 400 and 700 nm. Begin using increments of 20 nm, but reduce them to 10, 5, and 2 nm as you near the peak absorbance in order to observe the shape of the peak accurately and pinpoint the maximum absorbance. **Remember at each wavelength setting to first set the LKB to 0.000 Absorbance with the blank. Be careful to do this after you reach the desired wavelength, not before.**

Plot the Spectrum

Plot the absorbance vs. wavelength for this solution and discuss your results.

PART 2. Identifying the Dyes in Kool-Aid

In this part of the experiment, you will separate the dyes in purple Kool-Aid using column chromatography and identify them based on their wavelengths of maximum absorption. We will defer a complete discussion of chromatography until a later experiment. For now, understand that chromatography simply makes use of different physical properties of compounds in a mixture such as polarity or boiling point to separate them. Here, we will pack a column with silica gel, an inorganic compound that is much more polar than the organic compounds one is typically interested in separating (such as food dyes). As more polar components of a mixture have a greater affinity for silica than less polar ones,

they take a longer time to pass through a column. With a column that is sufficiently long, it is possible to completely (or almost completely) isolate the different components of a mixture.

Chromatographic Separation of Kool-Aid Dyes

You will prepare 2 miniature chromatography columns from Pasteur pipettes.

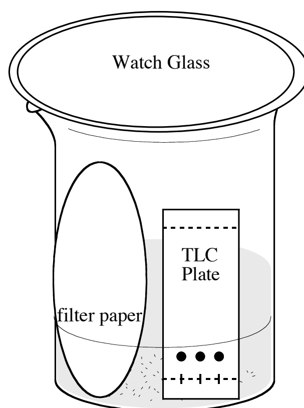
1. Carefully push a small piece of cotton down to the narrow part of the pipette. Clamp the pipette with a thermometer clamp and add about 0.5 cm of sand (use a second pipette as a funnel).
2. In a small beaker, dissolve enough silica gel in 10 mL distilled water to make a thick slurry. Make sure the slurry is well-stirred and pipette about 5 cm into your column. Handle the silica gel in the hood **as it is a carcinogen when inhaled**.
3. To pack the column and rinse excess silica gel off the sides of the column, force water through the column using a small pipette bulb until the top of the silica is level.
4. Add another 0.5 cm sand to the top of the column. Load the column by pipetting about 0.5 mL of your concentrated Kool-Aid solution to the top of the column. The Kool-Aid will separate into fractions as it progresses through the column. Elute them with distilled water and collect in separate test tubes. **Make sure your column never runs dry** by adding distilled water as necessary. To speed the process you may push the solvent through with a pipette bulb. Make certain that your mentor demonstrates this technique before attempting it!

Determining the Purity of your Fractions by TLC

1. With a pencil, *lightly* mark a baseline on a TLC plate, usually about 1 cm from the bottom. Do not touch the silica face of the TLC plate with your fingers, as the oils on your skin will contaminate the plate. On the very top of the TLC plate, label the spots in pencil according to what is being spotted. Spot the plate by dipping the micropi-

pette into the solution and touching it **lightly** to the TLC plate. Spot each of the fractions as well as the original solution. Be sure to use the matte (coated) side and not the plastic side.

2. In a beaker, add a small amount of water and a piece of filter paper as in the diagram below. The level of the solvent in the jar must be below the level of the spots, and the atmosphere in the jar should be saturated with solvent vapors. This is the purpose of the filter paper. If the jar is not saturated with solvent vapors, the solvent will not run all the way up the plate. Gently place your plate in the chamber. When the *solvent front* is near the top of the plate, immediately remove the plate from the beaker with forceps and mark the solvent front with a pencil before the solvent evaporates.



3. Calculate the R_f (distance traveled by the spot divided by distance traveled by the solvent) for your fractions.

Identifying the Dyes

1. You will be provided with stock solutions of two red dyes and two blue dyes. Using TLC, determine the R_f values of the dyes and make a hypothesis as to which of the dyes is contained in purple Kool-Aid.

2. Using an LKB spectrometer, determine the peak absorptions of the components you separated on your column. Based on their colors, choose an appropriate range of wavelengths to scan.
3. Prepare an approximately 1×10^{-5} M solutions of each dye and determine its peak absorption. Use your results to confirm your hypothesis of which dyes are contained in Kool-Aid.

RESULTS

Kool-Aid Spectrum

Tabulate the data you obtained for Kool-Aid as follows:

Wavelength (nm)	**Absorbance**	**Wavelength (nm)**	**Absorbance**	**Wavelength (nm)**	**Absorbance**

Maximum absorbance(s) of Kool-Aid peak(s):

Peak Absorbances and R_f Values of Dyes

	Name of Dye	Max. Absorbance of Dye	R_f
1			
2			
3			
4			

Dyes present in KoolAid:

EXPERIMENT 6

Quantitative Analysis of Kool-Aid, UV Spectroscopy of Sunscreens

You have already obtained the wavelength(s) of maximum absorbance for the dyes present in the KoolAid. In the next part of this experiment we will determine the concentration of the dyes present in the KoolAid and the number of glasses per day that would be unsafe to consume based on the established limits of the food dyes. You should also research the MSDS for the dyes contained in the KoolAid.

Experiment:

Preparing the calibration solutions

Stock solutions of the dyes will be provided to you: $1x10^{-4}$ M solution of Red dye and $1x10^{-5}$M solution of Blue dye. Using pipettes, test tubes and Parafilm (for mixing). Prepare the following solutions.

Tube #	RED DYE (mL)	Vol. distilled H_2O (mL)
1R	3.0	7.0
2R	2.0	8.0
3R	1.0	9.0
4R	0.5	9.5
5R	0.3	9.7
6R	0	10

Tube #	BLUE DYE (mL)	Vol. distilled H_2O (mL)
1B	10.0	0.0
2B	7.0	3.0
3B	5.0	5.0
4B	3.0	7.0
5B	1.0	9.0
6B	0	10.0

Using a new square of Parafilm for each, gently mix the solutions.

Plotting the calibration curves

For each dye, set the spectrophotometer at the wavelength of maximal absorbance and set the absorbance to 0.000 with your blank. Record the absorbance of all the samples (blanking between readings of samples). **The readings may fluctuate so it is best to take each reading immediately.**

Plot a calibration curve for each of the dyes and obtain the equation of the lines and the correlation coefficients.

Use the equations of the lines to calculate the concentration of the dye solutions and use the molecular weight to calculate the number of grams in a regular glass (250mL) of KoolAid using the stock concentration of 7.2g/100mL as the concentration of drinkable KoolAid in a glass.

PART 2. UV Spectroscopy of Sunscreens

Ultraviolet violet spectroscopy will be used to create a spectrum of a solution that is colorless to the human eye.

Your instructor will demonstrate the use of the ultra-violet (UV) spectrometer. The sunscreen is ethylhexyl-methoxycinnamate (EHMC) will be used for this analysis. Quartz cuvettes are required in the UV region since plastic ones absorb UV light. The spectra is taken from 200 nm-400 nm

Sunscreens are prepared using the following procedure:

- You will be given a stock solution of 5-7 mg of EHMC dissolved in 50 mL of 2-propanol.
- Put 1 mL of that solution in a 10 mL vol. Flask and dilute to the mark.
- Take the UV spectrum between 200 and 400 nm.

RESULTS

Red	Dye(mL)	H_2O(mL)	Conc (M)	Abs
1R	3.0	7.0		
2R	2.0	8.0		
3R	1.0	9.0		
4R	0.5	9.5		
5R	0.3	9.7		
6R	0	10		

Blue	Dye(mL)	H_2O(mL)	Conc (M)	Abs
1B	10.0	0.0		
2B	7.0	3.0		
3B	5.0	5.0		
4B	3.0	7.0		
5B	1.0	9.0		
6B	0	10.0		

Concentration of dyes in the diluted solutions:

Amount of dye in a glass of KoolAid.

In what range of wavelengths does EHMC absorb the most light? Find out whether this range is classified as UV-A, B, or C.

EXPERIMENT 7

IR Spectroscopy

BACKGROUND

What is unique to chemistry is the molecule and the chemical bond. All molecules are made up of atoms held together by chemical bonds. These atoms vibrate with respect to each other with the bonds acting much like vibrating springs connecting them. Each molecule has a characteristic set of vibrational frequencies in the same range as the infrared frequencies of electromagnetic radiation. Infrared is the portion of electromagnetic radiation that extends beyond the visible into the microwave region. It is divided as follows:

-Near infrared (NIR) or overtone region from the visible region to 4000 cm^{-1}.

-Mid infrared, or fundamental region from 4000 to 400 cm^{-1}. This is the segment of the infrared most often used by organic chemists.

-Far infrared (FIR) is the low frequency region, which ranges from 600 - to 10 cm^{-1}.

Infrared spectral analysis gives the scientist a permanent, step-by-step record of his or her work. In carrying out a complex synthesis, for example, one can determine the identity and purity of reactants, follow changes taking place during conversion to products, determine yields and product purity. Furthermore, there is never any fear of having insufficient sample to proceed to the next stage of a synthesis or process since infrared analyses are nondestructive. The analytical sample is fully recoverable and reusable. Engineering processes such as distillation, oxidation and polymerization can be monitored on-line by direct sampling. In the near infrared (NIR) region, fiber optics are used to conduct radiation to and from sample and spectrometer.

Types of Vibrations

If infrared radiation of a given frequency strikes a sample composed of molecules having a vibrational frequency the same as that of the incident radiation, the molecules absorb radiation

and the energy of the molecule is increased. However, if the incident frequency differs from the characteristic frequencies of the molecule, the radiation passes through undiminished, or in some experiments, it is reflected. The vibrational frequencies for a particular molecule are determined primarily by the masses of the atoms in the molecule and the strengths of the bonds connecting them. For a diatomic molecule, this frequency can be derived from Hooke's law and is written as:

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

Where ν is the frequency of vibration; k is the bond force constant (dyne/cm) and μ is the reduced mass of the atoms in the bond:

$$\mu = \frac{m_1 \times m_2}{m_1 + m_2}$$

Furthermore, the proximity and spatial geometry of various groups of atoms often influence vibrational frequencies. Collectively, these characteristic vibrational frequencies "fingerprint" molecules, uniquely distinguishing them. The IR spectrum of a chemical compound can be considered as an inherent physical property.

The most useful vibrational frequencies are the **fundamental vibrations**, those that occur when molecules absorb infrared radiation that promotes them to the first vibrationally excited state. On excitation to the second or third excited states (or beyond), the **overtone** vibrational frequencies are observed. That is true for the frequencies of all mechanical oscillators. Coupling of fundamental vibrations produces new vibrations at frequencies above and below those observed in the absence of coupling. **Fermi resonance**, due to interactions between fundamental vibrations and overtones, is not uncommon in the infrared. This effect was first described for the vibrational spectrum of CO_2.

Molecular vibrations are also classified according to whether the vibrations are associated

with **stretching**, **bending or torsional** motions. Stretching motions can be symmetric or asymmetric. Bending motions are sometimes called deformations and are sub classified as scissoring, twisting, wagging, and rocking motions. Stretching and bending frequencies are dependent upon the masses of the vibrating atoms (or groups of atoms) and the stiffness of the spring-like chemical bonds connecting them (Fig. 1).

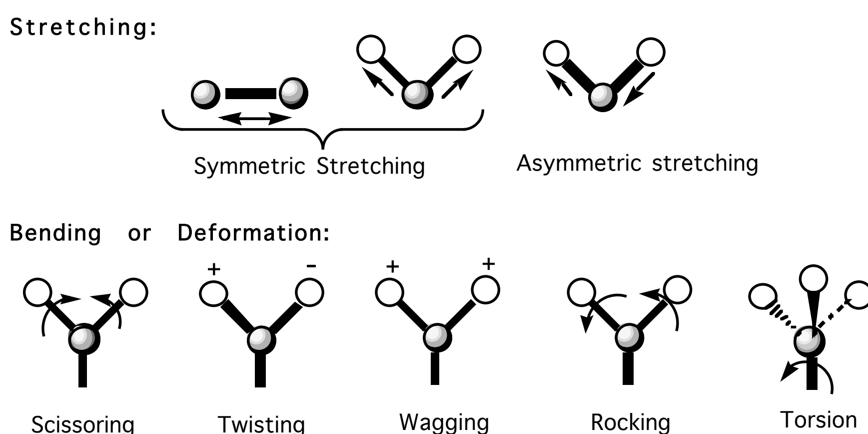


Figure 1. Types of vibrations.

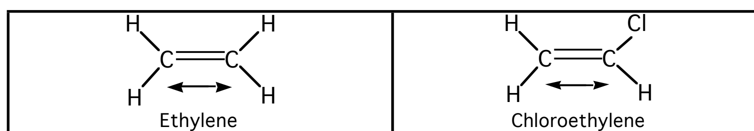
Stretching vibrations always have higher frequencies than bending vibrations of the same group (it's easier to bend a molecule than to strecht it). The smaller the masses of the atoms, the greater the vibrational frequencies. Likewise, the stiffer the bonds, the higher the frequencies. A bond's stiffness as measured by its force constant, is proportional to bond strength, which in turn is proportional to the bond order. That is, for groups with atoms of the same mass, a triple-bonded group has a higher vibrational frequency than a double-bonded group, and the double-bonded group has a higher vibrational frequency than a single-bonded group. A group bonded with a single bond that has partial double-bond characteristics, such as often results from resonance, has an intermediate vibrational frequency between those of the same group with "pure" double and single bonds.

Vibrations of groups where one atomic nucleus is a proton have the highest frequencies of all molecular vibrations. All stretching vibrations of hydrogen atoms occur above 2250 cm^{-1}. No other groups have fundamental absorptions in this region although overtones from lower frequency vibrations are sometimes observed above 2400 cm^{-1}.

Groups with triple bonds absorb in the next highest region of the spectrum, from 2300 to 2100 cm^{-1}. The only other principal group to have fundamental absorptions in this region are those with cumulated double bonds (2350 - 1930 cm^{-1}) such as ketene, $H_2C=C=0$. Cumulated double bonds absorb at higher frequencies than other double bonds (1900 - 1580 cm^{-1}) due to coupling between the bonds. Coupling, or mechanical interaction, occurs when two groups having similar frequencies are close to each other in the same molecule. In effect, resonance is established between the two vibrating groups, and vibrational energy flows back and forth between them so that the vibrations of the two groups modify each other. Another way of viewing this is that the coupling groups lose their individuality and vibrate together. Coupling is strongest when the two interacting groups share a common atom and the frequencies of the two groups are very close. Carbon dioxide is such a molecule. The stretching frequencies of most carbonyl groups are somewhere near 1700 cm^{-1}. In carbon dioxide the two carbonyl groups vibrate together with the asymmetric stretching vibration occurring at about 2350 cm^{-1} and the symmetric bending vibration occurring at approximately 1330 cm^{-1}. The symmetrical stretching vibration is actually a complex, widely spaced doublet due to Fermi resonance between an overtone and a bending vibration. The symmetrical stretching vibration itself does not cause an infrared absorption because no change in dipole moment occurs.

Selection Rules

If a pair or group of atoms is to absorb infrared radiation, it must undergo a change in dipole moment during the vibration. The changing dipole moment couples the vibration of the molecule with that of the radiation in much the same way as air couples the motion of a moving fan with another that is close by. IR inactive vibrational frequencies occur when the molecule contains a center of symmetry, which is retained upon vibration. The stretching of the carbon-carbon double bond in ethylene does not absorb infrared radiation because there is no change in dipole moment during the vibration.

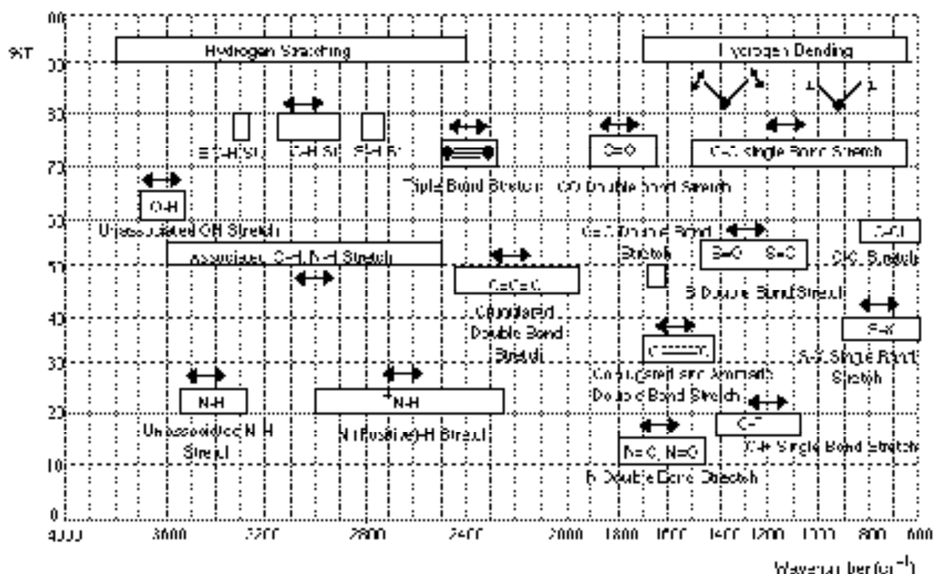


The stretching of the carbon-carbon double bond in chloroethylene causes a significant change in dipole moment, and this double bond has a strong infrared absorption. The changing dipole moment couples the electromagnetic radiation with the vibrating carbon atoms.

Qualitative Analysis

Infrared spectrometers measure the vibrational spectrum of a sample by passing infrared radiation through it and recording which wavelengths have been absorbed and to what extent. Since the amount of energy absorbed is a function of the number of molecules present, such instruments provide both qualitative and quantitative information. The recorded spectrum is a plot of percent transmittance (or absorbance) versus wavenumber or wavelength of the radiation. Since the spectrum is a fundamental property of the molecule, it can be used both to qualitatively characterize the sample and to quantitatively determine concentration. The fingerprint provided by the infrared spectrum contains basic information about the composition and structure of a compound. Organic compounds contain functional groups such as -OH, $-NH_2$, $-CH_3$, -CO, -CN, -C-O-C-, -COOH, -CS, etc.... each of which has characteristic absorption frequencies in the infrared that are usually relatively unaffected by the remainder of the molecule. When they are affected by the rest of the molecule, additional information about its structure can be obtained. The regions and the spectral correlations for some representative vibrations are summarized in Fig. 2. Consequently, an unknown compound can often be characterized by observing the presence of the absorption frequencies associated with such groups.

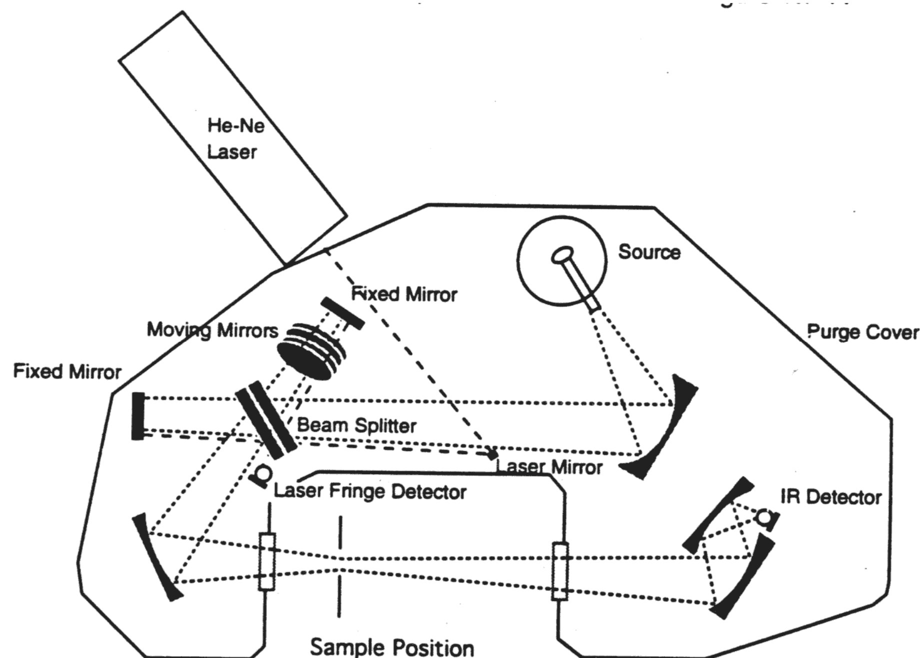
Spectral correlation charts of characteristic absorption bands for most of the common



Absorption of different regions of the Mid IR spectrum

functional groups make it possible to determine the gross structural features of an unknown by band identification. Commercial instruments contain large data files with more than 150,000 spectra, which may be searched to select the best matches between the unknown and the library (Aldrich Library, etc). If, however, the investigator does not have access to a reference spectrum of the compound under investigation, it can often be identified by functional group analysis together with a few easily determined physical and chemical properties such as melting point, index of refraction, and chemical analysis. A great advantage of infrared spectroscopy is that the interpretation of the infrared spectrum of a compound is based directly on the nature of the chemical bonds in the molecule.

Fourier Transform Infrared Spectrometer



OVERVIEW

Interferometry for chemical applications was first applied to the far infrared region. The main factors that influenced this were the relatively low energies emitted by the IR sources, relative simple optical requirements, and simplified mathematical requirements due to the fact that relatively few data points were required to perform the Fourier transformation. The development of a fast computational method (Cooley-Tookey algorithm) and microelectronics expanded the use of interferometers to the NIR and fundamental Mid IR regions. The purpose of this section is to highlight the principles behind the functioning of Fourier Transform Infrared (FT-IR) spectrometers and therefore facilitate your use of the instrument in the laboratory.

OBJECTIVES

After finishing this session and completing the individual write-up, you should be able to:

- Explain in your own words the theory behind interferometry.

- Summarize the operation of the FT-IR Spectrometer.
- Set the main parameters to collect the infrared spectrum of a given sample.

INTERFEROMETRY

Most Fourier Transform Infrared (FT-IR) commercial spectrometers are based on the Michelson interferometer. Michelson designed this device and together with Morley used it to disprove the existence of the ether, the hypothetical medium through which light waves were thought to propagate.

The **Michelson interferometer** is essentially an optical assembly composed of a beamsplitter, a moving mirror, and a stationary or fixed mirror. The interferometer is used to split a beam of radiation into two beams and then recombining them after a path difference is introduced. The division of the beam is achieved with the **beamsplitter**, which transmits about 50% and reflects about 50% of the radiation.

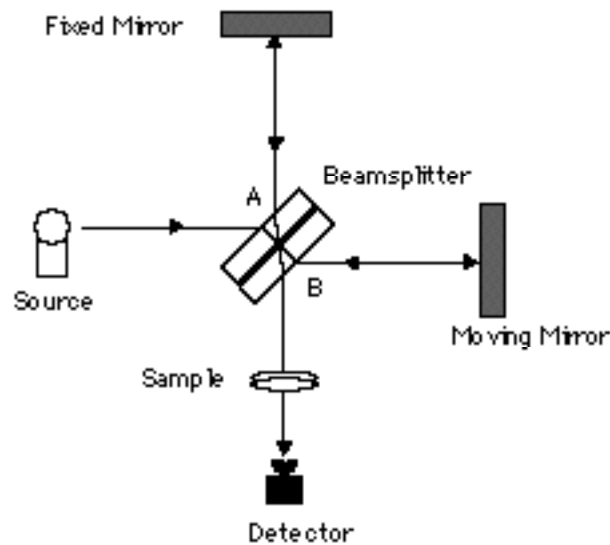


Figure 3. Michelson Interferometer

If radiation from an infinitely narrow line source hits the beamsplitter (Figure 3), one part of the beam (A), goes to the fixed mirror and the other beam (B), to the moving mirror. When the beams are recombined, an interference pattern is obtained as the Optical Path Difference (OPD) or the retardation δ is varied (OPD = 2 x mirror displacement). If the position of the moving mirror is such that beam (B) travels exactly the same distance as beam (A) before reaching the detector then the two beams will be in phase and will reinforce each other. Constructive interference occurs every time the value of the retardation δ is an integer multiple of

the wavelength or $\delta = n\lambda$; therefore, the energy arriving at the detector as a function of retardation $I(\delta)$ will be a maximum. On the other hand, if the position of the moving mirror is such that the optical pathlength of beam (B) is different from that of beam (A) by

$$\left(n+\frac{1}{2}\right)\lambda \text{ (where } n = 0, \pm 1, \pm 2, \ldots)$$

then the two beams are 180° out of phase and will exactly cancel each other on destructive interference. In this case the energy that reaches the detector is a minimum. Thus, as the moving mirror travels a certain distance, an interferogram as in Figure 4 will result. The intensity of the radiation at the detector, $I(\delta)$, varies as a cosine function of the optical retardation δ.

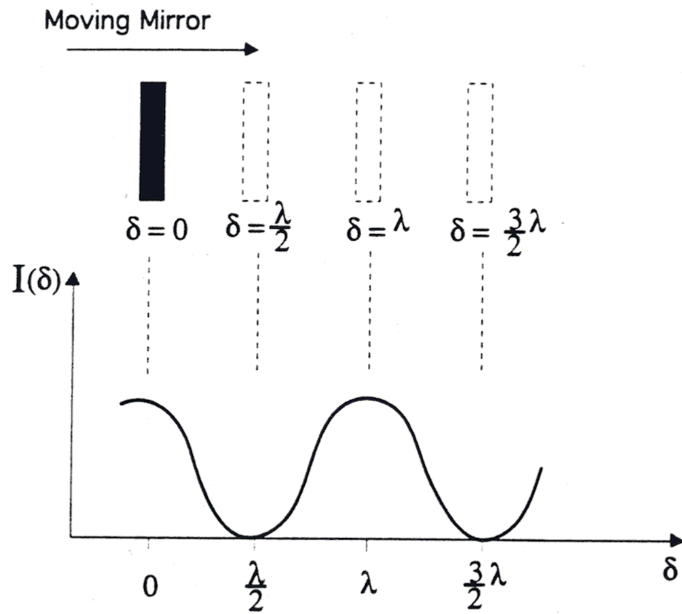


Figure 4. Interferogram of a monochromatic, infinitely narrow line source

The resulting interferogram is described as an infinitely long cosine wave defined by the equation:

$$I(\delta) = B(\tilde{v})\cos\left(2\pi\frac{\delta}{\lambda}\right) = B(\tilde{v})\cos(2\pi\tilde{v}\delta) \qquad (1)$$

$I(\delta)$: Intensity of the detector signal as function of retardation, δ

$B(\tilde{v})$: Intensity of the detector signal as function of wavenumber, $\tilde{v}$

If the source emits more than one frequency (polychromatic radiation) every frequency is treated as if it resulted in a separate train of cosine waves. The resultant interferogram is obtained after adding the cosine waves geometrically, as in Figure 5. Mathematically this can be expressed as:

$$\sum_{\tilde{v}_1}^{\tilde{v}_n} B(\tilde{v}_i)\cos(2\pi\delta\tilde{v}_i) \qquad (2)$$

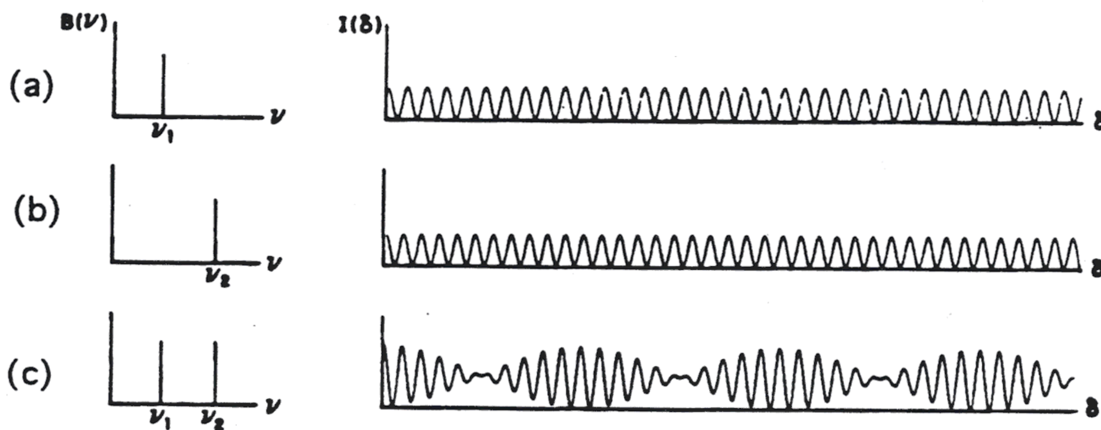


Figure 5. The interferogram of a single frequency source is a cosine function with a periodicity that varies with the frequency of the emitting source (a and b). The interferogram of a two-frequency source may be calculated by geometrically adding the cosine functions corresponding to each of the individual lines in the source (c).

As the moving mirror changes positions, it causes the waves that compose the infrared radiation undergo constructive and destructive interference when they recombine at the beamsplitter, producing an interferogram plot of the detector response, intensity in volts vs. the position of the moving mirror or retardation in cm (Figure 6).

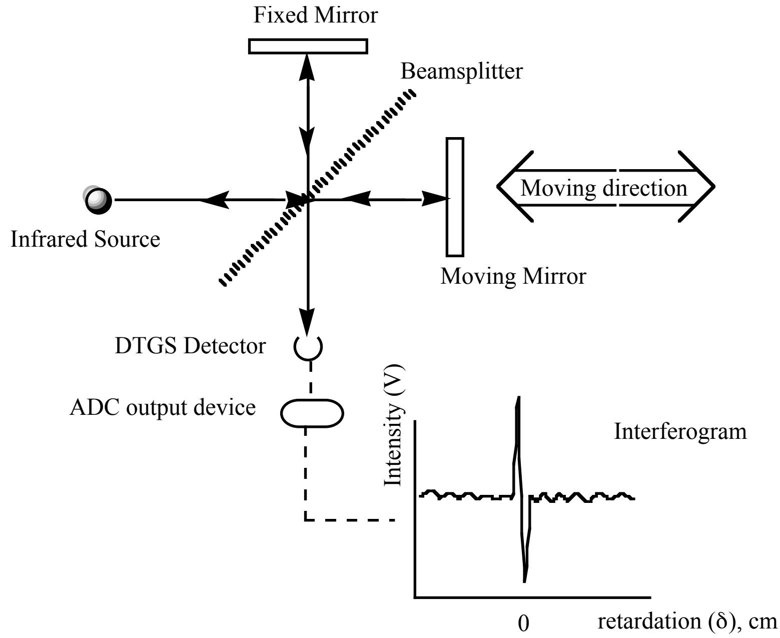


Figure 6. Interferogram generation from a polychromatic IR source

For an IR source such as a nichrome wire electrically heated, the interferogram can be interpreted as a sum of cosine waves from each contributing frequency. For a continuous infrared source, the sum (2) goes over to an integral (3):

$$I(\delta) = \int_{-\infty}^{\infty} B(\tilde{v}) \cos\left(2\pi \frac{\delta}{\lambda}\right) = \int_{-\infty}^{\infty} B(\tilde{v}) \cos\left(2\pi\tilde{v}\delta\right) d\tilde{v} \qquad (3)$$

Resulting the interferogram shown in figure 7, the primary data obtained with a FT-IR spectrometer:

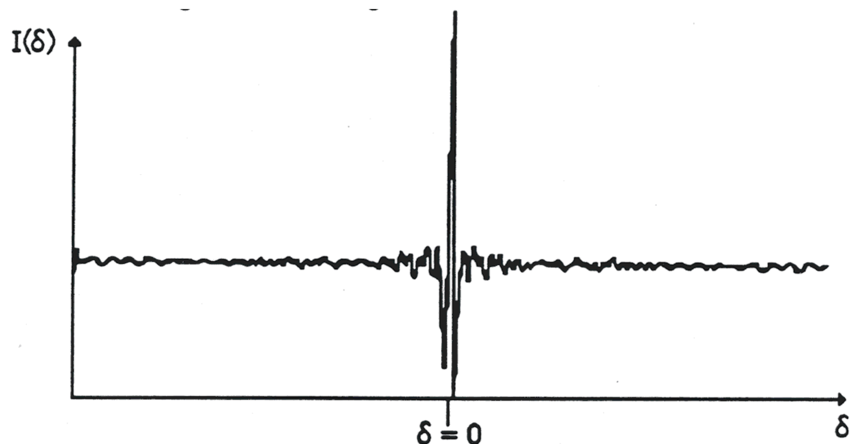


Figure 7. Interferogram of a typical infrared source

Note that at zero retardation, δ = 0, all the waves are in phase, this maximum is called the *centerburst* or the Zero Path Difference (ZPD) of the interferogram. Moving away from the ZPD in either direction, causes the intensity of the interferogram, I(δ), to die off as contributions from the various frequencies go in and out of phase with each other. The "spectrally meaningful" information is extracted from the wings of the interferogram as an infrared spectrum (plot of absorbance or %transmittance vs. reciprocal cm) by an operational amplifier that performs the mathematical operation called Fast Fourier Transform (FFT, conversion of the interferogram into a sum of sine and cosine functions).

The interferogram is measured by recording the detector signal as a function of the optical path difference between the two beams. The signal has to be sampled at precise intervals corresponding to equal steps in path difference. For signal averaging, successive interferograms have to be measured at exactly the same points. This is achieved by using a helium-neon laser as a reference. Radiation at exactly 632.8 nm traverses the same optical path as the IR beam. A separate detector measures the interferogram produced, giving a sinusoidal signal with maxima separated by the laser frequency at 15,803 cm^{-1}. This signal is used to trigger the sampling of the IR signal very reproducibly.

An interferogram contains all of the IR absorption information albeit in a form not useful to a chemical spectroscopist. The Fourier transform of this interferogram gives the conventional spectrum. The relationship between the interferogram and the spectrum is given by equation (5).

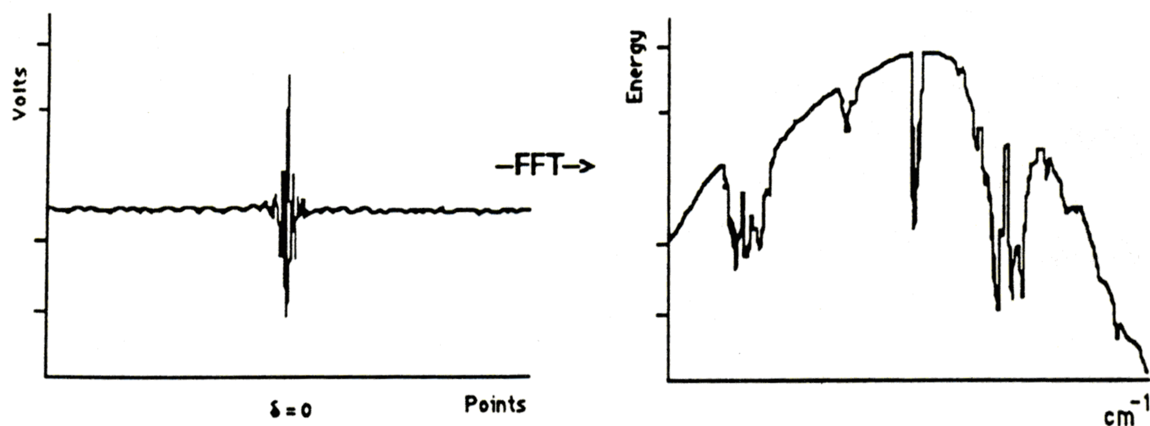
This equation is the Fourier Transform pair of equation (3) given above.

$$B(\tilde{\nu}) = \int_{-\infty}^{\infty} I(\delta)\cos(2\pi\tilde{\nu})d\delta \qquad (5)$$

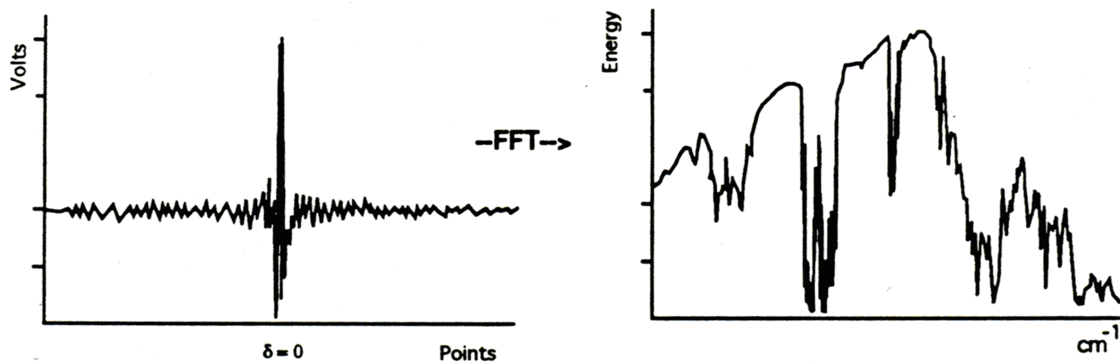
The Fourier Transform operation changes the domain of the original data into its reciprocal; in this way the space domain data (interferogram) is turned into a reciprocal space domain data (the spectrum) as shown below. Notice that he slope of the single beam spectra obtained accurately resembles the intensity distribution of a black body radiator with the absorptions due to sample and air respectively embedded in the distribution.

Generation of a FT-IR spectrum

The FT-IR spectrometer generates the infrared spectrum of a given sample by calculating the ratio of the signal obtained by scanning air (empty beam) to the signal obtained by scanning the sample. The process is schematically illustrated in the figures below (Figs 8-9): first an interferogram of the source (background) is scanned, transformed into a single beam spectrum, and stored in computer memory:



The sample, in this case a polystyrene film, is then placed in the sample holder and the process is repeated:



The ratio between the two single-beam spectra, in computer memory, is calculated and the "double beam" presentation with a flattened baseline is produced. The features present in the background spectrum correspond to the emission profile of the source, the optical efficiency, or detectivity of the detector, the absorption of the film optical coatings, atmospheric water, and gaseous CO_2. The ratio process compensates for these bands and they don't appear in the spectrum of the sample as shown below.

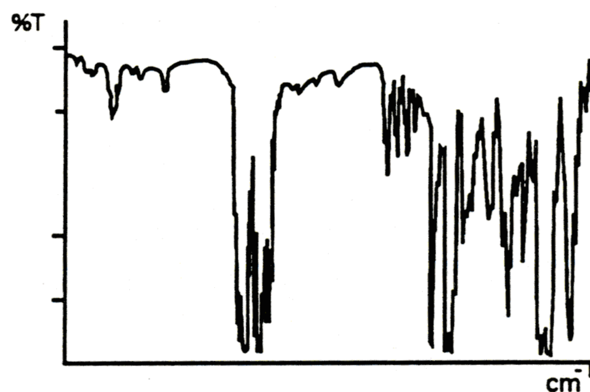


Figure 10. IR spectrum of polystyrene.

(a) Source

The most frequently used sources in the Mid IR region are Nernst Glowers, cylinders composed of a mixture of zirconium, yttrium and thorium, Globars which are silicon carbide rods, and incandescent wires of nichrome, or rhodium sealed in a ceramic cylinder. All of these sources exhibit the energy distribution of a theoretical black body radiator. However, the thermal sources are inefficient in the far infrared region, and the mercury arc, a quartz jacketed tube filled with Hg vapor has to be used.

(b) Beamsplitter

For the Mid–IR range 4,400 to 450 cm^{-1}, the beamsplitter consists of a thin film of Germanium entirely deposited on a potassium bromide plate, except on its center for transmission purposes, this plate is covered by a clear potassium bromide plate. Plates of CsI covered in the same way with Silicon are also used. A film of iron (III) oxide deposited on $CaCl_2$ plates is used for Near IR, and for Far IR applications a thin film of Mylar sandwiched between two plates of a low refractive index solid is used.

A Mid IR spectrometer should never be turned off, which would cause water condensation over the hygroscopic alkali beamsplitter, which will obscure the center of the plate impeding the transmission of light. If the instrument needs to be moved, it has to be packed with a dehydrating agent such as calcium sulfate to avoid damage to the beamsplitter and alkali windows.

(b) Detector

FT-IR spectrometers use either pyroelectric or photoconductive detectors. Pyroelectric detectors are a special kind of thermal detector consisting of single, thin, pyroelectric crystals such as deuterated triglycine sulfate (DTGS) or LITA (lithium tantalate). When a pyroelectric material is polarized by an electric field, it remains polarized after the field is removed due to an effect called residual electric polarization. This residual polarization is sensitive to changes in temperature.

Photoconductive detectors show an increase in electrical conductivity when illuminated with IR radiation. They have a rapid response and high sensitivity. The most commonly used photoconductive detector is the MCT (Mercury Cadmium Telluride), which must be cooled to liquid nitrogen temperatures for proper operation.

FT-IR Scanning parameters

- **Scanning Time**: One scan taken with an FT-IR spectrometer is equivalent to a complete displacement of the moving mirror from the initial position to the final one. This change of position is referred to as the Optical Path Difference (OPD) or retardation because it

sets the difference between the paths of the beams of light that come from the fixed mirror and from the moving mirror and recombine at the beamsplitter as explained above. The largest the distance traveled by the moving mirror, corresponds to the highest resolution that can be achieved. For this reason, the scanning time increases with resolution.

- **Signal to Noise Ratio (SNR)**: Indicates the quality of the baseline of the sample's infrared spectrum; mathematically, the SNR is a comparison of the size of the noise to the size of the signal. The SNR improves with the number of scans acquired because of the averaging nature of the data acquisition: after averaging each scan the signal increases in size while the noise diminishes. The dominant noise in FT-IR is the detector-limited noise, which varies as the square root of the number of scans ($\sqrt{N}$ for N scans). Hence, combining these two factors, the SNR varies with the number of scans N as $\frac{N}{\sqrt{N}} = \sqrt{N}$.

  Acquiring a larger number of scans will improve the SNR or a cleaner baseline spectrum. For transmission experiments of condensed phase samples, 4 scans will suffice to obtain a good SNR, because of the high energy involved in the measurement. For gases, at least 16 scans must be acquired to assure good SNR. When reflectance accessories such as Diffuse, Specular, or Total reflectance are used, a minimum of 64 scans must be collected due to the limitation on the energy that reaches the detector or optical throughput.

- **Resolution** (spectral resolution): Indicates the ability of the spectrometer to distinguish two closely spaced vibrational or rotational modes. The resolution of the interferometer increases with δ and therefore with mirror travel. To a good approximation, the best resolution is given by

$$\Delta\tilde{\nu} = \frac{1}{\Delta} cm^{-1} \qquad (4)$$

  Where: $\Delta = \delta_{max}$ is the maximum mirror travel distance. Since this mirror displacement cannot be infinite in an actual spectrometer, the resolution is somewhat less, as discussed below. For condensed samples acquired in transmission mode, a resolution of 8 cm^{-1} will

resolve most vibrational modes, which full width at half height (FWHH) is about 10 cm^{-1}. This resolution also allows the acquisition of a large number of scans at a relatively short time improving the SNR. If the rotational fine structure is required for the calculation of molecular parameters for gas samples, the best resolution of the spectrometer must be utilized together with the acquisition of a large number of scans for good SNR. Apodization functions limit spectral resolution, as it will be explained below, so for gas samples a simple truncation function called boxcar must be used.

- **Apodization**: The mathematical form of the Fourier pair, equations (3) and (5), sets the integration limits to retardation values from $-\infty$ to $+\infty$. However, integration is physically impossible because the moving mirror travels a finite distance from $-\Delta$ cm to $+\Delta$ cm, which is determined by the design of the mirror drive. Setting these limits is equivalent to truncating the interferogram, or setting it equal to zero for all values greater than Δ and less than $-\Delta$. This is known in mathematics as convoluting the complete interferogram ($-\infty < \delta < +\infty$) with a function that has the value 1.0 for ($-\Delta < \delta < +\Delta$) and zero for values of δ outside these limits (Figure 11). Because of its rectangular shape, this function is called **boxcar** and the process is called boxcar truncation.

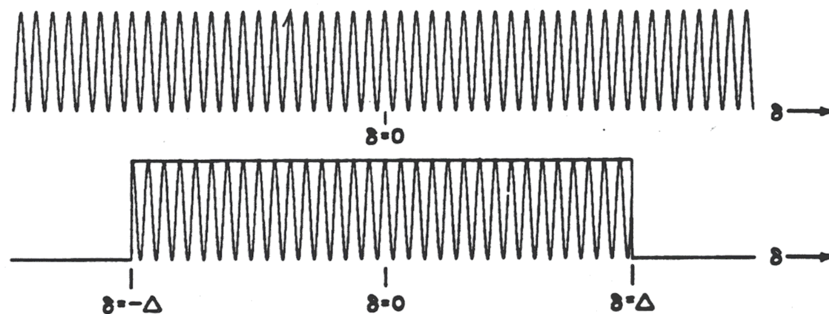


Figure 11. Boxcar Truncation on a monochromatic infinitely narrow line.

When the Fourier transform with boxcar truncation is performed, an infinitely narrow monochromatic line takes on width and acquires positive and negative side lobes, known as ringing (Figure 12). Mathematically this function is of the form sinx/x, which is known as sincx function. The full width at half height (FWHH) of the obtained function is $0.6/\Delta$.

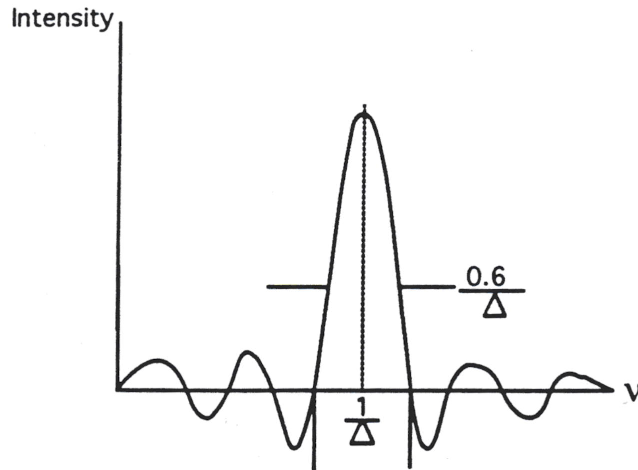


Figure 12. Effect of boxcar truncation on an infinitely narrow line.

The first minimum has a negative value, which is about 22% of the amplitude of the central positive frequency. If a weak line were present at the frequency value of this minimum, it could be lost from the computed spectrum. The elimination of the side lobes that result after truncating the interferogram with a boxcar function is known as apodization, and functions used for this purpose are called apodization functions. Triangular apodization is one of the simplest functions used. In Figure 13 boxcar truncation is contrasted to triangular apodization for the Fourier transformation of an infinitely narrow monochromatic line.

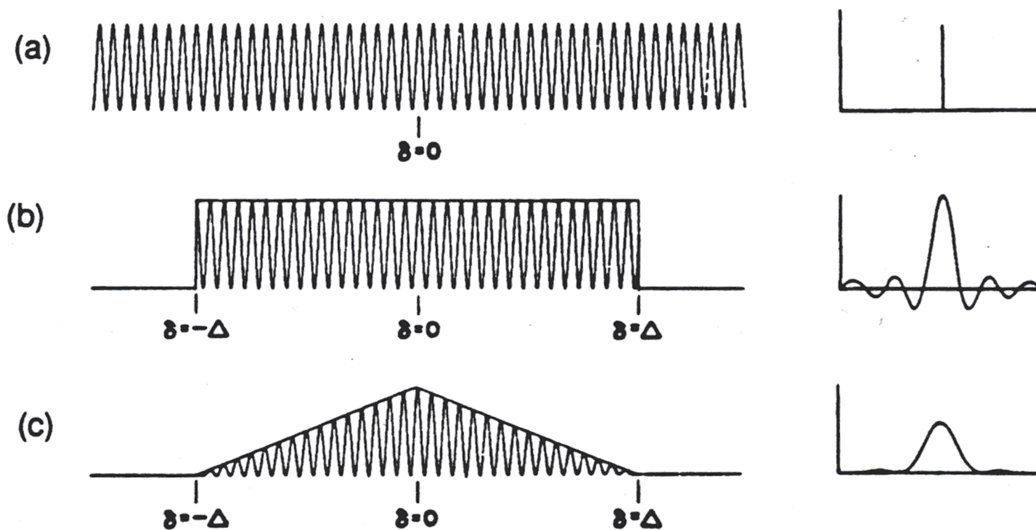


Figure 13. Comparison of triangular apodization and boxcar truncation.

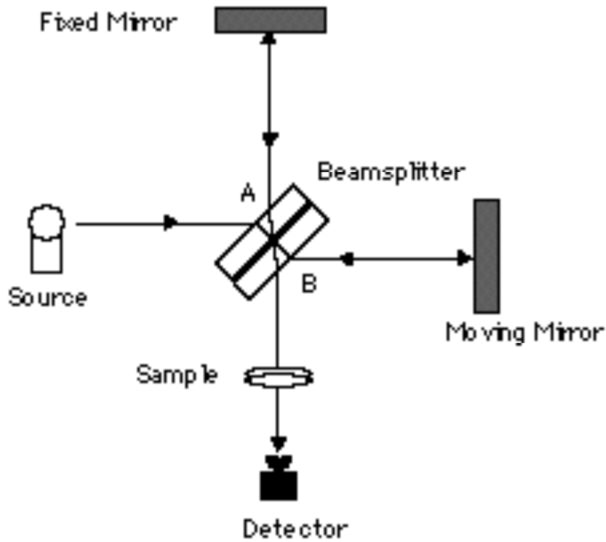
The source is an infinitely narrow line. The interferogram (a) is an infinitely long cosine train, which transforms faithfully to return the infinitely narrow line. When boxcar truncation is used (b) the transformed line takes on width and exhibits positive and negative lobes. Triangular apodization (c) further increases line width but reduces the magnitude of the side lobes and makes them all positive. Note that although the triangular function decreased the side lobes, the resultant line width is considerably increased, affecting the resolution. There are several apodization functions, which employ a degree of non linearity or curvature in an effort to maintain resolution while reducing the ringing, these include Gaussian and Lorentzian functions, a raised cosine function (Hanning apodization), Happ-Genzel apodization and Norton-Beer functions.

When sampling liquids and solids in transmission mode, an apodization function of medium strength such as triangular that does not seriously affect resolution should be used. For gas sampling when rotational fine structure is required, no apodization function should be used. The boxcar truncation will maximize the resolution although the baseline will be affected. In this case a greater number of scans will ultimately favor the SNR of the spectrum.

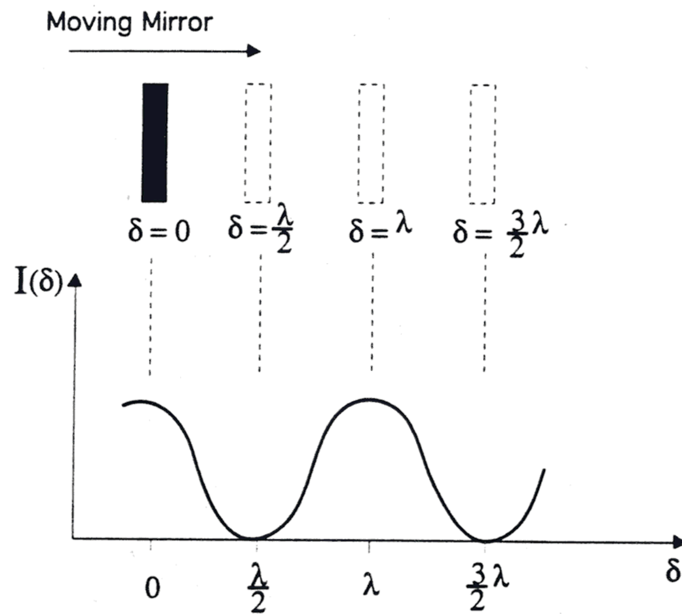
ASSESSMENT

I. INTERFEROMETRY

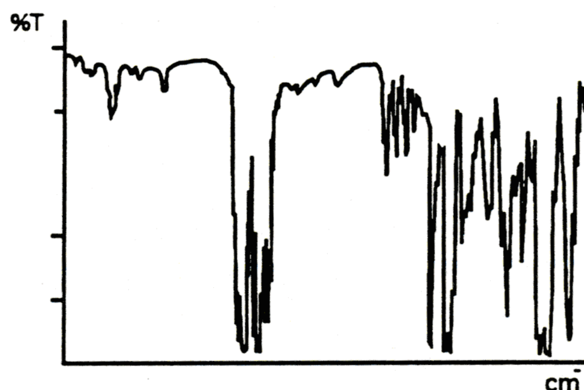
1. Name and explain the functioning of the assembly shown and label the components.



2. In your own words, explain the following diagram. Use equations to illustrate your point.



3. Explain how the following graph was obtained. What does it represent?



II. SPECTROMETER COMPONENTS AND FUNCTIONING

4. What is a DTGS and how it works?
5. Is it a good practice to turn off a mid IR spectrometer? Explain.
6. Why is it necessary to collect a larger number of scans when using reflectance accessories than for transmission measurements?
7. Indicate the scanning conditions needed to resolve the fine rotational structure of the infrared spectrum of a sample of carbon monoxide with optimal SNR.
8. What effect will have on the appearance of the spectrum if the conditions used in the previous question were applied to a sample of liquid acetone? Explain answer using SNR, scanning time, and resolution?

EXPERIMENT

You will record an IR spectrum of hexane, hexanol, hexanoic acid, cyclohexanone, and benzoic acid. Research the structures of these compounds before coming to lab and identify the functional groups present in each.

EXPERIMENT 8

Chromatography

INTRODUCTION

Separation of a mixture into its pure components is an essential part of organic chemistry. For example, a chemist may want to purify the crude extract of a medicinal plant, isolate the pure product(s) of a chemical reaction from the reaction mixture, or identify foreign compounds in a sample. You may have noticed that the standard operating procedures in this course covered basic separation and purification techniques: recrystallization, distillation, and extraction. This experiment will add a fourth: chromatography.

BACKGROUND

All of these techniques are similar insofar as they take advantage of differences in physical properties between various compounds. Recrystallization and extraction take advantage of the fact that compounds may have different solubilities in a particular solvent. Distillation uses variations in boiling points between liquids. As you will see, chromatography will primarily use a different property, polarity, to separate a mixture.

Chromatography is one of the most ubiquitous methods of analyzing and purifying organic compounds. Flash column chromatography (occasionally *CC*) separates large quantities of compounds under air pressure while thin layer chromatography (usually referred to as *TLC*) is more useful for qualitative analysis and small-scale separations. Gas chromatography (usually called *GC*) is purely an analytic technique.

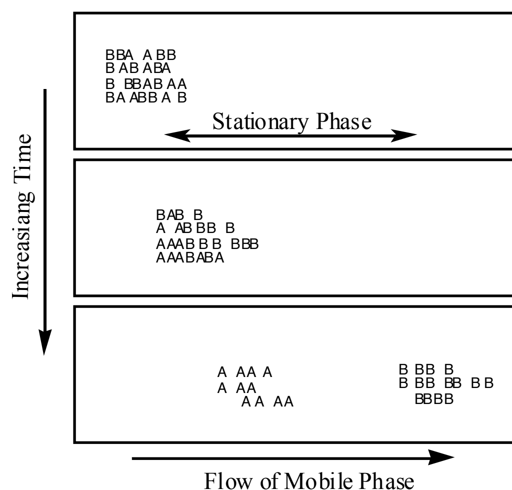
The fundamental principle of chromatography is the *distribution equilibrium* that forms when a compound is either dissolved in a *mobile phase* or adsorbed on a *stationary phase*.

When a compound is dissolved in the mobile phase it is carried along the direction of flow, but when it is *adsorbed,* or stuck, on the stationary phase it does not move. If compound B

spends more time in the mobile phase than compound A, B will move further along the column than A. The longer the mobile phase travels, the better the separation between A and B.

Stationary phases are usually very polar, while mobile phases vary widely in polarity, but are less polar than the stationary phase. This is called *normal phase (NP)* chromatography. The exception is *reverse phase (RP)* chromatography, in which a polar mobile phase, and a less polar stationary phase are used.

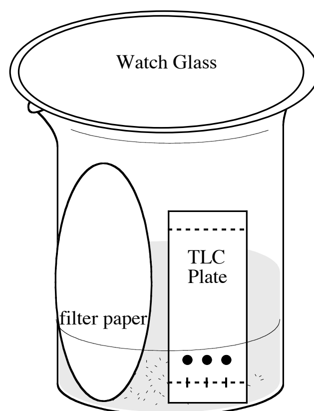
In normal phase chromatography, where the stationary phase is polar, polar molecules will spend more time adsorbed on the stationary phase, while less polar ones will be carried more quickly by the non-polar mobile phase. In the diagram below, assuming that the setup is normal phase, B represents a less polar compound, while A represents a more polar one.



1. Thin Layer Chromatography

This technique is performed on a glass or plastic plate that is coated with a thin layer (thus the name) of dry adsorbent. Usually these plates are pre-coated with a layer of *silica gel* or *alumina*. These are inorganic materials and are very polar compared to nearly all organic structures. The sample mixture is *spotted* on the plate near the bottom, and the plate is put in a closed beaker or jar with a small amount of the appropriate solvent or solvent mixture.

Capillary action draws the solvent up the plate. When the *solvent front* is near the top, the plate is removed from the beaker and a separation of the sample's components may be observed.



Visualizing the TLC Plate

If the compounds are colored, the plate can be read easily. If the compounds are not colored then they can be visualized using an ultraviolet lamp or a chemical stain. There are a wide variety of chemical stains based on the functional groups present. The following methods of detection will be used in this laboratory:

Ultraviolet Light Detection: A nondestructive visualization technique that will show any compounds that absorb UV light. Compounds containing benzene rings or conjugated systems usually absorb UV light. Commercial TLC plates have phosphor in the adsorbent, which fluoresces in short-wave UV light. If a compound is present on the plate it blocks the glow and appears as a dark spot. (This is technically true only for compounds that quench the fluorescence). Some organic compounds also fluoresce themselves, and will show up as bright spots under short-wave UV light.

Iodine vapor: Iodine vapor is also a non-destructive visualization technique. A few crystals of iodine are placed in a closed chamber, such as a capped jar containing silica gel, and the slide is placed into the chamber to collect on the spots by a weak electronic attraction. Iodine forms a

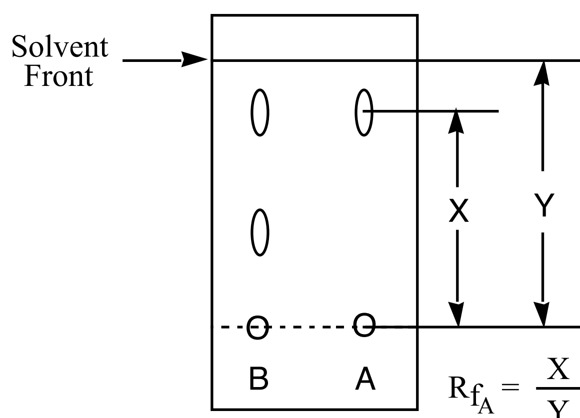
yellow or brown complex with most organic compounds containing double bonds, even isolated ones. The reaction is reversible, so that I_2 staining can be followed by another chemical stain if the plate is allowed to sit in air for several minutes so that the iodine can sublime off the plate.

Please note that while these two methods will show any compounds containing double bonds, any compounds without double bonds will not show up on your plate (unless they are themselves colored, in which case you will be able to see them with your naked eye). Other TLC visualization methods exist for these compounds, but they are usually strong oxidizers and for that reason not safe for use in an undergraduate lab.

R_f Values

For each spot on the TLC plate, a characteristic value R_f (ratio to the front) can be calculated. **R_f** is defined as the ratio of the distance traveled by a spot (measured from the center of the spot) to the distance traveled by the solvent (See Figure below)

$$R_f = \frac{\text{Dist. travelled by compound}}{\text{Dist. travelled by solvent}}$$



A = pure compound
B = mixture

Although the R_f is characteristic for a given compound, it depends greatly on the solvent and the type of adsorbent used. Consequently, the *CRC Handbook of Chemistry and Physics* and *Merck Index* do not contain tables of R_f values. The <u>difference</u> in R_f values between two spots on a plate, ΔR_f, which also varies with the solvent, is used as a measure of the performance of the separation. The bigger the difference, the better the separation.

Note that all R_f's are measured from the baseline (the line on which the spots were placed), and not the bottom of the plate, and measured to the solvent front, not the end of the plate.

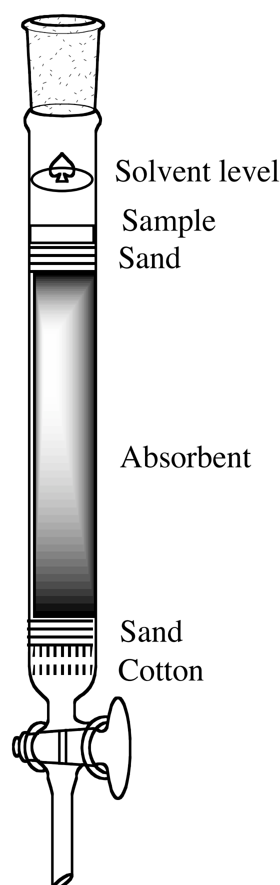
The choice of solvent system is crucial for good separation. If the developing solvent has a high polarity, all of the spots will run to the top of the plate, and ΔR_f will be zero. With a very non-polar solvent, the spots will not move at all from their initial positions, and again $\Delta R_f = 0$. The best separation is often achieved by using a mixture of a non-polar solvent with a polar solvent. The polarity of the developing solvent is adjusted by changing the ratio of polar to non-polar solvents in the mixture. The best developing solvent should give an R_f value of 0.3 to 0.7 for the desired compound and a ΔR_f of at least 0.1 between the desired compound and any impurities.

Because the fundamental action of chromatographic separation remains the same, an appropriate solvent mixture is often determined via TLC and then used to run a column in column chromatography.

2. Column Chromatography

This technique is performed by packing a glass tube with an adsorbent as shown in the figure below. There are many different types of adsorbents (solid phase) that are used in column chromatography, and the choice of adsorbent depends on the types of compounds to be separated. The most common adsorbents used are silica gel and alumina. Silica gel is used to separate a wide variety of compounds such as hydrocarbons, alcohols, ketones, esters, acids, azo compounds, and amines. Alumina is also used extensively, and comes in three forms: acidic,

basic, and neutral. Acidic alumina is used for separating acidic materials such as carboxylic acids and amino acids. Basic alumina is used to separate amines, while neutral alumina can be used to separate non acidic and non basic compounds. Likewise, cellulose, starch and sugars are used to separate natural products, and magnesium silicate is used in the separation of acetylated sugars, steroids and essential oils.



In the diagram above, cotton is added to prevent the sand and silica from running out the bottom. The sand is added on top to create a level surface for the silica to rest on. Next, the silica is added, followed by another layer of sand. This layer of sand protects the silica underneath from being jostled by the addition of sample and solvent later on.

It is important to keep silica and all adsorbents both level and moist while running a column. You want your adsorbent to be as uniform as possible all the way around. If the surface is not level, compounds traveling down one side of the adsorbent will get through faster than those traveling down the other side, which can lead to overlapping bands (think of the side with less

silica as giving its compounds an unfair head start, which might allow slower-moving, more polar components to travel as fast as faster-moving, less polar components traveling down the other side). Letting the column go dry will create cracks in the adsorbent, which act as similar shortcuts.

A column may be packed either 'wet' by pouring a solvent-adsorbent slurry into the tube or 'dry' by filling it with dry adsorbent. If it is packed dry, it must still be kept wet once solvent has been added. The mixture to be purified is then dissolved in a small amount of the appropriate solvent and added carefully to the top of the solid adsorbent. It is added carefully to ensure that the packing is not disturbed. The column is developed by adding more solvent to the top, then collecting the fractions of *eluent* (the compound-containing solution) that come out the bottom. For 'flash' column chromatography, moderate air pressure is used to push the solvent through the column. The success of the separation and the contents of the fractions can be determined by spotting the fractions along with the initial mixture on TLC.

A column may be developed with a single solvent or a **solvent gradient** (a solvent system which gradually increases in polarity.) For example, a column may be developed first with a low polarity solvent such as hexane, and as fractions are collected the developing solvent is changed to 10:1, 5:1, and 1:1 hexane-methylene chloride. A polarity gradient is used for mixtures of compounds with very different polarities.

Solvents: A common non-polar solvent for both thin-layer and chromatography is hexane. It can be used with a variety of polar solvents. The following solvents are listed in approximate order of increasing polarity: cyclohexane, petroleum ether, pentane, carbon tetrachloride, benzene, toluene, chloroform, ethyl ether, ethyl acetate, ethanol, acetone, acetic acid, and methanol. The higher the percentage of polar solvent, the faster compounds will elute.

Elution sequence: An approximate elution sequence, based broadly on polarity, for compounds from fastest to the slowest is hydrocarbons, olefins, ethers, halocarbons, aromatics, ketones, aldehydes, esters, alcohols, amines and acids.

Note that the more polar the solvent, the faster compounds elute, regardless of the compounds polarity. This means changing the solvent polarity cannot change the order compounds

elute from a TLC or column. This may seem non intuitive, as it would seem that a polar solvent would move a polar compound farther than a nonpolar compound.

To help visualize this concept, consider that solvents will compete with compounds for sites on the stationary phase. A less polar solvent will not compete well, allowing the compounds to remain bound to the stationary phase, resulting in slow elution. A polar solvent will compete well with molecules and will occupy sites on the stationary phase. This will force compounds into the mobile phase, and result in faster elution/increased travel distance.

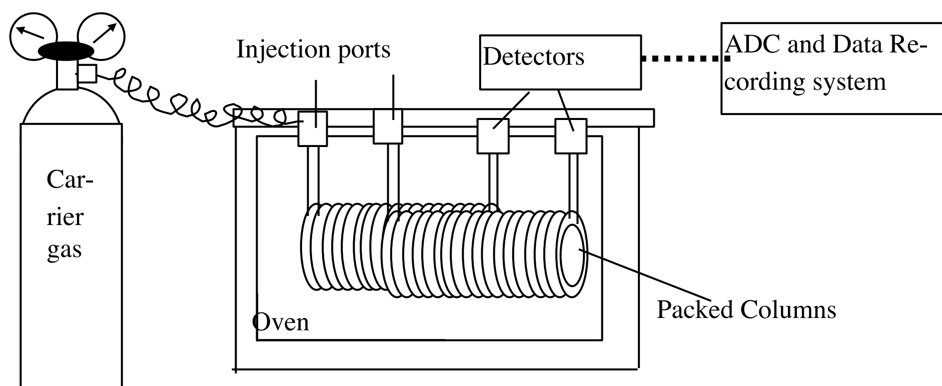
It may also be helpful to remember that alumina and silica are much more polar than any organic solvent. Therefore, the stationary phase will always be more polar than the mobile.

3. Gas Chromatography

As in other types of chromatography, the analytes exist in equilibrium between the stationary and mobile phases. The analytes can be 'stuck' on the adsorbent as a liquid, or moving with the carrier gas as a vapor.

GC is somewhat different from the other two methods explored in this experiment in that here the *boiling point* is the primary property on which the separation depends. However, you will see that if two compounds have similar boiling points but very different polarities, they can be separated by polarity via GC.

The gas chromatograph contains a long (6 ft. or longer) stainless steel tube packed with adsorbent (usually a liquid phase on an inert solid support) and put in a temperature controlled oven. The solid phase used in the 8400 Perkin-Elmer Gas Chromatograph is carbowax, a carbon backbone polymer $OH\text{-}(CH_2\text{-}CH_2\text{-}O\text{-})_n\text{-}H$, on silica. This is non-polar like thin layer chromatography and column chromatography. An inert gas, helium in our lab, is passed through the column at a controlled *flow rate* and serves as the mobile phase. A small amount (about one microliter) of a liquid sample is injected into the tube, and compounds are detected as they emerge from the outlet.



The detector response is plotted vs. time, and this is called a *chromatogram*. The time it takes an analyte to emerge from the column is called the *retention time, RT*, and is analogous to R_f for TLC. The detector response is proportional to the amount of compound passing through it, so the area under a peak is roughly proportional to the total amount of compound in the sample. Hence, the ratio of areas in a single chromatogram is approximately equal to the ratio of compounds in the mixture. In the old days these areas were measured by cutting out and weighing the paper for each peak! Our GCs are computer controlled and automatically calculate the retention time and area for each peak.

Basic Theory

It is easiest to imagine a GC as a miniature distillation. A small amount of a mixture of liquids is injected into one end of a long capillary tube. They all heat at the same rate, until the temperature rises to the boiling point of the lowest-boiling liquid in the mixture. It becomes a vapor and is carried along by the helium carrier gas towards the detector. As it travels, the second-lowest-boiling component may boil and begin traveling down the column as well, behind the first fraction. When the first component reaches the detector, a peak is recorded. If the separation was good, there should be as many peaks as components in the mixture.

Because the column is polar, components will not travel straight through but will be slowed down more or less based on their own polarity. More polar compounds will adsorb on the stationary phase and travel more slowly, leading to longer retention times. However, this effect is generally minor. So long as your liquids have boiling points that differ by ten degrees or so or

more, you are likely to see your components come out in order of boiling point. If, however, you have liquids that boil within a few degrees of one another, polarity may come into play.

Temperature

Rather than choosing a solvent as is done for TLC and column chromatography, one chooses an oven temperature for GC. The oven temperature is analogous to the polarity of the solvent in TLC. A high temperature leads to short RT and little separation because all compounds are vaporized and they move at the same rate as the mobile phase. A very low temperature leads to long or nearly infinite RT since the compounds remain adsorbed on the solid phase. In addition, diffusion causes the peaks to spread out as the RT increases, so compounds that are retained in the column for a long time give broad, ill-defined peaks.

The temperature of the injection port and detector are controlled separately from the oven temperature. The injection port must be hotter than the oven to insure that the entire sample goes into the column rather than condensing in the injector. The temperature of the detector is also set higher than that of the column so that compounds do not condense in it.

Gas Flow

The carrier gas flow rate is set for optimum column performance but is not regularly adjusted. A fast flow rate leads to short retention times but can give poor resolution. A slow flow rate gives long retention times and broad peaks. To obtain a good compromise between high separation efficiency and quick chromatographic separations it is usually better to use a high flow rate (20-30 mL/min) and a low temperature.

Columns

There are two kinds of columns: wide bore and capillary. A wide bore column is usually 1/4"-1/8" diameter stainless steel and is packed with adsorbent. A capillary column has a much narrower diameter and the adsorbent is coated on the inside surface. Capillary columns give much better resolution but they have much lower capacity. These columns require a *splitter*,

which allows only a fraction (1/50) of the injected sample into the column. Long columns always give better separation than short columns.

Detectors

There are two common types of detectors: thermal conductivity (TCD) and flame ionization (FID).

For TCD a hot filament is placed at the column exit port. Helium cools the filament as it flows over it, but when the helium is diluted with an organic compound, the filament is cooled differently and the conductivity changes. A Wheatstone bridge, an electronic device that senses the conductivity difference between two filaments, measures the actual response. A reference filament is attached to a second column in the same oven. The reference column is structurally the same as the sample column but it is never used for the sample. Pure helium is the only gas flowing through. It provides the baseline of the chromatograph. Injecting sample into the reference column will result in 'negative' peaks or no peaks depending on the system.

Flame ionization detection is done by burning the sample in a flame and measuring the ions produced. It does not require a reference column. FID is used with capillary columns, where the low flow of helium makes it impractical to use TCD.

4. High Performance Liquid Chromatography (HPLC)

The technique is based on the same principles as GC (i.e. partitioning between phases, the main difference is that the analyte is pumped by the mobile phase through the column instead of being pushed by the carrier gas in GC. HPLC is applicable to over 80% of all known compounds and is the method of choice over gas chromatography (GC) for the separation of non-volatile and thermally unstable compounds.

In an HPLC system, a pump shoots the mobile phase through the system at the desired flow rate (Figure. 1). At the injector, the user inserts the sample for analysis. The injector is usually found right before the column. From there, the mobile phase picks up the sample and carries it through the column where it is separated into its various components. The separated components then travel through a detector cell where, based on the individual components' prop-

erties like UV absorption, fluorescence, or electrical conductivity, the amount of each component is determined. A recorder or another data-handling device translates the detector's electrical signals into peaks of various sizes. The most popular mode of HPLC is reversed-phase chromatography (RP-HPLC). In this technique, the stationary phase is non-polar (e.g. carbon chain bonded to silica) and the mobile phase is polar (e.g. methanol, water, acetic acid).

This is opposite to normal phase HPLC where the stationary phase is polar (e.g. silica, alumina) and the mobile phase is non-polar (e.g. hexane). The length of the carbon chain of the stationary phase used in RP-HPLC is typically eight (C_8) or eighteen carbons (C_{18}). The separation is based on partitioning of the sample components between the mobile and stationary phases.

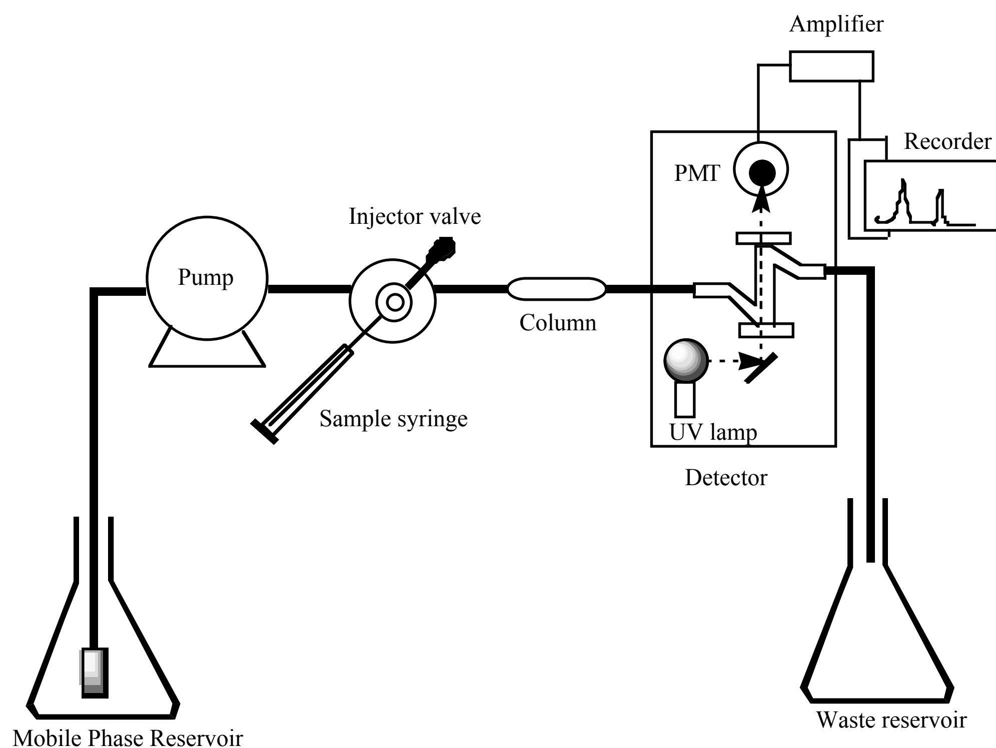


Figure 3. Schematic diagram of an HPLC System

HPLC Operating Parameters

In this experiment you will be able to modify all the following parameters with the exception of the column packing.

1. Pump parameters:
    a) Mobile phase composition
    b) Flow rate
2. Column parameters:
    a. Stationary phase (packing) reversed-phase vs. normal-phase
    b. Mobile phase composition
3. Injector volume
4. Detector parameters:
    a. Detector wavelength
    b. Attenuation (AUFS, Absorbance Units Full Scale)
    c. Pump flow rate

EXPERIMENTAL OUTLINE

You will determine the composition of a mixture of ethanol and cyclohexanol using GC, and of caffeine and acetaminophen using HPLC.

PROCEDURE

PART 1. Gas Chromatography

The composition of an unknown mixture of ethanol and cyclohexanol will be determined using GC. The mixture will be separated and the peaks obtained on the chromatogram identified by determining the retention times of pure ethanol and cyclohexanol. The experiment will be performed using a Thermal conductivity detector (TCD) Gas Chromatograph. Your TA will give you guidelines for operating this instrument. One important thing to remember is that the injector port on a GC is hot. It is usually 50 degrees or so hotter than the oven, which may itself be 200 ^{o}C or more. The injector port is exposed on the top--be careful not to burn yourself!

You will choose the GC sequence titled “HSSP07” and note the parameters this sequence uses. The mixture will be injected and the parameters varied to effect a separation if necessary.

In general assume that all syringes are dirty. You should first rinse out each syringe with the sample you plan to inject two or three times to make sure there is no residue left from the last sample injected. You will be loading the syringe with 1 micro liter injections of your sample. Once the peaks are separated you will inject pure components to identify your peaks.

PART 2. High Performance Liquid Chromatography (HPLC)

HPLC will be used to analyze a mixture of acetaminophen and caffeine. The effect of parameters on the separation of peaks in the chromatogram will be investigated and an optimal separation obtained. Pure components will be used to identify the peaks in the mixture. A calibration curve will then be created to determine the concentration of acetaminophen in the mixture.

Sample Mixture Preparation

1. Fit a plastic filtering syringe. Remove the cap on the tip, fit the bottom of the syringe with a filter, and pull out the piston.
2. Pour about 0.75 mL of caffeine and 0.25 mL acetaminophen into the top of the syringe. This solution will be used for parameter investigation as well as preparation of a calibration curve.
3. Place the syringe over an open, clean glass vial and filter the liquid into the vial by pushing the piston back into the syringe shaft.
4. Repeat the above steps for solutions of the following composition for the calibration curve:

   0.10 mL acetaminophen, 0.90 mL of caffeine
   0.50 mL acetaminophen, 0.50 mL of caffeine
   0.75 mL acetaminophen, 0.25 mL of caffeine

Instrumental Parameter Investigation

1. Following your mentor's demonstration with regard to the proper handling of the Hamilton syringe, perform an initial trial with the following suggested settings:

   flow rate: 1.2 mL/min %methanol = 30%
   chart speed: 30 mm/min, AUFS (attenuation): 1.

Be sure to inject more than 20μL of the sample as this instrument has a 20μL sample loop.

2. Examine the number of peaks, peak quality (are the peaks overlapping or are they separate, "resolved?"), and baseline quality (is the baseline straight? Does it drift up or down?)
3. Label the chromatogram with the set of parameters used. Investigate the effect of varying flow rate, chart speed, AUFS and %methanol on the quality of separation. Be sure to record the settings on the chart and change each parameter back to the initial setting before using a new set of parameters.

Separation Optimization

Using your knowledge of how instrumental parameters affect the appearance of a chromatogram, experiment with different settings to achieve the best resolution of the components in the mixture. Remember to record the parameters that provide the optimal separation. Record the column length in your notebook.

Peak Identification

Under the conditions used for optimization, inject filtered samples of the pure components to identify the peaks on the chromatogram. Be sure to rinse the syringe with the incoming mixture to remove all traces of the previous mixture

Peak Calibration

Using the optimized conditions, inject the four different acetominophen-caffeine mixtures you prepared (three times each so you can average the data obtained). Be sure to rinse the syringe with the incoming mixture to remove all traces of the previous mixture

Unknown Determination

Inject the unknown sample into the HPLC using the same conditions as for the peak calibration.

Peak measurement

Measure the peak height (h) and peak width (W_l) for each of the peaks in the calibration as well as the unknown. Then use these to calculate peak are using the approximation to a triangle 1/2 x W_l x h. You should average the same injections to that your final data involves 4 points (from the calibration) and 1 point from the unknown. Now plot a calibration curve for area vs. concentration, obtain the equation of the fit, and determine the concentration of the unknown.