Chemistry 2273a
Organic Chemistry I:
Structure and Spectroscopy

September 2010
Laboratory Manual

SAFETY GUIDELINES

SEE *The UWO Occupational Health and Safety Manual for General Laboratory Practices (http://www.uwo.ca/humanresources/facultystaff/h_and_s/h_and_s_index.htm)*

A chemical laboratory is potentially a dangerous place. Safety is EVERYONE'S concern. The following notes are provided to assist you in avoiding unnecessary accidents, and to indicate the action to take should an accident occur. Study this material carefully.

No laboratory work is permitted unless a demonstrator is present. Smoking, eating, or drinking in the lab is forbidden, as are unauthorized experiments, practical jokes, or horseplay.

ATTIRE: The University safety policy mandates "maximal skin coverage" for laboratory personnel – including you! Closed-toed shoes must be worn; open-toed shoes, sandals, ballerina slippers or similar footwear that leaves skin exposed are not permitted. Socks cannot take the place of footwear. Long pants must be worn; no shorts, skirts or dresses are permitted. The wearing of a lab coat is now REQUIRED as Departmental Policy. Long hair must be confined as well as loose clothing to prevent it from becoming entangled in experimental apparatus or dangling into dangerous conditions such as flames, hotplates or solutions. Topcoats, windbreakers, etc. should be properly stored, along with book bags etc, in the cupboard under your fumehood.

**********Anyone who does not comply with the dress requirement will be asked to leave the laboratory and will be assigned a zero grade without the possibility of making up the lab at another time.* *********

Please note: Please keep your laboratory coat in a separate plastic bag to keep hazardous material away from your other personal items/books when not in use.

EYE PROTECTION: SAFETY GLASSES or GOGGLES of approved design must be worn at all times by everyone in the laboratory. Ordinary prescription eye glasses alone are NOT acceptable as eye protection; they must be covered by additional safety glasses such as Eyeguard "Tuff-Specs" or have side-shields. A good alternative, but more expensive, is prescription safety glasses which possess side guards.

CONTACT LENSES can be worn under approved safety glasses. However, in the case of accidents they often cannot be removed rapidly if liquid splashes into the eye. So, if you must wear contact lenses, identify yourself to the laboratory demonstrator before the first lab commences.

Any student not wearing safety glasses or goggles will be required to leave the lab immediately and will be assigned a zero grade.

GLOVES: will be made available during portions of experiments where they are needed. It is unnecessary to wear disposable gloves at all times because the experiments have been constructed to minimize hazardous contact with chemicals. Carefully read the experimental procedures for each lab and note where the protection of gloves is required.

Learn the location and method of operation of the FIRE EXTINGUISHERS, the SAFETY SHOWERS and the EYEWASH FOUNTAINS. Note the EXIT ROUTE from the lab and from the building.

FIRE AVOIDANCE: No open flames are necessary in this laboratory; turn off all hotplates when not in use. Check the area near you for flammable solvents before turning on a hotplate. Check around for "hot spots" before using a flammable solvent. Be particularly careful when using volatile flammable solvents such as: diethyl ether, petroleum ether, methanol, ethanol, acetone.

CHEMICALS: Handle all chemicals with care; avoid spillage and wastage. Replace caps after use, clean up spills immediately when they occur. Do not inhale fumes or vapours of organic solvents. If a chemical is spilled on the skin or clothing, do NOT try to remove with organic solvents (which are absorbed through the skin). Wash with water and seek attention at once. NEVER taste a chemical. NEVER pipette any solution by mouth. Smell chemicals or vapours CAUTIOUSLY and only if you are specifically told to do so.

GLASSWARE: If glassware is hot, use tongs or rubber finger protectors to handle. Chipped or cracked glassware is a hazard and must be exchanged at the storeroom without delay. Dispose of broken glass only in the containers provided. See your Demonstrator immediately for proper clean up of broken thermometers.

FUMEHOODS: The fumehoods are among the most up-to-date models currently being used in an undergraduate laboratory in Canada. They make it possible for students to work with hazardous materials that would otherwise not be possible.

Remember:

1) Keep all hazardous materials in the fumehoods.
2) When transporting hazardous materials outside the fumehood make sure they are contained/covered or corked as instructed.
3) Dispose of hazardous materials in the appropriately labeled containers provided.
4) Use acetone (in red-topped squeeze bottles) to rinse remnants of material into the waste container prior to a soap and water wash at the sink.

DISPOSAL:

Waste

Waste chemicals or broken glass placed in regular garbage bins can cause injury to the persons required to dispose of that garbage. For this reason all laboratory waste must be put in the appropriate container.

At all times, please try to avoid waste by taking no more of each reagent or solvent than that required in the experimental procedure. However, once a chemical has been removed from its original container it becomes your responsibility to properly label, handle and dispose of it and its by-products.

Liquids: Organic chemicals and solvents must be disposed of in the receptacles provided, NOT down the sink. Aqueous solutions may generally be disposed of down the sink, EXCEPT where noxious elements (e.g. heavy metals) are being used and a special disposal container is provided for wastes. If a mixed organic/inorganic liquid mixture is used, e.g. aqueous ethanol, your demonstrator will advise you on its correct disposal.

Solid chemicals (including used filter papers, etc.): Solids must be disposed of in the special container provided, NOT in the general garbage. Used melting point capillaries should be placed into the labeled plastic bench top containers.

GLASS (boxes labeled for glass disposal).

All waste glass (with the exception of thermometers containing mercury, unknown sample vials and melting point capillaries and slides) must be placed in glass disposal bins. The glass must be clean.

BROKEN THERMOMETERS (main fume hood).

See TA immediately for proper clean-up.

IN CASE OF ACCIDENT

FIRE: Alert the Demonstrator at once and obey his/her instructions. Immediately unplug all hotplates and melting point apparatus; remove combustible materials and solvents. SMALL fires in beakers, etc., can be extinguished by covering the container with a watch-glass, or large beaker, if necessary. DO NOT use water. Use a fire extinguisher directed at the base of the flames. If there is the slightest risk that the fire will get out of control, evacuate the lab and pull the fire alarm; this automatically summons the Fire Department. We do NOT expect students to act as fire-fighters!

NOTE: misuse of the fire alarm is a serious offence.

THE FIRE ALARM SOUNDS like a ringing bell. If it sounds, then you must leave the building. Collect your personal items quickly and exit, because there is no guarantee you'll be back quickly.

In the event of an evacuation, extinguish all heat sources, unplug apparatus and leave the building IMMEDIATELY by the nearest exit. The time to speculate about "false alarms" is when you are OUTSIDE the building. Do not return until told to do so by your instructor or other responsible official.

********Treat every evacuation as an authentic emergency. There is no time for delay or to finish your laboratory work. ********

PERSONAL INJURY: All personal injuries, however slight, must be reported AT ONCE to the Demonstrator and to the Lab Technician.

Major Chemical Spill on Clothing or Person, or Clothing on Fire: IMMEDIATELY put the victim under the shower to extinguish flames or wash off chemicals. If acid or chemicals are spilled on clothing, IMMEDIATELY remove all affected clothing (speed is vitally important, this is NOT the time for modesty)! Keep victim warm, get medical attention (University Hospital) at once.

NEVER use fire extinguisher on a PERSON.

Minor burns (chemical or thermal) should be flushed with cold water for several minutes, followed by medical attention, if necessary.

Chemicals splashed in face or eyes: Get the injured to the eyewash IMMEDIATELY and irrigate the eyes with cold water for 15-20 minutes, holding open the eye to ensure thorough washing. Always seek further medical attention at the hospital in the case that your eyes are exposed to hazardous chemicals and take a current copy of the MSDS with you.

Minor Cuts: Minor cuts from glassware, etc. should be washed thoroughly with cold water, removing any glass fragments. The storeroom has a supply of "band-aids", an accident report form MUST be completed.

For more serious cuts, apply pressure to stop bleeding, keep victim warm, and immediately summon medical assistance.

PREVENT ACCIDENTS - Stay Alert at All Times!

OTHER RESPONSIBILITIES

The laboratory is a shared work environment; the way you work affects fellow students in your class and other lab courses, the laboratory demonstrators and technicians. It is important that you maintain good habits and good chemical hygiene including keeping the work area clean and free of hazards.

Always wipe down and clean your bench and fumehood with a wet paper towel/soap. Leave your work area and glassware clean.

Wash your hands before leaving the laboratory.

Failure to adequately clean your workstation can result in a zero grade for the entire lab.

Safety compliance waiver form

This form must be completed, signed prior to undertaking the first laboratory experiment.

Arrive at the lab ahead of time and be well prepared. If you arrive late, or are not adequately prepared or attired, you will not be permitted to perform the lab and will receive at mark of zero. No opportunity for a make-up will be available.

Attend only your designated lab section. Section changes are not permitted and Laboratory Demonstrators will not admit any student to a section that does not belong or have written permission from the course coordinator.

Laboratory Demonstrators and the Laboratory Technicians have the authority to eject students from the lab, resulting in a mark of zero, for unsafe conduct or practices, or not following instructions pertinent to the keeping a safe, clean laboratory.

A pre-experiment discussion, given by your Laboratory Demonstrator will provide you with additional information.

I have read and will comply with all of the regulations and safety rules pertaining to Chemistry 2273a as outlined in the attached safety guidelines. I realize that I alone will take full responsibility if I do not obey them.

Name:	
Student I.D.:	Lab Day, Time, Section:
Signature:	Date:

Personal Copy. Please fill in this form and keep for your records.

Safety compliance waiver form

This form must be completed, signed and handed to your demonstrator prior to undertaking the first laboratory experiment.

Arrive at the lab ahead of time and be well prepared. If you arrive late, or are not adequately prepared or attired, you will not be permitted to perform the lab and will receive at mark of zero. No opportunity for a make-up will be available.

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Name:	
Student I.D.:	Lab Day, Time, Section:
Signature:	Date:

Departmental Copy: **Complete this form and give to your TA before your first laboratory experiment.**

LABORATORY RECORDS AND REPORTS

Your written work in this course comes under two headings:

(i) Your personal notebook containing records of all your lab work.
(ii) A data sheet or formal report handed in for marking at the conclusion of selected experiments.

Your Laboratory Notebook

One of the most important aspects of laboratory work is maintaining an accurate and complete record of your experiments. This should be done in a laboratory notebook that you must acquire and bring with you to **every** laboratory period.

A suitable notebook (8”x10” hardcover is recommended) in which to make a permanent record of all your lab work. This book is for your exclusive use; it will not be marked, but will be examined by your TA or instructor. It is most important that all your observations, such as weighings, results of qualitative tests, etc., be recorded on the spot AT THE TIME THEY ARE MADE. It is **unacceptable** to make notes on paper towels, scrap paper, this laboratory procedure sheet, etc. with the intention of copying them "neatly" into your notebook later on. This only wastes time and introduces errors, and in laboratory practices in industrial or research labs is forbidden.

Some additional rules are as follows:

1. Always sign and date each page of your notebook for each day you work on an experiment.
2. Write in ink, never in pencil. Mistakes are crossed out with a single line.
3. Make all entries directly into your notebook. Do not write on scraps of paper!
4. Make sure your notebook is legible. If the person looking at your cannot read what you have written, it defeats the purpose of keeping accurate records.

The following format is suggested:

1. Date. If you work on an experiment for two days, there should be two separate dates throughout your notebook pages for that experiment.
2. Experiment Title.
3. Reaction Scheme and Reaction Table. This is included for synthetic experiments only. You must complete these *before* coming to lab. (Note: If you are performing a multi-step synthesis, you may have more than one Reaction Scheme and Reaction Table.)
4. Pre-lab Calculations. Some experiments require you to calculate amounts for starting materials, reagents, glassware size, etc. For such experiments, you must complete these calculations *before* coming to lab.

5. References. A list of the reference materials used for the experiment. The source for anything you look up to help you with the experiment becomes a reference.
6. Chemical List. A list of all chemicals (including pertinent data) used. If you have not used the chemical previously, you must draw the chemical structure. For synthetic experiments, the Chemical List is augmented by a reaction table.
7. Apparatus. A list of all equipment to be used for the experiment. All instrumentation used is also listed here (instrument model number and, if applicable, instrument parameters).
8. Procedure. A description of experimental manipulations performed in the experiment (be specific enough that someone can repeat the experiment without the aid of any other information). Include detailed operational procedures for any specialty equipment or instrumentation used. This should be what you actually did in lab, not a "copy" of the experimental procedure from the source (your laboratory experiment handout). This can be point form. You also record any observations here. For example: "On addition of acid, the solution turned cloudy".
9. Data/Data Analysis/Calculations. Calculations include data for graphs, area calculations, calculations for spectroscopic analysis, and reaction yields. Data includes a list of all data, including observations, recorded during the experiment. Some of this information may need to be "gleaned" from other sections (especially the procedure section) and *re-written* here. Rough notes on spectral assignments.

This notebook is used to prepare formal reports.

Obviously, if your notebook is beside you on the bench all the time, it will acquire water stains on the cover and dog-eared corners to the pages. Don't worry about it; this is a working laboratory, not a museum! At the same time, try to keep your records neat and legible for future use. Students often fear that they may write in the notebook something which later turns out to be "wrong". Again, don't worry, just cross it out (neatly, by drawing one line through it, don't waste time with erasers or "liquid paper") and write in the correction.

Be especially careful to record data given to you in the lab. On occasion you will be given "unknowns" to investigate. These will have code numbers for identification, which you should record immediately when you receive them. We suggest that you use the right-hand page only for recording observations. Later on, you will start roughing out equations, calculating yields, working up analytical results, etc., and this may conveniently be done on the left-hand page opposite the original data. All results will then be coordinated when you come to prepare your Data Sheets or write your Formal Report.

Collection of Data:

At times in C2273a, and if you continue in C2283g, you will be required to collect experimental data and spectra. You are expected to make your own observations, collect your own data and spectra. Whenever possible, label your data (or spectra) with

your name. Only when explicitly indicated in the laboratory manual or by permission of your instructor may you share data and results. When sharing of data is done, the source must be acknowledged.

Data Sheets

Experiment 1a does not require a formal report only completion of Data Sheets. The data sheets are included in this lab manual.

Formal Reports

Experiments 1b, 2 and 3 will be reported using formal reports. A generalized format for formal reports is shown below. Specific requirements for the formal reports are found at the end of each experiment.

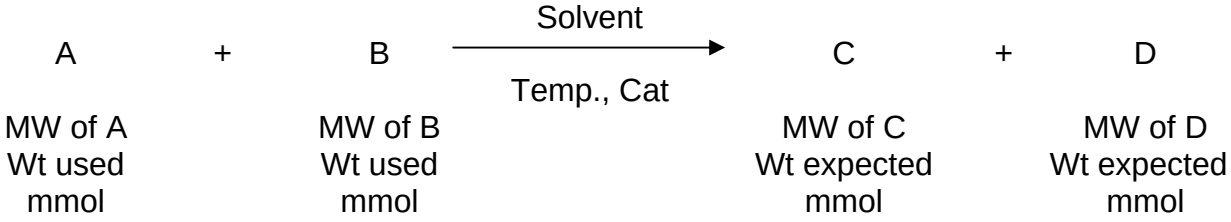
A maximum length of 5 pages, excluding attached spectra

The principal sub-sections should include:

1. INTRODUCTION – a brief statement of purpose and/or general objectives

2. REACTION EQUATION (S)

- a full equation of reactants/products - but not a mechanism which appears in the Discussion.
- It is appropriate (and encouraged) to include data on properties e.g., B.P., M.P., Density; M.W.; Weights used (g and mmol)



3. PROCEDURE

- A brief outline of what you did: Reaction; isolation; purification; or cite this lab manual.
- where appropriate, "Flow Charts" of, for example, complicated extractions, separations
- Comment if, how and why your procedure differed from that in the manual.

4. RESULTS

Give for each product isolated:

- Yield – In weight and percent (show calculation) – always for the **pure** product; sometimes (where appropriate) crude yield will also be required
- Tables of Results of qualitative tests
- Physical Properties

-Appearance (liquid/solid, crystalline character, color)
-bp., mp., CITE LITERATURE VALUE FOR COMPARISON
-Spectra with peaks labeled
-Results of any tests

5. DISCUSSION OF RESULTS: This will depend on particular case, but may include:

- Evidence that correct product was obtained (mp, bp, spectra)
- Nature of reaction (role of reagents; conditions (temp., etc.))
- Yield; explanation of high/low; side reactions
- "Special Features": e.g., extraction; chromatography; spectra; stereochemistry;
- Mechanism: One general mechanism with curly arrows will suffice.

EXPERIMENT 1

Thin-Layer Chromatography and Column Chromatography: Extraction and Separation and Plant Pigments and Common Analgesics

Relevant sections in the text: WADE pages 155-163; 198-205

General Concepts

Chromatography is a common and extremely useful method used to separate and analyze complex mixtures. Using this technique the course of a reaction can be followed, and the products separated and isolated. In this method, the components within the mixture are distributed between two phases: a *stationary phase* and a *mobile phase* (which moves through the stationary). Chromatography works on the principle that different compounds will have different solubilities and adsorption to the two phases between which they are to be partitioned. The material to be separated is placed onto the stationary phase and is then carried along by the mobile phase. The components of the mixture are absorbed by the stationary phase to different degrees and it is thus the various rates of migration for each component on the adsorptive materials that allows separation; the stronger the adsorption the slower the material passes through the system. In this experiment you will learn the techniques of two types of chromatography: column (also known as Adsorption and Partition) chromatography and thin layer chromatography.

Column chromatography will be used to separate the pigments present in spinach leaves, namely the green chlorophylls, orange carotenes and yellow xanthophylls. The pigments will be isolated from the leaves by solvent extraction and then separated by the two types of chromatography.

Prior to performing a bulk separation and isolation using column chromatography, analysis of the plant extract by thin-layer chromatography (TLC) will be performed in order to find the best solvent system to be used in the large-scale separation. TLC with plant extracts produces several spots; easily identified by colour. More commonly in organic chemistry is the separation of complex mixtures of colourless components. In these cases other methods of visualization must be used. In this experiment, you will also perform TLC analysis of common analgesic drugs to determine which components are present. The organic components in these drugs are colorless and produce colourless spots on the TLC which require alternative techniques for identifying spots. Ultraviolet/visible absorption spectroscopy will be used to detect TLC spots

that are otherwise colourless. During the course of Chemistry 273a and 283g you will learn other methods of visualization.

Principles of Chromatography

The separation of the components of a mixture depends on the phase each component remains in and the rate at which each travels. The stationary phase does not move, while the other mobile phase travels past the fixed (stationary) phase. Due to the interaction of the various functional groups present in the solute molecules with the stationary phase, each compound travels at a different speed. The strength with which an organic compound binds to an adsorbent depends on the strength of the following types of interactions: ion-dipole, dipole-dipole, hydrogen bonding, dipole induced dipole, and van der Waals forces.

Different types of chromatography use various types of stationary and mobile phases. In this experiment, the solid phase is silica gel while the mobile phase is an organic solvent (may be a single type or a mixture of solvents). This means that the compounds to be separated must choose between being absorbed to the solid silica gel or moving along in the organic solvent. The silica gel is either packed into a column or adhered to a sheet of glass or plastic, depending on the type of chromatography.

When a column is used, the compound mixture is placed on top and the solvents are run down the column separating the mixture along the way. With the silica gel on a plate, the compounds are placed close to the bottom and the mixture is separated as the solvent travels up by means of capillary action. Since silica gel is a porous form of SiO_2, the surface of gel contains Si-OH and Si-O-Si functional groups. With silica gel, the dominant interactive forces between the adsorbent and the materials to be separated are of the dipole-dipole type. Highly polar molecules interact fairly strongly with the polar Si—O bonds of these adsorbents and will tend to stick or adsorb onto the fine particles of the adsorbent while weakly polar molecules are held less tightly. Weakly polar molecules thus generally tend to move through the adsorbent more rapidly than the polar species. Figure 1 illustrates the general column chromatography set-up and

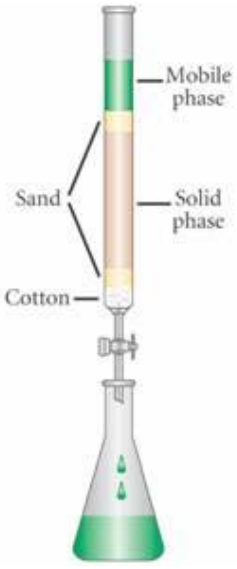


Figure 1. Column chromatography set-up

Figure 2 illustrates the movement of a compound mixture in a column and their separation and isolation.

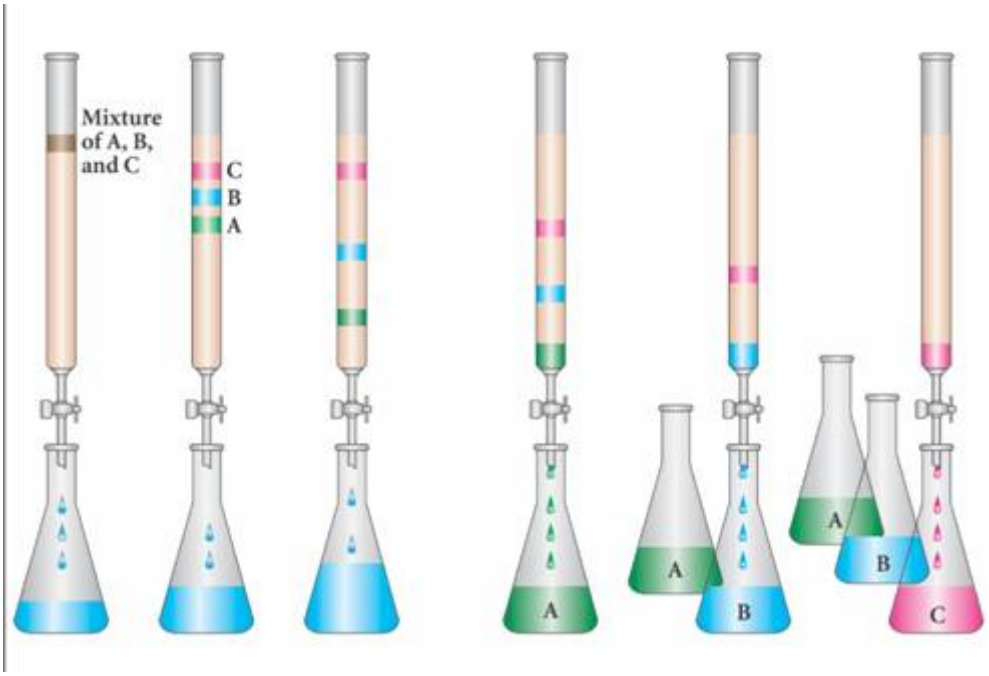


Figure 2: Illustration of a column chromatography separation of a mixture of 3 components, A, B and C. Compound A is the least polar and compound C is the most polar and the separation occurs as solvent is allowed to flow through the stationary phase.

Another factor that establishes the rate at which a compound travels past silica gel is the polarity of the solvent. A polar solvent will compete for silica absorption sites, disallowing the compounds to do so. This promotes a faster rate at which *all* compounds travel. The order in which the compounds move remains the same, while moving faster as the polarity of the eluent (solvent system) increases.

Column Chromatography

A column is prepared by placing a small plug of glass wool in the bottom of the cylindrical glass column, followed by a small layer of sand. The column is then packed with the solid adsorbent phase (silica gel). A slurry of adsorbent in solvent is prepared with the same solvent later used in TLC analysis. The slurry is carefully and slowly poured into the column after it is partially filled with solvent in order to prevent disturbance of the sand. The solvent is allowed to drain as the silica packs tightly. Once the solvent just barely becomes level with the silica (without drying it!), another small layer of sand is applied carefully without disturbing the silica.

The compound mixture is dissolved in a minimum amount of solvent (same as in the column) and very carefully added to the top of the column using a Pasteur pipette. After allowing the compound to absorb into the column, solvent is continually added to the top of the column until each band resolves and is carefully collected.

With coloured substances, the bands may be directly observed and collected as they run off the column. However, with colorless compounds, the development can be observed by collecting many small fractions of the eluting solvent and testing each by thin layer chromatography.

Thin Layer Chromatography (TLC)

TLC involves the same principles of separation as column chromatography but the apparatus and technique for development is different. Instead of a column, the silica (or alumina) is adhered to a plate of plastic or glass. A capillary spotter is used to apply the dissolved sample onto the plate about 1 cm from the bottom (a line with *pencil* is drawn). Once the solvent has evaporated, only the sample remains. The plate is carefully placed into a closed developing chamber, which has a shallow layer of solvent that does *not* submerse the spot. The chamber is lined with a folded piece of filter paper to ensure a uniform and saturated atmosphere of solvent vapour.

The plate is removed when the solvent front has reached about 0.5 cm from the top, and is quickly marked with pencil. The capillary action of the solvent causes the initial spot to be separated into individual components that may be visualized by colour identification or with the following techniques for colourless compounds:

(i) Irradiation with ultraviolet light
(ii) Reversible staining with iodine vapour (formation of brown spots which fade)
(iii) Spraying with a reagent that irreversibly colors the spots, e.g. H_2SO_4, $KMnO_4$

Figure 3 illustrates the general principles showing the separation of the same mixture described for column chromatography in Figure 2.

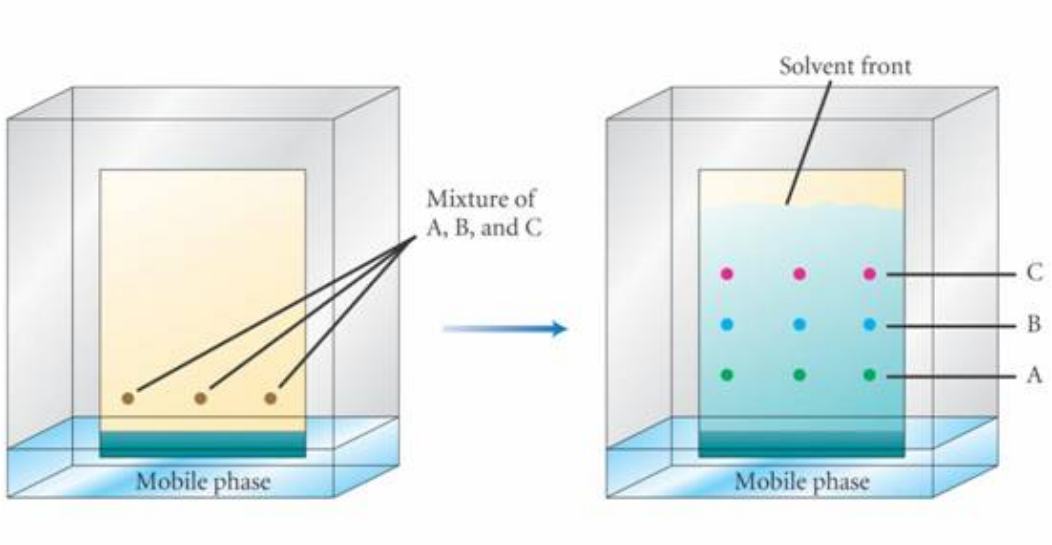


Figure 3: Thin-Layer Chromatography, illustrating the separation of the same mixture described for column chromatography in Figure 2.

The rate at which a compound moves in respect to the solvent front, the retention factor (R_f), is characteristic of that compound under standard conditions. The R_f value is calculated by dividing the distance each spot has traveled (measured from the pencil line to the middle of the spot) by the distance the solvent front traveled from the pencil line. This is illustrated in Figure 4.

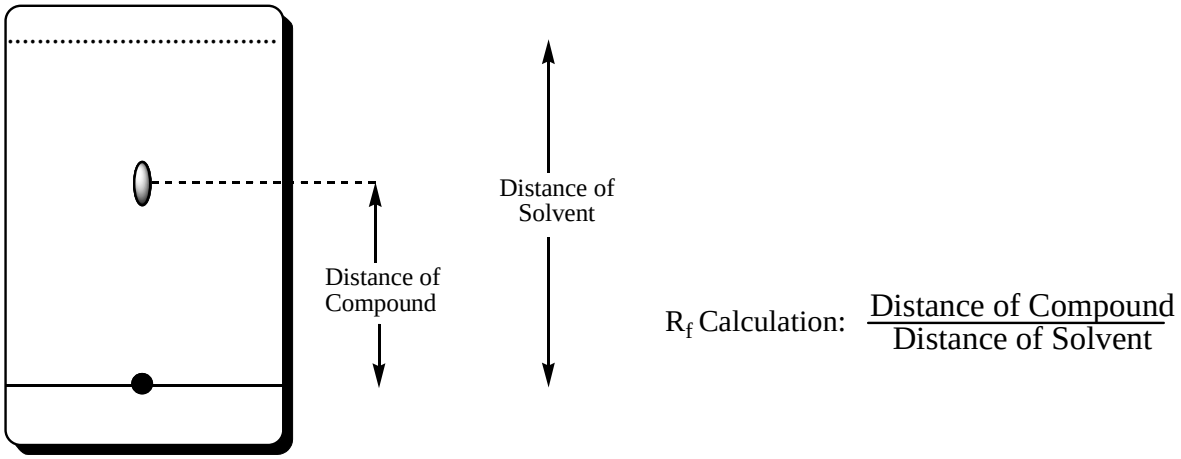


Figure 4: Illustration of a Developed TLC Plate and R_f Calculation

The advantages of TLC are the very small quantities of sample required and the great ease and rapidity with which it is resolved. For these reasons, it is often used to monitor the progress of a reaction by running the crude sample beside the reaction sample on the same plate. It is also

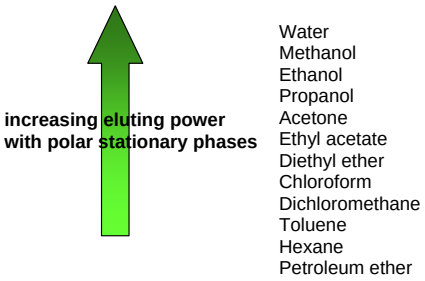
used to determine the best developing or eluting solvents for subsequent separation by column chromatography. The following are some common uses of Thin-Layer Chromatography:

1. To determine the number of components in a mixture.
2. To determine the identity of two substances.
3. To monitor the progress of a reaction.
4. To determine the effectiveness of a purification.
5. To determine the appropriate conditions for a column chromatographic separation.
6. To monitor the progress and separation achieved by column chromatography.

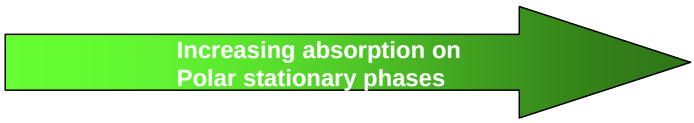
Factors Affecting Resolution of Separation

(i) Adsorbents: Weight ratio of adsorbent to sample is important to obtain accurate separation. If too much sample is applied, the active adsorbing sites will be saturated and the column will be flooded, resulting in poor separation. In most cases, a ratio of 20 to 1 is satisfactory but sometimes up to 100 to 1 is necessary. Even the ratio of column height to diameter is important; about 8 to 1 is considered optimal.

(ii) Solvents: The solvent (or solvent mixture) is important to the compound separation, keeping in mind that the more polar the solvent, the faster the compounds move. In some cases, a solvent system may increase in polarity by gradually changing the composition of the solvent mixture.



(iii) Functional Groups: Compounds with highly polar groups are strongly adsorbed and eluted less readily than less polar (or polarizable) compounds. The strength of adsorption for compounds having the following types of polar functional groups decreases in the order listed below. However, variations may occur depending on the overall structure of each specific compound.



RH, R-X, alkenes, $R\text{-}OCH_3$, $R\text{-}CO_2R'$, $R^1R^2C{=}O$, RNH_2, R-OH, RCO_2H

LIQUID-LIQUID EXTRACTION OF ORGANIC COMPOUNDS

Laboratory experiment #2 has an emphasis on liquid-liquid extraction techniques, but we will use a quick extraction at the beginning of Lab 1, thus the technique is briefly explained below.

The extraction or separation of neutral organic compounds from inorganic, ionic or otherwise water-soluble compounds is based on differential solubilities. Many organic compounds are of low polarity and preferentially dissolve in solvents of low polarity, whereas inorganic salts and highly polar organic compounds preferentially dissolve in solvents of high polarity, such as water. If the proper choice of solvents is made, one organic and water, then the two solvents make an immiscible pair (i.e. they will not mix or dissolve each other), and form layers – like oil and vinegar salad dressing! If a solid compound is then introduced to the solvents, and shaken vigorously, it will tend to dissolve to a greater degree in the solvent that is closest in polarity. In this scenario, inorganic salts and highly polar organic compounds (like sugar) may be separated from compounds of low polarity. Once the compounds have been partitioned between the solvents, the layer are separated by use of a separatory funnel and the solvent can be removed (by evaporation) to yield the solids (or high-boiling liquids), Figure 5.

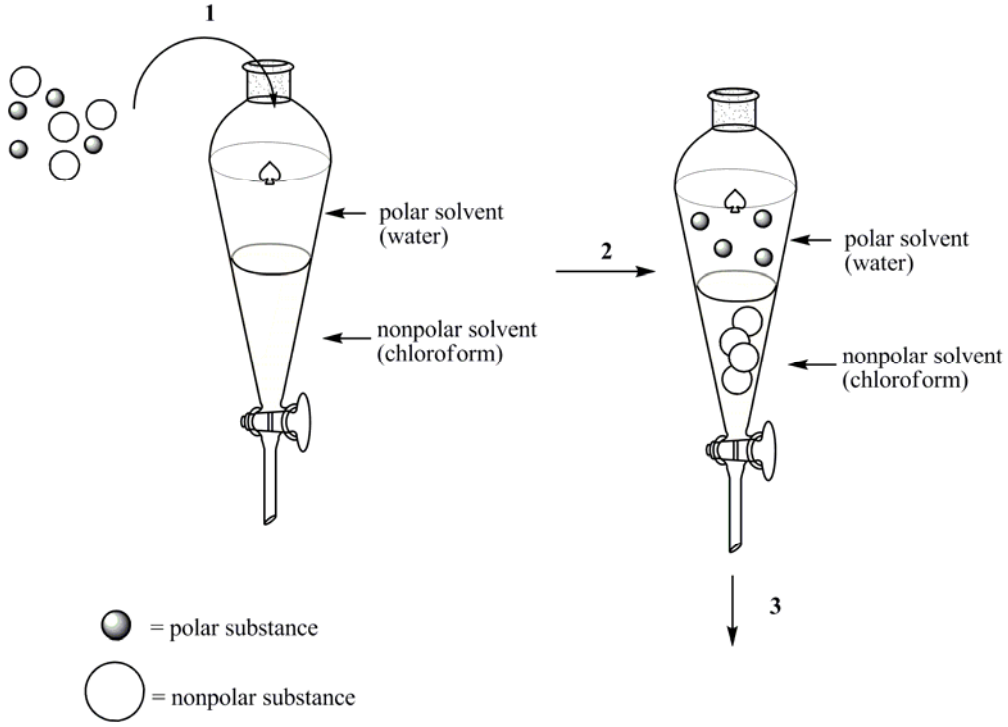


Figure 5: illustrating the principles of solvent-solvent extraction. 1) mixture of compounds introduced to solvent pair, 2) compounds partition into solvent of like polarity, 3) drain individual layers to isolate the separated compounds.

Apparatus and Method

Extraction is made possible since water and organic solvents are immiscible, which form two distinct layers (based on their specific density) in the separatory funnel. The glass bulb has a stopcock and stem at the bottom, as well as a plug at the top. The whole device is supported with a ring clamp covered in rubber tubing.

The solution is poured into the separatory funnel with the stopcock closed and a beaker under it just in case of a leak. A small amount of extracting solvent is added to the funnel (the flask should never be more than ¾ full) and with the upper opening and stopcock closed, the funnel is shaken with both hands. The opening should always be facing away from you into the fume hood away from others (Figure 6).

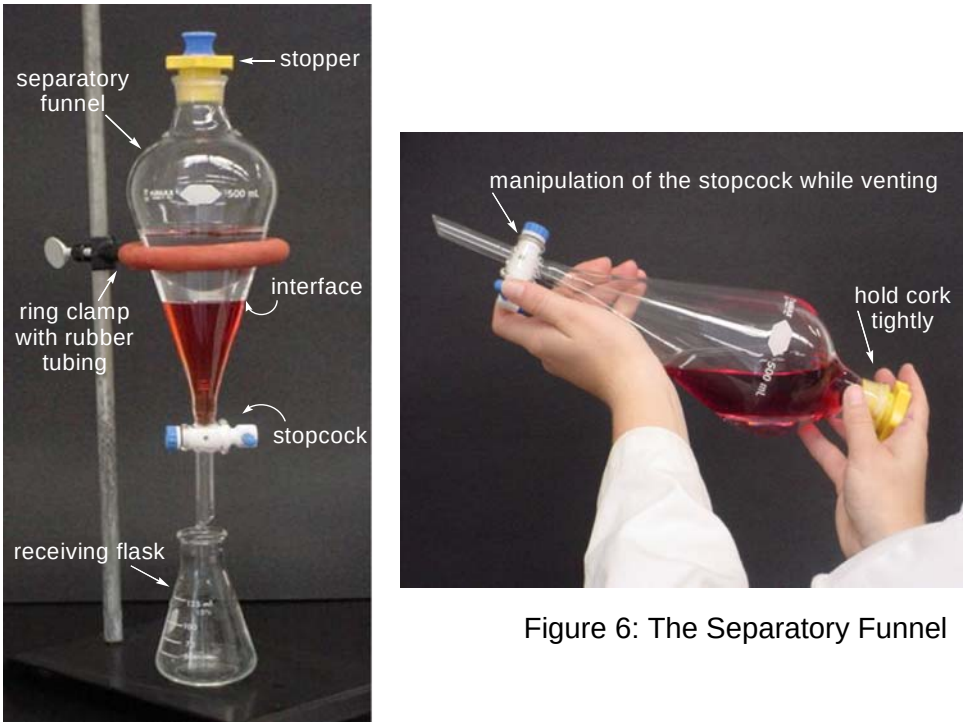


Figure 6: The Separatory Funnel

Shaking the flask is important since it maximizes the surface area of each component and allows intimate mixing. Every couple seconds the stopcock is slowly opened while pointing up to "vent", which allows built-up pressure to be released. The flask is put back on the ring clamp (with the stopcock closed!) and the top plug removed. The two layers are allowed to settle and the bottom layer is removed at a slow rate into a labeled flask. Just before the last couple drops of the lower layer are collected, swirl the funnel to collect any compound that may have been on the walls. ***Collect any last amounts of the lower layer and remove the top layer by***

pouring it out from the top. (bottom layer always out of the bottom, top layer always out of the top)

*****************Never discard any layer until you are certain you do not need it.*** ****************

If you are unable to identify which is the organic or aqueous, drop a small amount of water into one and if it dissolves it is aqueous. Normally, three extractions are performed to have an efficient extraction. To remove any last races of water in organic solvent, anhydrous magnesium or sodium sulfate is used before removing solvent by rotary evaporation.

Part A: TLC Analysis of Analgesic Drugs

Experimental Objectives:

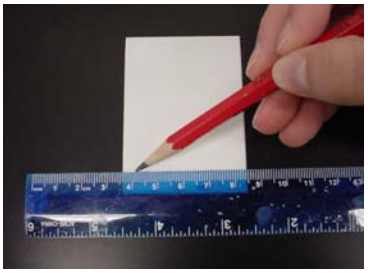
- Introduction to solvent-solvent extraction.
- To learn the technique of TLC and the visualization of colourless components.
- To resolve the components of commercial painkillers using TLC and identify the components found in each.

Procedure:

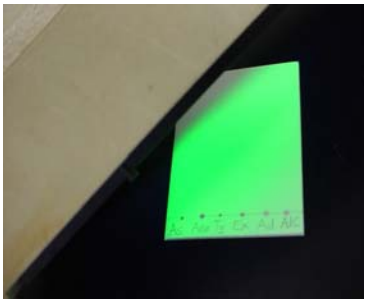
- Your TA will help you coordinate with other students at your bank of fumehoods to share and receive the solutions for TLC analysis. You will personally only prepare the solution for one drug and receive the other four from your colleagues.
- Obtain one tablet* of only one of the following common drugs and record the name in your notebook: (i) Aspirin©, (ii) Tylenol©, (iii) Excedrin©, (iv) Anacin©, (v) Advil©

  *Half of a tablet is sufficient – share the other half.

- Add 10 mL of chloroform into the mortar and dissolve thoroughly and then stir for about 10 seconds.
- Add 10 mL of water and thoroughly mix, stirring for about 10 seconds.
- Add the mixture to a small separatory funnel and isolate the organic fraction. Dry with a small amount of anhydrous sodium sulfate and use the supernatant for the TLC analysis.

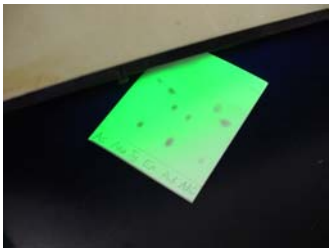


- Obtain a TLC plate. Draw a light pencil line about 1 cm from the end of the chromatographic plate.
- By sharing with the people around you, spot the plate with the 5 different analgesics. ***Use a separate capillary tube for each sample,*** so that you do not cross contaminate. Make each spot as small as possible (preferably less than 5 mm in diameter). Examine the plate under the ultraviolet (UV) light to see that enough of each compound has been applied; if not, add more.



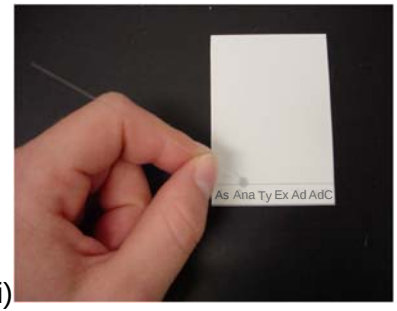
- Prepare a developing chamber as indicated in the picture using a 250 mL beaker as the chamber, a half-piece of filter paper inside, and foil or plastic wrap to cover.

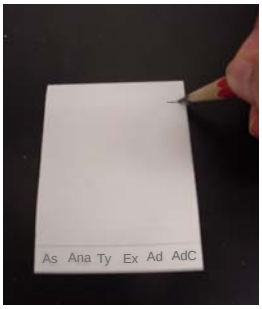
- Pour the eluent, 95:5 mixture of Ethyl Acetate/Glacial Acetic Acid, into the beaker to a depth of under 1 cm (~10 mL). Place the prepared TLC plate in the developing chamber, ensuring the solvent level is below the pencil line.
- After the solvent has risen to near the top of the plate (about 0.5 cm from the top), remove the plate and mark the solvent front with a pencil. Do not use a plastic ruler! Allow the solvent to evaporate from the plate in the fumehood and then examine it under UV light.



- Outline the spots with a pencil. The spots may also be visualized by putting the plate in an iodine chamber for a couple minutes (although this is usually not necessary).
- Show your TA the plate and if everything is in order then sketch the plate in your notebook and measure/calculate the R_f values for each spot, before leaving the lab.
- Determine the ingredient(s) of your analgesic contains based on the R_f values below.

The entire procedure is summarized in Figure 7.

(i) 

(ii) 
(iii) 

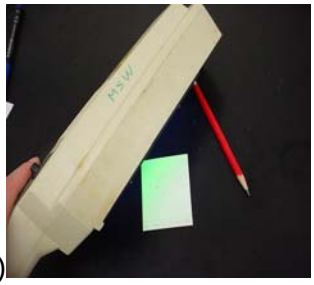
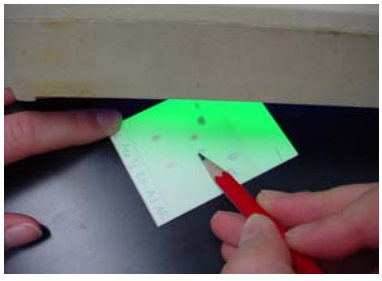
(iv) 
(v) 

Figure 7: (i) spotting the labelled TLC plate; (ii) eluting in the developing chamber; (iii) marking the solvent front (iv) visualizing the spots with the UV Lamp; (v) marking each spot.

TLC Troubleshooting (these hints will be useful throughout your undergraduate/graduate career)

With practice and experience, you will come to find that TLC is a very easy and useful technique. Suggestions are given below on how to solve common problems you may come across with TLC:

(i) The compound runs as a streak rather than a spot.

- The sample was most likely too concentrated. Run the TLC again, but try either diluting the sample or applying less sample to the plate. Your sample may also contain many components or your experiment did not proceed as expected, which could cause the spots to appear as a streak.

(ii) The sample runs as a smear or an upward crescent.

- Strongly acidic or basic compounds (amines and carboxylic acids) may appear as a smear. With the addition of a few drops of ammonium hydroxide (amines) or acetic acid (carboxylic acids) to the eluting solvent, the spots should appear clearer.

(iii) The sample runs as a downward crescent.

- This shape is characteristic of disturbed adsorbant caused during spotting. Hold the capillary spotter very carefully while applying the sample, ensuring the compound is only located on the desired spot by visualizing the plate under UV light before development.

(iv) The solvent front does not run in a straight line.

- Without a straight solvent front, R_f values cannot be accurately calculated. This problem can be solved by ensuring the edges of the TLC plate are smooth and not chipped by running your fingers along the sides and bottom of the plate. Also, make sure that the plate is not touching the sides of the container (or the filter paper) as the plate develops.

(v) Many random spots are seen on the plate.

- An organic compound such as solvent may have dropped on the plate. If the capillary spotter chipped or broke while applying the spots, excess sample may have splashed on the plate. Also, if pen was used on the plate, blue spots would appear.

(vi) Few or no spots are seen on the plate.

- Most likely, an insufficient amount of compound was applied. Try concentrating the solution or spot it several times in one place, allowing the solvent to dry between applications. If the solvent level in the chamber was deeper than the spotting line, the eluent would have dissolved the samples, preventing them to travel up the plate. Also, try visualizing the plate with iodine if the spots did not appear under UV light. Lastly, your experiment may not have proceeded as expected.

EXPERIMENT 1A: DATA SHEET

TLC of Analgesics

Name:	
Demonstrator:	

Due before the start of your laboratory the week of Oct. 4, 2010.

---

1. Draw a representation of your TLC plate after it has eluted and calculate the Rf values.

2. Identify the components of each of the analgesics from the tlc and complete the following table. (place an **X** if the drug contains the medical ingredient)

Components of Several Commercial Painkillers

		Medical Ingredient and its Rf Value			
		Acetylsalicylic Acid (Rf= 0.8)	Acetaminophen (0.6)	Caffeine (0.2)	Ibuprofen (0.9)
Analgesic	Anacin				
	Aspirin				
	Excedrin				
	Tylenol				
	Advil				

3. Look up and draw the chemical structures of each of the organic medical ingredients listed in the Table below (use any source you can find). Do the relative Rf values make sense to you? Explain your reasoning.

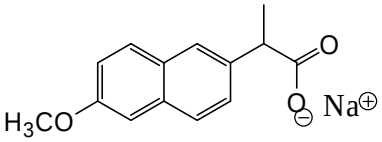
Medical Ingredient	Rf	Chemical Structure
Acetylsalicylic Acid	0.82	
Acetaminophen	0.62	
Caffeine	0.20	
Ibuprofen	0.93	

Rationale for relative Rf values:

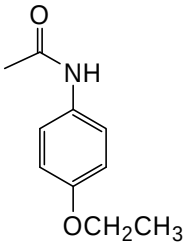
4. Drugs exert their effects by binding to specific proteins and receptors. To be recognized by the receptor, the molecule must contain certain atoms positioned at the right distances with respect to each other.

Besides all being powerful analgesics (pain-killers) some of these drugs also act as antipyretics (fever reducer) and/or anti-inflammatory agents (reduces swelling). For example ibuprofen acts as an analgesic, antipyretic and anti-inflammatory, but acetaminophen has little value as an anti-inflammatory.

Given the above statements what drug effects do you expect from the drugs naproxen and phenacetin whose structures are shown below? Explain briefly your reasoning.



naproxen

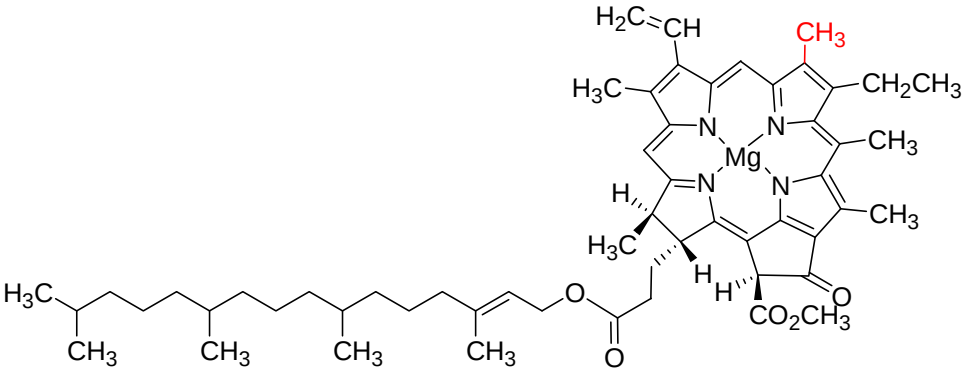


phenacetin

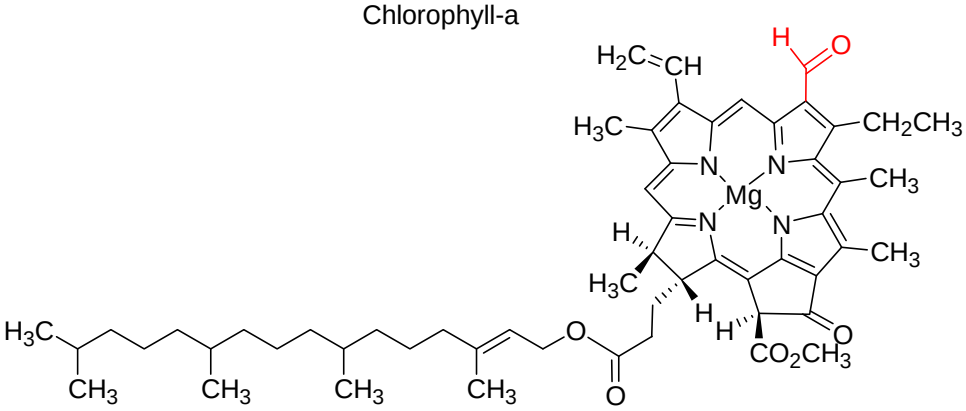
Week 2

Part B: Column Chromatography and TLC Analysis of Spinach Extract

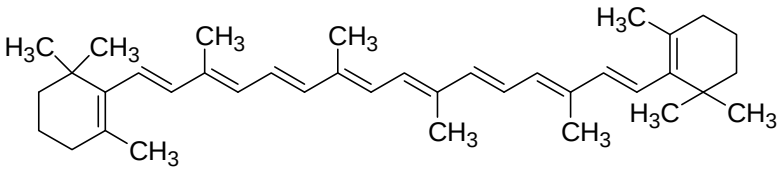
Column chromatography will be used to separate the following pigments found in spinach extracts. Some of these are shown in Figure 8.



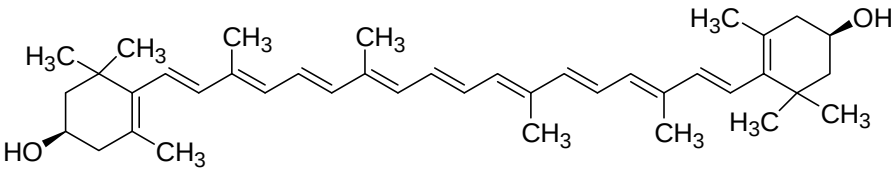
Chlorophyll-a



Chlorophyll-b



β-carotene



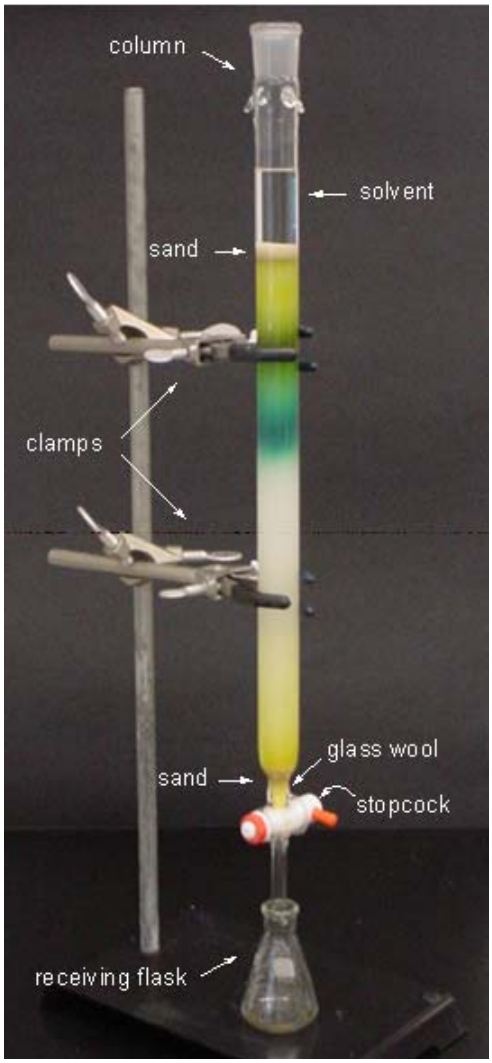
Lutein (a xanthophyll)

Figure 8: Structures of some of the pigments found in spinach leaves.

Experimental Procedures

(i) Column Preparation

- Obtain a 60 cm chromatography column, 40 mL of deactivated silica gel and 250 mL of the developing solvent mixture (petroleum ether (bp 60-80^{o}C)/acetone; 8:2).**
- Prepare a slurry of the silica adsorbent with 70 mL of solvent in a 250 mL Erlenmeyer flask.
- With a piece of glass tubing push a small plug of glass wool or cotton into the constriction at the bottom of the column. These glass tubes must be shared amongst the class.
- Using two clamps as shown, fix the column in a vertical position and add a 1 cm layer of sand on top of the glass wool or cotton (at the bottom of the column).
- Ensuring that the stopcock of the column is closed, pour in 15 mL of the 8:2 solvent mix. After the sand settles, quickly agitate and then decant all of the slurry through a funnel into the column.
- Open the stopcock and allow solvent to drain (2-3 drops/second) into a large flask while tapping the walls of the column with the ends of a folded piece of rubber tubing.



NOTE: The majority of packing occurs in the first 5 minutes and regular tapping of the column is particularly important in this period. However, over the next 20-30 minutes as the solvent drains out, additional settling occurs which can be monitored by marking with a felt tip pen or masking tape.

****Extraction of pigments should be completed during this time.**

DO NOT allow the silica to run dry!

- Once the solvent level is within 6 cm of the top of the adsorbent, the packing should be essentially complete. Carefully add a 1 cm *level* layer of sand on the adsorbent.
- Drain off excess solvent until its level is precisely on top of the sand (no lower!) and close

the stopcock.

(ii) Extraction of Spinach Pigments

- Shred about 2-3 medium sized leaves of fresh spinach and crush the shreds in a clean porcelain mortar containing a little sand and about 15 mL methanol.
- Filter the methanol solution into a 250 mL separatory funnel (with the stopcock closed!) supported by a ring clamp through a stemmed funnel and filter paper. Keep the spinach in the motar and press the solvent from it. Grind the macerated spinach again with a mixture of 10 mL of methanol and 25 mL of petroleum ether (bp 30-60^{o}C).
- Filter the mixture again into the separatory funnel. Regrind the spinach collected in the filter with a second portion of 15 mL of methanol and 25 mL of petroleum ether (bp 30-60^{o}C), and again filter the mixture into the separatory funnel. You may need to change the filter paper before this second filtration step if the solvent flow through it is too slow.
- At this point, you should have a dark green and a light green layer in the separatory funnel.
- Wash the petroleum ether layer (which contains the pigments) by adding 25 mL of water to the separatory funnel. With the stopcock closed and the top corked, invert the funnel and point the stopcock away from you and into the fume hood. Gently shake several times, and vent by slowly opening the stopcock while the flask is still inverted to relieve pressure. Shake and vent an additional two times.
- Allow the layers to settle with the flask on the ring clamp and remove the top cork. Emulsions may be broken up with the addition of 25mL aqueous NaCl. Slowly allow the bottom aqueous layer to collect in a labelled Erlenmeyer flask. Add another 25 mL of water to the organic layer in the separatory flask and complete the removal of methanol by washing again. Combine the ether layers into a labelled Erlenmeyer flask and dry by adding a small (approx. 1-2 g, there is no need to weigh this out) quantity of anhydrous sodium sulfate. After swirling and allowing to dry for 2-3 minutes, gravity filter off the drying agent and concentrate the pigment solution by evaporation to a volume of about 2 mL using a rotary evaporator.

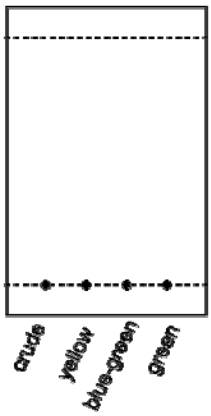
NOTE: This sample is used for TLC and Column Chromatography

(iii) Development and Elution

- Saving sufficient pigment extract to complete the TLC analysis (approx. 2-3 drops), carefully transfer the remaining extract to the top of the sand layer with a dropper. Avoid wetting the upper walls! Drain off the solvent until the pigment solution is just below the top of the sand. Rinse the walls with about 1 mL of fresh solvent and drain until the level is once again below the top of the sand. Repeat the rinsing of the walls until the solvent above the silica gel is virtually colorless.
- Very carefully fill the column with fresh solvent, and allow solvent to drain at a rate of 2 drops/second.
- Observe the separation of bands as the column develops. When the edge of the first band (yellow) reaches the lower part of column make a drawing of the various bands. DON"T STOP RUNNING THE COLUMN AS YOU DRAW! Label the various bands and their colour. Compare the separation with that observed by TLC with the same solvent.
- Collect the three fractions (yellow, blue-green, and green) by replacing the 250 mL flask with a 125 mL Erlenmeyer flask just before a new band is eluted. As soon as the band is collected, replace the flask with the original containing just clear solvent. Change the flask whenever a new band is about to run off. Clear solvent may be discarded in the proper container.
- Concentrate each fraction to a small volume by evaporation (**rotoevaporator**) for analysis by TLC.

(iv) Thin Layer Chromatography (TLC) with Spinach Extract

- Obtain a three (3) TLC silica gel plates and lightly draw a line about 1 cm from the bottom with a pencil and ruler. With a short length of capillary tubing, dip it into the original spinach extract sample (crude). The solution will be drawn up the tubing by capillary action. Touch the tip of the capillary against the plate at the line and solution will drain onto the coating. Apply a sample of each other concentrated fractions isolated by column chromatography (yellow, blue-green, and green in the same way, repeating application to each spot until each is about 2 mm in diameter.



- Prepare three (3) developing chambers by lining the wall of a 4-oz bottle with a folded 12.5 cm filter paper**.** Add sufficient developing solvent (5-8 mL) to saturate the paper liner and form a layer in the bottom of the bottle about 4mm deep at the shallow

centre.

- Develop the one TLC plate in each of the following solvent mixtures:
  - (i) petroleum ether (bp 60-80 ^{o}C)/acetone (9:1)*
  - (ii) petroleum ether (bp 60-80 ^{o}C)/acetone (8:2); already prepared
  - (iii) petroleum ether (bp 60-80 ^{o}C)/acetone (7:3)*

*Make about 10 mL quantities as needed.

- Carefully lower the spotted plate into the bottle and stand it on the bottom centre against the wall, ensuring that the spots are above the solvent level.
- Recap the bottle and observe the development of the solvent and sample mixture up the plate. When the solvent front reaches ~5 mm from the top**,** remove and make a pencil marking of the solvent level before it quickly evaporates.
- Record the color, intensity and location of each spot visible. Then note the presence of any additional spots by standing the plate in a bottle containing a few crystals of iodine for a few minutes. Quickly outline any additional spots with a pencil.

(v) Ultraviolet/Visible Spectra

- The electronic absorption spectrum of each fraction in cyclohexane over the range 350-700 nm, will be provided to you.
- Include a discussion of the visible spectrum in your report.

LAB REPORT GUIDELINES: CHEMISTRY 2273a – 2010

Due: in class by Friday, Oct. 15

A formal report of no more than 5 pages (excluding spectra)

Experiment 1b: Column Chromatography and TLC Analysis of Spinach Extract

1. Title, Date, Name

2. Objective: purpose of lab – 1 sentence

3. Introduction: brief description of technique(s) used in lab – 2/3 sentences

4. Procedure: a) cite lab manual with proper reference, note any changes

5. Results and Discussion:

    a. Present the drawings of the TLC plates developed with different eluents and the calculation for Rf values.
    b. Comment on the number of pigments in spinach and identify them
    c. Present the drawing of the column chromatography at a point that shows separation of the components
    d. Explain the rate of elution of the pigments based on their chemical structures.
    e. Discuss the Rf values based on chemical structures and explain which solvent systems is best and why.
    f. Discuss the UV-vis spectra in relation to the observed colour of the pigments.
    g. This technique is very useful for separating reaction mixtures. Explain briefly how this technique can be used to separate a mixture of colorless components.

Total: 20 marks

EXPERIMENT 2

(i) Liquid-Liquid Extraction – Separation of Organic Acid, Organic Base and Neutral Components of a Mixture

(ii) Recrystallization as a means of purification of organic solids

Purity, Purification and Physical Properties

Naturally occurring compounds are seldom found in a 'pure' form but more commonly as mixtures with a number of other compounds. Similarly, chemical reactions lead invariably to mixtures of products and unreacted starting materials. Clearly methods must be available, or devised, for the separation, isolation and purification of the molecules of interest. The most important methods that have evolved over a period of time are based primarily on **differences in physical properties** between compounds.

The various physical properties of compounds are, in large measure, determined by intermolecular attractive forces, either between the 'like' molecules of one compound or the 'unlike' molecules of two different compounds. This applies not only to the aggregate physical state of a compound (solid, liquid, and gas) but also to intrinsic properties such as density, vapor pressure, solubility, etc. The most commonly used methods of separating and purifying compounds are based on differences in only three particular physical properties:

VOLATILITY: the 'escaping tendency' of molecules from the liquid or solid state to the vapor state; 'vapor pressure' is its quantitative measure.

SOLUBILITY: the extent to which one compound (solid, liquid or gas) will 'dissolve' in a second compound (most commonly a liquid) to form a single, homogeneous phase.

ADSORPTION: the tendency of foreign molecules to be attracted and held in a mono-molecular layer on the surface of a solid; involves weak Van der Waals forces similar to those determining solubility.

Summarized below, are the four methods of separation and purification that are commonly used in the laboratory;

Method	**Physical Property**	**Type of Material**
Distillation	Volatility	Liquids
Recrystallization	Solubility	Solids
Extraction	Solubility	Solids or Liquids
Chromatography	Adsorption/Solubility	Solids or Liquids (or gases)

Although by definition a pure compound is one that is completely free of any impurity, in practice this is never possible if for no other reason that it is impossible to measure very small amounts of impurities. Compound A, for example, may be contaminated by 20%, 1%, one part in a thousand, or even one part in a million of a compound B. The purity required of a compound is often dependent on its intended use. In this laboratory course, compounds that are 99% pure are usually quite satisfactory reagents, but in specialized work, such as quantitative analysis, compounds of much higher purity, e.g. 99.999% may be required.

As mentioned above, the concept of purity implicitly assumes the ability to measure it. Thus, not only are methods of purification needed, but also **methods of determining purity**. The methods used are based on those physical properties. For **liquids**, the common criterion of purity is the **boiling point**, but it suffers many disadvantages. For **solids**, the **melting point** is the physical constant commonly used. Chromatographic techniques and spectroscopic techniques also play an important and major role for this purpose, especially in research laboratories.

In the first experiment you learned the general technique of separation using absorption techniques, namely thin-layer and column chromatography and solvent extraction. This experiment will demonstrate **solvent extraction** as practice in the technique for the separation and isolation of the components of a mixture. As outlined briefly in the following discussion, the utility of this simple technique is enormously enhanced when used in conjunction with the acid/base properties of the compounds involved. Another problem commonly associated with the separation of mixtures is that the identity of one or more of its components will be unknown and must be determined experimentally. That identification may be as simple as determining the melting or boiling point. Typically, of course, much more information will be required. Various spectroscopies, especially Infrared (IR), Ultraviolet (UV) and Nuclear Magnetic Resonance (NMR) are particularly useful. In this experiment you will be introduced to the measurement of **Infrared spectra** and their use for structural elucidation. During the course of the year you will learn and use NMR spectroscopy, which is a much more powerful technique for structural elucidation of unknowns.

BASIC PRINCIPLES

The technique of extraction, or, more accurately, solvent extraction, is probably the most widely used method for either the initial isolation of natural products from their source materials or the preliminary separation of products from reaction mixtures. In general terms, separation by extraction is based on the principle of phase distribution. This involves selective transfer of one or more components of a mixture to a second, immiscible phase in contact with it. In practice, the mixture is either solid or liquid and the second phase is always a liquid solvent. In an **ideal** case, the desired compound would be completely transferred to the new liquid phase, while all impurities or other components are left behind. More commonly, particularly with two immiscible liquid phases, the desired compound is distributed or partitioned between the two phases i.e. part of it remains in the original phase and part is transferred to the new phase. The partitioning is the consequence of differing relative solubility of the component(s) in the two immiscible solvents. (See the Appendix for a discussion) In those instances in which it is the impurities that are removed, leaving the desired compound in the original phase, the extraction process is more commonly referred to simply as "washing".

LIQUID-SOLID EXTRACTION

This simplest form of extraction is well known and practiced by most of us each day (or at least by those at Tim Horton's or Starbuck's) in brewing a cup of coffee or tea. A finely divided solid mixture is stirred, and usually warmed, with a suitable solvent to effect selective dissolution of one or more components of the solid. After mechanical separation of the liquid solution from undissolved solids by filtration, the resultant solution may be used for some purpose (drinks, drugs, etc.) or, if desired, the extracted components may be isolated, usually by evaporation of the solvent (instant coffee powder!).

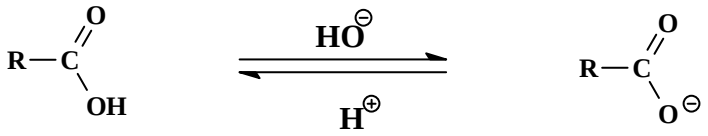
The extraction of alkaloids from leaves and barks, flavoring extracts from seeds, perfume essences from flowers and sugar from sugar cane are typical examples of separations of this type. The solvents most commonly used for this purpose are water, various alcohols, ether, chloroform, methylene chloride (a.k.a dichloromethane), acetone, and benzene or other hydrocarbons.

LIQUID-LIQUID EXTRACTION OF ORGANIC ACIDS AND BASES

The extraction or separation of organic acids and organic bases is based on the observation that acids and bases can be interconverted from their uncharged (neutral) form to a charged form upon treatment with strong acids or strong bases. In general, charged species (e.g. ions) are soluble in water and are insoluble in organic solvents (e.g. methylene chloride, diethyl ether). On the other hand, neutral, uncharged species tend to be water insoluble and soluble in organic solvents. Thus, if a charged species has its choice between water and an organic solvent, it will go into the water; a neutral species will choose an organic solvent over water.

Like all generalizations, there certainly are exceptions to this rule. For example, while sodium chloride dissolves in water, silver chloride does not. There are quite a few ionic compounds that are water insoluble. However, there are very few ionic compounds that dissolve in organic solvents. Similarly, there are several neutral compounds that dissolve readily in water. Most of these are small polar molecules capable of hydrogen bonding to the water. Examples include sugar, methanol, ethanol, acetic acid and acetone.

There are very few types of organic compounds that are acids and the most common contain either the carboxylic acid group (-CO_2H) or the sulfonic acid group (-SO_3H). The one you will encounter in this experiment contain the carboxylic acid group and most of these are weak acids with a pKa ~ 5. This consequence is that an excess of hydroxide will deprotonate the uncharged acid and convert it to its charged conjugate base form. Treatment of the charged conjugate base form with excess hydrochloric acid will protonate it and convert it back to the uncharged carboxylic acid form.



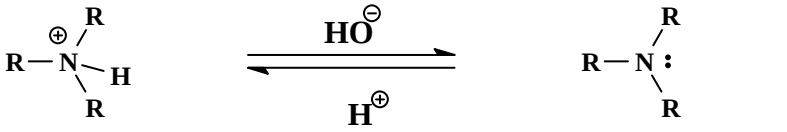
uncharged
organic soluble

charged
water soluble

If you now recall the solubility behavior discussed above, this means that an organic acid can be moved back and forth from water to an organic solvent by treatment with excess hydrochloric acid or excess sodium hydroxide. When excess hydrochloric acid is used, the organic acid exists in the uncharged form and will select an organic solvent over water; with excess hydroxide, the organic acid exists in the charged form and will select water over an organic

solvent.

Analogously an organic base, typically an amine (RNH_2, R_2NH or R_3N), can exist as either a charged or uncharged species depending upon the pH of the solution. However, the behavior is the opposite of an organic acid. In acidic solution, the base is protonated and exists as an ammonium salt and is charged. If the solution is made basic, the ammonium salt is deprotonated and the base becomes uncharged. Thus under acidic conditions, the charged base will prefer to dissolve in water and under basic conditions, the base will choose an organic solvent.



charged
water soluble

uncharged
organic soluble

The great majority of organic compounds are neither acids nor bases and these 'neutral' organic compounds will not be protonated or deprotonated. Consequently, these neutral compounds remain uncharged species that greatly prefer to dissolve in organic solvents in preference to water.

It is this differing behaviour between acids, bases and neutral organic compounds that forms the basis for their separation by extraction. You will be given a mixture of an organic acid, an organic base and a neutral organic compound that you will separate by solvent extraction and tested appropriately to determine the identity of each component.

Apparatus and Method

Extraction is made possible since water and organic solvents are immiscible, which form two distinct layers (based on their specific density) in the separatory funnel. The glass bulb has a stopcock and stem at the bottom, as well as a plug at the top. The whole device is supported with a ring clamp covered in rubber tubing.

The solution is poured into the separatory funnel with the stopcock closed and a beaker under it

just in case of a leak. A small amount of extracting solvent is added to the funnel (the flask should never be more than ¾ full) and with the upper opening and stopcock closed, the funnel is shaken with both hands. The opening should always be facing away from you into the fume hood away from others (Figure 1).

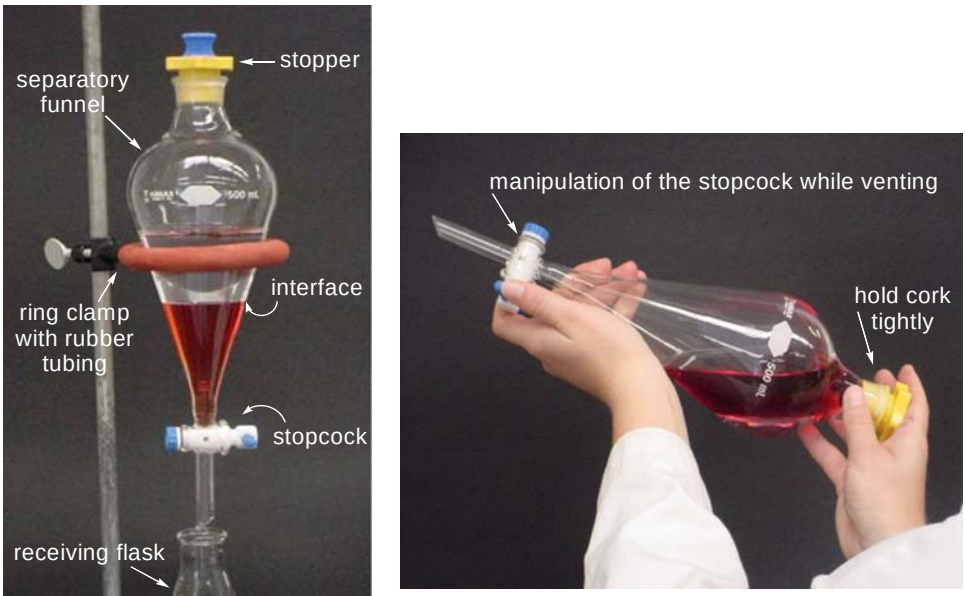


Figure 1: The separatory funnel

Shaking the flask is important since it maximizes the surface area of each component and allows intimate mixing. Every couple seconds the stopcock is slowly opened while pointing up to "vent", which allows built-up pressure to be released. The flask is put back on the ring clamp (with the stopcock closed!) and the top plug removed. The two layers are allowed to settle and the bottom layer is removed at a slow rate into a labeled flask. Just before the last couple drops of the lower layer are collected, swirl the funnel to collect any compound that may have been on the walls. ***Collect any last amounts of the lower layer and remove the top layer by pouring it out from the top***. (bottom layer always out of the bottom, top layer always out of the top)

Never discard any layer until you are certain you do not need it. If you are unable to identify which is the organic or aqueous, drop a small amount of water into one and if it dissolves it is aqueous. Normally, three extractions are performed to have an efficient extraction. To remove any last races of water in organic solvent, anhydrous magnesium or sodium sulfate is used before removing solvent by rotary evaporation.

Experimental Procedure

DO ALL PROCEDURES IN THE FUMEHOOD!

DO NOT WORK OUTSIDE OF THE HOOD, except for the melting points.

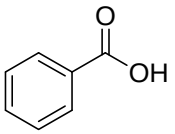
You will be using a lot of different solutions and flasks, so LABEL THEM APPROPRIATELY and be organized

When a procedure mentions to use water, use deionized water (from blue plastic pipes)

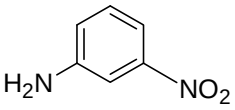
BEFORE COMING TO THE LABORATORY COMPLETE THE FLOW CHART THAT APPEARS AT THE END OF THIS PROCEDURE. IT WILL HELP CLARIFY THE VARIOUS STEPS AND WILL BE A USEFUL GUIDE DURING THE EXPERIMENT.

Step A: Dissolve the mixture

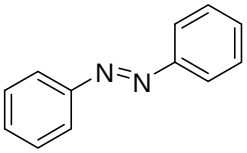
- You will be given approximately 1.0 g mixture that contains equal portions of benzoic acid, azobenzene, and 3-nitroaniline. Weigh the mixture to <u>obtain an accurate mass</u> and then dissolve it in ~25 mL of dichloromethane in a 50 mL Erlenmeyer flask.



Benzoic Acid (mp 122.4 ^{o}C)



3-nitroaniline (mp 114 ^{o}C)



Azobenzene (mp 69 ^{o}C)

Step B: Extraction of the Organic Base

- Place the dissolved mixture into a separatory funnel. Make sure the stopcock is closed. Proper technique is to be sure a large beaker is placed below the sepratory funnel in case of any spillage or leaking from the stop-cock. Add ~15 mL of 3 M hydrochloric acid to the dichloromethane solution in a separatory funnel. Invert the funnel with the stopcock closed and top corked. Point the flask into the fume hood and shake gently, while regularly venting. Remove the two layers into labeled, clean 250 mL Erlenmeyer flasks. The lower organic layer is replaced back into the funnel, where it is extracted two more times with additional 3 M HCl (~15 mL portions). All aqueous layers are combined in a 250 mL Erlenmeyer flask and set this flask aside until **STEP E**. The organic layer is carried forward to **Step C.**

Step C: Extraction of the Organic Acid

- To the dichloromethane solution (you may want to add a bit more dichloromethane), add ~15 mL of 3 M sodium hydroxide in a separatory funnel and extract the two layers. The lower organic layer is washed two more times with additional 3 M NaOH (~15 mL portions). Both layers are collected in clean and labeled flasks. All aqueous layers are combined in a 250 mL Erlenmeyer flask and set this flask aside until **STEP F**. The organic layer is carried forward to **STEP D**.

Step D: Extraction of the Neutral Organic

- Lastly, wash the dichloromethane solution in a separatory funnel 3 times with ~10 mL distilled water. The combined upper aqueous layers may be properly discarded.
- Place the dichloromethane solution in an Erlenmeyer flask (you may have to add more dichloromethane). Add an appropriate amount of anhydrous sodium sulfate to this solution (until you see the snow globe effect) and allow it to dry for ~5 min. Gravity filter the solution through a fluted filter paper into another *pre-weighed* dry Erlenmeyer flask containing a boiling stone.
- Evaporate the dichloromethane solution (until less than 1 mL remains) on a hot plate (on a low setting) with one boiling stone. Allow the solution to cool to room temperature and then put in an ice bath.
- Weigh the flask and crystals to determine the mass of product. Record the colour and melting point of the product. Use this is **Step G**.

Step E: Isolation of the Organic BASE

- *To be sure you have he right flask, check the pH using litmus paper. It should be acidic to start; you will neutralize it by adding base.*
- Neutralize the combined acidic aqueous extracts (containing the organic BASE) by adding 6 M NaOH dropwise (with swirling) until the solution is alkaline. This may be monitored by using litmus paper. The litmus paper will change from red to blue. **Do not add too much base!** Notice the color change! (What is going on?)
- Cool the flask in an ice bath for 10 minutes and collect the solid precipitate by vacuum filtration using a Buchner funnel. Wash with 2 mL of cold distilled water.
- Dry and weigh the crystals. Record the colour of the product and determine its melting point.
- The dried filtrate should be recrystallized from a suitable solvent until pure (constant melting

point) and kept for identification. It should be weighed before and after recrystallization to determine the purification yield as well as the overall yield. Use the melting point for identification.

- Obtain an IR spectrum of the dried, recrystallized product. (second lab day). The TAs will assist with collection of the spectra.

Step F: Isolation of the Organic ACID

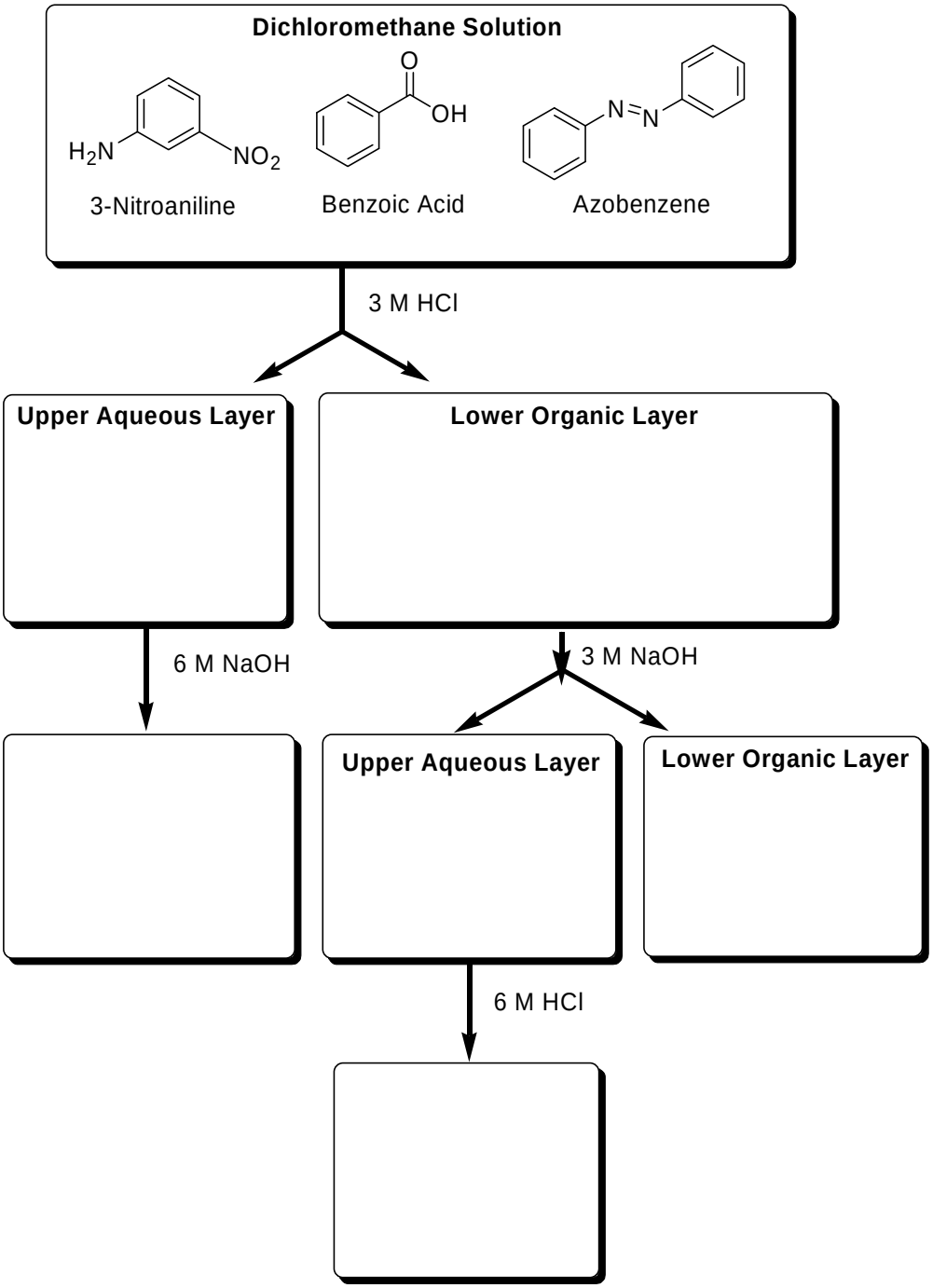
- *To be sure you have the right flask, check the pH using litmus paper. It should be basic to start; you will neutralize it by adding acid.*
- While on ice, neutralize the combined basic aqueous extracts (containing the organic ACID) by adding 6 M HCl dropwise (with swirling) until the solution is acidic. This may be monitored by using litmus paper. The litmus paper will change from blue to red.
- Continue to cool the flask in an ice bath for 10 minutes and collect the solid precipitate by vacuum filtration using a clean Buchner funnel. Wash with 2 mL of cold distilled water.
- Dry and weigh the crystals. Record the colour of the product and determine its melting point.
- The dried filtrate should be recrystallized from a suitable solvent until pure (constant melting point) and kept for identification. It should be weighed before and after recrystallization to determine the purification yield as well as the overall yield. Use the melting point for identification.
- Obtain an IR spectrum of the dried, recrystallized product. (second lab day)

Step G:

- The neutral component will be impure (why? - think about the extraction process; is it 100% effective? Read the appendix) To purify this component, you will recrystallize it from an appropriate solvent. (details to be given)
- Obtain an IR spectrum of the dried, recrystallized product. (second lab day)

***Waste:** Combine all the aqueous layers throughout the experiment and adjust the solution to neutral using the acid or base provided before pouring down the sink with lots of water. The neutral organic flask is rinsed with acetone in the organic waste container.

Draw the appropriate chemical structure (acid, base, neutral, conjugate base, conjugate acid) in the appropriate box in the following flow chart.



Appendix 1: Solvent Partitioning in Liquid-Liquid Extractions

In organic chemistry, most commonly one of the solvents is organic and the other is aqueous. Inorganic compounds can usually be separated from organic compounds in this way; the former dissolve in the aqueous phase and the latter in the organic solvent. In such cases, a single extraction may be sufficient to effect satisfactory separation. However, many organic compounds (particularly oxygen – or nitrogen – containing compounds, such as aldehydes, alcohols, esters, and amines, which can form hydrogen bonds) are partially soluble in water. They distribute themselves between the aqueous phase (w, for water) and the organic solvent (o) in proportion to their relative solubilities (S) in the two solvents. In this sense the extraction can be considered a competition between two immiscible liquids for the solute, with the solute partitioning between these two liquids.

The ratio of the concentrations of a substance in the two solvents (C_o /C_w) at equilibrium is called its distribution coefficient, K_D, and can be expressed mathematically:

$$\mathbf{K_D = S_o/S_w = [A] \text{ in organic solvent}/[A] \text{ in water} = C_o/C_w}$$

For example, suppose the solubility of compound A is 0.60 g/100 mL in ether and 0.12 g/100 mL in water. K_D is then 0.60/0.12 = 5.

To illustrate how the distribution coefficient K_D can be used, let us calculate the amount of A that is removed from a solution containing 80 mg of A in 80 mL of water by extracting with 150 mL of ether. If we let *x* be the number of milligrams of A extracted into the ether layer, then (80 - *x*) represents the milligrams of A remaining in the water. The equation for K_D is, therefore,

$$\mathbf{K_D = C_o/C_w = 5 = (x/150)/((80\text{-}x)/80)}$$

Solving for *x*, we find that 72.3 mg of A will be extracted by the ether and, consequently, that 7.7 mg of A (80 – *x*) will remain in the water.

It is easy to show that if we had extracted A twice with 75 mL of ether instead of once with 150 mL of ether, we would have removed 65.9 + 11.6 mg = 77.5 mg of A from the water. In general, performing several extractions using smaller volumes of solvent is more efficient than performing a single extraction using a larger volume of solvent.

Practical Considerations

The selection of the appropriate extraction solvent is a key to the success of the technique of isolating and purifying compounds. An extraction solvent:

1. must not react in a chemically irreversible way with the components of the mixture.
2. must be immiscible, or nearly so, with the original solution
3. must readily dissolve the substance to be extracted
4. should extract only the desired substance or as small an amount as possible of any other substance present (related to the relative K_D's)
5. should be easily separated from the desired solute after extraction. This last requirement can be met if the solvent is low-boiling and easily removed by distillation or evaporation.

Common organic solvents that fulfill these requirements include many hydrocarbons and their chloro derivatives, such as benzene, petroleum ether (not a "true" ether, but rather a mixture of low-boiling alkanes), dichloromethane, chloroform, and carbon tetrachloride. Another common solvent is diethyl ether (a "true" ether and usually referred to as just ether, although this is incorrect). Diethyl ether is highly flammable and is slightly water-soluble (about 7 g/100 mL) but since most organic compounds are highly soluble in it and because of its low boiling point (35° C), ether is frequently used despite its drawbacks and its cost. Remember that all organic solvents are potentially harmful, but they can be used safely if we carry out operations in an efficient fume hood and take care to avoid getting them on the skin.

The above discussion focused on partitioning of one substance between two immiscible solvents. The same principles apply if one has a mixture of two or more compounds

Appendix 2: Recrystallization

Impure crystalline substances can be purified by recrystallization from a suitable solvent or solvent mixture. This process depends on the fact that most compounds are more soluble in hot solvents than in cold ones and on the fact that the impurities present have solubilities different from those of the desired compounds. The procedure involves:

1. Dissolving the impure material in a minimum amount of boiling solvent,
2. Filtering the hot solution to remove insoluble impurities,
3. Allowing the solution to cool and to deposit crystals of the compound,
4. Filtering the crystals from the solution (called the mother liquor),
5. Washing the crystals with a little cold solvent to remove the mother liquor, and
6. Drying the crystals to remove the last traces of solvent.

General Concepts:

If recrystallization is to be effective, the solvent must be properly selected. A good recrystallization solvent should:

1. dissolve a moderate quantity of the substance being purified at an elevated temperature, but only a small quantity at low temperatures,
2. not react with the substance being purified,
3. dissolve impurities readily at a low temperature or not dissolve them at all, and
4. be readily removable from the purified product. This last requirement usually means that the solvent should have a fairly low boiling point and should evaporate readily. If a single solvent cannot be found that meets all these requirements, it is possible to use a mixture of two solvents.

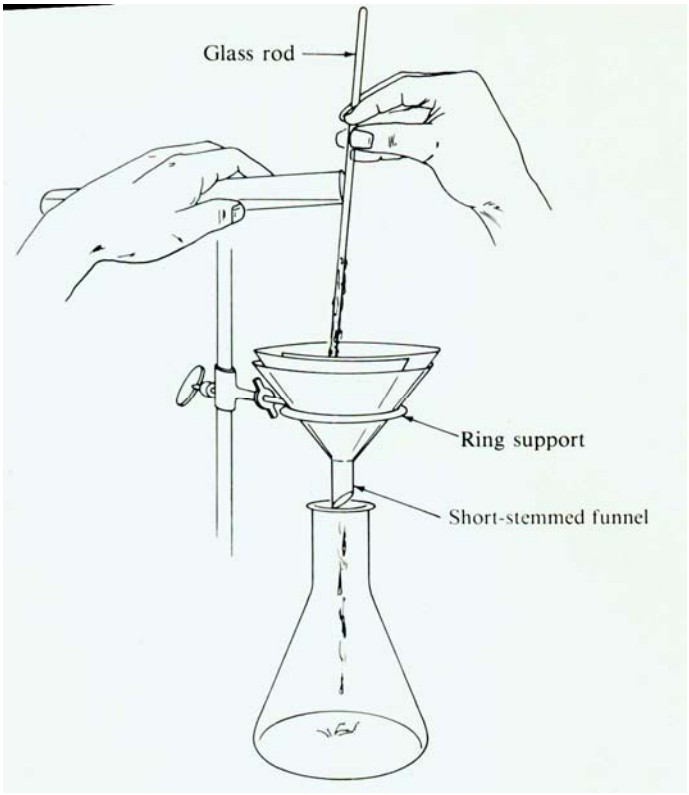
Solvents suitable for recrystallizing a known compound are generally reported in the chemical literature. If none is reported, or if the substance is a new compound, several solvents can be tested in the following way. Place about 10 mg (a small spatula tip-full) of the substance to be purified in each of several 10 × 75 mm test tubes, and add about 0.25 mL of a different solvent

to each. Then observe the solubility of the sample in each solvent, when cold and when heated. Also note whether abundant, well-formed crystals are produced as the hot solution cools.

To obtain a good recovery of purified material, it is best to avoid using unnecessarily large volumes of solvent. Dissolving the substance in the smallest possible amount of hot solvent minimizes the amount of pure material lost by retention in the mother liquors. In practice, 3-5% more solvent than the minimum required is used so that the hot solution will not be quite saturated. This helps to prevent separation of the crystals and clogging of the filter paper during filtration of the hot solution.

Traces of coloring matter or resinous impurities can sometimes be removed with selective absorbents, such as finely divided charcoal. To do this, add a small amount of decolorizing charcoal to the warm solution before filtering it. (*Do not add decolorizing charcoal to a hot solution. If a solution is at or near its boiling point, the addition of finely divided charcoal (which acts as thousands of boiling chips) will cause rapid boil over.) Avoid using excess decolorizing agent, however, because it may also adsorb appreciable amounts of the substance being purified.

Some substances readily form supersaturated solutions, and crystallization may not occur spontaneously when the hot solution is cooled. In such situations, it is sometimes possible to initiate crystallization by scratching the walls of the vessel beneath the surface of the solution with a stirring rod. The best way to induce crystallization is to "seed" the cold solution with one or two crystals of the substance being purified. It is necessary to filter the solution while it is still hot, otherwise the solution will cool rapidly and solids will form prematurely.



Apparatus for gravity filtration – when done hot, a fluted filter paper should be used – your TAs will show you how.

Apparatus for Hot Filtration and Vacuum Filtration

To remove insoluble impurities and decolorizing charcoal, it is necessary to filter the solution while it is hot using a fluted filter paper. (Figure 2) Vacuum filtration is generally used to remove soluble impurities and solvent from the crystals of the purified substance. A Hirsch (or Buchner) funnel is fitted to a filter flask with a rubber adapter. A disk of filter paper just large enough to cover all the holes in the funnel is placed in the funnel and moistened with some of the solvent used in the recrystallization. The filter flask is then connected to the aspirator by thick-walled rubber tubing through a water trap, and a vacuum is applied. (set-ups are shown below). Hirsch funnels are generally used for smaller quantities and Buchner funnels for larger quantities. When the filter paper is drawn tightly to the funnel, the solution and crystals are transferred to the funnel. The solution passes through the paper, while the crystals deposit on the paper.

Set-us for vacuum filtration: (left: using Buchner funnel, right: using Hirsch)

Far right is shown the Hirsch Funnel (left) and Buchner Funnel (right), respectively.

LAB REPORT GUIDELINES: CHEMISTRY 2273a – 2010

Due: in class by Friday, Nov. 12

Experiment 2: Liquid-Liquid Extraction and Recyrstallization

Style: Formal report of 5 or less pages (excluding spectra).

1. Title, Date, Name

2. Objective: purpose of lab – 1 sentence

3. Introduction: brief description of technique(s) used in lab – 2/3 sentences

4. Procedure: a) cite lab manual with proper reference, note any changes
   b) include extraction flow chart

5. Results:

   a. Yield of Acid, Base, & Neutral
      i. weight of crude (after extraction) and purified (after recrystallization)
      ii. percent yield of crude based on weight of mixture
      iii. percent yield of purified based on weight of mixture
      iv. percent recovery of purified based on crude

   b. Physical Properties

      i. Appearance – liquid/solid, crystalline character, colour
      ii. Melting Points – report as range, cite literature values for comparison
      iii. IR Spectra – label important peaks with respect to functional groups present

6. Discussion:

   a. Discussion of Yield
      i. Reasons for less (or more) than 100% yield (A+B+N) from crude (do not include human error)
      ii. Reasons for less than 100% recovery from each recrystallization (do not include human error)

   b. Discussion of Physical Properties – <u>evidence that the correct compounds were obtained for A, B, & N</u>
      i. Discussion of melting points for A, B, & N
      ii. Discussion of IR spectra for A,B,& N

   c. Why should neutral compound be the most impure?

Total: 20 marks

EXPERIMENT 3

Practical NMR and IR Spectroscopy and Mass Spectroscopy

Relevant parts of the text:

Chapter 12: Infrared Spectroscopy and Mass Spectrometry
Chapter 13: NMR, ^{13}C and ^{1}H

General Concepts

Nuclear Magnetic Resonance (NMR) Spectroscopy is the most widely used and powerful analytical tool for structural elucidation of molecules. It is used extensively by chemists to follow the course of reactions and to properly identify the structure of molecules. It is also commonly used in natural product chemistry to solve the structure of unknown molecules obtained by isolation from natural sources. In addition to its use to elucidate structure, the technique is also utilized to study dynamic processes such as kinetics and chemical equilibrium processes. Outside the realm of chemistry, biochemists make use of NMR spectroscopy to solve the 3-D structure of proteins, peptides, DNA and polysaccharides. The principles of NMR are identical to those of Magnetic Resonance Imaging (MRI), which is a well-known technique used in medicine.

The purpose of this experiment is to learn and apply the principles of NMR spectroscopy. You will be given an unknown organic molecule for which you will obtain the ^{13}C, ^{1}H NMR and IR spectra and be provided with its molecular formula mass spectrum and then you will analyze the data to solve the structure.

As part of the experiment, you will prepare the NMR samples and the spectra will be collected at the NMR facility. You will also have the opportunity to tour of the department's multimillion dollar NMR and MS facilities, information on the facility can be found at:

http://publish.uwo.ca/~chemnmr/

http://www.uwo.ca/chem/resources/MassSpectrometry.htm

Experimental Procedure

Obtain an unknown compound from your TA and record the unknown number. You will be given the mass spectrum. Your compound contains only C, H and O. You will obtain Infrared Spectra and NMR Spectra (^{13}C, ^{1}H) of your unknown. For this experiment, all samples are liquids but the details for both solids and liquids are provided for future reference.

Part A: Proton and Carbon NMR Spectroscopy

(i) Preparing the NMR Sample

BACKGROUND INFO:

- NMR tubes are precision pieces of glassware that are rather fragile, handle them carefully. The NMR tube cannot be used if it is chipped or broken. The tube must be at least 7 inches long, shorter ones will cause the instrument to malfunction.
- To run a ^{1}H NMR spectrum typically about 10-15 mg of a solid (for liquids 1-2 drops) are dissolved in an NMR solvent so there is 50 mm (in terms of the height) of solvent (approximately 0.6 mL, or 5 cm of liquid) in the NMR tube.
- To run ^{13}C NMR spectra, you will need about 5 times as much material (5-10 drops) with the same amount of solvent (^{13}C NMR spectra are less sensitive and take more sample to measure in a reasonable amount of time – why?).
- Your compound should be soluble in the deutero-chloroform NMR solvent that is provided. Usually, you would first check to make sure that your compound is soluble in the NMR solvent before you prepare your tube. There should not be any floating particles in the NMR sample solution!
- There are many NMR solvents to choose from, but the key considerations are solubility (your compound must be soluble) and then cost of the solvent. The NMR solvent must be perdeuterated, so that the protons of the solvent do not overwhelm the spectrum. Deuterated solvents are expensive. A typical NMR solvent that has good solubility properties and has a relatively low cost is $CDCl_3$ –deuterated chloroform. This solvent is still expensive so do not waste it. Also, it is important not to contaminate the solvent bottle. Any impurities will appear in the NMR spectra and complicate your analysis.

WHAT YOU NEED TO DO:

- ***In this experiment you are going to run both a ^{1}H and ^{13}C NMR spectrum on the same sample, in the same tube. Dispense ca. 5 drops of your unknown into an NMR tube, then fill your NMR tube up to 5 cm using $CDCl_3$.***

- ***Clearly label your tube using only the labels provided and a fine tip pen or permanent marker. The label should include your initials, class number, and unknown number. For example Superb O. Chemist is in Chemistry 2273 and is running her unknown # 12 so she would label her tube as: SOC227312. This is important so that you can identify and process the data from your unknown.***

(ii) Running NMR experiments:

Routine proton and carbon spectra samples are run by an automated sample changer attached to the Varian Mercury 400 MHz spectrometer. The robot places all of the samples into the magnet and the spectra are run automatically. We have already toured the NMR facility, so you should be familiar with the instrument configuration. The TAs will set up the NMR experiments.

You will process your own NMR data using the instructions below.

(iii) Retrieving NMR data:

The NMR data will be transferred from the Mercury 400 computer to a PC in the lab using an FTP program. Please do not change directories - the software is setup for a specific location on the disk so that bookkeeping is manageable.

A) Right Click on the **Start** button and open the start menu.
B) Click **NMR FTP** file folder icon to load the software
C) Click on the '**Profiles**' folder and select '**NMR Data**'.
D) Type in the password **MSA0216** and click '**OK**'. Click on the **Data** file folder then your course file folder.
E) Find your file name in the list in the right window and drag it to the left side.
F) When it asks if you wish to replace the existing file, click on **Yes**.
G) The file has been transferred to C:\NMR Data
H) After you have all of the files your need, close the FTP software.

(iv) Processing NMR data: BRING A USB KEY TO TAKE AWAY YOUR SPECTRA

The data transferred from the spectrometer computer to the PC are in a raw unprocessed form (fid). In the end, you will want to have a spectrum with chemical shifts, integrals (if ^{1}H), peak lists, and possibly expansions of regions. This can be accomplished using the instructions below. You will want to print off expansions of key parts of your spectra to make your spectra easier to see and solve.

A) Click on the **Start** button.

B) Click **NMR Processor** (upper left of start panel) to load the software.

C) Click File → Import (not open, open is for already processed NMR data) → From a 1D NMR directory.

D) Find your file name and double click on it. In this directory you will find a directory called **PROTON.fid** (and/or **CARBON.fid** if a carbon was run). Double click on this folder. Double click on **fid** in the left window to load the raw data.

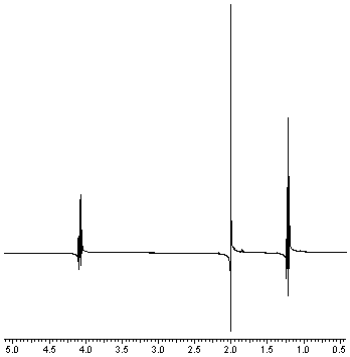
E) **FOUREIR TRANSFORM:**

- Press the **Fourier Tr.** button to Fourier transform the data from the time domain to the frequency domain.

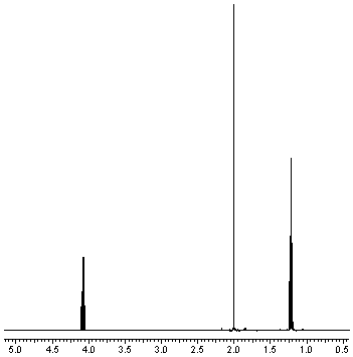
F) **PHASE CORRECTION:**

a. If phasing is good, move to part G. If phasing is bad, continue to next point.

b. Press the **Phase Corr.** (correction) button to phase the spectrum. Press the **Auto Simple** button. This should fix the phase distortions in 99% of your spectra. Once you have finished phasing the spectrum press the green checkmark on the far left of the toolbar to save the changes to the phase constants.



POOR PHASING



GOOD PHASING

G) **BASELINE CORRECTION:**

- Press the **BLine Corr.** (baseline correction) button to improve the baseline of the spectrum. Press the **Auto** button followed by the **Result** button. Once you have finished the baseline correction, press the green checkmark to save.

H) **REFERENCE:**

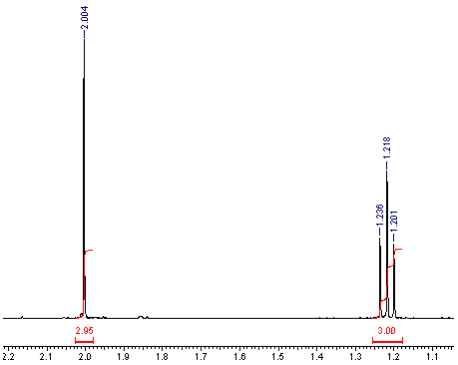
Press the **Reference** button. Use the cursor to select the signal closest to 0 ppm. This is TMS (tetramethylsilane), which is a reference compound included in the chloroform-d. Select TMS from the list of solvents that appears and click OK. This should put the signal at exactly 0.00 ppm. You should also have a singlet at 7.25 ppm (^{1}H NMR Spectrum) or a triplet at 77.00 (^{13}C NMR Spectrum), which is your chloroform-d signal.

I) **PEAK PICKING:**

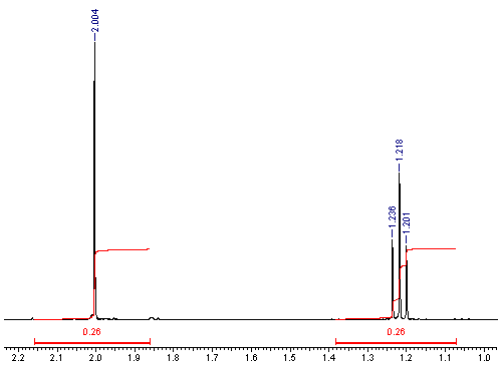
To perform peak picking (to get chemical shifts and J-couplings) press the **Peak Picking** button. Press the **Peak Level** button. Now place your cursor on the spectrum to the height of the smallest peak you want to select (pick) and press the mouse button. All peaks of this height and higher will be picked. If you pick too high, just move the cursor down a bit and press the mouse again. If you have picked too low and have selected too many peaks, press the **Clear All** button and start over. Once you have finished peak picking, press the green checkmark to save.

J) **INTEGRATION (^{1}H NMR SPECTRA ONLY):**

- Integration is done to determine the relative area of each signal. Integration is best done as follows:
- First **zoom in** on the peak you wish to integrate. To do this, move the cursor to the left hand side of the peak (or peaks) you wish to zoom in on. Right click the mouse – a magnifying glass will replace your cursor. Then left click, hold, and drag to the right hand side of the peak(s) and release the button. A magnified view of that peak(s) will now appear on the screen.
- Press the **Integration** button. Press the **Manual** button. Move the cursor to the left hand side of the peak you want to integrate. Click and hold the mouse button. Move the cursor to the other side (right) of the peak and release the button. This will integrate over the area that you held the mouse.
- To **zoom out** back to the full spectrum (in order to integrate other signals), click on the picture of a magnifying glass with a minus sign inside it (toolbar menu). Zoom in and integrate all the remaining signals.
- Integration values should be expressed as whole numbers, your TA will show you how to do this.
- Once you have finished integration, press the green checkmark to save.



WELL DONE INTEGRATION



POORLY DONE INTEGRATION

K) **PRINT (SAVE AS .PDF):**

- To print (save as .pdf and print at home) the spectrum, press the icon that looks like a printer. There are a number of options that you can change depending on what you want for output (including peak lists, integration tables, titles, etc.). Under the "TEXT" tab, input your name in the "USER INFORMATION" box. Press the **OK** button. This will call up the "SAVE AS" window, save your spectrum to your USB key.
- In addition to printing your whole spectrum, you should also print expansions. Zoom in on the desired areas (as described above) and repeat the printing/saving procedure.

L) When you have finished processing and saving your data, double click on the **X** in the upper right hand side of the software to close the program and press **NO** to saving the data.

(v) Solving the Structure:

Once you have obtained your spectra you can begin to solve the structure of your unknown. Use the molecular formula to determine the sites of unsaturation of your molecule. This may aid in finalizing a structure.

When writing up your experiment you will need to present your data in such a way as to convince the reader how you have solved the structure from the spectra. Assign the IR spectrum, being sure to indicate the key absorptions for functional group analysis.
For the NMR spectra you will need to report *Chemical Shifts* and the structural information obtained from it. For the proton spectra, in addition to reporting chemical shifts, you need to report and discuss *integration* and *splitting (coupling).* This might be best accomplished by setting up a chart/table. No formal introduction, experimental is required. Your report should be a clear and concise analysis of your spectra and a proposed structure of your unknown.

The chemical shifts of solvent signals observed for ^{1}H NMR and ^{13}C NMR spectra are listed in the following table. The multiplicity is shown in parentheses as 1 for singlet, 2 for doublet, 3 for triplet, etc.

Table 1: NMR Solvent Signals

Solvent	**^{1}H NMR Chemical Shift**	**^{13}C NMR Chemical Shift**
Acetic Acid	11.65 (1) , 2.04 (5)	179.0 (1) , 20.0 (7)
Acetone	2.05 (5)	206.7 (13) , 29.9 (7)
Acetonitrile	1.94 (5)	118.7 (1) , 1.39 (7)
Benzene	7.16 (1)	128.4 (3)
Chloroform	**7.26 (1)**	**77.2 (3)**
Dimethyl Sulfoxide	2.50 (5)	39.5 (7)
Methanol	4.87 (1) , 3.31 (5)	49.1 (7)
Methylene Chloride	5.32 (3)	54.00 (5)
Pyridine	8.74 (1) , 7.58 (1) , 7.22 (1)	150.3 (1) , 135.9 (3) , 123.9 (5)
Water (D_2O)	4.8	

Listed below are the chemical shift positions of the water signal in several common solvents. Note that water is seen in aprotic solvents, while HOD is seen in protic solvents due to exchange with the solvent deuteriums.

Table 2: NMR Water Signals

Solvent	**Chemical Shift of H_2O (or HOD)**
Acetone	2.8
Acetonitrile	2.1
Benzene	0.4
Chloroform	**1.6**
Dimethyl Sulfoxide	3.3

Part B: Infrared Spectra

During the course of the experiment you need to obtain an IR spectrum of your unknown. Since all unknowns in this experiment are liquids you will obtain a spectrum of a thin film. Your TA will remind you how to properly obtain an IR spectrum.

(i) Sample Preparation for Infrared Spectroscopy

- **Liquids:** Place a drop (or less) of the neat liquid directly onto an IR salt plate to form a thin continuous film. Measure the spectrum, ensuring that you wipe off excess compound (with a Kimwipe) if the absorptions are too intense.
- **Solids:** Solid samples are often difficult to analyze, particularly if they cannot be reduced to a fine powder or are not soluble in common infrared solvents. The solid sample (powder form) must be distributed evenly in the spectrometer in order to minimize light scattering effects and to eliminate distorted band shapes.
- **Cast Films:** A cast film is used if a solid material cannot be easily reduced to a fine powder but can be dissolved in a volatile solvent, such as acetone or chloroform. The sample is dissolved in a minimum amount of solvent and the solution is spread over a window where the solvent is evaporated, leaving only the solid sample.
- **Mulls:** An oil mull is prepared by grinding the solid to a very fine particle size and suspended in Nujol (spectra between 1330 cm^{-1} to lower frequencies) or Halocarbon oil (4000 cm^{-1} to about 1330 cm^{-1}). The solution is spread between two infrared windows, forming a thin continuous film that produces a spectrum virtually free of interfering bands of the mulling agents.

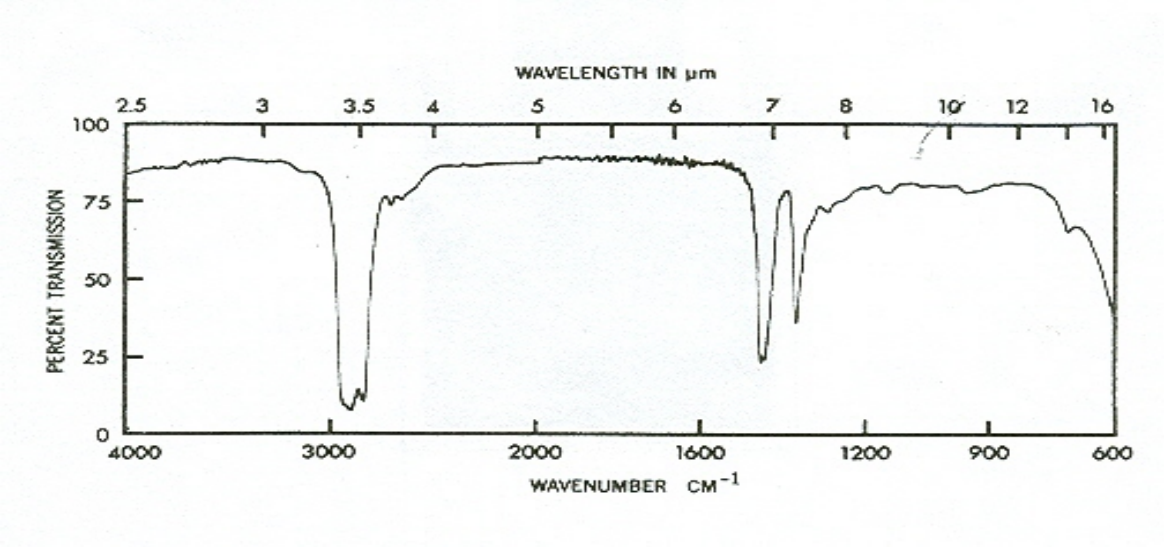


Figure 1: Spectrum of Nujol

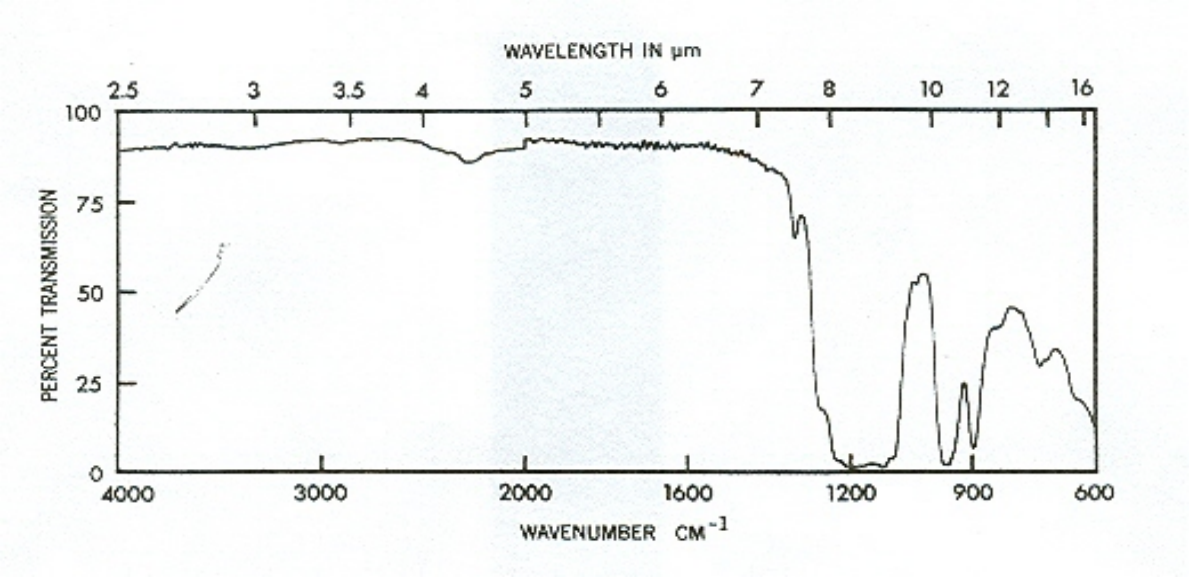


Figure 2: Spectrum of Halocarbon

(ii) Operating Instruction for FTIR Spectrometers

Note: All spectral files should be saved to your personal USB flash drive. Only save to the computer temporarily if you have forgotten to bring one.

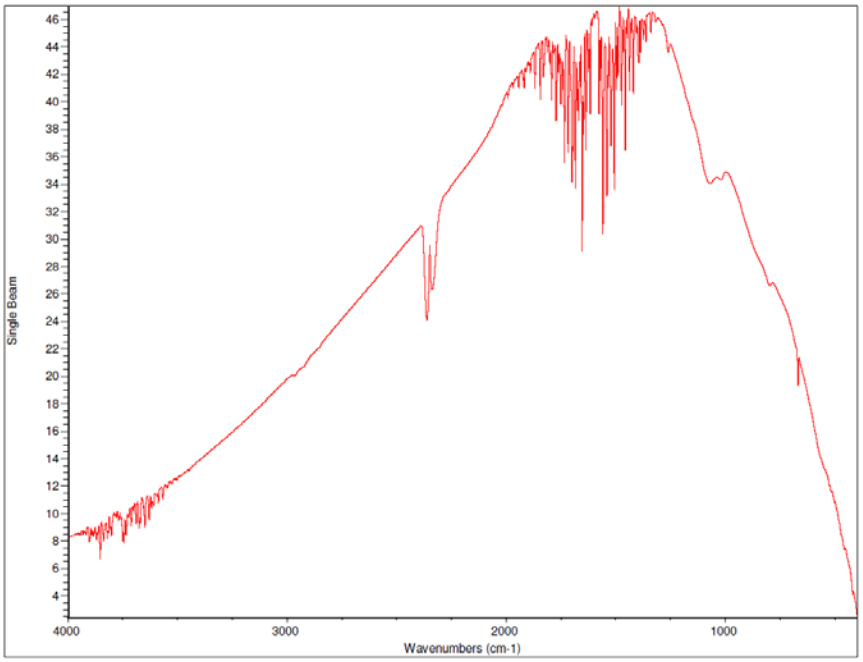
If the spectrometer's software is not already present on the screen, activate it by right clicking on the '**Start**' button in the lower left corner of the screen, open the start menu and select '**FTIR Spectrometer**'.

To collect a BACKGOUND spectrum – this only needs to be done by the first user of the day or when prompted by the instrument (approximately every 60 minutes).

1. Make sure the sample compartment door is empty and closed, then click on the '**COL BKG**' icon. A Dialogue Box appears—'Please prepare to collect a background sample'.

2. Click the '**OK**' button and let the instrument finish scanning (scanning progress can be monitored in the lower left screen). When it has finished a Dialogue Box appears asking if you want the background spectrum added to the current window. Choose the '**No**' option.

The background spectrum will appear like this:



To Collect a Sample Spectrum

1. Click on the **COL SMP** Icon – A dialogue box appears with the correct date and time. **Type in your name and sample name to replace the date/time information**, then click **OK.** Another dialogue box appears saying – '**Prepare to collect Sample'** –do **not** click 'OK' yet.

2. Insert your prepared sample into the compartment, close the lid.

3. ***Note*:** Wait several minutes before recording your spectrum to allow the atmosphere in the spectrometer to equilibrate, and then click '**OK**'.

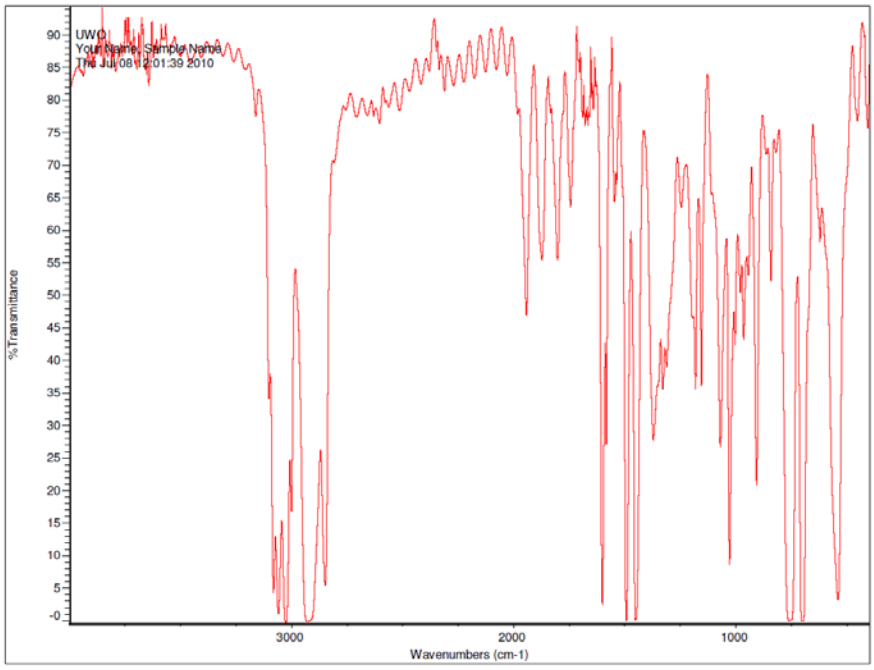
4. Let the instrument finish scanning (scanning progress can be monitored in the lower left screen). When it has finished a Dialogue Box appears asking if you want the sample spectrum added to the current window. If the spectrum looks acceptable choose the '**Yes**' option otherwise choose the '**No**' option and prepare a new or modified sample.

5. When you have acquired an acceptable spectrum you may print directly to a file in .pdf format or do some further manipulation of the spectrum, such as **Peak Find** or selecting different areas of the spectrum to enhance. Your TA will instruct you further in regard to this. After manipulation of the spectrum you must click on the '**REPLACE**' button to save your changes.

6. When satisfied with the displayed spectra click on the '**PRINT**' icon. A dialogue box will appear, click on '**OK**'. Your spectra will now be sent to a .pdf format file for you to name. When finished click on the '**Clear**' icon to leave the instrument ready for the next user.

The absorptions of a good quality spectrum fills the spectral window and uses the full scale of % transmittance. If the signal is too weak or strong, simply add or remove sample and re-measure by following the directions above.

Your sample spectrum should have strong, well-defined signals and a level baseline such as:



7. When data collection is complete, click [YES] to place the spectrum in a window for data manipulation and printing.

8. Peaks may be labeled individually by using the [T] annotation button in the lower left toolbar or you can use the [Find Peaks] icon on the main toolbar and use the cursor to set a peak threshold below which all peaks will be labeled. *After setting a peak threshold <u>be sure</u> to click on the [Replace] button just above the spectral window.*

(iii) Printing

Use the [Print] icon on the main toolbar to save a *.pdf copy of your spectrum that you must transfer off the computer .

Note: *Attendance at Week 2 of the laboratory is REQUIRED, even if you have obtained all your spectra. The time will be used to start to solve your spectra. The TAs will be available to help you work through the data. They will not know the identity of your unknown.*

Part C:

Make a photocopy (or print out a duplicate) of all your spectra before writing on them. If you have an even unknown number, swap your data with someone who has an odd unknown number and vice versa.

NOTE – ONLY SWAP THE SPECTRA (NOT THE SOLUTION), YOU ARE RESPONSIBLE FOR SOLVING THE STRUCTURE FOR TWO COMPOUNDS

YOU WILL NEED TO HAND IN THE SOLUTION TO BOTH UNKNOWNS.

LAB REPORT GUIDELINES: CHEMISTRY 2273a – 2010
Due in class by Wednesday, Dec. 8

Experiment 3: Practical NMR/IR Spectroscopy

A short formal lab report in the following format:

Title, Date, Name

Lab section (day) and TA name

Objective: purpose of lab – 1 sentence

For each unknown you need:

- units of unsaturation calculation and molecular formula
- data tables accounting for major signals (IR, ^{1}H NMR, ^{13}C NMR, MS)
- all spectral data, **including expansions**, that clearly illustrate important spectral features such as integration, coupling patterns, etc.
- structure of unknown
- discussion of how data lead to structure of unknown

example:
^{1}H NMR Data Table ($CH_3CH_2OCH_2CH_3$)

Chemical Shift in $CDCl_3$ (ppm)	Chemical Environment	Integration (# ^{1}H's)	Splitting and # of Neighbours
1.21	$\underline{C}H_3$-CH_2	3	triplet 2 neighbours
3.48	CH_3-$\underline{C}H_2$-O	2	quartet 3 neighbours

NOTE: You are responsible for solving the structure of **two** unknown compounds – the one you were assigned and another from exchange with a fellow student. *You will solve one odd-numbered unknown and one even-numbered unknown*.