

CHEM 202 LAB
ORGANIC CHEMISTRY LABORATORY I
Fall 2008

Monday, Tuesday, Wednesday, Thursday 1:15 – 4:05pm
Carr Lab LL04 and LL10

1. COURSE INSTRUCTORS

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3. RULES FOR THE COURSE

Goals: Chemistry 202 Lab provides an important technical foundation for first-hand experimental work in classical and modern organic chemistry. The course emphasizes invaluable techniques and skills such as recrystallization, extraction, and chromatography.

Contract: The Laboratory Class Participation Rules appear on the next two pages. You must sign both copies. Remove the first copy and hand it to your laboratory instructor. Keep the second copy in the manual for your records.

Department of Chemistry Laboratory Class Participation Rules

The laboratory classes that accompany courses in the chemistry department have been developed to provide full exposure to the range of experimental techniques that all chemistry students should experience. To fully acquire, develop and practice these skills requires full and active participation in all elements of the laboratory classes. Adherence to the following rules by all participants in laboratory classes is mandatory:

1. Students must fully complete ALL of a course's laboratory experiments in order to pass the course: this means full, punctual, participation in all laboratory exercises and timely submission of all accompanying laboratory reports. Only in extraordinary circumstances, and with a written note including a valid explanation, will a student be excused for not conducting an experiment and submitting the corresponding laboratory report.
2. Laboratory reports are due at 1:15 pm, one week after the experiment is performed. All laboratory reports handed in late without a legitimate excuse will have 10% of the maximum grade possible deducted from the total grade for each day it is late. Thus, a report handed in several days late will have a very low recorded grade, but note that submission of all lab reports is still required to pass the course.
3. Attendance in a laboratory class other than the one to which a student has been assigned is NOT permitted, unless both the instructor of the assigned lab session and the instructor of the lab session the student wishes to attend receives notification *via* email, phone, or in writing from the student's doctor, advisor, or course instructor BEFORE the lab session they wish to attend.
4. Compliance to all safety rules is mandatory.

By signing below you confirm that you have read and understood these rules and will abide by their implications. Sign this page and hand it to your laboratory instructor.

Printed name: _____ Class year: _____

Signature: _____ Date: _____

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4. LAB SAFETY GUIDELINES

In the organic laboratory, we will work with a wide range of solvents, organic molecules, acids, and bases which could be harmful if you were to come into direct contact with them. **The following safety rules must be followed without exception:**

- (a) Be aware of the location of exits from the building, and the location of eye wash stations, the fire extinguishers, and safety shower. The emergency phone number is **1911**.
- (b) You must **always wear safety goggles** in the lab.
- (c) **Do not wear sandals** in the lab. Anyone wearing any form of open-toed shoes will be sent back to their room to change.
- (d) **Do not wear shorts, skirts, or dresses in the lab.** Anyone wearing any form of clothing that leaves the legs uncovered will be sent back to their room to change.
- (e) **No food or drink is allowed** in the lab.
- (f) **Never leave a reaction unattended.**
- (g) No work can be done unless an instructor or TA is present.
- (h) **Do not touch common items with your gloved hand(s).** This includes doorknobs, computer keyboards, the hallway, etc.
- (i) You should **never** pour solvents in the sink, or put solid chemicals into a trash can. All non-halogenated waste must be poured into the non-halogenated waste container, and halogenated waste must be poured into the halogenated waste container. Solid organic materials are discarded in the solid waste container. There is also a container for broken glassware in the laboratory.
- (j) Be alert to hazards and prepared for emergencies. If you are ever unsure of whether something is safe, immediately consult with your instructor or TA.

5. LABORATORY REPORTS

Lab Preparation Write-ups (Pre-Labs): It is necessary that you study the experiment that you are to do prior to arriving to the laboratory. You should read the lab manual carefully and try to understand the overall content and purpose. Your performance in the laboratory will benefit enormously with proper advance work, and so will your grade!

Report Format: Reports will be written down in a laboratory notebook containing carbon-copy duplicate pages (these notebooks are available from the Blanchard campus store). The original copy will remain in your notebook for reference later in the course (and as a back-up copy), and the carbon copy will be turned in for grading.

7. GRADING

Your grade is based on 400 total points. Reports are **due at the beginning** of the following week's lab. Late lab reports will be reduced by two points for every day it is late. ***You must complete and submit all 11 lab reports in order to pass this course.***

(a) Lab reports: 20 points for each lab, 11 lab reports

- Total 220 points for lab reports

(b) Lab performances: 20 points for each lab and 15 points for overall performance

- Total 180 points for lab performances

Note: Lab performances include the quiz (5 points for each) and lab skills (10 points for each). The lab quiz will exactly be done at 1:25 PM. If you were late, you cannot take it and lose those 5 points, and you will also lose the points for the pre-lab part (parts a and b).

Schedule for Chem 202 Lab: Fall 2008

Week	Date	Title of Experiment
1	Sept. 8 - 12	Exp. 1 Check-in / Safety / Melting Point Determination
2	Sept. 15 - 19	Exp. 2 Recrystallization
3	Sept. 22 - 26	Exp. 3 Chromatography Part A: TLC
4	Sept. 29 - Oct. 3	Exp. 3 Chromatography Part B: Column Chromatography
5	Oct. 6 - 10	Exp. 3 Chromatography Part C and D: HPLC and GC
6	Oct. 13 - 17	No Experiment --- Mid Semester Break (Oct. 11 - 14)
7	Oct. 20 - 24	Exp. 4 Liquid- Liquid Extraction Exp. 5 Resolution of Enantiomers (1)
8	Oct. 27 - 31	Exp. 5 Resolution of Enantiomers (2)
9	Nov. 3 - 7	Exp. 6 Bromination of Stilbene
10	Nov. 10 - 14	Exp. 7 Substitution: Kinetics of Solvolysis of t-Butyl Chloride
11	Nov. 17 - 21	Exp. 8 Elimination: Competitive Product Formation in a Dehydration Reaction
12	Nov. 24 - 28	No Experiment --- Thanksgiving Recess (Nov. 26 - 30)
13	Dec. 1 - 5	Exp. 9 IR Spectroscopy and Mass Spectroscopy
14	Dec. 8 - 12	Check-out / Evaluations / Review

Last day of classes: Dec. 12 (Fri.)

Final exams: Dec. 15 - 19

December recess: Dec. 20 - Jan. 1

Spring semester starts: Jan. 29 (Wed.)

Experiment #1: Melting Point Determination

PURPOSE

To introduce the technique of melting point determination.

INTRODUCTION

The melting point of a solid can be easily and accurately determined with only small amounts of material and, in combination with other measurements, can provide rapid confirmation of identity. The most accurate method of determination is to record a cooling curve of temperature versus time. However, this approach requires quite large amounts of material and has been exclusively replaced by the **capillary method**.

Capillary Melting Point Determination

The method involves placing a little of the sample in the bottom of a narrow capillary tube that has been sealed at one end. The determination is made using a **Melting Point Apparatus** that simultaneously heats both the sample tube and a thermometer. The temperature **range**, over which the sample is observed to melt, is recorded. Some pure materials have a very narrow melting range, perhaps as little as 0.5–1.0 °C, while more typically a 2–3 °C range is observed. Data is typically recorded as, for example, mp 232–234 °C. Though formally denoting the melting range, this piece of data is almost universally referred to as a **melting point (mp)**.

The **rate** of heating, controlled by a dial, should be kept relatively low, especially for samples with a low melting point, to ensure that the thermometer reading represents, as accurately as possible, the true temperature experienced by the sample tube (since the transfer of heat within the apparatus is relatively slow). With this fact in mind, it is sensible when recording a melting point of an unknown material to perform a **trial run** where the temperature is increased relatively rapidly in order to ascertain a **rough** melting range. The determination is then repeated by heating rapidly to within around twenty degrees of the expected melting point and then very carefully (slowly) increasing the temperature the remaining few degrees until the melting point is reached. Of course, if the melting point of the material is known with some confidence, for instance if the determination is being made to confirm identity, then the trial run is unnecessary.

Although a pure solid might be expected to have a single, sharp melting point, most samples are observed to melt over a narrow range of a few degrees

Celsius. The observation of a melting range may be a result of the heating process involved in capillary measurements (mentioned above), may reveal the presence of inhomogeneities in the macroscopic nature of the solid sample, or may indicate the presence of other substances in the sample (contaminants or by-products of the method used to prepare the materials).

Melting Points as Criteria of Purity

Thermodynamics tells us that the freezing point of a pure material decreases as the amount of an impurity is increased. The presence of an impurity in a sample will both **lower** the observed melting point, and cause melting to occur over a **broader range** of temperatures. Generally, a melting temperature range of 0.5–1.0 °C is indicative of a relatively high level of purity. It follows that for a material whose identity is known an estimate of the degree of purity can be made by comparing melting characteristics with those of a pure sample.

Melting Points as a Means of Identification and Characterization

For pure samples a clear difference of melting point between two materials reveals that they must possess different arrangements of atoms, or configurations. If two materials are found to have the same melting point then they may, but not necessarily, have the same structure. Clearly, the recording of a melting point is a desirable check of purity and identity but must be combined with measurements from other analytical techniques in order to unambiguously identify a material and assess the purity. Part of the need for additional verification derives from the subjective nature of capillary melting point determination. Even when heating is very finely controlled, to ensure consistency of sample and thermometer temperature, the human element of visual inspection of the melting point introduces significant variation.

Mixed Melting Points

Mixtures of different substances generally melt over a range of temperatures that concludes at a point below the melting point of either of the **pure** components—each component acts as an impurity in the other. Two pure substances, with sharp melting points, can be shown to be different by mixing them and recording the **lowered melting point range**. This type of experiment provides a means by which to confirm a proposed identity for an unknown sample: if a **sharp** melting point is observed for a **mixture** of the unknown with a genuine sample then it is highly likely that the samples are **identical**.

Melting Points and Molecular Structure

Melting points are notoriously difficult to predict with any accuracy or confidence. Systematic variations of melting point with variation in structure are not as obvious or predictable as with boiling points. However, the sometimes

surprising variations are often only highlighted in very closely related molecules and the obvious general rule can be applied: melting points do generally increase with increasing molecular weight.

The difficulties involved in predicting melting points are a result of the problems associated with predicting molecular packing in crystals. Many, potentially conflicting, factors play a role in determining melting points including molecular shape, interactions between groups within the molecule, and the degrees of freedom the molecule possesses within the crystal.

PROCEDURE

For the melting point determinations you will use the melting point instruments housed in the laboratory. There are three experiments to perform. For the first two experiments you should work in pairs. The capillary melting point tubes should be filled by crushing the sample to a fine powder on a watch glass with the end of a glass rod, and then introducing this powder to the tube by pressing the open end into the powdered sample. Once a 2–3 mm depth of powder has been introduced the tube should be inverted and the sealed end tapped on a hard surface until the sample rests squarely at the bottom. Your laboratory instructor will demonstrate these techniques.

Part 1

One member of each pair should determine the melting point of **urea**, the other the melting point of **cinnamic acid** (recall that by melting point we imply the range over which melting is observed to take place). For both samples the range should be very close to 132.5–133.0 °C. Record your own and your partner's values.

Part 2

Mixtures of urea with cinnamic acid have been prepared in **molar percentage** ratios of 100:0, 97:3, 95:5, 80:20, 60:40, 50:50, 40:60, 20:80, 5:95, 3:97 and 0:100 ratios: each student pair in your laboratory class will be assigned two of these sample ratios. Carefully record the melting point range for your mixed samples, paying particular attention to carefully note the exact point at which melting begins, the exact point at which melting is complete, and any changes in appearance at or near the melting temperature range. Record your data on the chalkboard along with that from each of the other groups. Once all the data has been collected plot both the beginning of melting and complete melting temperatures versus the molar percentage ratios of urea to cinnamic acid.

Part 3

Working **individually**, determine the melting point of an unknown sample provided by your TA or instructor (**reminder:** a crude melting point run will be necessary to determine the approximate melting temperature). Confirm the proposed identity of your unknown sample by performing a mixed melting point determination with an authentic sample of the suspected material. Recall that the melting point **will not be lowered or broadened** if the materials are identical, i.e. **if you have correctly identified your unknown**. Your sample will be one of the compounds listed in Table 1-1.

Table 1-1. Melting point data for the possible unknown samples.

Material	Melting Point (°C)
Acetanilide	113.5 – 114.0
Fluorene	114.0 – 116.0
Cyanoacetamide	121.0 – 122.0
Benzoic acid	121.5 – 122.0
Benzamide	128.0 – 130.0
Glucose pentaacetate	131.0 – 134.0
Benzilic acid	150.0 – 153.0
Adipic acid	152.0 – 154.0
Salicylic acid	158.5 – 159.0
Sulfanilamide	165.0 – 166.0
Succinic acid	184.5 – 185.0
<i>p</i> -Terphenyl	210.0 – 211.0

CLEAN UP

Put all used tubes in the solid waste container marked for this purpose.

REPORT

Record all of the melting point ranges for the three parts of the experiment in the results section of the report. In the discussion section describe what is revealed by each measurement about the sample in question (refer to the discussion in this script for some reminders).

QUESTIONS

1. Why should samples for melting point determination be finely powdered?
2. Why is it important that the heating rate be carefully controlled once near the suspected melting point of a sample?
3. Describe and analyze the trend lines for the initial and final melting temperatures versus composition revealed by the graph you generated in Part 2 of this experiment. To what extent do you think it would be possible to employ this graph to determine the composition of an unknown urea/cinnamic acid sample?

Experiment #2: Recrystallization

PURPOSE

To introduce the technique of recrystallization through the recrystallization of crude cholesterol. To recrystallize an unknown compound and determine the identity *via* melting point determination.

INTRODUCTION

Purification of a solid by recrystallization from a solvent relies on the fact that different substances are soluble to differing extents in a given solvent. In the simplest case all of the impurities present in a solid sample will be so much more *insoluble* in the chosen solvent that all that remains in solution is the pure dissolved product (the solute). It has often been said that recrystallization is an art: the master crystallizer knows just when to add a little more solvent, just when to stop heating, and seems to be able to conjure beautiful crystalline samples from nasty materials. This is all true. However, most of us are able to achieve good results by following some simple guidelines.

Recrystallization

The process of recrystallization can be broken down into a number of discrete steps:

1. Choosing the solvent

The choice of solvent is crucial, but how do we decide which solvent will work best? An essential characteristic of a successful solvent is that the compound be ***soluble*** in the hot solvent but ***insoluble*** when the solvent is cold. The impurities are either insoluble in the solvent at all temperatures or remain at least moderately soluble in the cold solvent. In order to select a solvent system, tests can be performed with small amounts of material in test tubes: a few drops of solvent are added and if the material proves insoluble then the tube is heated to see if the material will dissolve at a higher temperature—if so, then a good solvent for recrystallization of that material may have been identified. If the material appears insoluble at high temperature a little more solvent can be added, drop by drop, to ascertain whether dissolution of the solute will occur in a larger volume of solvent. A solvent should be ***rejected*** if: the material appears readily soluble in the cold solvent, is not soluble to any appreciable extent in the hot solvent even when the volume of solvent is increased, or requires an impractically large volume in order to fully dissolve the crystals. An appropriate choice of solvent for the crystallization process will pretty much ensure success, all that follows is manipulation.

2. Dissolving the sample

An Erlenmeyer (conical) flask should be used of such a size that it is only filled to around half-way when all the solvent has been added. The solid sample is introduced, together with around 75 % of the amount of solvent thought to be required, it is always advisable to err on the side of using **less solvent** at this stage. The flask is heated on a hotplate until the solute has dissolved, additional solvent can be added to the hot solution as necessary to ensure complete dissolution. A boiling (wooden) stick should be added to provide a point for bubbles to form and facilitate an even boiling process. By a process of gradual addition of solvent to the flask, until all the material has dissolved, you will ensure that the hot solution is saturated and will deposit crystalline material once it is cooled. Do not forget that some insoluble impurities may be left suspended in solution, you must judge when all of the desired material has dissolved and only the unwanted impurities are left as a suspension.

3. Hot filtration

If some insoluble impurities remain suspended in solution it is necessary to filter the solution while it is still hot, see Figure 2-1. To ensure that the funnel is kept hot, some of the solvent is placed in an Erlenmeyer flask with a boiling stick, on a hotplate. The funnel, equipped with filter paper, is placed above the flask, using a ring clamp, and the vapor from the warm solvent is allowed to warm the funnel. Care should be taken to allow some space between the filter funnel and the flask to avoid a build up of pressure in the flask. The hot solution is gravity filtered through the warm funnel and a watch glass may be placed on top of the filter funnel to prevent heat loss during filtration.

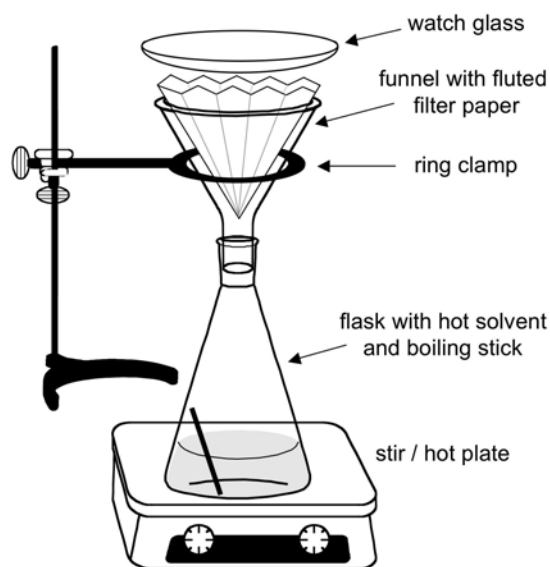


Figure 2-1. Hot filtration set up.

4. Cooling

Cooling the filtered solution will allow crystals to form. The rate of cooling has a role in determining the size of the crystals: fast cooling will tend to generate more crystals of relatively small dimensions, slow cooling might allow larger crystals to form. Usually the best compromise of speed, convenience, and crystal quality, is simply to let the solution cool to room temperature on the bench. To ensure maximum recovery of material the solution should be cooled in an ice–water bath after the solution has been allowed to cool to room temperature. In some particularly stubborn cases, crystal formation can be encouraged by providing a nucleation point, from which the crystals may grow. This can be achieved by scratching the inner wall of the flask with a glass stirring rod (or spatula), or if available a small amount of pure crystals can be added to the flask.

5. Cold filtration

When the crystallization process is judged to be complete the crystals need to be collected by **suction filtration**, see Figure 2-2. Both the funnel and suction flask should be chosen so that neither will become more than half full during the filtration process. It is preferable that all of the crystalline material is transferred to the funnel as a suspension in the crystallization solvent, however it is sometimes hard to get all of the crystals moving freely by swirling the flask and occasionally it will be necessary to add more ice–cold solvent in order to transfer the last of the crystalline material. It may also be necessary to dislodge crystalline material from the sides of the flask with a spatula prior to filtration.

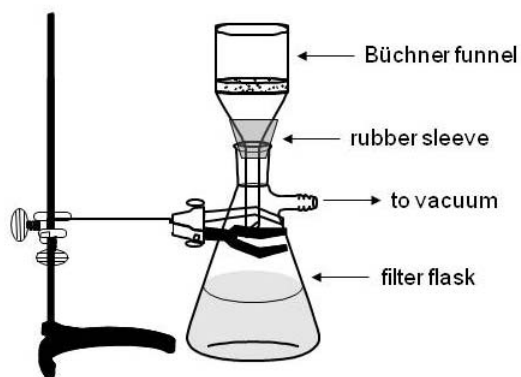


Figure 2-2. Typical set up for suction, or vacuum filtration.

6. Washing the crystals

Once the suction filtration process is complete the collected crystals should be washed with a little more *ice-cold* solvent to remove final soluble impurities which would otherwise be left on the surface of the crystals. The solvent used for this final washing should be *as cold as possible* to minimize losses from the crystals redissolving: recall that your precious crystals are soluble in warm solvent so you'll lose material if you wash with solvent that is not cold.

7. Drying the crystals

Once the crystals have been collected on the suction funnel they can usually be satisfactorily dried by continuing to draw air over them for a few minutes. The almost dry crystals should then be spread on a filter paper to allow the last traces of volatile solvent to evaporate.

The descriptions given above are long and detailed and cover most of the pitfalls that can be encountered when faced with recrystallizing a material. They can be summarized to form a more useful running order for a typical procedure:

1. Identify a good candidate solvent.
2. Dissolve the sample in the **minimum** volume of hot solvent.
3. If solid impurities remain suspended in solution, filter the hot saturated solution under **gravity** keeping the filter funnel **hot**.
4. Cool the saturated solution, first in air and then in an ice-water bath.
5. Filter the cold solution under vacuum, if necessary dislodge and transfer remaining crystalline material with a little more **cold** solvent.
6. Wash the crystals with a small volume of **cold** solvent.
7. Dry the crystals under vacuum, and then in the air.

PROCEDURE

There are two parts to the experiment. You will work in pairs throughout.

Safety Considerations: *No solvents or solids should ever be poured into the sinks or thrown in the trash. All solvents must be discarded in the appropriate waste container. Ask your instructor or TA if you are ever unsure.*

Part 1: Recrystallization of Crude Cholesterol from Methanol

Add 1.0 g of crude cholesterol to a 125 mL Erlenmeyer flask, add around 10 mL of methanol and heat the flask with swirling on a hotplate* (remember to place a wooden stick in the flask to ensure that the mixture boils evenly and safely). In a second flask heat around 50 mL of methanol to boiling (again, use a wooden stick). Add the boiling methanol in small portions (around 5 mL) to the flask containing the cholesterol. Keep heating, adding more solvent, and swirling, until all the cholesterol appears to have dissolved.

Remove the solution from the hotplate and allow it to cool slowly to room temperature. The wooden stick should be left in the solution in order to provide a point at which crystal nucleation (growth) can begin. The pure cholesterol will deposit as white needles. To complete the crystallization process the flask should be immersed for 5–10 minutes in an ice–water bath, a second flask containing an additional 5 mL of methanol should also be cooled in the bath. The crystals should be collected by suction filtration using the Büchner funnel and vacuum flask as demonstrated at the beginning of the laboratory class. Before attempting to filter your crystals use a spatula to dislodge any crystals from the sides of the Erlenmeyer—try to ensure that as much crystalline material as possible is moving freely in the solvent. The spatula head can be used to press solvent from the crystals before washing them with the additional 5 mL of cold methanol. Maintain the vacuum for a few minutes to draw air through the crystals and aid their drying, and then transfer them to a piece of filter paper to dry in the air. Record the weight and a melting point of your dried crystals.

Part 2: Recrystallization and Identification of an Unknown Compound

Obtain a sample of an unknown compound from you Instructor or TA and record its identification number in your notebook. The aim of this experiment is for you to determine a suitable solvent to use for its recrystallization, perform the recrystallization, and determine the materials' identity by recording its melting point. All of the unknowns will crystallize from one, or more, of the following solvents: water, methanol, or ethanol. Refer to the start of this experiment for notes on selecting a good solvent, and use a small beaker of water on your hotplate to warm the test tubes in which you perform your solubility experiments. If you are still unsure which solvent to use after performing tests with the three suggested solvents consult your TA or instructor. ***The unknown will be one of the materials listed in Table 2-1.***

Once you have identified a good recrystallization solvent, recrystallize 0.5 g of the unknown sample using the techniques you have been practicing. Record the

* As methanol is a flammable solvent only an explosion proof hotplate should be used. Check your hotplate to ensure you have the correct kind and consult your TA or instructor if unsure.

weight of the dried product and record its melting point. Establish the identity of the unknown sample by recording a mixed melting point with an authentic sample (available from your instructor or TA).

Table 2-1. Melting point data for the possible unknown samples.

Material	Melting Point (°C)
Acetylsalicylic Acid	134–136
Adipic Acid	152–153
Benzamide	128–129
Benzil	94–95
Benzilic Acid	150–153
Benzoic acid	122–123
Cholesterol	147–149
2-Nitrobenzoic Acid	140–142
Salicylamide	140–144
Salicylic Acid	158–160
Sulfanilamide	164–166
<i>trans</i> -Stilbene	122–124

CLEAN UP

All solvent wastes from vacuum filtrations should be placed in the non-chlorinated waste container. All of your solid samples should be placed in the solid organic waste container. Wash all of your glassware and return to your drawer.

REPORT

In the observations/data section of your report record the solvent types you chose, the method of adding solvents, the method of cooling down solutions, the dried weight, percentage of recovered material, and melting points, for the two parts of the experiment. Also include descriptions of both the crude and recrystallized crystals. For part 2, record the steps you took to select the solvent for recrystallization of the unknown. In the discussion/conclusions section describe how you made your solvent selection for recrystallization of your unknown and whether your percentage of recovery indicates that you made a good choice, correctly identify the unknown, and make a decision about if the recrystallization process can purify low quality materials.

QUESTIONS

1. What properties should ideally be possessed by a recrystallization solvent?
2. How might the melting point of a material differ before and after recrystallization?
3. Why are crystals washed with additional solvent when collected? Why cold solvent?
4. What information was gained about the unknown substance by recording a mixed melting point at the end of Part 2?

Experiment #3: An Introduction to Chromatography

PURPOSE

To introduce four chromatography techniques and to learn how to manipulate these techniques to both separate mixtures of compounds and to analyze materials.

INTRODUCTION

Chromatographic analysis is used to separate complex mixtures of compounds. First used in the early 1900s chromatography got its name because it was used to separate different mixtures of colored compounds. By the 1930s the popularity of chromatography had increased as chemists realized that this experimental technique could also be used to separate mixtures of colorless compounds. All chromatographic systems have two phases, a mobile phase and a stationary phase. The **mobile phase** is a liquid or a gas that carries a sample through a solid **stationary phase**. As the sample in the mobile phase passes through the stationary phase the compounds in the mixture will separate because of differences in their affinities for the stationary phase, and differences in their solubilities in the mobile phase.

In the following three-week experiment you will examine four different types of chromatography: thin layer chromatography (TLC), liquid (column) chromatography (LC), high-pressure liquid chromatography (HPLC), and gas-liquid chromatography (GC). In the first week of the experiment you will complete part A, thin layer chromatography. In weeks 2 and 3 you will complete parts B, C, and D (in any order) where you will gain experience with the other three chromatographic techniques.

In **thin layer chromatography (TLC)**, glass, metal, or plastic plates are coated with a thin layer of a stationary phase, usually a polar adsorbent such as silica gel or aluminum oxide. The mobile phase consists of a developing solvent or mixture of solvents, carefully chosen based on an assessment of the polarities of the sample compounds. Generally, less polar developing solvents are used for less polar compounds and more polar solvents are used for more polar compounds. As the solvent travels up the plate, the components in the sample move with it and separate from each other as they reversibly adsorb to, and desorb from the plate according to their differences in polarity.

Liquid or column chromatography (LC) is used to separate mixtures of compounds of low volatility. The stationary phase is packed into a hollow vertical

glass column: as the sample travels down the column, compounds adsorb reversibly onto the stationary phase and the components of the mixture are separated. More polar compounds will bind more tightly to the stationary phase and will stay on the column longer; the non-polar compounds will **elute** (exit) from the column first. Fractions of solvent are collected as the compounds are eluted from the bottom of the column. Unlike TLC, which is a micro scale and purely qualitative technique, column chromatography can be used to separate and purify large amounts of sample.

In **high performance liquid chromatography (HPLC)** the basic principles are the same as those for liquid chromatography. A column of small, tightly packed particles serves as the stationary phase. The small particles have a large surface area, allowing for better separation of the components of a mixture. High pressure is used to force the mobile phase through the column because the column is so tightly packed. A detector and computer are used for sample detection and monitoring so that each eluent (fraction) can be collected such that it is free of the other components in the mixture.

Finally, **gas chromatography (GC)** is used to analyze small amounts of mixtures of volatile organic compounds. The stationary phase is a nonvolatile liquid with a high boiling point and the mobile phase is an inert gas such as helium or nitrogen. Once more, mixtures separate based on how their components interact with the mobile gas phase and the liquid stationary phase. Generally, compounds with lower boiling points will elute from the GC column more quickly than compounds with higher boiling points.

The target compounds

You will work with two sets of samples as you learn about the techniques described above. The first set of compounds is a series of closely related organometallic compounds called ferrocenes, the second set are organic molecules that constitute the active ingredients in a series of modern, over the counter, painkillers.

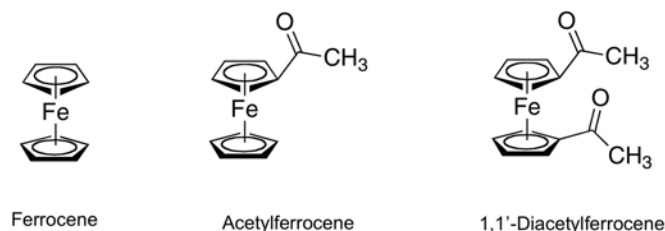


Figure 3-1. Set 1: Ferrocenes.

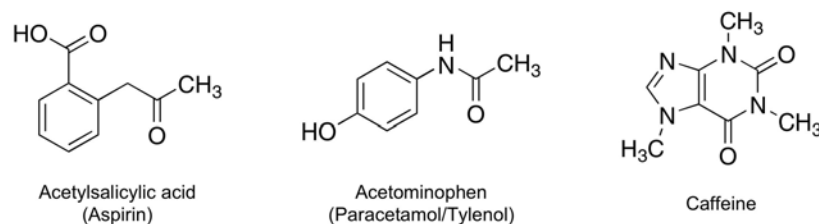


Figure 3-2. Set 2: Painkillers.

Part A: Thin Layer Chromatography (TLC)

INTRODUCTION TO TLC

Though organic chemistry looks neat and tidy on paper, in practice this is rarely the case. Most reactions, conversions of one substance into another, usually generate more than one product, and often many. **Thin layer chromatography (TLC)** is a useful method for quickly assessing how many different products have been formed, and for identifying a set of conditions by which a large scale separation of a set of materials may be achieved.

The theoretical and practical basis for thin layer chromatography is the same as for “full scale” preparative chromatography. A stationary solid phase **adsorbent**, spread as a thin layer (0.25 mm depth) on a plastic or glass sheet, is treated with the subject material or mixture of materials, the **adsorbate**. The adsorbent has different affinities for different materials. As the plate is developed (solvent is eluted through the solid-phase) those molecules that adsorb most strongly to the adsorbent spend comparatively little time in solution and do not move very far as the solvent front advances. In contrast, those molecules that do not adsorb so strongly spend comparatively little time stuck on the solid phase and most of their time in solution, as a consequence they move farther. This is the principle behind all forms of chromatography.

A range of **stationary phases** (adsorbents) are available but, by far the most widely used in organic chemistry is silica gel (SiO_2). However, the **mobile phase** (solvent or solvent mixtures) varies widely, and must be matched to the system under investigation in order to obtain good results. Silica gel contains many relatively polar silicon–oxygen bonds and therefore, has high affinity for polar molecules: **the more polar the molecule the shorter the distance it will travel during TLC development.**

In practice

Plastic TLC plates will be available in the laboratory, pre-cut into small rectangles. The sample is applied to the layer of adsorbent as a solution in an appropriate solvent using a small glass applicator called a **spotter**. Spotters are available in the lab and are made by stretching an open-ended capillary tube that has been heated in a flame. The aim is to deposit a small concentrated spot a few millimeters from the bottom of the plate. Once the application solvent has evaporated from this spot the material is left adsorbed on the solid phase and the plate is ready for development. A few mLs of the chosen solvent, or solvent mixture, are poured into the bottom of a beaker. The prepared TLC plate is then placed so that it stands up in the beaker and its bottom edge is in the puddle of solvent (see Figure 3-3). The solvent is drawn up the layer of adsorbent—if the placing of the plate is executed carefully a perfectly horizontal solvent front should result. The solvent passes over the spot of applied material and causes a separation of the components of the spot according to their relative polarities. When the solvent front has reached around 1 cm from the top of the plate it is removed from the beaker and the point of the furthest extent of the solvent is marked as a faint line with a pencil. **Do not** use a pen when making any marks on TLC plates: the dyes in the ink will adsorb to the plate, separate and interfere with the results obtained. Graphite from a pencil marking will not dissolve in the developing solvent or in any other manner affect the results of TLC. Also, be careful to mark gently; the layer of adsorbent is easily scratched and will flake from the surface.

Because the distance traveled by a substance relative to the distance traveled by the solvent front depends upon the molecular structure of that substance, TLC can be used to separate and identify substances. The distance relationship is expressed as an R_f value given in Equation 3-1.

$$R_f = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent front}} \quad (\text{Eq. 3-1})$$

Due to the small size of the TLC plates we use a small ruler to make these distance measurements. When measuring the distance traveled by the spot be sure to measure from the middle of the spot, especially if the spot has smeared as it traveled up the plate (see Figure 3). The **ratio to the front** (R_f) value for a given substance depends on both the nature of the adsorbent and the solvent used to develop the plate. Though consistency should be obtainable if both these factors are replicated, in practice there is not sufficient accuracy in these measurements to allow for the unique identification of substances. However, TLC is a very useful **comparative** technique when the identity of a substance is suspected and a genuine sample is available for comparison.

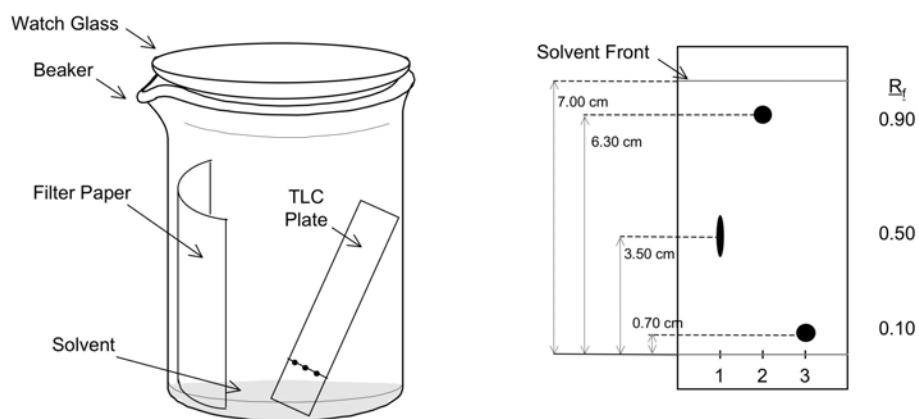


Figure 3-3. Example TLC chamber (left panel) and TLC plate (right panel). The distances traveled by each spot and by the solvent front are shown along with the R_f value for each spot.

Note: TLC plates are pretty small so measurements with a standard ruler can only provide R_f values to two decimal places. This is the level of accuracy that is routinely reported and the level you should use throughout these experiments.

The practice of TLC can be summarized in three discrete steps:

1. Sample Application

A small amount of sample is dissolved in a small volume of a solvent in which it is readily soluble to provide a spotting solution. A glass spotter is dipped in this solution and then applied to the plate to form a small concentrated spot at a marked position around 1 cm from the bottom of a TLC plate. The spot is made as small as possible to ensure, in as far as this is possible, that all of the applied material starts the journey up the TLC plate from the same point. The amount of material deposited on the plate is also very important: if the sample spot is too concentrated then the adsorbent can become overloaded and the features in the TLC will streak and spread (making it hard to measure their position, see Figure 3-3), if the spot is rather weak it may be hard to see the features on the plate after it has been developed. Preparing a good TLC plate requires practice and experience.

2. Development

The container used for development is most often a simple beaker covered with a watch glass. Sometimes a piece of filter paper is placed along the wall of the beaker to ensure saturation of the atmosphere with solvent vapor. Once a

suitable solvent system for plate development has been found, a few mLs are added to the beaker to provide a depth of a few millimeters. The plate is placed in the beaker so that the bottom edge (the edge nearest the applied spot of material) is immersed in the puddle of solvent. Care should be taken to ensure that the **solvent level is below that of the spot itself**. A watch glass, which serves as a crude lid, is placed on top. The solvent is allowed to run up the plate until the solvent front has reached around 1 cm from the top edge of the plate. At this point the plate should be removed and the furthest extent of the solvent front marked.

3. Visualization

The solvent is allowed to evaporate from the adsorbent on the plate. If the substances under investigation are colored, i.e. they absorb light in the visible region of the spectrum, then both during and after development of the plate you will be able to see where the spots are and how they have moved relative to one another and the solvent front. Direct measurement can then be made of the R_f values of the components. If the components of the sample are not colored, and this is most often the case for typical organic materials, you will not see much happening during the development of the plate. In these cases an Ultra-violet (UV) lamp may reveal the location of the spots and their positions may then be marked with a pencil. Molecules containing multiple bonds are typically revealed by use of a UV lamp. Alternatively, the staining of a TLC plate with iodine vapor is among the oldest methods for the visualization of organic compounds. This technique exploits the fact that iodine has a high affinity for both unsaturated and aromatic compounds. There are also chemical methods for revealing the positions of materials on plates that cannot be located by eye, UV lamp, or the iodine staining technique.

PROCEDURE

Perform all the experiments in the hood.

Part 1: TLC Analysis of Mixtures of Ferrocene Derivatives

You will gain some familiarity with the technique of TLC by examining the behavior of some colored compounds on silica TLC plates using a set of solvents and solvent mixtures that differ in polarity. The compounds are called ferrocene, acetylferrocene, and 1,1'-diacetylferrocene (See Figure 3-1). Don't worry about the ferrocene part, just note that the acetyl group contains a relatively polar carbon-oxygen bond. Therefore the compounds **increase** in polarity from ferrocene (no acetyl group) to acetyl ferrocene (1 acetyl group) to diacetylferrocene (2 acetyl groups). Each material is provided as a solid. You should take small samples of each (a small spatula tip of material is sufficient)

and dissolve each material in around 1 mL of methylene chloride (dichloromethane) in three small, readily identified, sample vials.

Draw a faint pencil line around 1 cm from the bottom edge of the plate. Using a fresh spotter for each sample, apply each of the three solutions to evenly spaced points on this line aiming to prepare a colored spot of material no more than 3 mm in diameter. Allow the application solvent to evaporate for a few moments, leaving three small colored spots. Develop the plate in neat methylene chloride. Remove the plate and mark the maximum extent reached by the solvent front. Measure the R_f values of each material.

Repeat the experiment using some of the mixtures of methanol and methylene chloride available in the lab, measure the R_f values on each occasion. Keep trying mixtures until you have identified that which you think gives the best results, i.e. the one which gives the best separation (resolution) of the three materials. Keep in mind that if the three components are bunched together at the top or bottom of the plate then they haven't really separated—you are looking for a situation where the spots are spread about a median line about halfway up the plate.

Now, as before, take small amounts of each of the solid compounds and add them to a **single** sample vial. Dissolve the mixture in around 1–2 mL of methylene chloride. Spot the mixture at one side of a TLC plate and then alongside spot the three “pure” materials from before. Develop the plate in your chosen solvent system (from above). Note the results.

Sketch all of the TLC plates in your notebook, and label them carefully with the solvent mixture, the material in question, and the R_f values of all of the components.

Part 2: TLC Analysis of a Pharmaceutical

In the previous experiment identity of the compounds were known along with their relative polarity. The intention was to gain some experience in running and analyzing the data from TLC experiments in a well behaved and readily visualized system. The next experiment provides a more interesting application of TLC and involves probing the constituents of some commercial pharmaceuticals, mostly painkillers.

Spot a plate with each of the three standard solutions: caffeine, acetaminophen, and aspirin (available at the front of the lab). Develop the plate with neat ethyl acetate. Examine the plate under UV light and mark the positions of the spots you observe. Now place the plate in an iodine chamber for about 10 minutes and record and note any differences in the nature of the spots. **Be sure to keep the iodine stained plates in the hood.** Calculate the R_f values for each of the three components examined.

Prepare a second plate exactly as before with each of the three standard solutions. Develop the plate with a mixture of 3 mL of ethyl acetate plus one drop of glacial acetic acid. Examine the plate under UV light and mark the positions of the spots you observe. As before, now place the plate in an iodine chamber for about 10 minutes and record and note any differences in the nature of the spots. Calculate the R_f values for each of the three components examined. Compare the results of the two runs and decide which experiment gave the better results.

Next, obtain a tablet of an unknown painkiller from your instructor or TA. Place the tablet in a 10 mL Erlenmeyer (conical) flask, add around 5 mL of methanol and crush the tablet with the end of a glass rod. Allow the undissolved material to settle out (this is mostly starch). Spot some of the resulting solution on a TLC plate and let the solvent evaporate (you will need room for four spots in total so make sure to leave room). Check that the concentration of sample on the plate is appropriate by looking at the plate under the UV light. Remember that if the sample is too concentrated then poor results will be obtained, but too little sample will make it hard, or impossible, to measure the R_f values of the components. If the concentration appears low, i.e. you can't see much material where you spotted, then spot several times more in exactly the same place. If the concentration appears too high then dilute a couple of drops of the solution with some more methanol (about, 1 drop of solution and 10 drops of methanol) and re-spot on a fresh plate. Spot the three standard solutions you looked at earlier in the remaining places and develop the plate under the conditions you found to work best for the first part of the experiment. Measure the R_f values of the components and conclude which of the three components were present in your unknown tablet. Check your conclusions with your TA or instructor.

CLEAN UP

All of the liquid samples should be poured into the halogenated solvent waste container. The different solvents and solvent mixtures used for TLC should be poured into the appropriate solvent waste container, either halogenated or non-halogenated. Dispose of all vials and spotters in the glass waste container.

REPORT

In the results section include sketches of all of your plates, and label all features with their R_f values and identities. Describe the behavior of the ferrocene compounds when the plates are developed with different solvents, and how you arrived at your decision of the optimal solvent system for separation of these materials. Use the TLC evidence you obtain in Part 2 to indicate which materials were present in your pharmaceutical. Discuss/rationalize the presence of any other TLC features that you observed during this second experiment.

QUESTIONS

1. Does the solvent in which the samples are dissolved for plate preparation (i.e. spotting) have any bearing on the observed R_f values? Explain.
2. Why is it not possible to *uniquely* identify a material by its R_f value? (Hint: think about the maximum accuracy with which an R_f value might be recorded).

Part B: Column Chromatography: Separating Ferrocene Compounds

INTRODUCTION TO COLUMN CHROMATOGRAPHY (LC)

Column chromatography is one of the most common and useful ways to separate mixtures of materials and isolate large amounts of the individual components in pure form. You will isolate three ferrocene derivatives from a mixture and determine the effectiveness of this technique in terms of the purity of the products.

Thin Layer Chromatography (TLC) was used last week to analyze the components of a mixture. In the TLC analyses, the stationary phase was silica (SiO_2 , affixed to a plastic plate) and the mobile phase was a solvent or mixture of solvents, chosen to optimize the separation of compounds in a given mixture. Silica gel contains many relatively polar silicon–oxygen bonds and therefore has a high affinity for polar molecules. As a result, it was observed that the more polar the molecule the shorter the distance it traveled up the TLC plate.

In this experiment **liquid chromatography** will be employed to separate the components of a mixture. Much like TLC, the stationary phase will be silica gel and the mobile phase will be a solvent, or mixture of solvents. In contrast to TLC, where the components of a mixture were separated from top to bottom using capillary forces to draw solvent onto the bottom of a TLC plate, column chromatography is performed using gravity to draw solvent downward through a glass tube containing the silica gel. Column chromatography allows separation of relatively large amounts of a product mixture into pure individual compounds, each of which can be isolated, characterized, and perhaps used for other experiments or syntheses. This type of purification and isolation is crucial to all synthetic organic chemistry and the modern pharmaceutical industry could simply not exist without it. Various types of liquid chromatography are also widely used in separations and preparations of biological macromolecules such as proteins, carbohydrates, and DNA.

In practice

Using column chromatography, you will separate a mixture of ferrocene derivatives (ferrocene, acetylferrocene, and 1,1'–diacetylferrocene), and then confirm their identities using TLC.

To begin, you must first determine the most effective solvent system for separating the three compounds. The solvent, or solvent mixture you select, should allow good separation of the three compounds from one another, and also

cause all three materials to elute from (that is, exit) the column in a reasonable time. Additionally, the desired solvent, or solvent system must be **non-halogenated**, in order to minimize exposure to large quantities of (nasty) chlorinated solvents, and to reduce the volume of toxic and expensive waste. Please note that this restriction means you **cannot** use the solvent conditions you carefully worked out in the previous experiment, although those conditions would likely work very well.

To determine the best solvent, you *could* run a bunch of trial columns, but that would be expensive, wasteful, and very time-consuming. Instead, you will test several solvent mixtures by TLC, just as you did in the previous experiment. Since the stationary phase, silica gel, is the same for both TLC and column chromatography, the separations observed by TLC will accurately reflect the separations seen on the column.

Once you have determined which solvent, or solvent mixture, you will use in this separation, you will set up your column by neatly packing it with silica and solvent. The mixed sample will be added to the column and its components will move through the column at rates determined by their polarities and that of the mobile phase (the solvent) that you have selected. Since the three compounds are colored, you will be able to see at all times where they are on the column, how well they are separating, and when they are about to elute from the bottom. Once you have collected the separate “fractions”, each containing a pure sample of one of the three compounds, you can confirm the identity of each one by comparative TLC with an authentic sample.

PROCEDURE

Perform this entire experiment in the hood.

A. Determine the conditions for running the column using TLC

1. Using a spatula, transfer a tiny amount of the mixture of ferrocene compounds to a small vial and dissolve in a few drops of methanol. Prepare a TLC plate with a spot of this mixture exactly as you learned in the previous experiment. Consult last week’s experiment for additional details if you need them.
2. Prepare 5 mL of a 1:1 mixture of hexanes and ethyl acetate and use this mixture to develop the TLC plate. Once the solvent front nears the top, remove the TLC plate from the beaker and immediately mark the final position of the solvent front in pencil. You may need to use the UV lamp to confirm the location of the products (though they are colored the spots can be very faint). Sketch the TLC plate in your notebook and note the characteristics of the separation: which product, or products, moved most along the plate with the solvent? Which

adsorbed strongly to the stationary phase (that is, did not move very far)? Did the three products separate well from each other?

3. Repeat this experiment with different ratios of solvents, making careful note of the results of each in your notebook. Think carefully about how you might modify the mixture based on your first run. For example, if a mixture causes all three components to run toward the top of the plate, your mixture is **too polar** and you will want to reduce the proportion of the more polar component in the solvent mixture (the ethyl acetate). Of course, the opposite will hold if your components remain stuck to the bottom of the plate.
4. Based on these TLC runs, decide which solvent mixture, or mixtures, you will use to achieve the best separation and elution of the three products. Keep in mind that you may change mobile phases during the course of running the column to optimize the separation. For example, you may need to elute the first two products in solvent mixture "A", **then change** to solvent mixture "B" to elute the final product. You will want to use the least polar solvent mixture first, then switch to successively more polar mixtures to "chase" the products from the polar surface of the silica.

B. Prepare the column

5. Clamp your column securely but gently to a ring stand, leaving enough space at the bottom to allow for easy switching of the flasks or beakers you will use to collect the eluted solvent. Try to clamp the column such that it is as straight as possible so the compounds will run evenly down the column. Place a funnel on the top of the column and place a beaker underneath. Also have ready several clean, labeled sample vials for collection of the compound containing fractions.
6. You will use a crude dry-pack method to fill your column. Weigh 2.3 g of silica gel into a small beaker and pour into the column. Using 20 mL of your **initial** solvent mixture (as determined above) add portions of 1–2 mL at a time to the column, letting the solvent adsorb to the silica until the column is saturated. You may need to use a glass rod to poke down in the column and force out air bubbles, and use small batches of the solvent to wash any remaining silica gel from the inside surfaces of the column.
7. Very carefully open the stopcock and let some of the solvent drip through (both the column and stopcock assembly are quite fragile so please be careful here). As the solvent collects in the waste beaker below you may recycle it (as needed) to aid in washing all of the silica gel into an even, tightly packed, cylinder, within the column. Please note that it is very important that the column does not **ever run dry** during this process. Little cracks and channels are formed when air is allowed to enter the column, and separation is greatly compromised as a result.
8. Close the stopcock when the solvent level is just covering the top of the column. If the top surface of the packed silica appears crooked, tap gently on the side of the column until it flattens out.

C. Run the column and identify the fractions

9. Dissolve around 20 mg (0.02 g) of the mixture of ferrocene derivatives in ~0.5 mL of your starting solvent mixture. Also prepare 20 mL of fresh solvent mixture to begin the column.
10. Using a glass pipette and rubber bulb, start to add your dissolved sample to the top of the column. Try to add the solution gently, and evenly, to avoid displacing the silica gel, an ensuring that the sample starts running down the column from an even level. Open the stopcock and allow the sample to run onto the column, adding drops of the dissolved sample slowly and gently to the top via the pipette so that the column will not run dry and ensuring that a few millimeters depth of solvent is **always** maintained above the level of the silica gel. Leave any undissolved material behind in the vial (for the purposes of this experiment it is better to keep the volume of added solvent small, rather than try to get every last bit of material onto the column).
11. Once the sample is loaded onto the column, you can (very) gently add ~5 mL of fresh solvent mixture to the column and start the separation. Add more solvent as needed, at all times ensuring that there is no danger of the column running dry. Try to run the column continuously without closing the stopcock *via* regular additions of solvent. If you have elected to change solvent systems during the separation then at some point in your running of the column you will need to switch solvent systems.
12. Collect the three colored fractions (ferrocene, acetylferrocene, 1,1'-diacetylferrocene) in clean glass vials, and label each clearly. All of the colorless solvent fractions can be collected in a beaker to be disposed of as non-halogenated waste.
13. Once you have collected all of the samples, empty the silica into a beaker by turning the column upside down and gently tapping. Dispose of the liquid in the non-halogenated waste (by decanting), and the silica gel in the solid waste container.
14. Confirm the identity and purity of each of the fractions by running TLC analysis against pure samples of each material. Your fractions are relatively dilute so you may need to examine the developed plates under the UV lamp (the spots may be too faint to be directly visible). If your spots are difficult to see, and analysis of the results is ambiguous, you should choose one (or more if you wish) of your samples for evaporation: transfer to a small round bottomed flask, remove the solvent using a rotary evaporator (consult your instructor or TA), and then redissolve the obtained solid material in a tiny amount of solvent to make a concentrated TLC sample. Repeat the TLC analysis using this concentrated sample to achieve clearer results.

CLEAN UP

All of the solvents and samples left over from the TLC runs and liquid chromatography should be poured into the non-halogenated solvent waste container. Silica gel should go into the solid waste container. Dispose of all spotters in the glass waste container. Vials should be thoroughly rinsed with acetone, the washings discarded in the non-halogenated waste container and left in the hood to dry for the next lab group.

REPORT

In the report you should include sketches of all of your TLC plates, and label all features with their R_f values and identities. You should describe the behavior of the ferrocene compounds when the plates were developed with different solvents, and how you arrived at your decision of the optimal solvent system for separation of these materials on a column. Discuss the purity of your isolated final products, as determined by comparative TLC with authentic samples.

QUESTIONS

1. Last week we separated the same mixture of ferrocene compounds by TLC using dichloromethane/methanol solvent mixtures. Why did we use ethyl acetate and hexanes instead of dichloromethane/methanol for column chromatographic separation? What property of these solvent mixtures must be similar?
2. Even if the components of the mixture had not been distinctly colored, you could still have used liquid chromatography to separate and isolate them. Describe the practical measures you would need to employ in order to collect and identify the correct fractions?

Part C: High Pressure Liquid Chromatography (HPLC)

INTRODUCTION TO HPLC

In recent years the technology used in chromatographic analysis has greatly improved. Advances in this area have led to the common use of High Performance Liquid Chromatography, a laboratory technique that allows for the very efficient separation of small amounts of the components of a mixture. The technique has essentially the same operational basis as liquid chromatography, but HPLC allows for far better separation of the components of a mixture. The column in an HPLC instrument contains tiny particles of only about 5 μm diameter, ensuring a very large surface area to which molecules may adsorb. These particles comprise the **stationary phase** of the chromatographic system. Because these tiny particles are so tightly packed, the **mobile phase** solvent must be forced through the column under very high pressure. A detector and a computer are connected to the HPLC instrument in order to signal when eluents are coming off the column and fractions should be collected. The diagram in Figure 3-4 illustrates the main components of an HPLC instrument.

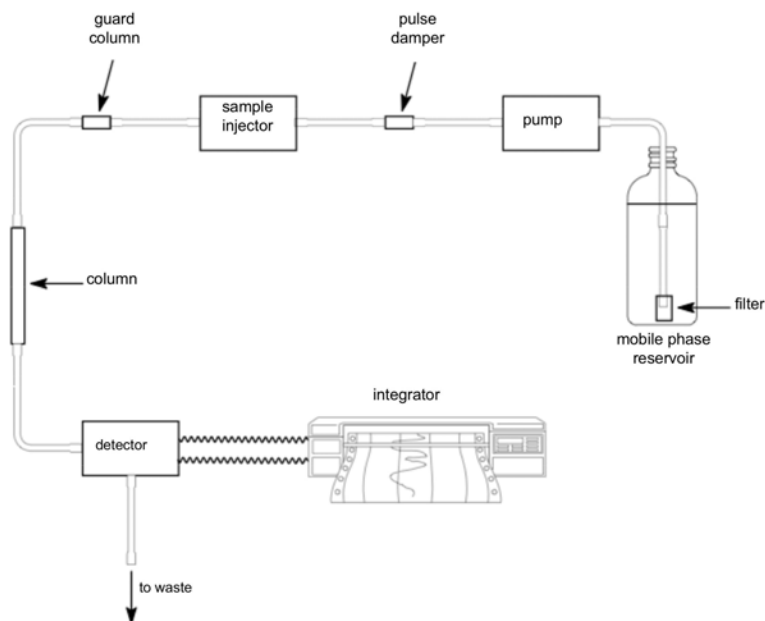


Figure 3-4. Block diagram of high pressure liquid chromatography.

In Practice

Once more, we will address separation of our mixture of ferrocene derivatives (ferrocene, acetylferrocene, and 1,1'-diacetylferrocene), this time using high pressure liquid chromatography. As with the liquid chromatography employed in Part B, the **stationary phase** is a tightly packed silica gel column, and the **mobile phase** (the solvent) is a mixture of hexanes (non-polar) and ethyl acetate (fairly polar). The initial solvent mixture will be 75% hexanes and 25% ethyl acetate. Throughout the run the percentage of ethyl acetate will be slowly increased (thereby increasing the polarity of the solvent) in order to elute the more polar ferrocene derivatives (acetylferrocene and 1,1'-diacetylferrocene) from the column. By comparing the retention times of the compounds, i.e. how long they stay on the HPLC column, with the results from your TLC experiments (Part A) you will be able to determine which compounds elute from the column at which times.

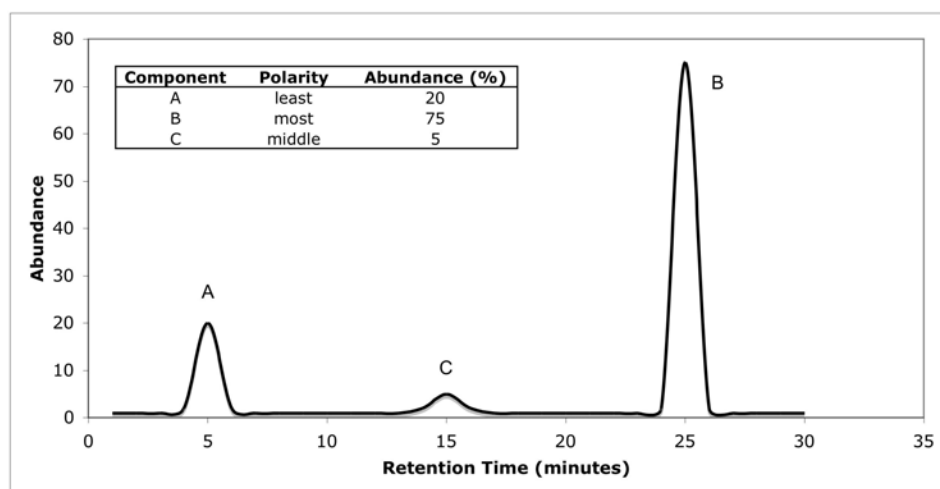


Figure 3-5. Example HPLC chromatogram.

PROCEDURE

Note: A mixture of the three ferrocene derivatives dissolved in a 3:1 ratio of hexanes to ethyl acetate is available in the laboratory for your use.

1. On the computer monitor for the HPLC there should be a green “ready to inject” window open. When your laboratory instructor or TA verifies that the instrument is ready you will prepare for the sample injection.
2. Go to “Run control” and click “Sample info...”. A new window will open: enter your name as “operator name”, your sample as “sample name”, and

add any other comments you wish under “comments”. DO NOT make any other changes.

3. Thoroughly rinse the microsyringe with the solvent mixture (the 3:1 ratio of hexanes to ethyl acetate) expelling the solvent into a Kimwipe.
4. Rinse the microsyringe with the sample mixture (the three ferrocene derivatives dissolved in a 3:1 ratio hexanes:ethyl acetate), expelling the solvent into a Kimwipe. Fill the syringe with a fresh 20 μL of the sample solution. Ensure that there are not any air bubbles in the syringe: if there are any air bubbles in the microsyringe, expel the sample into the Kimwipe and refill the syringe.
5. On the injection port of the HPLC, turn the knob upward and to the left toward the “load” label.
6. Wipe the needle of the microsyringe with a Kimwipe and gently press the needle into the HPLC injection port (no real force should be necessary). When the syringe is part of the way in, you will feel some resistance. Gently push past this point until the syringe is all the way in. Press down on the syringe plunger in a smooth, rapid motion to inject the 20 μL sample. With the syringe still in the injection port, turn the injection port knob down and to the right towards the word “inject”. Withdraw the syringe and rinse several times with solvent, expelling the solvent into a Kimwipe.
7. When the run is complete, print out a copy of the completed chromatogram.

REPORT

Include the chromatogram you obtained and explain how you interpreted the peaks. Explain the reasoning you used to determine which peak in the chromatogram corresponded to each ferrocene derivative in the original mixed sample.

QUESTIONS

1. Based on your experiences of running the various ferrocene derivatives on TLC plates, how would the results you obtained from this HPLC experiment vary if the amount of ethyl acetate in the solvent mixture were increased?
2. How is HPLC related to the other chromatographic methods, which you have studied (TLC, liquid chromatography)? You might note in your answer those practical elements they share in common, and those that are quite distinct.

Part D: Gas–Liquid Chromatography (GC)

INTRODUCTION TO GC

Gas–liquid (GC) chromatography provides a quick and simple method for analyzing volatile organic mixtures, both qualitatively and quantitatively. Often, the gas-liquid chromatographic instrument is coupled with an IR, NMR, or mass spectrometer in order to combine the excellent separation capabilities of GC with the superior identification capabilities of spectroscopy.

Figure 3-5 shows the main components of a gas chromatograph. The experiment begins by injecting a volatile liquid through a rubber septum into a heated injection port, which vaporizes the sample. The sample is swept through an open tubular column by a carrier gas, and the separated eluents (the compounds exiting the column) flow through a detector, whose response is displayed on a computer screen. The column must be hot enough to produce sufficient vapor pressure for each solute to be eluted in a reasonable time. The detector is maintained at a higher temperature than the column so that all the solutes are gaseous at the point of detection.

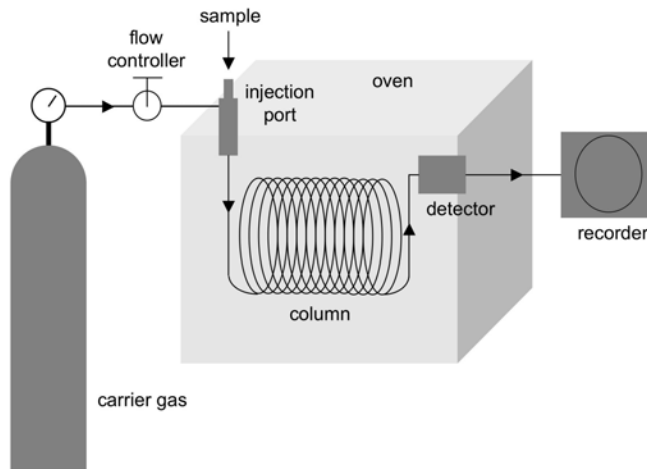


Figure 3-6. Diagram of gas chromatograph

The column is a hollow capillary tube 30 m in length with a 0.25 mm inner diameter that has a thin polymeric film of solid stationary phase, which lines the inner wall. The **stationary phase** in this experiment is a 0.25 μm thickness film containing phenylmethylsiloxane. The **mobile phase** (the carrier gas) flowing through the column is helium. Because different compounds in a mixture have

differing affinities for any particular stationary phase, compounds can be separated (resolved) as a mixture of compounds travels through the column since the column retains some of the compounds longer than others. The time required for a separated compound to elute from the column is called the **retention time** and is effected by several experimental factors: the boiling point of the eluent, the column temperature, the column flow rate of the mobile phase, and the polarity of the stationary phase. Compounds may be identified by their retention times, as long as all the other experimental variables are kept constant, while the area below each peak corresponds to the relative abundance of the compound in the sample mixture. An example of a GC chromatogram is shown in Figure 3-7 for a three-component mixture. Note that the least polar component also has the lowest boiling point, while the most polar component has the highest boiling point. Since this will not always be the case, GC instruments allow the temperature of the column to be varied (usually increased) during the run.

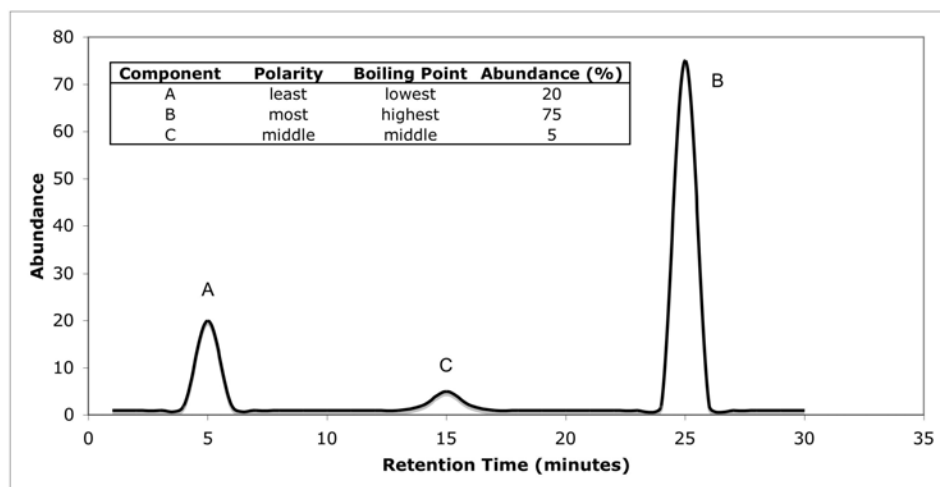


Figure 3-7. Example GC chromatogram.

In Practice

For this experiment we will be using a gas chromatograph with a flame ionization detector (GC-FID). The flame ionization detector recognizes the presence of a material and in response produces an electric signal. In the GC-FID, the eluents are separated by the gas chromatograph and then burned in a micro-flame produced by a mixture of H_2 and air. Carbon atoms (from the solute) produce CHO^+ ions in the flame. These ions are collected, and the detector response is directly proportional to the amount of solute present. A schematic of a FID is shown in figure 3-8.

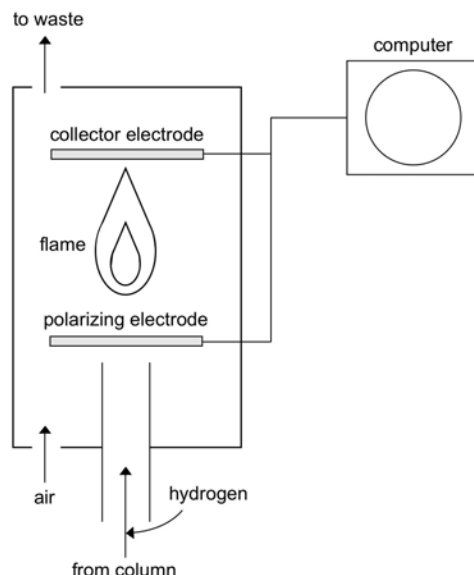


Figure 3-8. Schematic of a flame ionization detector

Samples of 4 pharmaceuticals will be available in the laboratory. The pharmaceuticals will be dissolved in anhydrous methanol in the following concentrations: Aspirin, 5 mg/mL; Ibuprofen, 1 mg/mL; Nabumetone, 1 mg/mL; and Caffeine, 1 mg/mL. With your TA or instructor's assistance, you will use a small hypodermic syringe to inject 1 μ L of each sample through a sealed rubber septum into a stream of helium flowing through the heated injection port of the GC-FID (these very small amounts of sample are injected to avoid overloading the column). The sample will be immediately vaporized and the helium gas will carry it onto the column. The sample's components will be separated on the column and pass into the flame detector. You will then be able to print out a graph of the retention time for each of these standard solutions. It is important to thoroughly rinse the syringe with the solvent before and after each injection to avoid contaminating any of the subsequent injections. After you have printed out chromatograms for each of the known samples, you will be given an unknown mixture that contains one or more of the pharmaceuticals. You will run your unknown sample through the GC-FID and determine the contents of your unknown by comparing the retention times of the unknown sample to the retention times for the known pharmaceuticals.

PROCEDURE

A. The standard solutions

1. On the computer screen for the GC–FID click on “Start Run” and in a few seconds the “Ready” window should appear.
2. Thoroughly rinse the microsyringe with the anhydrous methanol solvent, expelling the solvent into a Kimwipe.
3. Rinse the microsyringe with the standard solution of Ibuprofen dissolved in anhydrous methanol and then load the syringe with **at least 7 μL** of sample. There must not be any air bubbles in the syringe. If there are any air bubbles present expel the Ibuprofen sample into a Kimwipe and refill.
4. On the GC–FID machine push the “prep run” button and wait until the red “not ready” light disappears.
5. Expel sample from the syringe into a Kimwipe until the syringe contains **only 1 μL** of sample. Wipe the needle with a Kimwipe and insert the syringe all the way down into the GC injection port. Be careful as the injection port is hot and the syringe must be placed in the port close to vertically. Attention to this proper injection technique is important in order to get well resolved peaks in the chromatogram. Quickly press down on the syringe in a smooth, rapid motion to inject the entire 1 μL sample. Press the start button on the GC and carefully remove the syringe from the injection port. The run will stop when the temperature program is completed (after about 10 minutes).
6. While the run is in progress rinse the syringe with solvent several times, expelling the solvent into a Kimwipe.
7. When the run is complete print out a copy of the total ion chromatogram, noting the retention time of each peak.
8. Repeat the procedure (steps 1–7) for the remaining two standard solutions.

B. Determination of the composition of an unknown mixture

9. Obtain from your instructor a solution of unknown composition. Make sure to write down the number of your unknown sample in your lab notebook.
10. Repeat steps 1–7, this time injecting your unknown sample into the GC–FID.
11. Compare the total ion chromatogram for your unknown sample to the chromatograms of the standards in order to determine the contents of your unknown. Check your conclusions with your lab instructor or TA.

CLEAN UP

The unknown solution can be poured into the non-halogenated waste container.

REPORT

Include all of the total ion chromatograms, which you printed out, for each of the known samples and for your unknown. Explain how you interpreted the peaks in the total ion chromatogram of your unknown sample and employed this reasoning to determine the contents of your unknown.

QUESTIONS

1. What functions do the mobile phase and stationary phase perform in GC?
2. When injecting your sample into the GC–FID, why is it essential that the syringe be carefully rinsed before and after each use?

Experiment #4: Extraction of Spinach Pigments

PURPOSE

To introduce the techniques of liquid-liquid extraction, reflux, and rotary-evaporation, while isolating chlorophyll from spinach.

INTRODUCTION

This experiment employs two sequential extraction techniques to isolate some of the compounds responsible for the intense green color of spinach leaves. The colored pigments in green plants contain a mixture of chlorophylls (Figure 4-1), molecules that absorb sunlight and through this mechanism play a crucial role in photosynthesis. As research strives to identify alternatives to oil as energy sources, scientists are currently investigating the exact mechanism of photosynthesis with the goal of someday synthetically mimicking the process to provide a viable energy resource. A key step in these investigations is to first isolate the molecules that participate in the photosynthetic process. Today you will perform this crucial first step while learning the powerful technique of liquid-liquid extraction, learning how to set up a reaction under reflux, and learning how to use the rotary evaporator.

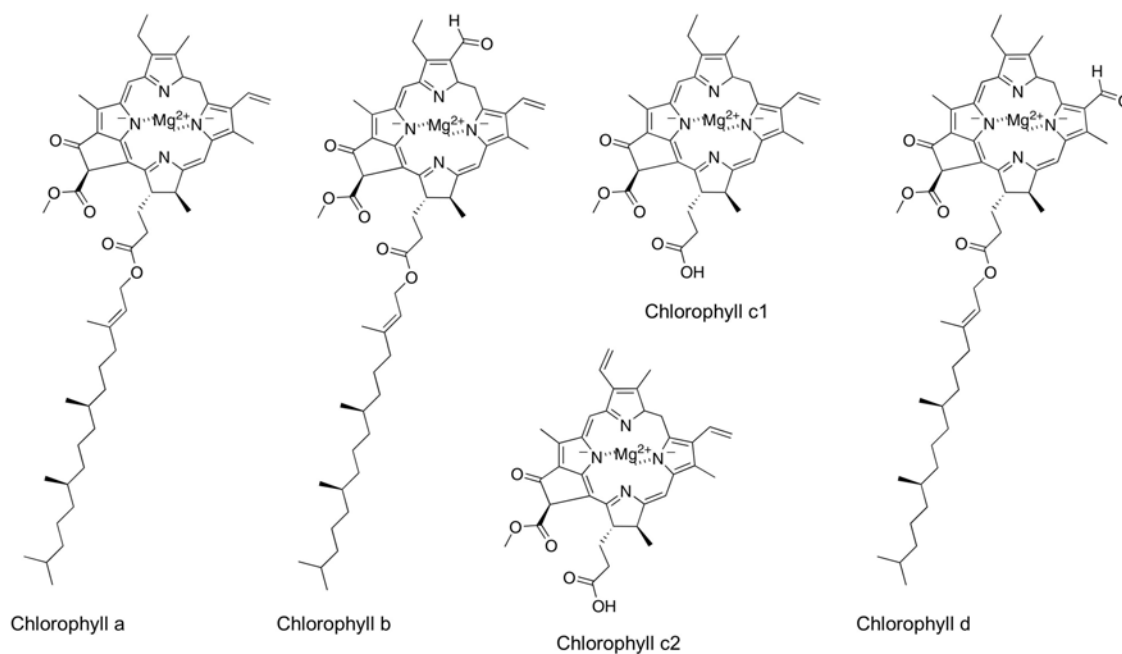


Figure 4-1. Structure of chlorophyll molecules. Spinach contains chlorophyll a and chlorophyll b.

The first extraction technique involves removal of the chlorophyll a and chlorophyll b pigments from the leaves by stirring in a hot organic solvent, under reflux. The second technique, a classical and important one in organic chemistry, is liquid–liquid extraction. You will use this second technique to separate the colored pigments from accompanying water–soluble compounds. The resulting organic extracts will then be evaporated under reduced pressure (rotary-evaporation) and the highly colored pigment residue analyzed by thin layer chromatography.

Reflux

A system is in **reflux** when sufficient heat is provided to make the liquid form vapor, as the vapor rises it is cooled to a liquid and allowed to return to the reaction pot. In this way heat is provided to a system without the loss of the liquid components in the form of vapor. A condenser is normally attached to the reaction flask to provide the cooling mechanism. Cold water is allowed to run through the condenser, however, to achieve the desired cooling effect a constant flow of water must be maintained. Therefore, water should enter the condenser at the bottom, and exit at the top. Figure 4-2 shows the typical set up. Notice that the reaction flask is clamped such that it could be raised out of the heating bath in the event that it became over heated.

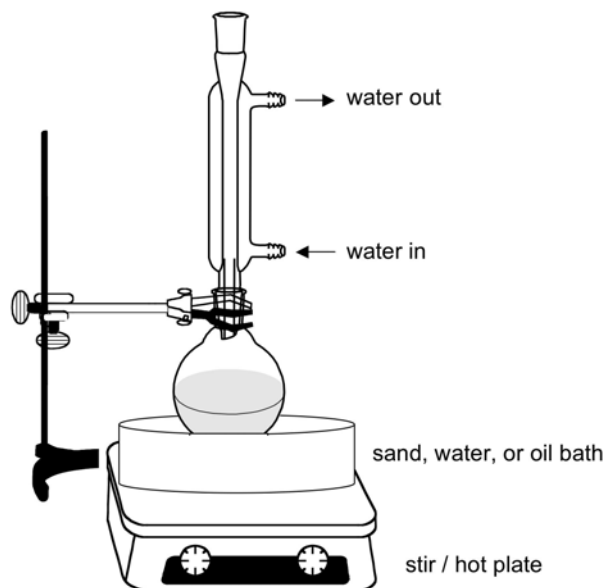


Figure 4-2. Typical set up for reflux.

Liquid-Liquid Extraction

Liquid-liquid extraction is a technique used to separate compounds by employing differences in their solubility in various solvents. This technique is important because it is very commonly used as a routine separation/purification step at the end of an organic reaction. Strictly speaking the ability to separate two materials by this technique relies on the material in question being very unevenly distributed between two immiscible solvent layers. Almost always, one of the layers is aqueous and the other is an organic solvent with which the water will not mix (**immiscible**), such that two layers are formed and can be cleanly separated. Since polar inorganic material will generally be water soluble, and the much less polar products of organic reactions will be soluble in an organic solvent, a clean separation of polar from non-polar material can be achieved by separating these two liquid layers.

The separatory funnel is a glass vessel with a stoppered top, tapered construction (to allow a clear view of the interface between different solvent layers), and a stopcock to allow controlled release of the liquid contents. It is supported by means of a metal ring on a laboratory retort stand: **DO NOT** clamp the neck of the funnel, see figure 4-2 for the typical set up. With the stopcock closed (tap in horizontal position) the solution to be extracted is introduced *via* the neck of the funnel, followed by the extracting solvent. The two-layer mixture is now vigorously shaken to thoroughly mix the two layers, your instructor will demonstrate the technique. A period of ten to thirty seconds of mixing is sufficient to establish equilibrium, returning the funnel to the stand for a few seconds usually results in clean separation of the two solvent layers.

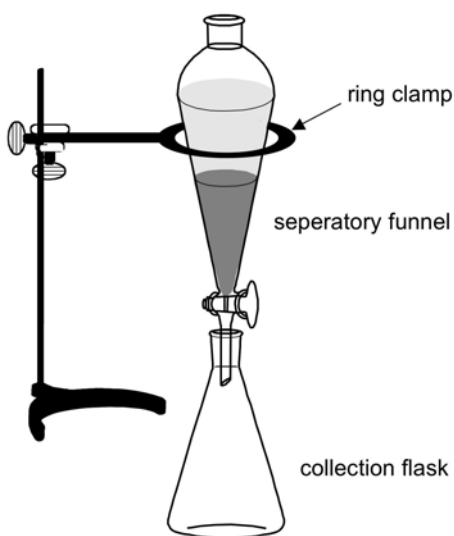


Figure 4-3. Typical liquid-liquid extraction set up.

If the density of the extracting solvent is higher than that of water then it will form the lower layer, the opposite is true if the density is lower than that of water. In the former case, the lower layer, in which the desired organic material is now dissolved, can be collected by removing the stopper from the neck of the funnel and turning the stopcock so that this layer drains into an Erlenmeyer flask. If the top layer is desired then the aqueous layer must first be drained off before the organic layer is collected.

Hints & Tips

- In all extractions with volatile solvents the establishment of the equilibrium vapor pressure of the solvent will cause the pressure to rise in the stoppered funnel. This pressure **MUST** be released by inverting the funnel and gently releasing the pressure *via* the stopcock, while holding the stopper in place, in the funnel neck. Solvents such as diethyl ether and methylene chloride are very volatile (have relatively low boiling points) and the build up in pressure can be considerable. For this reason the pressure should be released after just a few gentle shakes of the funnel and then every few shakes after that. You will find that the difference in pressure diminishes rapidly with each successive mixing process. ***In general, great care should always be taken when using volatile extraction solvents. In particular, ALWAYS point the funnel away from you (and your partner!) and into the hood.*** This will ensure that any vapor will be expelled away from you and into the hood.
- In situations, which are common, where more than one extraction is to be performed on an organic solution then it is preferable, if possible, for that solvent to have a *lower* density than water. In this situation the organic layer will always remain on the top of an aqueous solution and need never be removed and replaced in the funnel.
- Extraction can, of course, be performed in batches. In this manner large volumes of aqueous solution can be extracted with organic solvent and the organic portions combined at the end.
- A little solid material is frequently found suspended at the interface between the two layers. This is not a problem and such solid is easily removed by simple filtration at a later stage in the purification process.

Rotary Evaporation

An important and common method for concentrating substances is rotary evaporation. The solvent is removed under reduced pressure while the flask containing the substance is rotated in a water bath. The temperature of the water bath is controlled and the vaporized solvent is collected in a separate flask

after condensing on a cold finger or water condenser. Your instructor or TA will demonstrate the use of this instrument.

In Practice

By treating some chopped spinach leaves with a refluxing organic solvent you will extract a complex mixture of materials that, you will further refine using the liquid–liquid extraction technique. At the end of the process you will have a small amount of a concentrated mixture of materials whose composition you will explore by performing TLC.

PROCEDURE

Safety Considerations: *This experiment uses the flammable solvents acetone and hexane and you should perform all possible manipulations in the hood. Be aware of all possible ignition sources.*

1. Weigh out 16 g of chopped spinach and transfer to a 100 mL round-bottomed flask. Add 20 mL of acetone and 20 mL of hexane to the flask along with a stirrer bar.
2. Using figure 2-1 as a guide, clamp your flask securely to the stand and attach a cold water reflux condenser. Ensure that your water hoses deliver water at the bottom of the condenser and allow it to exit from the top. Be aware that the water needs to fill the condenser for it to work effectively and air bubbles should be avoided as much as possible. Ensure that the exit hose is secure in the sink, in your hood. A spare clamp can be useful to weight the end of the hose to make sure it stays put.
3. Adjust the height of the clamped apparatus so that the bottom of the flask is sitting in the sand bath, below which is the stirrer. Turn on the stirrer and the heating power, and adjust the control to about 60 %. Watch patiently for the solvent to start to boil. In time you will see vapor condensing on the inside surface of the condenser and dripping back into the flask. This is the reflux situation you should maintain, adjusting the power as necessary. Allow the mixture to reflux for 10 minutes.
4. Turn off the power and let the flask cool. To aid the cooling, raise the flask and remove the sand bath. During this time set up the vacuum filtration apparatus, recall that you learned how to do this in Experiment 2, ensuring that the filter flask is clean. Remember to include a piece of filter paper in the bottom of the funnel.
5. Filter the spinach mixture by removing the spinach from the round-bottomed flask with a spatula. Once all the spinach is in the filter funnel use the bottom

of a beaker to press down on the leaves and squeeze out as much solvent as possible: allow the vacuum time to do its work and after a short while you will find that most of the solvent has been collected. Transfer your collected filtrate to a clean beaker and discard the spinach leaves and filter paper in the trash.

6. Set up a ring stand to safely hold your separatory funnel, ensure that the tap is in the closed (horizontal) position and carefully pour in your filtrate. Two layers should rapidly become established: a lower, more dense, aqueous layer and an upper, less dense, organic layer. Both layers will be colored so be careful to ensure that you know the position of the interface between the two layers. Slowly drain the lower layer into a beaker—proceed very slowly near the point where the two layers meet to ensure good separation—and label “aqueous extracts”. Drain the remaining liquid (formerly the upper layer) into a second beaker and label “hexane extracts”.
7. Return the aqueous extracts to the separatory funnel and add 10 mL of fresh hexane. Stopper the funnel, remove it from the ring stand, and begin mixing the two layers by gently inverting and swirling the funnel. After a few gentle swirls vent the funnel by opening the stopcock to release any pressure that has built up during mixing. Close the stopcock and repeat the mixing procedure a few times. Return the separatory funnel to the ring stand, remove the stopper and allow the two layers to form. Drain the aqueous lower layer into the same small beaker as before (the one you labeled “aqueous extracts”), and put safely to one side. Add the new hexane extracts that remain in the funnel to your “hexane extracts” beaker.
8. The next step is to remove all the traces of water from your hexane extracts. Return the hexane extracts to the separatory funnel and add 10 mL of brine (saturated sodium chloride solution). Gently mix the two layers as before by inverting the funnel, swirling, and occasionally releasing the pressure. Set the funnel back on the ring stand and allow the two layers to separate. Drain the lower aqueous layer into your “aqueous extracts” beaker and drain the hexane layer into a clean 50 mL Erlenmeyer flask. To complete the drying process add a small amount of anhydrous sodium sulfate to the hexane solution (check with your instructor or TA if you are unsure how much to add), swirl the solution gently a few times, and then let it sit for five minutes. You should find that you obtain a clear solution with the solid material resting on the bottom. A little more drying agent can be added if the solution retains any cloudiness at this stage.
9. This final solution must now be concentrated to a small volume before TLC analysis. Carefully decant the dried organic solution from the drying agent into a clean and dry 50 mL round-bottomed flask, ask your TA or instructor to concentrate the solution by a rotary evaporator. Allow the flask to cool before beginning your TLC analysis.

10. Prepare a TLC plate in the manner you learned in a previous lab, ensuring that you have established a small concentrated spot of your green solution on the plate (the spot needs to be quite concentrated in order for you to clearly see the results).
11. Develop the plate in the solvent system available in lab (a 1:1:6 ratio of 95% ethanol:acetone:hexane). Remember to mark the distance on the plate the solvent has traveled when you remove it from the developing chamber and the position of any spots that are visible. Then examine the plate under a UV light to see if there are any additional components of the spinach extract. Sketch your plate in your notebook and record the R_f values of all the significant features.

CLEAN UP

Discard your aqueous extracts in the aqueous waste container and your small volume of organic extract in the non-chlorinated waste container. The dried spinach leaves and filter paper may be discarded in the trash.

REPORT

Record all of your observations through the various stages of the experiment including the reflux step and extraction step. Provide an accurate sketch of your TLC plate in your notebook, making sure to indicate the number, intensity, and color of all the features that you observe.

QUESTIONS

1. Your TLC analysis will have revealed the presence of several/numerous materials in the isolated sample. What does this observation allow you to conclude about the selectivity of the extraction process?
2. What property of an organic solvent determines the position of the immiscible layer it forms with water? When the following solvents are used for extracting an organic compound from an aqueous solution, will the organic solvent form the upper or lower layer: chloroform, benzene, heptane, methylene chloride?

Experiment #5: Resolution of Racemic 1-Phenylethylamine *via* Diastereomer Formation With (2*R*, 3*R*)-Tartaric Acid

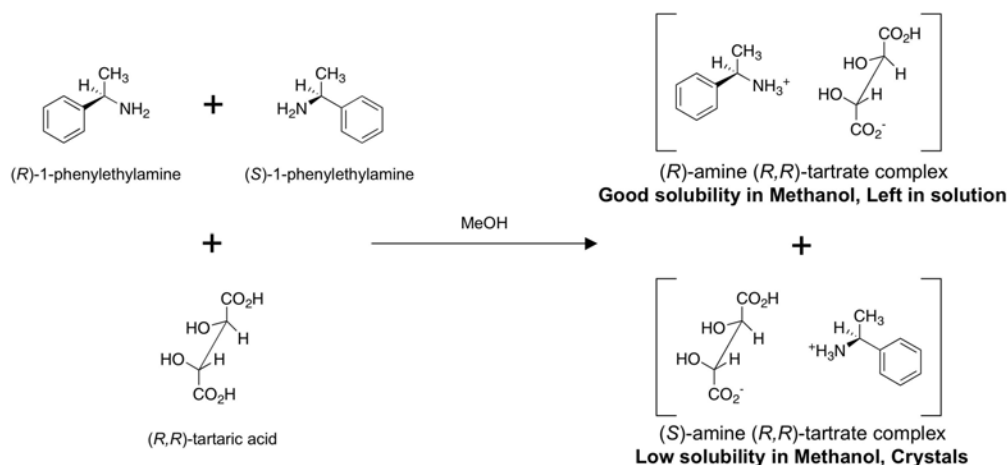
PURPOSE

To separate the enantiomers of phenylethylamine and to learn the technique of measuring optical rotation with a polarimeter.

INTRODUCTION

The separation of the enantiomers, **resolution**, of phenylethylamine can be achieved by exploiting the very different solubilities in methanol of their diastereomeric complexes with a single diastereomer of tartaric acid.

Recall that enantiomers have identical physical properties, while those of diastereomers differ. When a hot solution of **racemic** (*R,S*)-1-phenylethylamine and an equivalent amount of (*R,R*)-tartaric acid in methanol is allowed to cool, the less soluble (*S*)-amine (*R,R*)-tartrate complex crystallizes out of solution (see Scheme 5-1).

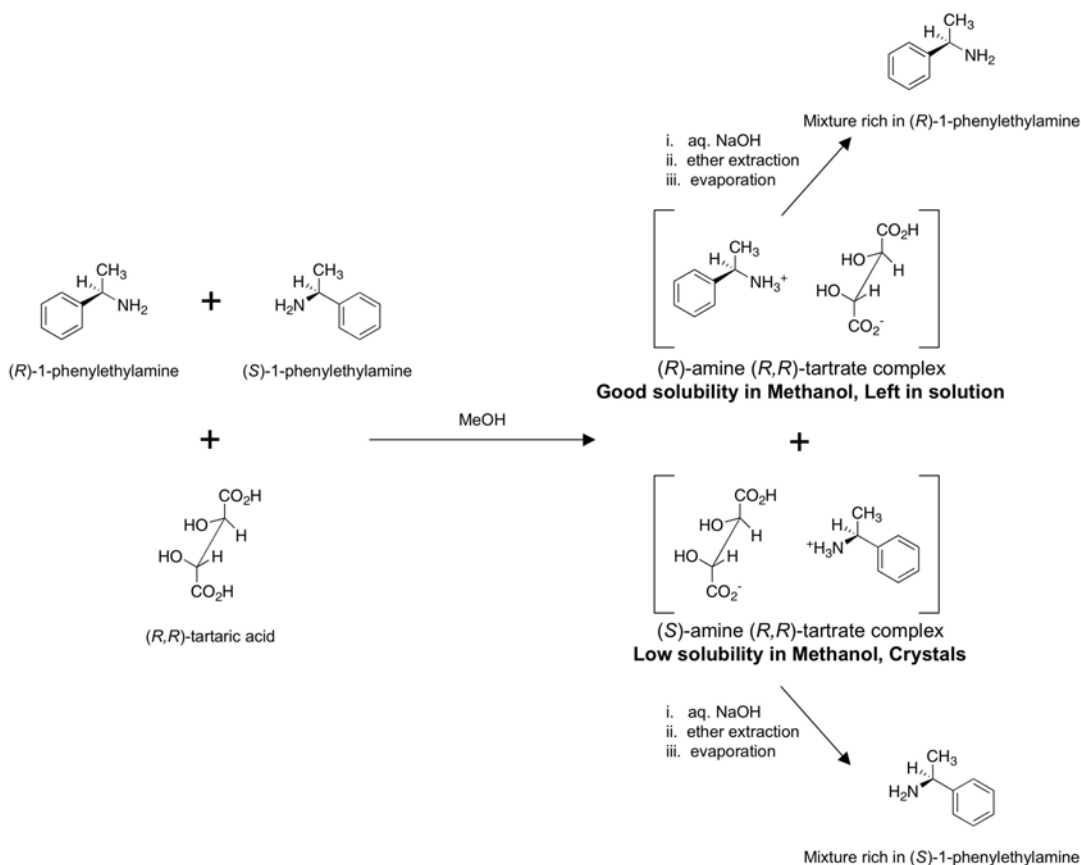


Scheme 5-1. Solubility of (*R,R*)-tartrate salt complexes in methanol.

Isolation of this crystalline material and treatment with excess aqueous sodium hydroxide allows liberation of the (*S*)-amine from the tartrate. Just as with the extraction experiment you have already done, we now have a situation where we can separate a relatively non-polar organic material (the amine) from a

polar one (the tartrate salt). After extraction the pure (*S*)-amine is obtained by evaporating the methanol solvent on a rotary evaporator.

In theory, the more soluble diastereomeric complex that remains dissolved in the filtrate can also be isolated by treating the filtrate with sodium hydroxide solution, extracting the liberated amine into an organic solvent to separate it from tartrate, and evaporating the methanol solvent. Scheme 5-2 details the steps involved in this resolution experiment.



Scheme 5-2. Resolution and isolation of phenylethylamine enantiomers.

It is very rare for a single crystallization of this kind to prove sufficient to fully separate two enantiomers. Invariably, the complex containing the (*S*) enantiomer crystallizes along with some of the complex containing the (*R*) enantiomer, and not all of the (*S*) enantiomer complex crystallizes. Thus we are left with a mixture of some of the (*S*) containing complex and most of the (*R*) containing complex in solution. If it was required to achieve absolute and complete separation of a pair of enantiomers the crystallization experiment would be performed several times, in sequence, gradually enriching the percentage of pure single enantiomer.

PROCEDURE

Work in pairs throughout this experiment. Though the first part of this two-week experiment is rather short, the best results are obtained by being careful at this stage and performing the simple operations cautiously.

Safety Considerations: *The racemic amine mixture has a strong and irritating odor. Dispense this material and perform all manipulations in the hood. The sodium hydroxide solution you will use during week 2, will cause serious burns if it comes into contact with your skin. Wear gloves and eye protection at all times when handling this material.*

Week 1: Preparation of the tartrate salt complexes.

Weigh 6.25 g (41.6 mmol) of (*R,R*)-tartaric acid into a 250 mL Erlenmeyer flask, add 100 mL of methanol, and heat the mixture gently on a hotplate for a few minutes. To the warm solution slowly add 5 g (5.3 mL, 41.2 mmol) of (*R,S*)-1-phenylethylamine. **Care!** Too rapid addition will cause the mixture to boil over. Once the addition is complete, let the solution boil very gently on the hotplate for at least 15 minutes (use a boiling stick). Let the solution cool to room temperature, seal the flask with parafilm, label it with your names and laboratory section, and place it in the location indicated by your TA or instructor. **The solution must now be left to slowly crystallize until next week.**

Week 2: Resolution of enantiomers.

Part 1: Regenerating the S-amine

1. Collect the crystals by vacuum filtration and wash the crystals with methanol. Dry the crystals on a piece of large filter paper and record the yield. **Do not discard the filtrate!**
2. Dissolve the crystals in 50 mL of distilled water in a 100 mL beaker and add 4.5 mL of 50 % NaOH solution. **Caution: concentrated NaOH is very caustic.** Ensure that the solution is basic by testing with pH paper.
3. Transfer the aqueous solution to a separatory funnel, add 30 mL of diethyl ether (the density of diethyl ether: 0.71 g/mL). Perform the extraction technique.
Diethyl ether is extremely volatile and flammable. When using it for extractions release the pressure after just a few shakes of the funnel and do extractions in the hood.
4. Return the aqueous layer to the separatory funnel and repeat the extraction with a second 30 mL portion of ether.
5. Collect the second portion of ether, combine with the ether extract from the first extraction, and then add more anhydrous Na₂SO₄. Dry the ether layer for 10 min. **NOTE:** Diethyl ether will form H-bonds with water, so there is more

water in the ether layer. We have to add more drying agent and wait for a longer time to remove water.

6. Weigh a dry, clean, and empty 250 mL round-bottomed flask and record the weight. Decant the dried extracts into this flask and give it to your TA who will demonstrate the operation of the rotary evaporator to remove the solvent.
7. Weigh the round bottomed flask again after removing all the solvent and record the weight. The difference of the weights is the amount of your S-amine.
8. Prepare the following solution of S-amine in a clean vial: 0.1 g S-amine and 9.9 g methanol.
9. Measure the optical rotation (the specific rotation).

Part 2: Regeneration of the R-amine:

1. Weigh a dry and clean 250 mL round bottomed flask and record the weight. Transfer the filtrate to this flask and evaporate the solvent on the rotary evaporator.
2. Weigh the round bottomed flask again after removing all the solvent and record the weight. The difference of the weights is the amount of your R-amine-(*R, R*) tartrate.
3. Add 50 mL of distilled water and 4.5 mL of 50 % NaOH solution. **Caution: concentrated NaOH is very caustic.** Ensure that the solution is basic by testing with pH paper.
4. Repeat steps 3-7 of the Part 1 procedure for regenerating the S-amine.
5. Prepare the following solution of R-amine in a clean vial: 0.1 g R-amine and 9.9 g methanol.
6. Measure the optical rotation (the specific rotation).

With the assistance of your instructor record the optical rotation of your two samples using the polarimeter in the central instrument room. In the same manner, also record the optical rotation of the original *racemic* mixture, (*R,S*)-1-phenylethylamine.

CLEAN UP

Your purified amines, methanol filtrate, and any ether left over from the extraction should be disposed of in the non-chlorinated waste solvent container. The basic layers from the extraction should be placed in the aqueous waste container.

REPORT

In the observations/data section describe the crystals you obtained. Record the dried weight of the crystals, and the weights of the two samples of amine you isolated. The preparation of each solution for the optical measurement should be described and the optical rotation for each sample clearly stated.

In the discussions/conclusions section calculate the percentage yield for the crystalline material you isolate and the yield of the free amine. With reference to the optical rotations you recorded on the polarimeter, discuss the effectiveness of your attempt to separate the two amine enantiomers (optical rotations for the pure *single* enantiomers, in other words the theoretical values, are: (S) -38.2° and (R) $+38.2^\circ$).

QUESTIONS

1. The (S) enantiomer is isolated *via* a crystalline salt and, as crystals grow in a “pure” form, this enantiomer can potentially be isolated in a very pure state. Isolating the (R) enantiomer from the residue left in solution inevitably involves isolation of that portion of the (S) amine that did not crystallize. The presence of this (S) amine has an effect on the optical rotatory power of the sample isolated in this part of the experiment. What effect?
2. It is possible to separate the two enantiomers comprising a racemic mixture using the various forms of chromatography you have met (TLC, column, GC, HPLC). These techniques share a common operational basis, but what modification is required if separation of enantiomers is to be achieved?

Experiment #6: Preparation of Stilbene Dibromide

PURPOSE

To prepare stilbene dibromide and determine the mechanism of the reaction from inspection of the reactant and product structure.

INTRODUCTION

Stilbene can exist in *cis* or *trans* isomeric forms, shown in Figure 6-1. Addition of bromine to an alkene generally proceeds *via* a mechanism involving a cyclic bromonium ion which is subsequently ring opened from the opposite face by bromide ion to form a 1,2-dibromo product. You have encountered this reaction in class and solved many problems involving the addition of bromine across a double bond. The stereochemical demands of this mechanism ensure that the arrangement of groups in the starting alkene is directly reflected in the product dibromide. Experiments of this type, the inference of mechanistic pathway from examination of reactant and product structure, remain a very important tool in the continuing development of mechanistic organic chemistry.

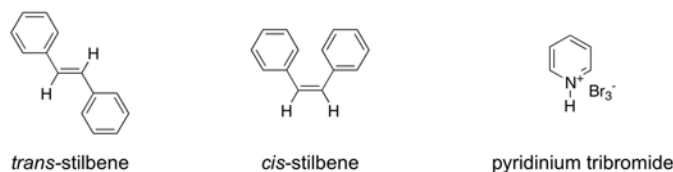


Figure 6-1. Structures of stilbene and pyridinium tribromide.

In Practice

You will prepare 1,2-dibromo-1,2-diphenylethane, the product of addition of Br₂ across the double bond of *trans*-stilbene (*trans*-diphenylethene). The Br₂ is delivered in the form of pyridinium tribromide, a convenient solid source of bromine, see Figure 6-1 for the structure. Think of this material simply as a solid form of bromine (Br₂). You will deduce the stereochemical outcome of your reaction by determining the melting point of your product. In turn you will be able to confirm that the mechanism of addition of bromine across the double bond of stilbene occurs *exclusively via* a bridged intermediate (**A**, in Figure 6-2), and does **NOT** involve participation of an intermediate carbocation (**B**, in figure 6-2).

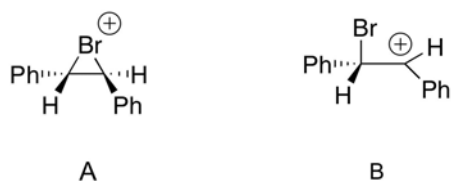


Figure 6-2. Structures of possible intermediates.

Table 6-1. Physical constants for the materials in this experiment.

Material	Mol. Wt. (g/mol)	Mp (°C)
trans-stilbene	180	122–124
pyridinium tribromide	320	–
glacial acetic acid	60	16
racemic-(1R,2R)/(1S,2S)- 1,2-Dibromo-1,2-diphenylethane	340	113–114
meso-1,2-Dibromo-1,2-diphenylethane	340	237

PROCEDURE

Safety Considerations: *Pyridinium tribromide is corrosive and lachrymatory (causes the eyes to tear). Use gloves when handling this reagent and perform all manipulations in the hood.*

In a 10 mL Erlenmeyer flask dissolve 0.25 g of *trans*-stilbene in 6 mL of glacial acetic acid. Warm gently on a hotplate until all the material has dissolved. Add to this solution 0.50 g of pyridinium tribromide, in small portions over a couple of minutes. Continue to heat gently, and over the next ten minutes swirl the flask periodically to thoroughly mix the reagents. Allow the flask to cool for 10 minutes and then cool it in a cold water bath to fully complete precipitation of the product. While the flask is cooling put 2–3 mLs of methanol in a test tube and cool in a small beaker of ice. Collect the crystalline product on a Hirsch funnel and wash the crystals on the filter with the cold methanol. Let them dry on the funnel by drawing air through them for around 15 minutes. Record the isolated yield and the melting point of the dried crystals. Calculate the percentage yield for the overall reaction. Propose a mechanism for this reaction that is consistent with your result.

CLEAN UP

Dispose of all filtrates in the acid waste container provided. Discard your products in the solid organic waste container once you have recorded a melting point and yield.

REPORT

In the observations/data section describe how you added pyridinium tribromide to the flask and what changes happened after adding it. Describe your observations for each stage during the reaction and record the weight and melting point of the crystals.

QUESTIONS

1. By drawing careful mechanistic/structural diagrams identify the possible product(s) of addition of Br_2 to *trans*-stilbene if the reaction proceeds (a) *via* a cyclic bromonium ion intermediate (**A**, Figure 6-2), and if the reaction (b) involves a carbocation (**B**, Figure 6-2).
2. The melting point you recorded for your material will have indicated that only one type of cationic intermediate is involved in this reaction. Which one? How is it that you can be very confident of your answer?
3. Imagine that you are now going to repeat the entire experiment starting with *cis*-stilbene, in place of *trans*. What would you expect the melting point of your product to be? Fully explain your reasoning.

Experiment #7: Kinetics of Solvolysis of *t*-Butyl Chloride

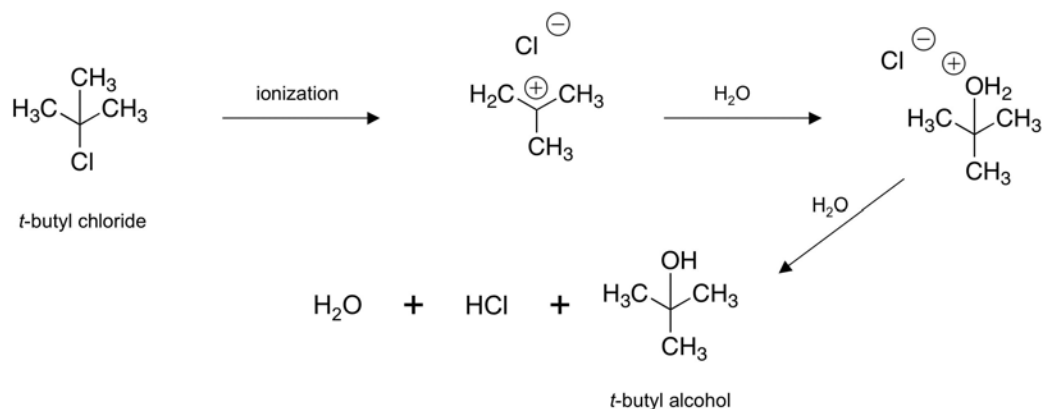
PURPOSE

To study solvolysis reactions of alkyl halides by examining the kinetics of the solvolysis reaction of *t*-butyl chloride in water.

INTRODUCITON

Alkyl halides are excellent compounds for substitution reactions as halogens make good leaving groups. In the first step of an S_N1 (substitution, nucleophilic, unimolecular) reaction the leaving group departs, creating a carbocation. Recall that the S_N1 mechanism is favored for alkyl halides where the loss of halide results in a stabilized carbocation. For tertiary alkyl halides the S_N1 mechanism is the only one observed.

In an S_N1 solvolysis reaction the initially formed carbocation is captured by a nucleophilic solvent molecule to produce the product. If the solvent is water the product will be an alcohol. This process is illustrated for *t*-butyl chloride in Scheme 7-1.



Scheme 7-1. Solvolysis of *t*-butyl chloride in water.

The overall ease with which the initial carbocation is formed can be modulated by addition of acetone (dimethylketone) to the water used in this solvolysis experiment. Acetone is far less able than water to solvate the cation–

anion pair produced in the first step of the S_N1 reaction, and as this step is rate determining a change in overall rate for the reaction will be noted.

One method to study the rate of a reaction is to monitor formation of the product, or products, of the overall process. There is no obvious, and inexpensive, way to measure production of *t*-butyl alcohol, but for this process we have the good fortune to also produce one mole of HCl for every mole of *t*-butyl alcohol. Thus, monitoring of HCl concentration, by titration to determine hydrogen ion concentration, with respect to time provides a direct quantitative measure of the extent of production of alcohol. As it's been a while since you've thought about kinetic phenomena, a quick refresher is in order.

Kinetics Refresher

The rate of a reaction is measured by monitoring the change in concentration of a species with respect to time. For consumption of an alkyl halide, RCl, we would write:

$$\text{rate} = \frac{-\Delta[\text{RCl}]}{\Delta t} \quad (\text{Eq. 7-1})$$

The rate of a **first order** reaction, one that depends on the concentration of a single species (in this case RCl), is equal to a rate constant, *k*, multiplied by the current concentration of RCl. Therefore, we may write:

$$\frac{-\Delta[\text{RCl}]}{\Delta t} = k[\text{RCl}] \quad (\text{Eq. 7-2})$$

Integrating and rearranging gives the following familiar form:

$$\ln[\text{RCl}] = -kt + \ln[\text{RCl}]_0 \quad (\text{Eq. 7-3})$$

Equation 7-3 describes a straight line of slope $-k$. Therefore, a plot of $\ln[\text{RCl}]$ vs. time, should fit a straight line if the reaction is indeed first order. [**Note:** Using the titration method, we cannot measure $[\text{RCl}]_t$. Instead, we measure the volume of NaOH solution required to neutralize the reaction mixture at time *t*, and these volumes are directly proportional to concentration].

Equation 7-4 can be developed from Equation 7-3, which expresses the mathematical relationship between the NaOH solution volume and time.

$$\ln(V_{\infty} - V_t) = -kt + b \quad (\text{Eq. 7-4})$$

In Equation 7-4, V_{∞} is the volume of NaOH neutralized after all the *t*-butyl chloride has reacted. Therefore, $(V_{\infty} - V_t)$ represents the concentration of *t*-butyl chloride remaining at time *t*. The plot of $\ln(V_{\infty} - V_t)$ vs. *t* should be a straight line with a slope of $-k$.

In Practice

The purpose of this experiment is to determine the kinetics of solvolysis of *t*-butyl chloride, thereby providing evidence for the operation of the S_N1 mechanism in the aqueous solvolysis of this alkyl halide. There are two parts to the experiment and you should work in pairs throughout.

Before beginning any kinetic measurements it is essential that the apparatus you will use is entirely free from contamination. Therefore, the **very first thing** you need to do is ensure that your burette drains are entirely clean by filling with around 10 mL of 5.00×10^{-3} M aq. NaOH and allowing this solution to empty. Repeat this rinsing step two more times. Next, fill the burette completely with the NaOH solution. Drain any excess solution until the level just below the zero mark and record the exact volume that corresponds to the position of the meniscus.

PROCEDURE

Safety Considerations: Acetone/water mixtures will evolve heat and develop pressure, so be sure to release pressure after each inversion of the volumetric flask by releasing one edge of the film.

1. Gather three 125 mL Erlenmeyer flasks and label 2 of these with an infinity (∞) sign and the 3rd with "50:50". Add 20.0 mL of room temperature deionized water to each infinity flask and 10.0 mL of acetone to your 50:50 sample flask. Add 3 drops of phenolphthalein indicator to each flask and thoroughly mix by swirling the flask.
2. Add 10.0 mL of 0.1 M *t*-butyl chloride stock solution and 15.0 mL of acetone to a 50 mL volumetric flask. Prepare a beaker containing 50 mL of room temperature deionized water. Add water from the beaker to the volumetric flask until it is around three-quarters full, swirl and **begin timing** (Don't stop the stopwatch until you finish step 7). Add further water until the volume of the flask reaches the 50 mL line. Cover the neck of the flask with parafilm and invert three times to thoroughly mix the liquid contents. Move onto the next portion of the experiment **immediately**.

3. Use a 5 mL calibrated pipette, add 5.00 mL of the reaction mixture prepared in step 2 to each of the two “infinity” flasks. Swirl each of these, cap them, and set them aside (You have to constantly swirl them). As soon as possible after this add 5.00 mL of the reaction mixture to your “50:50” sample flask and record the time when half of the mixture has drained into the flask. Swirl thoroughly to mix the contents.
4. Titrate your “50:50” sample flask with aq. NaOH until the color of the indicator changes pink. Remember to do this slowly and carefully so as not to overshoot the volume of solution you add. Accurately record the volume of aq. NaOH you used. This is your first value of V_t , i.e. volume required at time t .
5. Discard the solution from this first titration in a large beaker (you will use this for the waste from all of your titrations). Rinse the Erlenmeyer flask with acetone, shake out any excess liquid, and refill with 10.0 mL of acetone and 3 drops of phenolphthalein indicator. At the 6 minute mark after the reaction mixture was added to the first sample flask, add 5.00 mL of the reaction mixture to the new sample flask. Once again record the time at which half the liquid has drained into the flask, titrate, and record the volume of aq. NaOH used. This is your second value of V_t .
6. Repeat step 5 an additional six times at intervals of 6 minutes: therefore, in addition to your first two measurements, you will make additional measurements at 6, 12, 18, 24, 30, and 36 minutes, eventually recording seven values of V_t .
7. Finally, add 10.0 mL of acetone to each of the infinity flasks. Titrate each of these infinity samples as you have the others and record the volume of aq. NaOH used for each sample. Average the two volumes you obtain and use this as your value for V_∞ in the analysis that follows.

CLEAN UP

Dispose of leftover reaction solution and sample/titration wastes in the halogenated waste container. Excess aq. NaOH should be disposed of in the basic waste container. Thoroughly rinse your burette and place it upside down in the stand, with the stopcock open, to dry.

REPORT

Turn in the data sheet. The time and volumes should be recorded with proper significant figures.

You should include a graph which plots one set of data points for $V_{\infty}-V_t$ vs. time, and a second for $\ln(V_{\infty}-V_t)$ vs. time.

Determine the negative slope of the lines for $\ln(V_{\infty}-V_t)$ versus time: recall from the kinetics refresher that this value corresponds to k , the rate constant for the reactions.

In the discussions/conclusions section discuss if your plot of $\ln(V_{\infty}-V_t)$ vs. time is a straight line? If so, what does this reveal and prove?

QUESTIONS

1. What do the plots of $\ln(V_{\infty}-V_t)$ versus time reveal concerning the order of reaction of *t*-butyl chloride with water? Explain your answer with reference to both the graphs you have plotted and the rate equation for the reaction.
2. How would you expect the rate of solvolysis of *t*-butyl chloride to change if you were to (a) triple the concentration of *t*-butyl chloride, and (b) triple the concentration of water? Fully explain your answers with reference to the rate equation for the reaction.
3. How would you expect the rate of solvolysis of *t*-butyl bromide with water to compare to that you've measured for *t*-butyl chloride? Explain your answer.

Experiment 7: Kinetics of Solvolysis of t-Butyl Chloride

name: _____

section: _____

Data Sheet

time (clock reading)	t (elapsed time)	buret reading (mL)	V _t (volume of NaOH solution added) (mL)	V _∞ -V _t (mL)	ln(V _∞ -V _t)

volume of NaOH neutralized when reaction is completed, mL

V_{∞1} =

V_{∞2} =

the average volume, mL

V_∞ =

rate constant, min⁻¹

k =

Experiment #8: Competitive Alkene Formation via Alcohol Dehydration

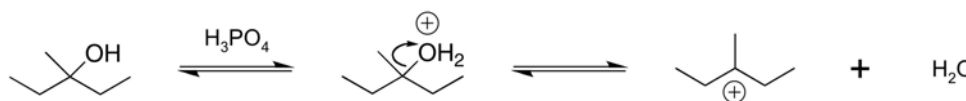
PURPOSE

To examine the competitive intermediates involved in the dehydration of 3-methyl-3-pentanol by analyzing the reaction products, while learning the techniques of distillation and GC-MS.

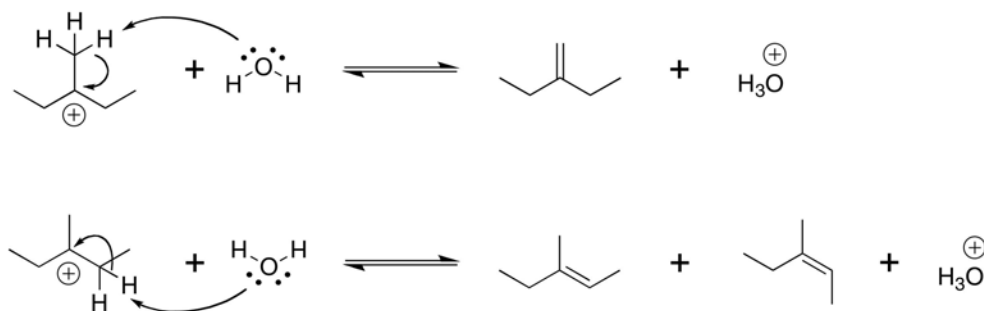
INTRODUCTION

This experiment examines competitive alkene formation *via* acid-catalyzed dehydration of an alcohol. The product mixture of alkenes and water may be separated and analyzed by Gas Chromatography/Mass Spectrometry (**GC-MS**). You have encountered GC before, and this new technique simply feeds the materials of a gas chromatographic separation into a mass spectrometer. In this manner we obtain both composition and structure information from a single analysis.

There are many examples of simple alcohols for which different product alkenes can be obtained upon dehydration. A common example is 3-methyl-3-pentanol, for which the mechanism of the acid-catalyzed dehydration is shown in Schemes 8-1 and 8-2. In the first step a tertiary carbocation is formed through protonation and subsequent loss of water (Scheme 8-1). In the second step two distinct sites for deprotonation lead to two distinct products (Scheme 8-2).



Scheme 8-1. First step in the acid-catalyzed dehydration of 3-methyl-3-pentanol.



Scheme 8-2. Second step in the acid-catalyzed deprotonation of 3-methyl-3-pentanol.

Formation of the three product alkenes is competitive: the pathways (i.e. transition states) leading to their formation are different in energy, leading to a difference in their rate of formation, and different extents of production. In this experiment you will examine the outcome of this competitive dehydration by analyzing the alkene mixture produced using GC–MS.

In Practice

The elimination reaction will be performed on a small scale, using a specialized piece of glassware called a Hickman still (essentially, a very small scale distillation apparatus) to collect the mixture of product alkenes. A sample of this mixture will be analyzed by GC–MS to determine composition and confirm identity. You will also perform some simple molecular modeling calculations to predict the stabilities of the product alkenes.

PROCEDURE

Work in pairs for this experiment. At the beginning of lab your instructor will divide the class in two: half the group will perform the practical part of the experiment first, while the other completes the molecular modeling exercise.

Safety Considerations: *Phosphoric acid is corrosive and will cause severe burns if it comes into contact with your skin. Wear eye protection and gloves at all times when handling this material. Inform your instructor or TA immediately of any spills or instances of contact.*

Table 1. Physical constants for the materials you will use or prepare

Material	Mol. Wt. (g/mol)	Boiling Pt. (°C)
3-methyl-3-pentanol	102	123
<i>trans</i> -3-methyl-2-pentene	84	70-72
<i>cis</i> -3-methyl-2-pentene	84	67-68
2-ethyl-1-butene	84	64-65

Part 1: Alkene Formation via Dehydration

1. Add 1.25 mL of 3-methyl-3-pentanol (density: $d = 0.824\text{g/mL}$) and a magnetic stirrer bar to a 5 mL round-bottomed flask. Clamp the flask securely above the sand bath, which in turn should be stacked securely on top of the magnetic stirrer unit and lower until the bottom of the flask makes good contact with the sand.
2. Start gentle stirring and add, dropwise, 0.25 mL of 85 % phosphoric acid to the flask. After the reagents have thoroughly mixed, attach a Hickman still to the flask (place a very small amount of grease on the neck of the still to ensure that it will not become stuck). Attach a water-cooled condenser to the top of the Hickman still.
3. Turn on the power to the sand bath and watch for liquid to collect in the still: be patient as this may take a little time and try to resist the temptation to crank the sand bath power way up (consult your TA or instructor at any stage). After a short while, distillate will begin to collect in the collar of the still. Transfer the distillate to a capped vial using a Pasteur pipette and continue heating and transferring until about 0.5 mL of liquid remains in the flask (to minimize evaporative loss of your collected liquid keep the vial capped).
4. Add a small amount of anhydrous CaCl_2 to the vial containing the collected distillate and recap the vial promptly. Let the vial sit for a few minutes to allow all traces of water to be absorbed to the drying agent. During this time collect two clean Pasteur pipettes and use the tip of one to push a small cotton wool plug down into the top of the narrow part of the other (consult your TA or instructor if you need guidance).
5. Collect a clean, capped, vial and record its weight. Arrange the plugged pipette above it so that any contents will drain into the vial. Use the unplugged pipette to carefully withdraw your dried liquid, avoiding as far as is possible any solid material, and expel the liquid into the neck of the plugged pipette. You may use the pipette bulb to apply a little pressure to the top of the plugged pipette and speed up the filtration process. Cap the vial as soon as all the liquid has been collected.

6. Weigh the vial and determine your yield.

Part 2: GC–MS Analysis

Next, you will employ gas chromatography–mass spectrometry to analyze your product mixture. To a vial labeled with your names, add 10 mL of *n*–pentane and **one** drop of your product mixture. Mix thoroughly and consult your TA on running your sample through the GC–MS instrument.

Once the analysis is underway peaks should be recorded as the product mixture separates and elutes from the column (remember that as the products of this reaction are isomers they will have identical molecular weights). Decide which peak in the chromatogram corresponds with which alkene in the product mixture by comparing the boiling points of the expected alkene products.

CLEAN UP

All liquid waste can be deposited in the non–halogenated waste containers. The calcium chloride you used as drying agent should go into the solid waste container. Used Pasteur pipettes should be discarded in the glass disposal containers in the laboratory.

REPORT

In your report you should include a careful description of the dehydration process and the collection and isolation of the alkene products. Record the yield (in grams) of the alkene mixture and calculate the percentage yield this represents. Use the data collected from the GC–MS analysis to identify which component of the analysis corresponds to which alkene isomer, and therefore determine which alkene was the dominant product.

QUESTIONS

1. Are the results of your experiment and analysis in agreement with the reactions that you have studied in lecture? Fully justify your answer.
2. Draw a complete energy versus reaction progress profile for this reaction, paying close attention to the energy of the various intermediates and products and label accurately the positions of the reactant, common intermediates, and products.
3. Sketch structures of all the transition states involved in this reaction.

Experiment #9: Mass Spectrometry and Infrared Spectroscopy

PURPOSE

To analyze and identify unknown compounds using mass spectroscopy and infrared spectroscopy, while developing and practicing your spectra interpretation skills.

INTRODUCTION

The determination of the composition and structure of an unknown compound is an essential aspect of organic chemistry. There are a wide variety of analytical and spectroscopic techniques that may be employed to probe aspects of a molecule's structure and properties, from a simple technique like TLC (which provides a crude assessment of polarity) to a complex technique such as nuclear magnetic resonance (NMR) spectroscopy (a technique that provides very detailed structural information, and one that you'll learn a great deal about at the beginning of Organic II).

In this experiment you will be introduced to two very important techniques for determining molecular structure: mass spectrometry and infrared (IR) spectroscopy. As implied by its name, the former technique provides molecular weight information, you've already observed some of the results of the application of this technique in experiment 8. The nature of the process that provides this weight data also leads to the formation of fragment ions. The weights of these ions, and the relative intensity of the peaks in the spectrum to which they correspond, can also provide useful structural information. The second technique, infrared spectroscopy, uses radiation in the infrared range of the electromagnetic spectrum to probe the structure of a molecule. Groups of atoms in a molecule that are connected to one another in a particular fashion (for example, the carbonyl group, $\text{C}=\text{O}$) absorb radiation in a narrow band of frequencies that correspond to the frequency of movement of that particular group of atoms. These movements can be stretches, bends, or twists, and are specific to a particular group. Thus, absorption at a particular frequency allows us to consult tables of data and determine which group is present.

The combination of these techniques does not allow determination of all the details of a molecule's structure, that will have to wait until you learn NMR spectroscopy. However, you can use these two techniques to explore the structural differences between closely related molecules and, given a list of possibilities, decide which sample corresponds to which structure.

In Practice

Your task in this final laboratory exercise is to assign the correct identities to a small number of materials. There are four materials in the first set, all of which are monobrominated or dibrominated alkanes, and you will examine these materials using mass spectrometry. There are six materials in the second set, all constitutional isomers of the formula C_6H_8O , which you will examine by infra-red spectroscopy. The structures of the two sets of materials are shown in Figures 9-1 and 9-2.

Your laboratory instructor will tell you how many samples your pairing should examine for each set. It is not necessary for you to record the spectra of every material since the whole class is working with the same set of unknowns: once you have recorded the spectra of the samples to which you have been assigned you will be directed to make copies for the remainder of the class members. In turn, you and your partner will receive copies of those spectra you need to complete your set.

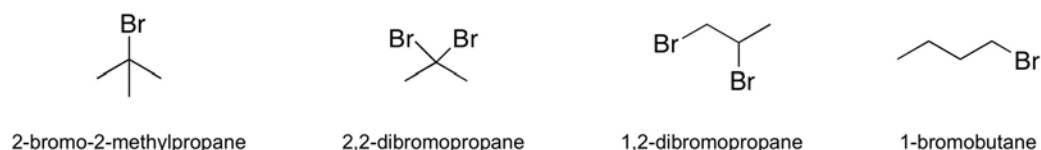


Figure 9-1. Set 1 compounds for analysis by Mass Spectrometry.

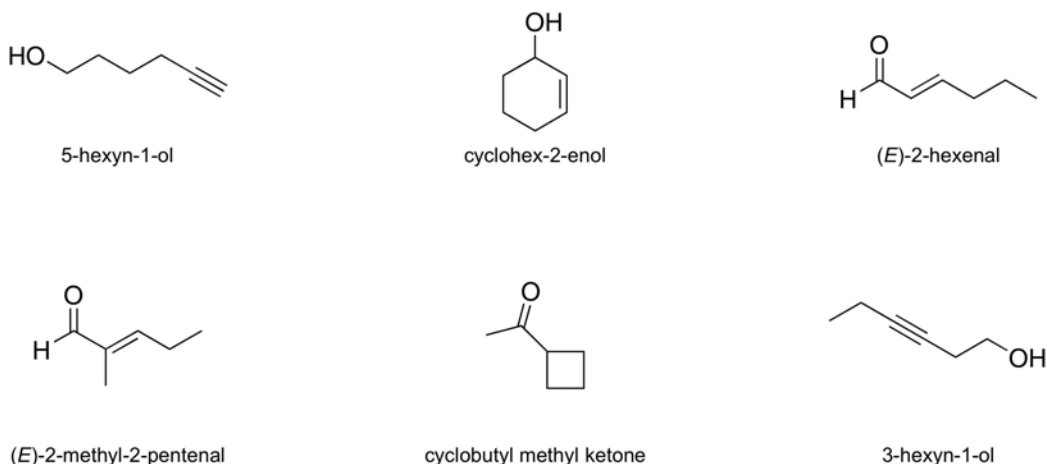


Figure 9-2. Set 2 compounds for analysis by Infrared Spectroscopy.

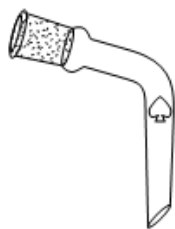
PROCEDURE

For the first set of materials you will use the GC–MS instrument you used last week. For your designated unknowns prepare a sample solution containing one drop of the unknown in 10 mL of pentane. For the second set of unknowns you will use the unknown materials in their pure liquid form for IR analysis. Your TA or instructor will demonstrate how to place a small volume of the liquid unknown between two sodium chloride plates to prepare for spectrum acquisition (sodium chloride is used as this material has no IR absorption in the range of frequencies of interest to us).

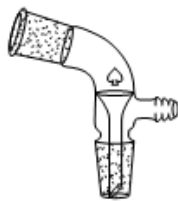
REPORT

For this final experiment it is up to you to decide how best to analyze the spectra you have collected and to assign identities to the unknowns. Some conclusions will, of course, be obvious. For example, the materials in set 1 are not all of the same molecular weight, so it's (potentially) easy to tell a mono-bromo from a di-bromo compound, but less obvious how to distinguish, for example, the two dibrominated compounds from one another. Likewise, the set of IR spectra are full of clues that will allow you to solve the problem of assignment, but you will have to think carefully about how to use the data contained in your spectra. For example, if you believe two of the six materials to contain one particular functional group then, for those two, an additional difference in their spectra should be enough to confirm identity. Happy hunting.

Appendix A: Common Glassware



distillation adapter, simple



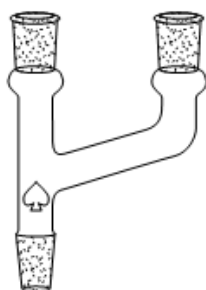
distillation adapter, vacuum



condenser



separatory funnel



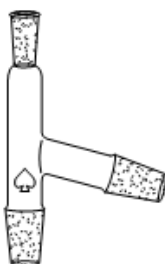
Claisen distilling head



round-bottomed flask



Keck clamp



distillation head



Büchner funnel



Hirsch funnel

Appendix B: Melting Point Apparatus Heating Curves

