Experiment 1: Recrystallization of Acetanilide

Learning Objectives

- Understand principles behind purification and recrystallization
- Critical analysis of recrystallization technique
- Analyze data to assess purity and success of experiment
- Understand the role H-bonding plays in dissolving, precipitation, and melting point
- Predict sources of intrinsic error and understand their effects on results

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How to Prepare for Lab + Assignments - Follow Canvas Exp 1 Module…

Before Lab

- Read this PDF – background, procedure, safety, pre-lab and in-lab questions
  - Option to listen to **Exp 1 Podcast** on Canvas = Caitlin reads this document ☺
- Attend **lab lecture** and take notes on **templates** provided on Canvas
- Practice the lab online *via* **Slugs@home platform** - sites.google.com/ucsc.edu/slugshome/home
- Complete the **pre-lab questions** at the end of this doc - incorporated into Canvas quiz ☺
  - **Pre-lab quiz** on Canvas due midnight Monday before your enrolled section
- Download the **Exp 1 worksheet** on Canvas and prepare your **lab notebook**…

Lab Notebook Preparation – *Required before lab; worksheet provided as suggested template on Canvas*

- **Purpose:** one-sentence summary of the main lab goals plus the recrystallization scheme (**Figure 2**).
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info!
- **Procedure with Diagrams** – complete before starting lab; sample on Canvas
  - Use the procedure that follows to create your hand-drawn experimental instructions
    - Simple sketches & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!
    - Avoid copying the procedure word-for-word.
    - Make it easy for anyone to follow your procedure without referring to this document.
  - **Slugs@home Exp 1 website** - Equipment & Safety pages; pictures & videos of the whole lab
  - The **class notes** include useful diagrams as well

During Lab (Tu-Th)

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A
- Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

After Lab – each partner submits separate, individual assignments

- Upload **Notebook Pages** to Canvas by midnight on lab day – graded on completeness / participation
- Complete & upload the **Lab Report** on GradeScope (GS) – due date on Canvas
  - Guidelines at end of this document

Background Theory

Paracetamol, the active ingredient in **Tylenol®**, is commonly used for pain relief (analgesic) and to reduce fevers (**Figure 1**). The synthesis of pharmaceutical agents like Tylenol requires pure starting materials to avoid complications from impurities. Purification is a tedious part of synthetic organic chemistry. Often product recoveries are sacrificed in favor of more pure materials. Solids can be purified *via* recrystallization or sublimation and liquids *via* distillation. Acetanilide has a similar structure to Tylenol and is also an analgesic. Acetanilide is no longer marketable due to toxic effects when ingested, but it is safe to use in the organic teaching lab as it is only a mild skin irritant. Acetanilide recrystallizes well from water due to its high solubility in hot water and low solubility in cold water. The purification of acetanilide serves an excellent introduction to recrystallization (**Figure 2**).

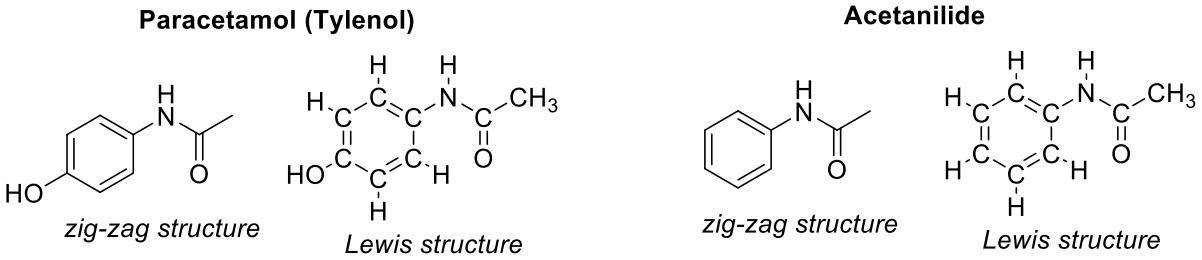

Figure 1. Structures of paracetamol and acetanilide

The basic steps of recrystallization are as follows.

1. Choose a good recrystallization solvent
2. Dissolve the sample in the *minimum* amount of boiling solvent
3. Hot filtration to remove insoluble impurities
4. Cool the solution to induce crystallization
5. Cold filtration to separate the solid from the solution (mother liquor or filtrate)
6. Wash the solid with a small amount of cold solvent
7. Dry the solid to remove traces of solvent

The crude, impure solid is dissolved in the smallest possible amount of solvent of choice; in this case, the solvent is water. Acetanilide (Ac) has a much higher solubility in hot water than in cold water. If too much hot solvent is added in the beginning, little-to-no Ac will recrystallize from cold water at the end of the experiment. Activated charcoal is added to remove **colored impurities**. These impurities are often non-polar organic compounds that have an affinity for activated charcoal, a non-polar fine, black powder. Any **insoluble impurities** (including those that have adsorbed onto the charcoal) are removed during the **hot filtration** step, while acetanilide remains in solution (filtrate). This solution is gradually cooled in an ice bath to induce crystallization. **Cold filtration** is performed under reduced pressure (vacuum). Any **soluble impurities** remain in the filtrate and the recrystallized solid is collected from the filter paper.

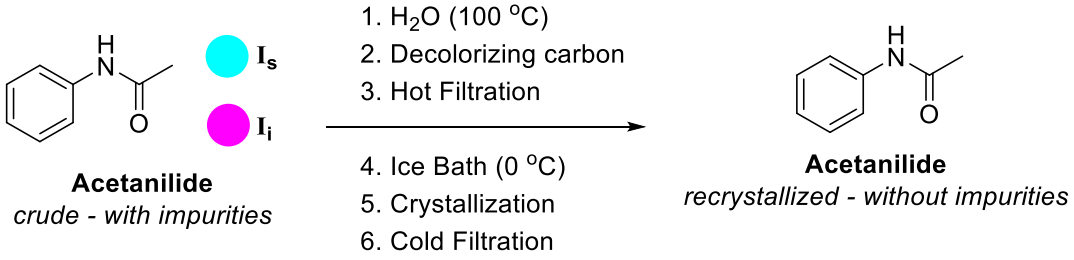

Figure 2. Recrystallization of acetanilide overview – *use this in the Purpose of lab notebook*

Finding the proper **recrystallization solvent** has to be determined experimentally. This can be tricky if there is no literature precedent for the compound (luckily it is known that water works for acetanilide!). A successful recrystallization requires that the compound be *highly soluble at the solvent's boiling point* and significantly *less soluble at low temperature*. The masses of recrystallized product (m_{recrys}) after cold filtration and the original crude starting material (m_{crude}) are used to calculate the **percent recovery of recrystallization** according to **eq 1**.

$$\% \text{ Recovery} = \frac{m_{recrys}}{m_{crude}} \times 100\% \qquad \textbf{(eq 1)}$$

A **theoretical recovery** represents the maximum amount of material that can be obtained if the experiment were carried out flawlessly; assuming no product is lost in transferring solutions, particularly in the hot and cold filtrations. It is unrealistic to carry out this 'perfect' experiment due to the nature of recrystallization. Mass recoveries are expected to be quite low. This is generally acceptable due to the high purity of recrystallized material.

Theoretical recovery is calculated using the known solubilities of acetanilide in water at a cold temperature (S^c = 0.53 g Ac / 100 mL at 0 °C), and high temperature, (S^H = 5.50 g Ac / 100 mL at 100 °C). The maximum mass recoveries from the hot and cold filtrations are calculated by multiplying each solubility by the solvent volume used in the experiment. The **theoretical mass recovery (g)** is the difference between the resulting S^H and S^c, accounting for solvent volume.

It is useful to know how much recrystallized sample could be recovered in a perfect experiment as a measure of experiment success, or at least set a more realistic expectation than 100%. This **theoretical % recovery** is determined by dividing the theoretical mass recovery by the initial mass of crude sample (m_{crude}), expressed in **eq 2**. Note that the percent *recovery* and theoretical *recovery* are different from the theoretical *yield*. Theoretical *yield* applies to a chemical reaction and *recovery* refers to a physical process.

$$\text{Theoretical \% Recovery} = \frac{S^H - S^c}{m_{crude}} \times 100\% \qquad \textbf{(2)}$$

The purity of commercially available (crude) and recrystallized acetanilide will be assessed by **MelTemp analysis**. Colligative properties predict that impurities lower melting temperature and increase melting ranges. The melting range is the temperature recorded when the solid begins to melt and again when all is converted to liquid. The recrystallized product should have fewer impurities and a more ordered structure based on intermolecular forces (IMFs) like hydrogen bonding (H-bonds) (**Figure 4**). The impurities interfere with H-bonds, creating weaker IMFs that take less energy (lower temperature) to break and cause the phase change. Purity may also be apparent in the appearance of the solid before and after the experiment.

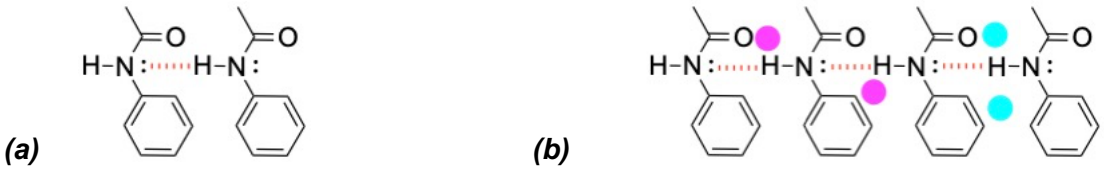

Figure 4. H-bonding patterns in **(a)** pure acetanilide and **(b)** acetanilide with impurities.

LAB PROCEDURE

Students work in pairs on the lab work and complete the Exp 1 notebook pages & report individually.
Pro-tip for notebook prep: circle / highlight each all equipment & chemical names with amounts.

Part 1. Dissolving the Sample. Place *approximately* 2 g of crude acetanilide in a labeled 125-mL Erlenmeyer flask. Record the actual mass obtained. Please ***DO NOT LEAVE ANY SOLID ON OR AROUND THE BALANCES.*** Add 35.0 mL water and two *black* boiling chips. Bring the mixture to a boil on a hot plate (hot plates should *never* go past a medium setting). Stir the system frequently with a glass rod. Allow the solution to boil for a few minutes then, if necessary, add more water drop-wise (up to 5 mL max) until all solid dissolves. Adding more water may decrease the percent recovery, however, solvent evaporates as the solution boils so more water may be necessary. *Record the total amount of water added.* Some material may melt (aka "oil out") and oily droplets appear on the top of the solution. Not to worry – proceed to the next step.

Addition of Activated Charcoal. Once all acetanilide has dissolved, remove the flask from the hot plate using **two hot mitts** and place on the counter to cool (do not add charcoal to a boiling solution or risk creating a volcano!). Slowly add a spatula-ful of activated charcoal to create a black, opaque suspension. It is normal for crystals to form at this stage. Place the flask back on the hot plate and boil the solution to re-dissolve solid. In the meantime, follow the instructions below to set up the ***hot filtration*** apparatus.

Part 2. Hot Filtration. Label two clean 125-mL Erlenmeyer flasks ("filtrate" and "water"). Place two boiling chips and 5 mL of water into each. Place a small (~1 inch) piece of copper wire, bent into a U, and over the lip of the filtrate flask, then add a short-stem funnel with a fluted piece of filter paper. This will provide space for steam to escape between the "filtrate" flask and funnel. Heat both flasks on medium while charcoal

suspension warms. Keep in mind that as steam escapes, the water is evaporating! Be sure that these flasks do not boil to dryness or the glass will crack. Once the steam has heated the funnel, immediately before filtering the acetanilide-charcoal suspension, pour some of the hot water from the second flask through the funnel to heat the filter paper.

Swirl the acetanilide-charcoal suspension, hold the bottom of the flask with **hot mitts,** and quickly transfer into the fluted filter paper *portion-wise.* Use a glass rod to direct the solution into the funnel and prevent dripping down the side of the flask. Fill the funnel no more than half full at any given time, being careful not to poke or tear the filter paper. Frequently place the flasks back on the hot plate to maintain the temperature of both solutions. Rinse the pre-mature crystals on the filter into the filter with small portions of boiling water. It may be necessary to restart the hot filtration if too much solid is on the filter. The bulk of the acetanilide should be dissolved in the filtrate.

Cooling Down. After completing the hot filtration, discard the filter paper in solid waste and allow the flask containing the filtrate to cool to *room temperature* then place it in an ice-water bath. If the system cools down too quickly, small crystals form and adsorb a large amount of impurities from the filtrate. Place 5 mL of distilled water in the ice bath to wash the crystals later during the cold filtration. Allow crystals to form for 10 minutes (note initial time of crystal formation as it may not happen immediately). Scratch the inside bottom of the flask with a glass stir rod to release seed crystals. Drawing a star and circle across the bottom of the flask tends to do the trick! Otherwise, do not disturb the flask once crystal formation has begun.

Part 3. Cold Filtration. After crystallization is complete, collect the crystals by vacuum filtration. Attach thick-walled vacuum tubing to a 125-mL filter flask then securely clamp the filter flask to a ring stand. Place a rubber “filter vac” seal on top to create a seal with a porcelain Buchner funnel. Obtain the correct size filter paper that covers all the holes of the filter but does not fold up the walls. Pre-weigh the filter paper, position it on the funnel, turn the vacuum on, and wet the filter paper with 5-10 mL of *cold* water. This will adhere the paper to the funnel and prevent it from moving during the cold filtration. Gently swirl the contents of the Erlenmeyer flask then pour the suspension into the funnel. Keep the liquid filtrate until the end of lab, then dispose in liquid waste.

Wash and Dry the Solid. Turn off the vacuum once the entire solution has been transferred and the liquid stops dripping from the funnel. Add 2-5 mL of ice-cold water to the funnel to wash the crystals. Turn the vacuum on and press the crystals with a spatula (tip should be slightly bent) to remove as much water as possible. Let the solid dry on the filter with the vacuum on for 20 minutes and proceed to ***melting point analysis*** while the solid continues to dry. If you hear a *hissing* sound, the vacuum seal is not tight. Re-adjust until the hissing stops. Keep the vacuum on to dry the remaining solid for an additional 30 minutes, until solid is dry. Transfer the solid and filter paper to a pre-weighed watch glass. Spread out the solid and carefully remove the boiling chips with tweezers. Weigh the dried solid and calculate the mass of pure acetanilide by difference. Calculate

percent recovery and *record a description of the product*. If the recovery is greater than 100%, the solid requires further drying in the Buchner funnel.

Part 4. MelTemp Analysis. Once the solid has been drying with the vacuum on for 20 minutes, take a small sample for MelTemp analysis (negligible effect on mass recovery). Spread the solid on a porous plate with a spatula for at least one minute to remove water. This is essential for accurate MelTemp determination, as water will significantly lower the melting temperature.

Obtain the melting ranges of very small samples of crude solid simultaneously with recrystallized solid. The settings on the MelTemp indicate the *rate of temperature increase.* Use a medium setting until the temperature is 20 degrees below the melting point of acetanilide, then lower the setting for a slower temperature increase. Closely observe and record the melting ranges of both samples: record the temperature when the sample begins to melt (looks like it is sweating) and again when the entire sample is in the liquid phase. Dispose of recrystallized product in the solid waste after analysis.

Clean-up and Waste	**Safety Hazards**
* *Solid waste*: Filter paper, acetanilide (crude and recrystallized), and used capillaries * *Liquid waste*: Filtrates aka mother liquor * Rinse the *charcoal-containing flask* with water into the liquid waste. Fill ~$^1/_3$ full with soapy water and warm (not boil) on hot plate before cleaning with a large brush.	* Be careful with hot glassware. Use both hands in hot mitts to handle hot glassware. Do not use clamps, paper towels, or bare hands. * Do not let acetanilide come in contact with eyes, mouth, or skin (irritant).
* Wipe down all bench tops with a sponge then dry with paper towel – no solid left behind.	
* Stack hotplates and ring stands neatly. Separate clamps from holders and return to proper drawer.	
* Remove gloves to wash glassware. Conserve soap and water when washing. Rinse cleaned glassware twice with tap water and once again with distilled water. Let it dry on a paper towel for a few minutes before drying further by hand and returning equipment to drawer.	

Pre-lab Questions & Quiz

The pre-lab quiz is due before lab – check Canvas for due date

- The quiz incorporates the questions below - the questions may be reworded.
- **Be prepared with your responses to the pre-lab questions *before* starting the quiz.**
- There is a 20-minute time limit on the quiz and you get two attempts.
  - Make sure you have enough time to complete the quiz - you can't save and come back later.
  - If you choose to re-take the quiz, your grade will be the highest of the two attempts.
  - Email your TA if you have technical issues and need an extra attempt at the quiz.

Though we encourage collaboration in this class, this is an individual quiz.

- The responses should be a product of your original work so that you are assessed on *your* understanding of the material.
- Sharing your quiz or your responses in any format (screenshots, email, CHEGG, social media, text, carrier pigeon, etc.) is in violation of the UCSC academic integrity policy.

Pre-Lab Questions

1. List the *basic steps* in the recrystallization of acetanilide in order. Include the **identity of the solvent** that will be used and what is added before hot filtration.

2. What **bonds** are present in acetanilide (ex. C-C)? Identify each as **polar or non-polar**. Use this information to **explain why water** is a good recrystallization solvent for acetanilide.

3. Why should a **minimum amount of *hot* solvent** be used for dissolving the crude solid? Why should the recrystallized solid be **washed with *cold* solvent**?

4. The solubility of acetanilide in hot water is 5.50 g/100 mL at 100 °C and its solubility in cold water is 0.53 g/100 mL at 0 °C. Use the following steps to calculate the theoretical percent recovery for a recrystallization that uses with 2.00 g of crude acetanilide (m_{crude}) and 35.0 mL of water, assuming no product loss or solvent evaporation.

   - How much acetanilide (g) should dissolve in the **hot water** before hot filtration? This result is S^H.

   - How much acetanilide (g) should remain in the **cold water** before cold filtration? This result is S^c.

   - What is the **maximum amount of acetanilide (g)** that could be recovered in a perfect (unrealistic) experiment - assume no solvent evaporation or product loss in transfers. This is ($S^H - S^c$).

   - Use equation **(2)** and the steps above to calculate the theoretical % recovery of acetanilide in a perfect (unrealistic) experiment.

$$\text{Theoretical \% Recovery} = \frac{S^H - S^c}{m_{crude}} \times 100\% \qquad \textbf{(2)}$$

5. What effect does an **impurity** have on the **melting point** of organic compounds? What effect does an impurity have on the **boiling point** of a solvent?

LAB REPORT – upload to GradeScope / Canvas
No abstract for the Exp 1 report.

In-lab Questions – numbered responses that incorporate the questions, no need to include the exact question

1. **Describe what happens to the molecules** when acetanilide dissolves in hot water. Are any **covalent bonds** broken or formed in the recrystallization?
    a. Include **diagrams** to depict the hydrogen-bonding (H-bond) patterns involved before and after Ac dissolves in water. These should be original, hand-drawn figures - do not copy / paste from online sources. *Hint: review lecture notes and the Exp 1 lab-site.*
        i. solute
        ii. solvent
        iii. solution

2. What is the **role of the activated charcoal** in this experiment? What is another **application** of activated charcoal in everyday life?

3. After hot filtration removes insoluble impurities, the remaining solution (filtrate) is cooled in an ice bath without being disturbed. Explain what happens to the acetanilide molecules during the **crystallization process** and what happens if **crystals form too quickly**. Use a few sentences and diagrams in your response.

4. Report the starting **mass of crude acetanilide (Ac)** and **total volume of water used**. Re-calculate the **theoretical recovery (g)** and **theoretical percent recovery** from *your* recrystallization experiment, taking into account the actual mass of crude acetanilide and the amount of water used in the experiment through the hot filtration. **Show your work** for the theoretical percent recovery calculation – steps outlined in pre-lab #2 (typed or by hand).

5. Report the **mass of recrystallized acetanilide (g)** obtained at the end of the lab. Calculate the **percent recovery** of the recrystallization of acetanilide from water. Show your work.

6. Report the **melting temperature ranges of the crude and recrystallized acetanilide**. Compare this to the **literature melting point** of acetanilide (is it higher or lower?). *Briefly* explain your results in terms of expected **colligative properties** (what's supposed to happen to the melting point when impurities are present vs. removed?).

Experiment 2: ISOLATION AND ANALYSIS OF CITRUS OILS
A GREEN-CHEMISTRY APPROACH

Learning Objectives

- Understand principles behind distillation and gas chromatography (GC)
- Critical analysis of distillation, liquid-liquid separation, and GC technique
- Analyze data to assess percent recovery & composition of essential oil
- Understand the role of intermolecular forces as they relate to volatility and boiling point

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How to Prepare for this Two-Part Lab + Assignments - Follow Canvas Exp 2 Module…

Before Lab

- Read this PDF – background, procedure, safety, pre-lab and in-lab questions
  - Option to listen to **Exp 2.1 & 2.2 Podcasts** = Caitlin reads this lab in 2 parts ☺
- Attend **lab lecture** and take notes on **templates**
- Practice the lab online *via* **Slugs@home** - sites.google.com/ucsc.edu/slugshome/home
- **Pre-lab questions** after each part (distillation & GC)
  - **Two pre-lab quizzes** on Canvas due the day before each lab

Lab Notebook Preparation – *Required before lab*

- Use the **worksheets** to prepare your **lab notebook**, one day at a time…
- **Purpose:** brief summary of the main lab goals and structures of citrus oil components
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info
- **Procedure with Diagrams** – hand-drawn using procedure in this PDF
  - Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!
  - **Slugs@home website** - Equipment & Safety pages; pictures & videos of the whole lab
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During Lab (Tu-Th)

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A
- Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

After Lab – each partner submits separate, individual assignments

- Upload **Notebook Pages** to Canvas by midnight on lab day – graded on completeness / participation
- Complete & upload the **Lab Report** on GradeScope (GS) – due date on Canvas
  - Guidelines at end of this document

BACKGROUND: CITRUS OIL DISTILLATION AND GAS CHROMATOGRAPHY (GC) ANALYSIS

Essential oils are mixtures of volatile compounds made by plants to communicate with their environments. They are used to attract insects and other animals to help in the fertilization and propagation processes. Some are also herbicides used by plants to defend their territory from aggressive vegetation. In this experiment students will isolate the essential oil of oranges. This same procedure could be used to isolate most other citrus oils and fragrant plants – lemon, grapefruit, lavender, and spearmint! Steam distillation will be used to obtain the oil from citrus peels and gas chromatography (GC) used for analysis.

Distillation of freshly grated citrus peels with water will produce a mixture that consists largely of water and small amounts of citrus oil. The oil is immiscible with and less dense than water. The distillate may look cloudy because of the emulsification of the oil in water, but if left to settle, it will separate into two layers: a large aqueous bottom layer and a small oily upper layer. The separation of the oily upper layer from the bottom aqueous layer can be difficult, especially if the volume of oil is small. To circumvent this problem, the oil is usually separated from the water by liquid-liquid extraction with an organic solvent (most commonly, ether). This process is very effective in removing the oil from the water but requires the use of organic solvents, which are usually toxic, expensive, and contribute significantly to the waste stream. This is of great cost to the planet.

Green chemistry is the quest for finding safer and more environmentally friendly chemicals to carry out common laboratory operations. One of the principles of green chemistry is to use safer solvents or eliminate solvents altogether if possible (reduce, reuse, recycle!). It is common industry practice to trap, purify, and reuse solvents and reagents as much as possible. Unfortunately, waste is an inevitable part of research even with extensive recycling efforts. Steam distillation is a green chemistry method for collection of citrus oil without organic solvents. Enough oil is produced that it can easily be separated from water using a 50-mL buret (the same piece of glassware used in titrations). All waste from this procedure (citrus water) is safe to put back into the environment!

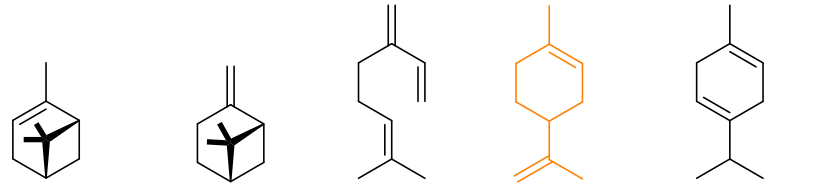


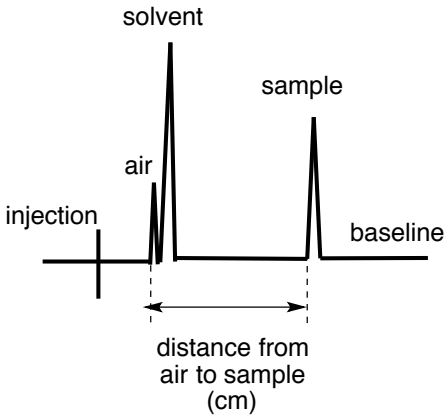
Figure 1. Monoterpenes found in citrus oils and their normal boiling points

Citrus oils consist largely of volatile hydrocarbons and small quantities of aldehydes, alcohols, and other oxygenated compounds. Any *essential oil* has a particular combination of chemicals that makes its smell unique and often times easily recognizable. Spearmint, orange, and lemon oils have very similar components but very distinct fragrances! It is common for natural plant extracts to contain compounds having carbon atoms in multiples of five. This broad class of compounds is called **terpenes**. They are biosynthetically derived from two or more *isoprene* units. There are several different ways for plant metabolism to combine two five-carbon isoprene units into a ten-carbon *monoterpene*, hence the occurrence of many different isomers. The most prominent components in citrus oils are α-pinene, β-pinene, myrcene, limonene, and γ-terpinene (**Figure 1**).

The isolated oil will be analyzed by gas chromatography (GC) to determine its composition. The components of the oils usually travel through the heated GC column in the order of their boiling points. More volatile, lower boiling compounds travel fastest and elute first (earliest to exit the column and create a peak on the GC chart). Less volatile compounds travel slower and elute last. There are exceptions to this relationship so authentic samples of each compound are injected individually. The authentic samples are called **standards**.

Retention times are calculated and used to compare the standards' relative elution (exit) order from the GC column. The standard retention times are compared to those of the peaks in the citrus oil. The areas under the peaks will be calculated to determine the percent composition of the oil.

At a given set of GC conditions (temperature and type of column) *on the same instrument,* one compound will consistently exit the column in the same amount of time after injection. This is known as the **retention time, t_R** (the amount of time the compound is retained on the column). For example, students inject pure limonene and observe one major peak. There is no timer on the recorder, but it moves the paper at a certain speed (2.5 cm/min). The distance (cm) from the start of the injection to the beginning of the peak can be converted to its retention time in minutes (**eq 1**) then converted into seconds. For better precision, students should calculate **corrected retention times (t_R')**, where the distance is measured using the air peak as the beginning of the run. You can expect there to be several peaks in the chromatogram of citrus oil, which correspond to the various components isolated in the distillation step. If the citrus oil contains any limonene, then expect to see a peak at the exact time as the standard. Once the peaks are identified, calculate the percent composition of each component in the citrus oil using the area under the curves (integration) of each of the peaks (**eqs 2 & 3**).



$$t_R' \text{ (sec)} = \text{distance from air to sample (cm)} \times \frac{\text{chart speed } \mathbf{1 \text{ min}}}{\mathbf{2.5 \text{ cm}}} \times \frac{\mathbf{60 \text{ sec}}}{\mathbf{1 \text{ min}}} \quad \textbf{(1)}$$

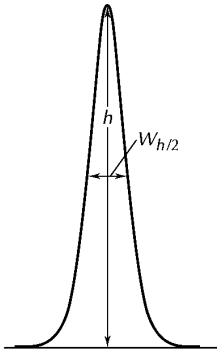


FIGURE 20.13
Determining peak area: h = height; $W_{h/2}$ = width at half-height.

Integration: Peak Area = h x $w_{h/2}$ **(2)**

$$\% \text{ Composition of A} = \frac{\text{Area of component A}}{\text{Total area of all components}} \quad \textbf{(3)}$$

PROCEDURE, PART 1 - ISOLATION OF CITRUS OILS

1. Prepare peels before lab: Select whole fruit with strong citrus fragrance - 5 oranges, 6 grapefruit, 8 lemons, or 8 limes per group. No smell means low terpene content and little-to-no recovery. Fruit fresh from the tree gives best results! Clementines or cuties with thin peels tend to give very low recovery. It is ideal for the colorful part of the peel to be grated as small as possible the morning of lab, leaving behind the pith (white part). If a grater is not available, carefully cut the colorful peel away from the pith, then finely chop to rice grain size. Prepare the peels the night before and keep in the fridge if necessary, but the fresher the better! Transport peels to lab in a leak-proof plastic bag (no Tupperware). *Cutting tools will not be provided and you cannot bring grating or chopping utensils into lab*.

2. Distillation Assembly: Tare a large beaker and transfer the prepared citrus peels into it. Record the mass of peels. The mass should be no less than 100 g and no more than 150 g. Transfer the peels into a 500-mL round bottom flask (RBF) set on a cork ring using a wide-mouth plastic funnel and stir rod to poke the peels through. **Add 150-175 mL of distilled water** (depending on your mass of peels) to the beaker, swirl to pick up any bits of peel, and gradually transfer into the 500-mL RBF. Assemble a simple distillation apparatus, beginning with the 500-mL RBF (**Figure 2**). If you forget to add the distilled water before heating, it will scorch the flask. *Pay attention to the in-lab demo. Start the set up with the peels and water already in the flask.*

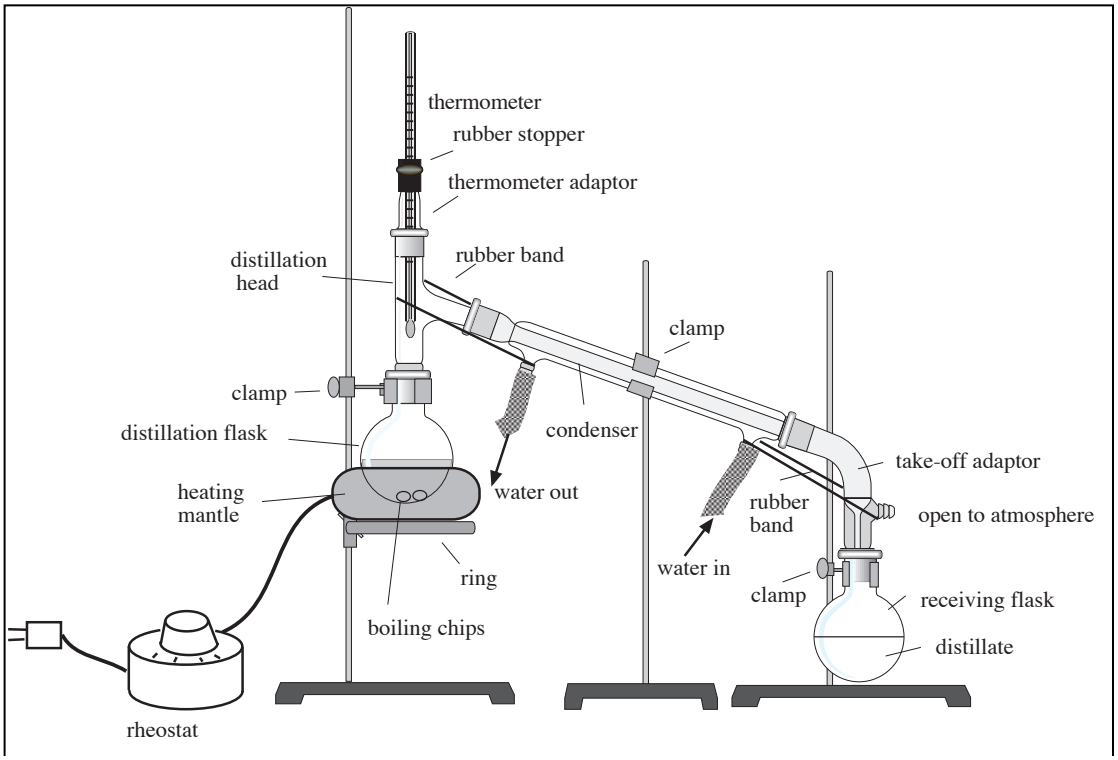


Figure 2. A simple distillation apparatus used for steam distillation. *Sketch this labeled diagram into the procedure portion of your notebook (omit boiling chips).*

In addition to the supplies in your drawer, obtain **three ring stands, 3 clamps, 1 support ring,** and **4 clamp holders**. Center the ring stands in front of the water hoses at your bench. Secure the 3 clamps and support ring onto separate clamp holders. Clamp the support ring approximately halfway up the ring stand. This must be secure enough to hold the weight of the **heating mantle** and boiling orange peel suspension. Attach one of the clamps to the same ring stand above the heating handle, leaving plenty of room for the **500-mL RBF**. Hold the RBF *containing orange peels and water* with one hand and pinch the clamp around the neck of the RBF with the other hand. Keeping the pressure on the clamp, let go of the RBF and securely

tighten the wing nut of the clamp. The flask should be steady and there should be no space in between the clamp, clamp holder, and ring stand. Carefully raise the heating mantle by loosening the clamp holder from the ring stand. The flask should sit in the heating mantle with a **small air gap** in between. Tighten the clamp holder to the ring stand. It may be necessary to adjust the position of the clamp horizontally (closer or farther from the ring stand). **Have your TA check your setup**, then turn the heating mantle to LOW while finishing the apparatus.

Add a small dab of **grease** to the joints of the **distillation head**. Attach the distillation head to the clamp and turn to spread the grease. Separately, insert a **thermometer** into a **rubber thermometer adaptor** (not a stopper) using water as a lubricant (not grease), then secure the rubber adaptor around the **glass thermometer adaptor**. Grease and connect the adapter joint to the distillation head and spin to spread the grease. Loosely drape two **rubber bands** around the **condenser** *in between the hose adaptors*. Connect the '**water in**' hose (labeled blue) to the bottom **water inlet** using pressure and a circular motion, then secure with a small wire clamp. The hose must be at least over two of the bumps on the hose adapter. Repeat with the '**water out**' hose (labeled red) and the top **water outlet**. The rubber bands should still be in between the hoses on the condenser.

Wrap the first rubber band around the **distillation head**, attach the condenser to the distillation head, and turn to spread the grease. Position a clamp *loosely* around the condenser with the second ring stand. This clamp is a secondary support and the condenser should rest on the clamp, rather than the clamp being secured. Wrap the second rubber band around the **take-off** adaptor and connect this to the condenser with grease. The rubber bands should be securing the set-up as shown below. Loosen the water clamp to begin water flow.

Pour 70 mL of water into a **250-mL RBF** and mark the level of the liquid with a marker. Pour the water out and use this as the **receiving flask** for the distillate. Attach to the take-off adaptor then clamp the flask once in position. Your TA must OK your apparatus before increasing the heat. Heat the distillation flask using a heating mantle, starting at a medium setting. Adjust the heat supply so that the liquid distills at an approximate rate of one drop per second. Distilling to quickly with too high heat will severely decrease recovery of oil. Be patient - it may take up to 20 minutes for the first drop. Collect approximately 70 mL of distillate. Read and record the temperature throughout the distillation (first drop and the final temperature).

During the distillation, review notes on Gas Chromatography (GC) and work on the sample GC calculations.

Stop the distillation by turning off and unplugging the heating mantle then carefully lowering the ring support for the heating mantle. You will need a spotter to help with this. You may take off the receiving flask, but wait for the entire system to cool before disassembling. Place a beaker under the take-off adapter to collect any further distillate.

3. Separation and Collection of Citrus Oil: In the meantime, fasten a 50-mL buret to a ring stand using a buret clamp. Use a funnel to add distilled water to leave a few milliliters of space above the liquid in the buret. *Slowly* add a few milliliters of the orange distillate to the buret, being careful not to overflow. Allow the liquid to settle, then drain to the 45 mL mark, catching the liquid in a 125-mL Erlenmeyer flask. Repeat the add – settle – drain process until all of the distillate has been transferred. Add 10 mL of water to the flask, swirl to dislodge any drop adhered to the wall and transfer the liquid portion-wise to the buret. Repeat this operation with another 10-mL portion of water. This process should limit the formation of emulsions, allowing the orange oil to neatly rest on top of the water.

Let the system settle for at least 5 minutes, or until phase separation of oil on top of water is apparent. There may be some air bubbles or oil droplets stuck along the buret walls, however, we have found that attempting to collect this oil has an overall negative effect on recovery. The level of the liquid should be near the 50-mL marking so that you can collect the oil from the top layer by pipet. Label a small screw-cap vial with your name and the name of the oil. Weigh it and keep it handy.

Use a glass pipet and pipet bulb to carefully collect the layer of oil from the top of the buret, avoiding the water as much as possible. You will likely not be able to collect all of the oil so just do your best! Students can expect to recover anywhere from 0.5 to 5 mL of citrus oil. Transfer the oil to the pre-weighed vial. Carefully remove any visible droplets of water in the bottom of the vial using a pipet. Weigh the vial with the oil and determine the mass by difference. Cap the vial and store in your drawer for GC analysis (Day 2).

Table 1. Clean-up and Safety – Part 1, Distillation

Clean-up	**Safety**
Allow the distillation apparatus to cool completely before disassembling. All ring stands, clamps, etc. should be put back in an organized manner. **Taking the hoses off of the condenser:** disconnect the condenser on both ends, clamp the hoses to stop water flow, then carefully remove the hoses OVER THE WATER TROUGH with a back-and-forth motion	
Strain the distilled orange peels through a strainer into the sink, using water to aid the transfer with shaking. When the strainer becomes full, transfer to the specified compost container.	Use caution with the heating mantle and distillation apparatus. Slowly increase heat, which should never go past a medium setting.
Dispose of the aqueous layer from the buret down the drain (orange water is safe for fishies!). Dispose of pipets in the glass waste box.	Myrcene, γ-terpinene, α-pinene, β-pinene are irritants.
Keep the citrus oil in a capped, labeled vial in your drawer for Day 2.	Limonene is a possible carcinogen.
Dispose of pipets in the glass waste box.	

Pre-Lab Questions, Day 1

The following questions are incorporated into the Canvas Exp 2.1 pre-lab quiz. Prepare your responses before starting this individual quiz. There is a 20-minute time limit with two attempts.

1. Define the term **terpene**. What is the approximate percent composition of each of the terpenes in **Figure 1** in orange oil (or whichever citrus you will be using)? A quick Wikipedia search is fine this time! There is no need to report components if not present in citrus oil.

2. What is “green chemistry”? Describe how the distillation procedure used in this lab is a more “green” approach than liquid-liquid extraction with organic solvents.

3. Why should the distillation apparatus have an opening to the atmosphere at the end?

4. Why are terpenes distilled along with water even though their boiling points are significantly higher than water?

PROCEDURE, PART 2 – GC ANALYSIS OF CITRUS OIL

*Write the **abstract** in your GC-wait-time using the writing guidelines on Canvas. Bring a **calculator** to lab.*

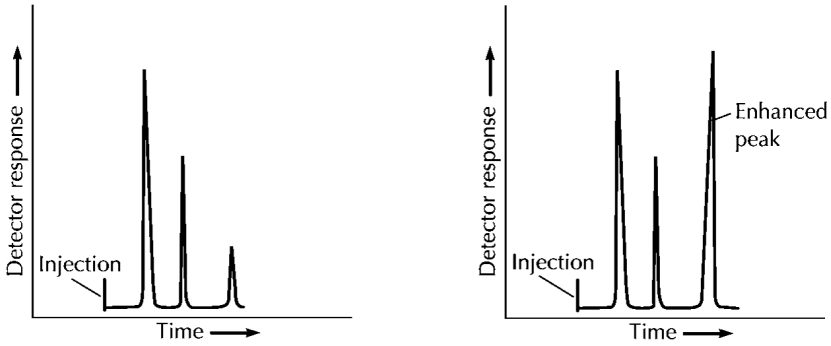
4. Sample Preparation: There will be demonstrations on proper sample preparation and the use of the gas chromatograph at the beginning of lab. Standards are injected first then your oil. Record the chart speed and all other conditions posted on the bulletin board above the instrument (oven temperature, etc.). Use care when handling the sharp, delicate needles. Rinse the syringe three times with the sample before each run to treat the syringe and remove air bubbles. Load 0.2 μL of sample into the syringe and pull back to the 10 μL mark with air. Write the sample name on the chart paper then turn on the chart recorder. Turn the nob on the recorder to mark the beginning of the run and quickly inject the sample with air into the HOT injection port. The air or acetone peak will be used as the starting point for the **corrected retention time**. Rinse the syringe three times with acetone and clean the outside of the needle with a Kim wipe to remove traces of liquid after each injection to avoid cross-contamination.

5. Injection of Standards, Citrus Oil, & Data Collection: Inject pure samples of **limonene, α-pinene, β-pinene, and γ-terpinene**, rotating with other groups for fair sharing of the instruments as instructed by the TA. Allow all the components to come out before performing the next injection. Myrcene is not injected due to unwanted interaction with the column. **Measure the corrected retention times of each standard (no integration) BEFORE PERFORMING THE INJECTION OF YOUR CITRUS OIL**. Check with your TA then inject your oil and identify the peaks by comparing corrected retention times to the standards. Determine which compounds are the main components of your oil. Spike your sample with a standard to confirm presence of minor components (procedure below). Calculate the percent composition of the oil. *This analysis must be done during this lab period in case further injections are necessary.*

Although not ideal, standard peaks can still be interpreted if the peaks level off at the top because only the retention time is needed. However, if the peaks from the citrus oil are too small to interpret or if sample peaks (not air or solvent peaks) level out at the top, repeat the injection. The intrinsic error is very high because the sample volume is so small. Often, **simply repeating an injection at the same volume will provide data that is easier to interpret. Do not increase sample volume beyond 0.2 μL.** Measure the retention times of the peaks and calculate the area under the curve (integration).

6. Sample Spiking with Data Collection: There is also intrinsic error associated with the measurement of retention times because the boiling points of certain citrus components are similar. Confirm the identity / absence of either of the pinenes and/or terpinene by injecting your oil plus **spiked** with the standards. Suppose you obtained the chromatograms of the oil and the standards and it is unclear whether a certain peak is α- or β-pinene (both have similar retention times)…

Spiking procedure: Rinse the syringe with acetone as usual then rinse with one of the standards, for example, α-pinene. Some will remain adhered to the inside walls of the needle and syringe. Wipe the needle with a Kim wipe to remove any α-pinene on the outside. Draw 0.2 uL of citrus oil into the syringe, being careful not to contaminate your sample with α-pinene. Draw air into the syringe. This oil sample is now contaminated with α-pinene. Inject your contaminated oil. If your oil contains α-pinene you *will observe an enhanced peak for this compound*, but if your oil contains β-pinene instead, you will see a peak for β-pinene plus another for α-pinene (the contaminant spike). If needed, repeat the same type of analysis, spiking your oil with β-pinene. The procedure could be applied to any standard to positively identify any or all of the peaks in the chromatogram of the oil, time permitting.



(a) Original chromatogram

(b) Chromatogram after addition of a known compound identical to a compound in the sample

Figure 4. Sample chromatograms for spiking the oil with a standard

Table 2. Clean up and safety, Part 2 - GC

Clean Up	Safety
Rinse syringes with acetone three times after each injection and clean needle with Kimwipe. Dispose of the oil in the same labeled vial in the container provided after all GC analysis is complete.	GC Needles are sharp, delicate, and expensive – handle with care.
Keep the instrument room clean and free of personal belongings. No more than 6 students should be in the instrument room at any given time. GC kits should be kept clean and organized. Cap the markers after completing all GC runs.	

Experiment adapted from Palleros, D. R. "Recrystallization of Acetanilide," *Experimental Organic Chemistry*, Wiley: New York, **2000**.

Pre-Lab Questions, Day 2

The following questions are incorporated into the Canvas Exp 2, Part 2 pre-lab quiz. Prepare your responses before starting this individual quiz. There is a 20-minute time limit with two attempts.

1. Define the following concepts and terms as they relate to gas chromatography.

a) Mobile Phase, AKA Carrier Gas
b) Stationary Phase
c) Integration
d) Retention Time
e) Dead Time
f) Corrected Retention Time

2. Report the boiling points of the GC standards and predict the order in which these compounds should exit the column.

LAB REPORT – due date on Canvas

Type, leave space to insert hand-written calculations, upload to GradeScope, "select pages"

Abstract – See **Writing Guidelines** on Canvas for HOW TO write a brief paragraph on the citrus lab…

- Purpose – what is accomplished in this lab?
- Methods – names of chemicals & techniques, NOT the procedure!
- main results – citrus oil distillation yield and GC percent composition
- and conclusions based on expected results.

In-lab Questions, Part 1

1. Report the **temperature range of distillation** during actual collection of distillate. Is the distillation temperature higher or lower than pure water? Explain this temperature difference (if there was one) on a molecular level.

2. Calculate the **% recovery** of citrus oil from the peels (chopped and grated). Show your work and state the recovery in a complete sentence.

% Recovery = (mass of oil) / (mass of peels) x 100%

3. Report the **observations** of the peels (size, color, etc.) and compare your recovery to that of the other distillation performed in the remote lab (bigger vs. smaller peels). Explain the observed **relationship** between peel size and % recovery on a molecular level (release of terpene molecules from peels).

In-lab Questions, Part 2

4. Show one **sample calculation** for corrected retention time (t_R') and report the final results in a typed table (see worksheet). Did these compounds elute in the **order predicted**?

5. For each peak in the GC chromatogram of the oil, calculate **corrected retention time, integration, and % composition**. Report your results in table format (see worksheet).

- **Number each peak** and **identify** each as one of the injected standards, where possible.
  - Corrected times may not match exactly but the order should be the same.
  - Recall that myrcene was not injected as a standard and there may be other unidentifiable components.
- Show one sample calculation each for integration and percent composition.

6. Which standards were used to **spike** the oil? Briefly discuss the result of the spiked chromatograms and what was learned about your oil, if anything. If results were inconclusive, please interpret the GC spiking sample data on Slugs@home.

7. What is the **major component** of your citrus oil? What are the **minor components**? How does this compare with the expected composition?

Name _____ Partner Name _____

TA Name _____ Section Day _____ Time _____

Experiment 2.1 Worksheet

Use as reference for notebook preparation – every student submits on Canvas individually after lab

Pre-Lab Requirements

1. **Dress for lab** – see safety rules – arrive a few minutes early
2. **Lab Notebook:** copy templates below into designated notebook
   - **Purpose, scheme,** and **reagent table**
   - **Procedure Diagrams** – must be complete before you can start the lab

A. Purpose, sketch of citrus, and structures of terpenes:

B. Reagent Table

Sample Name	**Amount** Fill in during lab	**Molecular Mass**	**mmoles** Fill in during lab	**Boiling or melting point**	**Density**	**Hazards**
Citrus Peels		n/a	n/a	n/a	n/a	Enter terpene hazards:
Water						

C. Procedure – diagrams of key procedural segments on as many pages as needed.

- Include all **labeled equipment, chemical names** with **amounts**, and pertinent **safety notes**.
- Leave space to record additional notes and observations within the procedure diagrams

Step 1. Preparation of peels from home

Step 2. Distillation Apparatus – copy **Figure 2** from Exp 2 PDF

- Diagram of complete distillation setup with labeled components and contents in flask
- Not necessary to show order of assembly 😊

Step 3. Separation and Collection of Citrus Oil

- Buret setup – addition of water and distillate

E. Data

Description of your peels & those of a neighboring pair (size, color, fragrance, texture, etc.):

Mass of citrus peels _____ g

Volume of water _____ mL

Distillation temperature: first drop _____ C final temperature _____ C

Mass of citrus oil _____ g Percent Recovery _____%

Calculation: % Recovery = (mass citrus oil) / (mass of peels) x 100%

F. Practice Calculations

GC chromatograms are on Canvas & in the lab for practice measuring retention times and integration.

- Show one sample calculation each for retention time, integration, and percent composition.
- Report your findings in the tables and show sample calculation below.

Table 1. Standard GC Retention times

Sample	**Corrected t_R (s)**
α-pinene standard	
β-pinene std.	
Limonene std.	
γ-terpinene std.	
Carvone std.*	
Citrals std.*	

* Carvone and citrals standards will not be injected in Exp 2.

Table 3. GC Analysis of Unknown Oil #4 – *do this simpler one first*

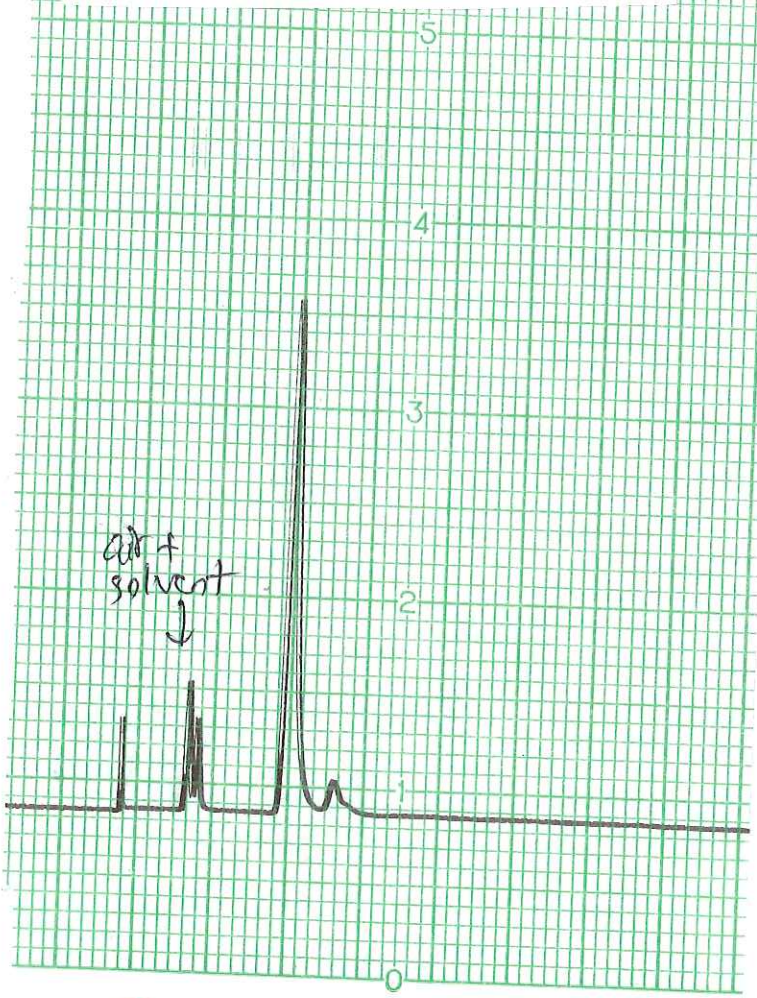
Peak #	**Peak ID****	**Corrected t_R (s)**	**Integration (cm^2)**	**% Composition**
1				
2				

Table 2. GC Analysis of Citrus Oil (Unknown Oil #1)

** Use corrected retention times to assign each peak to one of the standards.
Note that not all standards may be present, some peaks overlap, and other unknown peaks may appear.

Peak #	**Peak ID****	**Corrected t_R (s)**	**Integration (cm^2)**	**% Composition**
1				
2				
3				
4				
5				
6				

α-Pinene



α

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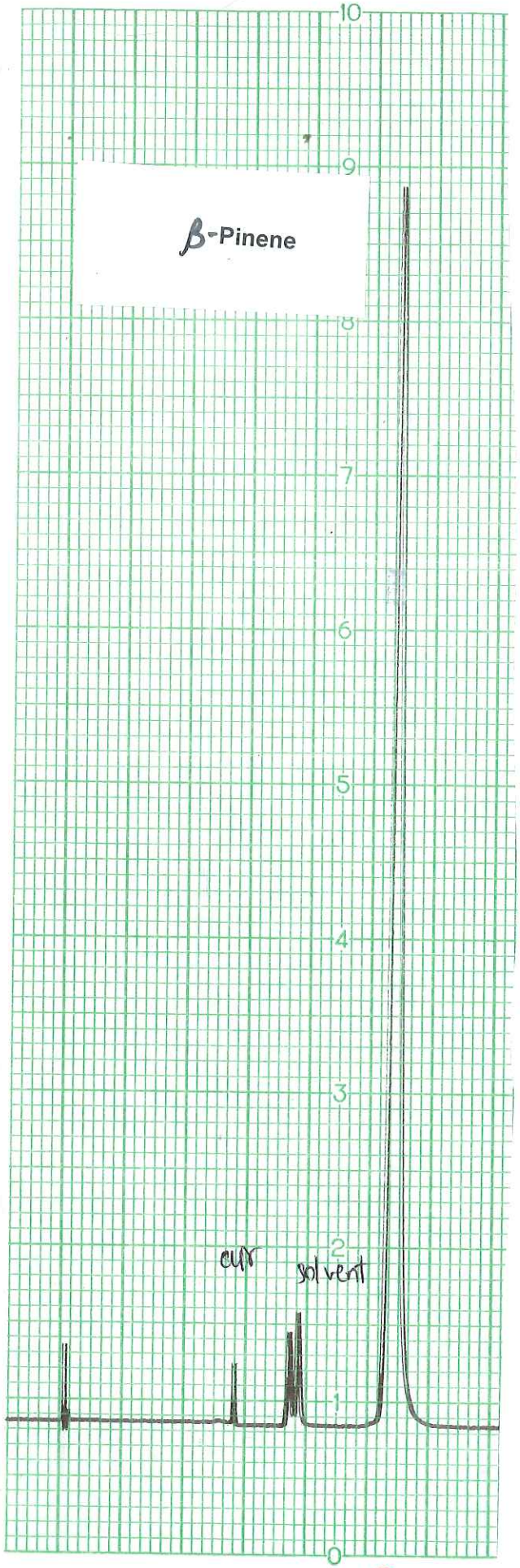
β-Pinene



β

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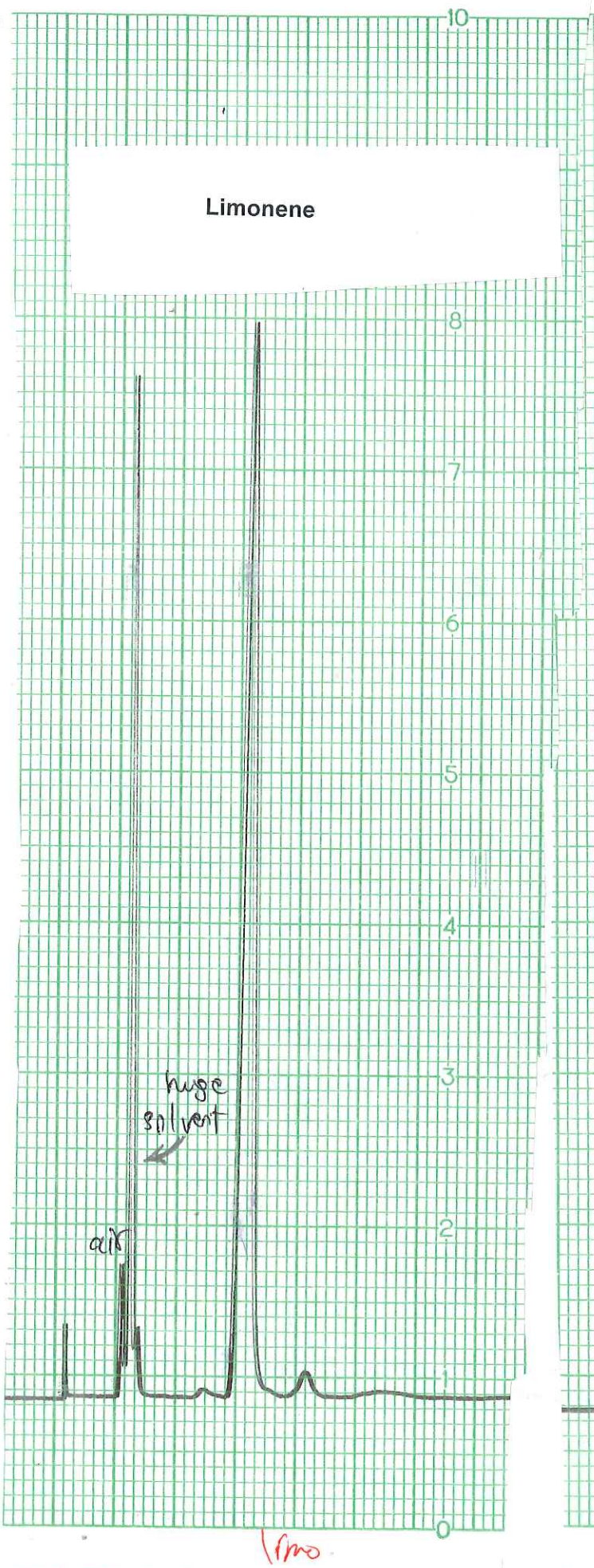
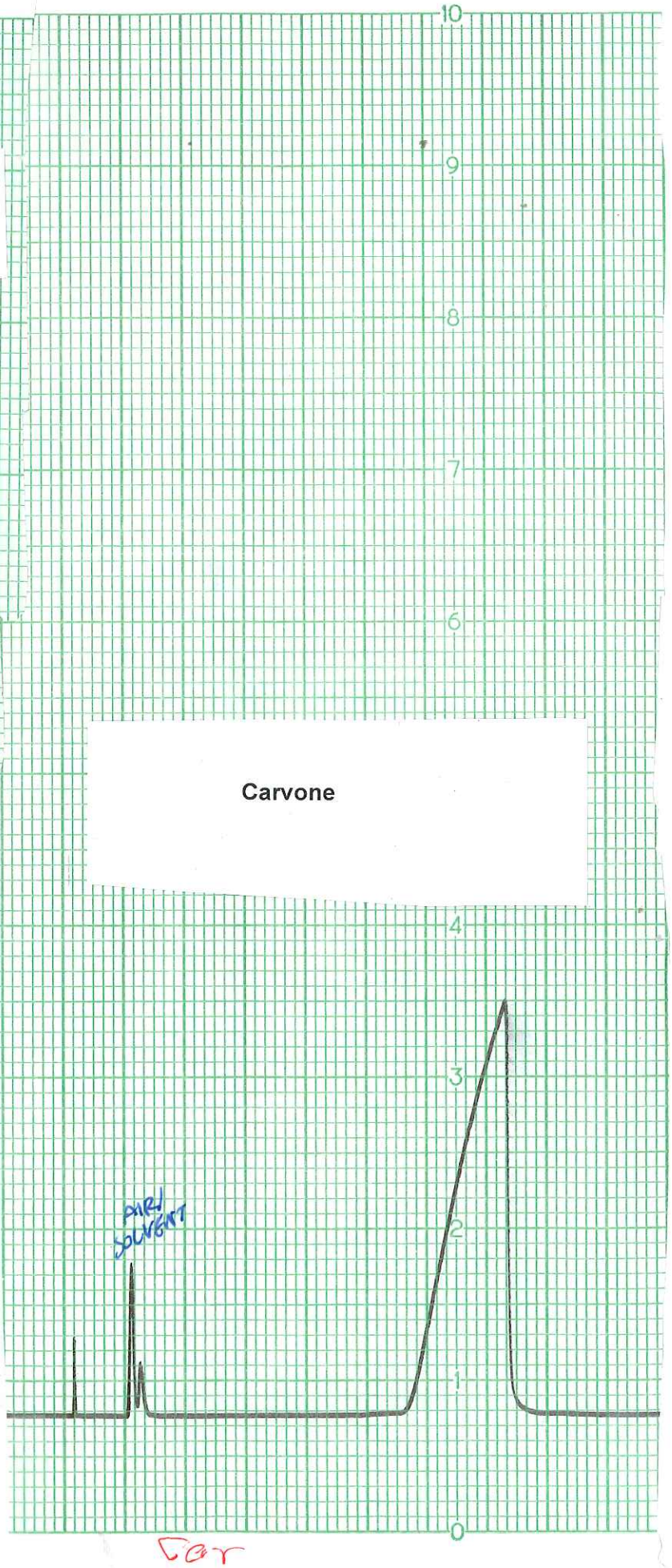
Limonene

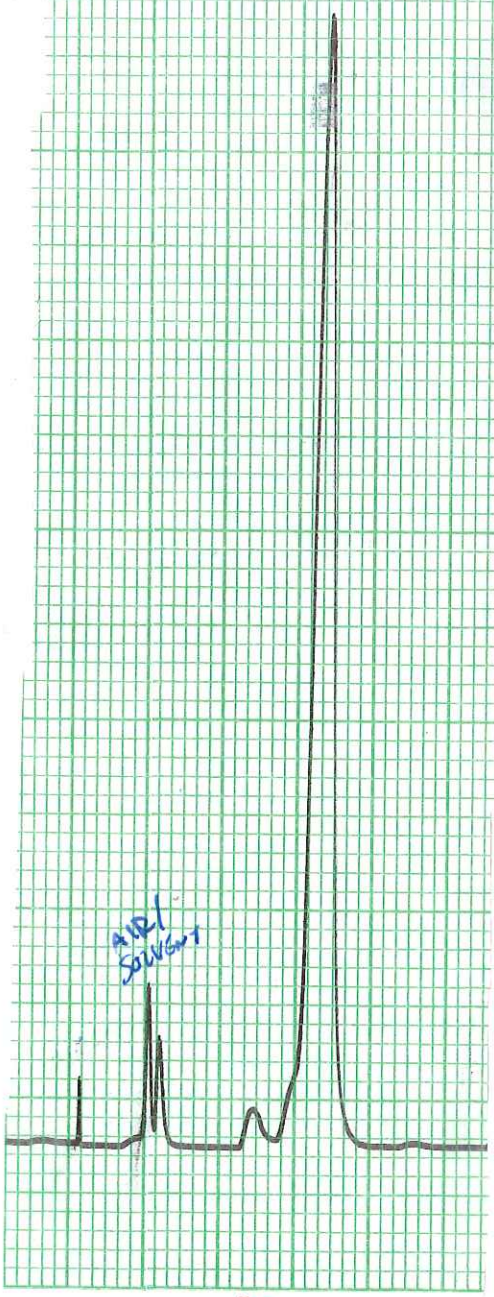


Carvone



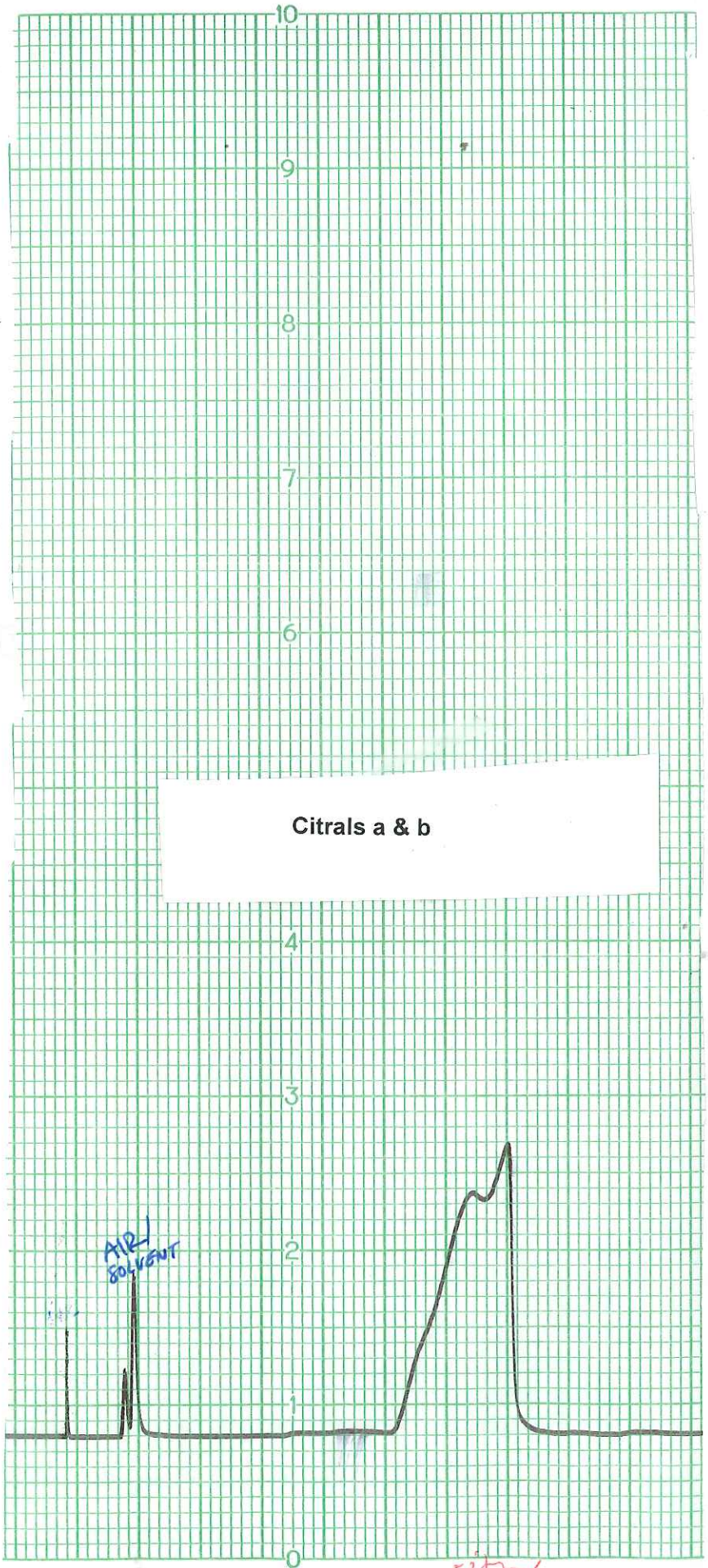
Scientific Co. Pittsburgh, PA 15219 Catalog No. 13-939-40

γ-Terpinene

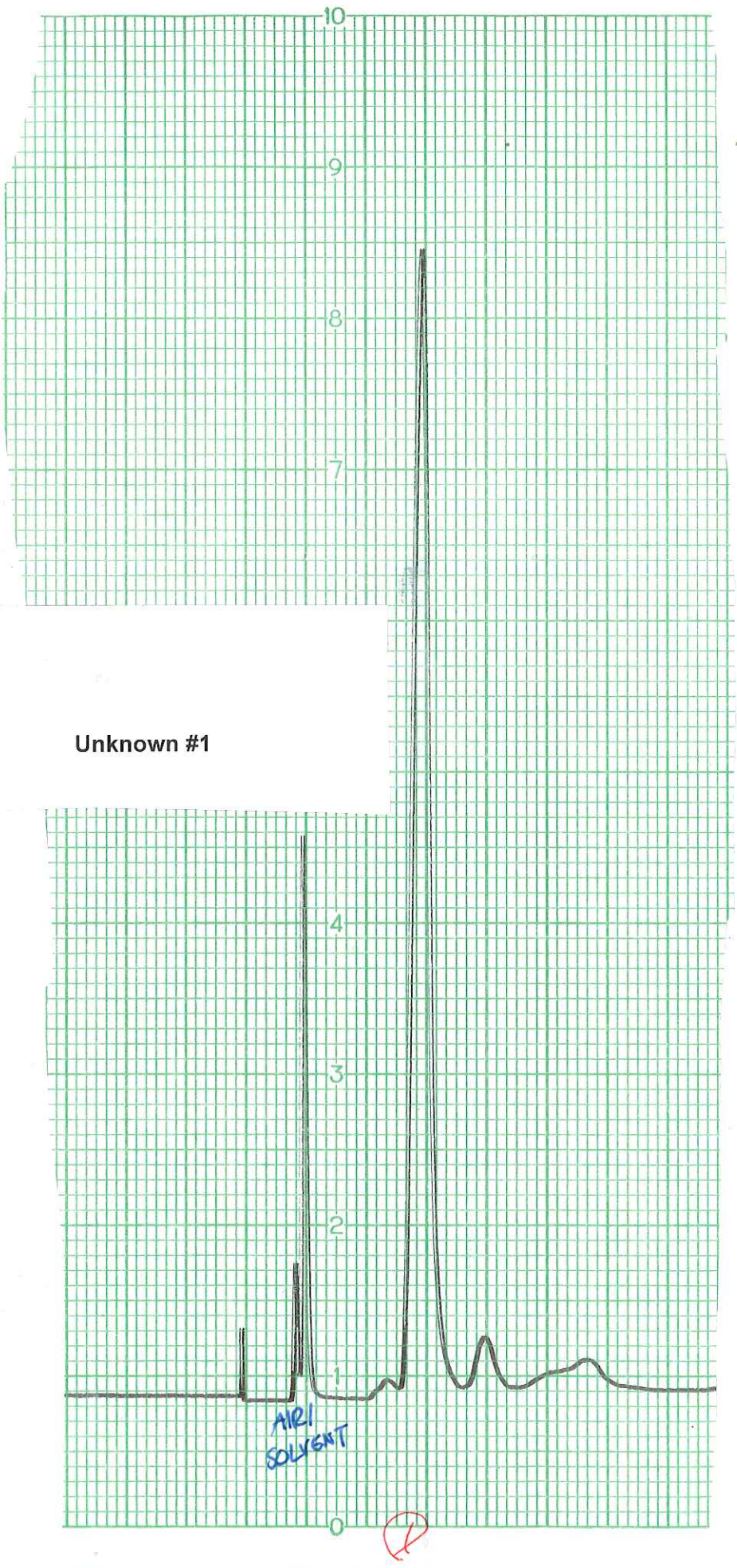


γ

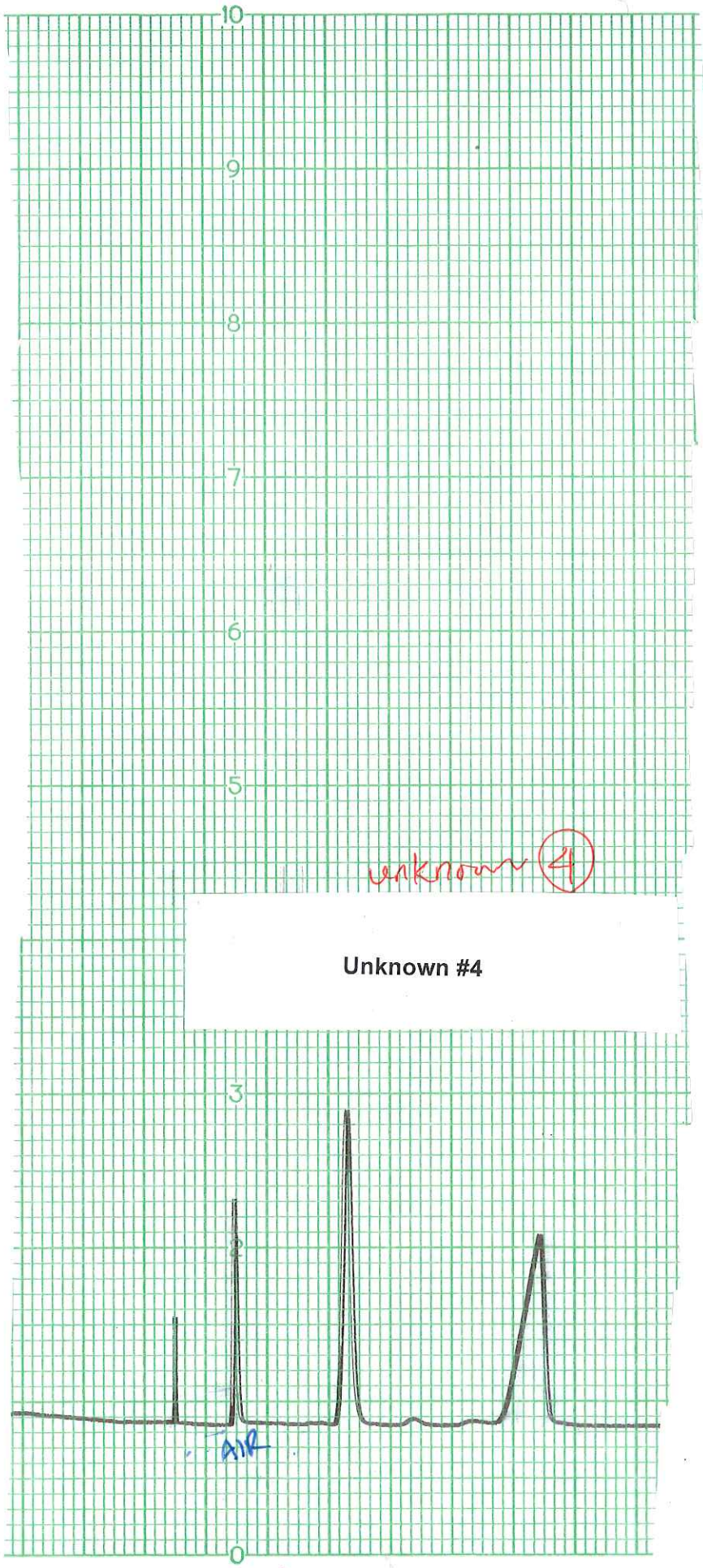
Citrals a & b



citral



Unknown #1



Name _____ Partner Name _____

TA Name _____ Section Day _____ Time _____

Experiment 2.2 Worksheet – Gas Chromatography (GC) Analysis of Citrus Oils

Use as reference for notebook preparation – every student submits on Canvas individually after lab

Pre-Lab Requirements

1. **Dress for lab** – see safety rules – arrive a few minutes early
2. **Lab Notebook:** copy templates below into designated notebook
   - **Purpose, scheme,** and **reagent table**
   - **Procedure Diagrams** – must be complete before you can start the lab

A. Purpose and structures of terpenes:

B. Reagent Table

Sample Name	Molecular Mass	Boiling point	Density	Hazards
alpha-pinene				
beta-pinene				
limonene				
gamma-terpinene				

C. Procedure – hand-drawn using procedure in lab PDF, class notes, & Slugs@home

- Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
- Leave space to record additional notes and observations within the procedure diagrams

Step 4. Sample Preparation

- Representative diagram for any 1 sample: steps for drawing liquid & air into syringe

Step 5. Injection of Standards, Citrus Oil, & Data Collection

- Identity and volume of each standard
- Transfer from needle to GC (one representative sample)
- GC diagram: injection port, oven, chart recorder & rough sketch of chromatograms

Step 6. Sample Spiking with Data Collection

- Steps for sample spiking, including how it differs from regular sample preparation
- Identity of components in syringe for both sample spikes

E. Data

Standard (pure) GC Retention times

Sample	Corrected t_R' (s)
α-Pinene standard	
β-Pinene std.	
Limonene std.	
γ-Terpinene std.	

GC Analysis of Citrus Oil
(add as many rows as needed)

Peak #	Peak ID	Corrected t_R' (s)	Integration (cm^2)	% Composition

Analysis of "spiked" chromatograms – pretreat syringe with any standard except limonene, then inject oil

Spiked with _____

Peak #	Corrected t_R' (s)	Peak ID

Spiked with _____

Peak #	Corrected t_R' (s)	Peak ID

What do each of these spiked chromatograms tell you about the composition of your oil?

F. Abstract Draft / Content

Use the writing worksheet on Canvas for step-by-step instructions!

Experiment 3 – TLC Analysis of Spinach Pigments

Learning Objectives

- Understand principles behind solid-liquid extraction, liquid-liquid extraction, and thin-layer chromatography (TLC)
- Critical analysis of extraction & TLC technique
- Analyze data to assess components of extract and success of experiment
- Understand the role polarity plays in extraction and TLC

How to Prepare for this Lab + Assignments - Follow Canvas Exp 3 Module…

Before Lab

- Read this PDF – background, procedure, safety, pre-lab and in-lab questions
  - Option to listen to **Podcast** = Caitlin reads the lab ☺
- Attend **lab lecture** and take notes on **templates**
- Practice the lab online *via* **Slugs@home** - sites.google.com/ucsc.edu/slugshome/home
- **Pre-lab questions** incorporated into **Pre-lab Quiz** – check Canvas for due date

Lab Notebook Preparation – *Required before lab*

- Use the **worksheet** to prepare your **lab notebook**, one day at a time…
- **Purpose:** brief summary of the main lab goals and structures of citrus oil components
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info
- **Procedure with Diagrams** – hand-drawn using procedure in this PDF, Slugs@home, & class notes
  - Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!

During Lab

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A
- Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

After Lab – each partner submits separate, individual assignments

- Upload **Notebook Pages** to Canvas by midnight on lab day – graded on completeness / participation
- Complete & upload the **Lab Report** on GradeScope (GS) – due date on Canvas
  - Guidelines at end of this document

BACKGROUND PART 1: Spinach Pigment Extraction

The most prominent types of spinach pigments are **chlorophylls, carotenoids, flavanoids,** and **tannins.** Chlorophylls contain a ring system formed by four pyrroles linked by four methine bridges, a Mg^{2+} ion in its center, and a long nonpolar hydrocarbon chain ($C_{20}H_{39}$). Chlorophylls are tetrapyrrole cousins of other biologically important molecules such as vitamin B_{12} and the heme found in hemoglobin. There are two main types of chlorophylls present in higher plants, **a** and **b** (**Figure 1**). Chlorophyll **a** is more abundant than chlorophyll **b** by a ratio of 3 to 1. The only structural difference between them is that a methyl group in **a** has been replaced by a formyl aldehyde group, CHO, in **b**.

Chlorophylls are found in the chloroplast in association with small proteins. These protein-chlorophyll complexes are crucial in photosynthesis. The chlorophyll molecule acts as an antenna for visible radiation. The light absorbed is used to make carbohydrates and oxygen using carbon dioxide and water as raw materials. Think about that for a minute: *every carbon in sugar comes from CO_2 in the air (woah)!*

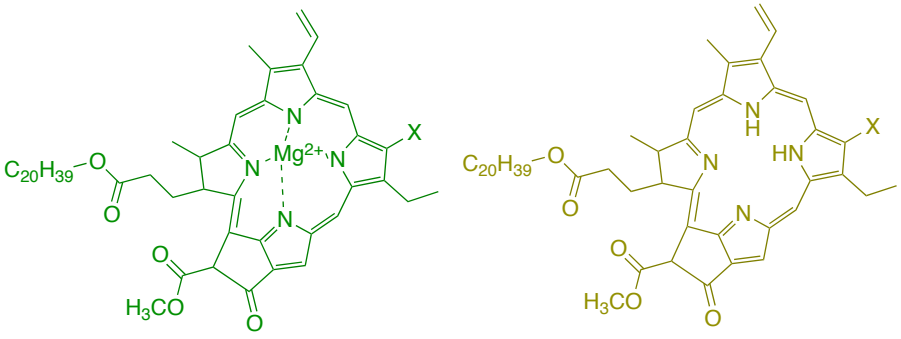


Figure 1. Structures of chlorophylls and pheophytins.

Chlorophylls are labile compounds, meaning that in the presence of acids, the central Mg^{2+} ion is easily replaced by protons and **pheophytins** are produced. In the summer months the leaves produce and degrade chlorophylls at a fast rate. Every fall, as the daylight dwindles, less chlorophyll is produced but its degradation continues, giving way the colors of autumn: yellows, reds, purples, and browns. These colors are largely due to the other leaf pigments, such as carotenes, flavins, and tannins (**Figure 2**), which are masked by an abundance of chlorophyll during the summer months.

Carotenes are polyunsaturated hydrocarbons that belong to the family of terpenes. Carotenes have 40 carbon atoms per molecule. They are found in the chloroplasts in association with chlorophylls and proteins where they play an important role in photosynthesis. They are auxiliary pigments in the light-harvesting process and protect the chloroplasts against photooxidation.

β-Carotene, found in carrots, sweet potatoes, and green leaves, is one of the most abundant members of the family, and the precursor of vitamin A. Other members include α-carotene, an isomer of β-carotene with only half of its vitamin A activity, and lycopene, a red pigment found in tomatoes and watermelons that has no vitamin A activity. Carotenes are easily oxidized, especially in processed food, and must be protected from light and air to maintain their dietary value. The oxygen-containing products derived from carotenes are called **xanthophylls**. Xanthophylls and carotenes form the **carotenoid** family. The most abundant xanthophylls found in green leaves are lutein, violaxanthin, and neoxanthin (**Figure 2**).

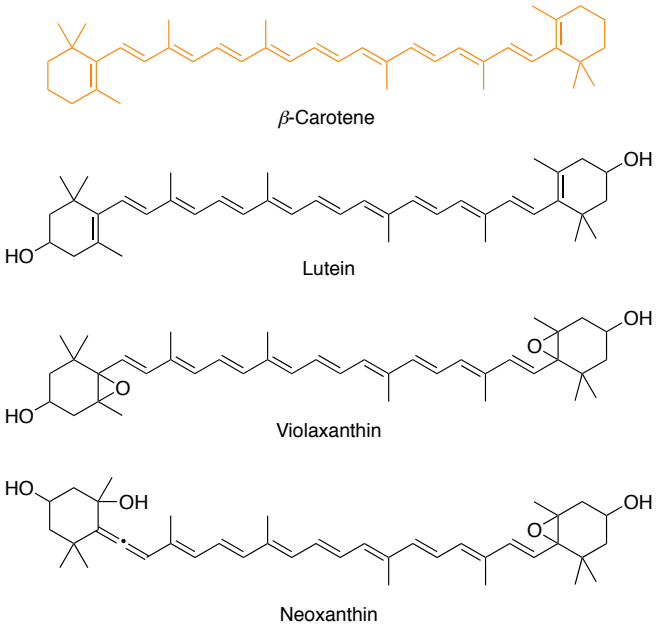


Figure 2. Structures of select carotenes and xanthophylls.

In this experiment, students will isolate pigments from spinach leaves *via* solid-liquid and liquid-liquid extraction. The procedure given below can be applied with minor modifications to the extraction of chloroplast pigments (chlorophylls and carotenes) from almost all types of leaves. It can also be used to isolate the pigment from red, yellow, or green bell peppers. As already mentioned, the pigments from green leaves normally include carotenoids (*β*-carotene, lutein, violaxanthin, and neoxanthin), chlorophylls a and b, and pheophytins a and b. Pheophytins may be present in the leaves, but they may also be generated during the extraction process. As the cell membranes are broken, naturally occurring acids from the cytoplasm and other cell organelles come in contact with chlorophylls from the chloroplasts, producing pheophytins.

The extraction is performed by first crushing the leaves with methanol, and then with a mixture of methanol and hexanes (two immiscible solvents). The first extraction with methanol breaks the cell membranes and removes water. This extract is discarded because it is poor in pigments. The second extraction with methanol-hexanes removes the pigments that go preferentially to the hexane layer. Methanol helps in detaching the pigments from their cellular complexes with proteins. The methanol-hexanes extract (solution of pigments) is separated from spinach pulp (mainly cellulose) by gentle vacuum filtration. The extract is washed several times with water to remove water-soluble pigments, which would interfere with TLC analysis. The extract is then dried with sodium sulfate (Na_2SO_4) and filtered. Depending on the concentration and amount of solvent remaining, it may be necessary to concentrate further using a rotary-evaporator (rota-vap).

BACKGROUND PART 2: Thin-Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a versatile and rapid form of qualitative analysis used regularly in synthetic organic chemistry labs. Analyses of complex mixtures can be successfully carried out in minutes with relatively inexpensive equipment. The composition of a mixture can be assessed relative to known standards. In TLC the separation takes place on a thin layer of **solid stationary phase** spread on a solid support such as a glass, aluminum, or plastic plate. A **liquid mobile phase,** an organic **solvent,** moves along the plate.

The principles behind this technique are not terribly different from GC. Separation is based on selective affinity for mobile and stationary phases. In GC, the mobile phase is a gas and the stationary phase is a liquid. In TLC, the mobile phase is a liquid and the stationary phase is a solid. The principles of TLC will later be applied to *column chromatography*, where larger sample volumes will be physically separated and isolated based on polarity of the components. In this lab, students will use solvents of different polarities to determine the optimal separation of the pigments found in spinach extracts on a TLC plate.

TLC separation of a mixture is based on relative polarity of components. The sample used for TLC is a nonvolatile liquid or a solution in a volatile solvent, in our case spinach pigments in hexane. It is applied to a polar silica (SiO_2) plate, the **stationary phase**, with the aid of a capillary tube. The sample is spotted about 1.5 cm from the lower edge of the plate, referred to as the **origin** (**Figure 3**). *The origin is marked with a pencil, never with ink because the components in ink may separate during the run and interfere with analysis.* After the solvent has evaporated from the spot, the plate is placed in a chamber that contains the **mobile phase** of choice to a height of about 0.5 cm. The solvent should be lower than the spot on the plate, or else the sample will dissolve in the solvent. The chamber is capped to avoid evaporation of the solvent and to ensure liquid-vapor equilibrium inside.

The sample components are selectively carried up the TLC plate by the solvent *via* capillary action. Different compounds travel at different speeds because they have specific interactions with the polar stationary phase. The TLC chamber is kept very still while the plate is running to allow the components to travel in one straight lane. Various solvents and solvent mixtures are used to determine optimal separation conditions. When the solvent has traveled 80-95% of the length of the plate, the plate is removed from the chamber, and the level reached by the solvent, called the **solvent front,** is marked. The solvent is allowed to evaporate, and the plate is analyzed. Regardless of solvent polarity, the separation order is the same because the stationary phase is always polar.

Non-polar compounds have a lower affinity for the polar stationary phase and travel farther from the origin than polar compounds.

In **Figure 3**, the original sample spot has been separated into two spots after interaction with the mobile phase, indicating that the sample contains at least two different compounds.

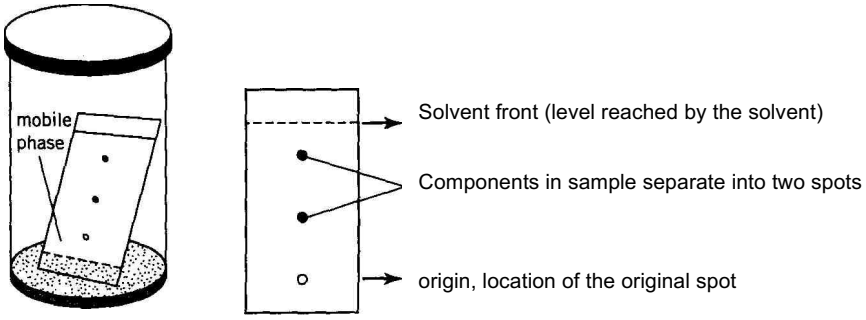


Figure 3. TLC chamber and developed plate

Absorption is a process by which molecules of a gas, liquid, or solid in solution (**solute**) interact with the molecules *on the surface of a solid,* called the **absorbent.** Absorption is strictly a surface process that depends on electrostatic forces between absorbent and sample. These forces arise from intermolecular forces including **dipole-dipole** and **ion-dipole interactions** as well as **H-bonds.** The surface of the absorbent is far from being perfectly smooth; it has crevices and crests with centers of positive and negative charge density. The solute binds to the absorbent through electrostatic attraction between its own centers of charge density and those on the surface of the absorbent. The places on the surface of the absorbent where the sample binds are called **binding sites**. Ions and molecules with permanent dipole moments readily bind to the absorbent.

The most commonly used absorbent in TLC is **silica gel.** Silica gel is obtained by hydrolysis of silicates. It has polarized Si—O and O—H bonds that interact with dipoles in the solute. It can also form H-bonds, especially with H-bond donors such as alcohols (R—OH), phenols (Ar-OH), amines (R-NH_2), amides (R-CO-NHR'), and carboxylic acids (R—COOH). The SiO_2 particles used for TLC have an average diameter of about 0.025 mm (teeny tiny!).

To understand how separation occurs on the surface of the absorbent, we should look closer at the interactions between the **absorbent** and **solutes** and between the **absorbent** and **mobile phase. Figure 4** displays a close-up view of a TLC plate cross-section showing the interactions on the surface of the absorbent. The sample has two different components, represented by ovals and triangles. The solvent molecules are shown as circles. Because there is a limited number of binding sites on the surface of the absorbent, *solute and mobile phase molecules must compete* with each other to bind to the absorbent; the stronger the interactions, the tighter the binding. In **Figure 4a**, the thickness of the lines between the shapes and absorbent indicates the strength of the interaction. The oval molecules have a very strong interaction with the absorbent as indicated by a very thick line, while the triangles have a weaker interaction as shown by the dashed line. The mobile phase molecules, on the other hand, have a stronger interaction than the triangles, but a weaker one than the ovals.

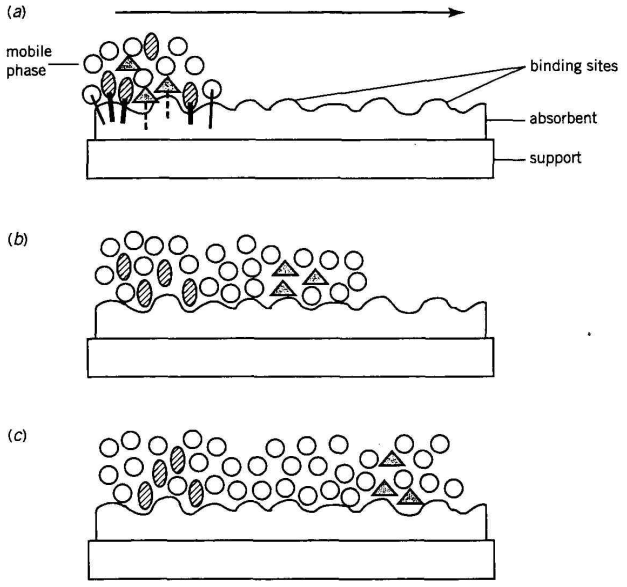


Figure 4. Separation of two compounds on a TLC plate (ovals and triangles). The arrow indicates the direction of the mobile phase flow (see text).

As the mobile phase ascends along the plate, *the mobile phase molecules close to the absorbent compete with the solutes for the binding sites.* If the interaction between mobile phase and absorbent is stronger than the interaction between absorbent and solutes, such as in the case of the triangle molecules, the mobile phase displaces the solute molecules from their binding sites, moving them farther away from the surface of the absorbent. As the mobile phase keeps flowing, these solute molecules are carried to a new location on the plate (**Fig. 4b**). Because the mobile phase has stronger interaction with the absorbent, the triangle molecules do not bind efficiently and keep traveling along the plate at high speed. At the end of the chromatographic run, the triangles are found near the solvent front (**Fig. 4c**).

If the interaction between solute and absorbent is stronger than the interaction between mobile phase and absorbent, the mobile phase still moves the solute in the direction of flow. This is a result of mass action; the mobile phase molecules, being much more numerous than the solute molecules, eventually displace the solute molecules from their binding sites, even if the solute has stronger affinity for the absorbent. However, the stronger the interaction between absorbent and solute, the more difficult it is for the solvent to move the solute. As shown in **Figure 4c**, the oval molecules, which display the strongest affinity for the absorbent, stay very close to the place where they were originally spotted.

Selection of solvent conditions

Table 1. Solvents and relative polarity

Solvent	Dielectric constant
Hexanes	1.89
Cyclohexanes	2.02
Toluene	2.38
Diethyl ether	4.34
Ethyl acetate*	6.02
Methylene chloride	8.93
Acetone	20.7
Methanol	32.7
Water	80.1

The *polarity of the mobile phase* plays a crucial role in the separations. The dielectric constant is usually taken as an indicator for polarity; however, other indices based on chromatographic separations have been devised. Some useful solvents for TLC are given in **Table 1** in order of increasing polarity. Solvent mixtures are very commonly used in TLC analysis. The polarity of the mobile phase can be changed within a wide range by mixing solvents of different polarities. For example, mixtures of hexane and ethyl acetate with increasing proportion of the latter provide a series of solvents of increasing polarity. A 7:3 mixture of hexanes / ethyl acetate is more polar than a 9:1 mixture, for example.

TLC and Functional Groups

Table 2. Functional Groups and Polarity

Family of compounds	Structure
aliphatic hydrocarbons	R–H
alkyl halides	R–X
unsaturated hydrocarbons	R–CH=CH–R
aromatic hydrocarbons	Ar–H
aryl halides	Ar–X
ethers	R–O–R
esters	R–COOR
ketones	R–CO–R
aldehydes	R–CO–H
amides	R–CO–NH_2
amines	R–NH_2
alcohols	R–OH
phenols	Ar–OH
carboxylic acids	R–COOH
amino acids	H_3N^+–CHR–COO^-

As we have seen, the separation in absorption chromatography is largely based on differences in polarity and the ability to form H-bonds. Compounds capable of donating an H-bond adsorb to silica more strongly than similar compounds with no H-donor capabilities. For example, carboxylic acids (R—COOH) are absorbed more tightly than esters (R—COOR'). Alcohols (R—OH) have a stronger interaction with the absorbent than ethers (R—O—R'). A list of different types of compounds in order of increasing polarity is given in **Table 2**. This list should be regarded only as a rough guide. The number of carbons in a molecule have a significant affect on polarity; more carbons make a compound less polar. In general, compounds that are H-bond donors (alcohols, phenols, carboxylic acids, amines, etc.) absorb more strongly than those that are only H-bond acceptors such as ethers, esters, ketones, etc.

To illustrate the relation between polarity and the success of the separation, consider three hypothetical mixtures, each containing two compounds of different polarity. In **Figure 5**, both compounds spotted on plates **A** and **B** are nonpolar, for example, an alkene and an aromatic hydrocarbon. A polar solvent does not separate the nonpolar mixture well (**Fig. 5 A**). Both compounds travel similar distances since the polarity of the solvent overrides the polarity of the plate. A nonpolar solvent, on the other hand, separates the two components (**Fig. 5 B**) due to greater interaction between non-polar solute and solvent molecules. The less polar alkene less polar travels farther than the slightly more polar aromatic hydrocarbon.

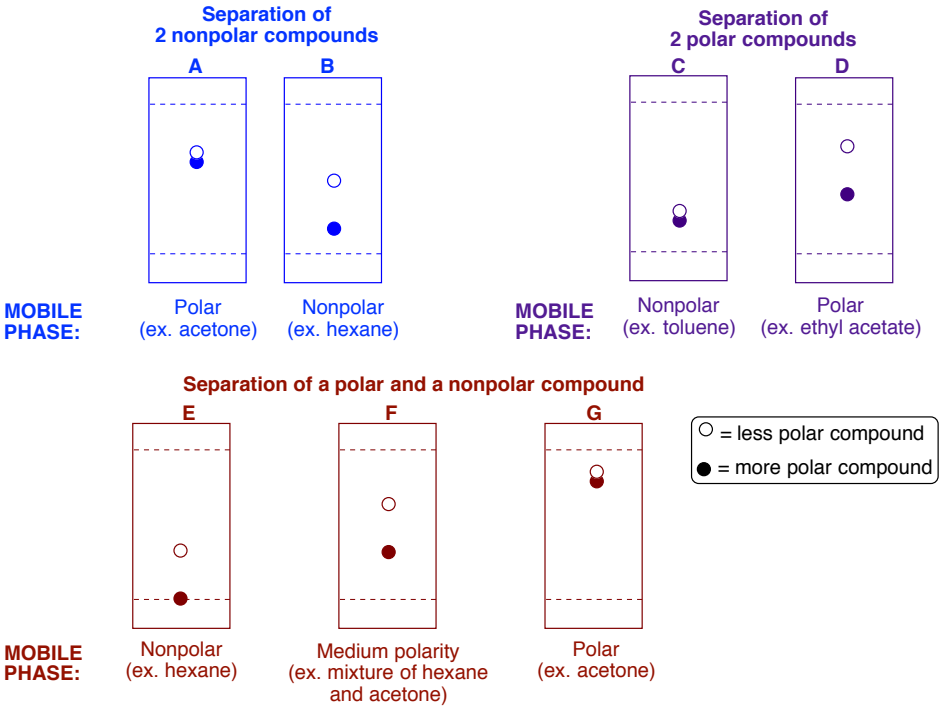


Figure 5. Separation of hypothetical mixtures.

The mixture second mixture on plates **C** and **D** contains two polar compounds, for example, an ester and an alcohol. Neither compound moves with a nonpolar solvent (**Fig. 5 - C**) since the polar compounds have a greater affinity for the polar plate. Both travel and separate when a polar solvent is used (**Fig. 5 - D**). The less polar ester travels farther than the more polar alcohol, which sticks to the plate.

The third case is most applicable to the separation of spinach pigments with a mixture of compounds of very different polarities. This type of mixture is best separated by medium polarity solvents, or a mixture of non-polar and polar solvents. For example, a mixture of an aromatic hydrocarbon and a phenol can be separated using a nonpolar solvent. The aromatic hydrocarbon moves farther and the more polar phenol stays at the origin (**Fig. 5 - E**). It is ideal for both compounds to move at least slightly from the origin to ensure complete separation, especially in the case of spinach pigments where there are more than two components. Using a solvent of medium polarity increases the distances traveled by both compounds (**Fig. 5 F**). A polar solvent causes both compounds to move at similar speeds and no separation is achieved (**Fig. 5 G**). The polarity of the solvent overrides that of the plate and both compounds travel upward, with the non-polar compound moving slightly more.

TLC CALCULATIONS: Analyzing the Chromatogram

Once the spots have been visualized, the distance traveled by the spot from the origin is measured along with the distance traveled by the solvent front. The ratio between these two distances is called **ratio to the front** or **retention factor (R_f)**:

R_f = (Distance traveled by spot) / (Distance traveled by solvent)

The distance traveled by the sample is measured from the origin to the middle of the spot. With very large and ill-defined spots, as in spots with "tails," R_f values are meaningless because the middle of the spot varies with the amount of sample applied to the plate. If the spot streaks or runs with a tail, it is an indication that **too much** sample was applied. A decrease in the volume of sample spotted should be tried first; if this does not correct the problem, then a different absorbent or solvent system should be tried.

The **R_f value depends on** several variables…
- the thickness of the absorbent
- the nature of the stationary phase and its degree of activation
- the mobile phase
- the amount of material applied

BACKROUND SUMMARY

Spinach pigments are extracted from fresh leaves with methanol and hexanes. Water-soluble pigments are removed from the solution by liquid-liquid extraction. The solution of pigments in hexanes is analyzed by thin-layer chromatography (TLC). The less polar carotene pigments travel farther up the TLC plate than the more polar xanthophylls. Chlorophylls and pheophytins are of medium polarity and travel more than the xanthophylls but less than the carotenes. Students investigate four different mobile phases (solvent mixtures) and determine their power in separating **chlorophylls (green), xanthophylls (yellow), pheophytins (green-gray), and carotenes (yellow-orange).** Optimal separation is achieved with a mobile phase that separates spinach pigments into 4-8 colorful, distinct spots on the TLC plate.

Supplemental Materials

- Filtration, extraction, and drying: Mohrig, J. R. *Techniques in Experimental Organic Chemistry, 4th Edition,* Chapters 9 – 11.
- Exp 3 Slugs@home labsite & Canvas / JoVe pre-lab videos
- Experiment adapted from Palleros, D. R. “TLC Analysis of Vegetable Extracts,” Experimental Organic Chemistry, **2000**. Wiley: Hoboken. p. 190-196.

PROCEDURE - spinach provided in the lab

PART 1. Solid-liquid and liquid-liquid extraction

Weigh 10 g of fresh spinach leaves and chop them using scissors. Obtain 12 mL of methanol using a graduated cylinder and pipet. Place the spinach in a large mortar along with the methanol and crush the leaves with the pestle gently for two minutes. This can get messy if you are careless. Use common sense in your crushing technique! With the aid of the pestle or a spatula, squeeze the spinach against the sidewall of the mortar to remove as much methanol as possible. Use a glass stir rod to decant the liquid into a 250-mL Erlenmeyer flask labeled "methanol-water" and set aside. The contents of this flask will eventually be discarded.

FUME HOOD PHASE 1: In a fume hood, extract the remains of the leaves with a mixture of 15 mL hexanes and 5 mL methanol, crushing the tissue with the pestle for about 2-3 minutes. The extract should be deep green. Leaving behind as much solid as possible, use a glass stir rod to decant the liquid (a mixture of hexanes and methanol) directly into a Buchner funnel set up for vacuum filtration in the fume hood. The vacuum should be low to prevent too much solvent from evaporating. Use an additional 2 mL of hexanes to rinse the spinach a final time and aid the transfer. Collect the filtrate directly in a small filter flask and discard the filter paper. Transfer the filtrate into a labeled screw-cap test tube, add 5 mL of water, and bring the contents back to your benchtop. Clean the funnel immediately before proceeding to the next step or it will make any future experiment turn green! Use a small amount of ethanol as instructed by your TA to initially rinse the funnel into the waste before washing in the sink.

FUME HOOD PHASE 2 (liquid-liquid extraction): When enough students are done with PHASE 1, take your turn back at the fume hood. Mix, vent, and allow the layers to separate. If a deep green upper organic layer is not apparent, add 5 mL of hexane, being careful not to overflow the test tube. Remove the lower aqueous layer using a long-stem pipet with bulb and transfer into the Erlenmeyer flask labeled "methanol-water." Wash the remaining hexane layer with 5 mL of water: add 5 mL of water, mix, vent, separate. Collect the aqueous layer along with any emulsion present in the "methanol-water" flask. Add a small spatula-full of granular sodium sulfate (Na_2SO_4) to the test tube to dry the organic layer. Cap the test tube and swirl occasionally back at your benchtop. After about 5 minutes, filter the suspension in the fume hood by using a clean and dry micro-funnel (2.5 cm in diameter) and a cotton plug. Collect the filtrate in a dry 50-mL round-bottom flask. Your TA will operate the rotary-evaporator (rota-vap) if necessary to evaporate the solvent from the extract until the volume is approximately 2-3 mL (size of a nickel). The color of the final extract should be deep green. Transfer the liquid by pipet to a labeled scintillation vial or test tube.

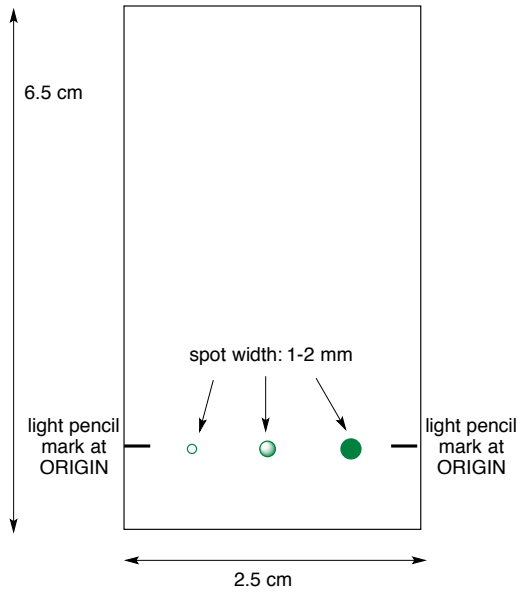
PROCEDURE PART 2. TLC Analysis

Locate, but DO NOT REMOVE, the TLC jars in the fume hood containing each mobile phase (solvent)…

- Hexanes
- 9:1 Hexanes-Ethyl Acetate (9:1 Hex/EtOAc)
- 7:3 Hexanes-Ethyl Acetate (7:3 Hex/EtOAc)
- Ethyl Acetate

Keep the jars covered with their lids except when opening to add your plate. ***Students are not permitted to add or remove any solvent from the TLC jars.*** Consult TA for assistance with adjusting solvent levels if necessary.

Obtain four silica gel TLC plates (2.5 x 6.6 cm). *Be extremely careful not to bend the plates or to scratch the silica surface (the chalky side). Handle plates by the edges.* Label them with the name of solvent on the very top of the plate with light pencil markings. Make two small pencil marks on either side of the plate to indicate the origin on the absorbent side of the plate at a distance of about 1 cm from the bottom edge.



Determine the Optimum Number of Applications

The "9:1 Hex / EtOAc" plate will be used to determine the optimum number of applications needed to visualize the separation of pigments. On this plate, the sample will be applied in three different lanes, each one with a different number of applications (1, 4, and 7). Dip a narrow capillary tube in the extract (not a melting point tube); the liquid will rise through capillary action. Apply the liquid to the plate by gently and *briefly* touching the plate with the tip of the capillary tube. Raise the capillary tube to stop the flow of liquid when the diameter of the spot is 1-2 mm. The spot should be very tight and small and at least 0.5 cm from the side edge of the plate. On the same plate and using the same capillary tube, apply a second and a third spot at a distance of about 0.5 cm from each other. The number of applications for the second spot should be 4, and for the third spot 7. Allow the solvent to evaporate between applications. Failure to do so will result in very large spots. *Conserve - only one capillary tube is needed per group for the entire lab!*

FUME HOOD PHASE 1: Using tweezers, hold the plate next to the jar to ensure the *spots are above the solvent level* of the 9:1 Hex / EtOAc jar. If the spots are too low, the sample will dissolve in the solvent and contaminate other experiments. *Gently lower and place* (DO NOT drop) the TLC plate in the chamber with 9:1 Hex / EtOAc. *Without moving the jar*, allow the solvent to run up the plate to a height about 0.5-1 cm from the top of the plate. ***Keep TLC chambers covered and in the fume hood***. Remove the plate from the chamber (use tweezers). *Immediately mark the solvent front with pencil* and allow the solvent to evaporate in the fume hood before bringing it to your bench.

Sketch the TLC plates to scale in your lab notebook, paying special attention to the colors of the spots. Are they yellow, pale green, deep green, gray, or orange? See p. 6-7 for structures: **chlorophylls (green),** the **xanthophylls (yellow),** the **pheophytins (green-gray),** and **carotenes (yellow-orange).** Notice and record any color spot at the origin. This analysis should be done without delay because the colors of the spots fade very quickly (within hours). Determine how many applications gave the best results, or in other words, clear and well-delineated spots without streaking. If all spots are too faint to be seen, the extract should be concentrated by further evaporation of the solvent. If the spots run with tails, too much sample has been applied. Either reduce the number of applications or dilute your extract with hexanes.

Analyze the Extract

FUME HOOD PHASE 2: Spot the extract on the remaining plates using the optimal number of applications determined on the 9:1 plate. Only one lane is necessary for each of the remaining plates. *Develop all remaining plates at the same time to the best of your ability.* Calculate the R_f values for each pigment in each solvent. Decide which solvent gives the best separation (most spots). Be sure to write down in your notebook all the data necessary to reproduce your R_f values (stationary phase, solvent, number of applications). Draw the plates in your notebook and indicate colors. Check first with your TA then dispose of all plates in the solid waste. Students who keep their plates will lose points. Dispose of your TLC plates and **consider the in-lab questions before leaving the lab.**

Clean-up and Waste **This has the potential to be a very messy experiment so take proper precautions.** **Clean and dry your work stations please.**	
TLC – Solid Waste	- All used TLC plates (return unused to TA) - Sodium sulfate, used cotton, capillary tubes, pipets - Let remains of spinach leaves dried in hood in mortar, then put in waste, NOT in sink or trash - Capped, labeled vial of spinach extract
Organic Solvents – TLC Waste	- Contents of "methanol-water" flask - Mobile phase from TLC chambers (only if asked) **- Rinse all glassware, including mortar & pestles, with ethanol into the waste before washing in the sink.**
General Clean-up	- Remove gloves when washing glassware - Wash glassware and mortar & pestles with soap & water after rinsing with solvent. TA's will perform a final ethanol wipe. - Clean vacuum filter funnel immediately after using. -*Thoroughly wipe down bench tops*
Safety: Hexane and ethyl acetate are flammable. Keep TLC jars in the fume hoods.	

Pre-lab Questions

1. Compare and contrast GC and TLC according to the following criteria:
   - Explain the differences in stationary and mobile phases (states of matter);
   - State the property on which each type of separation is based.

2. Arrange the following solvents/solvent mixtures in order of increasing polarity: hexanes, ethyl acetate, 7:3 hexanes-ethyl acetate, 9:1 hexanes-ethyl acetate.

3. Suppose a non-polar compound is spotted on a TLC plate. Which solvent from #2 will move the compound the farthest from the origin? Which solvent from #2 will move the compound the least from the origin?

4. What would happen if a TLC plate was placed in a jar where the solvent level was above the level of the origin (sample spot)? What should you do if you inadvertently spotted your plate too low (aside from consulting your TA)?

5. Why should ink be avoided in marking TLC plates?

6. Arrange the following compounds in order of increasing polarity: lutein (L), neoxanthin (N), β-carotene (C), violaxanthin (V). Which should display the highest R_f? Which should display the lowest R_f?

Take the **Canvas Exp 3 pre-lab quiz** *before* your enrolled section.

- The quiz incorporates the questions below - the questions may be reworded.
- **Be prepared with your responses to the pre-lab questions *before* starting the quiz.**
- There is a 20-minute time limit on the quiz and you get two attempts.
  - Complete each quiz in one sitting- you can't save and come back later.
  - If you choose to re-take the quiz, your grade will be the highest of the two attempts.

Though we encourage collaboration in this class, this is an individual quiz.

- The responses should be a product of your original work so that you are assessed on *your* understanding of the material.
- Sharing your quiz or the correct responses in any format (screenshots, email, CHEGG, social media, text, carrier pigeon, etc.) is in violation of the UCSC academic integrity policy.
  - Students in violation of this policy will go through the Academic Misconduct process.
    - *It's not worth the risk and is the least favorite part of Caitlin's job!*

LAB REPORT

Abstract

Refer to the writing worksheet for guidance in writing one paragraph: purpose, methods (NOT the procedure!), results, and conclusion.

The main result in this experiment is the determination of the **optimal TLC solvent**, being the one that separated spinach extract into the most spots. Retention factors (R_f) should not be included in the abstract. Use one sentence to comment on whether results were as expected – what polarity of solvent would you expect to best separate the spinach pigments based on their polarity?

In-Lab Questions

1. What roles does methanol play in each of the first two extractions?

2. Draw the TLC plates to scale. Indicate colors (brownie points for using crayons or colored pencils in the report) and identify as many pigments as possible on each plate (chlorophylls, xanthophylls, pheophytins, and carotenes). Not all pigments will appear as separate spots on all plates.

3. Calculate the R_f values for each spot on each plate and tabulate your results. Include the pigment identification (in-lab #2) for each spot. There is no need to calculate the R_f of a 'smear', but note in the table that a smear did occur. As you re-create the table from the worksheet in a word processing document, it may be appropriate to 'merge' cells when spots overlap. Alternatively, you can list the same R_f value for several pigments if they overlap.

4. Briefly discuss the overall separation power of the solvent using the points below based on your results. Consider the polarity of the solvents and pigments in your discussion.

- **(a)** Explain the differences observed in the plates run with hexanes and 9:1 hexane-ethyl acetate.
- **(b)** Was ethyl acetate a good solvent to separate the pigments?
- **(c)** Which solvent was best to separate just the carotenes from the other pigments?
- **(d)** Which was the optimal solvent for separation of the most pigments (greatest number of individual spots observed)?

5. Describe three procedural mistakes you actually made in the lab (aside from spilling) and/or what could have gone wrong (check out the mistakes on Slugs@home). Include at least one mistake from TLC. Explain what went wrong, how or why it affects the experimental results, and how to correct the mistake – does it require restarting the lab or can the experiment be saved?

Name _____ Partner Name _____

TA Name _____ Section Letter _____ Day _____ Time _____

Experiment 3 Worksheet – Extraction & TLC Analysis of Spinach Pigments

Use as reference for notebook preparation – every student submits on Canvas individually after lab

Pre-Lab Requirements

1. **Dress for lab** – see safety rules – arrive a few minutes early
2. **Lab Notebook:** copy templates below into designated notebook
    - **Purpose, scheme,** and **reagent table**
    - **Procedure Diagrams** – must be complete before you can start the lab

A. Purpose and pigment structures:

B. Reagent Table

Sample Name	**Amount** Fill in during lab	**Molecular Mass**	**mmoles** Fill in during lab	**Boiling or melting point**	**Density**	**Hazards**
Spinach		n/a	n/a	n/a	n/a	n/a
Methanol						
Hexanes						
Water						
Ethyl acetate	n/a					

C. Procedure – hand-drawn using procedure in lab PDF, class notes, & Slugs@home

- Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
- Leave space to record additional **notes and observations** within the procedure diagrams

DAY 1

STEP 1: Solid-liquid extraction – crush the leaves, addition and removal of solvents, vacuum filtration

STEP 2: Liquid-liquid extraction – step-wise transfer of solutions

DAY 2

STEP 3: TLC Analysis

a. **Optimal number of applications** – labeled diagrams of spotted plates before, during, and after running in the TLC chamber
b. **Optimal solvent / mobile phase** – labeled TLC chambers and sketches of all developed plates

E. Data

Sketches of all TLC plates. Describe the color of each spot and/or use colorful drawing tools.

Calculated **R_f values** for every spot on every plate. More rows may be needed, depending on the number of spots: Xanthophylls, chlorophylls, and pheophytins are classes of compounds and may separate into 2-3 spots.

Solvent → ↓ Pigment	Hexanes (R_f)	9:1 Hex / EtOAc (R_f)	7:3 Hex / EtOAc (R_f)	EtOAc (R_f)
Carotenes				
Pheophytins				
Chlorophylls				
Xanthophylls				

* EtOAc = ethyl acetate

Experiment 4 – IR Exercise

Learning Objectives

- Understand principles behind IR Spectroscopy as it relates to the stretching & bending of bonds
- Observe the inverse relationship between vibrational frequency and bond length
- Analyze spectra to predict functional groups and bonds in an organic molecule
- Understand the role resonance plays in vibrational frequencies
- Observe effects on sample preparation on quality of spectra

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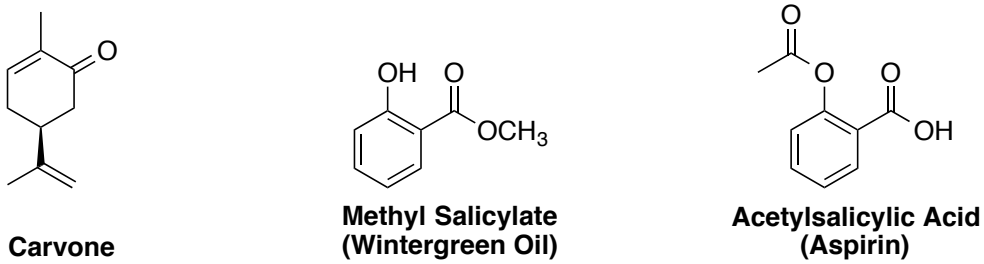
* Please find “How to Prepare & Assignments” after the procedure

In this experiment, students will study the infrared (IR) spectra of aspirin, carvone, and methyl salicylate (wintergreen oil). The purpose of the experiment is to become familiar with IR spectroscopy, including sample preparation and the interpretation of IR spectra for bond and functional group identification. Carvone is a monoterpenoid that is the oxidation product of limonene, the main component in citrus oil. Methyl salicylate is used in chewing gum for flavor and in muscle rubs for its cooling, pain-relieving (analgesic) properties. Carvone and methyl salicylate are liquids and their IRs are obtained directly from a very small drop of pure (aka “neat”) material. Methyl salicylate is the hydrolysis product of aspirin, a commonly known analgesic used for headaches. Aspirin is a solid and is diluted with the hydrocarbon mixture nujol to prepare the sample for IR analysis.



Carvone | **Methyl Salicylate (Wintergreen Oil)** | **Acetylsalicylic Acid (Aspirin)**

Figure 1. Structures of compounds to be analyzed by IR.

IR spectroscopy is similar to spectrophotometry, a common technique in the general chemistry labs used to determine the concentration of colorful samples based on absorbance of visible light. In spectrophotometry, a sample (ex. solution of red dye) *absorbs a specific wavelength of light* in the visible range of the electromagnetic spectrum (**Figure 2**). The spectrophotometer displays an absorbance value that is correlated to the concentration of the solution. **IR spectroscopy explores a different range of radiation in order to determine the types of bonds and functional groups present within a molecule**. Every molecule has a unique IR spectrum with multiple absorbances at specific frequencies, known as **wavenumbers (cm^{-1})**.

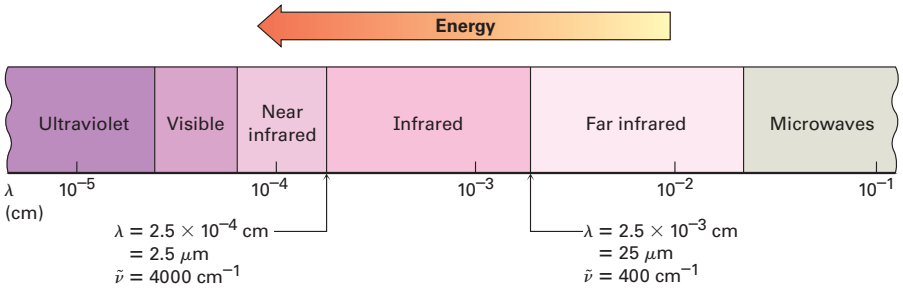


Figure 2. Electromagnetic spectrum, highlighting infrared (IR) region

Molecules are always in motion at rapid rates that are difficult to fathom! *Translational* motion is when a whole molecule moves to a different space. The rate of translational vibrations is highest for gases and lowest for solids. Bonds within molecules are constantly *rotating*, particularly sigma or single bonds. *Translational and rotational vibrations are not detected by IR spectroscopy.*

IR active bonds are those that exhibit **antisymmetric stretching** and **out-of-plane bending** (**Figure 3**). Stretching and bending of bonds in organic molecules occur at frequencies (wavenumbers) within the IR range, between 400 – 4000 cm^{-1}. Absorption of IR radiation in the spectrometer results in the *amplified* stretching and bending of bonds characteristic of its functional group.

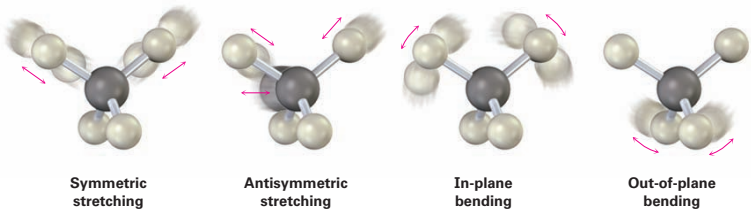


Figure 3. Stretching and bending vibrations.

$\bar{\nu}$ **Wavenumber** is the preferred unit for IR spectroscopy, simply because the values are easier to work with than wavelength (compare 400 cm^{-1} and 2.5 x 10^{-3} cm). Wavenumber is the inverse of wavelength **(eq. 1)**. Though it's not a typical frequency unit (cycles per second or s^{-1}), *wavenumber is proportional to frequency* so the terms are used interchangeably in IR discussions.

$$\bar{\nu} = \frac{1}{\lambda} \qquad \textbf{(eq. 1)}$$

The general rule in understanding IR spectra is that longer bonds go through a vibrational cycle less frequently and **shorter bonds vibrate more frequently.**

Longer Bond = Slower (↓) Stretching Frequency

	O-H	C-H	C=O
Bond Length (pm)	100	110	120
Stretching Frequency (cm^{-1})	3300	2900	1700

During IR analysis, energy is absorbed by each IR active bond and the remaining transmitted (not absorbed) IR frequencies are detected by the instrument and plotted on the spectrum – wavenumber vs. % transmittance (%T).

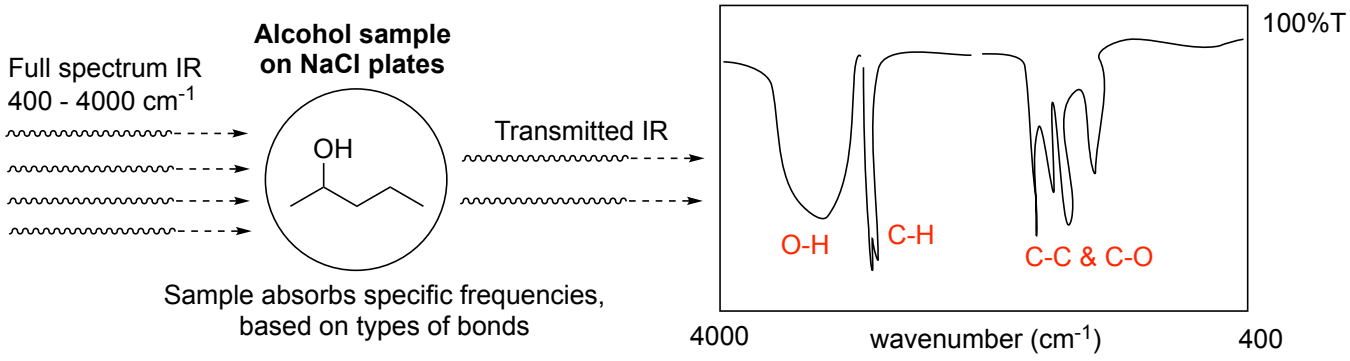


Figure 4. Crude diagram of IR spectroscopy and amateur sketch of alcohol spectrum.

Bonds **expand & contract (stretch)** at relatively higher frequencies (1000 – 4000 cm^{-1}) and **C-H out-of-plane bending** occurs at lower frequencies between 500 – 1000 cm^{-1}. Note that out-of-plane bending occurs within the **fingerprint region**, which displays characteristic signals for specific compounds, like its unique fingerprint. The region between *1000 – 1500 cm^{-1} is often ignored* due to complex overlap of the numerous C-C, C-N, and C-O bonds often present in organic molecules. A complete table of functional groups, bonds, and expected wavenumber ranges is at the end of this document and posted separately on Canvas. Typical examples and trends are discussed below.

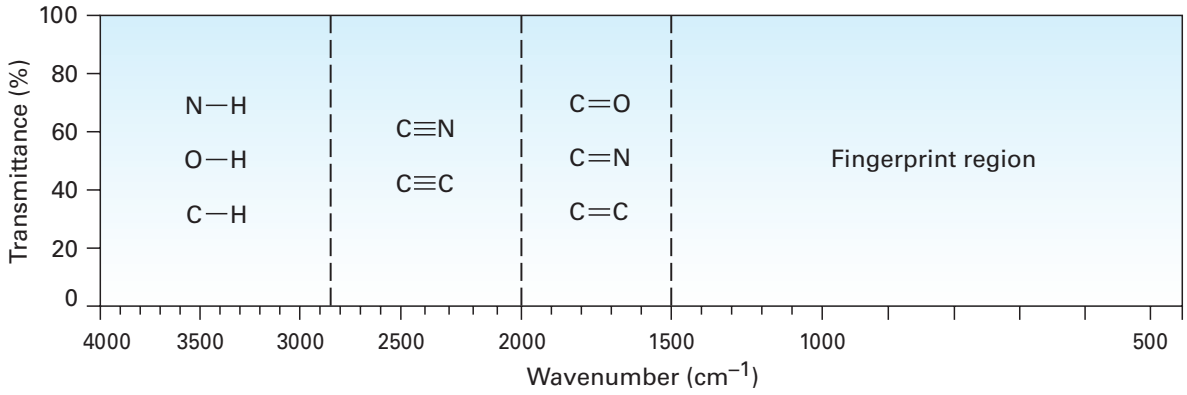


Figure 5. Four main regions of the IR spectrum.

The main *factors affecting bond length* and vibrational frequency are **atomic radius**, **hybridization** or type of bond, and **conjugation** (resonance). Single bonds to hydrogen are the shortest bonds because hydrogen is the smallest atom and thus have the highest stretching frequencies (2800 – 4000 cm^{-1}). Single bonds between sp^3 hybridized C-C, C-N, and C-O are the longest bonds and have the lowest stretching frequencies (1000 – 1500 cm^{-1}), though these signals are typically ignored in IR spectra as mentioned above.

Atoms that are sp^2 hybridized, typically double bonds, are held closer together and stretch more frequently (1500 – 2000 cm^{-1}) than corresponding single bonds. A sharp signal around 1700 cm^{-1} is characteristic of a carbonyl (C=O). The identity of the carbonyl functional group determines a more specific range of stretching frequency. This allows the observer to predict whether the carbonyl is within an aldehyde or carboxylic acid, for example. This trend continues with sp hybridized atoms, often triple bonds, which stretch between 2000 – 2800 cm^{-1}.

The ranges presented above are general guidelines. When the functional group is **conjugated**, or participates in resonance, the overall *bond length is increased* and the ***stretching frequency decreases***. This is exemplified in **Figure 6** below. The quickest way to spot a conjugated functional group is to look for alternating double-single-double- bonds.

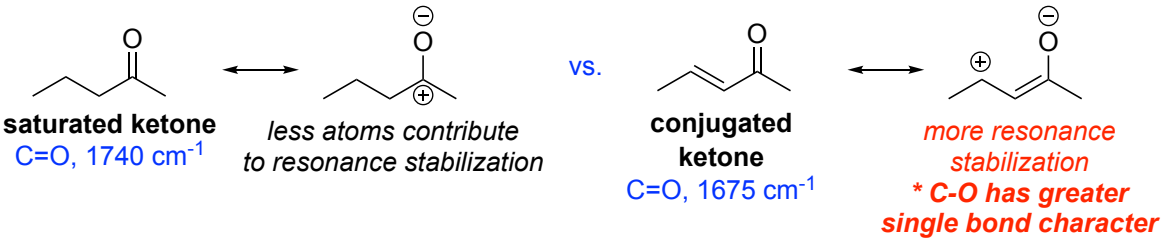
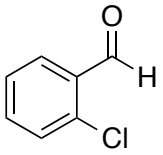


Figure 6. Comparison of stretching frequencies in a saturated vs. conjugated ketone.

This lab exercise will focus on using IR spectra to confirm the presence of functional groups in known compounds. Before running the IR sample, signals are predicted by making a **list of all functional groups** in the molecule. The IR table of values (end of this document) provides the expected range of frequencies for each type of bond within each type of functional group. A worked example of the predicted IR signals and assignment of a literature spectrum is presented below.

Steps for predicting IR spectra

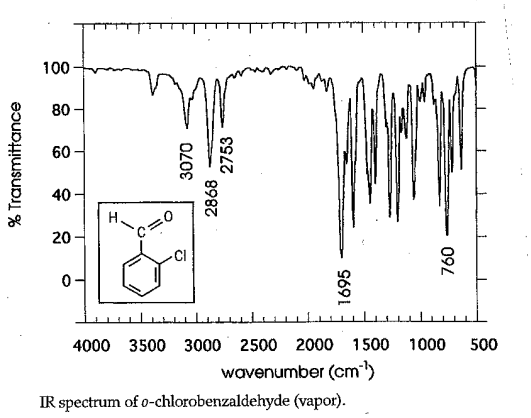
- Determine each **functional group** in the molecule (ex. *ortho*-chlorobenzaldehyde).
- Use the **IR Table** (end of this document) to find the IR active **bonds** within each functional group (FG) and its expected wavenumber range.
    - Be sure to list **all bonds and vibrations**.
        - FG's may have *multiple IR active bonds*.
        - Some bonds have two different vibrations (ex. C-H bonds in arenes stretch and bend).
    - Don't forget to determine whether any **double bonds** are **saturated** or **conjugated** (participate in resonance with a neighboring pi bond).
- **If alkenes or an aromatic ring is present:** Use **IR Table 2** to determine the more specific range of C-H bending frequencies. The **substitution patterns** of an alkene or arene ring affect the C-H bending vibrations.



***o*-Chlorobenzaldehyde**

Functional Groups:

- Aromatic Ring
- Aldehyde
- Aryl Chloride



IR spectrum of *o*-chlorobenzaldehyde (vapor).

Table 1. IR Analysis of *o*-chlorobenzaldehyde

* This column would be filled in after your actual IR sample is run. This is example will not be obtained in this lab.

ø Specific out-of-plane bending vibrations (Table 2 in IR reference sheet)

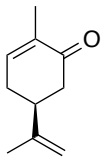
† Values approximated from the x-axis of the IR spectrum.

Functional Group	Bond Assignment (C=O, N-H, etc.)	Expected Wavenumber, cm^{-1}	Literature Wavenumber (cm^{-1})	Observed* Wavenumber (cm^{-1})
Aromatic ring *o*-disub	C-H stretch	3100 – 3000	3070	
	C=C	1625 – 1440	~1600†	
	C-H bend	900 – 680 (770 – 735)ø	760	
Aldehyde (conj.)	C-H stretch	2900 – 2800 & 2800 – 2700, doublet	2868 & 2753	
	C=O	1715 – 1680	1695	
Aryl Chloride	C-Cl	< 600 – 840	~800†	

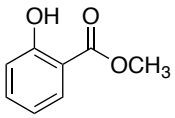
Properly identifying functional groups is half the battle! The IR tables give typical ranges, but remember that factors like conjugation could cause certain bonds to be outside of that range. An acceptable limit outside of the range is 40 cm^{-1} above or below, as long as the structure supports the outlier (ex. highly conjugated bonds may be outside the range). Otherwise, if no signal is observed within that range, it should be reported as "not observed." There are often multiple bonds in the same range, causing signal overlap. One signal may be assigned to two or more bonds along with a note to the reader. A final factor is the **variation in C-H out-of-plane bending frequencies**. Large ranges are listed for a C-H bend in an aromatic ring (900 – 680 cm^{-1}). Incorporating the substitution pattern of the ring narrows this range (770 – 735 cm^{-1}). More relationships are given in the tables at the end of this PDF.

PROCEDURE

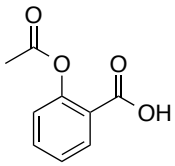
Overview: Predict the IR signals for carvone, wintergreen oil, and aspirin. Watch the demonstrations on preparing and running IR samples, then obtain the IR spectra of carvone, methyl salicylate, and aspirin. Use salt plates (pure NaCl) to support the sample. NaCl does not absorb IR radiation making it an ideal material to hold samples. The NaCl plates are very fragile and break easily. Handle them with care and never wash them with water (they will dissolve!). Instead, use small amounts of the acetone saturated with NaCl provided in the IR kit. Students will also complete an IR worksheet after interpreting IR spectra.



Carvone



Methyl Salicylate (Wintergreen Oil)



Acetylsalicylic Acid (Aspirin)

Predict spectra: Make three tables (one for each compound) with **functional groups**, **bonds**, and **expected wavenumber ranges**. Use the IR table and steps on the previous page for predicting IR bond vibrations and corresponding wavenumber ranges. Note that one functional group may have several IR active bonds (ex. carboxylic acid contains C=O and O-H) and that C-H bonds in alkenes and arenes have two vibrations (stretch and be nd).

Students will interpret two IR spectra per compound: one from the **literature** and one obtained by TAs (**observed**). Interpret the spectra by looking for a signal within each expected range and listing the corresponding wavenumber in the table. It is important to note that **not every signal in the IR spectrum will be assigned to a bond**! As discussed on the previous page, some signals may not be observed and some may be slightly outside the expected range.

IR of liquid samples - carvone and methyl salicylate: Touch the liquid with the tip of a pipet to pick up a small drop of liquid then touch the center of the salt plate with the tip of the pipet, using your thumb to apply pressure. This small amount of liquid should be enough to obtain a good IR. Cover the plate with the other half and spread the liquid by rotating the plates. Place the plates inside the plate holder, being careful not to break the plates, as demonstrated by your TA and obtain the IR spectrum. A "nice looking" IR will contain bands ending in sharp peaks rather than being flat at the bottom. Flat signals or a diagonal baseline are a result of too much sample being used.

IR of a solid sample: Place a microspatula-full of the solid in a mortar and add just one drop of Nujol. Grind the mixture with a pestle for about a minute to get a dense paste (aka "Nujol mull"). Grinding the solid very well is necessary since big solid particles will scatter IR light and lead to curved baselines and distorted spectra. Scoop some of the mull with the rubber policeman provided in the IR kit and spread it on one of the salt plates. Cover with the other plate and rotate them to further spread the mull. Obtain the IR the same way you did for the liquid sample. Keep in mind that Nujol absorbs IR radiation as well. Take a look at the IR of Nujol for reference and to avoid confusing Nujol peaks for sample peaks. It is fairly common for a student's first mull to contain too much Nujol, in which case the procedure is repeated (but you'll be better at it next time!).

Safety First!	**Clean- up**
- Wear gloves when preparing the sample using mortar & pestle. Gloves should be changed often and removed immediately after completion of the chemical operation.	- Clean the salt plates, the mortar and pestle, and the rubber policeman with a little acetone (sat. w NaCl) and tissue paper. Wear gloves when cleaning and remove when you're done.
- Methyl salicylate is toxic.	- Dispose of tissue paper in the trash and **pipets in solid-waste**.
- Carvone is an irritant.	

References

- Mohrig, J. R.; Hammond, C. N.; Schatz, P. F. "Infrared Spectroscopy" in *Techniques in Organic Chemistry*. Freeman: New York, **2006**.
- Palleros, D. R. "Infrared Spectroscopy" in *Experimental Organic Chemistry*. Wiley: New York, **2000**.

How to Prepare & Assignments - Follow Exp 4 Canvas Module…

Before Lab

- Read this PDF and/or listen to podcast
- Attend and/or watch **lab lecture,** taking notes on **lecture templates,** and the **pre-lab videos**
- Practice the lab online, including common mistakes, on the **Slugs@home platform**
- **Pre-lab questions** incorporated into **Pre-lab Quiz** – check Canvas for due date

Lab Notebook Preparation – *Required before lab;* Use the **worksheet** to prepare your **lab notebook** …

- **Purpose:** brief summary of the main lab goals and structures of carvone, wintergreen oil, & aspirin
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info
- **Procedure with Diagrams** – hand-drawn using procedure in this PDF, Slugs@home, & class notes
  - Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!

During Lab

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A; Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

After Lab – each partner submits separate, individual assignments

- Upload **Notebook Pages** to Canvas by midnight on lab day – graded on completeness / participation
- Complete & upload the **Lab Report** on GradeScope (GS) – due date on Canvas

Pre-lab Questions – incorporated into Exp 4 Pre-Lab Quiz taken individually on Canvas before lab

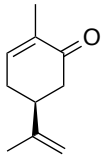
1. What happens when IR radiation is absorbed by an organic sample? How is the frequency of the radiation used to determine the functional groups in the molecule?

2. In IR spectroscopy, we normally talk about "frequencies" when in reality we are referring to wavenumbers. What is the mathematical relationship between frequency and wavenumber? Between wavenumber and wavelength? What are the units most commonly used for frequency, wavelength, and wavenumber?

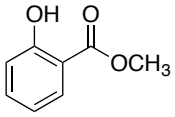
3. What is the range for the IR fingerprint region? Why are the bands in this region of limited use in structure elucidation?

4. What is Nujol? Where (what wavenumbers) does it absorb IR radiation?

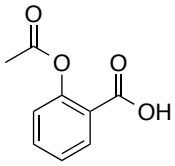
Examine these structures to answer #5-9…



Carvone



Methyl Salicylate (Wintergreen Oil)



Acetylsalicylic Acid (Aspirin)

5. Is the ketone in carvone classified as saturated or conjugated?

6. Is the ester in wintergreen oil classified as saturated or conjugated?

7. Is the carboxylic acid in aspirin saturated or conjugated? The ester?

8. What is the substitution pattern of each alkene in carvone (mono-, di-, tri-, or tetrasubstituted)?

9. What is the substitution pattern of the aromatic ring in wintergreen and aspirin (monosubstituted, ortho-, meta-, or para-disubstituted)?

Lab Report

- **IR Tables**
  - Revise and type three separate tables with predictions & interpretation of the IR spectra…
    - carvone,
    - methyl salicylate,
    - aspirin.

- **In-Lab Question: What is the relationship between bond length and wavenumber?**
  - Provide at least one example per compound's spectrum that supports this trend (ex. longer vs. shorter bond in carvone correlating to higher vs. lower wavenumber).

- **IR Problem Set** – next page

- There is no Abstract section for the Exp 4 report.

IR Problem Set

1. Briefly explain how one could use IR spectroscopy to distinguish between the following pairs of isomers: list the IR active bonds and expected wavenumber range to determine which signals are pertinent.
Hint: start by drawing the structures of each.

a) CH_3CH_2OH & CH_3OCH_3	**b)** cyclohexane & 1-hexene	**c)** $CH_3CH_2CO_2H$ (carb. acid) & $HOCH_2CH_2CHO$ (alcohol & aldehyde)

2. What **functional group(s)** might the following molecules contain?

a) A compound with a strong absorption at 1710 cm^{-1} _____

b) A compound with a strong absorption at 1540 cm^{-1} _____

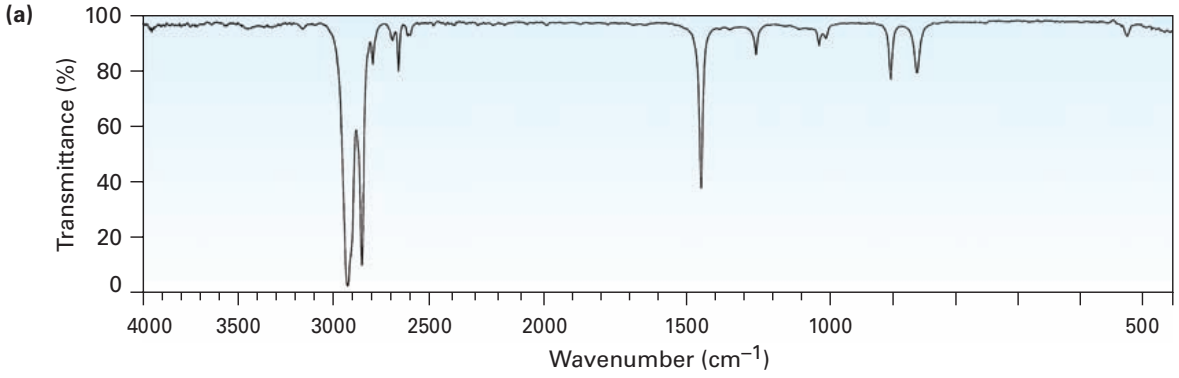
c) A compound with strong absorptions at 1720 cm^{-1} *and* 2500-3100 cm^{-1} _____

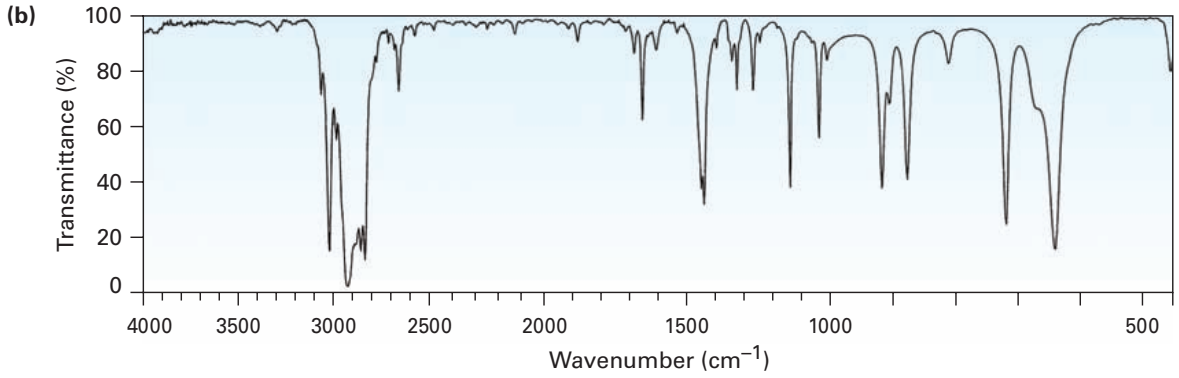
3. Acetone (CH_3COCH_3) and 2-propen-1-ol ($H_2C{=}CHCH_2OH$) are isomers. Draw the structures below. How could you **distinguish them by IR spectroscopy**? Reference expected wavenumber ranges from the IR table of values (last pages).

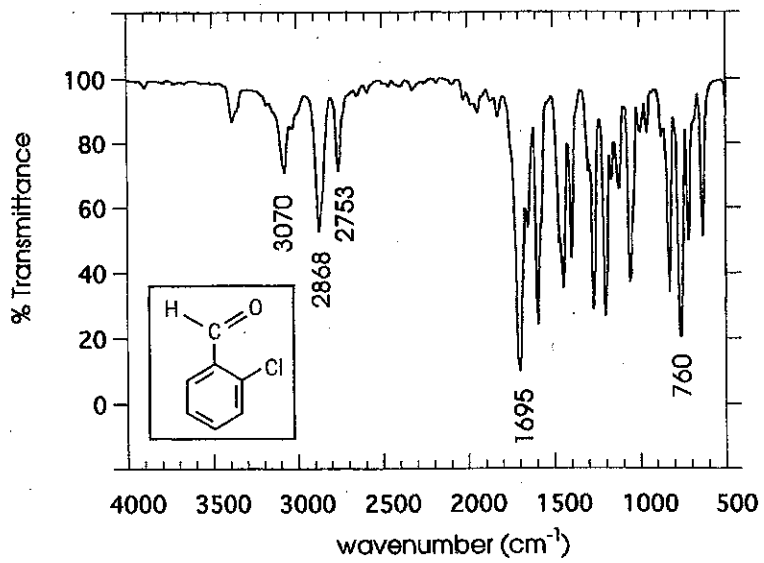
4. Propose at least two structures each for compounds that meet the following descriptions:

a)	**b)**
C_5H_8, with IR absorptions at 3300 *and* 2150 cm^{-1}	C_4H_8O, with a strong IR absorption at 3400 cm^{-1}

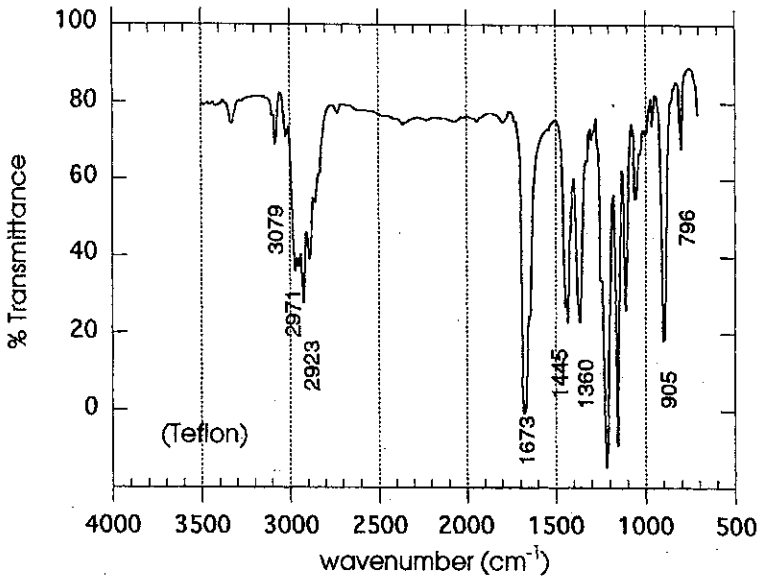
5. Consider the two IR spectra below. One is for cyclohexane and the other for cyclohexene. **Draw both structures and correlate to the spectra below.** Briefly explain your answer, referencing expected **wavenumber ranges** from the IR table of values (next page).



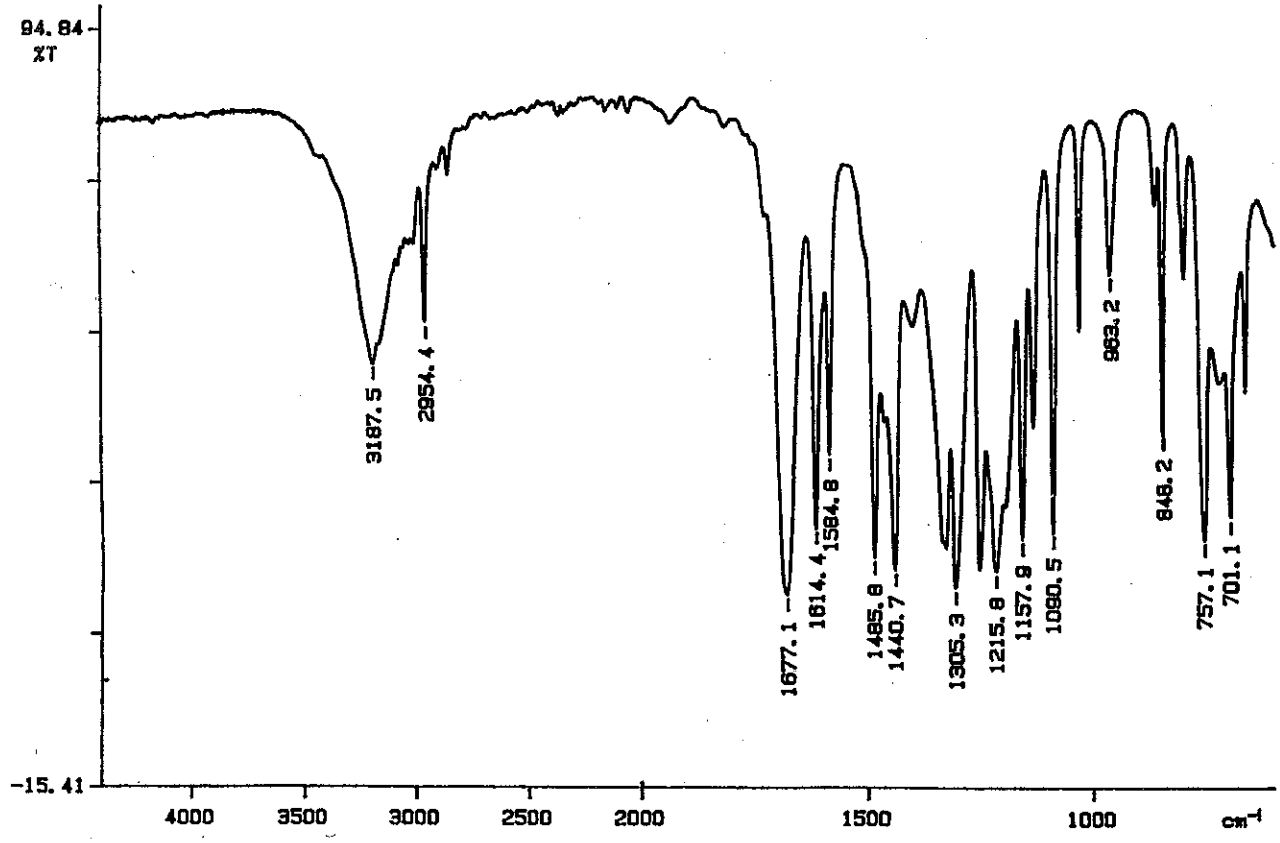




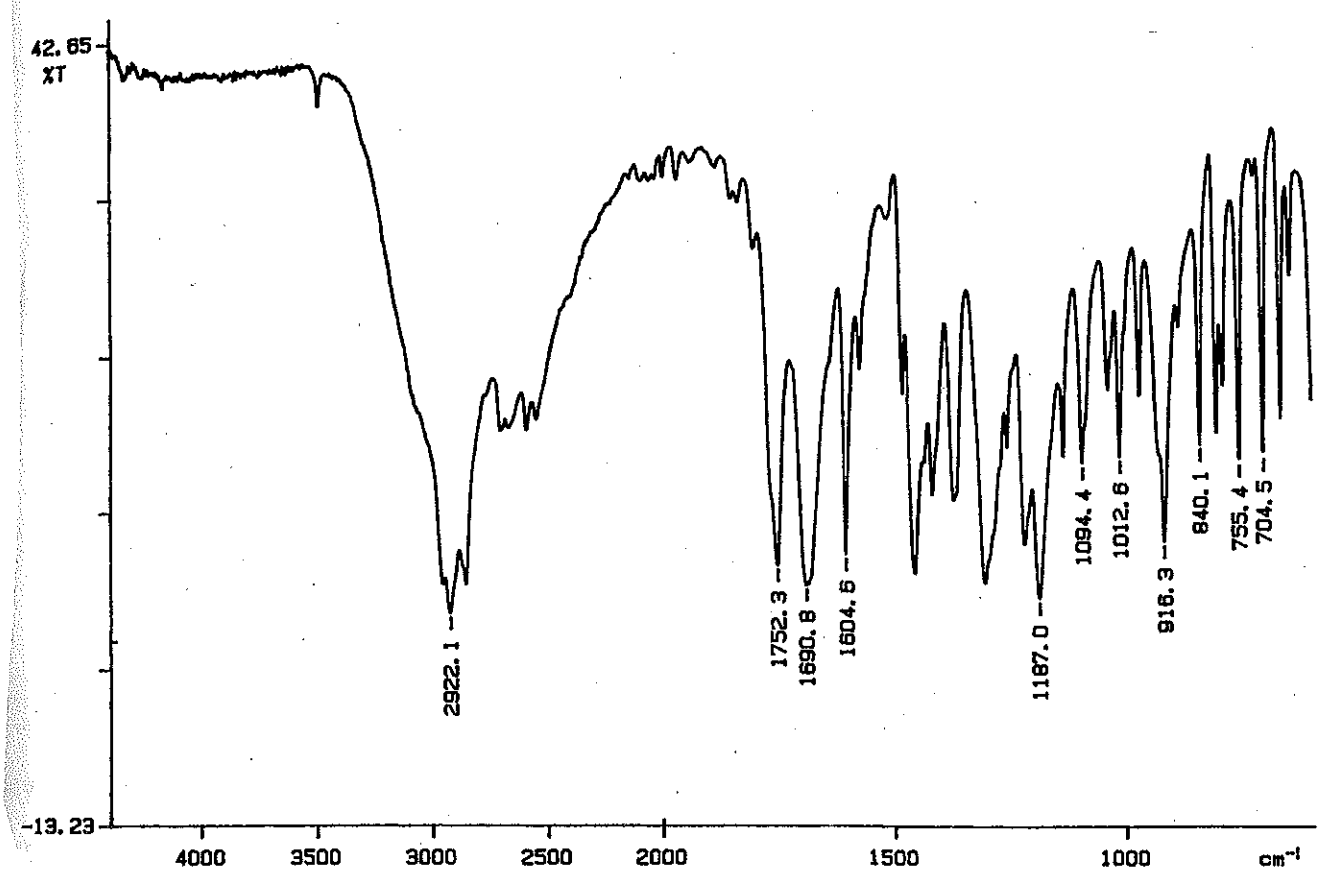
IR spectrum of *o*-chlorobenzaldehyde (vapor).



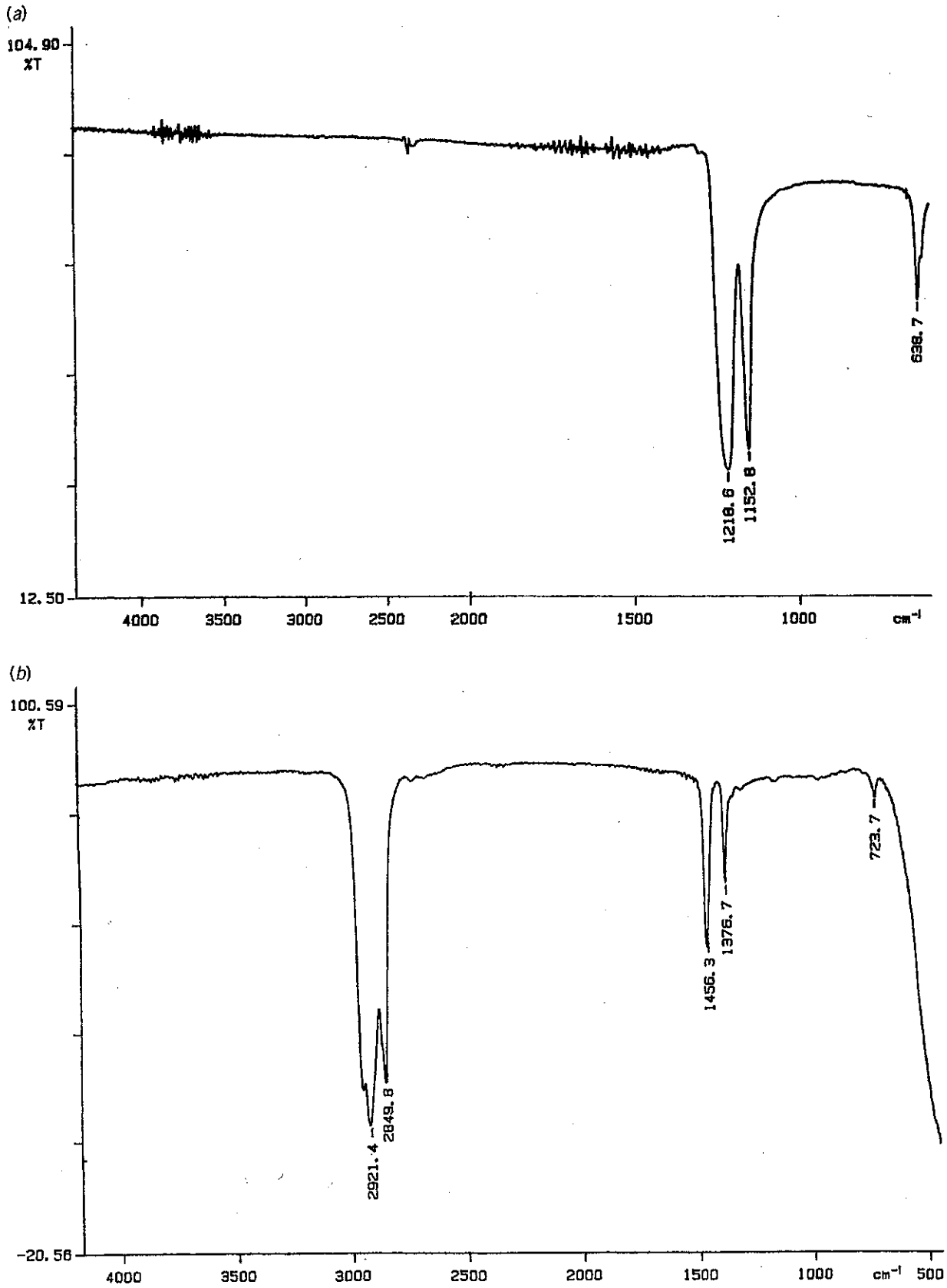
IR spectrum of carvone (Teflon).



IR spectrum of methyl salicylate (neat).



IR spectrum of aspirin (nujol).



a) IR spectrum of Teflon. *b*) IR spectrum of nujol.

Table 1. Characteristic IR Absorption Peaks of Functional Groups[i]

Functional Group & Bond Vibration	Position (cm^{-1})	Intensity*	Notes
Alkanes			
C-H stretch	2990 – 2850	m to s	
Alkenes			
=C-H stretch	3100 – 3000	m	
C=C stretch	1680 – 1620 (sat.) 1650 – 1600 (conj.)	w to m	
=C-H bend	995 – 685	s	See Table 2 for detail
Alkynes			
≡C-H stretch	3310 – 3200	s	
C≡C stretch	2250 – 2100	m to w	
Aromatic Compounds			
C-H stretch	3100 – 3000	m to w	
C=C stretch	1625 – 1440	m to w	Often hidden in fingerprint region
C-H bend	900 – 680	s	See Table 2 for detail
Alcohols**			
O-H stretch	3550 – 3200	br, s	Hydrogen bonded (typical)
Amines			
N-H stretch	3550 – 3250	br, m	Primary (two bands) Secondary (one band)
Nitriles			
C≡N stretch	2280 – 2200	s	
Aldehydes			
C-H stretch	2900 – 2800 & 2800 – 2700	s	H-C=O Fermi doublet
C=O stretch	1740 – 1720 (sat.) 1715 – 1680 (conj.)	s	
Ketones			
C=O stretch	1750 – 1705 (sat.) 1700 – 1665 (conj.)	s	
Esters**			
C=O stretch	1765 – 1735 (sat.) 1730 – 1715 (conj.)	s	
Carboxylic Acids**			
O-H stretch	3200 – 2500	br, m to w	
C=O stretch	1725 – 1700 (sat.) 1715 – 1680 (conj.)	s	
Amides			
N-H stretch	3500 – 3150	m	Primary (two bands) Secondary (one band)
C=O stretch	1700 – 1630	s	

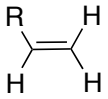
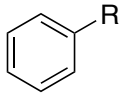
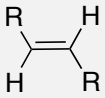
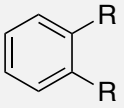
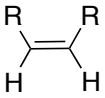
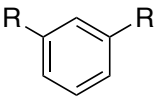
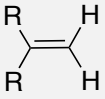
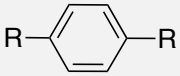
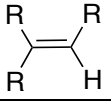
Table 1 cont'd

Vibration	Position (cm^{-1})	Intensity	Notes
Anhydrides**			
C=O stretch	1850 – 1800 & 1790 – 1740	s	
Acid Chlorides			
C=O stretch	1815 – 1770	s	
Nitro Compounds			
NO_2 stretch	1570 – 1490 & 1390 – 1300	s	
Thiols[ii]			
R-S-H stretch	2550 – 2600		
Alkyl & Aryl Halides†			
C-F stretch	1000 – 1400		Hidden in fingerprint region
C-Cl stretch	< 600 – 840		
C-Br stretch	< 700		
C-I stretch	< 600		

* Abbreviations: s = strong; m = medium; w = weak; br = broad; sat. = saturated; conj. = conjugated

** Alcohols, Esters, Carboxylic Acids, and Anhydrides also absorb in the fingerprint region due to the C-O stretch (1300 – 1000, s).

Table 2. Out-of-Plane C-H Bending Vibrations in Alkenes and Aromatics

Alkene Structure	Position (cm^{-1})	Phenyl Structure	Position (cm^{-1})
Mono-substituted 	997 – 985 & 915 – 905	Mono-substituted 	770 – 730 & 720 – 680
Disubstituted, *trans* 	980 – 960	Disubstituted, *ortho* 	770 – 735
Disubstituted, *cis* 	730 – 665	Disubstituted, *meta* 	810 – 750 & 725 – 680
Disubstituted, symm. 	895 – 885	Disubstituted, *para* 	860 – 800
Trisubstituted 	840 – 790		

[i] Adapted from…Mohrig, J. R.; Hammond, C. N.; Schatz, P. F. "Infrared Spectroscopy" in *Techniques in Organic Chemistry*. Freeman: New York, 2006.

Experiment 5 – Dehydration of Methylcyclohexanols

Learning Objectives

- Observe microscale distillation apparatus to collect products of a dehydration reaction
- Use gas chromatography to determine percent composition of products, exemplifying Zaitsev's rule
- Apply IR Spectroscopy to determine reaction success
- Interpret chemical tests to determine the presence or absence of alkene

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* Please find "How to Prepare & Assignments" after the procedure

As the name suggests, dehydration reactions involve the loss of water. A dehydration reaction is a type of elimination reaction with an alkene product (C=C double bond). The *regiospecificity* of the reaction is dependent on Zaitsev's rule, where the major product tends to be the more substituted alkene. When two different products are possible, these products are constitutional isomers of each other or in this case can be referred to as *regioisomers*. The type of *elimination mechanism* (E1 or E2) can depend on the type of reagent used as well as the substitution pattern of the starting material. In the case of the elimination of alcohols, reactions are performed under acidic conditions and therefore the E1 mechanism is favored. The exception would be for the dehydration of primary alcohols, which takes place *via* an E2 mechanism. Furthermore, when *isomerizable alkenes* are produced, the *stereochemistry* of the product (*cis* or *trans*) may be dictated by the type of mechanism taking place and the chirality of the starting material.

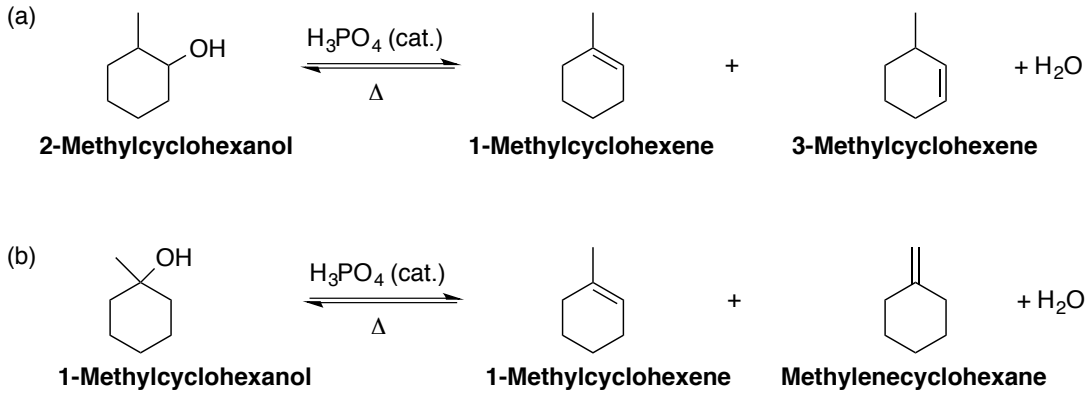


Figure 1. Dehydration of methylcyclohexanols

In this experiment, students will perform the acid-catalyzed dehydration of either **1-** or **2-methylcyclohexanol** (**Figure 1**). Students are assigned an alcohol starting material at the beginning of lab. The pre-lab questions should address both reactions to be prepared for either. The elimination reactions have two possible regioisomeric products. The major and minor products can be predicted according to Zaitsev's rule, which states that a more highly substituted alkene is more stable and favored. Students use gas chromatography (GC) to analyze the reaction mixture and compare to the retention times of commercially available standards to confirm their hypotheses. In addition, students confirm the presence of an alkene in the product with the potassium permanganate ($KMnO_4$) chemical test. $KMnO_4$ is lovingly called the "Barney reaction." It starts off dark purple and turns brown with the formation of a precipitate (MnO_2). Refer to the McMurry or Klein text for further discussion of the permanganate-mediated oxidative cleavage reactions.

PROCEDURE

Reaction Setup

Prepare a sand bath to reach a target temp of 150 °C on a hotplate at your station. Check the temperature periodically but *do not leave the thermometer in the sand bath*. Recall that hot plates should never be set higher than ½ of the maximum heat setting (med-low to medium is ideal). Keep an eye on temperature and adjust accordingly. It may be appropriate to turn off the hot plate for a little while. Do not touch the sand bath or container while it is hot!

The dehydration reaction will be run "neat" meaning no additional solvent is used. The alcohol acts as both the starting material and the solvent. Obtain 750 μL of the assigned alcohol using the provided pluringe and dispense into a pre-weighed 5-mL round-bottom flask, then obtain the mass of starting material by difference. 1-Methylcyclohexanol is a solid at room temperature (mp ~24 °C) and may be in a warm water bath. Carefully add 225 μL of concentrated phosphoric acid (H_3PO_4) using the pluringe provided. Note that conc. H_3PO_4 is an 85% w/w solution in water (85 g of H_3PO_4 per 100 g of solution; density = 1.685 g/mL). Add a boiling chip and clamp a Hickman still to set up the microscale distillation apparatus using a small amount of grease (include a sketch of this apparatus in the notebook). Use a plastic Keck clip to connect the flask to the still. Connect water hoses with clamps to a microscale condenser so that the water enters on the bottom and exits through the top. Attach the condenser on top of the Hickmann still to prevent product from evaporating. Immerse the flask a little more than half-way into the sand bath. It is okay to put the reaction in the sand bath before it reaches the target temperature.

Reaction Workup

As the reaction occurs, any alkene products that form will boil and collect into the Hickman still, driving the equilibrium to the right to make even more product! Keep a careful eye on the amount of liquid remaining in the flask. Turn off the heat once approximately half of the volume has been collected in the Hickman still or when half the volume in the flask is gone. Do not move the apparatus to cool as the distillate could drop right back into the reaction flask and the product must be distilled again. Using a pipet, carefully transfer the distillate to a screw-cap vial. Add small microspatula-fuls of Na_2SO_4 at a time until the drying agent runs free in suspension (snow globe-style). Cap the vial and let the system sit for 5 minutes. Filter through a pipet with a loose cotton plug as instructed by the TA and collect the filtrate into a pre-weighed dry screw-cap vial. Include a sketch of the filter pipet in the notebook. Record the mass. Recalculate the theoretical yield based on the mass of alcohol provided in lab and use this value to calculate % yield.

Analysis: IR, GC, and $KMnO_4$ Test – *perform in any order*

GC: Inject 0.2 μL of the product only. Compare with the retention times on the provided chromatograms to identify the peaks in your product. Integrate the peaks of the product mixture and calculate percent composition. Refer to the pages in your notebook for performing and analyzing GCs. You must analyze the chromatograms for retention time and percent composition before leaving the lab. Record your data in your notebook in table format.

IR: Obtain the IR spectrum of your product and starting material. Analyze both of these IRs in table format before leaving the lab. It is highly recommended, but not required, that you make an IR table for an alcohol and alkene before coming to lab (same format as in the IR exercise). At minimum, include in your notebook the expected stretches in alcohols and alkenes. The literature IR spectra for the alkenes are provided in a separate document online. Literature IR spectra for the alcohols are not provided and can be omitted from the table. Water can interfere with the IR spectrum of the product. If you observe an O-H stretch in the IR, it could either be from the presence of water or starting material. Analysis of the GC will be your definitive answer on that point.

Permanganate Test: Add 0.5 mL of the provided 0.5% $KMnO_4$ solution to four separate, 10 x 75 mm test tubes. Label the test tubes as follows:

1. Product 2. Cyclohexane 3. Cyclohexene 4. Alcohol (starting material)

Add 2-3 drops of product to tube #1. Carefully agitate the contents of the tube and record your observations (color change and/or formation of a precipitate). The formation of a black-brown precipitate is considered to be a positive test. Repeat the test adding cyclohexane to #2, cyclohexene to #3, and the starting alcohol to #4.

Table 1. Clean-up and Safety – copy into your lab notebook.

<table>
<tr><th>Clean-up</th><th>Safety</th></tr>
<tr><td rowspan="4">Liquid methylcyclohexanols waste:
*Remaining contents of the reaction flask (transfer by pipet)
* Product after analysis</td><td>H_3PO_4 is corrosive. Handle with care.</td></tr>
<tr><td>2-Methylcyclohexanol & Na_2SO_4 are irritants.</td></tr>
<tr><td>$KMnO_4$ is a strong oxidizer.</td></tr>
<tr><td>1-Methylcyclohexanol, cyclohexane, and cyclohexene are flammable.</td></tr>
<tr><td>Alkene tests waste:
*Use a pipet to transfer from test tube into waste.</td><td>Allow the sand bath to cool before breaking down. Handle warm/hot equipment with hot mitts provided in the lab, NOT paper towels.</td></tr>
<tr><td colspan="2">IR: Carefully wipe salt plates with salted acetone and return to the desiccator.
GC: Rinse GC needles 3x with acetone (regular, not salted) before and after injections.
DO NOT INJECT ALCOHOL as it will clog the syringe.</td></tr>
</table>

Experiment adapted from Palleros, D. “The Dehydration of Methylcyclohexanols” in *Experimental Organic Chemistry*. Wiley: New York, **2000**, p. 268 - 272.

How to Prepare & Assignments - Follow Exp 5 Canvas Module…

Before Lab

- Read this PDF and/or listen to podcast
- Attend and/or watch **lab lecture,** taking notes on **lecture templates,** and the **pre-lab videos**
- Practice the lab online, including common mistakes, on the **Slugs@home platform**
- **Pre-lab questions** incorporated into **Pre-lab Quiz** – check Canvas for due date

Lab Notebook Preparation – *Required before lab;* Use the **worksheet** to prepare your **lab notebook** …

- **Purpose:** brief summary of the main lab goals and dehydration reaction schemes
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info
- **Procedure with Diagrams** – hand-drawn using procedure in this PDF, Slugs@home, & class notes
  - Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!

During Lab

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A; Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

After Lab

- Individual: Upload **Notebook Pages** to Canvas by midnight on lab day – completeness / participation
- Work with your partner to complete the **Lab Report** – due date on Canvas
  - One student uploads the complete report to GradeScope (GS)
  - "**Select Pages**" then "**Add Group Members**" to include your partner's name

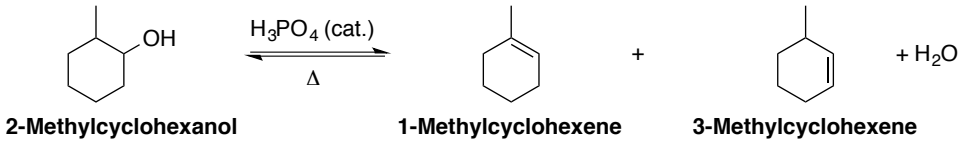
Additional Background Reading on Reactions

Reaction	**Sections in McMurry Organic Chemistry, 8th ed.**	**Sections in Klein Organic Chemistry, 3rd ed.**
Elimination Reactions	11.7-11.10	7.1, 7.6 – 7.7, 7.9, 7.11
Dehydration	17.6	12.9
Oxidative Cleavage of Alkenes – permanganate test	7.9	8.12

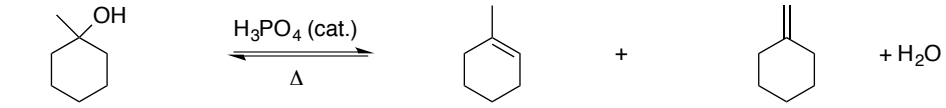
Pre-lab Questions

Incorporated into individual Canvas pre-lab quiz due the day before lab.

The acid-catalyzed dehydration of alcohols affords a mixture of two alkene isomers, along with water. Students carry out this reaction using with 750 μL of 1-methylcyclohexanol (density = 0.919 g/mL) or 2-methylcyclohexanol (density = 0.93 g/mL) and 225 μL of a concentrated solution of phosphoric acid (85% w/w, sol'n density = 1.685 g/mL). *Note: "w/w" = weight per weight, in this case 85 g of pure H_3PO_4 per 100 g of H_3PO_4 concentrated solution.*



2-Methylcyclohexanol **1-Methylcyclohexene** **3-Methylcyclohexene**



1-Methylcyclohexanol **1-Methylcyclohexene** **Methylenecyclohexane**

For 1-3: Show your work with units on every value using dimensional analysis – how units cancel.

1. **Convert the amounts of 1-methylcyclohexanol, 2-methylcyclohexanol, and phosphoric acid into mmols.** With the exception of molecular weights, all conversion factors are provided in the paragraph above. Calculate molecular weights (g/mol) using the structures above.

2. 1-Methylcycohexanol is the limiting reagent in the reaction (catalysts are regenerated, cannot be limiting). What is the **theoretical yield (in mg) of 1-methylcyclohexene** in this reaction? Both alkene products have the same molar mass. For simplicity, assume a 1:1 ratio of alcohol to 1-methylcyclohexene in the calculation.

3. 2-Methylcycohexanol is the limiting reagent in the reaction (catalysts are regenerated, cannot be limiting). **What is the theoretical yield (in mg) of 1-methylcyclohexene in this reaction?** Use the same assumption as in #2.

4. Do you expect the dehydration of 1-methylcyclohexanol and 2-methylcyclohexanol to proceed by an **E1 or E2 mechanism**? List **two factors** about these reactions to support your answer.

5. Draw the **products** for the reaction of **1-methylcyclohexene with $KMnO_4$** (McMurry Chapter 7.9 or see lecture notes – Klein text doesn't specifically cover oxidative cleavage with $KMnO_4$). Indicate the **by-product** that forms the **brown precipitate**.

6. What **compounds** do you expect to be in the **distillate** when the dehydration reaction is complete?

7. What is the **purpose of Na_2SO_4** in this experiment?

8. Look up the boiling points of **3-methylcyclohexene** and **methylenecyclohexane**. **Explain** why only one of these compounds is injected as a **standard for GC retention time**.

LAB REPORT

Complete the report with your lab partner – one student uploads to GradeScope

Abstract

Consult the writing guidelines to write a draft of the abstract after completing the in-lab questions and before leaving lab, time permitting. Either way, plan ahead to get help with the abstract by going to TA office hours or submitting a draft with the worksheet on Canvas.

- **Purpose** – read the introduction to this document to devise a one-sentence purpose
- **Methods** – include all chemical names, specialized glassware, and methods used for analysis
  - *Note:* this is NOT the procedure!
- **Results** – mass recovery (mg), percent yield, one main result for each method for analysis of product
  - GC: report percent composition only, not retention times & areas
- **Conclusion** – are the results consistent with predictions / purpose of the experiment? Restate the predicted major product and whether this was observed.

In-lab Questions

1. Report the **mass (in mg)** and **millimoles (mmol) of product** obtained (actual yield). Show your work for the mole calculation.

2. Calculate the **percent yield (% yield)** of the synthesis using the 'actual yield' from #1 and 'theoretical yield' from the pre-lab. Show your work.

% yield = [(actual yield) / (theoretical yield)] x 100%

3. Report the **retention times for the standards** in table format. Show your work for each calculation. Consider which reaction you were assigned (1-methyl or 2-methylcyclohexanol) to be sure you have retention times for both potential products. *Hint: refer to pre-lab questions for reminder on product retention times.*

4. Report the **GC retention times and integration for products. Identify each peak** in the chromatogram of the product.

5. Report the **percent composition of the products**. **Discuss** the distribution (ratio) of products in terms of the relative stability of the products.

6. Report and briefly discuss the results of the **permanganate test**.

7. Interpret the **IR results**. Include two typed tables for the detailed analysis of the IR spectrum of the starting material and product. Briefly discuss the **main identifying peaks** – how can IR be used to determine whether the reaction was complete?

8. The questions above were about individual forms of analysis. Now it's time to put it all together! Briefly discuss the **success of your dehydration reaction using chemical test results, IR, and GC data** combined. What is the **main product**? Are any other compounds present in your product mixture? If so, identify the impurities or by-products to the best of your ability.

9. What would be the substitution product of the reaction of **1-methylcyclohexanol** with a nucleophilic acid like **HCl** instead of H_3PO_4? Draw the full chemical reaction.

Name _____ Partner Name _____

TA Name _____ Section Day _____ Time _____

Experiment 5 – Dehydration of Methylcyclohexanols

Use as reference for notebook preparation – every student submits on Canvas individually after lab

Pre-Lab Requirements

1. **Dress for lab** – see safety rules – arrive a few minutes early
2. **Lab Notebook:** copy templates below into designated notebook
   - **Purpose, scheme,** and **reagent table**
   - **Procedure Diagrams** – must be complete before you can start the lab

A. Purpose and dehydration reactions:

B. Reagent Table

Name	Volume	Density	Mass	milli moles	Molecular Mass	Boiling or melting point	Hazards
1-methyl cyclohexanol*	750 µL						
2-methyl cyclohexanol*	750 µL						
Phosphoric acid, 85% w/w	225 µL	1.685					
Methyl cyclohexene product mixture	-	-					
Potassium permanganate, 0.5% solution	0.5 mL x 4	-	-	-		-	

* You'll be assigned either 1- or 2-methylcyclohexanol at the beginning of lab

C. Procedure Diagrams – hand-drawn using procedure in lab PDF, class notes, & Slugs@home

- Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
- Leave space to record additional **notes and observations** within the procedure diagrams

STEP 1. Reaction Setup – starting materials in flask and full microscale distillation apparatus
STEP 2. Reaction Workup – collect product, dry, filter, and weigh
STEP 3. GC Analysis – labeled sketches of each chromatogram
STEP 4. Analysis of IR spectrum – labeled sketches of IR spectra with main peaks labeled
STEP 5. Permanganate tests – contents and observations in each test tube

D. Accountability-Buddy Contract: Students work together to submit one report and get the same grade in GradeScope. Add your name to one box in part **(a)** and schedule a time to collaborate after lab in part **(b).**

(a) *Who's finalizing what?* Discuss the in-lab questions in the lab PDF with your partner during / after lab. Decide who will type the final abstract and type or draw the revised responses to which in-lab questions.

Name	**Report Component** **Abstract / In-Lab #**

(b) "DO" Date: _____ = when / how you'll meet or exchange work to discuss & proofread, at least 1-2 days before the DUE date to give each other time to review & revise responses.

Who will combine both sets of in-lab questions and submit as one PDF to GradeScope? _____

E. Exp 5 Data

Assigned alcohol _____

Alcohol volume _____ Theoretical Yield _____mg

Calculations:

Actual Product Yield _____ mg

_____ % Yield

Standard GC Corrected Retention Times

Sample	Corrected t_R' (s)

GC Analysis of Product

Peak #	Corrected t_R'	Peak ID	Integration (cm^2)	% composition

Permanganate Tests

Sample	Observations	Interpretation
1. Product		
2. Cyclohexane		
3. Cyclohexene		
4. Alcohol (starting material)		

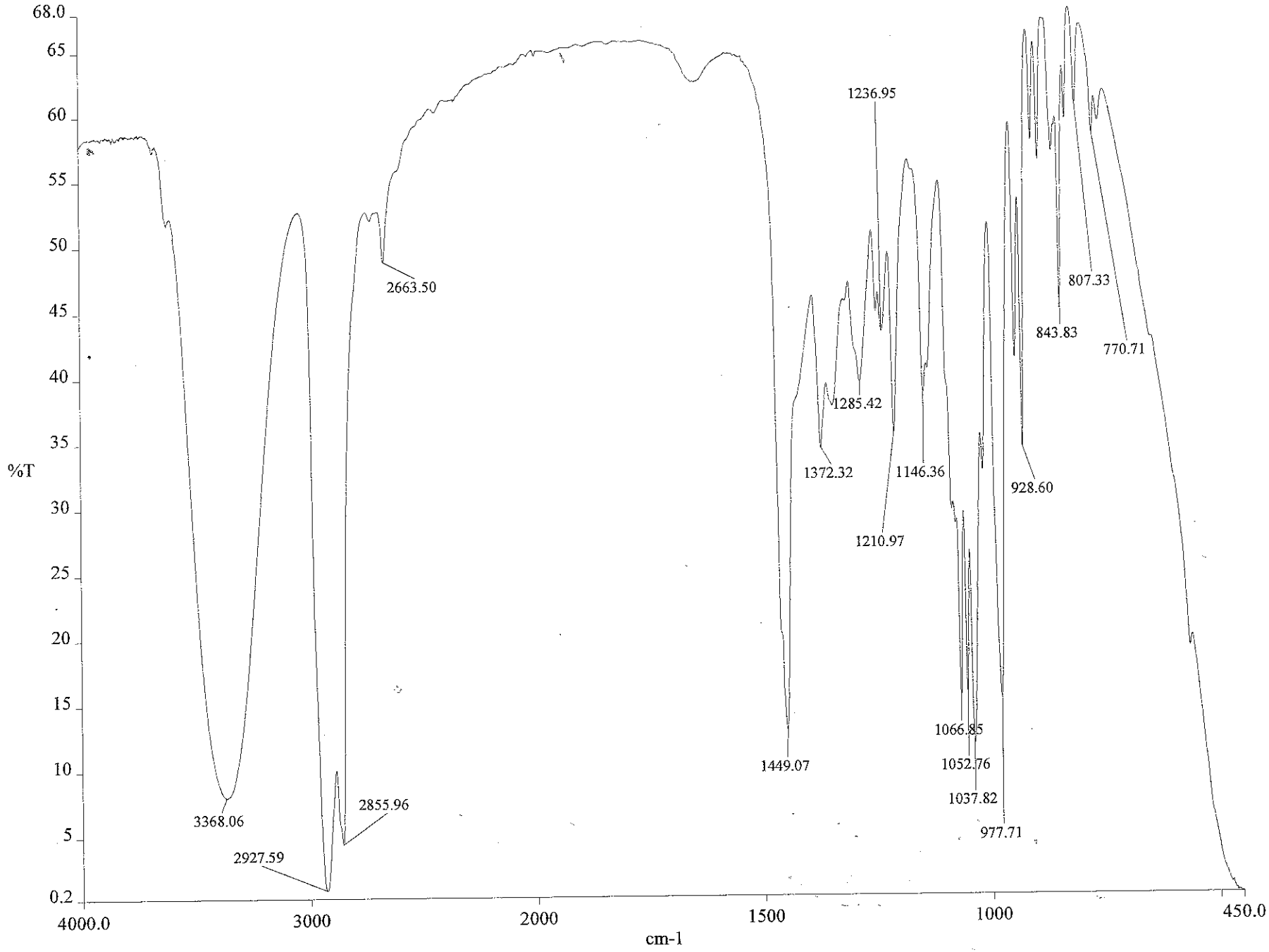
IR Spectrum - Alcohol

Functional Group	Bond Assignment (C=O, N-H, etc.) from IR Table	Expected Wavenumber Range (cm^{-1})	Observed Wavenumber (cm^{-1})

IR Spectrum – Major Product Only

Functional Group	Bond Assignment (C=O, N-H, etc.) from IR Table	Expected Wavenumber Range (cm^{-1})	Observed Wavenumber (cm^{-1})

Optional & encouraged: Submit a draft of the Exp 5 abstract for feedback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2663.50
1449.07
1372.32
1285.42
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1210.97
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Experiment 6 – Synthesis of *t*-Pentyl Chloride

Learning Objectives

- Observe liquid-liquid interface of two immiscible, clear liquids
- Perform a liquid-liquid extraction in a basic reaction "workup"
- Use gas chromatography (GC) to determine percent composition of products
- Apply infrared (IR) spectroscopy to determine reaction success
- Interpret chemical tests to determine presence or absence of alkyl halide

Alcohols are versatile starting materials in organic synthesis. They can act as an acid, base, nucleophile, or electrophile, depending on the reagent that they are paired! In the dehydration lab, students observed the acid-catalyzed elimination of alcohols at an elevated temperature (**Figure 1**). Elimination was the only product possible because the conjugate base of the acid (H_3PO_4 → HPO_4^-) is not a nucleophile. The addition of heat also promotes elimination over substitution.

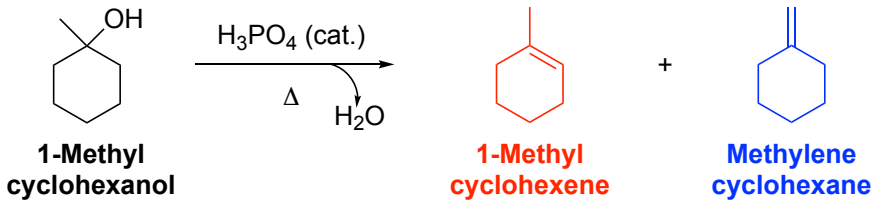

Figure 1. Dehydration of 1-methylcyclohexanol (Experiment 5)

When a haloacid (HX) like HCl is used, however, the reaction favors the substitution route (**Figure 2**). When the alcohol is protonated by HCl, a chloride ion (Cl^-) is formed as the conjugate base. The reaction in this lab occurs by an **S_N1 mechanism** because *t*-pentanol is a **tertiary alcohol**. An S_N2 reaction could *never* occur at a tertiary center due to steric hindrance. Alcohol protonation creates water as the **leaving group**. The C-O bond breaks spontaneously in a rate-limiting (slow) step to form a tertiary carbocation. Cl^- is the **nucleophile** carbocation to form a new C-Cl bond.

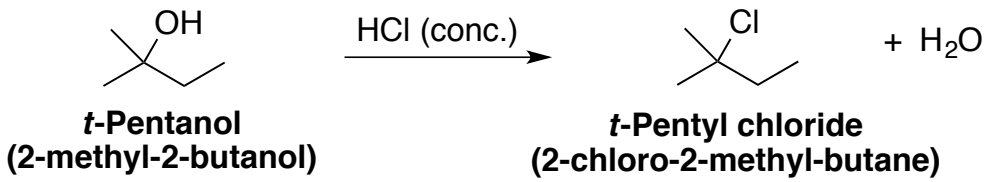

Figure 1. Synthesis of *t*-pentyl chloride

Competition with elimination is not an issue and alkene side-products are not formed because the reaction occurs at room temperature. The isolation of the product from solvent and by-products is called the **reaction work-up**. The crude reaction mixture is washed with water to dilute and remove excess HCl. Aqueous sodium bicarbonate neutralizes any remaining, unreacted HCl. These washes should be done relatively quickly to prevent hydrolysis of the product back to alcohol. If left to sit for 15+ minutes, the weakly basic $NaHCO_3$ solution begins to react with water to form ^-OH ions. Use of a stronger base would facilitate a much more rapid hydrolysis back to the alcohol so the weak base is preferred.

After work-up, the crude reaction mixture is analyzed by gas chromatography (GC), infrared (IR) spectroscopy, and chemical tests for alkyl halides (silver nitrate test and sodium iodide test). Time permitting, the product can be further purified by distillation. GC provides conclusive determination of reaction completion or percent composition if alcohol remains. IR is used to determine the presence or absence of the O-H and C-Cl bonds. The chemical tests determine the presence of an alkyl halide as evidenced by formation of a white precipitate, but cannot detect whether any alcohol or other impurity is present (**Table 1**).

Table 1. Reactions for positive chemical tests

Silver Nitrate in Ethanol Test	**Sodium Iodide in Acetone Test**
$RX_{(l)} + EtOH_{(l)} \rightarrow ROEt_{(l)} + HX_{(sol'n)}$ $AgNO_{3\,(sol'n)} + HX_{(sol'n)} \rightarrow HNO_{3\,(sol'n)} + AgX_{(s)}$	$RX_{(l)} + NaI_{(sol'n)} \rightarrow RI_{(l)} + NaX_{(s)}$
R = alkyl chain; X = Cl, Br, I	

The 'tails - Follow Exp 6 Canvas module

Before Lab

- Read this **PDF** or listen to **podcast** and watch the **pre-lab videos** on the Exp 6 Overview page
- Attend and/or watch **lab lecture** with **Exp 6 notes templates**
- Preview the lab on the **Slugs@home** platform!
- **Pre-lab questions** incorporated into **Pre-lab Quiz** – check Canvas for due date

Lab Notebook Preparation – *Required before lab;* Use the **worksheet** to prepare your **lab notebook** …

- **Purpose:** brief summary of the main lab goals and substitution reaction scheme
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info
- **Procedure with Diagrams** – hand-drawn using procedure in this PDF, Slugs@home, & class notes
  - Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!

During Lab

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A; Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

After Lab

- Individual: Upload **Notebook Pages** to Canvas by midnight on lab day – completeness / participation
- *Option* to work individually or with ONE partner to complete the **Lab Report** – due date on Canvas
  - One student uploads the complete report to GradeScope (GS)
  - "**Select Pages**" then "**Add Group Members**" to include your partner's name

PROCEDURE

Reaction set-up: Check the provided conical vial for leaks by adding a little water, closing, and inverting. Discard the water and dry the vial with a paper towel. Use a Pasteur pipet and pluringe to carefully transfer 1.00 mL of *t*-pentanol (2-methyl-2-butanol) and 2.5 mL of concentrated HCl (37% w/w, density = 1.2 g/mL) directly into a 5-mL conical vial.** Record the least count of the pluringe and determine the ILE. Cap the vial and let the mixture stand for a minute. Then carefully shake the mixture with occasional venting in the fume hood (partially unscrew the cap to vent, then close). None of the mixture should leak from the vial but do not tighten so much that you can't unscrew it later. When pressure builds, it will be harder to open.

** Change gloves after getting reagents, whether or not you think your gloves are contaminated!
** Do not cross-contaminate pipets and be extra careful not to spill HCl (corrosive).
** Keep all reagent bottles in the fume hoods.
** Recap reagent bottles immediately, even if someone is right behind you about to use it.

Reaction work-up: Allow 10-15 minutes for the two phases to completely separate. Remove the water using a pipet and save the layer containing alkyl halide. **Use the densities to determine which layer is aqueous.** Quench and wash the reaction mixture with water as follows: Add 1 mL of water, mix, allow the layers to separate, then remove the water. Add 1 mL of 5% $NaHCO_3$ solution. Carefully agitate and vent. **What gas is formed in this step?** Allow the layers to separate and remove the aqueous layer. Add 1 mL of brine (saturated NaCl), mix, then remove as much water as possible on this last wash.

Add a small amount of anhydrous sodium sulfate (Na_2SO_4) using a micro-spatula. Allow this drying agent to absorb water for at least 5 minutes. Make a filter pipet with a tiny piece of loosely packed cotton and weigh a labeled vial for product. Use a second pipet to filter the product mixture through the filter pipet to remove the (Na_2SO_4) hydrate and collect the product in the pre-weighed vial.

Analysis: **Weigh the product** and **calculate % yield**. Inject and analyze GC chromatograms of standards (*t*-pentyl alcohol and *t*-pentyl chloride) and the reaction mixture. Analyze the provided IR spectra of *t*-pentyl alcohol and obtain the IR spectrum of the reaction mixture to determine the absence of *t*-pentyl alcohol and presence of *t*-pentyl chloride.

Perform *either* the Silver Nitrate *or* Sodium Iodide chemical tests (not both) as described below. Chemical tests are performed in the fume hood. Obtain four clean, dry medium test tubes and label with #1-4 using the following designations. **Which do you expect to give positive vs. negative tests for alkyl halides?**

1. *t*-Pentanol (starting material)	**2.** Product mixture	**3.** Bromobenzene	**4.** Butylbromide

Silver Nitrate Test: Add 0.5 mL of 0.1 M silver nitrate in ethanol to each test tube and one drop of compounds 1-4 to the appropriate test tube. Gently agitate (tap) the test tubes and patiently wait 5 minutes to observe precipitation. If no solid forms, bring the solutions to a boil in the community water bath in the fume hood set to around 80 °C. Wait another 5 minutes to see if precipitation occurs. Record your observations. The formation of a precipitate is a positive test for alkyl halides.

Sodium Iodide Test: Add 0.5 mL of the provided sodium iodide solution (15% w/v in acetone) to each test tube followed by one drop of compounds 1-4 to the appropriate tube and gently agitate. If no precipitate is observed after 3 minutes, transfer the test tubes to a water bath set to about 50 °C and heat for 5 minutes. Record your observations. The formation of a precipitate is a positive test for alkyl halides.

Table 2. Clean-up & Safety

Liquid waste: For all the liquids, including product, after you're sure you're done with them! *Rinse the test tubes with a small amount of ethanol into the liquid waste before washing in the sink.	Concentrated HCl is very corrosive. It will burn through your clothes and/or skin. Take only what you need and keep the bottle in the reagent hood. *t*-Pentyl alcohol, butylbromide, bromobenzene, and acetone are flammable.
Solid waste: pipets	

Adapted from Palleros, D. "Synthesis of *n*-Butyl Bromide and 2-Chloro-2-Methylbutane" in *Experimental Organic Chemistry*. Wiley: New York, **2000**, p. 280 - 291.

Pre-lab Questions - incorporated into pre-lab quiz due before lab – see date on Canvas

1. What type of reaction mechanism is exemplified in this lab? Why is this mechanism favored and why is no elimination product observed?

2. What is the by-product of the substitution reaction of t-pentyl alcohol with HCl?

3. Reaction Calculations - Show your work!

- Calculate the mmoles of both starting materials (*t*-pentyl alcohol and HCl) using the amounts given in procedure.
- Indicate the limiting reagent in this 1:1 reaction.
- Calculate the theoretical yield of product in mmol and mg.

4. Why is the product washed with sodium bicarbonate after the reaction is complete? Show the chemical equation for the reaction of sodium bicarbonate with HCl.

5. Explain why sodium bicarbonate is used instead of NaOH in the extraction.

6. Show the chemical equation for the substitution reaction of *t*-pentyl chloride with sodium iodide in acetone. What compound precipitates as a white solid?

7. Show the chemical equation for the substitution reaction of *t*-pentyl chloride that occurs in a positive silver nitrate in ethanol test. What compound precipitates as a white solid?

EXP 6 LAB REPORT

- *Option* to work individually or with ONE partner to complete the **Lab Report** – due date on Canvas
  - One student uploads the complete report to GradeScope (GS)
  - "**Select Pages**" then "**Add Group Members**" to include your partner's name

In-lab Questions (no abstract for Exp 6)

1. Draw the full arrow-pushing mechanism for the reaction of *t*-pentanol with HCl. *Hint: this mechanism has three steps with two reaction intermediates.*

2. Restate the theoretical yield (mmol and mg) from the given volume of alcohol. Report the actual yield and calculate the percent yield. Show your work.

3. The reaction work-up involved a liquid-liquid extraction with an organic (ORG) and aqueous (AQ) layer. What molecule makes up the ORG layer and was it on the top or bottom? Explain which layer was which and why they are not miscible (created separate layers). *Hint: comment on the polarity of the two layers and include the density values for t-pentyl chloride and water.*

4. What was the purpose of adding aqueous sodium bicarbonate ($NaHCO_3$, baking soda) in the reaction work-up? Show the balanced chemical equation for the reaction of sodium bicarbonate with HCl and indicate the gas formed at this step. *Yes, this is very similar to a pre-lab question … revisit yo quiz* ☺

5. Report which chemical test was performed. Tabulate all chemical test results with observations and brief interpretation of each. Was the reaction successful based on these results alone? Explain why or why not, including comparison to standard test results.

Table x. Chemical Test Results – sodium iodide in acetone or silver nitrate in ethanol

Sample	Observation	Interpretation
1. *t*-Pentanol		
2. Product Mixture		
3. Bromobenzene		
4. Butyl bromide		

6. Draw the two chemical reactions that occurred in the two positive chemical tests reported in #5 above: starting material, reagent & solvent (either sodium iodide in acetone or silver nitrate in ethanol), and product. *Revisit your Exp 6 pre-lab quiz for related questions* ☺

7. Interpret the IR spectra of the starting material and product in table format (see worksheet). Which band(s) of the IR spectra are used to determine conversion of alcohol to alkyl halide and was the reaction successful based on IR data alone?

8. Interpret the GC charts of starting material and product. Calculate retention times and integration (area) to determine percent composition of products. Report your results in table format. Show your work for each calculation. Conclude with a statement about reaction success based on GC results in combination with chemical test and IR data above, including presence of by-products in your product mixture.

Name _____ Partner Name _____

TA Name _____ Section Day _____ Time _____

Experiment 6 – Synthesis of *t*-Pentyl Chloride

Use as reference for notebook preparation – every student submits on Canvas individually after lab

Pre-Lab Requirements

1. **Dress for lab** – see safety rules – arrive a few minutes early
2. **Lab Notebook:** copy templates below into designated notebook
   - **Purpose, scheme,** and **reagent table**
   - **Procedure Diagrams** – must be complete before you can start the lab

A. Purpose and substitution reaction:

B. Reagent Table

Name	Volume	Density	Mass	milli moles	Molecular Mass	Boiling or melting point	Hazards
t-pentanol (*t*-pentyl alcohol)							
HCl, 37% w/w		1.2 g/mL					
t-pentyl chloride (product)	-						

C. Procedure Diagrams – hand-drawn using procedure in lab PDF, class notes, & Slugs@home

- Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
- Leave space to record additional **notes and observations** within the procedure diagrams

1. **Reaction set-up** – adding chemicals to conical vial, mix, & sit
2. **Reaction work-up** – refer to extraction diagram in lecture notes
3. **Silver Nitrate or Sodium Iodide Test** – contents of each test tube & observations
4. **GC Analysis** – labeled sketches of each chromatogram
5. **Analysis of IR spectrum** – labeled sketches of IR spectra with main peaks labeled

D. Accountability-Buddy Contract: Students work together to submit one report and get the same grade in GradeScope. Add your name to one box in part **(a)** and schedule a time to collaborate after lab in part **(b).**

(a) *Who's finalizing what?* Discuss the in-lab questions in the lab PDF with your partner during / after lab. Decide who will type the final abstract and type or draw the revised responses to which in-lab questions.

Name	**Report Component** **Abstract / In-Lab #**

(b) "DO" Date: _____ = when / how you'll meet or exchange work to discuss & proofread, at least 1-2 days before the DUE date to give each other time to review & revise responses.

Who will combine both sets of in-lab questions and submit as one PDF to GradeScope? _____

E. Experiment 6 Data

Volume of *t*-pentyl alcohol _____ Theoretical yield _____

- What gas is released during the reaction workup? _____
- Is the product in the top or bottom layer? _____
- Notes on potential Product Loss:

Mass of product _____ % yield =

Chemical Tests: Silver Nitrate in Ethanol *or* **Sodium Iodide in Acetone** (circle one)

Sample	Observations	Interpretation
1. *t*-Pentanol		
2. Product mixture		
3. Bromobenzene		
4. Butyl bromide		

Draw the two chemical reactions that occurred in all positive chemical tests reported above: starting material, reagent & solvent (either sodium iodide in acetone or silver nitrate in ethanol), and product. *Revisit your Exp 6 pre-lab quiz for related questions* ☺

IR Spectrum - Starting material - *t*-pentyl alcohol, draw structure

Functional Group	Bond Assignment (C=O, N-H, etc.) from IR Table	Expected Wavenumber Range (cm^{-1})	Observed Wavenumber (cm^{-1})

IR Spectrum - Product mixture, draw product structure

Functional Group	Bond Assignment (C=O, N-H, etc.) from IR Table	Expected Wavenumber Range (cm^{-1})	Observed Wavenumber (cm^{-1})

- Is water potentially present in the product mixture? _____

GC Standards Chart speed: 2.5 cm/min

Peak ID	Corrected Retention Time, t_R' (sec)
t-Pentyl Alcohol	
t-Pentyl Chloride	

Product Mixture GC Results

Peak ID	Corrected Retention Time, t_R' (sec)	Integration (cm^2)	Percent Composition (%)

- Is starting material present in the product mixture? _____

Retention Time Calculations, t_R'

Integration / Area Calculations:

Calculation of Percent Composition: