CHEM 8L Lab Manual, Fall '25

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CHEM _____ Qtr _____ Section _____ TA _____

LABORATORY SAFETY RULES

Safety First!

The instructors wish students an enjoyable lab experience! The best way to set you up for success is to provide you with lab common sense and etiquette, as much of the surroundings and techniques will be new to you. In general, be respectful of the space and follow instructions and you'll be fine, however, we may need to enforce repeated violations of the rules with point penalties, either for an individual or the whole section, but we hope it won't come to that! Thanks for reading and doing your best as you learn.

1. **Safety goggles must be worn** at all times when anyone in the room is working with chemicals, especially yourself! You are welcome to step outside to defog goggles if necessary.

2. **NO food, drinks, or gum** are allowed anywhere in the labs or in your mouth while you're in the labs. All water bottles, snacks, etc. should be left on the table outside the lab, not hidden in your bag. You may step into the hallway for a quick drink or snack during appropriate times, as long as you're not neglecting the experiment or your partner. Please check with your TA beforehand.

3. **Appropriate lab attire** must be worn at every lab. Students cannot go home to change.
   - **OK LAB ATTIRE:** Pants or long skirt, short or long-sleeve shirt, closed-toe shoes that cover the entire top of the foot. Long hair and loose clothing are confined or tied back.
   - **NOT OK:** Shorts or short skirts (no exposed ankles), *leggings/tights*, cropped pants that expose ankles, ripped pants that expose skin, tank tops, sandals, ballet flats, or any other shoes that expose the tops of the feet (Crocs and Tom's are NOT OK!). High heels, baggy clothing, and dangling jewelry are strongly discouraged.

4. **Lab coats** must be worn over appropriate lab attire (see above).

5. **NO running, fighting, or other acts of mischief**.

6. **NO visitors**, including pets and side-kicks.

7. Know the **locations of emergency equipment** including fire alarms, fire extinguishers, chemical fume hoods, safety showers, and emergency eye washes.

8. **Notify your instructor immediately of any injury, spill, fire, or explosion.** You may clean up small spills (less than a few milliliters) yourself, but let the TA know. You're not in trouble unless you do it on purpose!

9. Keep your lab space **clean and organized throughout the experiment**. Backpacks, purses, jackets, phones, etc. are not allowed where chemicals are being used.

10. **Never leave an ongoing experiment unattended**. If you need to leave the room, be sure a neighbor is watching your experiment.

11. Unless otherwise specified, dispose of broken glassware in broken glassware boxes only, including ceramics and disposable glass pipets. NO paper or other items in the broken glass boxes. **NO PIPETS OR OTHER GLASSWARE IN THE TRASH!** That's not cool to the staff and you'll lose points.

12. **DO NOT TASTE ANYTHING IN THE LAB**. EVER.

13. **Never remove chemicals or equipment** from the labs or stockroom without permission.

14. **NO unauthorized experiments**. Stick to the given procedure.

CHEM _____ Qtr _____ Section _____ TA _____

15. Wash your hands and arms with soap and water before you leave the lab, no matter what!

16. Always know the **hazards** as well as the physical and chemical properties of the materials used. Your lab notebook should include a brief note on the safety hazards for each chemical being used based on the safety table within in the experiment.

17. **Read labels carefully and know the name of chemicals with which you are working**. Read labels twice before dispensing chemicals. Read labels twice ;)

18. **Label all containers** with chemical/mixture names, your name, and the date **before** anything goes into that container.

19. **Use pluringes and pipet bulbs with glass pipets**. NEVER pipet by mouth. It's gross.

20. Check all **glassware for cracks and cleanliness** before using…or you'll be sorry later that you didn't.

21. **Avoid contamination**. Take only what you need from reagent bottles. NEVER return unused chemicals to the original bottle that other students are sharing.

22. **Fume hoods** are used to minimize chemical exposure. Handle chemicals **six inches** into the hood, away from you.
- DO NOT PUT YOUR HEAD IN THE HOOD – this would cause you to breathe the chemicals you want to avoid!
- DO NOT KNEEL in front of the hood – that would be a tripping and contamination hazard.

23. **Wash all glassware** before leaving lab for the day and **return all shared equipment** to the designated space.
- DO NOT WEAR GLOVES WHILE WASHING GLASSWARE.

25. **Dispose of all waste as instructed in the lab handout or by the TA.** Waste containers are typically in the fume hoods. Read waste container labels carefully to be sure it's going to the right place. Let your TA know if a waste container is full. DO NOT OVERFLOW THE WASTE CONTAINERS!

26. **NO use of flame in the lab**. Nearly everything in the organic chemistry labs is flammable.

27. ***Wear gloves*** when appropriate in the lab and ***change your gloves*** if you get chemicals on them. <u>They're cheap</u>! Gloves are only a first line of protection. They do not make you invincible! Take off gloves and wash your hands before you leave the room. **DO NOT touch door handles, your face, computers, or phones with gloved hands.**

28. ***Minimize chemical exposure*** (ex. keep containers capped) and treat every chemical as if it were hazardous.

29. **No cell phones or electronic devices are allowed to be used in the labs.** If you'd like to take a picture or video of your experiment, ask your TA for permission, but take your gloves off first.

30. **Treat your TA and labmates respectfully.** Please adhere to your TA's instructions and additional safety guidelines announced. Reach out to instructors if you are having interpersonal issues in the lab and we'll help as much as we can.

31. Please restore your locker to its original status. All equipment must be clean and organized in the drawer, whether or not you used those items.
- Check the equipment list and reference the 'perfect drawer' pic on the bulletin board in the lab.
- Replace any missing or broken items with a quick trip the stockroom. Do not bring broken items with you – place them in the glass or solid waste instead.
- **Check for missing, dirty, broken, and extra items** that may have fallen into your drawer, or ones that belong in your partner's drawer.

Name _____ Section Day & Time _____

TA Name _____ TA Office Hours _____

TA Email _____ Thimann Labs Room _____

Organic Chemistry Lab Orientation Activity

Complete during the first lab meeting; upload to GradeScope after lab, by midnight

Welcome to the organic teaching labs! We want your time in the lab to be just as **enjoyable** as it is **educationa**l. You'll learn how to **handle equipment** properly to **eliminate or minimize contamination** and **chemical exposure**. Please keep your lab **space neat** and **organized**, similar to cleaning the kitchen dishes as you're cooking or baking. **Instrumentation** and **equipment** are **shared** with the rest of the class so we'll work together to **stay safe**. This three-part activity is designed to teach you **how to keep the labs** in smooth, working order to **set you up for success** in your experiments.

Lab Orientation Content

A. Equipment Locker Drawer
B. Lab Safety Scavenger Hunt
C. Lab Basics Activity – Intra- vs. Inter-molecular Forces

Part A. Equipment Locker Drawer

You'll be assigned a drawer that contains glassware and equipment for completing your experiments.

Drawer Number & Letter _____ **Locker Combination _____**

- On or near the bulletin board: find the laminated **locker equipment list**, pictures of **glassware**, and pictures of **how to organize the drawers**.
- At the end of each lab, **pick up each item** from your drawer to make sure there are **no cracks** or **chemicals** present, even if you didn't use that equipment that day.
- There should be no **missing, extra, dirty, or broken items** after lab.
- Clean & Organized Drawer = _____ Experiments 😊

Intro Activity: Review the equipment list and pictures of glassware. **Sketch and name at least five (5)** items from your drawer, especially if they are new to you.

Part B. Safety Scavenger Hunt

Get to know your space!

Work with your lab-mates to lab items and their corresponding tag with information on safe use, precautions, and etiquette. Fill in the blanks on the following pages with the information on the tags. Some items, like sinks, are in multiple location, only one of which has an info tag. Emergency items may be outside the lab.

Make a LAB MAP on the next page and mark the locations by number.

You must have a complete map & description before leaving the lab.

Emergency Response	**Day-to-Day**	**Equipment**
1. Fire Extinguisher **2.** Fire Alarm **3.** Safety Shower **4.** Eyewash Station **5.** **5A** – Evacuation Procedure **5B** – Power Outage Protocol **6.** First Aid Kit **7.** Broken Glassware Box, Dust Pan & Broom **8.** Spill Control Center	**9.** Balance Station **10.** Sink **11.** Chemical Waste Station **12.** Dry Waste Box **13.** Chemical Fume Hoods **14.** Reagent Station (Chemical Reacting Agents) **15.** Disposable Gloves and Shared Goggles	**16.** Equipment Room (GC & IR) **17.** Rota-vap **18.** Water Re-circulation Pumps (water lines) **19.** Ring stands **20.** Clamps, clamp holders, and support rings **21.** Vacuum tubing **22.** Hot / stir plates

23. Your TA – go say hi! **24.** One-word hazard definitions & precautions **25.** Fire Protection (NFPA) Labels **26.** Lab coats **27.** Stools	**28.** Cotton **29.** Filter paper **30.** MelTemp apparatus **31.** Glass pipets **32.** Tape and markers

Find the numbered **tag** in lab, label its **location** on your **map**, read the info tag, then **fill in the blanks**.

LAB MAP, Thimann Labs, Room _____

Mark the **locations** of all items numbered in your room. Start by adding the locations of the **door** and **your equipment drawer** relative to the student workspaces and hallway.

Student Workspaces

HALLWAY

Student Workspaces

Find the numbered **tag** in lab, label its **location** on your **map**, read the info tag, then **fill in the blanks**.

1. Fire Alarm - find the closest one in the hallway outside your lab

* Located at the _____building entrance and exit left of the elevator.
* Pull this alarm only if you see a fire. Don't assume someone else has called it in.
* Alert by-standers by yelling "_____**!**"
* Individuals should also notify the fire department by calling _____.
* Only _____ should attempt to extinguish a fire.

2. Fire Extinguisher - find the closest one in the hallway

* Located in the hallway between rooms ____/____ and ____/____.
* Report the fire, _____,_____, and EXIT the building.
* Only _____ should attempt to extinguish a fire

(…so you should not be using this, but it's good to know where it is).

3. Safety Shower

* To be used if student is splashed with _____
* DO NOT pull the lever to test it! It automatically _____water that _____quickly or easily. Only use it if you need it.
* If needed, _____**,** pull the lever, and stand under the shower for _____ to wash away the chemical and reduce contact (**everyone else leaves the room to provide that student privacy**).
* Call 911 anytime the safety shower is used.

4. Eyewash Station * You shouldn't need this because you should be _____!

* Hold the eyelid open in the running eyebath for _____ minutes.
* Call _____or go to the _____ if the injury requires further medical attention.

5A. Evacuation Procedure

* If there's a **fire or earthquake**, your TA will organize the evacuation. *Please stay with your section.* Do NOT take the _____. * **Intro activity:** Your TA will send you in groups to follow the evacuation route. **Take a selfie** at the **Emergency Assembly Area** to **upload to Canvas** with this worksheet. * **Evacuation Route:** exit the lab door and turn **right**. Walk to the end of the hall, turn **left**, then take the **stairs** down to the ground level. **Exit the door** on your **right** at the bottom of the stairs, turn **left**, then take a quick spin on the _____. Follow the path down the little hill and cross the street. Find the **Assembly Area** sign and snap a **selfie**.	Draw a mini-map from your lab to ASSEMBLY AREA LAB

Find the numbered **tag** in lab, label its **location** on your **map**, read the info tag, then **fill in the blanks**.

5B. Power Outage Protocol

* In the event of a power outage, emergency lights will turn on. Please wait _____ minutes for full power to return to continue the lab. _____ any equipment you are using in the meantime (ex. Hotplates).

If the power is not on after 5 minutes…

* Dispose of chemicals in the _____; rinse containers with a little water into the _____.
* Leave your rinsed glassware in a _____ to be cleaned by _____.
* Return the _____ to their place and _____ your equipment drawer.

6. First Aid Kit * Sufficient for _____& _____.

* All injuries must be reported to the TA followed by the completion of an "_____ Form" attained from the stockroom staff.
* For extensive injuries, student is escorted to the _____ before _____ or immediately call _____ if after _____.

7. Broken Glassware Box, Dust Pan & Broom

* Disposal container for _____ broken glassware.
* Please use the _____ to sweep up all glass; don't touch broken glass with your _____, even if you're wearing gloves. * Only _____ goes in here!

8. Spill Control Center * All spills must be _____.

* _____**(**_____**)** will neutralize solutions that are acidic or basic.
* _____ should be used for absorbing spilled solvents.
* For spills larger than a few milliliters, it may be necessary to _____.

(#9 on the next page)

10. Sink

* Before washing glassware, dispose of _____in the_____.
* Rinse any residual containers with _____ from a wash bottle.
* Only _____ down the drain! This is NOT a waste disposal area.
* _____ your gloves when washing glassware.
* Wash glassware with _____ and a _____, then rinse twice with DI water.
* Note on your map which sinks in this room, if any, have a **flood hose.**

11. Chemical Waste Station

* Waste bottles are kept here in _____(bin to catch spills).
* *Read the waste label* – there may be more than one type of liquid waste.
* Pour *into* the waste bottle using the _____provided.
* _____ if a waste bottle is full. Don't let the containers _____.

Find the numbered **tag** in lab, label its **location** on your **map**, read the info tag, then **fill in the blanks**.

9. Balance Station and Etiquette

HOW TO WEIGH A SOLID

(1) Bring a _____ or _____ and _____ from your equipment drawer.

(2) _____ in half on the diagonal and place on the balance pan.

(3) _____ by pressing the _____ or "zero" button.

(4) Use the spatula/scoopula to _____from its container onto the paper

a. Repeat until _____ on the digital balance.

b. _____ solid on separate weigh paper, *not back into the original container*

(5) _____, including all digits and _____

(6) _____ the container

(7) _____ into a separate, _____ container

a. DO NOT walk around the lab with solid just on weigh paper – it's likely to _____

(8) Dispose of weigh paper in the _____ and unused material in _____

(9) **CLEAN**! Brush spilled solids onto _____then to the _____

(10) Wipe down the counter with a _____or _____.

You may lose points if messes are left in the balance area.

Line at the balance?
Introduce yourself to the person in front of you. Ask them to tell you when they're done.
Please find something to set up or clean in the meantime ☺

12. Dry Waste Box

* For solid waste from experiments such as _____, _____, and _____.

* DO NOT put _____in the dry waste box.

* If it's a liquid, it doesn't go in here!

* _____ if you are unsure of what goes in the dry waste box AFTER reading the guidelines above and instructions in lab procedures.

Find the numbered **tag** in lab, label its **location** on your **map**, read the info tag, then **fill in the blanks**.

13. Chemical Fume Hoods

* Minimizes your _____ to _____
* Work with the chemicals at least _____ into the hood.
* Hood cover/sash should be _____ to the _____ or else!
* DO NOT put _____ in the hood!
* Keep surfaces clean – use paper towels or mat to absorb spills – the bench paper is NOT a spill mat.

14. Reagent Station (Chemical **Re**acting **Agents**)

* Take only what you need from bottles
* Keep surfaces clean – pick up spills.
* Prevent contamination: DO NOT return _____ to _____
* Bring a _____ and _____ for transfer – DO NOT walk around with a full, drippy pipet!
* Carefully read labels twice
* Carefully _____

15. Disposable Gloves and Shared Goggles

* This is a _____ line of defense
* Gloves do not make your hands _____!
* _____ gloves if you get chemicals on them
* Let your TA know if a box of gloves is _____ and place the empty box in the _____.
* Please **wash goggles** with soap and water after each use. Dry and hang back on its peg.

16. Equipment Room

* Mark on your map which room your section will use for GC and IR analysis.
* Ask your TA to point out the Gas Chromatograph (GC) and Infrared (IR) spectrometers.
* GCs can be hot! Don't leave _____ on top of them.
* Keep GC/IR kits _____.
* Clean up spills _____.

17. Rota-vap (CHEM 8M Students)

* Used to _____ samples: Round-bottom flask is attached, rotation prevents boiling over as _____ is applied to remove solvent, which is collected in the _____.
* Your TA will _____ and/or set this up for you the first couple times.
* Please _____ when not in use.
* Be respectful – empty that _____!

18. Water Pump & Water Lines

* Don't let pumps _____, check frequently
* Know your in's and out's – _____ is water in, _____ is water out
* Water lines run near _____ – watch where you point those things!
* Only use the clamps to adjust flow – please do NOT _____

Find the numbered **tag** in lab, label its **location** on your **map**, read the info tag, then **fill in the blanks**.

19. Ring Stands

* Used to secure _____ using _____.
* Stack them in an _____
* Remove all _____before returning

20. Clamps

* Separate clamps from _____
* Do not leave _____ or other items in this drawer
* If the threads on the clamp become worn and no longer work, please bring it to the _____ for them to fix.

21. Vacuum Tubing

* _____ tubing for connecting a vacuum line.
* Ask your TA where to connect to the _____.
* Return tubing when you are finished.
* Do not use for_____; vacuum only.

22. Hot/Stir Plates

* Note that there are separate dials for '_____' and '_____' and that different hotplates have different types of _____ settings.
* Mind where the cord lies to prevent_____
* Set heat on or below _____ setting. These hot plates get ridiculously (unsafely) hot on ___.
* When finished, put them away neatly, no leaning towers of hot plates please.

23. Your TA! Say hi, introduce yourself, and ask about their hobbies or passions _____. Share something similar with your TA 😊

(24-25 are on the following pages)

26. Lab Coats

* Worn over _____.
* Must be worn with _____ during all experimentation and cleaning.
* Contaminated lab coats are considered waste. Notify your TA and bring the coat to the _____ if you spill on your lab coat.
* Lab coats are shared with many sections – be considerate and do not leave _____in the pockets!
* Hang up coats neatly on the hanger labeled with the _____.

24. Hazard Definitions

In the case of exposure to any chemical, rinse the affected area immediately for _____and _____.

Copy the following precautions to be taken when handling the following types of chemicals then find the HAZARD TERMS below.

- *Irritant* – irritates the_____; minimize_____, wear _____ &_____
- *Flammable* – keep away from_____& potential_____, handle in_____ with_____ & _____
- *Lachrymator* – induces_____; wear _____&_____
- *Carcinogen* – linked to_____; minimize_____, wear _____&_____, handle in_____
- *Corrosive* – _____ and _____ the skin; minimize_____; wear_____& _____.

HAZARD TERMS

R S M E B X O A V M C H H W P
U W A Y L D Y H J A B T Y K Q
S S B F F B M E R O J J G U E
H Q H D E I A C X I S T R K T
S L U G R T I M I O B W O T P
N C D C C N Y N M Y K Z S N V
F F X I O L N F D A Y W C A Z
Z B X G Y L N R I K L N O T I
K O E B C B G X Z R N F P I T
T N V Y U W B U O M S R I R B
L A C H R Y M A T O R T C R W
Q G K W W A T B V A N Q W I T
X D M S E V I S O R R O C U J
J R O M A T H L T B J P M I Y
P K D E N H X C I J P K J N M

CARCINOGEN CORROSIVE FLAMMABLE

HYGROSCOPIC IRRITANT LACHRYMATOR

SAFETYFIRST SLUG TOXIC

Created by www.discoveryeducation.com

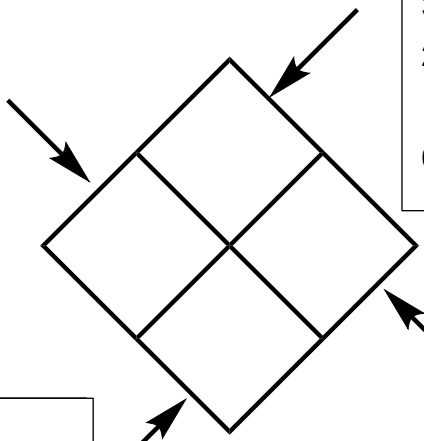
25 – NFPA Labels - Copy the full **NFPA code and label descriptions** from the bulletin board. Indicate the color or color them in if you have the equipment!

What does NFPA stand for? _____

Hazard Classifications – *write the missing information in the boxes*

Health Hazard
4 –
3 –
2 –
1 –
0 –

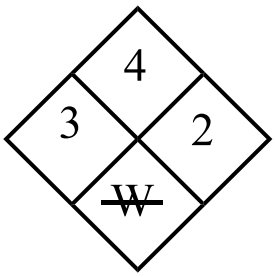
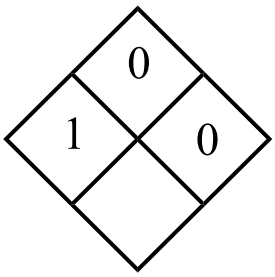
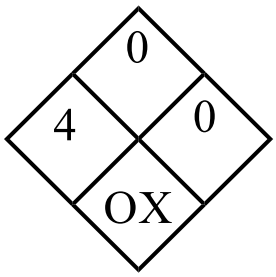
Fire Hazard
4 –
3 –
2 –
1 –
0 –



Specific Hazard
… ACID
… ALK
… COR
… OX
Radiation Hazard…
Use no water….

Instability Hazard
4 –
3 –
2 –
1 –
0 –

Classify each of the examples below using the ratings above.

Example			
Health Hazard			
Fire Hazard			
Specific Hazard			
Instability			

Find the numbered **tag** in lab, label its **location** on your **map**, read the info tag, then **fill in the blanks**.

27. Stools - This is your optional seating during lab ☺

- At the end of each lab, please _____ at the end of each lab.

28. Cotton - This is used instead of filter paper in certain applications.

- You will typically use _____ amounts of cotton.
- Dispose of soiled cotton in the _____.

29. Filter paper - Lab procedures will indicate which type of filter paper to use. There should be no need to cut filter paper. List the different types (size and thickness) of filter paper:

30. MelTemps are used to measure the _____ of a solid.

- Bring the MelTemp to your_____ instead of using it on the shared counter.
- Carefully lower the _____ into its slot on the MelTemp *before* turning the dial on.
- Follow TA demo to pack a tiny crystal into a small, glass _____
- Place the tube in one of the _____sample slots with the _____
- The dial indicates *relative rate of temperature increase*: low # = _____; high # = _____
- Closely monitor the _____in the viewing window and _____– do NOT leave unattended. CAUTION: the _____ when in use!
- After analysis, allow the apparatus to _____. Remove all samples and dispose in _____, then take out the _____and place in its designated bin.
- _____ around the MelTemp, then put it away.

31. Glass pipets

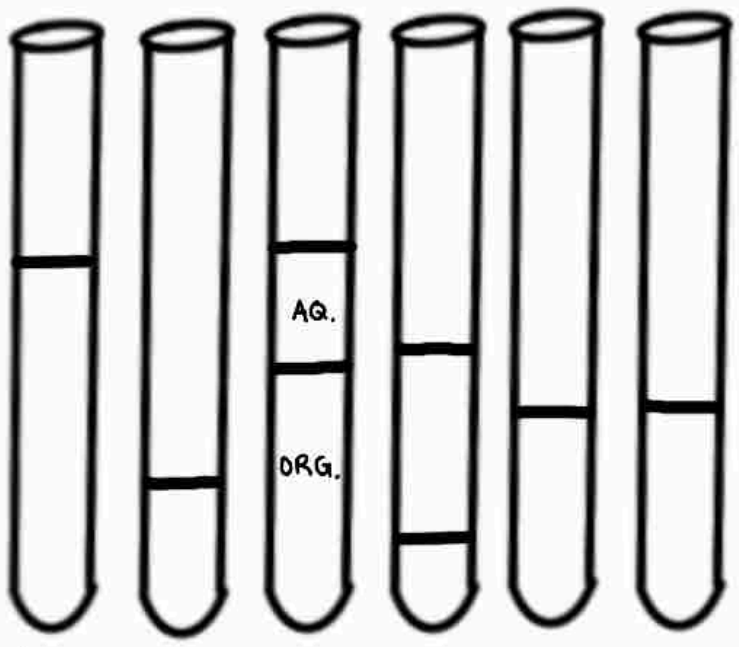
- Glass pipets used to _____with the _____ in your drawer.
- Dispose of used pipets in the _____box in the fume hood.
- **Broken pipets** should be disposed of in the taller _____ box in the lab.

32. Tape and markers

- Label all containers while they're _____, before adding any substances, even _____.
- Tape and markers are _____ - Please avoid taking them back to your work space.
- How to use: Tear off a one-inch piece of tape, stick it to the counter, and use the marker to write a descriptive label with _____.

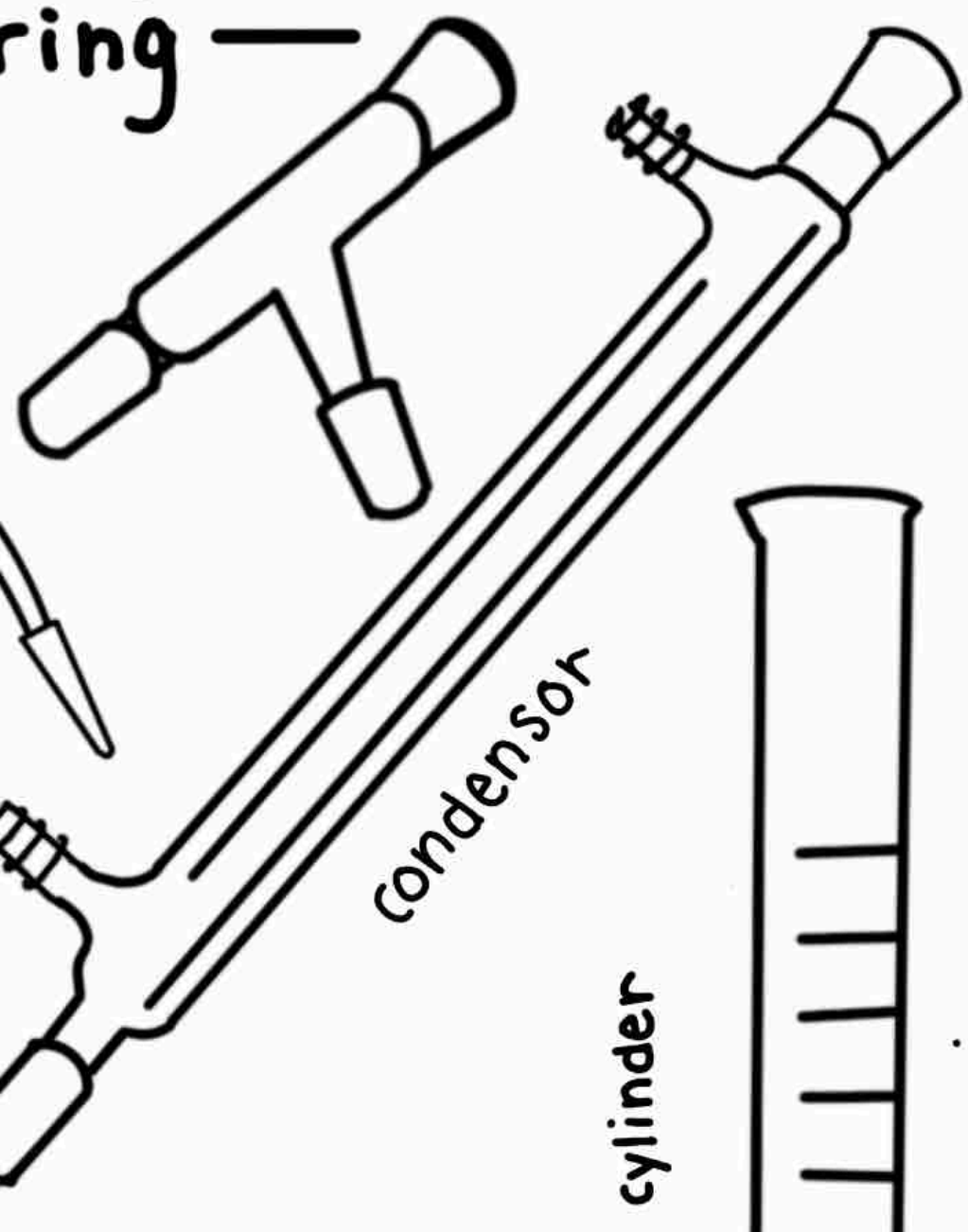
ORGANIC chemistry lab

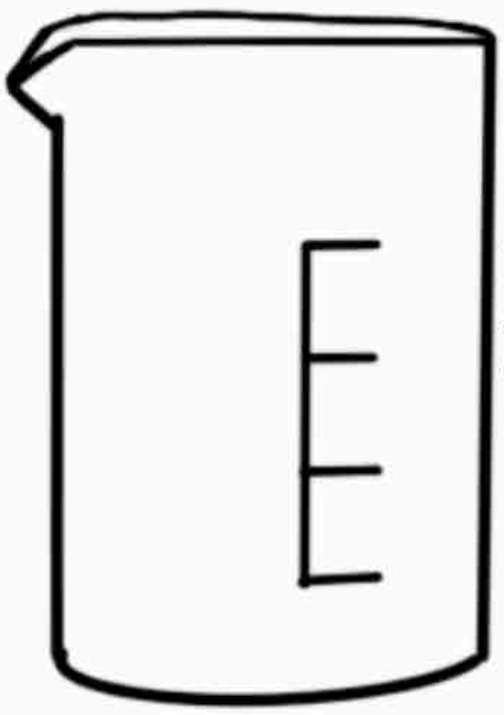
— coloring —

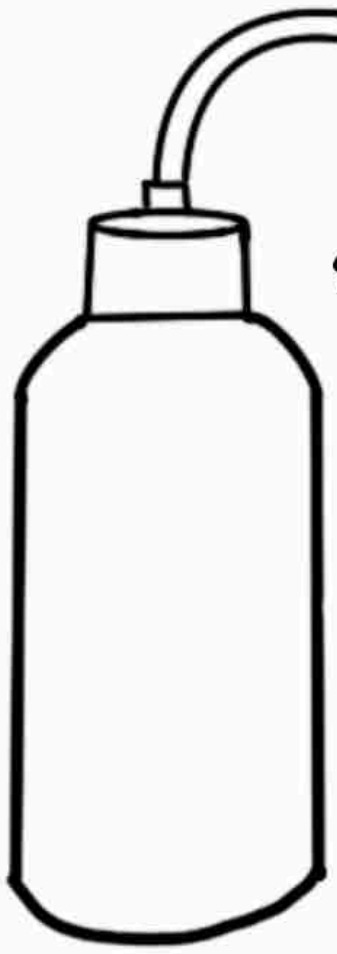


test tubes


Stopper

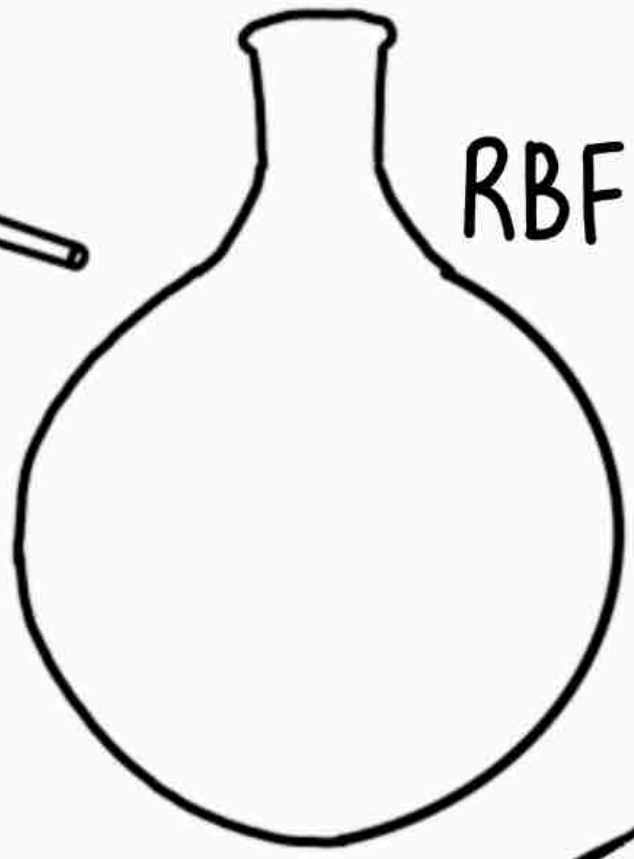

condensor

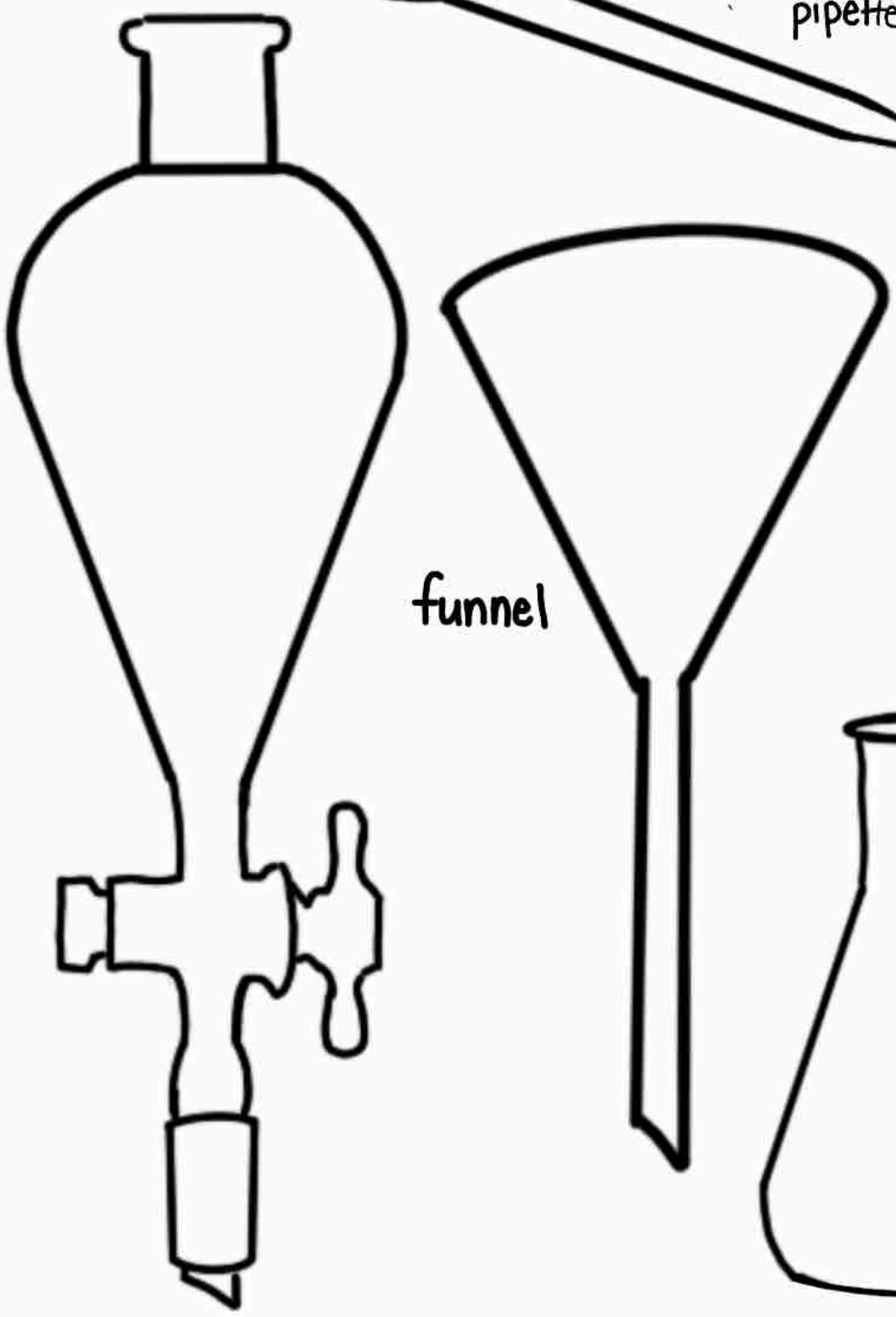

beaker

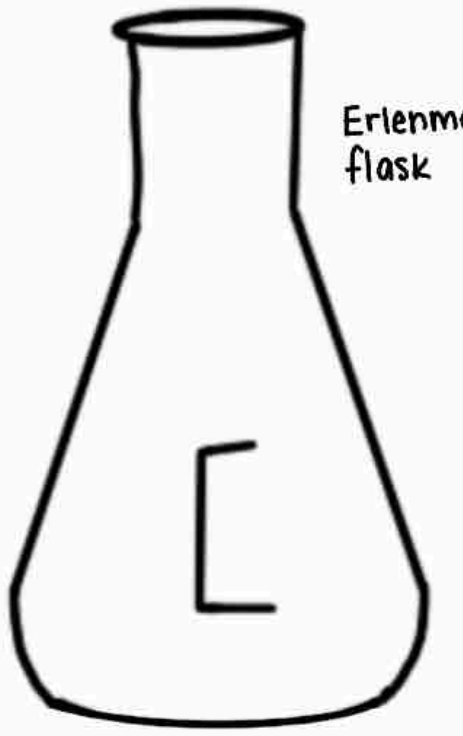

squeeze bottle

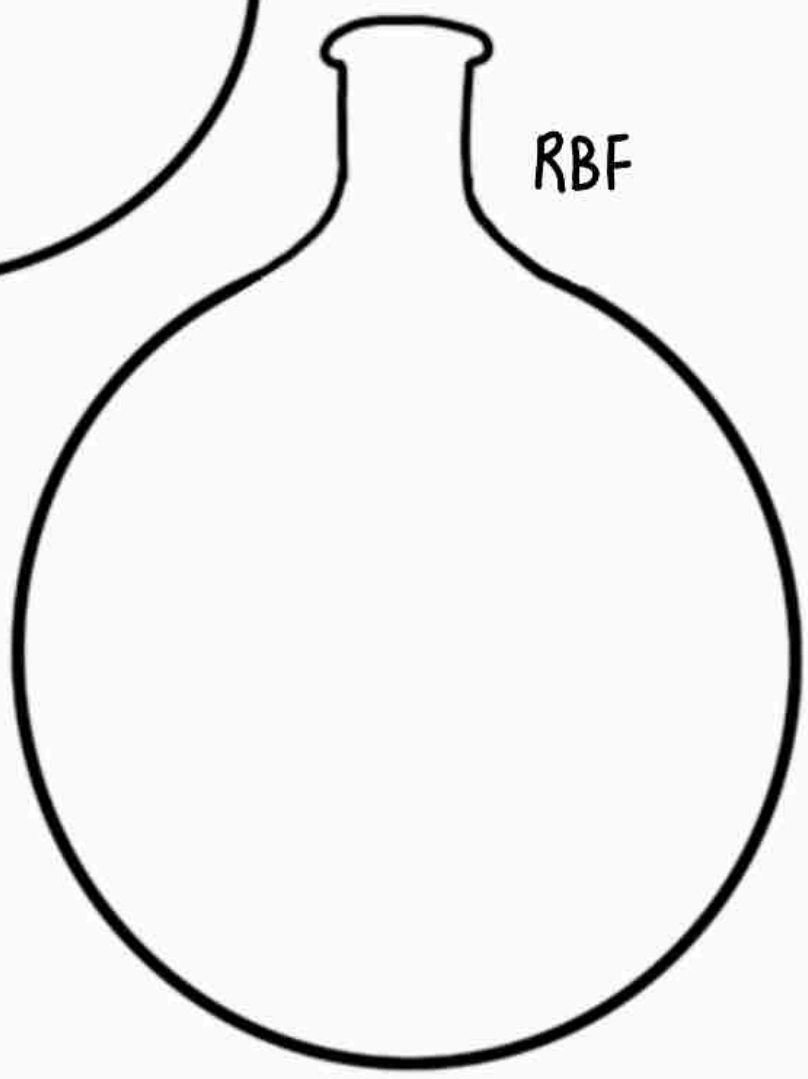

pipette

graduated cylinder


RBF


funnel


Erlenmeyer flask


RBF

Part C. Lab Basics Activity – Intra- vs. Inter-molecular Forces

1. *Skim* the safety hazard information in the **Material Safety Data Sheet (MSDS)** in the lab. The first page or two of the MSDS has all you need, but this is amount of data required for non-toxic materials!
2. Use the MSDS to enter the **molecular mass** and **boiling point** or **melting point** in the table below.
3. GO TO THE PROCEDURE. **Enter data** on the following pages and in the **table** below: amount measured for the **solid in milligrams** and for the **liquid in milliliters**.

	Chemical Name	**Amount**	**Molecular mass (g/mol)**	**Moles or mmoles**	**Boiling or melting point (°C)**	**Density (g/mL)**	**Safety Hazards**
Solid **Part C.1**	**Sodium bicarbonate**, baking soda	mg					
Liquid **Part C.2**	**Dihydrogen monoxide**, water	mL					

Part C.1. PROCEDURE - Measuring Solids

- The digital balances read in grams (g) so you'll need to convert to milligrams (mg).
- There are 1000 milligrams in every gram (10^3 mg / 1 g) or (1 g / 10^3 mg).
- **Convert 50 mg into grams:** 50 mg = _____ g

Follow the "Balance Etiquette" from the Safety Activity - weigh **approx. 50 mg (± 10 mg)** of the solid…

- Note (± 10 mg) means your measurement can be 10 mg higher or lower than 50 mg.

40 mg = _____ g 60 mg = _____ g

- **Record the exact mass measured**: _____ mg; *copy in data table above*
- **Convert mass (mg) to millimoles (mmol)** using the molecular mass of the solid. 10^3 mmol = 1 mol

_____ mmol

- **Transfer the solid** into a small beaker or Erlenmeyer flask labeled with chemical name.
- How many $NaHCO_3$ **molecules** are in the flask? **6.022 x 10^{23} molecules** are in **1 mole.**

_____ H_2O molecules

Part C.2. PROCEDURE - Measuring Liquids

Use the steps below to measure **15.0 mL of deionized water (diH_2O)** and make a solution with your solid. Practice these techniques as if the liquid were hazardous (pretend ☺) – use the water in reagent bottles in the fume hood; NOT water from the sink for this activity.

- **Label** the 25-mL graduated (grad) cylinder from your drawer, "diH_2O".
- Locate the diH_2O reagent bottle in the **fume hood**.
- **Pour a little under 15 mL** from diH_2O reagent bottle into the grad cylinder.
- **Use a glass pipet and bulb** to remove or add smaller volumes of water to get to exactly 15.0 mL.
    - Transfer any excess water into a **separate container**, NOT back into the reagent bottle.
- **Convert** the **volume (mL)** to **mass (g)** then to **moles (mol)**, using the density (1 g/mL) and molecular mass of water. *Copy into the data table.*

_____ g

_____ mmol

- How many H_2O **molecules** are in the grad cylinder? **6.022×10^{23} molecules** are in **1 mole.**

_____ H_2O molecules

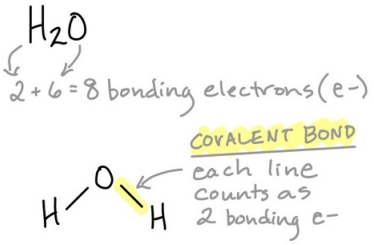
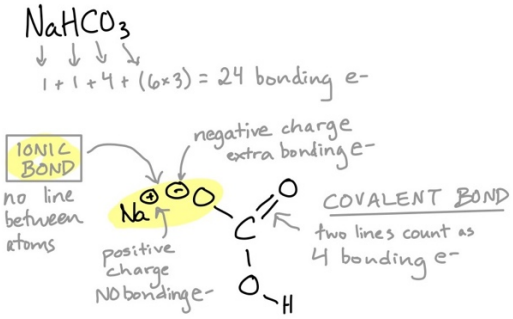
INTRA-Molecular Forces – within one molecule

Before you mix, revisit the molecules you're working with to see what's going on inside the liquid and solid.

- **Intramolecular Forces:** Molecules are made up of **ionic** and/or **covalent bonds** between **atoms**.

  Atoms use their outer-shell electrons to create bonds (**bonding electrons, e-).**

Complete the table below and think about how these **structures** relate to your **macroscopic observations**.

	Water, H_2O Covalent bonds	**Sodium bicarbonate, $NaHCO_3$** Ionic and covalent bonds
Structure & Explanation ADD the missing non-bonding electrons (lone pair) to ALL oxygen atoms in the line-bond structures		
Line-Bond Structure REDRAW the full structures of H_2O and $NaHCO_3$		

Part C.3. PROCEDURE – Aqueous Solutions

1. **Make the solution:** Transfer the diH_2O into the container with the weighed solid from **Part C.1**.
   - Swirl to dissolve completely, using a glass stir rod if desired.
2. **Calculate the solution concentration**. Molarity, M = (mmoles solute) / (mL of solution)

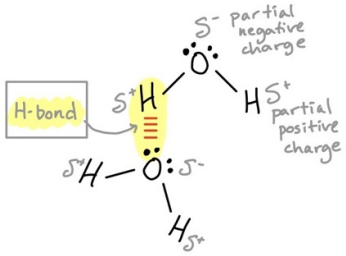
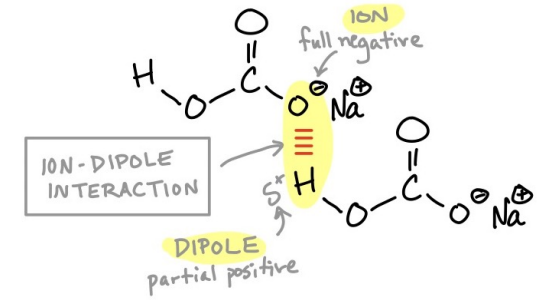
_____ M

3. **Sketch** each of your samples in their containers: **solute, solvent, and solution.**

INTER-Molecular Forces (IMFs) – between separate molecules

Your samples contain billions and billions of **molecules**!!! These molecules stick to each other like miniature magnets by **electrostatic interactions**, or simply "opposites attract."

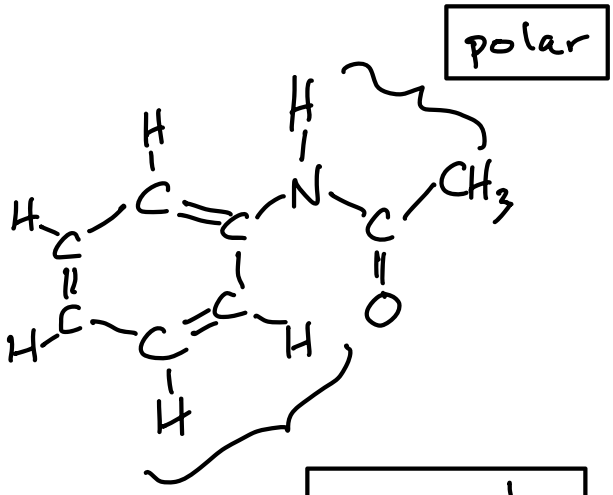
- Solvent: Individual **H_2O** molecules are held together by **hydrogen-bonds (H-bonds).**
- Solute: When in solid form, **$NaHCO_3$** molecules are held together by **ion-dipole interactions.**
- Solution: When **$NaHCO_3$** is added to **water**, the ions in $NaHCO_3$ dissociate from each other and become surrounded by water molecules. The **full charge** on one molecule is drawn to the **opposite, partial charge** on a different, called **ion-dipole interactions**.

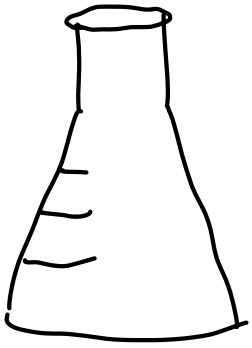
	Water, H_2O	**Sodium bicarbonate, $NaHCO_3$**
SAMPLE Intermolecular forces between 2 molecules		
ACTIVITY 1 REDRAW at least two bicarbonate ions with all lone pair and dissociated (separate) sodium ions.	Dissolved sodium bicarbonate:	
ACTIVITY 2 DRAW water molecules around the bicarbonate and sodium ions. ADD lines to represent the IMFs between them.		

Part C.4. CLEANUP PROCEDURE - Keep lab coat, and goggles ON

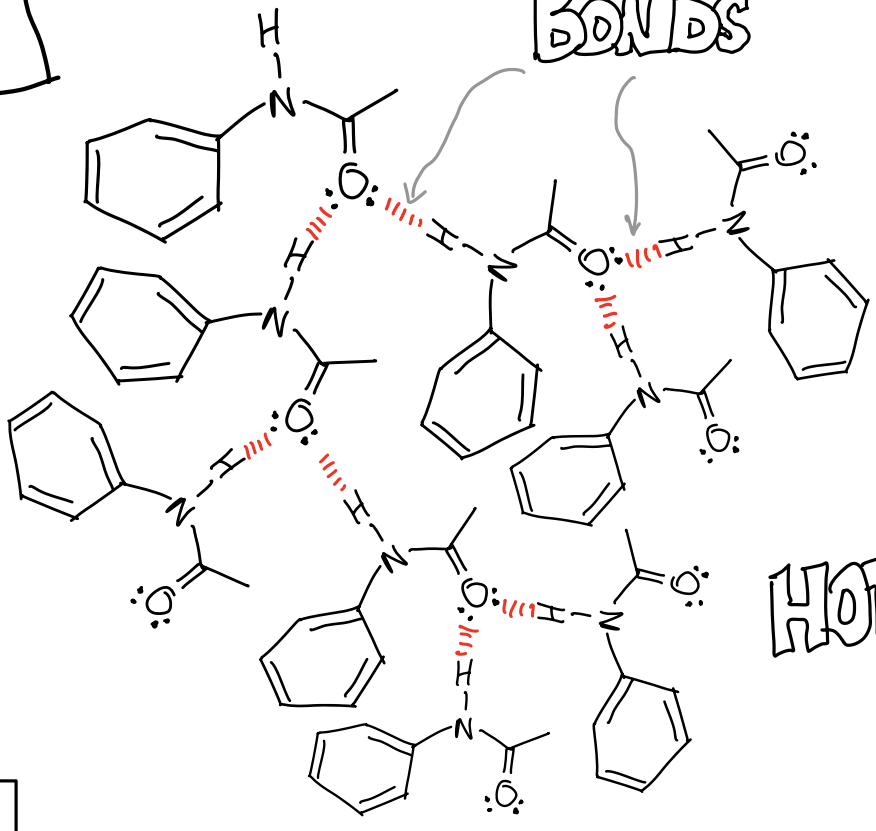
- Dispose of chemicals in appropriate **waste container**. Throw away your **gloves** in the regular trash.
- **Wash glassware** with soapy water and brush. Rinse with diH_2O. Leave to dry on paper towel.
- **Wipe down the counter** at your workstation with a wet sponge then dry with a paper towel.
- Ask your TA for a **community cleanup task**.
- **Put away glassware** in your drawer, leaving upside-down to dry if necessary and possible.
- **Wash your goggles** with soapy water. Rinse with diH_2O, blot to dry, then hang on the pegs for goggles.
- Take off your **lab coat**, empty the pockets, and place on the appropriate hanger by size.
- **Wash your hands.**
- **Ask your TA to check your workspace**. Wave goodbye to your **lab-mates**. *Congrats on your first lab!*

Recrystallization of Acetanilide

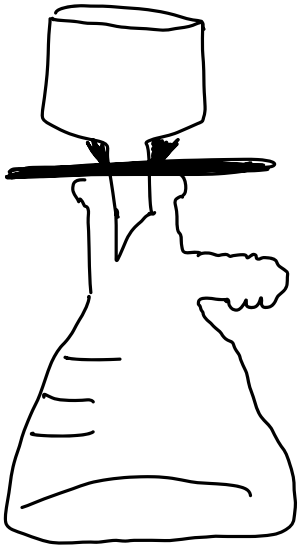


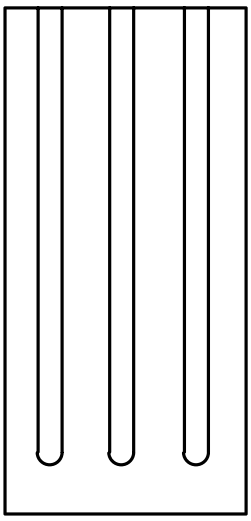


Hydrogen Bonds



Hot Filtration





Melting Point

Experiment 1 – Recrystallization of Acetanilide

Week 2 = Safety Scavenger Hunt & Lab Basics – Attendance Required
- Arrive on time, dress for lab, intro activity worksheet provided

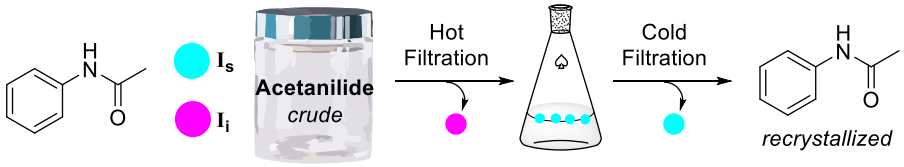
Week 3 = Perform Experiment 1 – quiz due Monday, prepare notebook before lab

---

Learning Objectives: By the end of this experiment, you will be able to…

- Describe the **steps of recrystallization** to purify a solid
- **Draw diagrams** to depict the role of **hydrogen-bonding (H-bonds)**
- Perform a **hot, gravity filtration** and a **cold, vacuum filtration**
- **Calculate percent recovery** from a recrystallization
- Assess the relative purity of a sample via **MelTemp analysis**

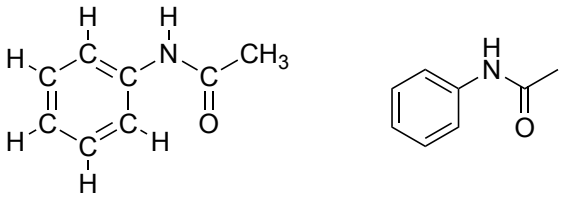
RECRYSTALLIZATION



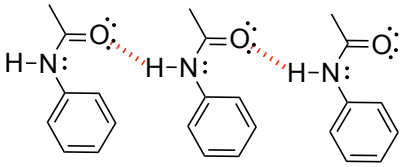
1. Dissolve _____
2. Filter _____
3. Remove _____
4. Precipitate _____
5. Filter _____
6. Analyze _____

Molecular Structure of Acetanilide

Intramolecular forces



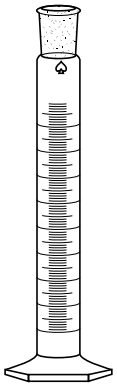
Intermolecular forces



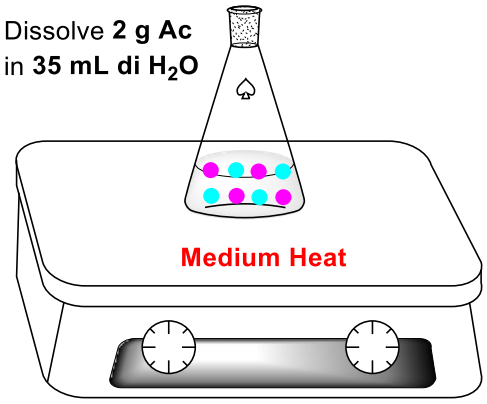
Experimental Procedure

1. Weigh the solid…

Measure recrystallization solvent (water)

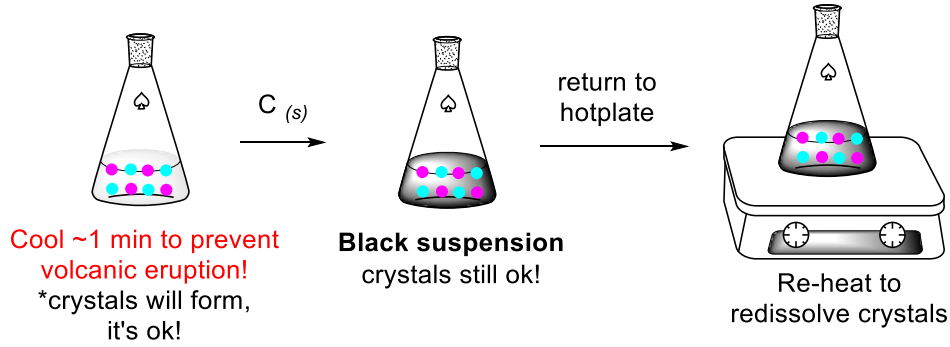


2. Dissolve sample in *minimum volume* of hot deionized water (di H_2O)

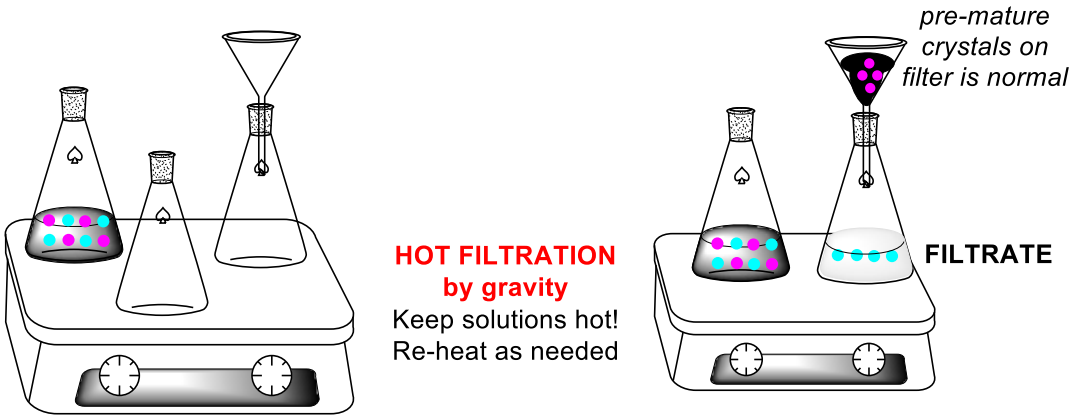


SAFETY FIRST
Use 2 hot mitts
to handle
hot glassware

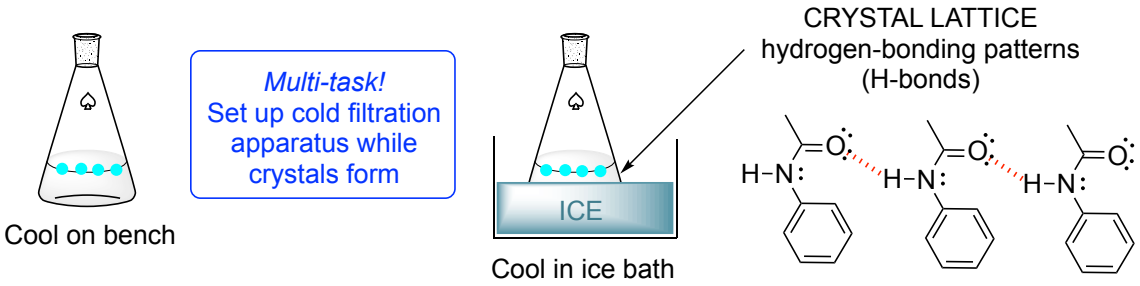
3. Filtering Agent: activated charcoal, $C_{(s)}$



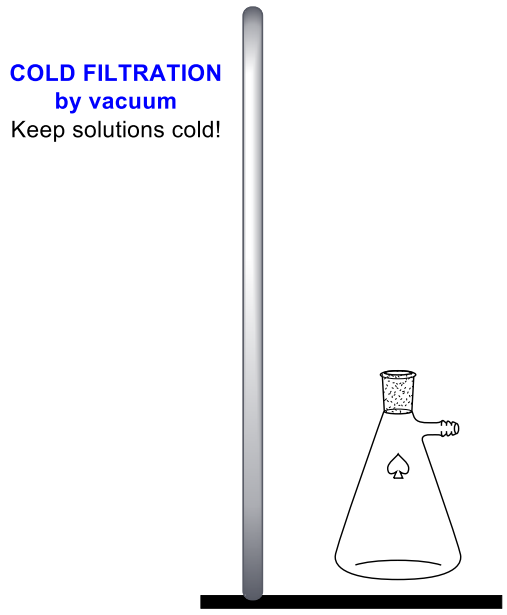
4. Hot Filtration



5. Crystallization



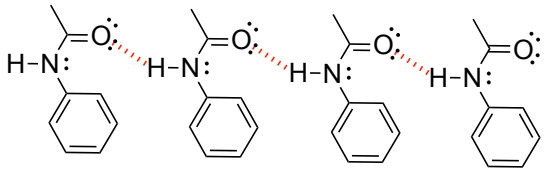
6. Cold Filtration: Removes insoluble impurities, $\mathbf{I_s}$ … Wash with cold di H_2O … Vacuum removes solvent from solid



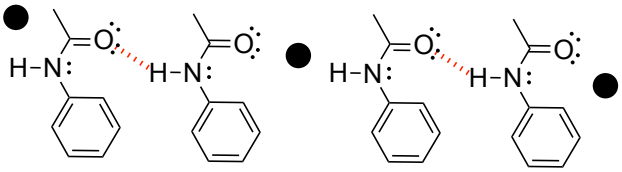
9a. Analysis: Percent (%) Recovery

$$\textbf{\% Recovery} = \frac{m_{recrys}}{m_{crude}} \times 100\%$$

9b. Melting Temperature Analysis



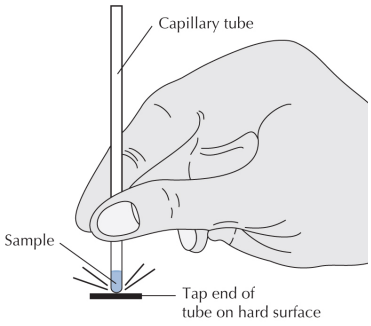
Pure Sample



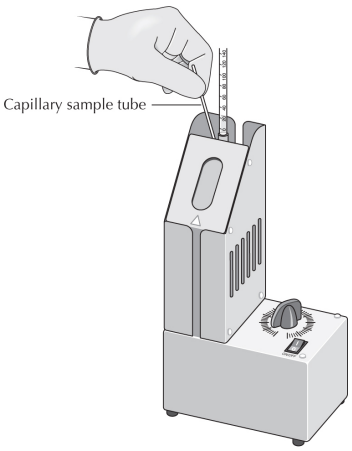
Impure Sample

MelTemp Sample Preparation

(a) Spread on porous plate to absorb water



(b)

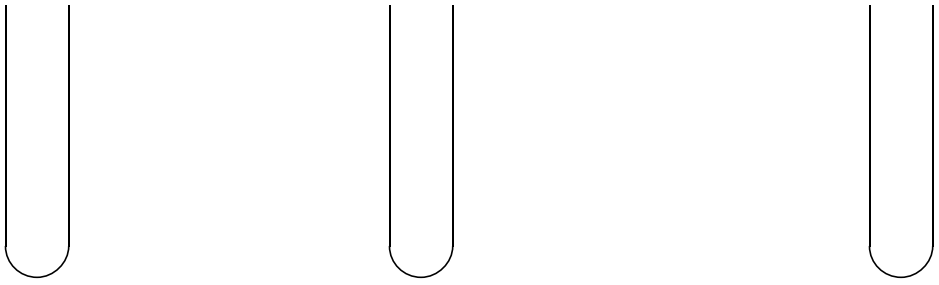


(c)

MelTemp, melting point apparatus

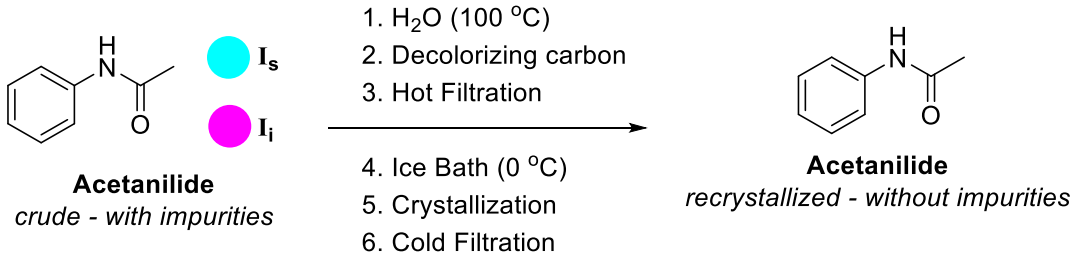
Through the viewing window:

Room Temperature	Beginning of mp range	End of mp range



Recrystallization Scheme – purpose section of lab notebook

The purpose of this experiment is to purify acetanilide by recrystallization from water and analyze the product's melting temperature.



Acetanilide
crude - with impurities

Acetanilide
recrystallized - without impurities

** Refer to **Exp 1 Notebook Templates** – purpose, reagent table, full lab procedure, clean-up & safety, data

Lab notebook must be prepared before lab or you'll be sent home :/

Key Terms for Recrystallization

Dissolve		Boil	
Filter		Melt	
Precipitate		Solubility	
Non-polar	Polar	Dipole	Solute
Bond Polarity	Covalent Bond	Hydrogen Bonding	Solvent
Molecular Polarity	Electronegativity	Physical Change	Solution

Review your notes – briefly describe each term and how it relates to this experiment

Experiment 1: Recrystallization of Acetanilide

Learning Objectives: By the end of this experiment, you will be able to…

- Describe the **steps of recrystallization** to purify a solid
- **Draw molecular diagrams** that depict the role of **hydrogen-bonding (H-bonds)**
- Perform a **hot, gravity filtration** and a **cold, vacuum filtration**
- **Calculate percent recovery** from a recrystallization
- Assess the relative purity of a sample via **MelTemp analysis**

Key Terms

Dissolve		Boil	
Filter		Melt	
Precipitate		Solubility	
Non-polar	Polar	Dipole	Solute
Bond Polarity	Covalent Bond	Hydrogen Bonding	Solvent
Molecular Polarity	Electronegativity	Physical Change	Solution

How to Complete this Lab + Assignments

See Canvas Exp 1 Module

Before Lab

- Read this document: background, procedure, safety, pre-lab and in-lab questions
- Attend **lab lecture,** fill in the class note **templates**
- Preview the lab online *via* **Slugs@home platform**
- Complete the **pre-lab questions** (towards the end of this document) - incorporated into Canvas quiz ☺
  - **Pre-lab quiz** due midnight, Monday before your enrolled section
- **You must have your lab notebook prepared to participate in lab…**

**** Lab Notebook Preparation**

- *Refer to the Exp 1 Lab Notebook Templates on Canvas – copy into notebook*
- **Purpose:** one-sentence summary of the lab goals and the recrystallization scheme (**Figure 5**)
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info!
  - Refer to the 'Cleaning & Safety' table after the procedure for one-word chemical **hazard**
- **Procedure with Diagrams** – examples in class notes
  - Draw simple sketches with labels: all **equipment, chemical names** with **amounts**, & **transfers**
  - Break up text with bullet-points & diagrams. Avoid copying the procedure word-for-word.
  - **Slugs@home Exp 1 website** – preview pictures & videos of the whole lab!
- **Cleaning & Safety** – copy the table from the end of the procedure
- **Data** – entries copied from template

During Lab (TuWTh)…

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- Please wait for your TA arrive before entering the lab
- Show your prepared **lab notebook pages** to your TA
  - You'll be sent home if not prepared :/
- Perform the experiment with a partner, fill out data & observations in your **lab notebook**

After Lab…

- Each student submits separate, individual assignments
- Upload **Notebook Pages** to GradeScope by midnight on lab day
- Complete & upload the **Lab Report** on GradeScope (GS) – due Friday, one week after lab

Recrystallization Background

Paracetamol, the active ingredient in **Tylenol®** (**Figure 1**), is commonly used for pain relief (analgesic) and to reduce fevers. 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. The purification of acetanilide serves an excellent introduction to recrystallization (**Figure 2**).

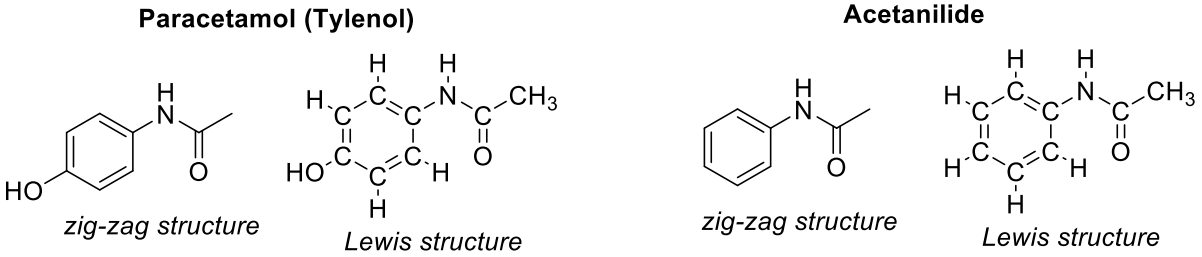


Figure 1. Structures of paracetamol and acetanilide

The basic steps of recrystallization are as follows.

1. Weigh the crude (impure) solid sample
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 weighed, then dissolved in the smallest possible amount of solvent. A successful **recrystallization** requires that the solid be *highly soluble at the solvent's boiling point* and significantly *less soluble at low temperature*. Acetanilide (Ac) has a much higher **solubility** in hot water (5.50 g dissolves in 100 mL of water at 100 °C) than in **cold** water (0.53 g Ac / 100 mL at 0 °C). Water is very **polar**, while acetanilide is **polar organic** (contains both polar and non-polar features), meaning Ac is much less polar than water.

The 2 grams of solid Ac used in this lab consists of billions upon billions of molecules, an unfathomable number to imagine! Each Ac molecule has the same **covalently bonded structure** of carbon, hydrogen, oxygen, and nitrogen atoms (**Figure 2a**). These covalent bonds are **intra**molecular forces, within the molecule, and are way too small to be seen with the naked eye. **Covalent bonds** are NOT broken or formed during this experiment.

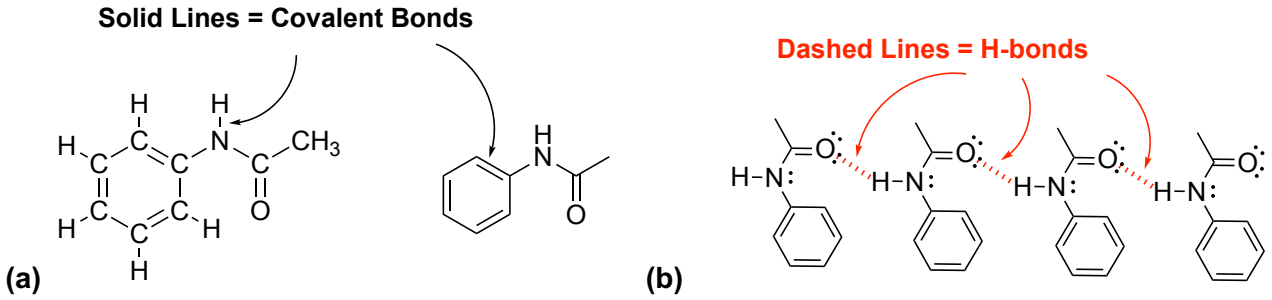


Figure 2. (a) Covalent bonds and **(b)** non-covalent H-bonds in acetanilide.

The solid Ac particles that you can see with your eyes are held together by **hydrogen-bonding (H-bonds)** between Ac molecules (**Figure 2b**). **H-bonds** are **inter**molecular forces that cause **molecules** to stick to each other. Imagine each molecule as a **sphere** and H-bonds are the **glue** that keeps the spheres close to each other. **H-bonds** are also the **intermolecular force** responsible for keeping water molecules together at the bottom of a glass, rather than flying through the air!

The term **"bond"** can mean very different things in chemistry, depending on the context. **"H-bonds"** are possible only in very polar compounds that contain covalent **O-H** or **N-H** bonds. Not all bonds to hydrogen are considered "H-bonds," which can be misleading. A "polar N-H bond" means the nitrogen and hydrogen are **covalently bonded**, and that their bonding **electrons** are **shared unequally**. Nitrogen is more "greedy" for electrons (electronegative) and has a **partial negative charge,** δ^-, leaving the hydrogen with a **partial positive charge,** δ^+. When a polar N-H bond is present in a molecule, the **lone pair** on a partially negative N-atom (δ^-) on one Ac molecule can "H-bond" with the partially positive H-atom (δ^+) on a *different* Ac molecule. Acetanilide also contains a polar C=O bond and the O-atom can serve as a **H-bond acceptor**, similar to the H-bonding patterns in peptides and proteins.

In recrystallization, a **minimum amount of liquid** water is added to solid Ac and the solution is heated. If too much hot solvent is added in the beginning of the lab, little-to-no Ac will recrystallize from cold water at the end of the experiment ☹ The increased temperature provides the energy to break **H-bonds** between individual Ac molecules, as well as the **H-bonds** in water. This frees up space for Ac to be **"solvated"** or surrounded by water molecules (**Figure 3**). Ac forms **H-bonds** with H_2O molecules for as long as the water stays hot. That is what it really means for Ac to be dissolved in water. Keep in mind that these **H-bonds** are temperature-dependent and reversible. Only **H-bonds** are affected by this experiment (covalent bonds do NOT change). Breaking/forming **covalent bonds** (like C-C or N-H) would be a **chemical change**, whereas breaking/forming H-bonds is a **physical change**.

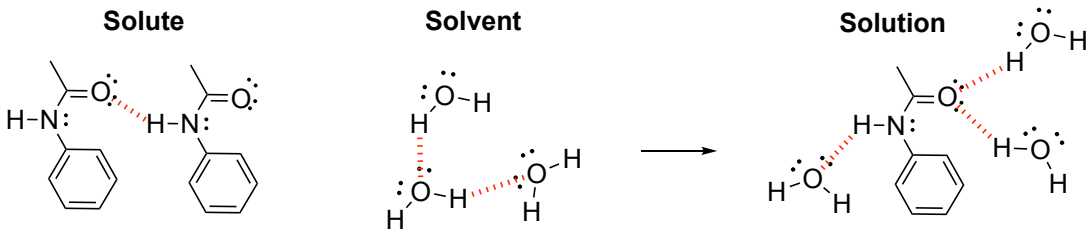


Figure 3. H-bonding examples in solute (acetanilide), solvent (water), and solution

Activated **charcoal** (aka decolorizing carbon) is a **non-polar,** black powder. It is added to the Ac solution as a filtering agent to absorb **insoluble impurities**. These impurities are **non-polar** organic compounds that stick to the small particles of activated charcoal. **Filter paper** is the barrier that physically separates any **insoluble impurities** (including the charcoal) from the solution during the **hot filtration** step, while acetanilide remains in solution (filtrate). The **filtrate** is gradually cooled to room temperature, then transferred to an ice bath to induce **crystallization**. If you were to put the hot or warm flask directly in the ice bath, instead of waiting, the crystals that form will absorb impurities from the filtrate.

During this time, the **H-bonds** between Ac and water break while **H-bonds** form between Ac molecules. The result is a pure, white, **crystalline** solid visible to the naked eye. On a molecular level, there is a highly **ordered pattern of H-bonds** between the billions and billions of Ac molecules in the sample. The purified solid is separated from the solution by **cold filtration** under reduced pressure (**vacuum**), using **filter paper** to trap the product. A small volume of cold water is added after transfer to remove **soluble impurities** from the crystals. Any **soluble impurities** remain in the cold filtrate solution. The vacuum remains on to pull air through the solid and **dry** the purified product by removing water molecules still **H-bonded** to Ac.

The **recrystallized**, dried solid is collected on the filter paper and weighed. Inevitably, Ac will be lost when transferring solutions to different containers, notably in the hot and cold filtration steps. The **percent recovery** indicates how much **pure Ac** was isolated from the **impure Ac**. The mass of **recrystallized** product (m_{recrys}) after cold filtration and the mass of the original, **crude** starting material (m_{crude}) are used to calculate the **percent recovery of recrystallization** according to **equation 1**. Recrystallizations sacrifice quality (purity) for quantity. In other words, percent recoveries tend to be low – better to have a small amount of very pure Ac than a larger amount of less pure Ac.

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

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 due to intermolecular forces like hydrogen bonding (H-bonds) (**Figure 4**). The impurities interfere with the ability of Ac molecules to form H-bonds. There are fewer H-bonds that take less energy (lower temperature) to break and cause the phase change from solid to liquid. Purity may also be apparent in the appearance of the solid before and after the experiment.

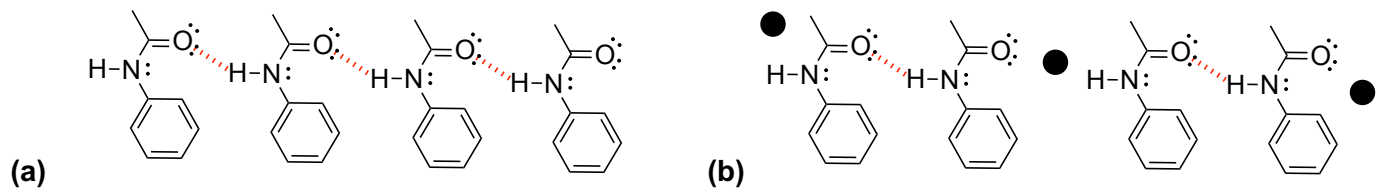


Figure 4. H-bonding patterns in **(a)** pure acetanilide vs. **(b)** acetanilide with impurities that interfere.

LAB PROCEDURE

Refer to **LAB NOTEBOOK TEMPLATE** on Canvas to prepare before lab. Students work in **pairs on wet-lab** experiment; assignments (quiz, notebook, and lab report) are completed **individually**.

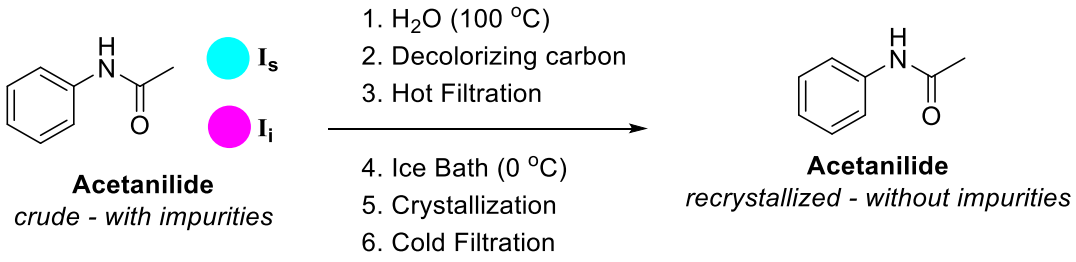


Figure 5. Recrystallization of acetanilide overview – *Purpose section of lab notebook*

Part 1. Dissolve the Sample. Place *approximately* **2 g of crude acetanilide** in a labeled **125-mL Erlenmeyer flask**. Record the actual mass obtained (between 1.900 – 2.100 g), including all decimal places and zeros. 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** at a medium setting (please avoid turning the heat above medium). Stir the system frequently and crush carefully the solid with a **glass stir rod**. Allow the solution to *gently* boil for a few minutes. If there is still visible solid after boiling, stirring, and crushing, do NOT turn up the heat! Instead, **add water drop-wise** (up to 5 mL max) with stirring and heat until all solid dissolves, or you've added a **total of 40 mL water**, whichever comes first. *Record the total approximate amount of water added.*

Caution: Adding more than 40 mL water will significantly decrease the percent recovery, however, solvent evaporates as the solution boils so keep that heat down! Some material may not dissolve, or it may melt (aka "oil out") and oily droplets appear on the top of the solution. Not to worry – proceed to the next step.

Activated Charcoal. 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-full of **activated charcoal** and stir to create a black, opaque suspension. It is normal for white crystals to form at this stage. Place the **flask** back on the **hot plate** and heat 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-shape, over the lip of the "filtrate" **flask**. Add a **short-stem glass funnel** with a folded piece of **filter paper**. The **copper wire** provides space for steam to escape between the "filtrate" **flask** and **funnel**. Heat both flasks on medium while the 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, 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 one hand protected by a **hot mitt.** With your other hand, hold a **glass stir rod** directly to the lip of the **flask** to guide the solution down into the **funnel** as you quickly pour the first portion. The **stir rod** should prevent the solution from dripping down the side of the flask, though it may take a few tries to get it right! Fill the **funnel** to below the level of the **filter paper** on each transfer and be careful not to overflow the **funnel**.

Use the glass stir rod to gently stir the content of the funnel *without poking or tearing* the **filter paper**. (Restart the hot filtration if the filter paper tears.) As needed, place the **flasks** back on the **hot plate** to keep the solutions hot and crystals dissolved. Rinse any **crystals** on the filter with the **hot water** used to rinse the **flask** on the **hotplate**. Repeat until all of the suspension has been transferred. (If there is too much solid is on the filter, you may re-do the filtration, but typically enough of the acetanilide is dissolved in the filtrate to continue.) Add **5 mL of water** to rinse the emptied charcoal flask and warm it on the hotplate for later. See special instructions for the **cleaning charcoal flask** in the **Clean-up & Safety** table following the procedure.

Crystal Formation. After transferring all of the black suspension to the funnel, discard the **filter paper** in **solid waste**. The **crystals** must be allowed to form slowly and **undisturbed** - no more stirring! Allow the **flask** containing the **filtrate** to cool to *room temperature* on the **benchtop***.* Use a plastic bin as an ice-bath to share with your lab mates. (The ice machine is down the hall – exit the lab door and turn left. At the end of the hall, turn left and the ice machine is immediately to your left.) **Label** the flask with your name and place in an **ice-water bath**. Place **5 mL of distilled water** in a labeled **test tube** in the **ice bath** to wash the crystals later. Allow crystals to form for at least 10 minutes. Note initial time of crystal formation as it may not happen immediately. If crystals do not form after 5 minutes in the ice bath, scratch the inside bottom of the **flask** with a glass **stir rod** to release seed crystals from the walls of the glass. Drawing a star and circle across the bottom of the flask tends to do the trick! Otherwise, do NOT disturb the **flask** to allow nicely ordered crystals (**H-bonds**) to form between Ac **molecules**.

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 an air-tight connection to 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 crystals in 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 **3-5 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 squeeze out as much water as possible. If you hear a *hissing* sound, the vacuum seal is not tight. Make small adjustments until the

hissing stops or lessens. Let the solid dry on the **filter** with the vacuum on for 20 minutes. [Proceed to ***MelTemp analysis*** after the solid has air-dried for at least 20 minutes, while the remaining solid continues to dry on the funnel.]

Meanwhile, keep the vacuum ON to dry the remaining solid for an additional **30 minutes (50 minutes total vacuum time**). Weigh a **watch glass** and record its mass. Transfer the **solid** with filter paper to the **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 (subtract mass of filter paper and watch glass). Calculate **percent recovery** and *record a **description** of the product (are the crystals white, gray, sparkly…?)*. If the recovery is greater than 100%, the solid should be placed back in the **Buchner funnel** with vacuum on, checking again at 10-minute intervals.

Part 4. MelTemp Analysis. Once the solid has been drying with the vacuum on for at least 20 minutes, take a small crystal 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 allow the porcelain plate to absorb water. This is *essential* for accurate MelTemp determination, as water will significantly lower the melting temperature of your sample and make it seem like your product is less pure.

Please ask your TA to help you set up your **MelTemp** apparatus with **thermometer**. Pack two very small samples (**crude Ac** and **recrystallized Ac)** into two **capillary tubes**. Lower them into separate lanes in the **MelTemp** with the closed end of the capillary tubes facing down. The numbers on the **MelTemp dial** indicate the *rate of temperature increase.* Use a **medium setting** until the thermometer reads about **90.0 °C**. Lower the MelTemp dial setting for a slower temperature increase and observe samples through the viewing window. Closely **observe** and **record** the **melting ranges** of both samples: record the **temperature** when the sample **begins to melt** (looks like it is sweat droplets on the inside of the glass) and again when the **entire sample is in the liquid phase**. For reference, the temperature difference may be around 10 °C for an impure sample (ex. 100.0 °C to 110 °C) and the highest possible melting point of pure acetanilide is **114.3 °C.** Record temperatures on the **thermometer** nearest **0.0 °C**, meaning you'll estimate one decimal place in between graduations (lines). Dispose of **recrystallized product** in the capillary tubes in the **solid waste** after analysis.

Please **clean your equipment and workspace**, then ask your TA for a **community task**.

Give your partner a **high-five**! You've just completed your **first organic chemistry lab** ☺

Cleaning and Waste Procedures – *copy this table into your notebook!*
* 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. * *Solid waste*: Filter paper, acetanilide (crude and recrystallized), and used capillaries * *Liquid waste*: Filtrates (liquid from cold filtration)
* **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. Further dry with a paper towel to the best of your ability and returning equipment to drawer. * 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 the proper drawer.
Safety Hazards * Be mindful not to touch **hot glassware**. Use both hands in hot mitts to handle hot glassware. Do NOT use clamps, paper towels, or bare or gloved hands. * Acetanilide is a mild **irritant**. Reduce chemical exposure by wearing a lab coat, goggles, and gloves throughout the experiment. Do not let acetanilide come in contact with eyes, mouth, or skin. * In the event of **chemical exposure**, rinse the affected area with **water for 15 minutes**. * Change gloves about once an hour and if the gloves come into contact with chemicals.

Pre-lab Questions & Quiz

The pre-lab quiz is **due the MONDAY before lab** – due date on Canvas

- The Canvas quiz incorporates the prompts 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 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.

Pre-Lab Questions

1. What are the basic **steps in the recrystallization** of acetanilide?
2. What are all the covalent **bonds** in acetanilide? See **Figure 1**, ex. carbon-carbon single bond.
   a. For each covalent bond in acetanilide, is it **polar or non-polar**?
3. Why is **water** a good **recrystallization solvent** for acetanilide?
4. Why should a **minimum amount of hot solvent** be used for dissolving the crude solid?
5. How does **activated charcoal** help with the hot filtration step?
6. What type of **impurities** are removed in the hot filtration?
7. Why should the recrystallized solid be washed with **minimal, cold solvent**?
8. What effect do **impurities** have on the **melting point** of organic compounds?

Full Name: **Lab Section:** [replace with day, time, room]

Lab Partner Name: **TA Name:**

EXP 1 LAB REPORT TEMPLATE, Page 1 of 3

Download the "Lab Report Template" – MS Word doc and edit; or **recreate** the template in a new word processing document. [Keep these instructions]

- Responses must be on the on **same part of the same page as this template** for grading.
- Provide all **TYPED, numbered** responses in **complete sentences**.
- Insert images of **hand-drawn diagrams** and **calculations**, where applicable.

1. Are any **covalent bonds broken or formed** in the recrystallization of acetanilide (Ac)? [Top of page 1]

2. Describe what **happens to the Ac and water molecules** *as the solid dissolves* in hot solvent to make the solution. [Two-to-three sentences, middle of page 1]

3. Draw three separate **diagrams** of the hydrogen-bonding (**H-bond**) patterns involved in solute, solvent, and solution molecules. These should be original, hand-drawn figures with full chemical structures - do not copy / paste from online sources. *Hint: review the background section.* [Bottom of page 1]

solute – draw 2+ molecules of solid Ac, H-bonded	**solvent** – draw 2+ molecules of water, H-bonded
solution – draw at least 1 molecule of solute and 2 molecules of solvent, H-bonded	

EXP 1 LAB REPORT TEMPLATE, Page 2 of 3

4. What is the **role of the activated charcoal** in this experiment? **When is it removed** from solution in the recrystallization? What is another **application** of commercially available activated charcoal in everyday life? [Top of page 2]

5. After hot filtration removes insoluble impurities, the remaining solution (**filtrate**) is cooled to room temperature, then in an ice bath *without being disturbed*.
   - What type of impurities can are present in the filtrate?
   - **Explain** what interactions break and form between the **acetanilide and water molecules** during the cooling process - recrystallization of Ac from the saturated solution. [middle of page 2]

6. Report the **mass of recrystallized acetanilide (g)** obtained at the end of the lab. Calculate the **percent recovery** of the recrystallization of acetanilide (**eq 1**). **Show your work**, including **units** on every value. [Bottom of page 2]

EXP 1 LAB REPORT TEMPLATE, Page 2 of 3

7. MelTemp Analysis
    a. Report the recorded **melting temperature ranges** of both **crude** and **recrystallized acetanilide**. Each melting range includes two temperatures with one estimated decimal place (ex. 95.0 °C). [Top of page 3]

    b. Report the **literature melting point** of acetanilide and **compare** it to the temperatures above (does each sample have a higher or lower melting temperature?). [Middle of page 3]

    c. Briefly explain *your results* in terms of expected **colligative properties**. In other words, how *should* impurities affect melting temperatures and is this *consistent with your data*?
    [Bottom of page 3]

Name _____ Partner Name _____

TA Name _____ Section Letter _____ Day _____ Time _____

Experiment 1 – Recrystallization of Acetanilide

Reference for notebook preparation – every student submits on GradeScope individually after lab

Pre-Lab Requirements

1. **Lab Notebook:** copy templates below into designated notebook
   - **Purpose, scheme,** and **reagent table**
   - **Procedure Diagrams** – must be complete upon arriving to lab
   - **Cleaning and Safety Table**
   - **Data**
2. **Dress for lab** – see safety rules – arrive a few minutes early

A. Purpose Sentence and Recrystallization scheme, see lab PDF

B. Reagent Table – look up and FILL IN PROPERTIES BEFORE LAB

Chemical Name	**Amount** Fill in during lab	**Molecular Mass**	**mmoles** Fill in during lab	**Boiling or melting point**	**Density**	**Hazards**
Acetanilide, crude						
Water						
Acetanilide, recrystallized		*(same as above)*				

C. Procedure – hand-written instructions & diagrams based on lab PDF, Slugs@home, and class notes

- Include all **labeled equipment, chemical names** with **amounts**, and **clean-up / safety notes**
- Indicate every **transfer** of chemicals from one container to another
- *Use as many pages as needed* - at least 3 pages is typical

Part 1. Dissolve Ac in hot solvent and add charcoal
- Weigh Ac, flask and its contents, heat, flask after charcoal addition

Part 2. Hot Filtration Apparatus
- Flask & filter contents before, during, and after filtration

Part 3. Cooling and Cold Filtration
- Flask & filter contents before, during, and after filtration

Part 4. MelTemp Analysis
- Sample identities & preparation, observations through viewing window

Part 5. Cleaning and Safety – copy the table from the end of the procedure

D is for Data

Record additional notes and observations within the procedure diagrams, including all potential sources of Ac loss as you carry out the procedure (ex. solid left behind in flask during hot filtration).

Mass of crude Ac _____ g

Initial volume of water _____ mL

Additional Water Added _____ mL

Approx. total volume of water = (initial) + (additional) = _____ mL

Cold Filtration

Mass of filter paper: _____ g

Mass of filter paper + recrystallized, dry acetanilide: _____ g

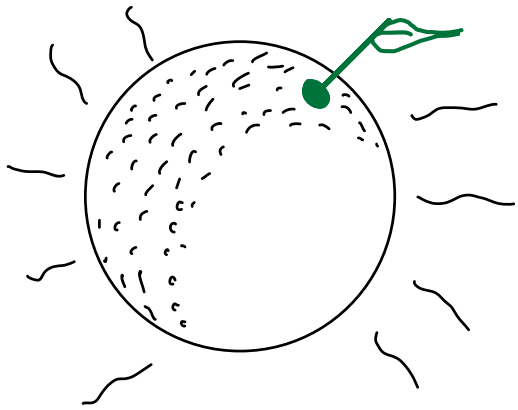
Actual **mass recovery** of recrystallized **Acetanilide**: _____ g

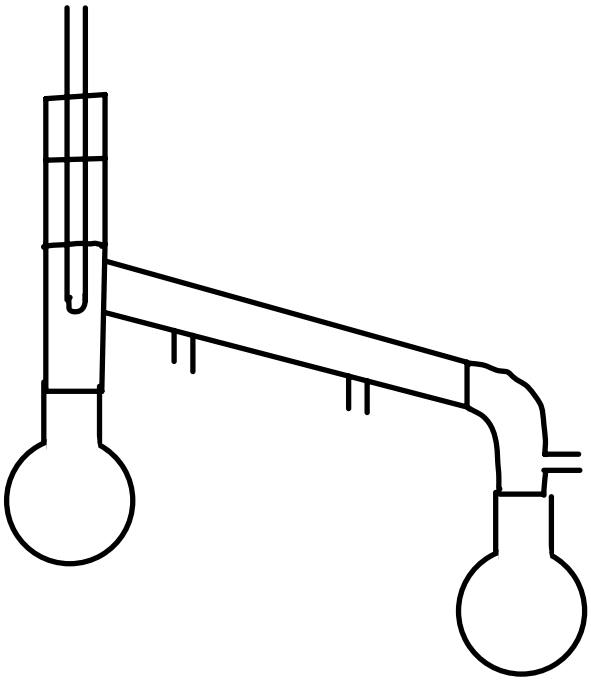
MelTemp Analysis

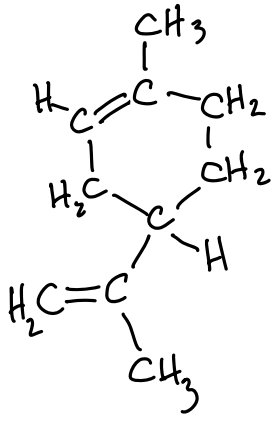
Melting Temperature Ranges, record with one decimal place, ex. 110.0 °C

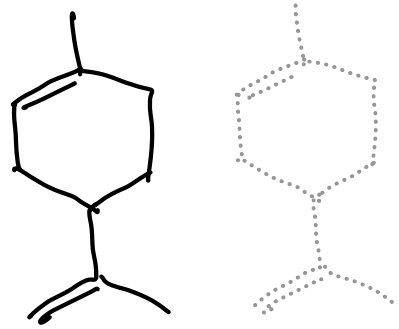
	Sweat (beginning)	**Melt (end)**
Crude Acetanilide	°C	°C
Recrystallized Acetanilide	°C	°C

Distillation of Citrus Oil





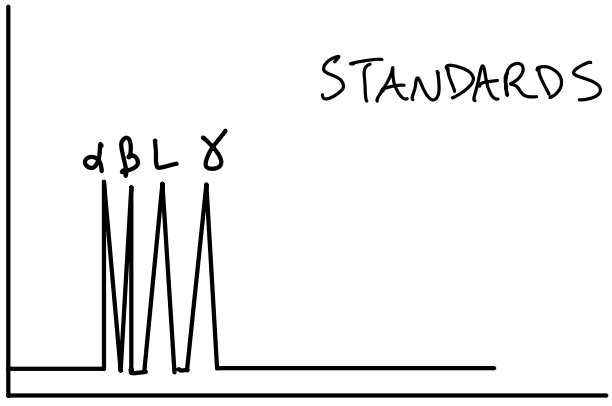


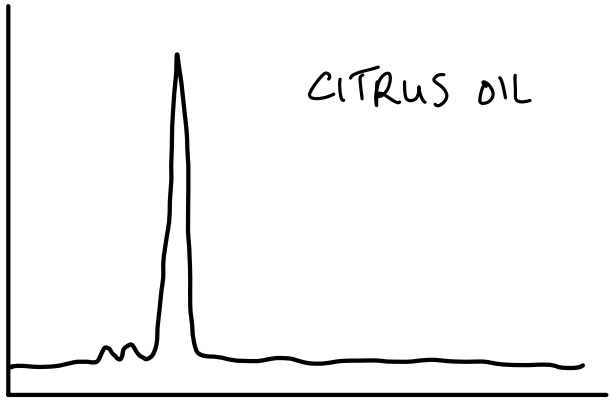


Limonene

GC

Gas Chromatography



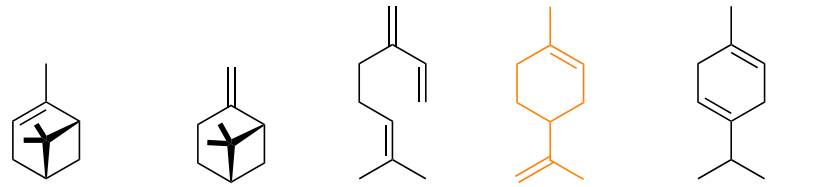


CHEM 8L, Experiment 2 – Isolation & Analysis of Citrus Oils

- Part 1 – **Isolation** of Citrus Oil **Steam Distillation** (AKA Co-Distillation): Percent Recovery & Temperature Range
- Part 2 - **Citrus Oil Analysis via Gas Chromatography (GC):** Terpene **Identification** and Percent **Composition**

Experiment 2, Part 1 – Distillation of Essential Citrus Oil

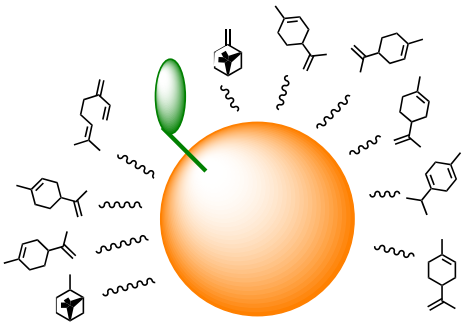
most volatile
(often evaporates before collection)



α-Pinene	β-Pinene	Myrcene	Limonene	γ-Terpinene
155 °C	165 °C	167 °C	175 °C	183 °C

Monoterpenes found in citrus oils and their normal boiling points

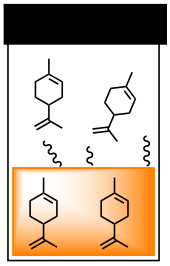
Terpenes are VOLATILE



BEFORE

vapor pressure
certain amount of
gas molecules above
solid / liquid
at a specific temperature

boiling point
occurs when the
vapor pressure of a
liquid is equal to the
pressure of surroundings



AFTER

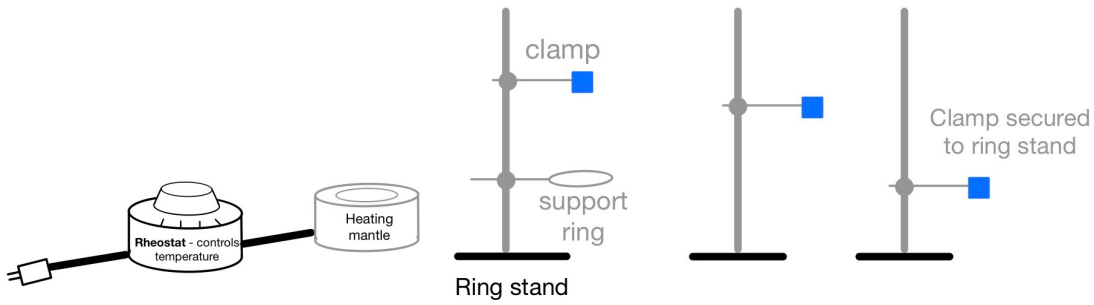
***Green = Environmentally-Friendly ***

- Water-based isolation of terpenes

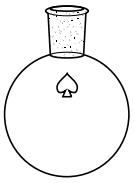
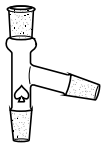
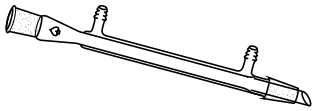
- Minimized waste (you eat the oranges, we compost the peels)

- Alternative to solvent-based extraction (toxic, wasteful; more on that in Exp 3)

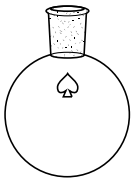
Steam Distillation of Citrus Oil

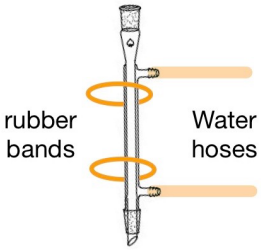


Glassware…

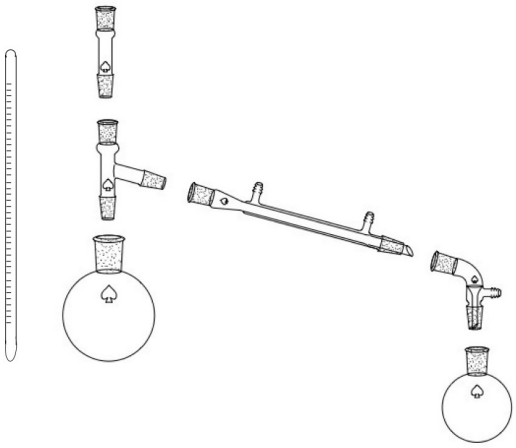
Round-bottom flask (RBF)	Distillation Head	Thermometer adaptor	Water-jacketed condenser	Take-off adapter
				

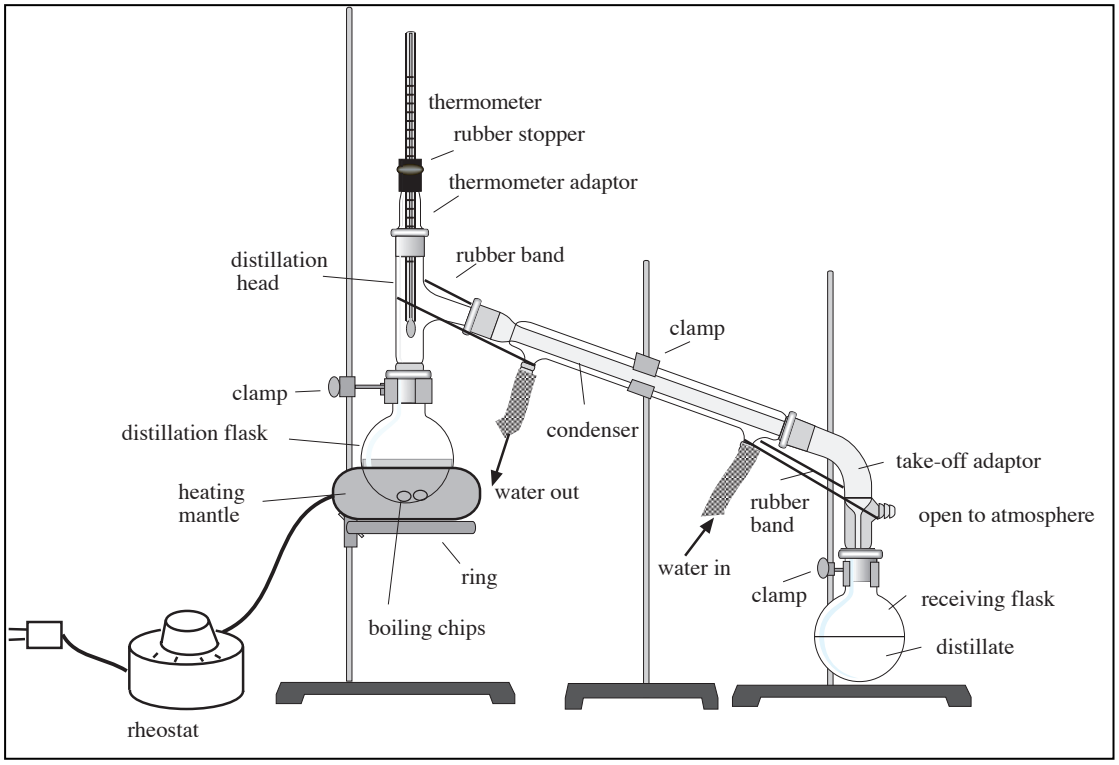
Pre-assembly…





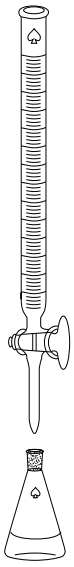
Distillation Apparatus…





Distillate Separation: Citrus Oil & Water

Isolation & Analysis



$$\text{Percent (\%) Recovery} = \frac{\text{(mass of oil)}}{\text{(mass of peels)}} \times 100\%$$

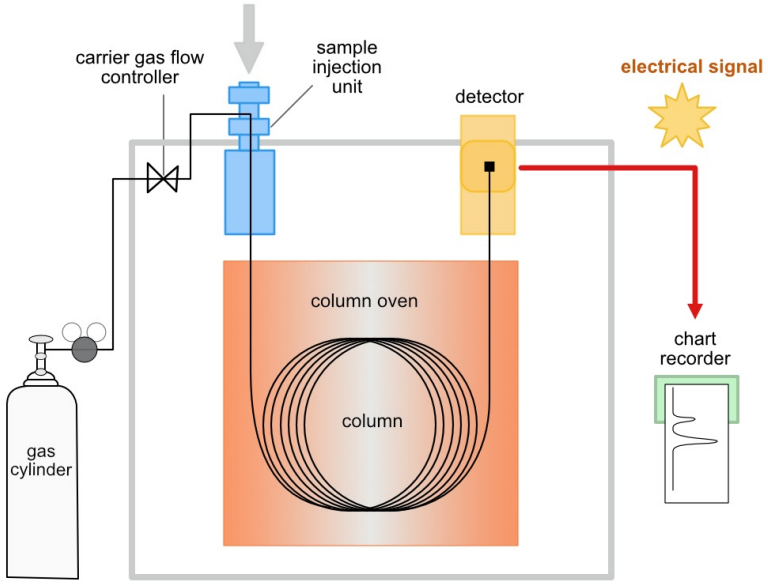
CHROMATOGRAPHY

Family of techniques for separating a **mixture** based on affinity for **mobile & stationary phases**

Exp 2.2 - Gas Chromatography (GC) – liquid mixture separated by boiling point

Mixture Stationary Phase Mobile Phase

Gas Chromatograph (GC)
Instrument Components



GC Data

1. Analyze Standard Terpenes

- Corrected Retention Times, t_R'

2. Analyze Citrus Oil

- Peak identification by t_R'

- Integration

- Percent Composition

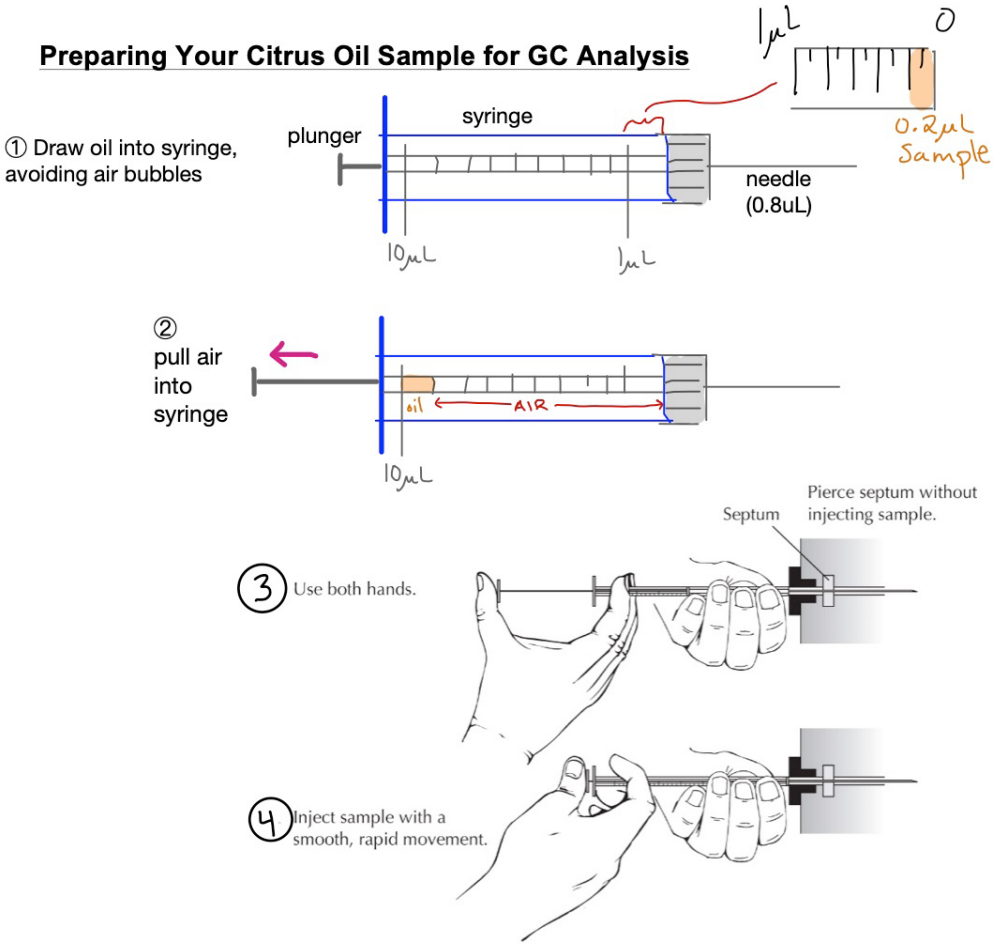
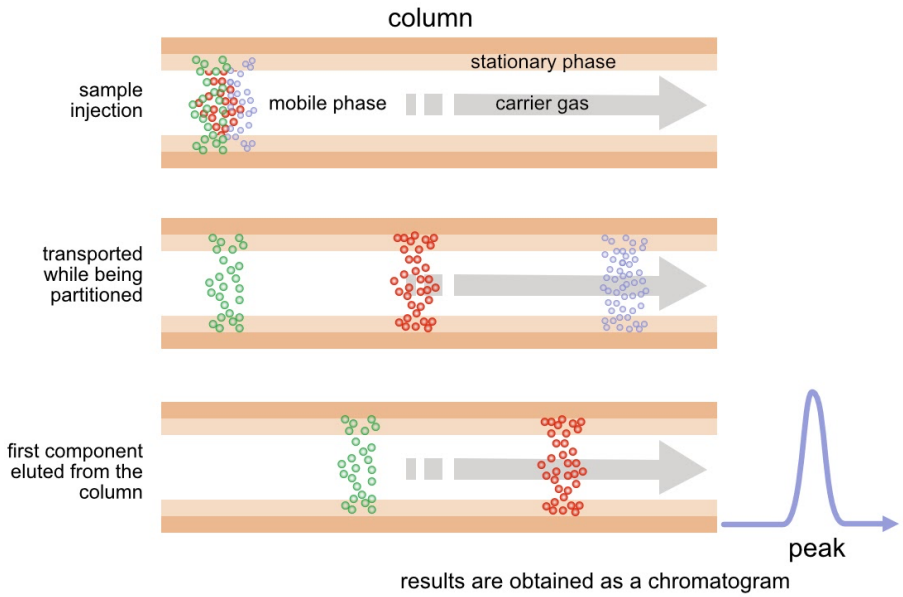


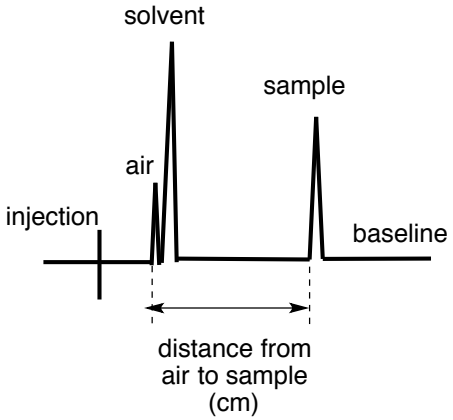
FIGURE 20.11 Injecting the sample into the column using a microliter syringe.

Separation inside the column

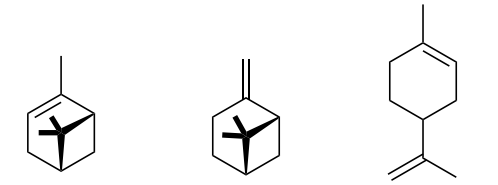


Chromatograph Analysis

(a) Corrected Retention Time (t_R') for Peak Identification



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



α-Pinene 155 °C

β-Pinene 165 °C

Limonene 175 °C

(b) Peak Integration and Percent Composition

Area = h x $w_{h/2}$

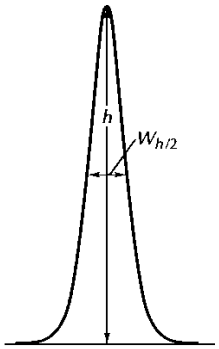
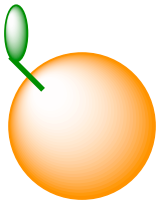


FIGURE 20.13
Determining peak area: h = height; $W_{h/2}$ = width at half-height.

$$\textbf{\% Composition, A} = \frac{\text{Area of component A}}{\text{Sum of all component areas}} \times 100\%$$

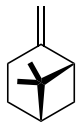
Citrus Lab Summary



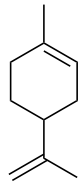
1. H_2O, heat
steam distillation

2. Buret separation
3. GC Analysis

α-Pinene
155 °C



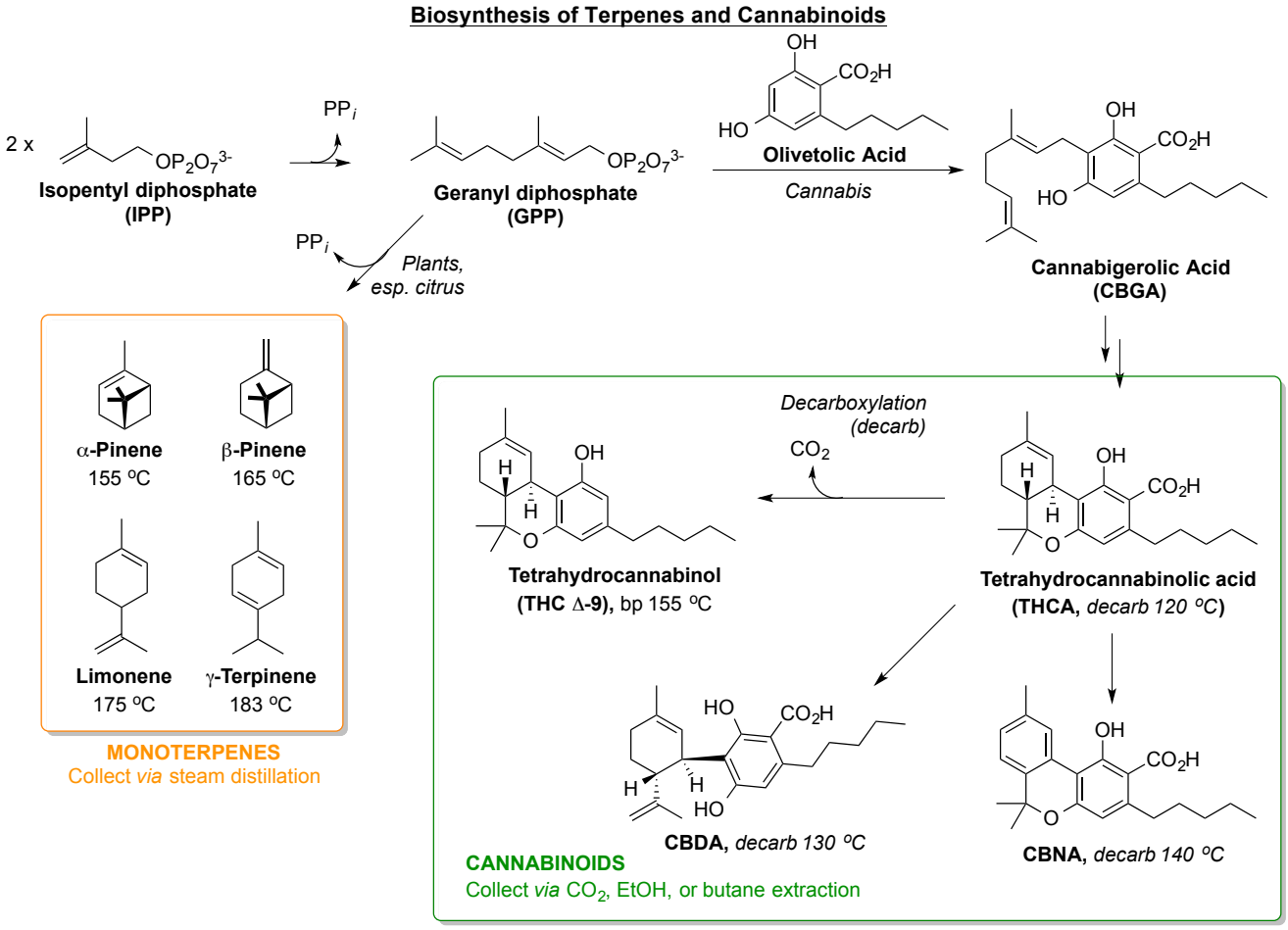
β-Pinene
165 °C



Limonene
175 °C

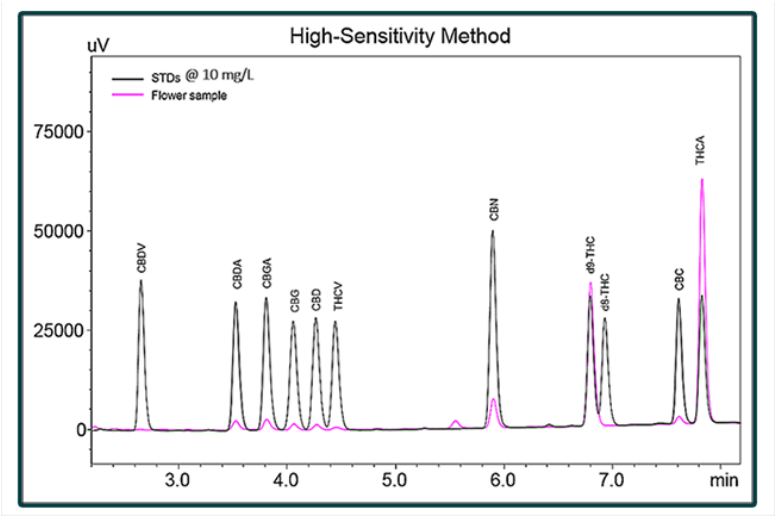
Exp 2.1 Results

Exp 2.2 Results



High Performance Liquid Chromatography (HPLC)

Room temperature separation and analysis of cannabinoids in an extract



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 Complete this Lab + Assignments

See Canvas Exp 2 Module

BEFORE LAB

1. 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 ☺
2. Attend **lab lecture** and take notes on **templates**
3. Practice the lab online *via* **Slugs@home** - sites.google.com/ucsc.edu/slugshome/home
4. **Pre-lab questions** – prepare your responses to the questions that follow the procedure
    - **Pre-lab quiz** on Canvas, due the Monday before lab – based on pre-lab questions

5. Lab Notebook Preparation – *Required at the start of lab or you'll be sent home :/*

- Refer to the **Lab Notebook Template** 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**
    - Instructions & sketches with all **equipment, chemical names** with **amounts**, & **transfers**
    - Break up text with bullet-points & diagrams. Avoid copying the procedure word-for-word.
    - *Feeling lost?* Go to the **Slugs@home website** - pictures & videos of the whole lab
    - **Cleaning & Safety** – copy the table from the end of the procedure
- **Data** – entries copied from template

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 prepared **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

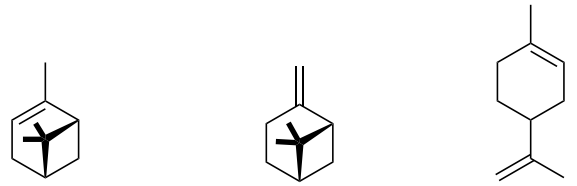
- Upload **Notebook Pages** to Canvas by midnight on lab day – graded on completeness / participation
- Complete & upload the **Lab Report** on GradeScope (GS) – due date on Canvas
    - Responses to questions & prompts - use the **template** at the end of this document

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) will be 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!



α-Pinene 155 °C | **β-Pinene** 165 °C | **Limonene** 175 °C

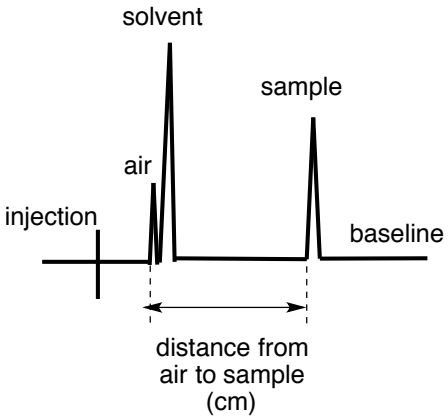
Figure 1. Terpenes commonly found in citrus oils and their normal boiling points (1 atm)

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 terpenes in citrus oils are **alpha-(α)-pinene, beta-(β)-pinene, and limonene**. The major terpene in citrus oil is **limonene, with 90 – 97% composition** (**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**).



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

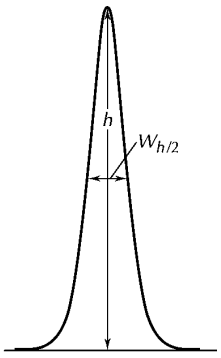


FIGURE 20.13
Determining peak area: h = height; $W_{h/2}$ = width at half-height.

Integration: Peak Area = $h \times w_{h/2}$ **(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. At Home: Prepare peels before lab. Use either **5 oranges, 6 grapefruit, 8 lemons, OR 8 limes** per group.

Select your citrus using the guidance below, or just bring what's available to you!

- Stronger citrus fragrance = higher terpene content = better recovery ☺
- No smell means low terpene content and little-to-no recovery
- Fruit fresh from the tree gives best results, if you happen to know a guy.
- Caution! Clementines or "cuties" with thin peels tend to give very low recovery ☹

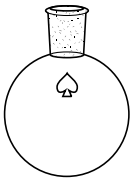
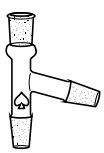
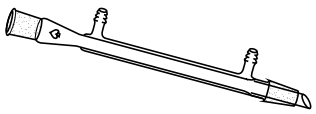
Chop or grate your citrus. Read bullet points below, then write what applies to you in your lab notebook.

- We strongly encourage you to **prep peels at home**, if at all possible.
  - **Only one grater** will be provided in each lab.
  - *Unable to bring citrus peels?* Please fill out the **Citrus Peel Request form** (Canvas Exp 2 module) by the **Friday before Exp 2.**
- **Prepare the peels the night before** and store in the fridge if necessary, but the fresher the better!
  - Transport peels to lab in a leak-proof plastic bag (no Tupperware).
- **OPTION 1, Grate:** It is ideal for the colorful part of the peel to be *coarsely* grated the morning of lab, leaving behind the pith (white part) to the best of your ability. It doesn't *need* to be a small zester, but that's ok too.
- **OPTION 2, Chop with sharp knife**: carefully cut the colorful peel away from the white pith, then further chop to approximately 1 cm^2 pieces.

2. Distillation Assembly: Tare a large beaker on the balance 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 (see **Figure 2** and more instructions on next page).

Caution!

- The peels *and water* should already be in the flask *before* clamping it to the ring stand.
- If you forget to add the distilled water before heating, the orange peels will scorch the flask ☹
- Do NOT transfer orange peels over the heating mantle. That would create burning orange peels later ☹

Round-bottom flask (RBF)	Distillation Head	Thermometer adapter	Water-jacketed condenser	Take-off adapter
				

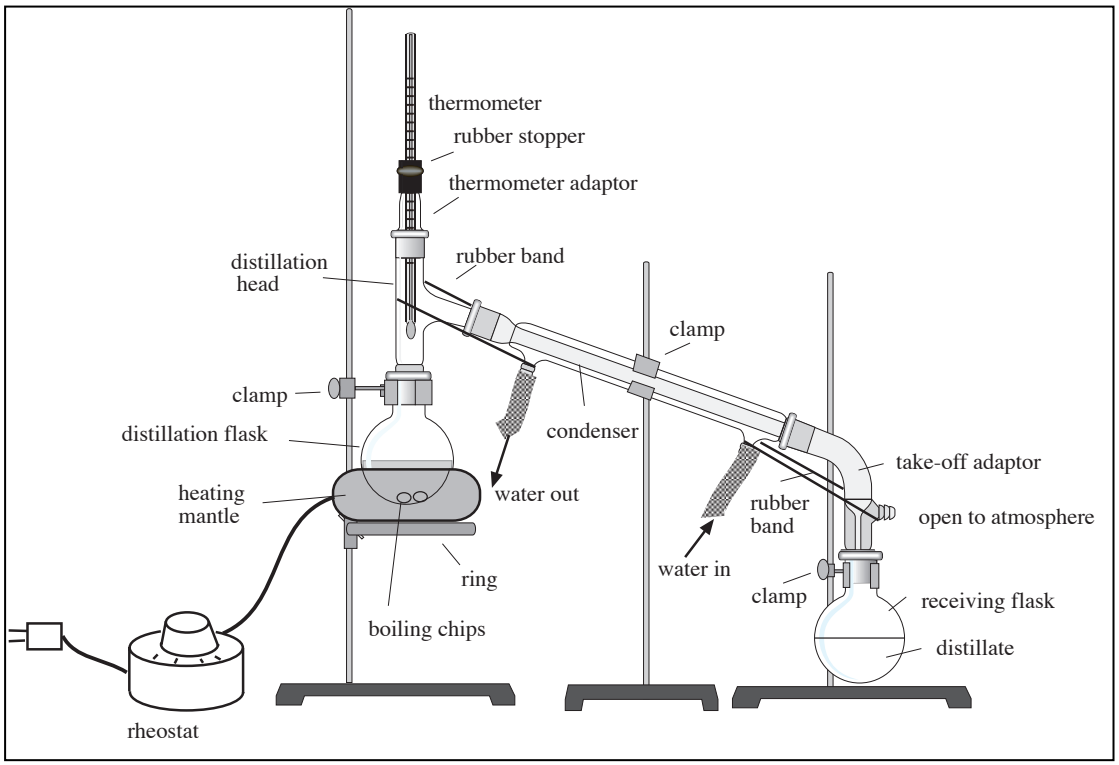


Figure 2. A 'simple distillation apparatus' used for steam distillation (omit boiling chips).

** See also the **Exp 2 class note templates** for step-wise diagrams to set up the distillation apparatus.
Copy these labeled diagrams by hand into the procedure portion of your notebook.
Your TA will demonstrate the distillation setup at the beginning of lab.

In addition to the supplies in your drawer, obtain **three ring stands, 2 rubber bands, 3 clamps, 1 support ring, 4 clamp holders,** and **2 hose clamps**. 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. Leave a **small air gap (1 mm)** in between the flask and the heating mantle. 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 assembling the rest…

Add a *small dab* of **grease** to the joint of the **distillation head**. Attach the distillation head to the clamp and turn to spread the grease. Separately, insert a **thermometer** into the red **rubber thermometer adaptor** (not a stopper) using water as a lubricant (NOT grease), then secure the rubber adaptor around the **glass thermometer adaptor**. Lightly 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 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 tightly around the condenser. 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. When the **first drop of distillate** enters the receiving flask, **record this initial temperature** on the thermometer, estimating one decimal place (ex. 97.0 °C). Thank you for your patience - it may take about 20 minutes for that first drop of distillate (mostly water, tiny bit-o-terpenes).

**** During the distillation down-time, carry out the GC calculations (see Part 2).**

- **Chromatograms (graphs) will be provided in the lab.**
- Prepare before lab - copy the GC data tables from the Exp 2 Lab Notebook Template.

[Back to distillation] Adjust the heat supply so that the liquid distills at an approximate rate of **one drop per second**. Distilling too quickly with too high heat will severely decrease recovery of oil and becomes dangerous. Collect approximately 70 mL of distillate. Record the **final temperature**, estimating 1 decimal place (ex. 99.0 °C). **Stop the distillation:** turn off and unplug the heating mantle, then carefully lower the ring support for the heating mantle. You will need two sets of hands to accomplish this safely. You may take off the receiving flask; place a **beaker** under the take-off adapter to collect any further distillate.

Wait for the entire system to cool before disassembling. Refer to the **Clean-Up & Safety Table** for taking apart the cooled apparatus.

3. Separation and Collection of Citrus Oil: Fasten a 50-mL buret to a ring stand using a buret clamp. Use a funnel to add distilled water to leave about 5-mL of space above the liquid. *Slowly* add a few milliliters of the orange distillate to the buret, being careful not to overflow. Allow the liquid to settle for 1-2 minutes, 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 oil, 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 this top layer of citrus oil 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 labeled vial with the oil and determine the mass by difference. Calculate the **% recovery** of citrus oil from citrus peels. Cap the labeled vial and store in your drawer for GC analysis (Day 2). **Share your peel observations** (3 descriptors – color, size, and fragrance), as well as **% recovery with a neighboring group**. You'll need this for the lab report!

PROCEDURE, PART 2 – GAS CHROMATOGRAPHY (GC) ANALYSIS OF CITRUS OIL

* *Time permitting*, TAs will guide students in the use of the **GC during distillation down-time**.

* **Sample charts** will be provided to ensure everyone gets GC data for the lab report (standards & citrus oil).

* Bring a **calculator**; take off gloves when using it. Do not contaminate your phone in the lab!

4. GC Sample Preparation: 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 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. GC Data Collection: Injection of Standard Terpenes & Citrus Oil

(time permitting; see notes on previous page)

Inject the terpene standard: **α-pinene, β-pinene,** and **limonene (1:1:1 mixture)**. Allow all the components to come out before performing another injection. Measure and calculate the **corrected retention times** of each standard (see page 3, **eq. 1**) *before* injecting your citrus oil. Although not ideal, **standard peaks** can still be interpreted if the peaks level off at the top (flat), because only the retention time is needed.

Ask your TA to inspect your oil for potential water contaminants. Then, **inject 0.2 μL of your citrus oil** into the **same GC instrument used to inject terpene standards**. Since the pinenes are such minor components in citrus oil, the tallest terpene peak (limonene) should be about 75% of the chart height. Repeat the citrus injection to reveal minor components, if needed – complete no more than 3 injections and consult your TA. 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.** It is relatively common for limonene to be the only peak in the citrus oil chromatogram.

Calculate the **corrected retention time** of each sample peak in your citrus oil chromatogram. Determine which **terpenes are in your citrus oil** by comparing to the corrected retention times of the terpene standards. Measure the height (**h**) and the width-at-half-height ($\mathbf{w_{h/2}}$) of each sample peak. Calculate the **area** of each peak (integration) from these measurements (page 3, **eq. 2**). Calculate the **percent composition** of the oil (page 3, **eq. 2**).

Set yourself up for success: *Copy the examples and equations from page 3 into your notebook. These calculations must be performed during this lab period and checked with your TA.*

**** Take a picture of your terpene standard chromatogram (chart)** and your best **citrus oil chromatogram** to include in your lab report. We cannot grade your results without your GC chromatograms! **

Table 1. Clean-up and Safety – Exp 2 - 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!).	α-pinene and β-pinene are **irritants**. Limonene is a possible **carcinogen**.
Dispose of **pipets** in the glass waste box.	Minimize chemical exposure by wearing a **lab coat, goggles, and gloves** during the entire experiment.

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 citrus oil in its labeled vial in the waste container bag 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

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

1. What is a **terpene** and what is the approximate percent composition of each of the terpenes from **Figure 1** in orange 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. How are terpenes collected in this steam distillation, even though their boiling points are significantly higher than water?

5. Define the following concepts and terms as they relate to gas chromatography.
   a) Mobile Phase, AKA Carrier Gas
   b) Stationary Phase
   c) Integration
   d) Corrected Retention Time

6. Look up the **boiling points** of the terpenes commonly present in citrus oil. Predict the order in which these compounds should exit the GC column: **β-pinene, limonene, α-pinene.**

Full Name: **Lab Section:** [replace with day, time, room]

Lab Partner Name: **TA Name:**

EXPERIMENT 2 LAB REPORT TEMPLATE, Page 1 of 4

Download the "Lab Report Template" from Canvas and edit the MS Word doc; or **recreate** the template in a new word processing document. [Keep these instructions]

- Responses must be on the on **same part of the same page as this template** for grading.
- For each question / prompt, provide **TYPED, numbered** responses in **complete sentences**.
- Insert images of **hand-drawn diagrams** and **calculations**, where applicable.

1. Report the **temperature range of distillation** during the collection (temperature at which first and last drop of distillate was collected). [Top of page 1]

2. Was the **initial** distillation temperature **higher or lower** than water's boiling point?
Explain why the **presence of the peels** affects the **boiling temperature of the water molecules**.
[Middle of page 1]

3. Report the **mass (mg)** and calculate the **% recovery of citrus oil** from the peels. **Show your work** with units on each value and state these results in a complete sentence.
[Bottom of page 1]

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

EXPERIMENT 2 LAB REPORT TEMPLATE, Page 2 of 4

4. Report the **observations** of your citrus peels (at least 3 characteristics – ex. size, color, aroma). [Top of page 2]

5. Report a different group's peel observations and % recovery. [Middle of page 2]

- Or compare your result to the "Slugs@home" remote lab: calculate the % yields for chopped or grated peels using **150.00 g** as the initial mass of peels. See links in Canvas Exp 2 module.

6. Consider how the **terpene molecules** are released from the **peels** and **explain** the observed **relationship between peel size and % recovery**. [Bottom of page 2]

EXPERIMENT 2 LAB REPORT TEMPLATE, Page 3 of 4

7. Gas Chromatography – Standard Terpene Analysis

Note: use the *GC data obtained in the lab OR the sample charts provided*; don't mix & match! Retention times of standards and citrus oil will only match if they're run on the **same instrument**.

(a) Conditions: Report the **GC Name** and how data was obtained (**sample charts or new injections**). [Top of page 3]

(b) Standards: Report the **corrected retention times (t_R')** for each terpene standard, table format below. [Middle of page 3]

Standard (pure) GC Corrected Retention times, t_R'

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

(c) Show any one **sample calculation** for **t_R'** of a terpene standard. [Bottom of page 3]

[insert image of GC standard chart analyzed]

EXPERIMENT 2 LAB REPORT TEMPLATE, Page 4 of 4

8. GC Analysis of Citrus Oil

(a) Citrus Oil: Using the table format below, Report the **corrected retention time, integration (area), and % composition** for each peak in the GC chromatogram of citrus oil. Assign a number to each sample peak. [Top of page 4]

(add as many rows as needed)

Peak #	**Peak (Terpene) Identification**	**t_R' (s)**	**Integration (cm^2)**	**% Composition**

(b) Show one **sample calculation** each for **integration (area)** and **percent composition**. [Middle of page 4]

5. What is the **major component** of the citrus oil you analyzed? What are the **minor components**, if any? How does this compare with the expected composition? *Hint: everyone should have the same major component!* [Bottom of page 4]

Name _____ Partner Name _____

TA Name _____ Section Day _____ Time _____

Experiment 2 Lab Notebook Template
Citrus Distillation and GC Analysis
Use as reference for notebook preparation – every student uploads to GradeScope after lab

Pre-Lab Requirements – must be complete *before* arriving to lab

1. **Lab Notebook:** copy templates below into designated notebook
    - **Purpose, scheme,** and **reagent table**
    - **Procedure Diagrams**
    - **Cleaning and Safety** – copy table from the procedure
    - **Data entries**
2. **Dress for lab** – see safety rules – arrive a few minutes early

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

B. Reagent Table – properties must be filled in before lab

Sample Name	**Amount** Fill in during lab	**Molecular Mass**	**mmoles** Fill in during lab	**Boiling or melting point**	**Density**	**Hazards** Refer to clean-up & safety table
Citrus Peels		n/a	n/a	n/a	n/a	n/a
Water						
Citrus Oil Enter properties for **limonene**						

List terpene hazards:

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

- Instructions, diagrams, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
- 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

- Include diagram of complete distillation setup with labeled components
- Step-wise procedure to assemble the distillation apparatus

Step 3. Separation and Collection of Citrus Oil

- Separation of citrus oil from distillate in a buret and collection in a vial

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

Cleaning & Safety – copy table from procedure

Cleaning and Safety – copy the tables from the end of the procedure

- Includes instructions for safely taking apart the distillation apparatus and returning shared equipment

D. Data

Description of your peels *and* those of a neighboring pair (3 characteristics, ex. size, color, fragrance, texture)

Mass of citrus peels _____ g Volume of water _____ mL

Distillation temperature: first drop _____ °C Final temperature _____ °C

- Estimate one decimal place, ex. 97.0 °C

Mass of citrus oil _____ g Percent Recovery _____%

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

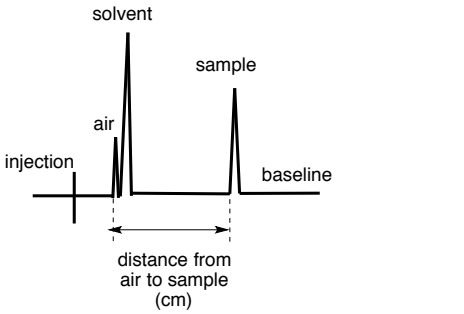
Record the **Observations** and **% recovery** of a neighboring group in your lab: _____ %

* you'll need this and comparison of your peels to their for the lab report (bigger or smaller pieces of peel?)

E. Data & Calculations: Gas Chromatography (GC)

- See Experiment 2 for background on GC; equations below.
- GC charts will be obtained in lab, time permitting, or sample charts provided.
- Copy the sample calculations and tables below into your notebook.

Corrected Retention Time, t_R'



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

Integration and Percent (%) Composition

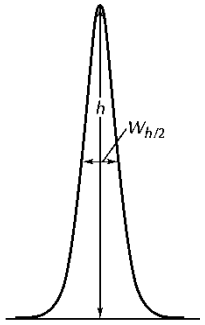


FIGURE 20.13
Determining peak area: h = height; $W_{h/2}$ = width at half-height.

Integration:

Peak Area = h x $w_{h/2}$

% Composition, A = $\frac{\text{Area of component A}}{\text{Sum of all peak areas}} \times 100\%$

Table 1. Standard Terpene GC Retention times

Sample	Corrected t_R (s)
alpha-pinene	
beta-pinene	
Limonene	

Retention Time Calculations:

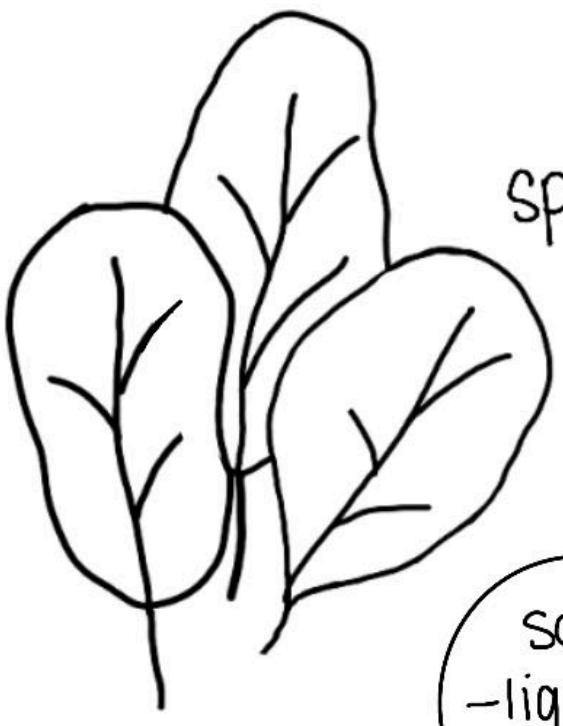
Table 2. GC Analysis of Citrus Oil

* Not all standards may be present in the oil, some peaks overlap, and other unknown peaks may be present. Add or remove rows as needed.

Peak #	Compound Name	Corrected t_{Rt} (s)	Integration (cm^2)	% Composition

Calculations (retention time, integration, % composition):

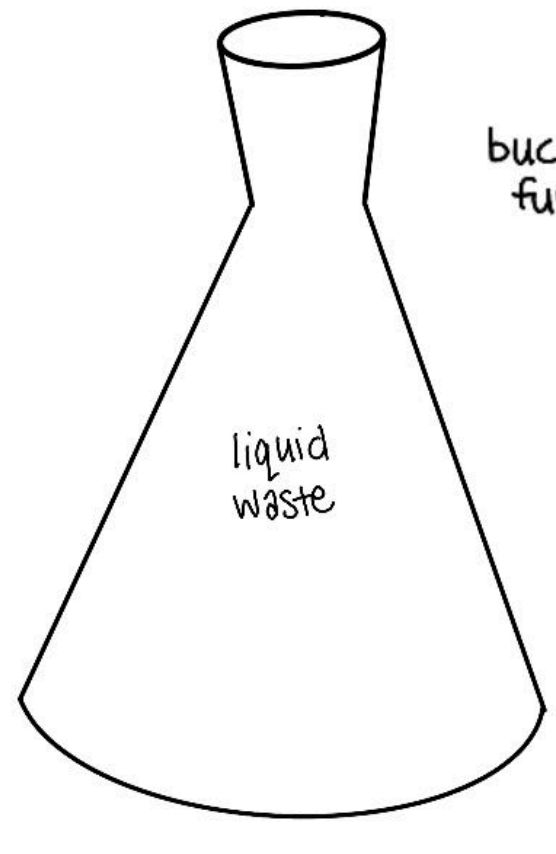
THIN-LAYER chromatography of SPINACH PIGMENTS



spinach

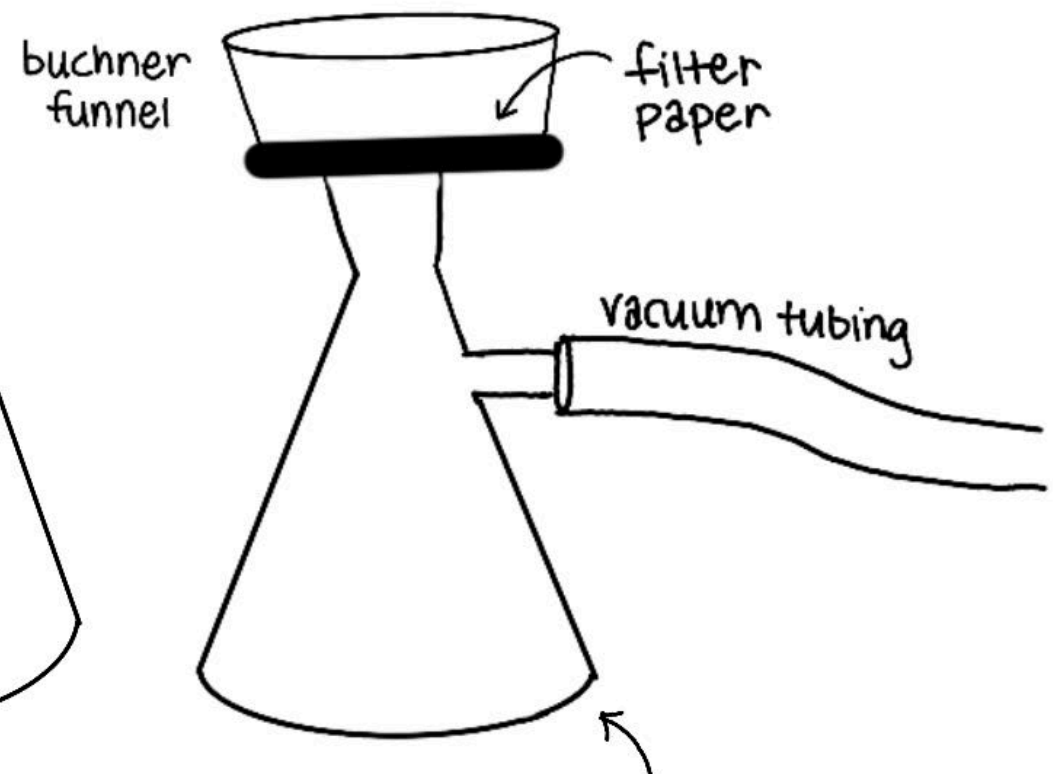
solid-liquid extraction

MeOH

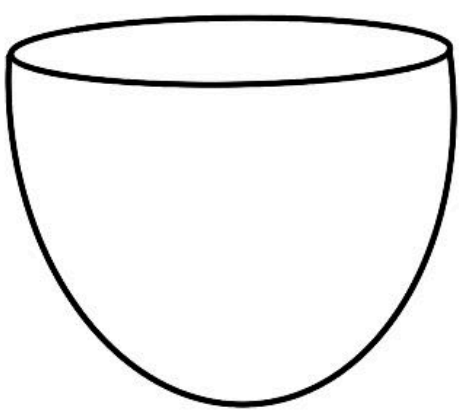


MeOH

vacuum filtration

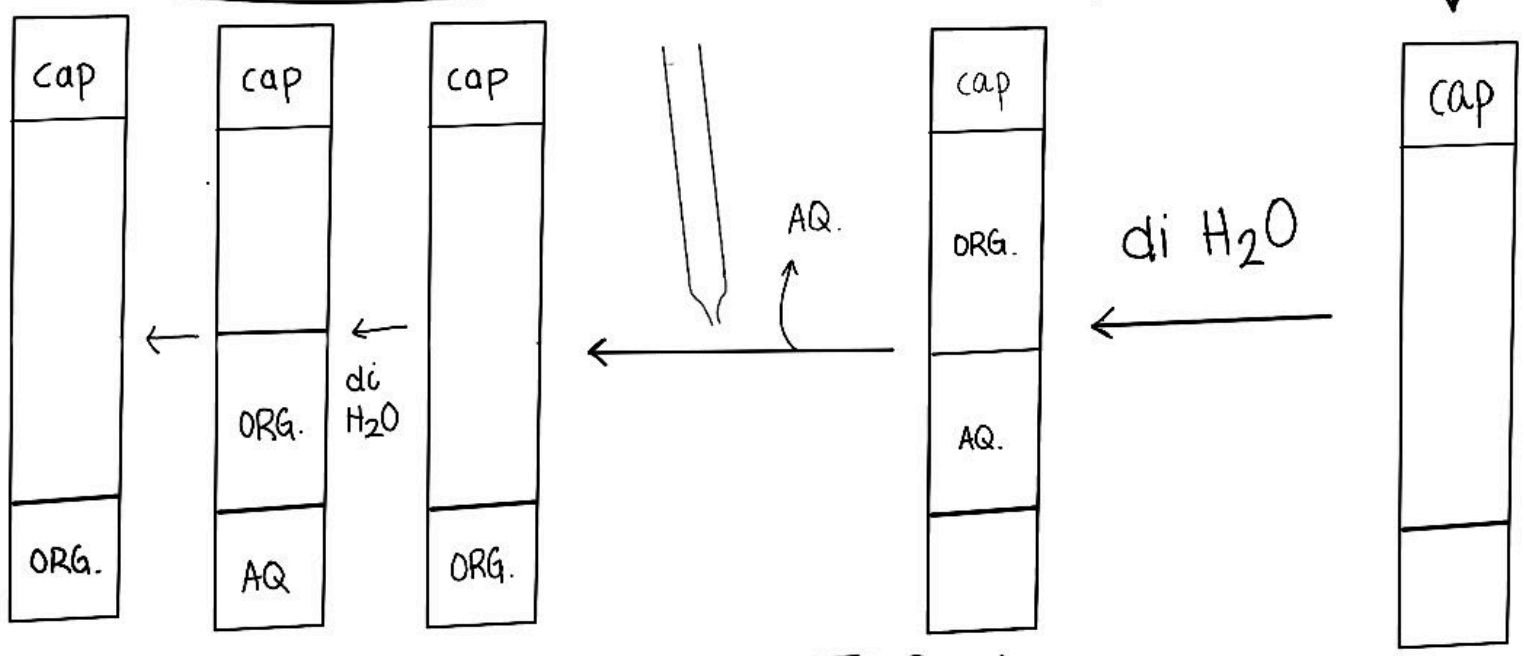


Hexanes

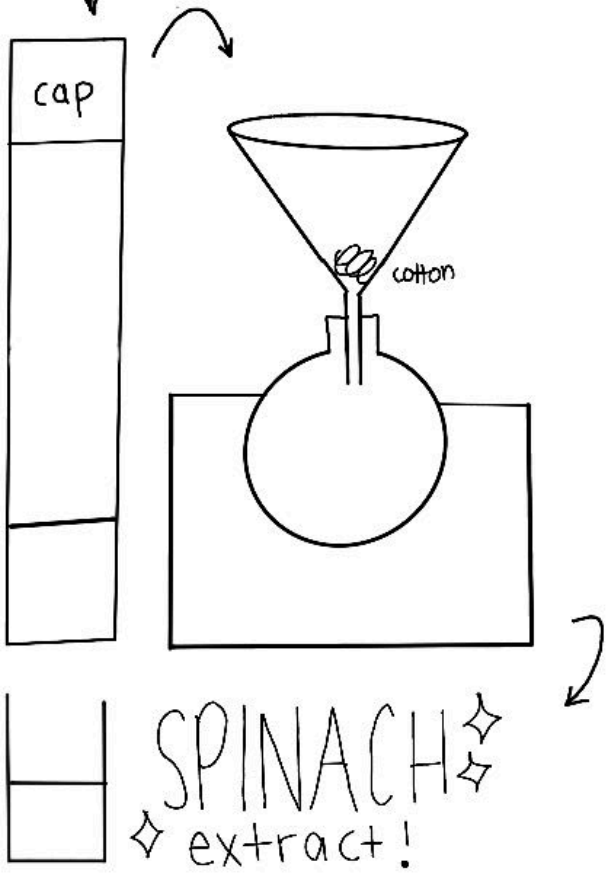


liquid-liquid extraction

screw-cap test tube

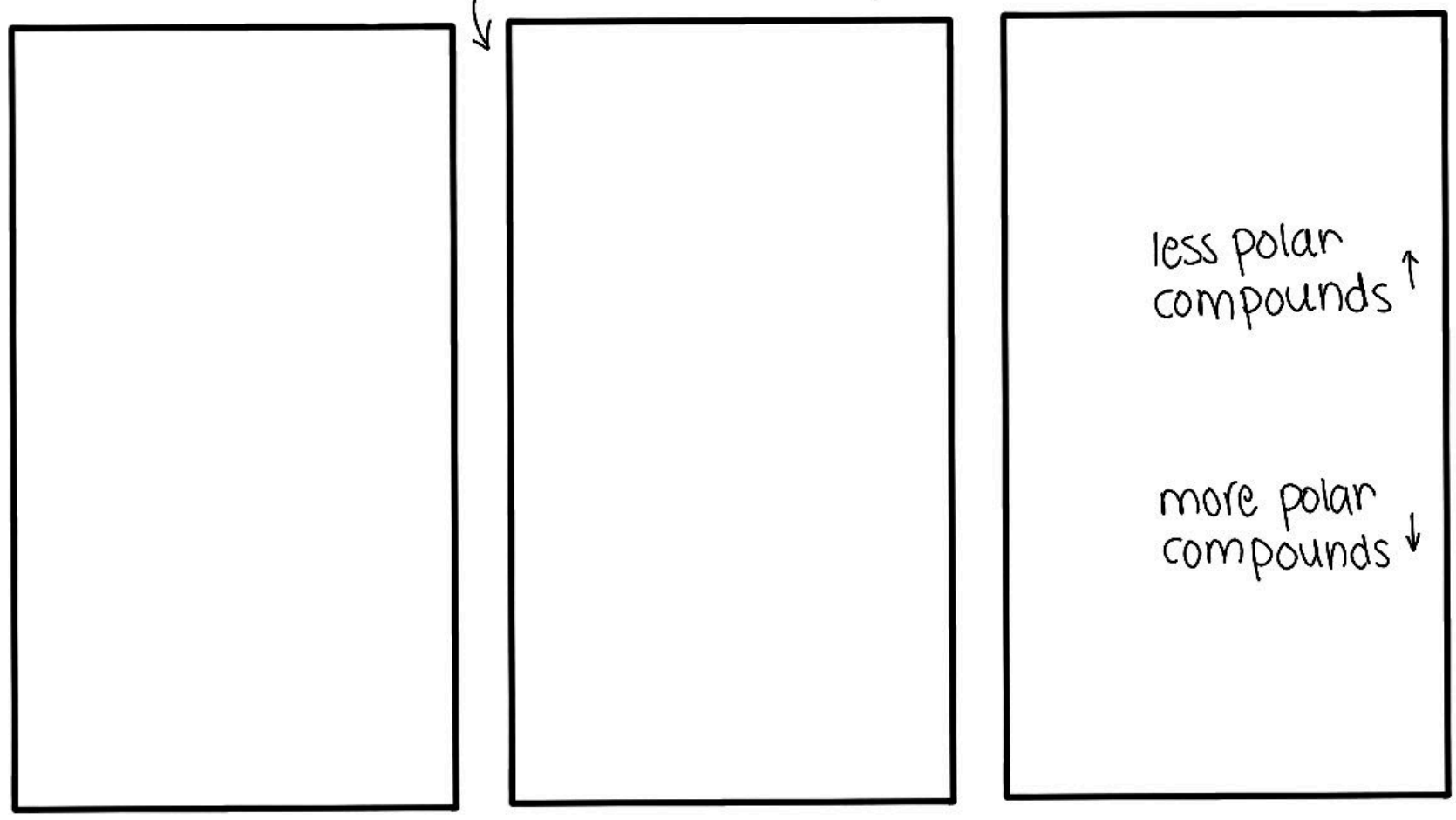


anhydrous Na_2SO_4



SPINACH extract!

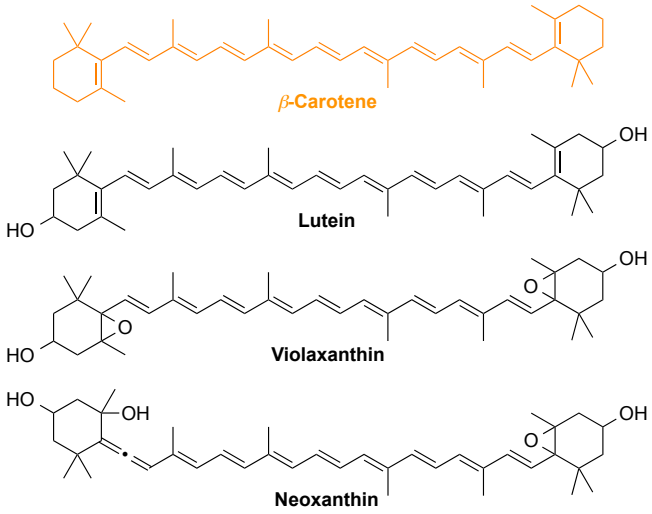
color in TLC plates



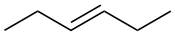
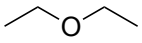
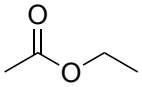
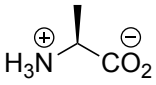
CHEM 8L, Experiment 3 – Extraction & Thin-Layer Chromatography (TLC) Analysis of Spinach Pigments

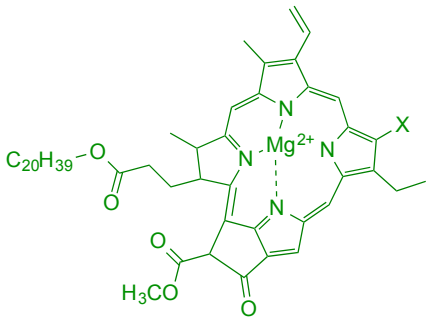
1. **Solid to liquid extraction** of pigments from spinach
2. **Liquid – liquid extraction** of pigments from unwanted water- soluble components
3. **TLC** analysis / separation of individual pigments

Spinach Pigments: carotenes, xanthophylls, chlorophylls, and pheophytins

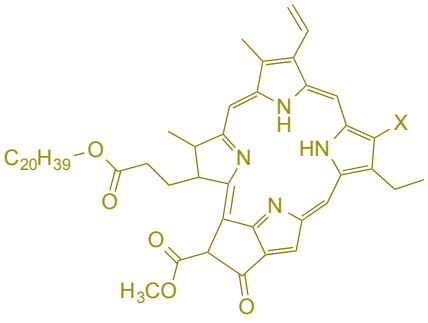


Relative Polarity

Functional Group	Example	
Alkanes	Hexane	
Alkenes	Hexene	
Ethers	Diethyl Ether	
Esters	Ethyl Acetate	
Ketones	Acetone	
Aldehydes	Formaldehyde	
Alcohols	Methanol	CH_3OH
Charged Compounds	Amino Acids	

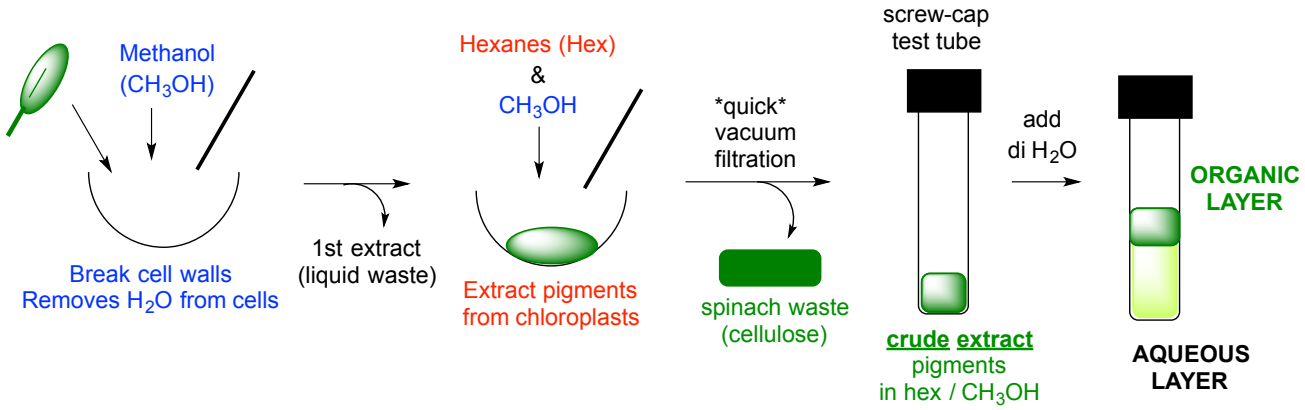


Chlorophyll a, X = CH_3
Chlorophyll b, X = CHO



Pheophytin a, X = CH_3
Pheophytin b, X = CHO

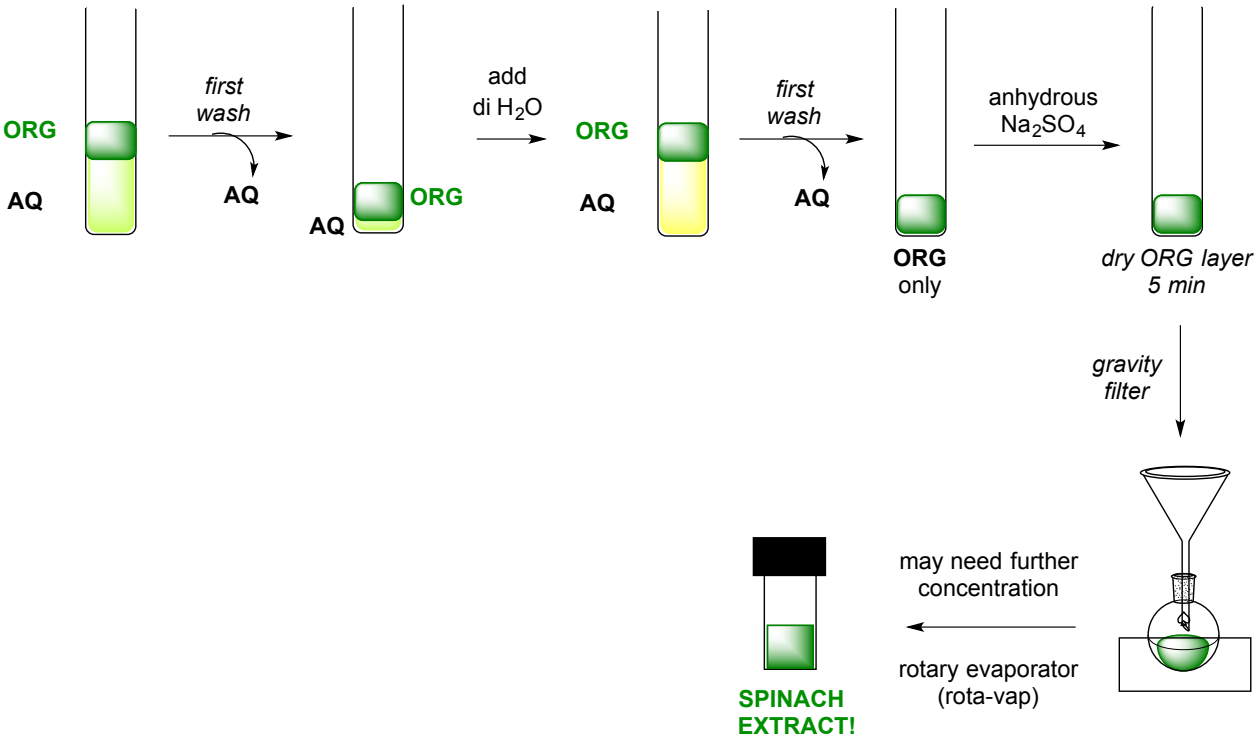
1. Solid to liquid extraction



2. Liquid – Liquid Extraction

Partition Coefficients ($K_{H/W}$) = Ratio of solubilities of pigments in organic solvent (ex. Hexane) vs. water

$K_{H/W}$ = (solubility of pigments in hexane) / (solubility in water) >> 1

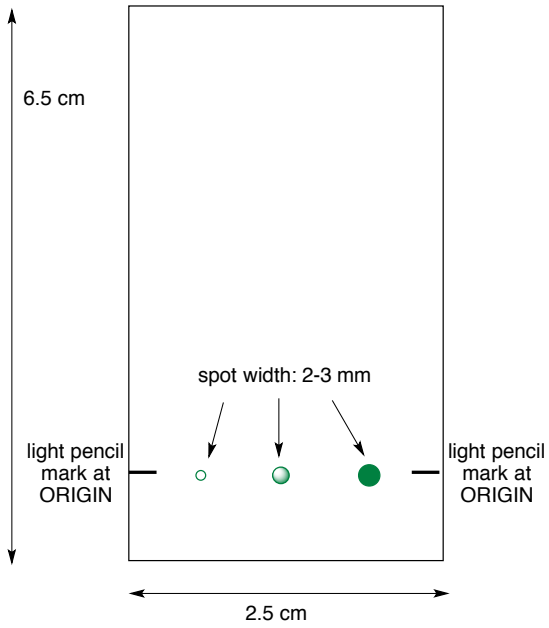


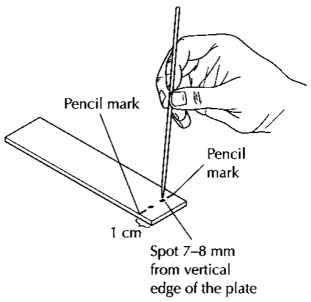
3. Thin-Layer Chromatography (TLC) Analysis

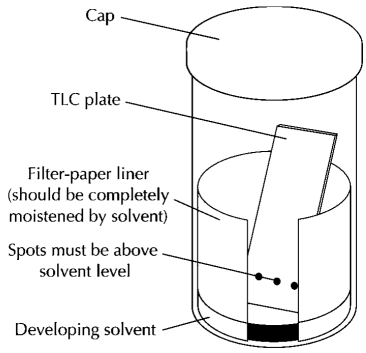
Chromatography Overview

	Classification	Nature of Stationary Phase	Nature of Mobile Phase	Property for Separation
GC	Partition			
TLC	Adsorption			

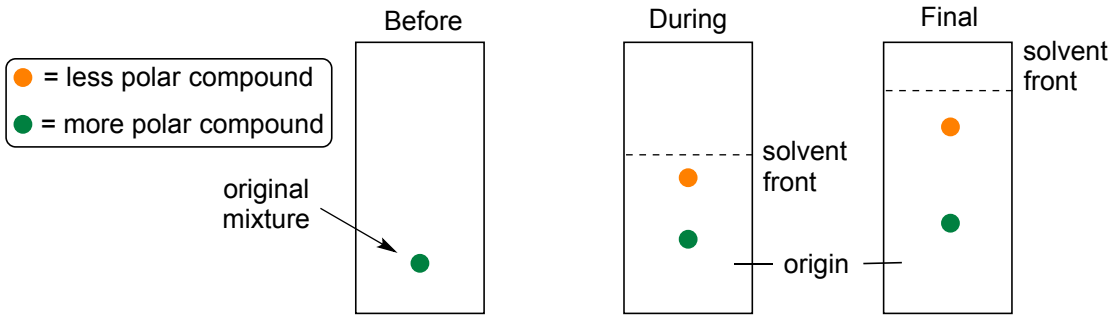
Spotting the plate







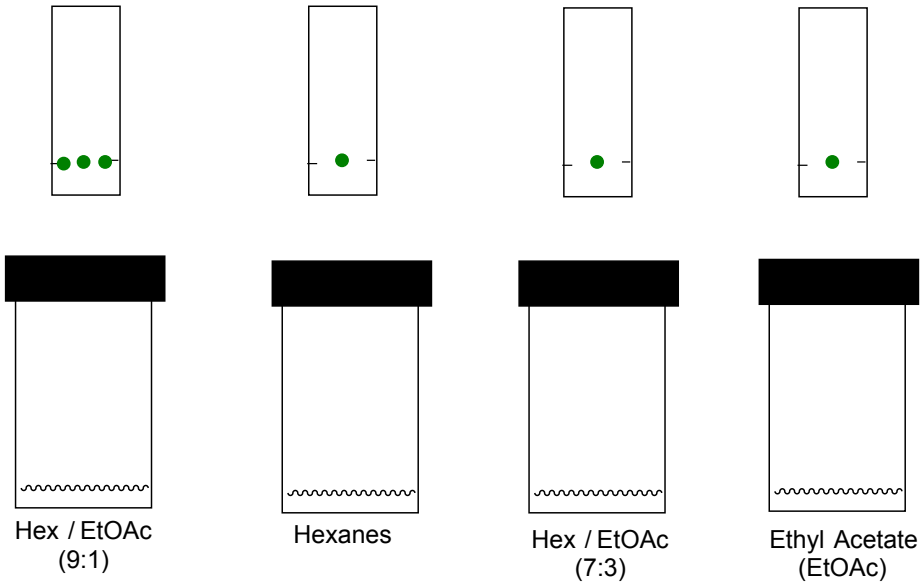
Running the plate



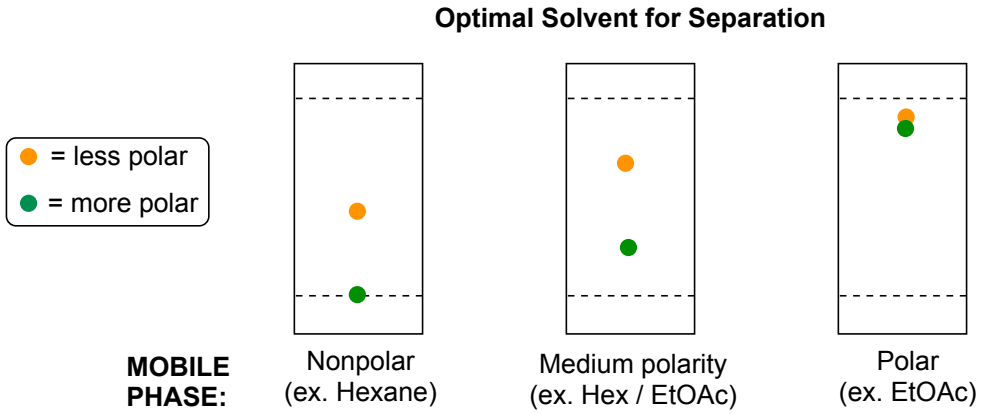
Calculate Retention Factor (R_f) for every spot…

$$R_f = \frac{\text{final distance from origin to } \textbf{spot} \text{ (cm)}}{\text{final distance from origin to } \textbf{solvent front} \text{ (cm)}}$$

Optimal TLC solvent (mobile phase) for pigment separation



TLC Separation of pigment mixture: polar, medium polar, and non-polar pigments



EXP 3 – SPINACH LAB SUMMARY

1. **Solid to liquid extraction**
2. **Liquid – liquid extraction**
3. **TLC**

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 Complete this Lab + Assignments

See Canvas Exp 3 Module

Before Lab

- Read this document: background, procedure, safety, pre-lab and in-lab questions
- Attend **lab lecture,** fill in the class note **templates**
- Preview the lab online *via* **Slugs@home platform**
- Complete the **pre-lab questions** (towards the end of this document) - incorporated into quiz ☺
    - **CPre-lab quiz** due midnight, Monday before your enrolled section (Canvas)
- You must have your lab notebook prepared to participate in lab…

**** Lab Notebook Preparation**

- *Refer to the Exp 1 Lab Notebook Templates on Canvas*
- **Purpose:** one-sentence summary of the lab goals and the recrystallization scheme (**Figure 5**)
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info!
    - Refer to the 'Cleaning & Safety' table after the procedure for one-word chemical **hazard**
- **Procedure with Diagrams** – examples in class notes
    - Draw simple sketches with labels: all **equipment, chemical names** with **amounts**, & **transfers**
    - Break up text with bullet-points & diagrams. Avoid copying the procedure word-for-word.
    - **Slugs@home Exp 1 website** – preview pictures & videos of the whole lab!
- **Cleaning & Safety** – copy the table from the end of the procedure
- **Data** – entries copied from template

During Lab (TuWTh)…

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- Please wait for your TA arrive before entering the lab
- Show your prepared **lab notebook pages** to your TA
    - You'll be sent home if not prepared :/
- Perform the experiment with a partner, fill out data & observations in your **lab notebook**

After Lab…

- Each student submits separate, individual assignments
- Upload **Notebook Pages** to GradeScope by midnight on lab day
- Complete & upload the **Lab Report** on GradeScope (GS) – due Friday, one week after lab

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)!*

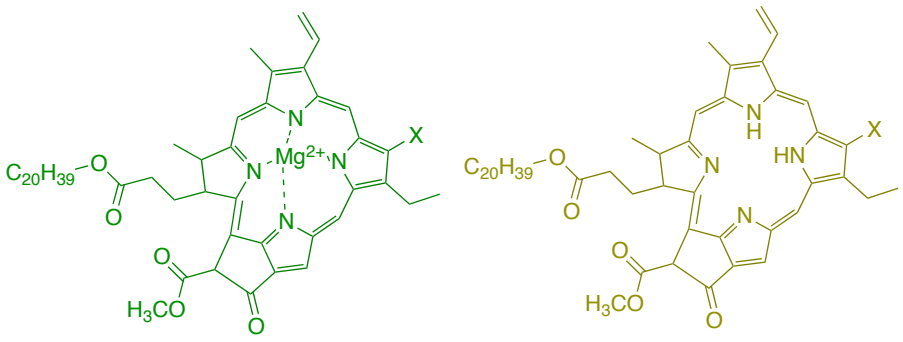


Figure 1. Structures of chlorophylls and pheophytins.

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**).

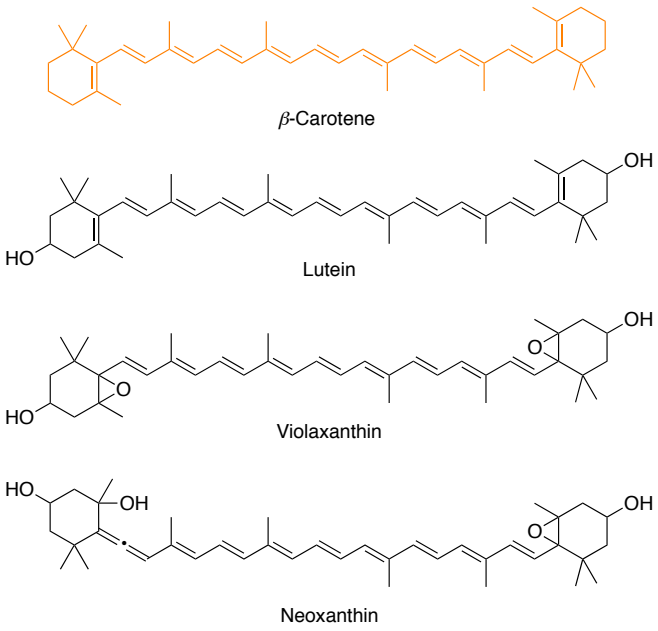


Figure 2. Structures of select carotenes and xanthophylls.

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.

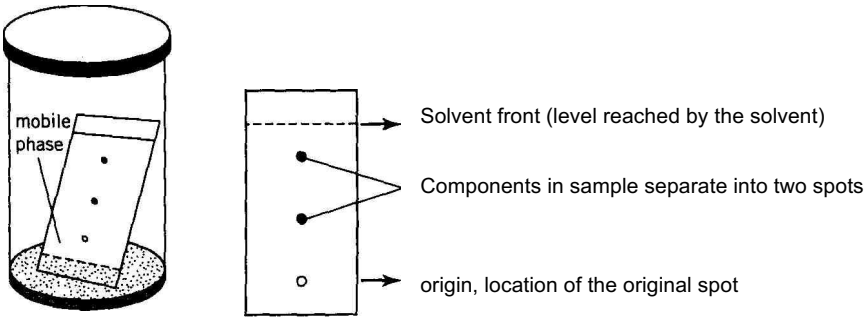


Figure 3. TLC chamber and developed plate

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.

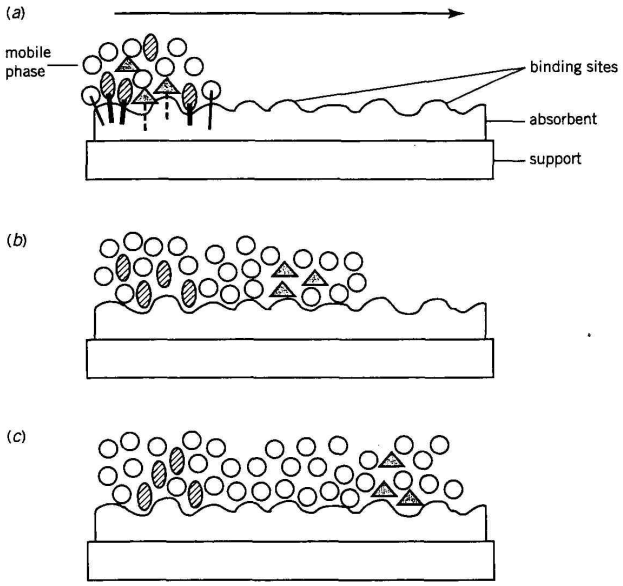


Figure 4. Separation of two compounds on a TLC plate (ovals and triangles). The arrow indicates the direction of the mobile phase flow (see text).

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 (more polar solvents have higher dielectric constants). 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.

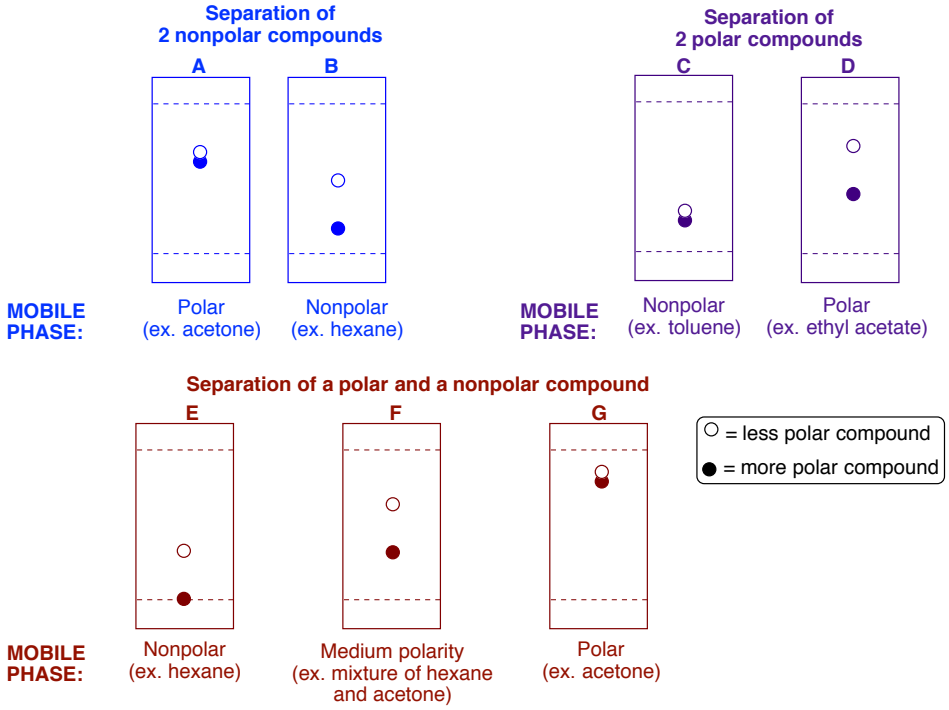


Figure 5. Separation of hypothetical mixtures.

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 farther up the plate.

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

PART 1. Solid-liquid and liquid-liquid extraction

Weigh 10 g of fresh spinach leaves (provided in lab) and chop 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.

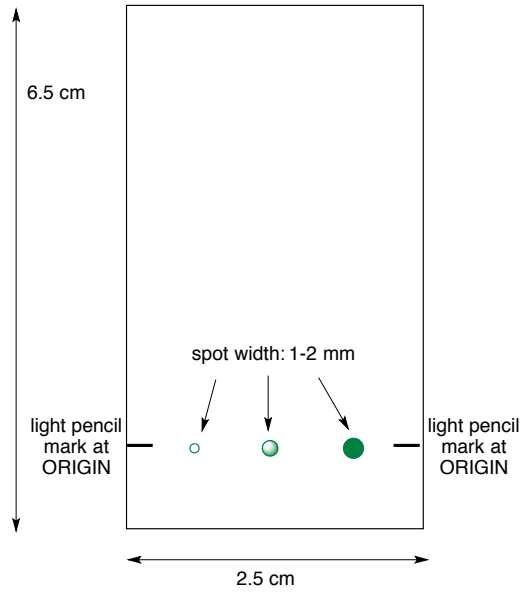
PART 2. Thin-Layer Chromatography (TLC) Analysis

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 3: 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 4: 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 **discuss the in-lab questions with your partner 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** (see page 3)

   lutein (L), neoxanthin (N), β-carotene (C), violaxanthin (V).

- Which pigment should have the **highest R_f** on TLC?
- Which pigment should have the **lowest R_f** on TLC?

Take the **Canvas Exp 3 pre-lab quiz** by the **Monday** *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!*

Full Name: **Lab Section:** [replace with day, time, room]

Lab Partner Name: **TA Name:**

EXPERIMENT 3 LAB REPORT TEMPLATE, Page 1 of 2

Download the "Lab Report Template" from Canvas and edit the MS Word doc; or **recreate** the template in a new word processing document. [Keep these instructions]

- Responses must be on the on **same part of the same page as this template** for grading.
- For each question / prompt, provide **TYPED, numbered** responses in **complete sentences**.
- Insert images of **hand-drawn diagrams** and **calculations**, where applicable.

1. What roles does **methanol** play in *each of the first two extractions*? [Top of page 1]

2. Draw the **TLC plates** to scale and report the **retention factor (R_f)** in a table. [Bottom of page 1]
   - Indicate **colors** (use crayons or colored pencils in the report, if available), 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.
   - Calculate the **retention factor (R_f)** values for each spot on each plate.
     - List the **same R_f value** for any pigments that overlapped on the TLC plate
     - List the **R_f value = 0** for any pigments that did not move from the origin.
     - Do not calculate the R_f of a smeared (very long) spot, but do note "smear" in the table.

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 3 LAB REPORT TEMPLATE, Page 2 of 2

4. Respond to each question and prompt below, including the relative **polarity of the solvents** and the **polarity of the pigments** within in each response.

(a) Explain the differences observed in the plates run with **hexanes** and **9:1 hexane-ethyl acetate**.
[Top of page 2]

(b) Was **ethyl acetate** a good solvent to separate the pigments? Explain why or why not.
[Middle of page 2]

(c) Which was the **optimal solvent** for separation of the most pigments (greatest number of individual spots observed)? How is the polarity of this **solvent** mixture related to the polarity of the **pigments**?
[Bottom of page 2]

Name _____ Partner Name _____

TA Name _____ Section Letter _____ Day _____ Time _____

Experiment 3 Lab Notebook Templates

Reference for notebook preparation – every student submits on GradeScope individually after lab

Pre-Lab Requirements

1. **Lab Notebook:** copy templates below into designated notebook
   - **Purpose, scheme,** and **reagent table**
   - **Procedure Diagrams** – must be complete upon arriving to lab
   - **Cleaning and Safety Table**
   - **Data**
2. **Dress for lab** – see safety rules – arrive a few minutes early

A. Purpose of the lab and structures of pigments (Exp 3, pages 2-3)

B. Reagent Table – look up and FILL IN PROPERTIES BEFORE LAB

Sample Name	**Amount** Fill in during lab	**Molecular Mass**	**mmoles** Fill in during lab	**Boiling point**	**Density**	**Hazards** Find one-word hazards for each in the safety table (after the procedure)
Spinach		n/a	n/a	n/a	n/a	n/a
Methanol						
Hexanes *use properties of *n*-hexane						
Water						
Ethyl acetate	n/a					

C. Procedure – hand-written **instructions & diagrams** based on lab PDF, Slugs@home, and class notes

- Include all **labeled equipment, chemical names** with **amounts**, and **clean-up / safety notes**
- Indicate every **transfer** of chemicals from one container to another
- *Use as many pages as needed* - at least 3 pages is typical

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 into and out of test tube

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

Cleaning & Safety – copy table from the end of the procedure

D. Data

Sketch each TLC plate to scale (with measurements), labeled with the solvent used to run the plate. Describe the color of each spot and/or use colorful drawing tools.

Calculate **R_f values** for every spot on every plate.

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

- Note: when pigments do not separate at the base-line, one spot contains multiple pigments – list the same Rf value for the overlapping pigments in the table
- 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

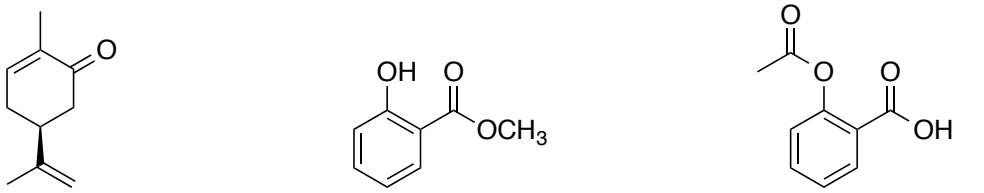
Experiment 4 – IR Exercise

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

*** 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})**.

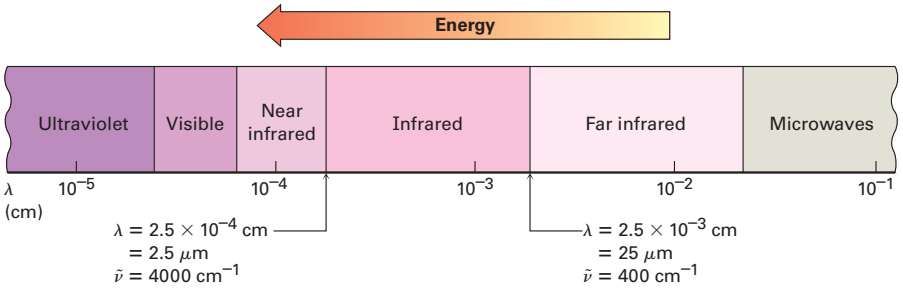


Figure 2. Electromagnetic spectrum, highlighting infrared (IR) region

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.

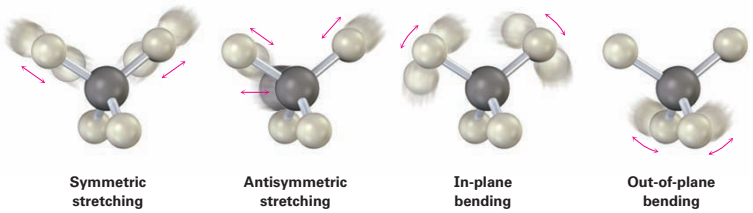


Figure 3. Stretching and bending vibrations.

$\bar{\nu}$ **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).

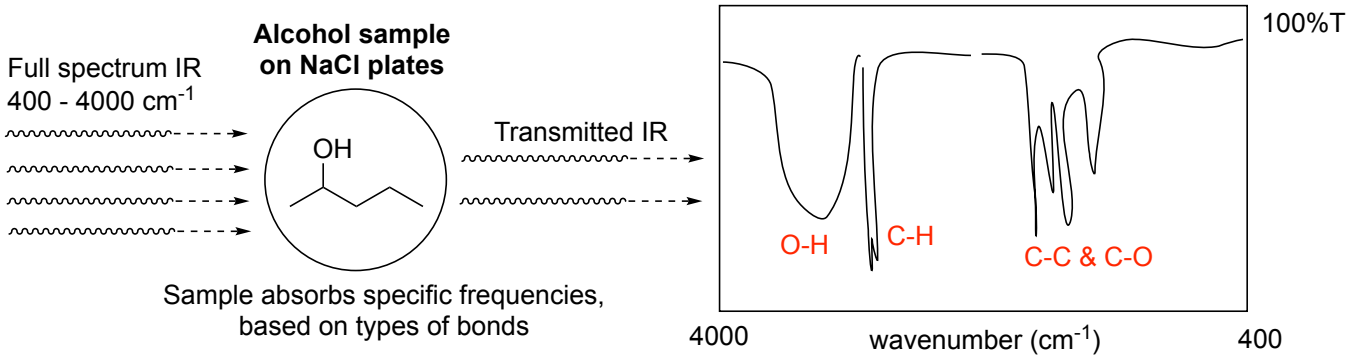


Figure 4. Crude diagram of IR spectroscopy and amateur sketch of alcohol spectrum.

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.

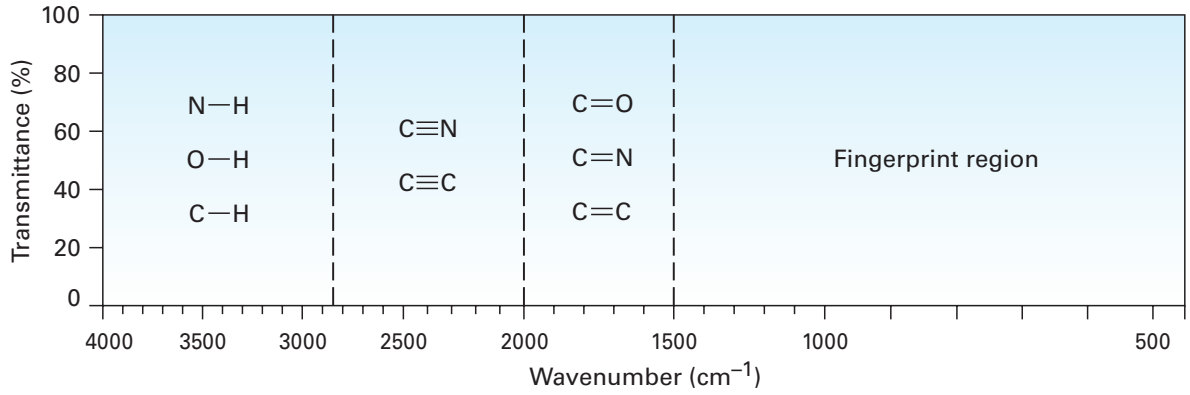


Figure 5. Four main regions of the IR spectrum.

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.

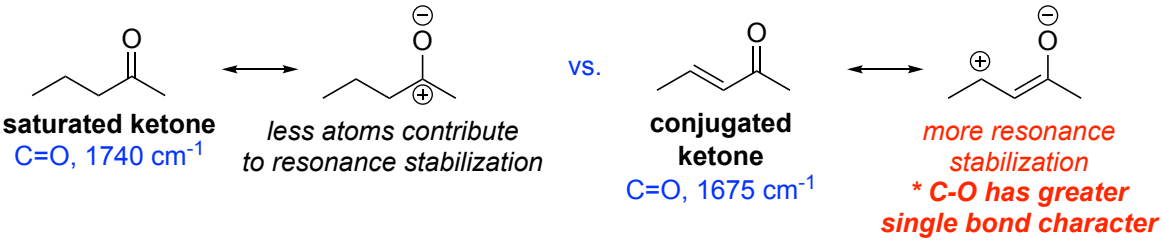
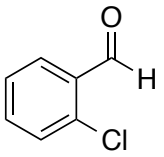


Figure 6. Comparison of stretching frequencies in a saturated vs. conjugated ketone.

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

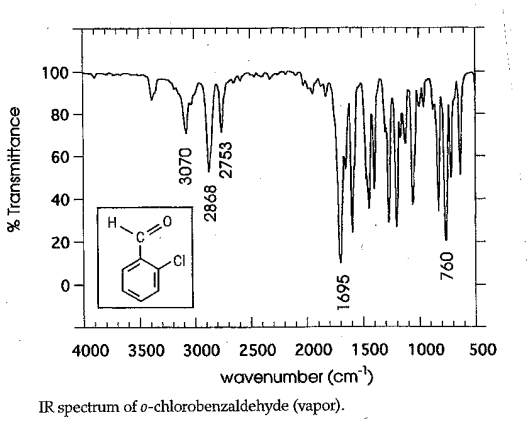
- Determine each **functional group** in the molecule (ex. *ortho*-chlorobenzaldehyde).
- Use the **IR Table** (end of this document) to find the IR active **bonds** within each functional group (FG) and its expected wavenumber range.
    - Be sure to list **all bonds and vibrations**.
        - FG's 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)

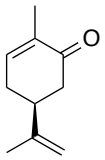
† Values approximated from the x-axis of the IR spectrum.

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†	

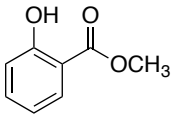
Properly identifying functional groups is half the battle! The IR tables give typical ranges, but remember that factors like conjugation could cause certain bonds to be outside of that range. An acceptable limit outside of the range is 40 cm^{-1} above or below, as long as the structure supports the 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

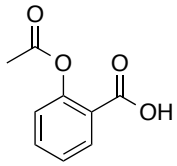
Overview: Predict the IR signals for carvone, wintergreen oil, and aspirin. Watch the demonstrations on preparing and running IR samples, then obtain the IR spectra of carvone, methyl salicylate, and aspirin. Use salt plates (pure NaCl) to support the sample. NaCl does not absorb IR radiation making 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**, see Exp 4 Lab Notebook Templates. Use the *IR table at the end of this document and steps on page 4* to predict IR bond vibrations and corresponding wavenumber ranges. Some functional groups have several IR active bonds (ex. carboxylic acid contains C=O and O-H) and that C-H bonds in alkenes or arenes have two vibrations (stretch and bend).

Students will interpret two IR spectra per compound: one from the **literature** and one obtained in lab (**observed**). Interpret the spectra by looking for a signal within each expected range, then report 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. Watch the pre-lab video on Canvas Exp 4 Overview page.
- Attend **lab lecture,** taking notes on **lecture templates**
- Preview 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 **template** 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 bullet-points, diagrams, and/or whatever works for you!
  - **IR tables** – filled in columns for functional groups, bonds, and expected wavenumber ranges
  - **Cleaning & Safety** – copy table from end of the procedure

During Lab

- Perform the experiment with a partner, fill out data & observations in **lab notebook**
- Complete the **IR Problem Set** - include in this in your **Lab Notebook** submission

After Lab – each partner submits separate, individual assignments

- Upload **Notebook Pages** to GradeScope by midnight on lab day – graded on completeness
- 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

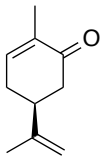
1. What happens when IR radiation is absorbed by an organic sample? How is the frequency of the radiation used to determine the functional groups in the molecule?

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?

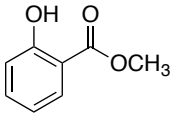
3. What is the range (cm^{-1}) for the IR fingerprint region? Why are the bands in this region of limited use in structure elucidation?

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

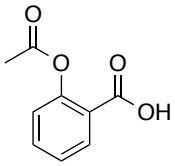
Use these structures to answer #5-9…



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 C=O in the **carboxylic acid** in aspirin **saturated or conjugated**? The C=O in 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 (**mono-, ortho-, meta-, or para-disubstituted**)?

Full Name: **Lab Section:** [replace with day, time, room]

Lab Partner Name: **TA Name:**

EXPERIMENT 4 LAB REPORT TEMPLATE, Page 1 of 2

Download the "Lab Report Template" from Canvas and edit the MS Word doc; or **recreate** the template in a new word processing document. [Keep these instructions]

- Responses must be on the on **same part of the same page as this template** for grading.
- For each question / prompt, provide **TYPED, numbered** responses in **complete sentences**.
- Insert images of **hand-drawn diagrams** and **calculations**, where applicable.

1. Create the three IR tables from lab (carvone, wintergreen oil, and aspirin) with all functional groups and bonds matched to expected wavenumber ranges from the IR Reference Table.

Table 1. IR analysis of **carvone** (draw structure)
ADD A ROW for each bond in each functional group in the molecule (see class notes)

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

[Top of page 1]

Table 2. IR analysis of **methyl salicylate**, wintergreen oil (draw structure)
ADD A ROW for each bond in each functional group in the molecule

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

[Bottom of page 1]

*** Literature spectra** are posted in the Canvas Exp 4 module

**** Observed spectra** will be obtained during lab

EXPERIMENT 4 LAB REPORT TEMPLATE, Page 1 of 2

Table 3. IR analysis of **acetylsalicylic acid**, aspirin (draw structure)
ADD A ROW for each bond in each functional group in the molecule

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

[Top of page 2]

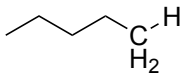
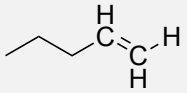
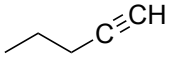
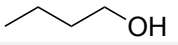
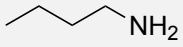
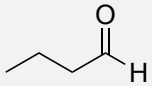
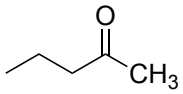
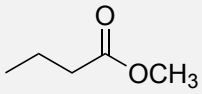
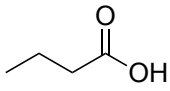
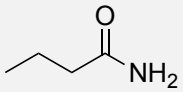
2. What is the general **relationship between the bond length and wavenumber** in IR Spectroscopy?

3. For each compound, **list a pair of bonds** (one longer, one shorter) and their corresponding **wavenumbers (cm^{-1})** that support the bond length vs. wavenumber trend.

- Include specific wavenumbers from the spectra you obtained, not expected wavenumber ranges.

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

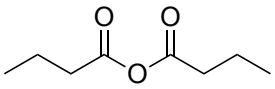
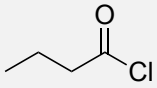
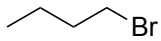
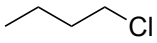
Use this table to predict the expected IR absorbance ranges for each bond within each functional group present in the molecule to be analyzed. Most absorbances for **C-C and C-O single bonds are omitted**, because they fall in the hard-to-distinguish "fingerprint region" between 1000 – 1500 cm^{-1}.

Functional Group & Bond Vibration	Absorbance Range (cm^{-1})	Intensity*	Sample Structure
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	[Table 2, next page]	s	
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	
C-H bend	[see Table 2, next page]	s	
Alcohols**			
O-H stretch	3550 – 3200	br, s	
Amines			
N-H stretch	3550 – 3250	br, m	
Aldehydes			
C-H stretch, 2 signals	2900 – 2800 & 2800 – 2700	s	
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, if present	3500 – 3150	m	
C=O stretch	1700 – 1630	s	

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

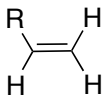
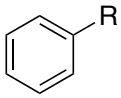
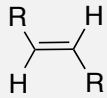
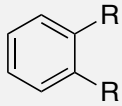
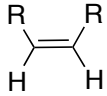
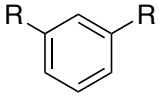
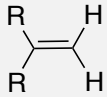
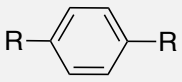
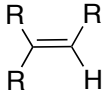
** Alcohols, Esters, Carboxylic Acids, and Anhydrides also absorb in the fingerprint region due to the C-O stretch (1300 – 1000, s). You can omit this from your analyses.

Table 1 cont'd

Functional Group & Bond Vibration	**Absorbance Range (cm^{-1})**	**Intensity**	**Sample Structure**
Anhydrides**			
C=O stretch, 2 signals	1850 – 1800 & 1790 – 1740	s	
Acid Chlorides			
C=O stretch	1815 – 1770	s	
Alkyl & Aryl Halides†			
C-F stretch	1000 – 1400	m	
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

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, symmetric 	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.

Name _____ Partner Name _____

TA Name _____ Section Day _____ Time _____

Experiment 4 Lab Notebook Template

Use as reference for notebook preparation – every student uploads to GradeScope after lab

* include IR Problem Set

Pre-Lab Requirements

1. **Lab Notebook:** copy templates below into designated notebook
   - **Purpose, scheme,** and **reagent table**
   - **Procedure Diagrams** – must be complete before you can start the lab
   - Cleaning & Safety
   - **IR Problem Set** – provided; complete during lab and submit with Lab Notebook Pages
2. **Dress for lab** – see safety rules – arrive a few minutes early

A. Purpose of the lab and structures of carvone, aspirin, and methyl salicylate

B. Reagent Table – Fill in properties BEFORE lab

Sample Name	Molecular Mass	Boiling or melting point	Density	Hazards
Carvone				
Aspirin (acetylsalicylic acid)				
Wintergreen oil (methyl salicylate)				
Acetone				

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 1: Liquid sample preparation – Carvone and/or methyl salicylate

STEP 2: Solid sample preparation – aspirin

STEP 3: Obtaining the IR spectrum – steps to run the spectrum &nsketch components of the instrument

(add details after in-lab TA demo)

Cleaning and Safety – copy table from the end of the procedure

D is for Data

Table 1. IR analysis of carvone (draw structure)
ADD A ROW for each bond in each functional group in the molecule (see class notes)

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

Table 2. IR analysis of methyl salicylate (draw structure)
ADD A ROW for each bond in each functional group in the molecule

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

Table 3. IR analysis of aspirin (draw structure)
ADD A ROW for each bond in each functional group in the molecule

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

* **Literature spectra** are posted in the Canvas Exp 4 module

** **Observed spectra** will be obtained during lab

IR Problems – provided in lab
Complete this during lab and upload with the Exp 4 Lab Notebook Pages

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 ranges** to determine which signals are unique.
Hint: start by drawing the structures of each.

a) CH_3CH_2OH vs. CH_3OCH_3	**b)** cyclohexane vs. 1-hexene	**c)** $CH_3CH_2CO_2H$ (carboxylic acid) vs. $HOCH_2CH_2CHO$ (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 broad absorption at 3200 cm^{-1} _____

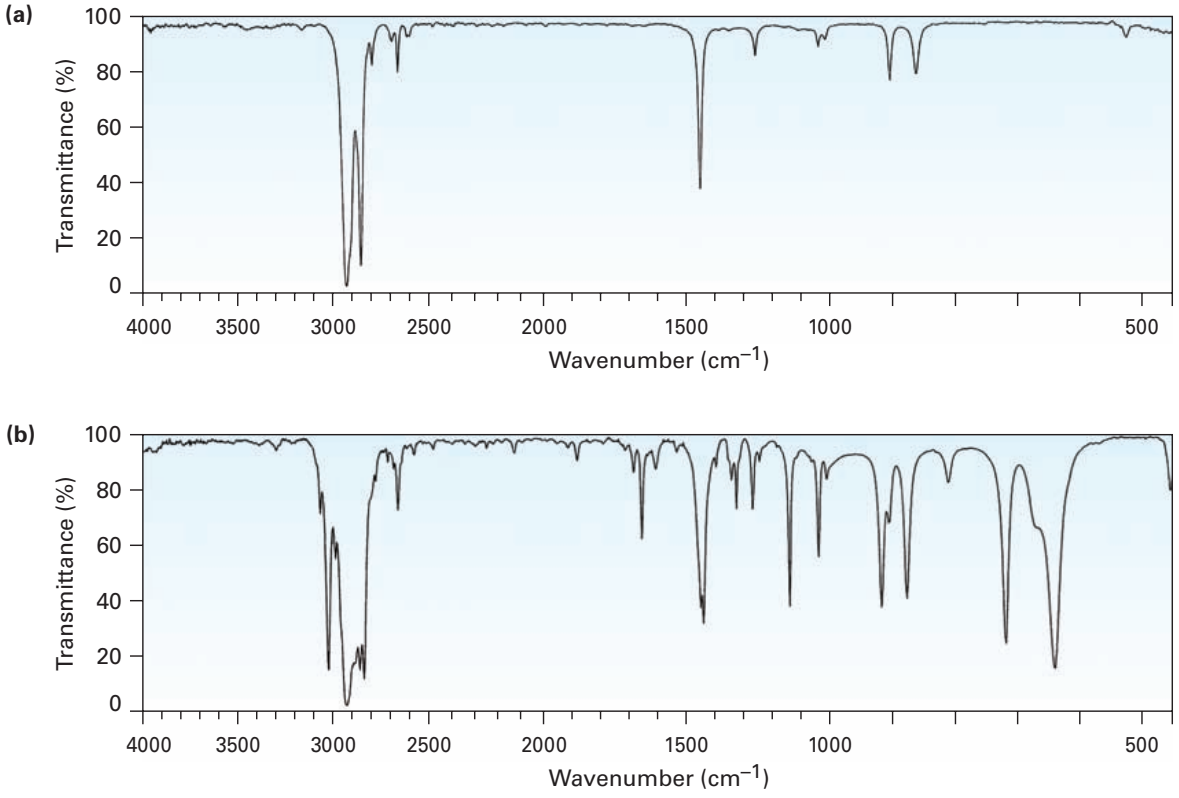
c) A compound with strong absorptions at 1720 cm^{-1} *and* 2500-3100 cm^{-1} _____

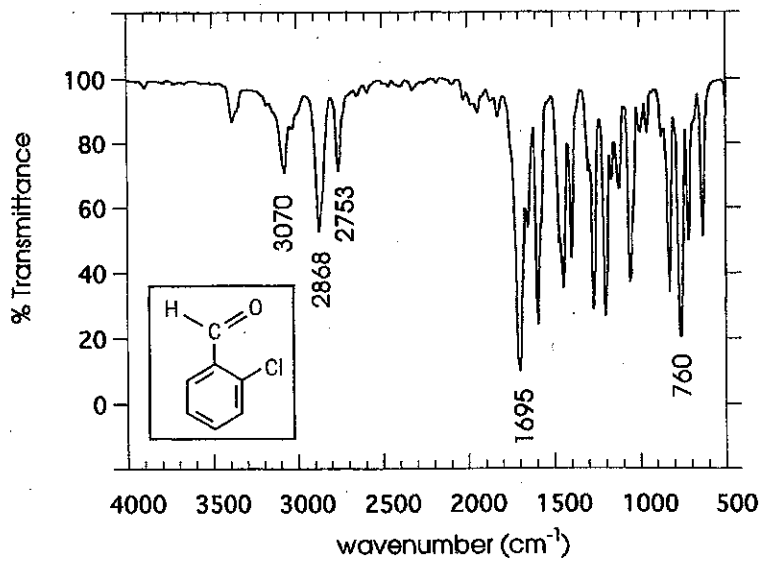
3. Acetone (CH_3COCH_3) and 2-propen-1-ol ($H_2C{=}CHCH_2OH$) are isomers. **Draw the skeletal structures** of both compounds below. How could you **distinguish** them by IR spectroscopy (unique signals for each)?

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

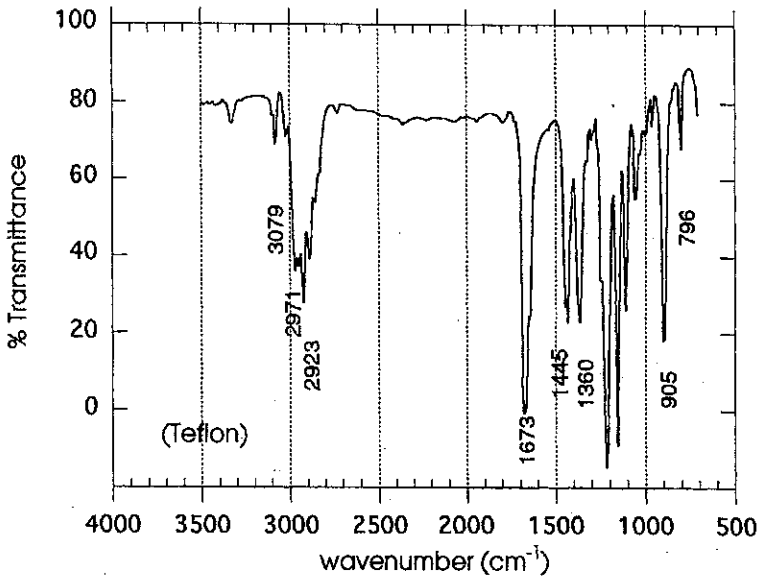
a)	**b)**
C_5H_8, with IR absorptions at 3300 and 2150 cm^{-1}	$C_4H_{10}O$, with a strong IR absorption at 3400 cm^{-1}

5. Consider the two IR spectra below. One is for cyclohexane and the other for cyclohexene. Identify them and explain your answer.

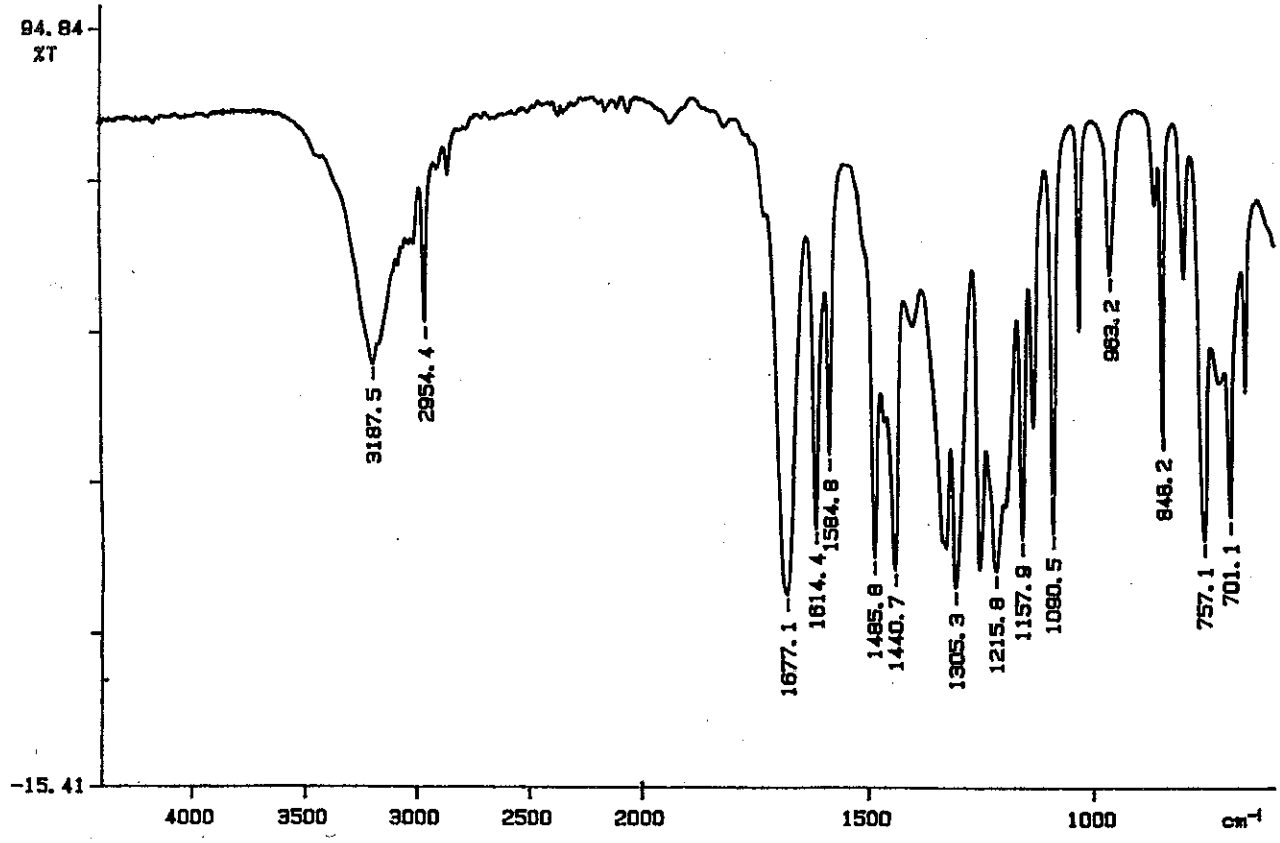




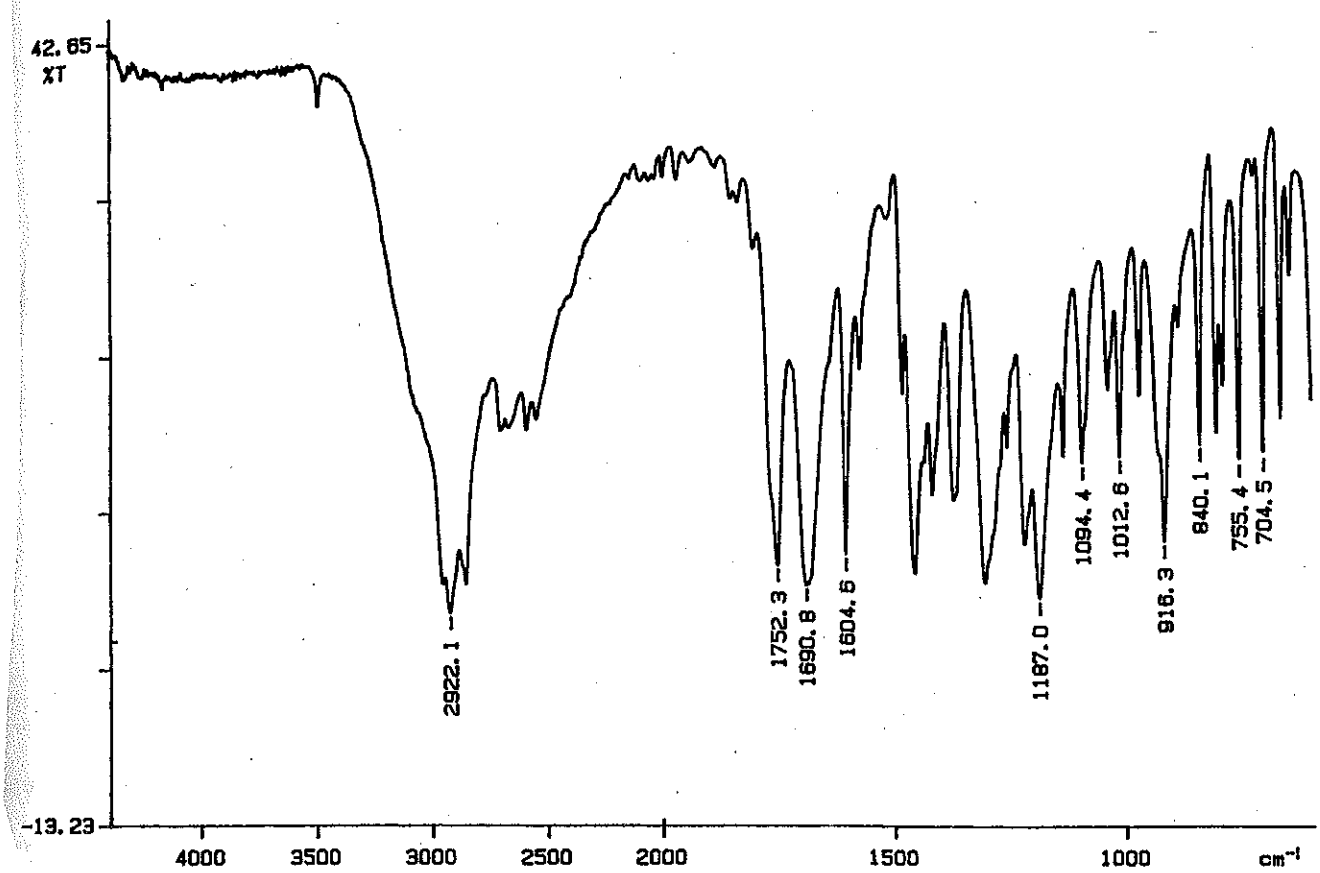
IR spectrum of *o*-chlorobenzaldehyde (vapor).



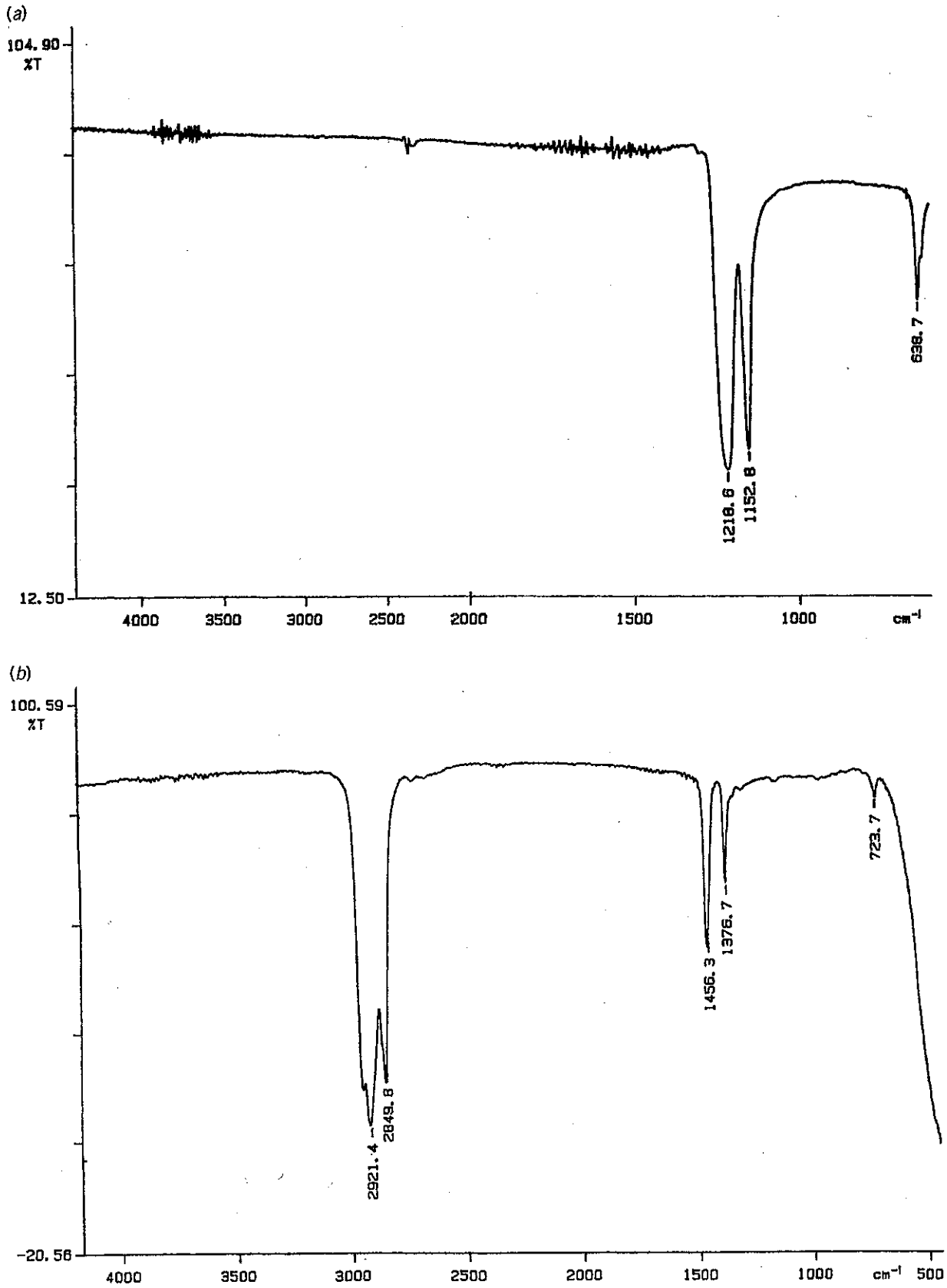
IR spectrum of carvone (Teflon).



IR spectrum of methyl salicylate (neat).

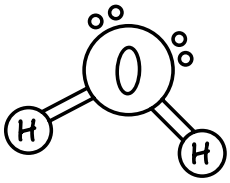


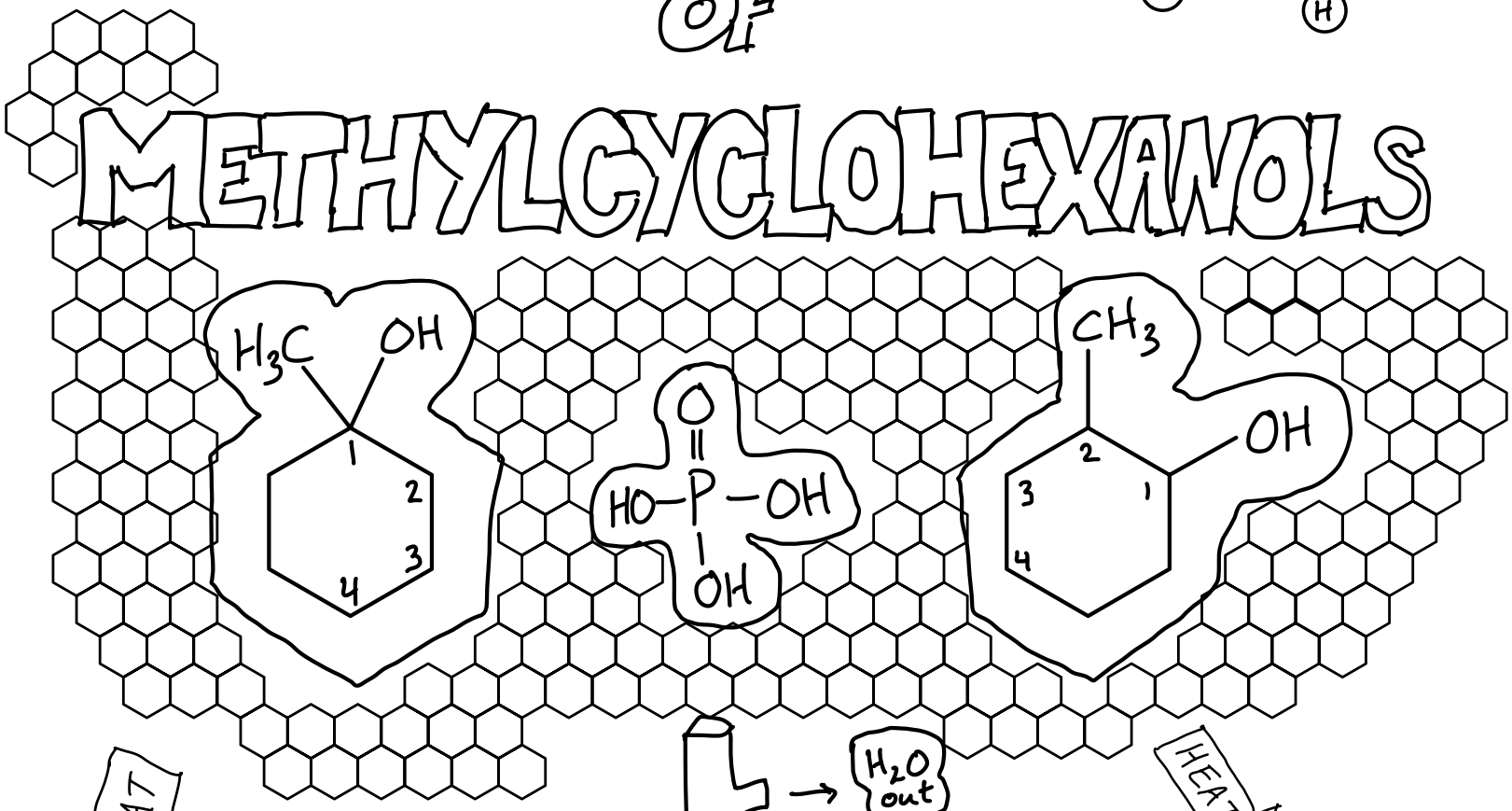
IR spectrum of aspirin (nujol).



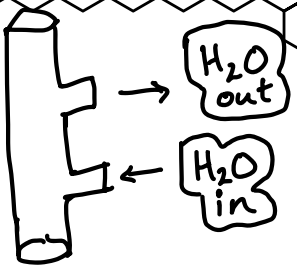
a) IR spectrum of Teflon. *b*) IR spectrum of nujol.

DEHYDRATION OF METHYLCYCLOHEXANOLS



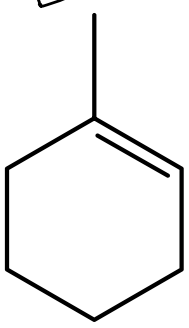


HEAT



HEAT

MAJOR PRODUCTS



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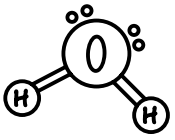
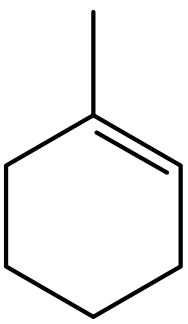


MAJOR PRODUCTS



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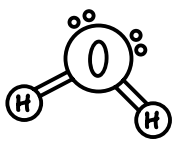
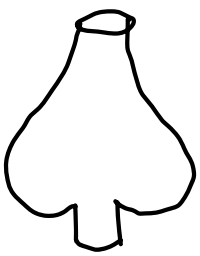
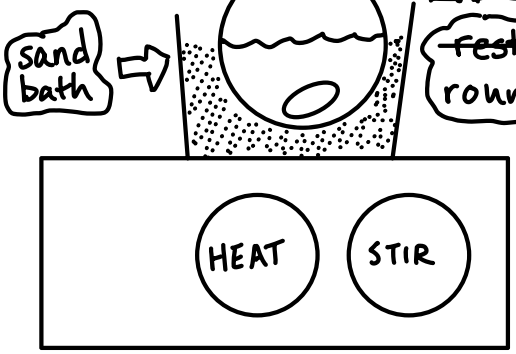




RBF

~~resting b~~ round-bottom flask

sand bath



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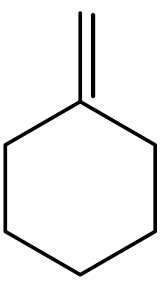
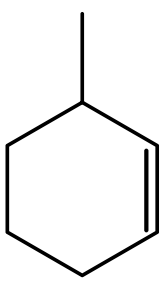


minor product

+

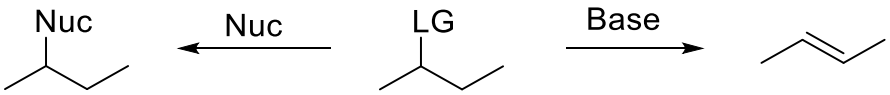


minor product

CHEM 8L, Experiment 5 – Dehydration of Methylcyclohexanols

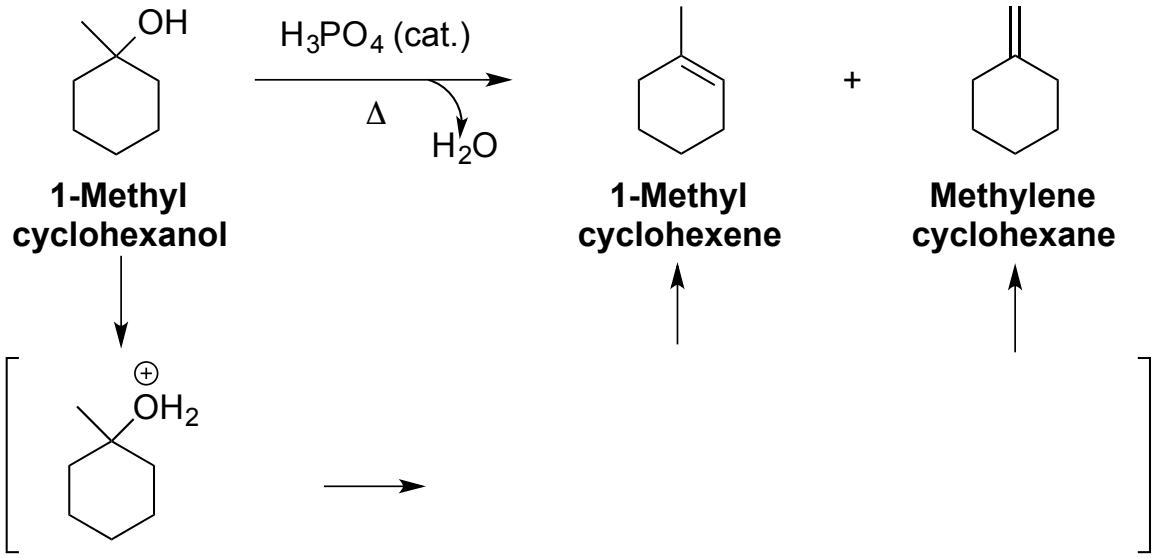
- Reaction Mechanism
- Zaitsev's Rule: product distribution
- Theoretical Yield Calculation
- Reaction Setup & Workup
- Analysis: (1) Percent Yield, (2) IR, (3) Chemical Tests, (4) GC

Substitution vs. Elimination

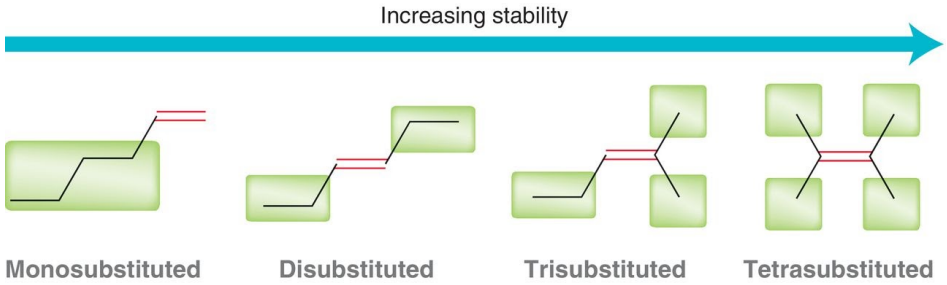


LG = leaving group

Dehydration of Primary & Secondary Alcohols - Unimolecular Elimination (E1) Mechanism

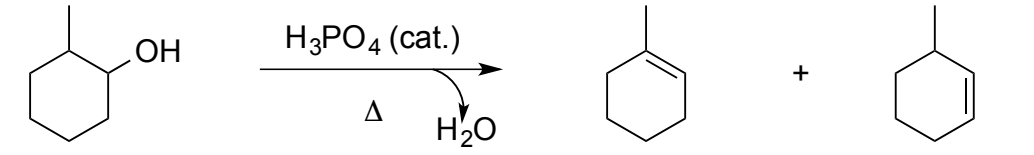


Zaitsev's Rule:



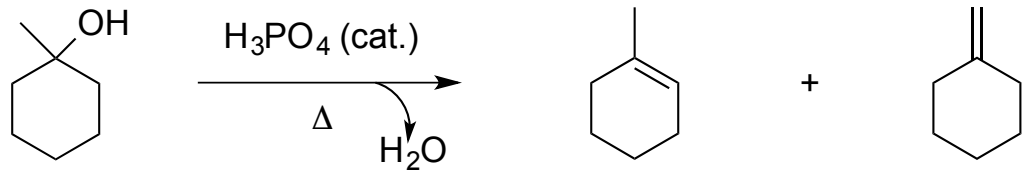
Klein, *Organic Chemistry, 3rd ed*. **Figure 7.13**

The other methylcyclohexanol…



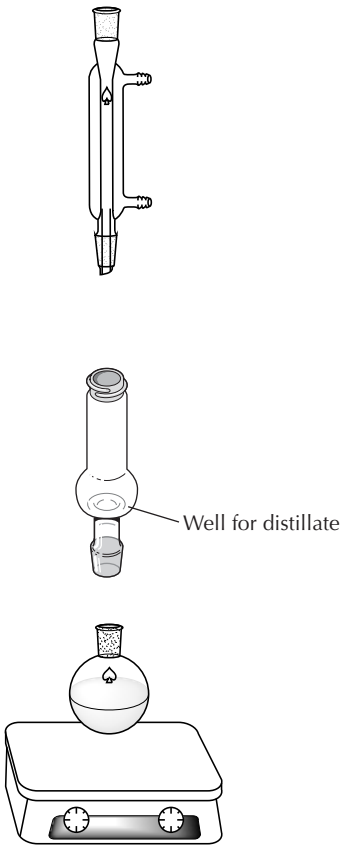
2-Methyl cyclohexanol | **1-Methyl cyclohexene** | **3-Methyl cyclohexene**

Theoretical Yield: how much product could be formed if 100% reacts and is collected?



Volume (mL) Theoretical Mass (mg)

Reaction Set-Up with Hickman Still



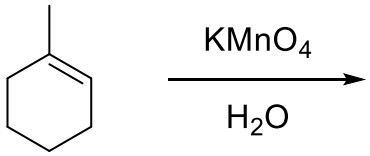
Reaction Work-up = Dry the Product

1. Transfer to test tube
2. Add anhydrous Na_2SO_4
3. Wait 5 min
4. Filter pipet

Analysis

$$\textbf{\% Yield} = \frac{\text{actual product mass}}{\text{theoretical mass}} \times 100\%$$

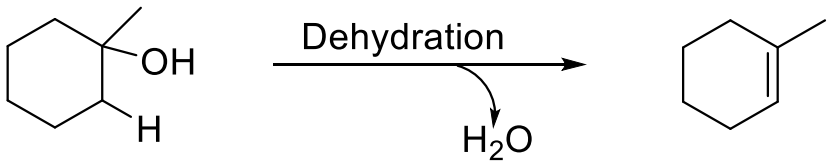
Potassium Permanganate ($KMnO_4$) Test



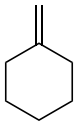
Test four samples

#1 – Product #2 – Cyclohexane #3 – Cyclohexene #4 Cyclohexanol

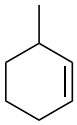
IR Spectroscopy used to assess reaction success



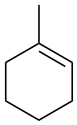
Great Success?? **Gas Chromatography (GC) analysis gives the final verdict!**



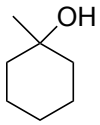
methylene cyclohexane
102 °C



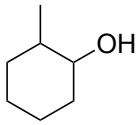
3-methyl cyclohexene
104 °C



1-methyl cyclohexene
110 °C



1-methyl cyclohexanol
155 °C



2-methyl cyclohexanol
163 °C

GC Standards Provided

- Mixture of 1- and 3-Methylcyclohexene (1:1)

- 1-Methylcyclohexanol

- 2-Methylcyclohexanol

- Reaction Mixture

EXP 5 SUMMARY – DEHDYRATION OF METHYLCYCLOHEXANOLS

1. Reaction set-up: microscale distillation
2. Reaction work-up: drying agent and filtration
3. Chemical Tests
4. IR Analysis
5. GC Analysis

Experiment 5 – Dehydration of Methylcyclohexanols

Learning Objectives

- Perform a microscale distillation with Hickmann still to collect products of a dehydration reaction
- Use gas chromatography to determine percent composition of products, exemplifying Zaitsev's rule
- Apply IR Spectroscopy to indicate reaction completion
- Interpret chemical tests to determine the presence or absence of alkene

*** 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.

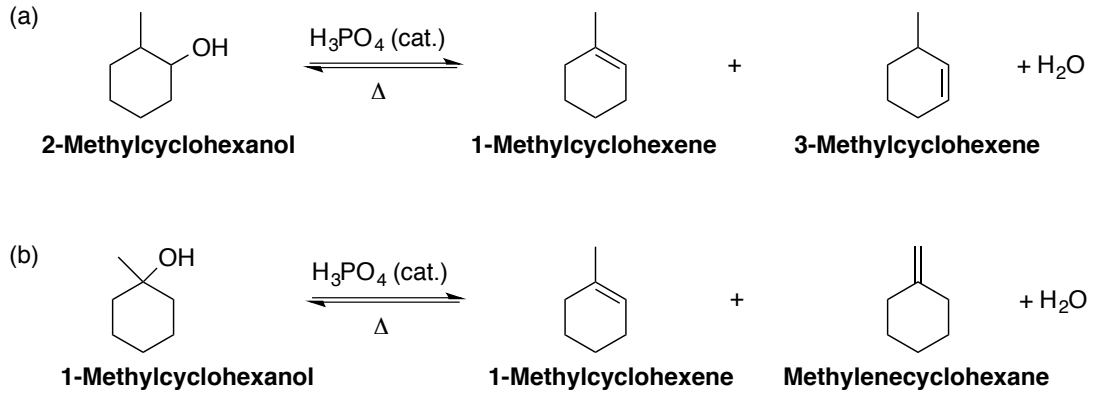


Figure 1. Dehydration of methylcyclohexanols

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.

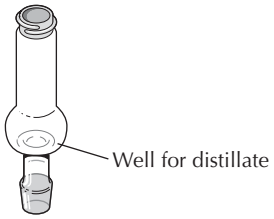
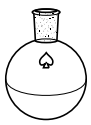
PROCEDURE

Reaction Setup

Prepare a sand bath to reach a target temperature 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 (0.75 mL) of the assigned alcohol using the provided pluringe and transfer into a pre-weighed 5-mL round-bottom flask (RBF). Obtain the mass of starting material by subtracting the mass of the RBF. [Note: 1-Methylcyclohexanol is a solid at room temperature (mp ~24 °C) and may be in a warm water bath.] Carefully add 225 μL (0.225 mL) of concentrated phosphoric acid (conc. H_3PO_4) to the RBF 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 to the RBF and clamp a Hickman still on top of the RBF, using a small amount of grease. Include a sketch of this microscale distillation apparatus in your notebook. Use a plastic Keck clip to connect the flask to the still. Connect water hoses with tiny hose 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.

	Microscale water-jacketed condenser
	Hickmann still
	Round-bottom flask (RBF)

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. The reaction is deemed "complete" once approximately half of the maximum volume of the Hickman still has been collected, or when half the volume in the flask is gone. Turn off the hot plate – do not take apart the apparatus. Use a spatula to carefully push the sand away from the flask and wait for the Hickmann still to be cool to the touch. If you move the apparatus, the distillate could drop right back into the reaction flask and the product must be distilled again ☹

Remove the condenser and use a pipet to carefully transfer the distillate into a test tube from the equipment drawer. Add a small microspatula-full of the drying agent, Na_2SO_4. Cap the vial and let the system sit for 5 minutes. Filter through a pipet with a loose cotton plug (your TA will demo this). Include a sketch of the filter pipet in the notebook (copy from class notes or leave space to add in the lab). Collect the filtrate into a pre-weighed, dry screw-cap vial. 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 (see lab notebook template).

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. 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.

Additional Background Reading on Pertinent 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

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** – due Monday before lab
- Prepare thy notebook…

Lab Notebook Preparation – *Required before lab*

Please refer to Lab Notebook Templates to prepare…

- **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!
- **Cleaning & Safety Table**
- **Data**

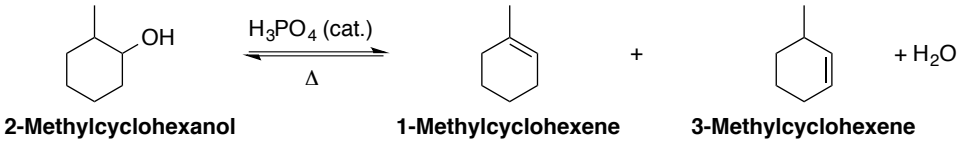
During Lab

- 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**

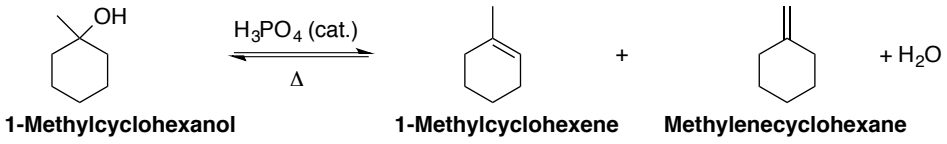
After Lab

- Individual: Upload **Notebook Pages** to GradeScope by midnight on lab day
- 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
    - Please **communicate** with your partner if you prefer to complete the report **individually**.

Pre-lab Questions



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



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

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.*

1. Convert the amounts given above 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 or look up online by their chemical names.

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

3. 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.

4. Draw the **products** for the reaction of **1-methylcyclohexene with $KMnO_4$** (McMurry Chapter 7.9 or see lecture notes – not all organic chemistry texts cover the oxidative cleavage with $KMnO_4$). Indicate the **by-product** that forms the **brown precipitate**.

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

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

7. 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**.

Full Name: **Lab Section:** [replace with day, time, room]

Lab Partner Name: **TA Name:**

EXPERIMENT 5 LAB REPORT TEMPLATE, Page 1 of 4

Complete the report **individually or with ONE lab partner** – both students contribute, one student uploads the full report to GradeScope for the same score.

Download the "Lab Report Template" from Canvas and edit the MS Word doc; or **recreate** the template in a new word processing document. [Keep these instructions]

- Responses must be on the on **same part of the same page as this template** for grading.
- For each question / prompt, provide **TYPED, numbered** responses in **complete sentences**.
- Insert images of **hand-drawn diagrams** and **calculations**, where applicable.

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

2. Calculate the **percent yield (% yield)** of the synthesis using the 'actual yield' from #1 and 'theoretical yield' based on the mass of starting alcohol weighed. **Show your work.** [Bottom of page 1]

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

3. GC Analysis

(a) Standard Retention Times (corrected) [Top of page 2]

- Report the **corrected retention times for the standards** in table format.
- **Show your work** for each calculation.
- Consider which **alcohol was reacted (1-methyl** or **2-methylcyclohexanol)** to **report the proper minor product** (methylene cyclohexene or 3-methylcyclohexene).
- The provided alkene standard was a **1:1 mixture of 1-methyl and 3-methylcyclohexene**.
    - **Methylene cyclohexane and 3-methylcyclohexene have the same retention time** because they have similar boiling points.

Standard GC Corrected Retention Times (provided, instrument room)

Sample	**Corrected t_R' (sec)**
[1- methylcyclohexanol or 2-methylcyclohexanol, based on reaction performed]	
1-methylcyclohexene	
[Methylene cyclohexane or 3-methylcyclohexene , based on reaction performed]	

(b) GC Analysis of Reaction Mixture – Products (and Reactant?) [Middle bottom of page 2]

- Report the **corrected retention times** and **integration** for the **product mixture.**
    - **Identify each peak** in the chromatogram of the product by chemical **name** and/or structure.
    - Report the **percent composition of the products**.
    - Show one **sample calculation** each of integration and % composition.

Sample calculations:

GC Analysis of Product Mixture

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

4. Discuss the distribution (ratio) of products (GC percent composition from #3 above) in terms of the relative **stability of the products**. Is this the **expected result** of the reaction? [Top of page 3]

5. Report the **results (observations) of the permanganate test** in table format and whether it is a **positive or negative test for alkenes** (interpretation). [Middle bottom of page 3]

Does the **"product" sample** contain alkenes?

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

6. Interpret the **IR results** for the analysis of starting material (alcohol) and product in table format.

Briefly discuss the **main identifying peaks** – does your IR data suggest the **reaction was successful** (complete)? [Page 4]

IR Spectrum - Alcohol

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

ADD ROWS for each functional group / bond

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})

ADD ROWS for each functional group / bond

Name _____ Partner Name _____

TA Name _____ Section Day _____ Time _____

Experiment 5 Lab Notebook Templates

Reference for notebook preparation – every student submits on GradeScope individually after lab

Pre-Lab Requirements

1. **Lab Notebook:** copy templates below into designated notebook
    - **Purpose, scheme,** and **reagent table**
    - **Procedure Diagrams** – must be complete upon arriving to lab
    - **Cleaning and Safety Table**
    - **Data**
2. **Dress for lab** – see safety rules – arrive a few minutes early

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- methylcyclohexanol 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

Cleaning & Safety – Copy the table from the procedure

D. Data

Assigned alcohol _____

Alcohol volume _____ Theoretical Yield _____mg

Calculations:

Actual Product Yield _____ mg

_____ % Yield

Name of Assigned GC Instrument _____

Standard GC Corrected Retention Times
(provided, instrument room)

Sample	**Corrected t_R' (s)**

GC Analysis of Product Mixture

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)		

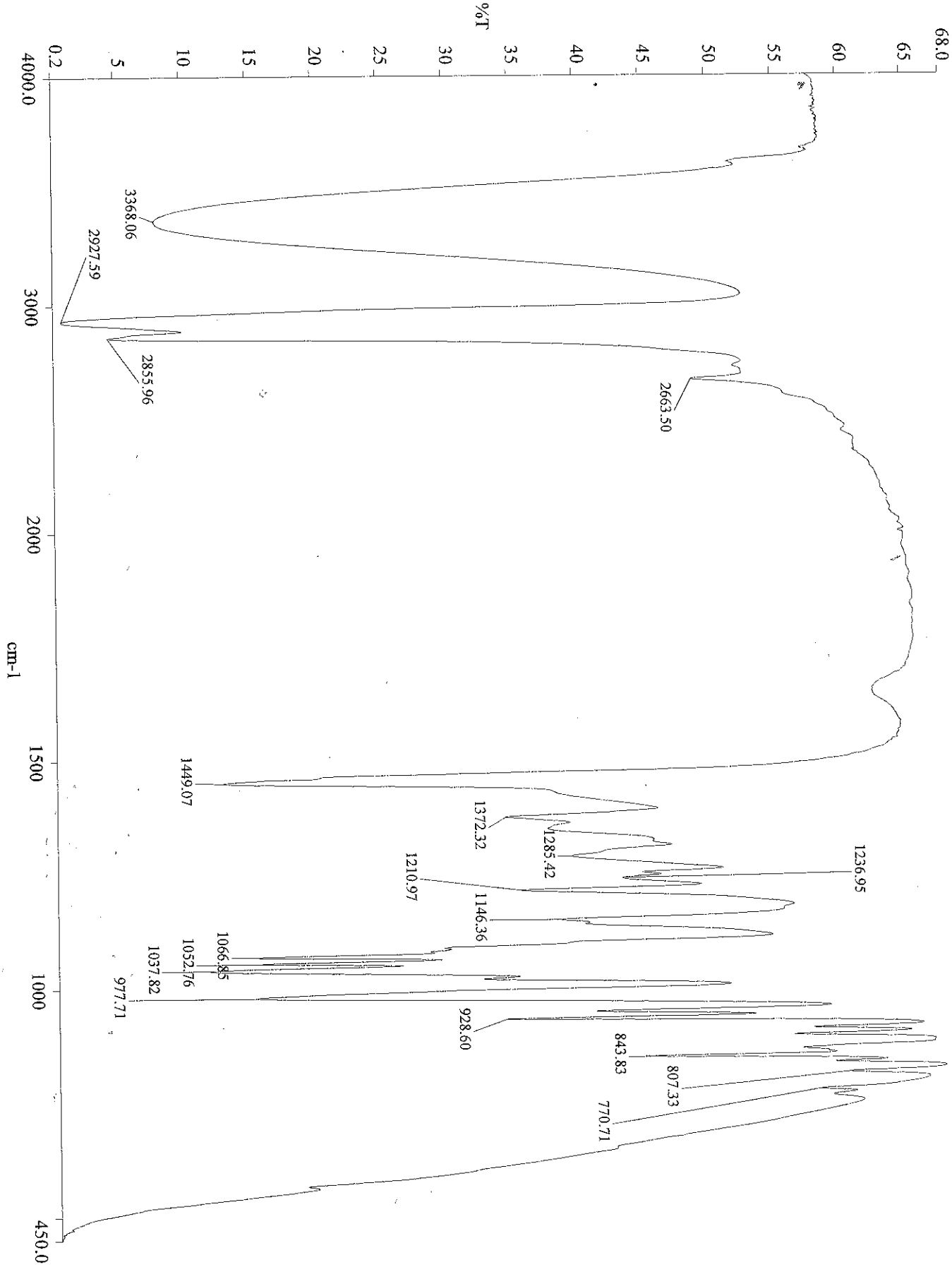
IR Spectrum - Alcohol

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

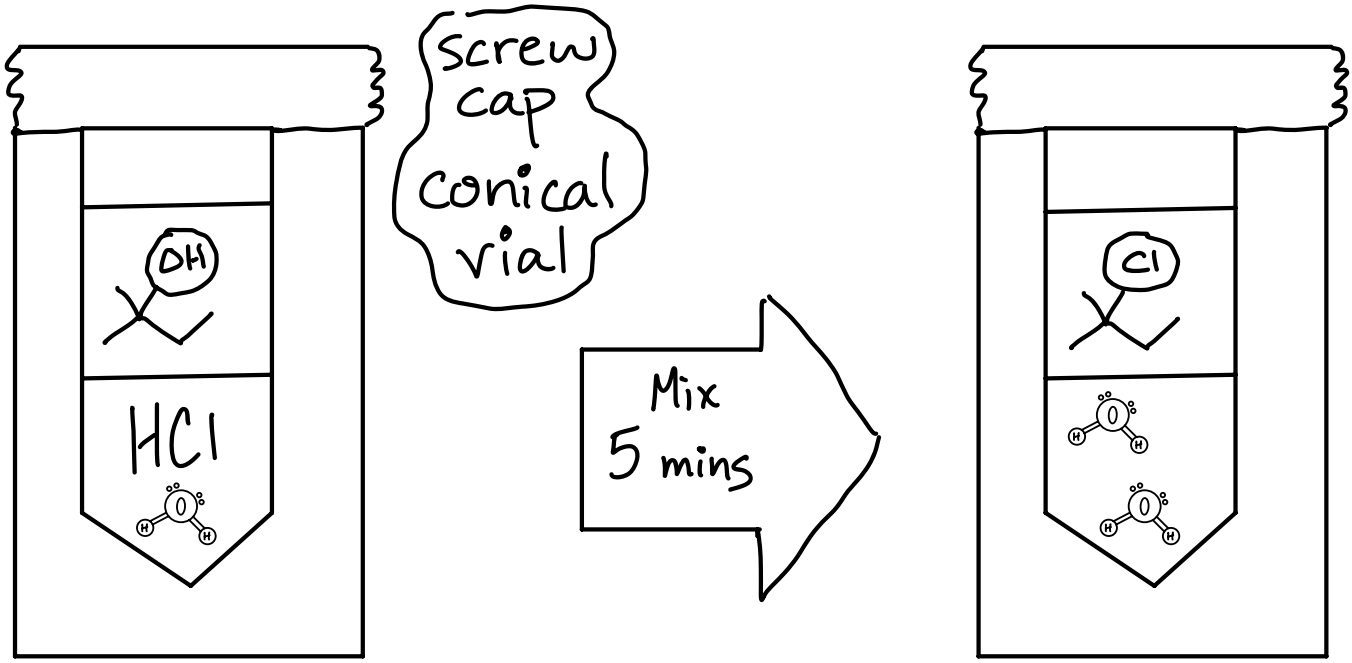
IR Spectrum – Major Product Only

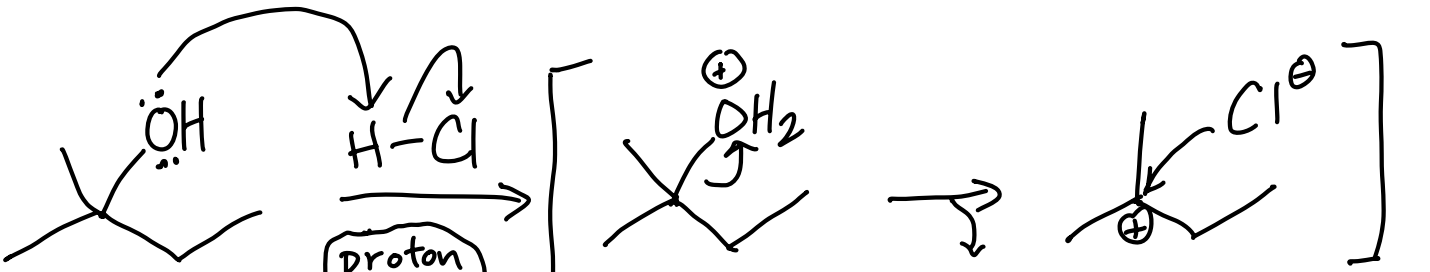
Functional Group	Bond Assignment (C=O, N-H, etc.) from IR Table	Expected Wavenumber Range (cm^{-1})	Observed Wavenumber (cm^{-1})

Methylcyclohexanol (starting material)

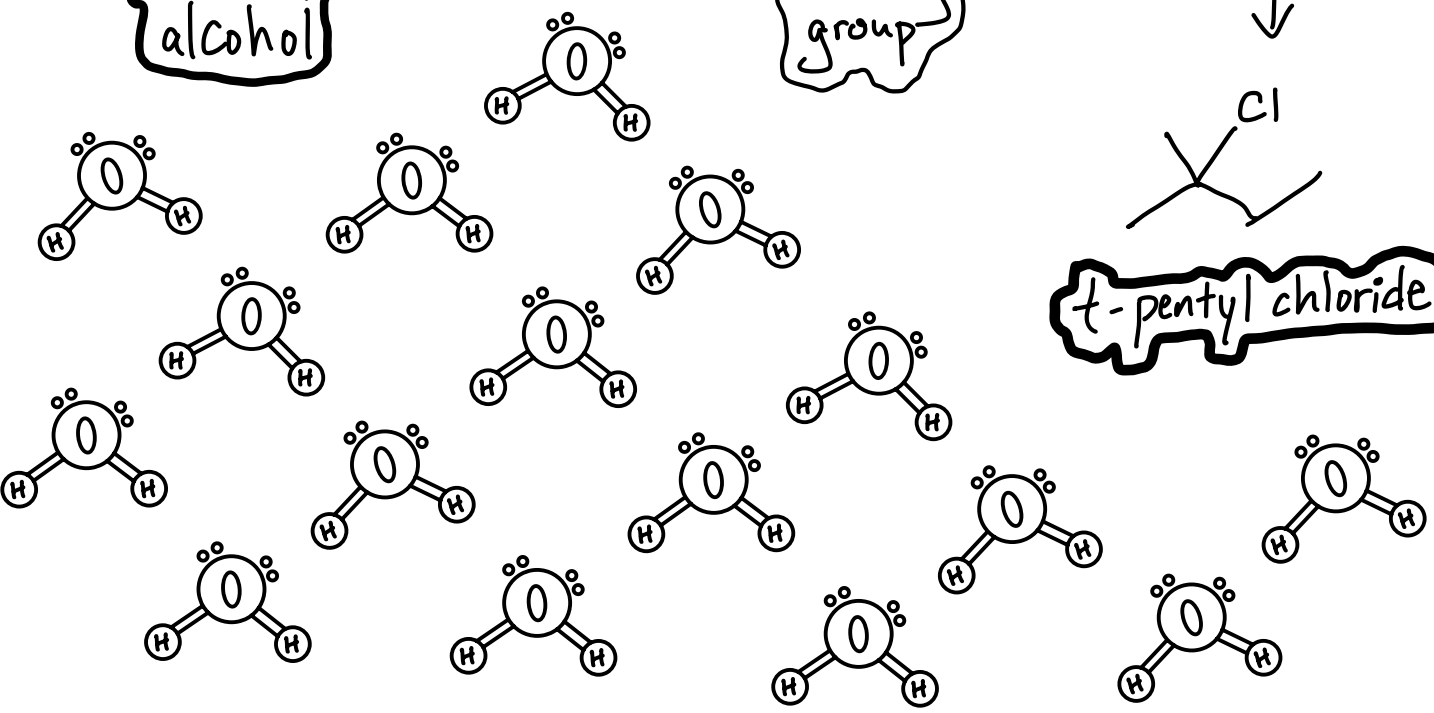


SUBSTITUTION





t-pentyl alcohol

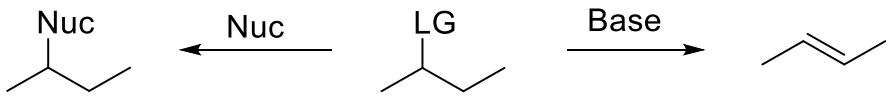


t-pentyl chloride

CHEM 8L, Experiment 6 – Synthesis of t-Pentyl Chloride

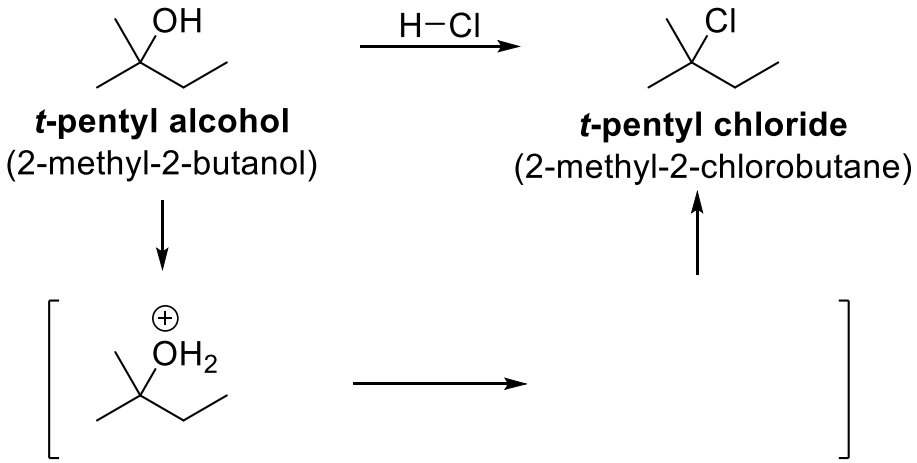
- Reaction Mechanism
- Theoretical Yield
- Reaction Setup & Workup
- Product Analysis: IR & GC

Substitution vs. Elimination

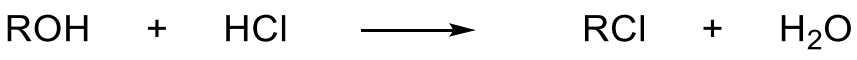


LG = leaving group

Unimolecular Substitution (S_N1) Mechanism



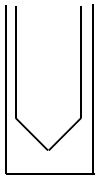
Theoretical Yield



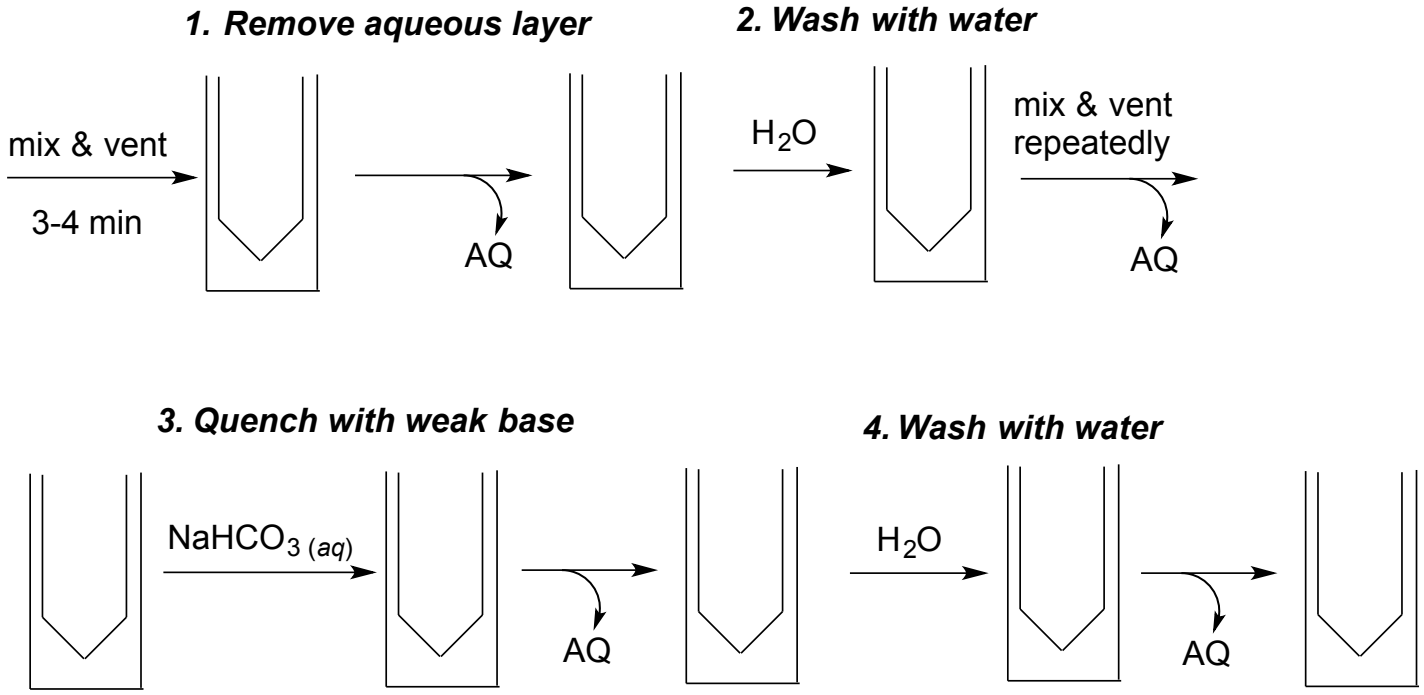
ROH	HCl	RCl
1.00 mL	2.50 mL conc.	106.59 g/mol
0.805 g/mL	1.2 g/mL	
88.15 g/mol	37% w/w	
	36.46 g/mol	

Reaction Set-up

Pipet + Pluringe Provided on Reagent Bottles! Conical Vial



Reaction Work-up



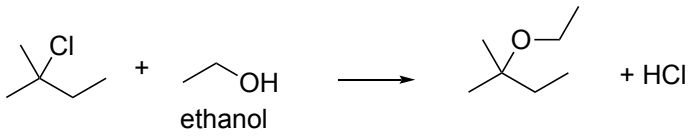
The Mildly Basic "Quench"

$HCl + NaHCO_3 \rightarrow$

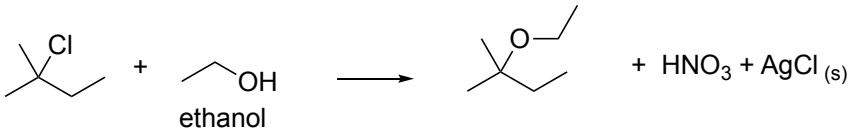
Weak base prevents hydrolysis

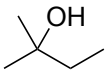
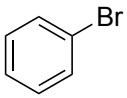
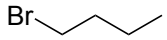
Chemical Test for Alkyl Halides

Silver Nitrate in Ethanol Test
RX *(l)* **+ EtOH** *(l)* → **ROEt** *(l)* + HX *(sol'n)* **$AgNO_3$** ***(sol'n)*** + HX *(sol'n)* → **HNO_3** ***(sol'n)*** **+ AgX** ***(s)***
R = alkyl chain; X = Cl, Br, I



$AgNO_3 + HCl \longrightarrow HNO_3 + AgCl_{(s)}$



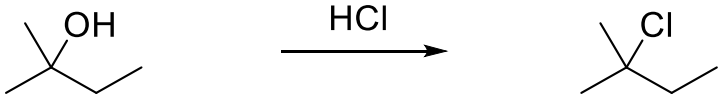
Sample	 *t*-pentanol	 bromobenzene	 bromobutane
Reaction with silver nitrate in ethanol			

GC Analysis

Alcohol & alkyl halide standard

Product mixture

IR Analysis



EXPERIMENT 6 SUMMARY: Substitution of t-pentyl alcohol

- Reaction set-up: strong acid + alcohol
- Reaction work-up: liquid-liquid extraction, wash with mild base
- Silver nitrate chemical tests for alkyl halides
- GC analysis of product – the search for starting material
- IR spectroscopy analysis

Questions???

CHEM 8L Overview

1. **Recrystallization** of Acetanilide

2. **Distillation** & **Gas Chromatography (GC)** Analysis of Citrus Oils

3. **Extraction** & **Thin Layer Chromatography (TLC)** Analysis of Spinach Pigments

4. **Infrared (IR)** Spectroscopic Analysis of Aspirin, Wintergreen Oil, and Carvone

5. Elimination / **Dehydration Reaction** – E1
   - Analysis *via* GC & IR
   - % Yield
   - Chemical Test: Permanganate Cleavage

6. **Substitution Reaction** – S_N1
   - Analysis *via* GC & IR
   - % Yield
   - Chemical Tests: Substitution w/ $AgNO_3$ or NaI

Please fill out instructor **evaluations** online (TA and CB). Help us help future students!

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.

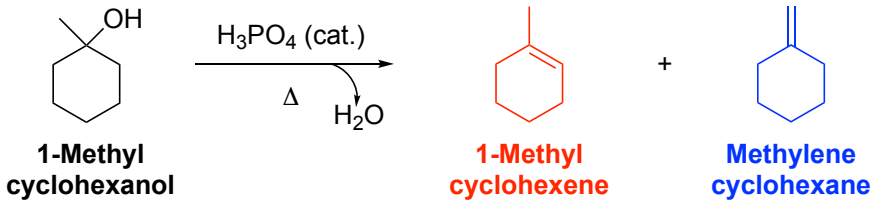


Figure 1. Dehydration of 1-methylcyclohexanol (Experiment 5)

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.

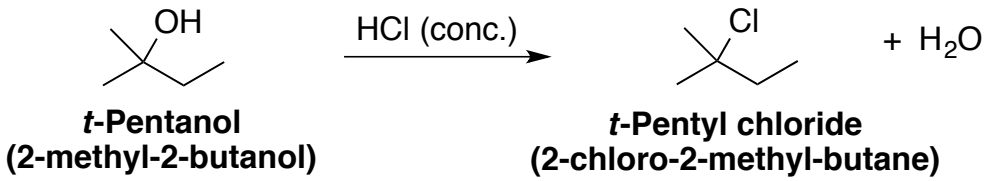


Figure 1. Synthesis of *t*-pentyl chloride

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 test for alkyl halide (RX)

Silver Nitrate in Ethanol Test
RX *(l)* **+ EtOH** *(l)* → **ROEt** *(l)* + HX *(sol'n)* **$AgNO_3$** *(sol'n)* + HX *(sol'n)* → **HNO_3** *(sol'n)* **+ AgX** *(s)* R = alkyl chain; X = Cl, Br, I

ASSIGNMENTS & HOW TO PREPARE: 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 GradeScope by midnight on lab day
- *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 *tert*-pentyl alcohol and obtain the IR spectrum of the reaction mixture.

Chemical Test for Alkyl Halides

Perform the silver nitrate chemical test in the fume hood with three standards and the product mixture. Obtain four clean, dry, medium-size test tubes and label with #1-4 using the following designations. Wrap the tape around the test tube so the tape sticks to itself.

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

Which do you expect to give positive vs. negative tests for alkyl halides?

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 (or not). Then, 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.

Table 2. Clean-up & Safety

Liquid waste: For all the liquids, including product, after you're sure you're done with them!	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.
*Rinse the test tubes with a small amount of ethanol into the liquid waste before washing in the sink.	*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

pre-lab quiz due Monday 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 – add all units to 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 that occurs in a positive silver nitrate in ethanol test. What compound precipitates as a white solid?

Full Name: **Lab Section:** [replace with day, time, room]

Lab Partner Name: **TA Name:**

EXPERIMENT 6 LAB REPORT TEMPLATE, Page 1 of 5

Complete the report **individually or with ONE lab partner** – both students contribute, one student uploads the full report to GradeScope for the same score.

Download the "Lab Report Template" from Canvas and edit the MS Word doc; or **recreate** the template in a new word processing document. [Keep these instructions]

- Responses must be on the on **same part of the same page as this template** for grading.
- For each question / prompt, provide **TYPED, numbered** responses in **complete sentences**.
- Insert images of **hand-drawn diagrams** and **calculations**, where applicable.

1. Draw the full **arrow-pushing mechanism** for the reaction of *t*-pentanol with HCl (insert image). This mechanism has three steps with two reaction intermediates. [Top of page 1]

2. Calculate the **theoretical yield** (mmol and mg) from the given volume of alcohol. Report the **actual yield** and calculate the **percent yield**. Show your work for both calculations. [Middle bottom of page 1]

EXPERIMENT 6 LAB REPORT TEMPLATE, Page 2 of 5

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).
    - Be sure to comment on the **relative polarity** of the two layers
    - Include the **density** values for t-pentyl chloride and water.

[Top of page 2]

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 your quiz* ☺

[Bottom of page 2]

EXPERIMENT 6 LAB REPORT TEMPLATE, Page 3 of 5

5. Tabulate all chemical test results with **observations** and **brief interpretation** of each (indicate the presence or absence of specific functional group). [Top of page 3]

Was the **reaction successful**? **Explain** why or why not, including **comparison to standard test results**.

Table 1. Chemical Test Results – silver nitrate in ethanol

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

6. Interpret the IR spectra of *t*-pentyl alcohol in table format. [Bottom of page 3]

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})

EXPERIMENT 6 LAB REPORT TEMPLATE, Page 4 of 5

6. Interpret the **IR spectra** of the **product** mixture in table format. [Top of page 4]

- **Which peaks(s)** of the IR spectra are used to determine **conversion of alcohol to alkyl halide**?
- Was the **reaction successful** (complete), based on IR data alone?

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})

7. Interpret the **GC charts of standards** in table format. [Bottom of page 4]

GC Standards Chart speed: 2.5 cm/min

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

EXPERIMENT 6 LAB REPORT TEMPLATE, Page 5 of 5

8. Calculate **retention times** and **integration** (area) to determine **percent composition of products**.
Show your work for each calculation.

- Briefly state whether the **reaction was successful (complete)** based on **GC** results, in combination with **chemical test** and **IR** data above,

Product Mixture GC Results

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

Retention Time Calculations, t_R'

Integration / Area Calculations:

Calculation of Percent Composition:

Name _____ Partner Name _____

TA Name _____ Section Day _____ Time _____

Experiment 6 Lab Notebook Templates

Reference for notebook preparation – every student submits on GradeScope individually after lab

Pre-Lab Requirements

1. **Lab Notebook:** copy templates below into designated notebook
   - **Purpose, scheme,** and **reagent table**
   - **Procedure Diagrams** – must be complete upon arriving to lab
   - **Cleaning and Safety Table**
   - **Data**
2. **Dress for lab** – see safety rules – arrive a few minutes early

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

Cleaning & Safety – copy table from the procedure

D. Data

Volume of *t*-pentyl alcohol _____(mL) Theoretical yield _____(mg)

- 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? _____

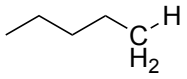
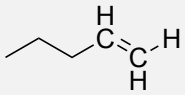
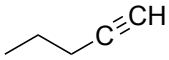
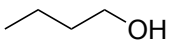
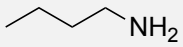
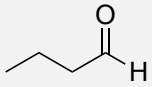
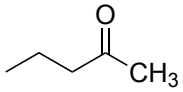
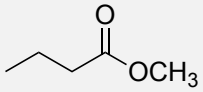
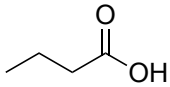
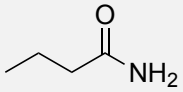
Retention Time Calculations, t_R'

Integration / Area Calculations:

Calculation of Percent Composition:

Table 1. Characteristic IR Absorption Peaks of Functional Groups*

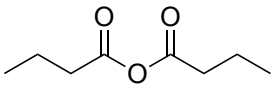
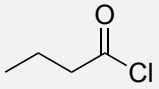
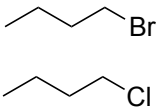
Use this table to predict the expected IR absorbance ranges for each bond within each functional group present in the molecule to be analyzed. Most absorbances for **C-C and C-O single bonds are omitted**, because they fall in the hard-to-distinguish "fingerprint region" between 1000 – 1500 cm^{-1}.

Functional Group & Bond Vibration	Absorbance Range (cm^{-1})	Intensity*	Sample Structure
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	[Table 2, next page]	s	
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	
C-H bend	[Table 2, next page]	s	
Alcohols**			
O-H stretch	3550 – 3200	br, s	
Amines			
N-H stretch	3550 – 3250	br, m	
Aldehydes			
C-H stretch, 2 signals	2900 – 2800 & 2800 – 2700	s	
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, if present	3500 – 3150	m	
C=O stretch	1700 – 1630	s	

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

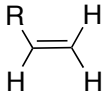
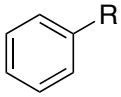
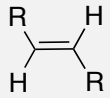
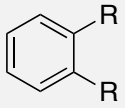
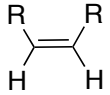
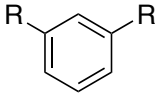
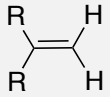
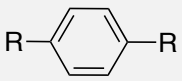
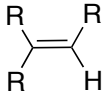
** Alcohols, Esters, Carboxylic Acids, and Anhydrides also absorb in the fingerprint region due to the C-O stretch (1300 – 1000, s). You can omit this from your analyses.

Table 1 cont'd

Functional Group & Bond Vibration	**Absorbance Range (cm^{-1})**	**Intensity**	**Sample Structure**
Anhydrides**			
C=O stretch, 2 signals	1850 – 1800 & 1790 – 1740	s	
Acid Chlorides			
C=O stretch	1815 – 1770	s	
Alkyl & Aryl Halides†			
C-F stretch	1000 – 1400	(Often 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

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, symmetric 	895 – 885	Disubstituted, *para* 	860 – 800
Trisubstituted 	840 – 790		

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

Useful Equations

Recrystallization

$$\% \text{ Recovery} = \frac{m_{recrys}}{m_{crude}} \times 100\%$$

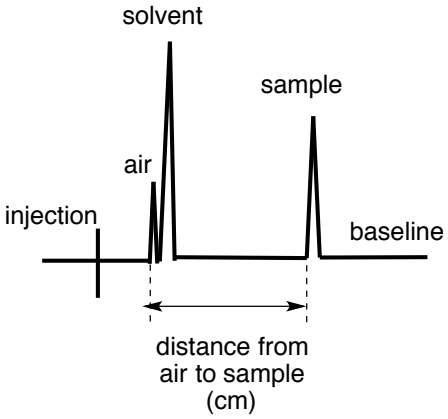
Distillation

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

Chemical Reaction

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

Gas Chromatography (GC) Calculations



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

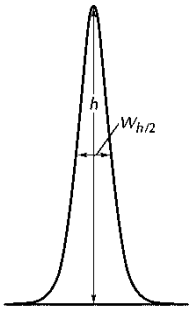


FIGURE 20.13
Determining peak area: h = height; $W_{h/2}$ = width at half-height.

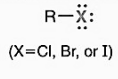
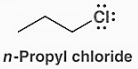
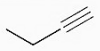
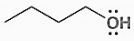
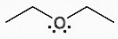
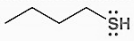
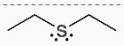
Integration: Peak Area = $h \times w_{h/2}$ **(2)**

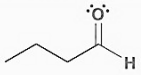
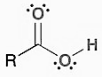
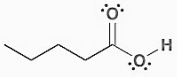
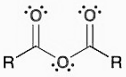
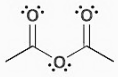
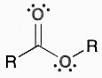
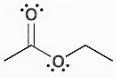
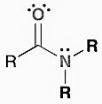
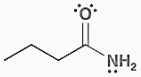
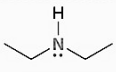
$$\% \text{ Composition of A} = \frac{\text{Area of component A}}{\text{Total area of all components}} \quad \textbf{(3)}$$

Retention Factors on TLC Plates

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

TABLE 2.1 EXAMPLES OF COMMON FUNCTIONAL GROUPS

FUNCTIONAL GROUP*	CLASSIFICATION	EXAMPLE	CHAPTER
 (X=Cl, Br, or I)	Alkyl halide	 *n*-Propyl chloride	7
	Alkene	 1-Butene	7, 8
	Alkyne	 1-Butyne	9
	Alcohol	 1-Butanol	12
	Ether	 Diethyl ether	13
	Thiol	 1-Butanethiol	13
	Sulfide	 Diethyl sulfide	13
	Aromatic (or arene)	 Methylbenzene	17, 18

FUNCTIONAL GROUP*	CLASSIFICATION	EXAMPLE	CHAPTER
	Ketone	 2-Butanone	19
	Aldehyde	 Butanal	19
	Carboxylic acid	 Pentanoic acid	20
	Acyl halide	 Acetyl chloride	20
	Anhydride	 Acetic anhydride	20
	Ester	 Ethyl acetate	20
	Amide	 Butanamide	20
	Amine	 Diethylamine	22

* The "R" refers to the remainder of the compound, usually carbon and hydrogen atoms.

Klein, D. (2019) Organic Chemistry, 3rd edition.