CHEM 110L – Review of Standard Lab Techniques & Etiquette

Chemistry majors will be held to a high standard in the organic labs. Your lab space should be kept impeccably neat and organized at all times. Equipment should be handled properly to eliminate or minimize contamination and chemical exposure. Instrumentation is shared not only with your lab section, but the rest of the class as well.

The student lockers, the reagents, the equipment, and instrumentation is all organized, set up, and otherwise maintained by the stockroom staff, all of which are very nice people. Do not take advantage of their kindness! As a general rule, **please leave the lab as you found it**.

Students can help keep everything working properly and keep themselves safe by following instructions in this packet, incorporating announcements made by TAs, and heeding the reminders on the signs in the labs. The TA will give at least a brief demonstration on each technique before you begin this exercise.

Even though you have performed these techniques before, it's important that you enter into this exercise with fresh eyes and an open mind – there is always more to learn! The idea is to *set you up for success today so that you can safely and efficiently perform experiments independently in future weeks*. Get into the habit of following lab protocols to make you a more desirable candidate for working in a research lab!

A. Pluringe: Measuring Liquids

A disposable glass pipet is connected to a syringe with rubber tubing (pluringe). This allows the synthetic chemist to obtain small volumes of liquids with reasonable accuracy and precision. The pipets needs only be inserted into the rubber tubing enough to stay put, not all the way, as it becomes difficult to remove later. The pluringe is re-usable as long as it does not come into contact with liquids. It is easy to avoid pluringe contamination:

- **DO NOT turn the pluringe upside-down when liquid is in the pipet,**
- **Make sure you are not obtaining more than the volume of the pipet itself!**

Only the glass pipet touches liquid. Contaminated pipets should be disposed of in the contaminated solid waste in the fume hood. Pipets can be put in the glass waste if the pipet was used to measure water only.

Under TA supervision (or a TA-appointed pluringe ambassador), slowly and carefully transfer two portions of water from one container into another without cross-contamination. Dispense any two separate volumes of water using the two pluringes in your drawer (1-mL and 3-mL). Record the volumes with proper significant figures and estimate the instrument limit of error (ILE) below. Use the same glass pipet to conserve resources for this exercise only (pretend you changed them). Otherwise, you would change the pipet when changing reagents. Cross-contamination is avoided when delivering the second portion of water into the second container – **do not touch the tip of the pipet to the water already in the flask.**

Measurement with 1-mL pluringe: _____±_____ mL

Measurement with 3-mL pluringe: _____±_____ mL

Draw diagrams of both pluringes, including graduation marks:

TA Initials _____

B. Separatory Funnel: Liquid-Liquid Extraction

Liquid-liquid extraction is a daily routine for the active synthetic organic chemist. Proper precautions make it easy to avoid chemical exposure and spills. You will practice this in the lab with water and your TA will supervise you to ensure this is done properly.

(1) Start with the cap off. Use a glass funnel to carefully transfer 50 mL of distilled water without spilling. Place the funnel on a kim wipe or paper towel in the fume hood.
(2) Put the cap on and twist to tighten.
(3) Hold the funnel with two fingers on the cap and the rest of the hand (or hands) holding the funnel. Slowly invert to test the cap is leak-proof.

- If it does leak, first clean up the spill. You would also change your gloves if anything other than water gets on your gloves.
- Take off the cap and try again after tightening. It may be necessary to try a different cap that does not leak.

(4) With a proven leak-proof cap, rotate once more (two inversions total). Hold the funnel upside-down (two fingers on the cap) with the **tip pointing away from your face and into the fume hood. Slowly open the stopcock to release the pressure.**
(5) Demonstrate step **(3)** as many times as necessary until your TA approves your technique: **2-3 inversions, vent, repeat.** A typical extraction step should take between 2-5 minutes, depending on the volume used and potential interactions taking place.
(6) Place the separatory funnel back on the support ring. Take off the cap and slowly drain the liquid into a container without spilling.

Draw a diagram of the separatory funnel set-up in the fume hood and label each component. Make a note of where each of these components is stored in the lab, including the organizational system of the ring stands.

TA Initials (diagram & technique)_____

C. Equipment Locker: Clean & Organized = Safe & Orderly Experiments

Your assigned station is your responsibility. Check that all your equipment is present in your drawer. Refer to the "GOLDEN RULE" in the lab safety agreement you signed. Make sure you know where to find the equipment list and the picture of the perfect drawer. Have your TA initial this page to confirm you understand how the drawers should be kept and the penalties for not doing so.

TA Initials _____

D. Infrared (IR) Spectroscopy: Functional Group Identification

Bonds in organic compounds vibrate at frequencies in the IR range (400 – 4000 cm^{-1}). These stretching and bending vibrations are characteristic of the types of bonds within specific functional groups (ex. O-H bonds stretch at a different frequency in an alcohol than in a carboxylic acid). When a sample is exposed to IR radiation, the energy is absorbed by the sample at those specific frequencies and peaks are absorbed in the IR spectrum. As a general rule, the longer the bond, the slower the stretching frequency (wavenumber, cm^{-1}). For example, C-H bonds are longer than O-H bonds because of carbon's larger atomic radius. Consequentially, C-H bonds stretch at a considerably slower frequency (alkanes, 2990 – 2850 cm^{-1}) than O-H bonds (alcohols, 3550 – 3200 cm^{-1}).

IR spectroscopy alone is not enough to determine the structure of a compound, of course! This technique must be used in combination with other analytical tools to gain the full structure. The use of IR in the organic labs is primarily to confirm the presence or absence of a suspected functional group, not to blindly look at numbers to guess the matching bond.

Students should know exactly which IR stretches to look for before running an IR sample. Complete all but the last column in the table below for the compound provided in the lab. Ignore any stretches expected in the fingerprint region (1500 – 1000 cm^{-1}).

Functional Group	Bond Assignment (C=O, N-H, etc.) from IR Table	Expected Wavenumber from IR Table	Literature Wavenumber (posted)	Observed Wavenumber (your IR)

Use salt plates (pure NaCl) to support your sample. Sodium chloride does not absorb IR radiation making it an ideal material to hold samples. The *NaCl plates are very fragile* and break easily so handle with care. **Prepare a nujol mull of aspirin by grinding a microspatula tip of solid with 1-2 drops of nujol for 60 seconds (count down!).** The final mull should resemble thin toothpaste. Transfer a small amount onto the plate with a rubber policeman; too much sample will cause the radiation to scatter. Spread the sample evenly by rotating the plates. Check that there is no sample in the instrument and perform a background scan. Place the plates inside the plate holder, being careful not to break the plates, and obtain the IR spectrum. *Take out your sample to allow the next student to obtain their spectrum when two kits are available. Be considerate of other students and classes waiting to use the instrument (your TA may facilitate a rotation).* Use small amounts of the **acetone saturated with NaCl provided in the IR kit (NOT the GC acetone and definitely not with water, or they will dissolve!).**

Compare to the IR of Nujol for reference and to avoid confusing Nujol peaks for sample peaks. Perform a quick comparison your "observed" IR's with the "literature" IRs posted in the instrument room. They should look similar to each other, though they may not be identical. Identify as many of the expected peaks as possible on your observed spectrum.

TA Initials (technique & assignments) _____

E. Gas Chromatography: Quantitative Separation & Analysis of Components in a Mixture

The gas chromatograph will be used in several experiments to confirm reaction completion and determine ratios of products. Corrected retention times are used to identify peaks relative to standards. Integration (area under the curve) is used to calculated percent composition of the components in the mixture. An essential oil containing limonene and carvone is available in the lab. A standard chromatogram of limonene is posted on the bulletin board above the GC for comparison and calculation of this standard corrected retention time. There is no carvone standard provided in this exercise, but it will be obvious which peak is carvone in the essential oil chromatogram.

After TA demonstration, start the chart recorder and inject 0.2 uL of essential oil into the GC as follows: *slowly insert the needle into the (hot) injection port, stabilizing it with the second hand, and inject the sample in a quick but controlled motion.* **Do not force the needle** if it does not readily enter the injection port. Ask for TA assistance if necessary. **Rinse the syringe** three times with the acetone designated for use with the GC while waiting for both peaks to elute and appear on the chart. The rinses can be ejected onto a Kim wipe and disposed of in the trash, assuming it is not highly contaminated (the acetone evaporates quickly). **Never use the acetone saturated with NaCl to rinse the GC syringes.** The NaCl can easily clog the syringes and damage GC column.

Show your work for calculating corrected retention times, integration, and percent composition below. Show these calculations, with final answers in the table, as well as the chromatograms to your TA to ensure you are using the correct measurements for these calculations.

Table 1. GC Results

Sample	**R_t (sec)**	**ID**	**Integration (cm^2)**	**% Composition**
Standard		Limonene	-	-
Oil				

TA Initials (injection technique & calculations)_____

F. Thin-Layer Chromatography (TLC): Qualitative Analysis of a Mixture

In this exercise, students will practice spotting and running a TLC plate using only acetone. This is for demonstrative purposes only, as acetone will evaporate. Students will not practice analysis of the TLC plate today because there will be no spots! Instead, emphasis is placed on proper handling and spotting of plates to ensure optimal separation in future experiments. Pay close attention to your TA's instructions then **demonstrate your ability to properly spot a plate, place it in the TLC chamber, let it run for a few seconds (demo only, plates take several minutes to run), then remove.**

TLC rules and procedure to live by…

- **Handle plates by the edges** only; avoid twisting or bending
- Use **light pencil markings** only to mark the origin; avoid scratching; no pen
- Touch the tip of a capillary tube into the sample then **briefly transfer onto the plate**
- Only **one application of sample** is typically necessary; keep **spots small**
- Keep **TLC chambers in the fume hoods** at all times.
- Hold the spotted plate next to the TLC chamber - **spots must be below solvent level**.
- Holding the jar with one hand and tweezers with plate in the other, **take your time in placing (NOT dropping) the plate evenly into the solvent** in the jar.
- The bottom of the plate should be resting in the middle of the jar and the top of the plate on the side of the jar before letting go with the tweezers.
- Place the cap upside-down on top of the jar – there is no need to screw on the cap
- **DO NOT move the jar** in any way while the plate is running.
- Once the solvent has run most of the way up the plate (not all the way), remove the plate and place it on a Kim wipe or paper towel to evaporate IN THE FUME HOOD before taking the plate to your bench for analysis (for future reference, remember there's no analysis in the introductory exercise).

Your TA or a TA-appointed TLC ambassador must sign-off on your TLC spotting and handling technique.

TA Initials _____

Discussion: Think about the potential risks of not following protocol and how to prevent accidents. What would happen if…

…you forgot to test the separatory funnel's seal with water and instead performed the extraction directly with a reaction mixture?

…you inverted more than twice before venting?

…you vented by taking off the cap instead of opening the stopcock?

…you opened the stopcock too quickly?

…you quickly pulled back the syringe to obtain liquid? Or if you quickly pushed the syringe to transfer?

…you were transferring a second portion of liquid into a container and dipped the tip of the pipet into the liquid in that container?

…you dispensed more liquid into the pluringe than the capacity of the pipet? What is the volume of a short-stem glass pipet?

…you threw away a glass pipet in the regular trash?

…you used the acetone designated for IR to rinse the GC syringe?

…you put your sample on top of the GC oven?

…you injected 2 uL instead of 0.2 uL into the GC?

…you forgot to turn off the chart recorder or forgot to take out the marker?

…you used the acetone designated for GC to rinse the IR plates? Or used water?

…you took the IR of your sample without knowing which peaks to look for?

…there were several students in the instrument room and you ran your IR sample without asking?

<u>Conceptual Questions</u>

- Explain the difference in stretching frequency of O-H vs. N-H vs. C-H bonds.
- What is the difference between a saturated and conjugated ketone? Look up the difference in IR stretching frequencies and explain.
- Why do C-H bonds have different stretching frequencies in an alkane vs. an alkene?
- On what property is GC separated based? TLC?