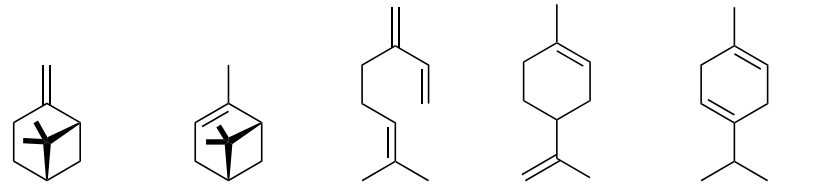
Experiment 2: ISOLATION AND ANALYSIS OF CITRUS OILS
A GREEN-CHEMISTRY APPROACH

Reading Assignment
Mohrig Chapters 12 & 20 (Distillation and Gas Chromatography)

In this experiment students will isolate the essential oil of one of the following citrus fruits: lemon, lime, grapefruit, or orange. Steam distillation will be used to obtain the oil from citrus peels. **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. These mixtures are called oils because they are immiscible with water and, being lighter than water, separate as the upper layer.

Distillation of freshly grated citrus peels with water will produce a mixture that consists largely of water and small amounts of citrus oil. 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. This process is very effective in removing the oil from the water but requires the use of organic solvents, which are potentially toxic and expensive and must be handled with special precautions.

The quest for finding safer and environmentally friendlier chemicals to carry out common laboratory operations is known as ***green chemistry***. One of the principles of green chemistry is the use of safer solvents, or their elimination altogether, whenever possible. In this experiment, you will obtain the citrus oil by a green chemistry method without organic solvents. Enough oil is produced in the steam distillation of citrus peels that it can easily be separated from water using a 50-mL buret (the same piece of glassware used in titrations) and a piece of copper wire.



α-Pinene	**β-Pinene**	**Myrcene**	**Limonene**	**γ-Terpinene**
155 ºC	165 ºC	167 ºC	175 ºC	183 ºC

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

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

The isolated oil will be analyzed by gas chromatography (GC) to determine its composition. The components of the oils usually come out of the GC column in the order of their

boiling points. More volatile compounds come out first and less volatile compounds last. There are exceptions to this rule. To identify the components of the oil, authentic samples of the compounds will be injected individually (these authentic samples are called **standards**) and compare their retention times with the retention times 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), 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, you will inject pure γ-terpinene and observe one major peak. There is no timer on the recorder, but it is set to a certain speed (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 γ-terpinene, then you will 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**).

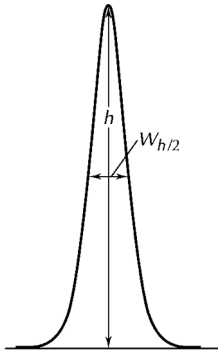


FIGURE 20.13
Determining peak area: h = height; $W_{h/2}$ = width at half-height.

t_R' = distance from air to sample (cm) x [1 / Chart speed (cm/min)] **(1)**

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

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

Notebook Preparation – OK to prepare one day at a time
Day 1 – Read Mohrig Chapter 12; Bring & work on Part C of Technical Writing Worksheet
Freshly grate citrus peels BEFORE coming to lab. Refrigerate if storing.

- *Purpose*: One-sentence description of the purpose of Day 1. The reaction scheme should begin with a picture of the citrus-of-choice, follow with boiling water over the reaction arrow and the structures of potential components (limonene, α-pinene, β-pinene, and γ-terpinene).
- *Reagent Table*: citrus peel (starting material) and citrus oil (product) in addition to the properties of limonene, α-pinene, β-pinene, and γ-terpinene (MW, bp, density, and one-word hazard). You can fill in the approximate amounts of the terpenes after GC analysis by using the percent composition and the mass of citrus oil after day 2.
- *Procedure*: Hand-written, step-wise, and in your own words; include **Figures 2** & **3**.
- *Safety & Clean-up* – Copy pertinent notes from the table after the procedure.

Day 2 – Read Mohrig Chapter 20

- *Purpose*: One-sentence description of the purpose of Day 2 (no scheme).
- *Procedure*: Hand-written, step-wise procedure in your own words.
- *GC Table*: re-create the tables from page 8, including table numbers and titles.
- *Safety & Clean-up* – Copy pertinent notes from the table.

Extra Credit: Polarimetry – can be completed Day 1 or 2, instructions at the end of procedure

PROCEDURE

Day 1 - ISOLATION OF CITRUS OILS

Coordinate with your lab partner and bring freshly grated citrus peels: 4 oranges, 5 grapefruit, *or* 7 lemons or limes for each group. It is important that the peels are finely chopped/cut/grated as small as possible the morning of lab. You can peel the night before and keep in the fridge, but freshly chopped peels are necessary for good recovery of citrus oil. *Cutting tools will not be provided and you will not be allowed to bring grating or chopping utensils into lab*.

Weigh approximately 150 g of the peel of finely chopped or grated citrus peel. Using a wide-mouth funnel for solids, transfer the crushed material into a 500-mL round bottom flask. **Add 150 mL of distilled water** to the flask and assemble a simple distillation apparatus (**Figure 2**). If you forget to add the distilled water before heating, it will smell terrible, *you will not be allowed to start over, and your report will be penalized*. In a 250-mL round-bottom flask, pour 70 mL of water and mark the level of the liquid with a marker. Pour the water out and use this flask as the receiving flask for the distillate. *Pay attention to the instructor's demo in the pre-lab talk. It must be set up in a particular order, starting on the side with the larger flask.*

Heat the distillation flask using a heating mantle, starting at a medium setting. Adjust the heat supply so that the liquid distills at an approximate rate of one drop per second. Distilling to quickly with too high heat will severely decrease recovery. Be patient - it may take up to 20 minutes for the first drop. Collect approximately 70 mL of distillate. Read and record the temperature throughout the distillation (first drop and the final temperature).

In your down time, read the textbook sections regarding Gas Chromatography (GC) and begin working on the abstract (part C of writing activity from day 1).

Stop the distillation by turning off and unplugging the heating mantle and carefully lowering the ring support for the heating mantle. You will need a spotter to help with this. You may take off the receiving flask, but wait for the entire system to cool before diassembling. In the meantime, with the help of a glass funnel, *slowly* transfer the distillate to a 50-mL buret fastened to a ring stand. After you have transferred about 50 mL, let the liquid layers settle

inside the buret for a few seconds, and then open the stopcock and *slowly* drain some of the water into a 125-mL Erlenmeyer flask to make room for the rest of the distillate. Add the rest of the distillate to the buret. Add 10 mL of water to the flask, swirl to dislodge any drop adhered to the wall and transfer the liquid to the buret. Repeat this operation with another 10-mL portion of water. You will eventually observe a layer of oil on top of the buret and bubbles along the walls (**Figure 3**). Some of these bubbles are trapped air bubbles and some are oil droplets that did not rise to the top. The more carefully the oil/water mixture is added to the buret, the easier the separation will be. The level of the liquid should be near the 50-mL marking so that you can collect the oil from the top later. Let the system settle for a couple of minutes. In the meantime, label a small screw-cap vial with your name and the name of the oil. Weigh it and keep it handy.

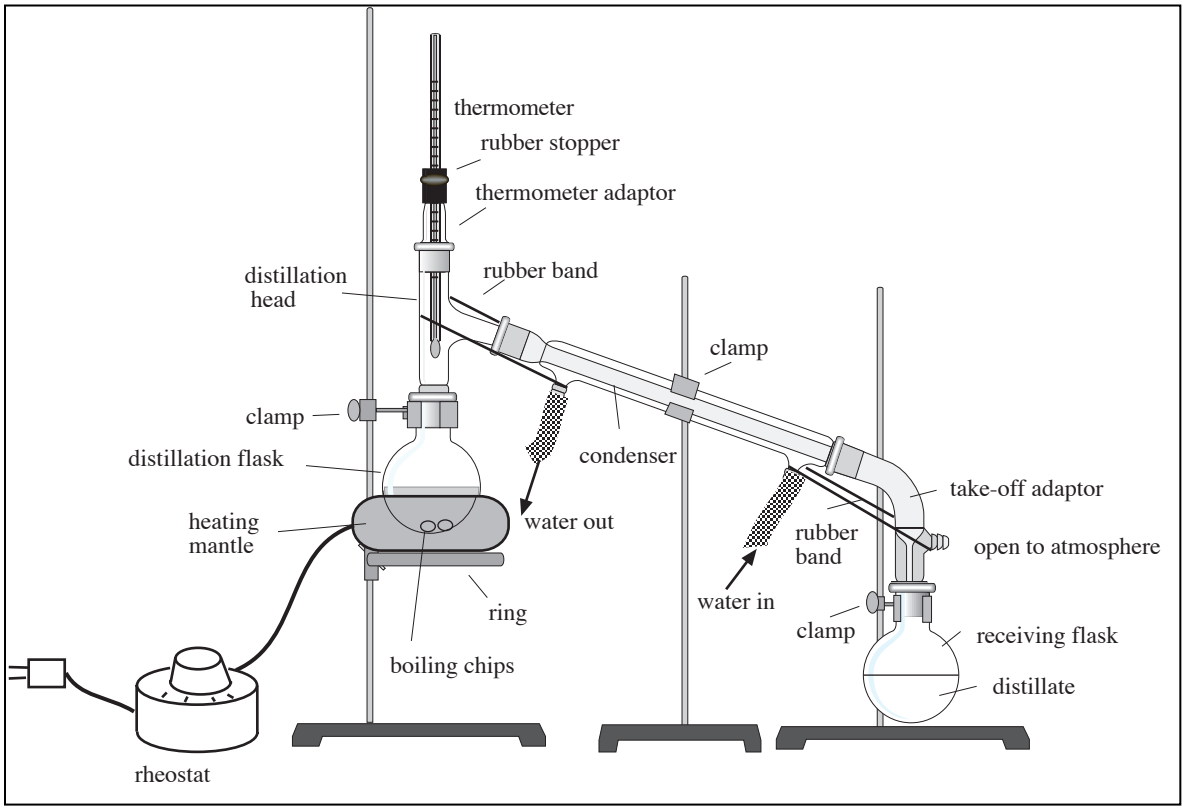


Figure 2. A simple distillation apparatus used for steam distillation. *Sketch this diagram into the procedure portion of your notebook.*

Cut a piece of copper wire about 6 inches longer than the buret (it may already be cut for you), and bend one of its tips to make a small loop that fits inside the buret. Remove the Erlenmeyer flask momentarily and lower the buret as much as possible. Insert the copper wire in the buret and carefully move the wire up and down making sure that the loop touches the wall of the buret (where you see trapped bubbles) and dislodge them. You will see a myriad of bubbles rising to the top. Remove the copper wire and let the system settle for 5-10 minutes.

Using a glass pipet and pipet bulb (not a pluringe), 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! For reference, students can expect to recover approximately 1-5 mL of citrus oil. Transfer the oil to the pre-weighed vial. Remove any visible droplets of water using a pipet per TA instructions. Weigh the vial with the oil and determine the mass of the oil by difference. Cap the vial and keep it for the GC analysis (Day 2).

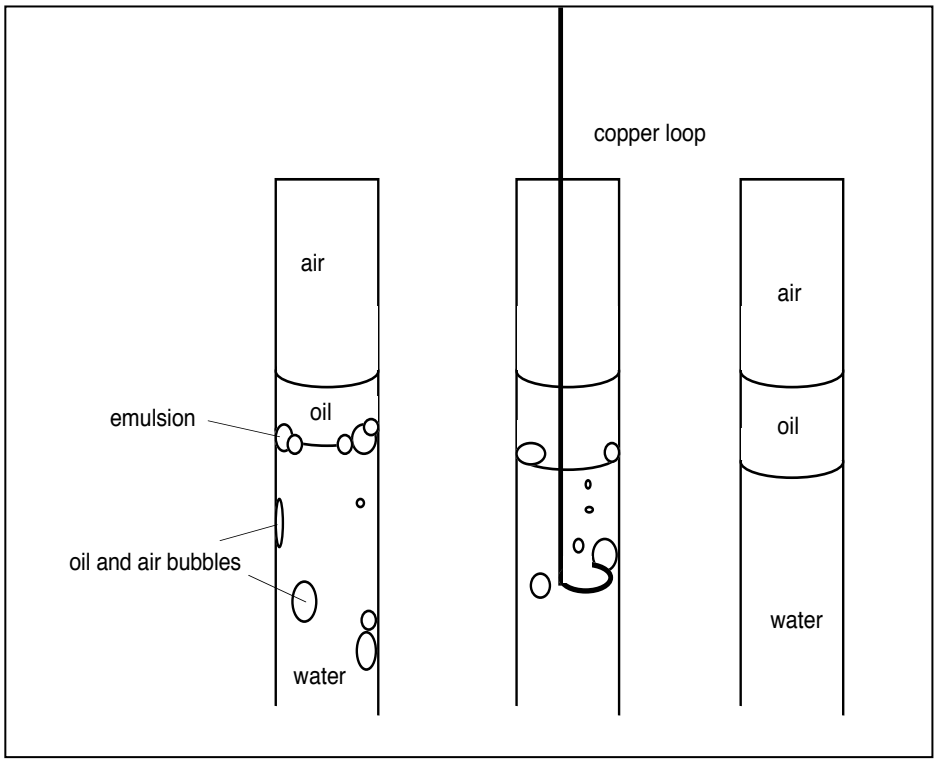


Figure 3. Buret with oil and water layers (liquid-liquid extraction).

Day 2 – GC ANALYSIS OF CITRUS OIL

Patiently await TA demo. The instrument room should have no more than 4-6 students at a time. In the meantime,

Before beginning the run, write down the sample's name, the chart speed (if applicable), attenuation on the chart, and all other conditions posted on the bulletin board above the instrument. 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 per TA instruction. Turn the nob on the recorder to mark the beginning of the run and quickly inject no more than 0.2 μL of each sample into the GC. The air or acetone peak will be used as the "zero" retention time and is used as the starting point for the **corrected retention time**. Rinse the syringe three times with acetone after each injection. This will avoid cross-contamination. Before you introduce the needle into a new liquid sample, clean it with tissue paper to remove traces of liquid from previous injections.

Inject pure samples of limonene, α-pinene, β-pinene, and γ-terpinene. Myrcene is not injected due to unwanted interaction with the column. Measure the corrected retention times of each standard(no integration). Inject your oil and identify the peaks by comparing corrected retention times to the standards. Determine which compounds are the main components of your oil. If any peaks are in question, spike your sample with a standard (procedure on the next page). Calculate the percent composition of your oil. *This analysis must be done during this lab period.*

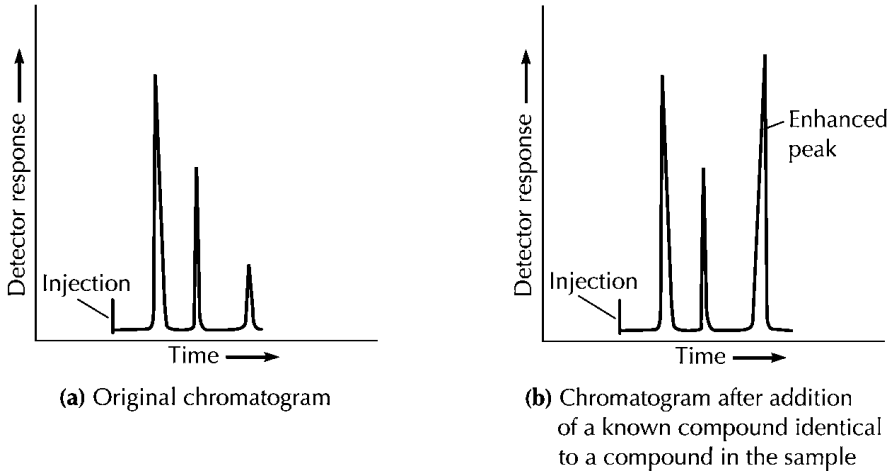
Although not ideal, standard peaks can still be interpreted if the peaks level off at the top because only the retention time is needed. However, if the peaks from the citrus oil are too small to interpret or if sample peaks (not air/solvent peaks) level out at the top, repeat the injection. Because the sample volume is so small, the intrinsic error is very high. Often, simply repeating an injection at the same volume will provide data that is easier to interpret. Do not

increase sample volume beyond 0.2 μL. Measure the retention times of the peaks and integrate them. Before you do a new injection, allow all the peaks to come out.

Because the retention times of the main components of citrus oils are not very different, there is an error associated with the measurement of their retention times. Confirm the identity of any citrus oil peaks in question by injecting your oil plus one of the standards simultaneously. This technique is called **spiking** and it's very useful in the identification of GC peaks. Suppose you obtained the chromatograms of the oil and the standards and it is unclear whether a certain peak in the oil is α- or β-pinene (both have similar retention times)…

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

Above is the procedure for spiking with α- or β-pinene. The procedure could be applied to any standard to positively identify any or all of the peaks in the chromatogram of the oil, time permitting.



(a) Original chromatogram

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

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

**Complete all analysis and write a draft of the abstract before leaving lab on Day 2.

Table 1. Clean-up and Safety

Clean-up	**Safety**
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.	Myrcene, γ-terpinene, α-pinene, β-pinene are irritants.
Keep the citrus oil in a capped, labeled vial in your drawer for Day 2. Dispose of the oil in the same labeled vial in the container provided after analysis on Day 2.	Limonene is a possible carcinogen.
Dispose of pipets in the glass waste box.	Acetone is flammable.
(Day 2) Rinse syringes with acetone.	(Day 2) GC Needles are sharp, delicate, and expensive – handle with care.
Thoroughly wipe down bench tops with a sponge and dry with a paper towel. Wash and rinse all glassware. Return shared glassware and equipment to where you found it in an organized fashion. All students in the section will lose points if even one student leaves a mess.	
(Day 2) 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.	

Abstract

Refer to the Technical Writing Guidelines. The main results in the abstract will be the recovery of oil (g and %) as well as the composition of the oil as determined by GC. For example:

"(Citrus) oil was isolated as a clear oil (xx mg, xx% recovery). GC analysis revealed the oil to contain α-pinene (xx%), β-pinene (xx%), limonene (xx%)…"

Introduction: Pre-lab Questions

Day 1

1. Define the term **terpene**. What is the approximate percent composition of each of the terpenes in **Figure 1** in orange oil (or whichever citrus you will be using)? A quick Wikipedia search is fine this time!

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

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

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

Day 2 - reproduce the tables from in-lab questions 5 & 6 as well as the following.

5. Briefly define the following concepts/terms related to gas chromatography: partition chromatography, mobile phase, stationary phase, solid support, integration, retention time, dead time, and corrected retention time.

6. Predict the order in which the GC standards should exit the column.

7. Show the calculations for corrected retention time of limonene and carvone using the sample GC chromatograms provided online. Integrate the sample peaks in the “unknown oil #4” chromatogram and calculate the percent composition. You may have already done this in lab during the distillation!

**Complete the tables on page 4 of the technical writing worksheet. Show your TA completed work before leaving lab on Day 2 for credit (5 points of the pre-lab). It is recommended that the bulk of this be finished on Day 1 or before coming to lab on Day 2, but it OK to finish this while you wait for your turn in the instrument room.

Results: In-lab Questions
Turn in typed responses all together on the report due date.

Day 1
1. Calculate the **% recovery** of citrus oil from the peel.

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

2. Report the **temperature range of distillation**, during actual collection of distillate. Is the distillation temperature higher or lower than pure water? Explain.

Day 2
3. Report the **GC conditions** posted on the bulletin board above the instrument.

4. Report the **ILE of the GC needle** and the **percent intrinsic error** for each 0.2 μL injection.

5. Show one **sample calculation** for corrected retention time (t_R) and report the final results in a typed table (see below).

Table x. Standard GC Retention times (reproduce in your notebook before Day 2)

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

6. For each peak in the GC chromatogram of the oil, calculate **corrected retention time, integration, and % composition**. Show one sample calculation for integration and one for percent composition and report all final results in a typed table (see below). **Number each peak** and **identify** each as one of the injected standards, where possible. Corrected times may not match exactly but the order should be the same. Recall that myrcene was not injected as a standard and there may be other unidentifiable components. Do not integrate solvent (acetone) and air peaks.

Table x. GC Analysis of Citrus Oil (reproduce in your notebook before Day 2)

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

*More or less rows may be required.

7. What is the **major component** of your citrus oil? What are the **minor components**? How does this compare with the expected composition (pre-lab #1)?

Exp 2 Isolation and Analysis of Citrus Oils

Name _____

Due Date for Report in Syllabus

Section Day _____ Time _____ TA Name _____

CHEM 8L GRADING RUBRIC – Use as cover page for report

SECTION	INSTRUCTOR COMMENTS	POINTS ASSIGNED
IN-LAB QUIZZES		**/ 10**
LAB REPORT		
ABSTRACT Purpose, procedure, main result(s), and conclusion(s) per Technical Writing Guidelines.		/ **20**
INTRODUCTION Each pre-lab question is addressed in its own paragraph using complete sentences. Structures and calculations are hand-written, where appropriate.		/ **35**
RESULTS The main results are stated, as outlined in the in-lab questions, using complete sentences.		/ **45**
NOTEBOOK PAGES Proper format: reaction scheme, chemical info table, procedure, waste and clean-up procedure. Procedure easy to follow, not copied directly from handout, includes pictures of glassware/equipment.		/ **25**
NEATNESS AND ORGANIZATION Proper grammar, order, and format (per instructions in syllabus).		/ **5**
LAB TECHNIQUE & CLEAN UP Lab space left clean, proper technique, instructions followed, checked in with TA before leaving.		/ **10**
	LAB REPORT TOTAL	**/ 150**