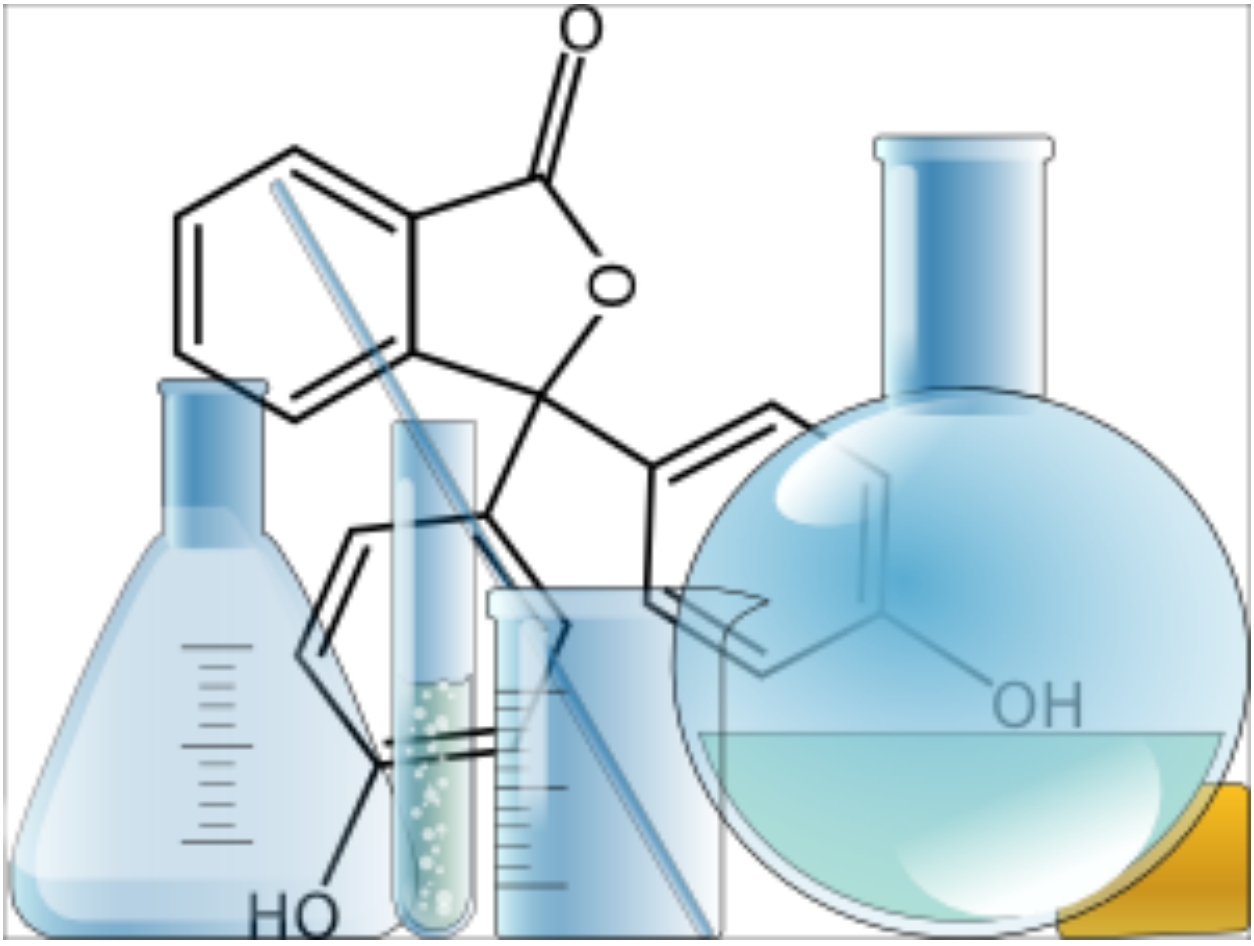
CHM2123

LABORATORY OF ORGANIC CHEMISTRY II



UNIVERSITY OF OTTAWA

FALL 2017

KATHERINE McGILVRAY

Table of Contents

Evaluation

Your final mark will be calculated as follows:

Pre-lab questions	5%
Lab Reports	30%
Post-lab questions	5%
TA Evaluation	10%
Practical (In-lab) Exam (Exps 1-3) Oct 16^{th}-20^{th}	10 - 15%
Final Written Exam (Exps. 1 – 7)	35 - 40%
	100%

A flexible grading scheme is in place such that the practical and final exams are worth a total of 50% of the final grade.

Learning Outcomes

Upon completion of CHM2123, students will gain an understanding of:

1. How to practice safety in the laboratory when handling laboratory equipment, glassware, and chemical reagents
2. How to interpret information of Material Safety Data Sheets (MSDS) for a given reagent, and how to dispose of chemical waste in a safe and responsible manner
3. How to work effectively as a member as a team, communicate productively and respectfully with lab mates, teaching assistants, and lab coordinator
4. How to use the scientific method to create, test, and evaluate a hypothesis through critical thinking
5. How to maintain a detailed scientific notebook containing MSDS information of chemical reagents, experimental procedure, raw data, observations, and calculations.
6. How to perform basic mathematical calculations to determine the limiting reagent, equivalents, concentrations, and yields
7. How to perform common laboratory techniques including glassware assembly, reflux, aqueous extraction, vacuum filtration, thin layer chromatography, cold-finger sublimation, recrystallization, and distillation
8. How to characterize organic molecules by physical and spectroscopic means, including mp, bp, IR, and ^{1}H NMR to determine the identity, purity, and yield of products
9. How to summarize results and communicate in a clear manner via laboratory reports
10. How to predict the outcome of several common organic reactions through a basic understanding of starting materials, functional groups, mechanism and reaction conditions

Practical In-Lab Examination

- A practical exam (10 - 15% each) will take place during your regular lab period during the week indicated above. The practical exam is 90 minutes long and performed independently such that one partner of each pair will perform the exam during the first 90 minutes, and the second partner will perform the exam during the remaining 90 minutes of the 180 min lab period. The practical exam will consist of six stations through which a student will circulate. A student will have ten minutes at each station to complete the task and submit their work to the TA, and the remaining five minutes to clean up and move to the next station. The practical exam will test your ability to assemble glassware, demonstrate/identify laboratory safe practices, perform various laboratory techniques, calculate yields, complete a mechanism and/or indicate missing reagents. There is no talking during the practical exam.

Final Written Exam

- A written final exam (35 – 40 %) during the regular exam period in December will summarize the key concepts, laboratory techniques, and reactions studied in the course. The exam is cumulative.
- A copy of the 2015 final exam can be found in the Supplemental Information section on BL.
- Special exam office hours will be posted on Brightspace before the final exam. Special review sessions will take place on December 5th and 6th with further details posted on Brightspace.

IMPORTANT

1) You must obtain **at least 50% in the practical aspects** (reports + TA evaluation + practical exam) in order to pass the course.
2) There is no supplemental exam for this course, nor are there any extra-credit assignments.
3) A student with three or more unexcused absences from the laboratory sessions will automatically receive a letter grade of E in the course.

Email Etiquette

It is important to be courteous and respectful when emailing your TA or the lab coordinator. Please follow these simple rules when sending an email.

1) Use your @uottawa.ca email address when corresponding with your TA or the lab coordinator. Please include CHM2123 in the subject line.
2) Begin each email with your name, student number, lab day, and your TA's name.
3) Always use formal language and be polite in your email. Coarse language, threats, or abuse of any kind will not be tolerated.

Your TA and the lab coordinator will respond to your email within 24 hours, provided that you have followed the above guidelines. We reserve the right to ignore your email if these requirements are not met.

Expectations

Students will	TA's will	The lab coordinator will
- Arrive on time for the lab	- Arrive 15 minutes early to the lab to open lockers	- Be available during the lab
- Have read the lab prior to coming in	- Provide a short demonstration or introduction	- Be available for office hours outside of the lab
- Communicate respectfully wth lab partner in and out of the lab		
- Submit reports on time one (1) week after the experiment	- Correct lab reports one (1) week after they have been submitted	- Be available to provide support for students and TAs as needed
- Complete the pre-lab questions prior to the lab	- Provide detailed and adequate feedback in correction of each	
- Prepare pre-lab in lab notebook	- Verify lab notebook for pre-lab	

Laboratory Schedule

The experiments have been designed such that they can be completed in the allotted time. It is expected, however, that you arrived well prepared. Your final clean-up of glassware and work area must begin no later than 15 minutes before the end of the lab period, and you must leave the laboratory no later than 5 minutes before.

Month	Monday (A00/A01)	Tuesday (A02)	Wednesday	Thursday (A03)	Friday (A04)
September	4 —	5 —	6	7 —	8 —
September	11 Meet & Greet	12 Meet & Greet	13	14 Meet & Greet	15 Meet & Greet
September	18 Exp. 1	19 Exp. 1	20	21 Exp. 1	22 Exp. 1
September	25 Exp. 2	26 Exp. 2	27	28 Exp. 2	29 Exp. 2
October	2 Exp. 3	3 Exp. 3	4	5 Exp. 3	6 Exp. 3
October	9 *NO LABS*	10 *NO LABS*	11	12 *NO LABS*	13 *NO LABS*
October	16 Practical Exam	17 Practical Exam	18	19 Practical Exam	20 Practical Exam
October *Reading week*	23 *NO LABS*	24 *NO LABS*	25	26 *NO LABS*	27 *NO LABS*
October/November	30 Exp. 4	31 Exp. 4	1	2 Exp. 4	3 Exp. 4
November ***Midterm Nov. 12th***	6 Exp. 5	7 Exp. 5	8	9 Exp. 5	10 Exp. 5
November	13 Exp. 6	14 Exp. 6	15	16 Exp. 6	17 Exp. 6
November	20 Exp. 7	21 Exp. 7	22	23 Exp. 7	24 Exp. 7
November/December	27 Exp. 8	28 Exp. 8	29	30 Exp. 8	1 Exp. 8

Note: Last day to drop a course: **November 17th, 2017**

Required Material

Please bring the following items to each lab period.

- Lab coat and safety glasses[1]
- Lab notebook[2], softcover (Hilroy or similar), as long as pages are bound
- Lab manual
- Pen, pencil, ruler, permanent marker (for labelling glassware), USB drive

Safety

Please note: anyone not following proper safety procedures will be asked to leave the lab and will receive a grade of zero for that experiment.

- Safety glasses and lab coat must be work at all times when in the laboratory. **Closed-toed shoes** that cover the whole top of the foot must also be worn. Long hair must be tied back.
- If you spill something on your hands, wash them immediately with cold water and consult with your TA.
- Many organic solvents and reagents go through disposable gloves within seconds. These gloves over limited protection and are not permitted in the lab, except under special circumstances.
- TAs are required to give warnings and penalties to students who do not follow safety rules. The penalty for the first warning will be at the discretion of the TA. A second warning will result in expulsion from the lab. The experiment may not be repeated at a later time.
- Dangerous behaviour will not be tolerated and will result either in penalties or expulsion from the laboratory.

[1] These items are on sale at the Science Students Association office located at MRN 023. Please note that lab supplies are no longer sold in MRN 301.

Pre-lab and Post-lab Questions

- Pre-lab and post-lab questions for each experiment are completed using Sapling Learning.
- Visit https://www.saplinglearning.ca/ibiscms/login/ to create your account for CHM2123. When prompted for the key code, use the 4 digit course number (2123) followed by the section code of your group (i.e. if a student is in 2123 section A02 – the Tuesday section – enter 2123A02as the key code).

Sapling Learning is an online homework system for quizzes custom-built to CHM2123 experiments. The web-based software provides organic chemistry problems in an active learning format with response-specific feedback. Features such as interactive 3D models, drag-and-drop syntheses, and chemical structure drawing with arrow-pushing build a foundation for mastering synthesis techniques. Tutorial prompts allow three tries per question with progressively decreasing credit (loss of 5% per try) and offer interactive mentoring. Sapling software has been shown to help students get better grades with targeted instructional feedback tailored to each individual student's responses. It's like having a personal tutor on call, ready to offer just the assistance needed.

- Question sets are made up of 5 questions.
- Pre-lab questions will be posted for one week, and must be completed **prior to performing the lab**.
- Post-lab questions will be posted for one week, and must be completed by the deadline for the submitted lab report.
- You may use the manual, textbook, online resources, work with a partner or a friend.

Reports

The lab experiments are performed in groups of two. Lab reports are to be handed in individually or in groups of two, depending on the schedule provided below.

Exp. 1	One report handed in by each student	Exp. 5	One report handed in by each student
Exp. 2	One report per pair	Exp. 6	One report per pair
Exp. 3	One report handed in by each student	Exp. 7	One report handed in by each student
Exp. 4	One report per pair		

A problem set will be posted on Blackboard Learn every week, together with an answer sheet. It is highly recommended to attempt to answer the questions without the assistance of the marking scheme, as these problem sets represent exam style questions.

As part of each pre-lab, you must prepare the following in your **lab notebook**: a table of reactants and solvents, a table of observations, and section of raw results, including TLC drawings. The

table of reactants must contain the following information: reactant/solvent name, molar mass, quantity, density (if a pure liquid), number of moles and number of equivalents (equivalents are not required for solvents). You will fill the table of observations throughout the lab period. The lab notebook component of your pre-lab will be evaluated by your TA each week.

Keeping a lab notebook

Purpose:

The purpose of a lab notebook is to have an accurate and permanent record of experimental methods and observations in the lab. Learning to keep a lab notebook in CHM2123 will establish good habits that will serve you throughout your career. Lab notebooks are essential to record keeping for patents and intellectual property. A good test for your quality of notebook keeping is the following question: Could someone else with equivalent skills and background knowledge use the information in your notebook to replicate your experiment and results?

Format:

- Bound notebook, 8.5'' x 11''
- Each page is dated at the top and numbered at the bottom
- Neat and legible handwriting
- Write in permanent ink only (not typed and printed/pasted)
- Record what you did and why you did it. It is good habit to record mistakes and difficulties as well.
- Cross mistakes out with a single line. White-out and blacking out is not acceptable
- Raw data (table of results, TLC, etc. is **initialed by TA** upon completion of the experiment.

Content of the Laboratory Notebook:

- Table of Contents
- Title
- Date
- Page Number

1. Title of Experiment and Date performed
2. Purpose (ie. to convert a primary alcohol to a carboxylic acid via Jones oxidation)

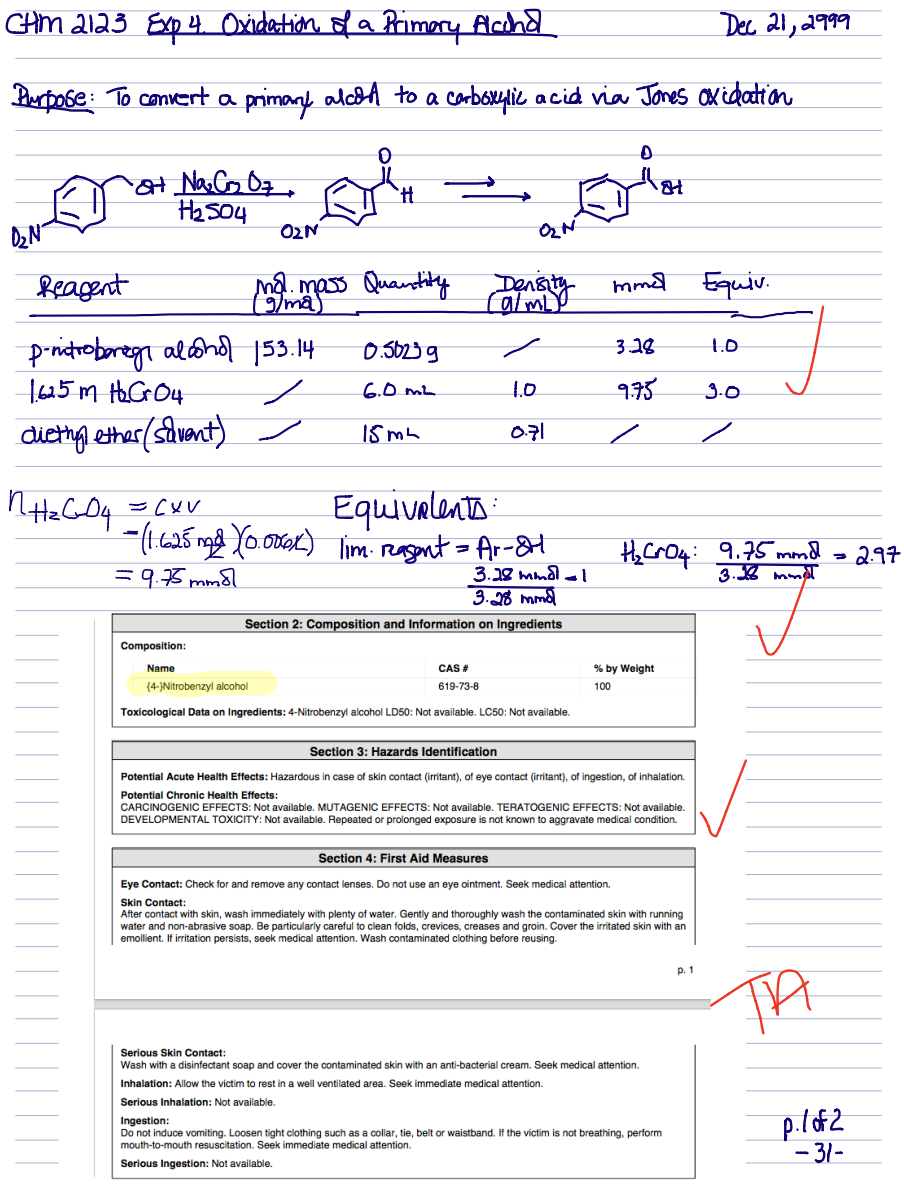
    - Materials and Methods
    - Table of reagents, complete with calculation of moles and equivalents
    - MSDS information for new reagents (taped or pasted into notebook)
    - Reference method to lab manual and any modifications posted on Brightspace
    - Calculations

3. Observations and Results

Everything that happens or doesn't happen is data!
Tables of data
Graphs
Print-outs
TLC plate sketches and Rf calculations (note: picture of TLC required in your report). Do NOT tape your TLC plates in your notebook.
Calculations

The lab notebook must be initialed by the TA prior to leaving the lab. No TA initials means a TA evaluation of 0 for that experiment!

Example of a lab notebook completion for pre-lab evaluation:



CHM 2123 Exp 4. Oxidation of a Primary Alcohol — Dec 21, 2999

Purpose: To convert a primary alcohol to a carboxylic acid via Jones oxidation

$Na_2Cr_2O_7$, H_2SO_4

Reagent	mol. mass (g/mol)	Quantity	Density (g/mL)	mmol	Equiv.
p-nitrobenzyl alcohol	153.14	0.5023 g	✓	3.28	1.0
1.625 M H_2CrO_4	✓	6.0 mL	1.0	9.75	3.0
diethyl ether (solvent)	✓	15 mL	0.71	✓	✓

$n_{H_2CrO_4} = c \times v$
$= (1.625\ mmol/mL)(0.006\ L)$
$= 9.75\ mmol$

Equivalents:
lim. reagent = Ar-OH
$\frac{3.28\ mmol}{3.28\ mmol} = 1$

H_2CrO_4: $\frac{9.75\ mmol}{3.28\ mmol} = 2.97$

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
(4-)Nitrobenzyl alcohol	619-73-8	100

Toxicological Data on Ingredients: 4-Nitrobenzyl alcohol LD50: Not available. LC50: Not available.

Section 3: Hazards Identification

Potential Acute Health Effects: Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation.

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. Repeated or prolonged exposure is not known to aggravate medical condition.

Section 4: First Aid Measures

Eye Contact: Check for and remove any contact lenses. Do not use an eye ointment. Seek medical attention.

Skin Contact:
After contact with skin, wash immediately with plenty of water. Gently and thoroughly wash the contaminated skin with running water and non-abrasive soap. Be particularly careful to clean folds, crevices, creases and groin. Cover the irritated skin with an emollient. If irritation persists, seek medical attention. Wash contaminated clothing before reusing.

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TA

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

Inhalation: Allow the victim to rest in a well ventilated area. Seek immediate medical attention.

Serious Inhalation: Not available.

Ingestion:
Do not induce vomiting. Loosen tight clothing such as a collar, tie, belt or waistband. If the victim is not breathing, perform mouth-to-mouth resuscitation. Seek immediate medical attention.

Serious Ingestion: Not available.

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TA Evaluation

Your TA will evaluation your performance at the end of each experiment. Each evaluation will be based on the following criteria:

- Pre-lab completion in lab notebook (2)
- Demonstration of good organizational and time-management skills. Respectful of cleanliness and lab safety (3)
- Ability to work independently and in a team (2)
- Practical and theoretical organic chemistry knowledge (2)
- Clear and concise communication in written reports (1)

A grade for each TA evaluation will be provided with report correction on Brightspace.

Report outline

The required sections for each lab report will be listed at the end of each lab. Shown below are all the possible sections that could end up in your report. Please consult the sample lab report on the next page for more specific marking guidelines.

i) **Title:** Your name and student number, title of the experiment, due date, your lab section, and the name of your TA.

ii) **Introduction**: Include a short description of the relevant theory required to understand the experiment and provide a description of the experiment goals and methodology.

iii) **Mechanism**: The mechanism of each reaction performed. A reaction mechanism shows the movement of electrons using curved arrows and shows all of the intermediates formed throughout the course of the reaction. You may use the ChemDraw software to create and copy/paste your mechanism into your report, or you can include a photo of a clear hand-drawn mechanism.

iv) **Table of reagents and solvents**: A table with the name, molar mass, quantity (in mL or g), density (if the compound is a pure liquid), number of moles, and equivalents of all reagents used in the experiment. The name and volume of solvents used *for the reaction only* must also be included in the table. Reagents used in the chemical workup and separation of products need not be included in the table.

v) **Experimental procedures**: Reference the pages in the lab manual and report any experimental modifications made.

vi) **Results and Observations**: Organize your data into one (or more) tables of observations, along with one (or more) tables of results. Be specific with your observations and results. Include a "table of products" identifying in mass, yield, melting point ranges, and molar mass of your product(s). Provide titles for each table.

vii) **TLC plates**: Include images or diagrams of your TLC plates. Specify the solvent or solvent mixture and ratio used (i.e. hexanes : ethyl acetate 8:2), Rf values (retention factor) of all spots shown in a table or on the plate, labeled lanes, and a legend clearly identifying what is on the plate.

viii) **Calculations:** If required, show the type of calculation performed in the lab (excluding Rf calculations).

ix) **Flow diagram:** A flow chart allows you to chart the separation of your compounds in your isolation steps, indicating the products of each extraction. Flow diagrams must include all purification steps (drying, filtering, evaporation, recrystallization, etc.).

x) **Discussion:** Your discussion will provide a rigorous analysis of your results. You should explain the importance of all key steps in the procedure. Compare your results with what is predicted by the theory and propose an explanation for any discrepancies. Give an analysis of your results, commenting on TLCs (what they indicate), melting point, yield, etc. Propose possible sources of error, if any. The discussion is where you will show your understanding of the experiment.

Your report should not exceed 6 pages in length, not including answers to the assigned questions. There is a sample lab report available on the next page.

Plagiarism

Submitting another person's words, ideas, images, ChemDraw mechanisms, etc. within lab reports or quizzes and passing them off as your own is considered plagiarism. Secondly, the complete or partial translation, as well as paraphrasing of a text written by someone else also constitutes plagiarism if you do not acknowledge your source.

If you write a report alongside your lab partner or others, be certain to express yourself in your own words and present independent ideas. Plagiarized lab reports will result in a grade of 0 on the lab report, and will be passed along to the lab coordinator. Further infraction can lead to serious sanctions such as expulsion from the University. You are strongly encouraged to review the University of Ottawa policy on plagiarism and academic integrity available at www.uottawa.ca/plaigiarism.pdf.

Sample Report

The points allotted to each section of the report will vary with each experiment. A rough guide for points is included at the end of each experimental protocol found in this lab manual. Any changes will be posted in an announcement on Brightspace prior to report submission.

Lab # 4 - The Oxidation of a Primary Alcohol

Philip J. Fry 7408141 and Bender B. Rodriguez 2716057

December 31, 2999

TA: Hubert J. Farnsworth

CHM 2123

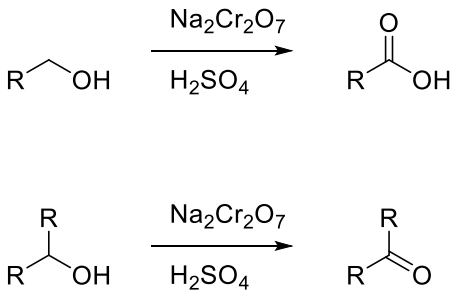
Introduction (6 points)

In your introduction, you should include theory relevant to the experiment and an overview of the experimental methods you will use and the overall goal of the experiment. The use of a reaction scheme can really help with your introduction.

6 points	- Theory is clearly presented and relevant to the experiment - Experimental goals are present and thoroughly explained - Methodology to be used in the experiment is present and thoroughly explained
4 – 5 points	- Theory is mostly present and mostly relevant to the experiment - Experimental goals are present, but explanation is missing 1 – 2 key points - Methodology is present, but explanation is missing 1 – 2 key points
3 points	- Theory is present, but connections to the experiment are unclear - Experimental goals are present, but explanation is weak - Methodology is present, but the explanation is weak
2 points	- Theory is poorly explained - Experimental goals are present, but are unclear - Methodology is poorly explained or missing
1 point	- Theory is very poorly explained - Experimental goals are very unclear, or missing - Methodology is missing
0 points	- No introduction is present

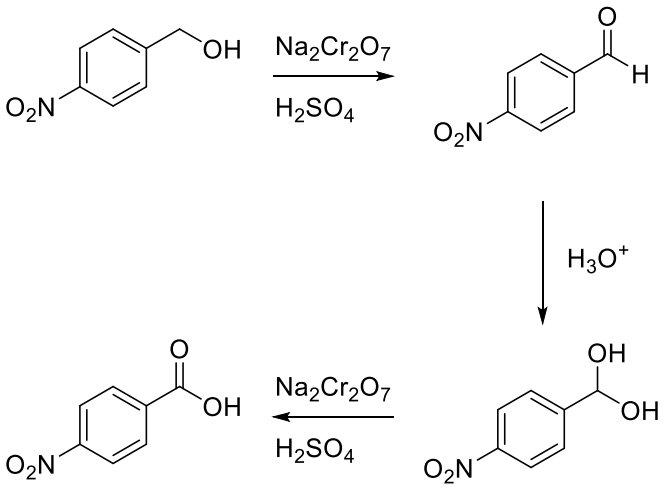
Oxidation is a key reaction in organic chemistry. Alcohols can be oxidized to aldehydes, ketones, or carboxylic acids. One of the most common methods of oxidation is the Jones reaction using chromic acid. The Jones reaction oxidizes primary alcohols to carboxylic acids and secondary alcohols to ketones.

Scheme 1: Jones Oxidation



The goal of this experiment is to oxidize *p*-nitrobenzyl alcohol to the corresponding carboxylic acid using the Jones reagent, $Na_2Cr_2O_7/H_2SO_4$. The mixture of sulfuric acid and sodium dichromate form chromium (VI) oxide in solution, which reacts with the primary alcohol to form a chromium ester. Once the ester has formed, the carbon-oxygen bond is oxidized to an aldehyde and the chromium is reduced to chromium (IV) oxide. In aqueous solutions, aldehydes are converted to diols. These diols react with another equivalent of chromium to repeat the oxidation step, forming a carboxylic acid. The purity of our compound will be analyzed using thin layer chromatography (TLC) analysis, using samples of *p*-nitrobenzaldehyde and *p*-nitrobenzoic acid provided.

Scheme 2: Experimental Overview



Mechanism (8 points)

Show the mechanism of all the reactions performed in the experiment. The points assigned for the mechanism will vary from experiment to experiment, depending on the complexity of the mechanism.

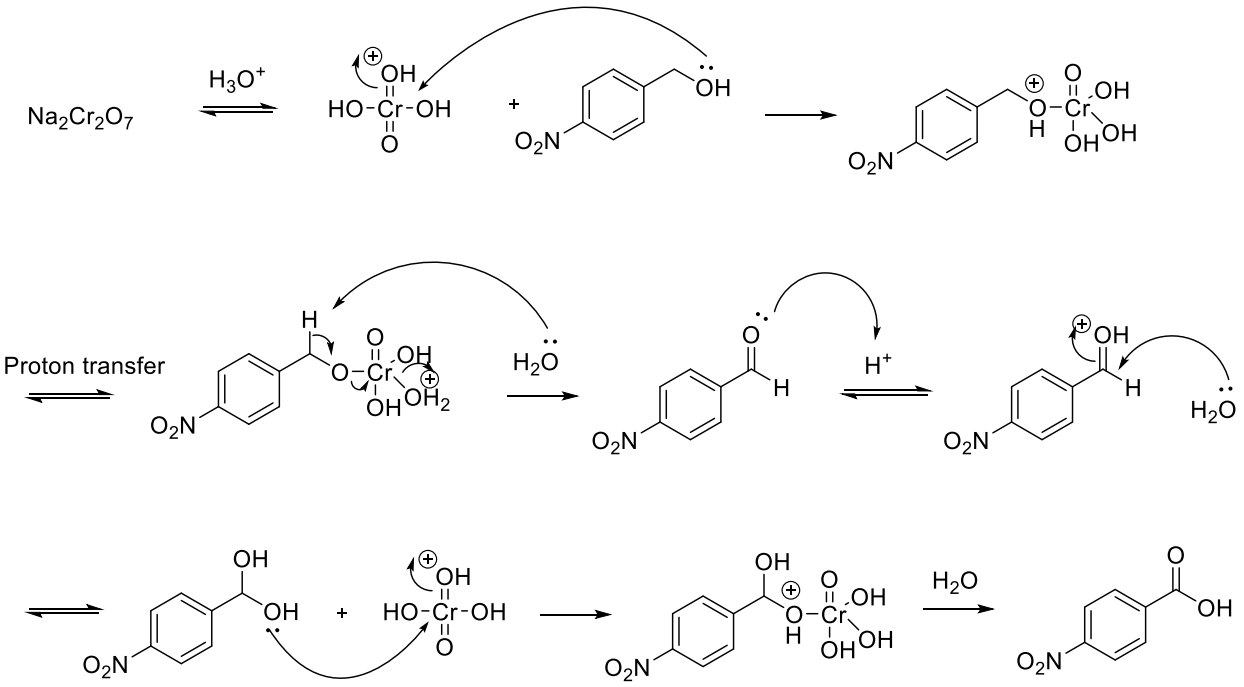


Table of Reagents (3 points)

Include a table with the name, molar mass, quantity, density (if compound is a liquid), moles, and equivalents of all reagents use. Include the volume of solvent used in the experiment (you do not need to include any other data with respect to solvents). Remember to use appropriate significant digits!

3 points	- All reagents are included with all relevant data properly calculated
2 points	- All compounds are present in the table - 1 – 2 small errors
1 points	- Some compounds are missing - 3 – 4 small errors or 1 large error/omission
0 points	- Table is missing - 5 or more errors

Reagent	Mol. Mass (g/mol)	Quantity (g or mL)	Density (g/mL)	Mmol	Equivalents
p-nitrobenzyl alcohol	153.14	0.5023 g	N/A	3.28	1.00
1.625 M H_2CrO_4 solution		6.0 mL	1.0	9.75	3.0
Diethyl ether (solvent)		15 mL	0.71	N/A	N/A

Note: Because the concentration of the H_2CrO_4 solution is given, you can omit the molar mass and density since the number of moles comes from the equation c = n/v or n = c·v = (6.0 mL)(1.625 mol/L) = 9.75 mmol.

Experimental Procedure (1 point)

In this section, you can mark "refer to CHM2123 lab manual" with appropriate page numbers, but be sure to report any experimental modifications made.

Modifications

- Prior to adding the chromate solution in step 2, the flask was cooled to 0 °C in an ice bath.

Observations and Results (4 points)

Use this section to present your results in a clear and concise manner. Organize your data into one (or more) tables of observations and results. Include the product name, mass, yield, and melting point (reported as a range). Sample calculations are also to be included in this section.

4 points	- All observations are clearly presented in a table - Results are clearly presented in table with proper calculations
3 points	- Most observations are presents in a table - Most results are presented clearly in a table - 1 – 2 small errors in calculations
2 points	- Some observations are present in a table - Results are not clearly presented or are not in a table - 3 – 4 small errors or 1 large error/omission
1 points	- Observations are minimal - Results are unclear - 5 or more errors
0 points	- Observation and Results are missing

Observations

Key Step	Observation
Addition of chromate solution	Solution becomes orange/brown Two distinct phases are visible
Wash ether layer with NaOH solution	Aqueous phase becomes green
Acidify aqueous phase with conc. HCl	White precipitate forms (product)

Table of results

Compound	Mol. Mass (g/mol)	Quantity (g or mL)	Mmol	Yield (%)	Melting point (°C)
p-nitrobenzoic acid (white precipitate)	167.12	0.3411 g	2.041	62.22 %	235 – 240 (lit. 237 – 240)

Calculation (2 points each)

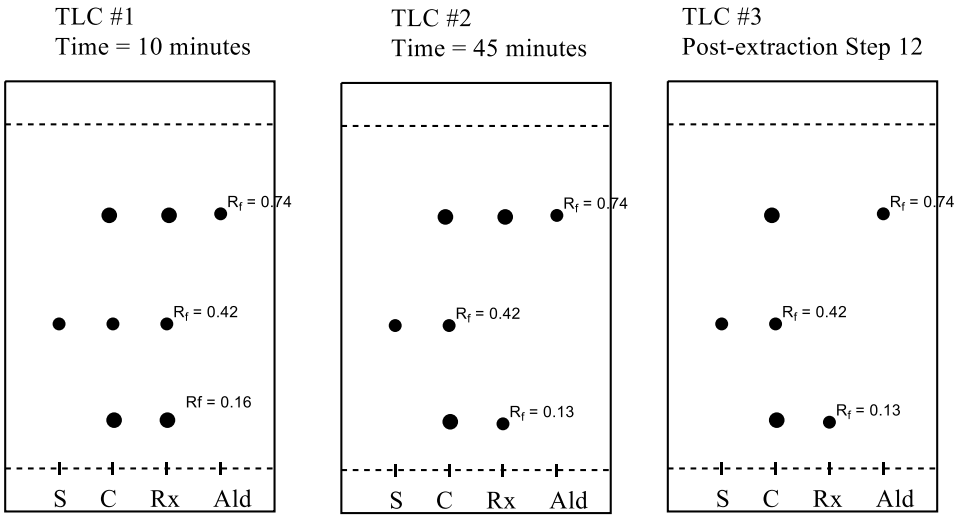
Show the calculations that are required.

$$\%\ yield = \frac{mol\ product}{mol\ starting\ material} \times 100\ \% = \frac{2.041\ mmol}{3.280\ mmol} \times 100\ \% = 62.22\ \%$$

TLC plates (4 points)

4 points	- Sketches of all TLC plates are included - All RF values are calculated and shown on each plate - Each lane is properly labeled on each plate - Solvent system is included for each plate
3 points	- All TLC plates are included - 1 – 2 pieces of data are missing from any of all RF values, all lanes labeled, and all solvent systems are included
2 points	- 1 TLC plate is missing **OR** - 3 – 4 pieces of data are missing
1 points	- More than 1 TLC plate is missing **OR** - 5 or more pieces of data are missing
0 points	- TLC plates are not included

Include sketches of all of your TLC plates. Be sure to include the solvent system used for eluting and labeling each lane and plate. Mark down the RF of the relevant spots.



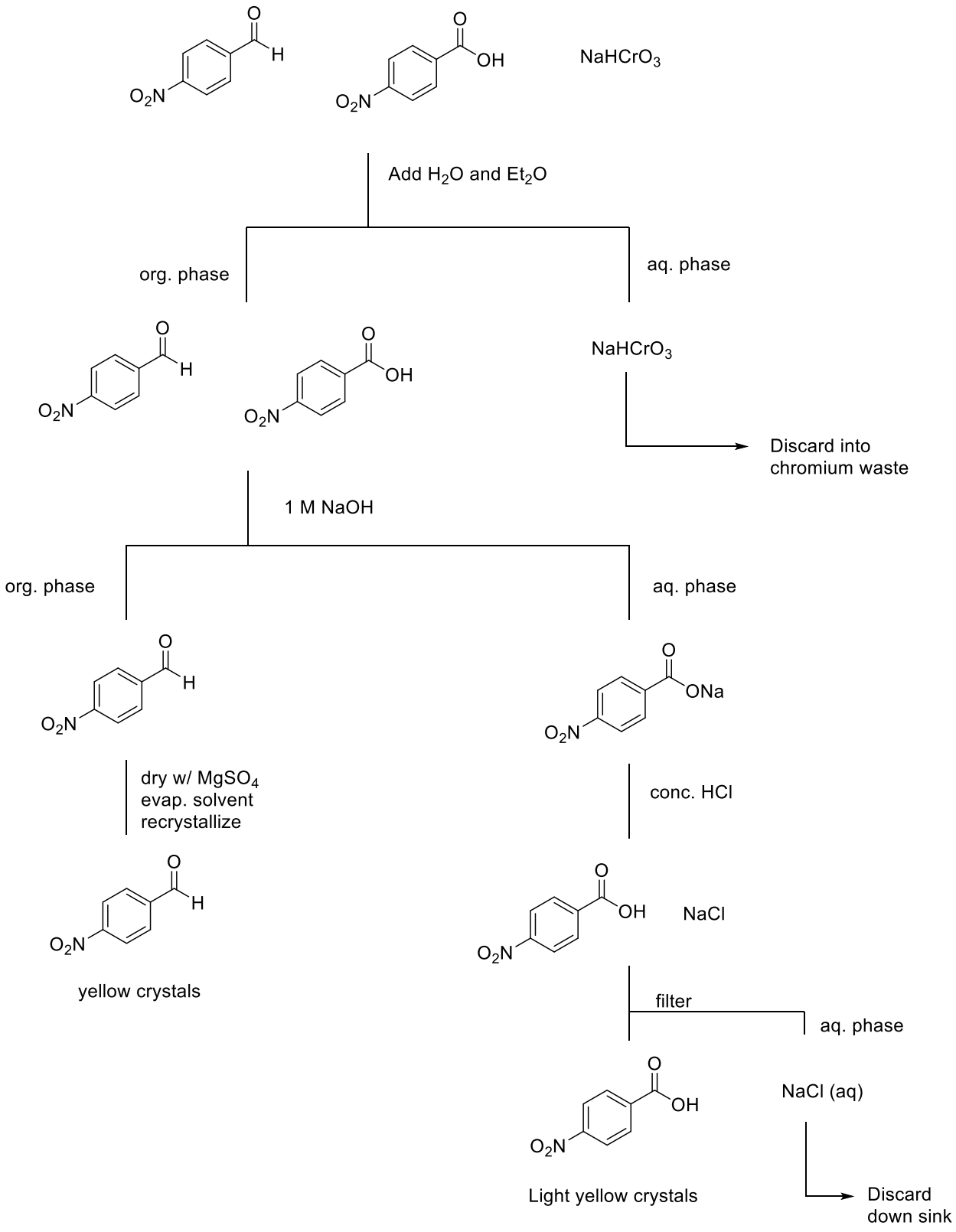
Solvent system is 1:1 hexanes:ethyl acetate for all plates

TLCs taken of the reaction. S = *p*-nitrobenzyl alcohol starting material, C = co-spot, Rx = reaction mixture, Ald = *p*-nitrobenzaldehyde reference.

Flow Chart (10 points)

Include a flow chart any time an extraction has been performed. Always remember to include every step, including drying with $MgSO_4$ (or Na_2SO_4), filtration, and recrystallization. Use structures as much as possible in your flow chart. Make sure your flow chart is specific with concentrations (i.e. 1 M NaOH or 10% HCl).

10 points	- Each step of the flow chart is correct with properly labeled arrows - Chemical compounds end up in the proper layers, with products and side-products from work-up steps designated in the appropriate steps - Molecules are drawn in the correct form (ie. a carboxylic acid washed with NaOH and H_2O is presented as $RCOO^-Na^+$. - All steps (drying, filtering, recrystallization, etc) are included.
8 – 9 points	- Flow chart is easy to follow - 1 – 2 mistakes or omissions
6 – 7 points	- Flow chart is incomplete - 3 – 4 mistakes or omissions
4 – 5 points	- Flow chart is incomplete and unclear or confusing - 5 – 6 mistakes or omissions
2 – 3 points	- Flow chart is sparse or very difficult to follow - Multiple mistakes and omissions
0 – 1 point	- Flow chart shows no coherent movement of molecules, is difficult to follow (1) - Flow chart is missing (0)


NaHCrO3
Add H2O and Et2O
org. phase
aq. phase
NaHCrO3
Discard into chromium waste
1 M NaOH
org. phase
aq. phase
dry w/ MgSO4
evap. solvent
recrystallize
yellow crystals
conc. HCl
NaCl
filter
aq. phase
NaCl (aq)
Discard down sink
Light yellow crystals

Discussion (10 – 20 points)

The discussion is where the bulk of the points for the lab report will be awarded. Be sure to explain your results, answer any questions in the procedure, and always answer the question "why?" Why was a reagent added? Why was the setup done as described? Why did the reaction work or not work the way you anticipated? The points assigned to the discussion will vary for each experiment.

> 80 %	- The discussion properly outlines why each step of the procedure was done - The discussion explains the role of each reagent used in the experiment - The discussion shows analysis of the observations and experimental results - The discussion connects the results and observations back to the theory relevant to the experiment - Proper discussion of TLC results, melting point, and other characterization techniques
70 % - 79 %	- Most of the above requirements are met - 1 – 2 errors or omissions
60 – 69 %	- Some of the above requirements are met - 3 – 5 errors or omissions
50 – 59 %	- Many of the above requirements are missing - 6 – 7 errors or omissions
20 – 49 %	- Multiple mistakes are present - Connections to theory are missing - Many errors and omission
< 20 %	- Discussion is missing (0) - Discussion is very sparse with little to no connections to theory or analysis present

The goal of this experiment was the oxidize *p*-nitrobenzyl alcohol to *p*-nitrobenzoic acid using the Jones reagent. The alcohol was dissolved in ether and a chromate solution was added drop wise to prevent the reaction from over-heating and exploding. The mixture was biphasic since the aqueous chromate solution is not miscible with ether, so vigorous stirring was required. After 10 minutes, we took our first TLC (TLC #1) and we observed that some starting material was still present in the reaction (spot at Rf = 0.42), as well as the appearance of two new spots at Rf = 0.74 and Rf = 0.16. Since the spot at Rf = 0.74 corresponded to the aldehyde sample provided by our TA, we could infer that some aldehyde had formed in our reaction mixture. After 40 minutes, we took another TLC (TLC #2) and observed that there was no spot at Rf = 0.42 in the reaction mixture lane, meaning that all the alcohol had been consumed. There was still some aldehyde present in the reaction mixture, as shown by the consistent appearance of the spot at Rf = 0.74. This means that not all of the alcohol had been converted to the corresponding acid.

The reaction mixture was poured into a separatory flask along with 5 mL of ether and 5 mL of water. The organic products (the aldehyde and the acid) will end up in the ether layer and the chromate salts and acid will end up in the aqueous layer, which was discarded in the chromium waste receptacle for environment reasons. The organic layer was washed three times with a 1 M NaOH solution to deprotonate the acid, forcing it into the aqueous layer and allowing us to separate it from the aldehyde.

We evaporated the solvent from the organic phase to obtain crude aldehyde, which we then recrystallized from ethanol to obtain the pure aldehyde product. To the aqueous phase, we added concentrated HCl to protonate the benzoate ion, making it insoluble in water (since most organic compounds are insoluble in water) causing it to precipitate out of solution. Cooling in an ice bath further decreased the solubility of the benzoic acid (since solubility is a function of temperature), allowing us to increase our yield. The precipitate was filtered and dried on a Buchner funnel to yield 0.3411 g of pale-yellow crystals. Our observed melting point was 235 – 240 °C suggesting that there may be some impurity in the sample. We took a last TLC to further examine the purity of our samples (TLC #3) and in the reaction lane, we did not observe any organic impurities since there was only one spot present in that lane. The larger melting point range could come as a result of a small amount of water still present in our reaction mixture; however, we were close enough to the range that we can assume our product was pure.

EXPERIMENT 1

SEPARATION AND PURIFICATION OF NATURAL PRODUCTS

SUGGESTED READING: The sections covering **POLARITY, PHYSICAL PROPERTIES OF MOLECULES, ACID-BASE REACTIONS,** of the Organic Chemistry textbook of your choice.
Recommended reading: Zubrick, *The Organic Chem Lab Survival Manual* 1st Ed., pp. 71-78, 91-107, 111-127, 197-208. *(.pdf on Blackboard Learn*)

Appendix I ……	Melting point apparatus
Appendix III ……	Extraction
Appendix IV ……	Chromatography

PURPOSE

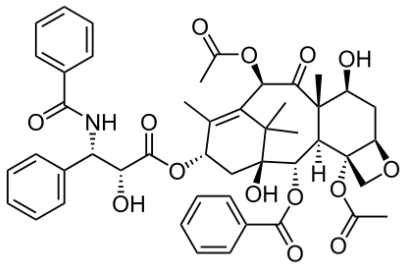
This experiment will provide a review of the techniques necessary to isolate and purify a product once a chemical reaction has finished. Three important techniques will be reviewed: **extraction, thin layer chromatography [TLC]**, and **sublimation**. Extraction and thin layer chromatography will be explored in the separation of pigments from spinach leaves, while extraction, TLC, and purification *via* sublimation will all be explored to isolate caffeine from tea leaves. These techniques are integral in any organic chemistry lab and will be used often in the rest of this course and in further organic chemistry lab courses.

INTRODUCTION

The purification of chemical compounds is very important in order to properly characterize the compound and use it for further reactions and avoid contaminants. When extracting interesting natural products from plant and animal sources, chemists go through a painstaking process to ensure that the product obtained is pure. These natural products often provide starting points for the synthesis of new medicinal compounds, such as Taxol shown below in Figure 1.1. There are many different techniques used to purify organic compounds and, in this experiment, we will review two techniques taught in CHM1321: extraction and thin layer chromatography (TLC), and use a cold finger (a bench-top sublimation technique).

Most purification techniques take advantage of the difference in physical properties between compounds. Extraction uses **two immiscible solvents** (usually water and an organic solvent, such as ethyl acetate or dichloromethane) to separate organic compounds. Compounds with high water solubility (most ionic compounds and very polar organic compounds) will end up in the aqueous phase, while the compounds with low water solubility (most organic compounds) remain in the organic phase. These two layers can then be separated and the products isolated.

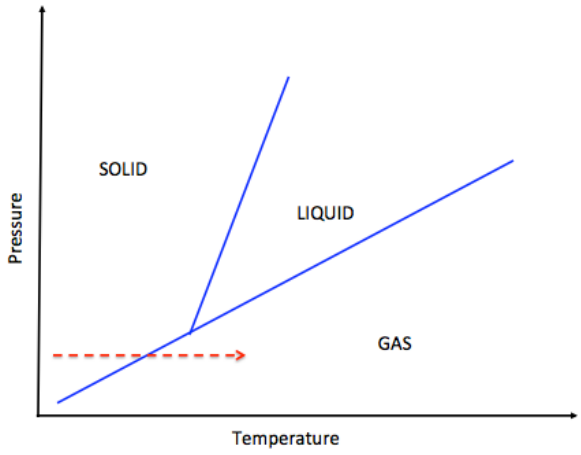
Figure 1.1: Taxol is a complex natural product and anti-cancer drug isolated from the bark of the Pacific yew tree.



Further, it is possible to separate compounds by taking advantage of their reactivity to acids and bases. Some compounds can react with an acid or a base to become ionic salts, making them soluble in the aqueous phase. Once isolated, the ionic salt is returned to the neutral state upon reaction with an acid or a base, rendering the molecule insoluble in water and easily isolated. Further details on extractions and reactive separations are available in Appendix III.

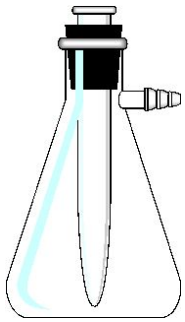
Once a target compound has been isolated, it can be purified by sublimation. Sublimation is the physical process in which a material transitions from the solid to the vapour phase without melting or going through a liquid state. If a compound possesses a high melting point as well as an appreciable vapour pressure under standard conditions (1 atm, 25˚C), it can be isolated from other non-volatile substances by condensation. Dry ice is a common example of material that sublimes in everyday life, where this solid form of carbon dioxide evaporates as it warms to room temperature without getting wet. Sublimation is used to freeze-dry coffee and food for camping and space travel. The phase diagram for caffeine is presented in Figure 1.2, where the dashed arrow indicates the phase transition at standard pressure.

Figure 1.2: Phase diagram of caffeine



Sublimation in the laboratory can be accomplished using an apparatus called a 'cold finger'. A cold finger, or test tube typically filled with ice, sits in a side-arm flask containing a loose, crude solid. As the flask is heated, molecules vapourize and condense as fine crystals on the surface of the cold finger, optimally positioned about 1 cm above the base of the flask. Applying reduced pressure can speed up the process.

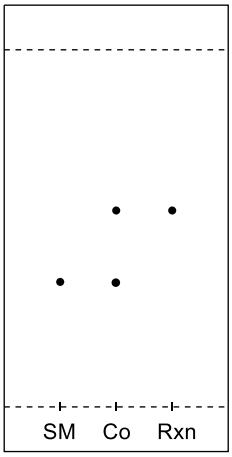
Figure 1.3 Cold finger apparatus used for sublimation.



Once the target compound has been isolated, thin layer chromatography, or TLC, can be performed to observe if any impurities are present in a product. Chromatography is used to separate a mixture according to the differences in distribution of its constituents between two phases. A mixture is dissolved in a liquid, or mobile phase, while the solution is passed over a stationary phase. The different components of a mixture will have a different affinity for the mobile or stationary phase, thus allowing the various compounds to be separated from one another. This technique takes advantage of the difference in polarity of molecules. TLC employs a plate covered with a very polar silica gel ($SiO_2 \cdot xH_2O$) as a *stationary phase*. The compounds in question are spotted onto a plate (~ 1 cm from the base) by dissolving the mixture in a solvent, typically 1 – 2 mg/mL, and using capillary tubes to fix the mixture onto the stationary phase. The plate is then placed in a developing jar containing a small volume (~ 10 mL) of developing solvent. This developing solvent acts as the mobile phase, moving up the plate *via* capillary action and pulling the analytes along with it. The distance a compound moves along the plate is dependent on its polarity. Molecules with polar groups can bind to the silica gel in two ways; through hydrogen bonding and through dipole-dipole interactions. The strength of the interaction of the compound with the solid phase is the sum of these two interactions. The rate of migration depends on the interaction of each compound with the two phases. Generally, more polar compounds interact more strongly with the polar silica gel and therefore migrate more slowly than less polar compounds. Less polar compounds generally migrate faster than more polar compounds.

Thin layer chromatography can also be used to monitor the progress of a reaction. A TLC plate is created with the starting material spotted in one lane and the reaction mixture spotted in another lane. A third lane is set up by spotting both the starting material and the reaction mixture together on the plate. Once the plate has been developed, the disappearance of the starting material over time can be observed, as shown in Figure 1.4. A more complete description of TLC, as well as how to report separations by calculation of a retention factor or R_f value, is available in Appendix IV.

Figure 1.4: A sample TLC plate showing a complete consumption of the starting materials (note the disappearance of the spot corresponding to the SM in the Rxn lane).



SM = Starting Materials

Co = co-spot of SM and Rxn

Rxn = Reaction mixture

In summary:

(1) The silica gel, the stationary phase, is polar: more polar compounds interact more strongly with this phase than less polar compounds.

(2) Increasing the polarity of the mobile phase (solvent) causes ALL compounds to migrate further on the plate.

(3) The compounds, once separated, can be visualized on the TLC plate using ultra-violet light or a staining method.

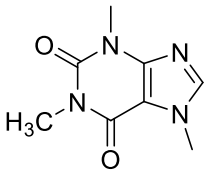
Observing the melting point of a solid is a quick and simple way to verify the purity of a product. When a chemical product is pure, the melting point range is very small, often less than 2 °C. Any amount of impurity that is present in the molecule, no matter how small, will lower its melting point by a few degrees and broaden the range. The impurity causes defects in the crystalline lattice of the molecule, making it easier to overcome the intermolecular interactions that hold the molecule as a solid.

PART A: EXTRACTION AND RECRYSTALLIZATION OF CAFFEINE FROM BLACK TEA

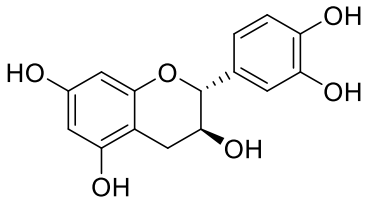
Tea is a popular beverage second only to water in worldwide consumption. Tea leaves are native to the southern regions of China, where it was discovered as a drink and medicine around 2737 BC. Tea leaves are composed of cellulose, chlorophyll, polyphenols, tannins, and caffeine. The medicinal properties of tea come from caffeine, as well as the antioxidant activity of polyphenols such as catechin and theaflavin. Black tea results from the oxidation of polyphenols during the fermentation process, while green tea is non-fermented. As a result, green tea possesses a greater concentration of

polyphenols over that in black tea. When tea is brewed, the polyphenols and caffeine are dissolved in the hot water, while the cellulose and other plant materials remain insoluble. Caffeine can be extracted from tea by using an organic solvent like dichloromethane. Caffeine is soluble in dichloromethane whereas the weakly acidic polyphenols are not. The industrial decaffeination of tea and coffee is done using either dichloromethane or chloroform as an extracting solvent.

Figure 1.5: The structure of caffeine and catechin (a polyphenol) found in tea leaves.



Caffeine
MW = 194.19 g/mol
m.p. 238 ^{o}C



Catechin
MW = 290.27 g/mol
m.p. 175 - 177 ^{o}C

Caffeine is a highly addictive alkaloid that is a stimulant for the central nervous system. It is found in many other plants, such as coffee beans and cocoa, and added to many drinks as a stimulant. Health Canada recommends no more than 400 mg/day for the average adult and 300 mg/day for young women of childbearing years. The caffeine content in various beverages is provided in table 1 below. Note that some coffee shops sell containers of up to 961 mL!

Table 1: Caffeine content in various beverages

Beverage	mg/250 mL
Coffee	80 – 125
Decaf coffee	2 – 6
Black tea	30 – 75
Green tea	20 – 50
Coca-Cola	32
Red Bull	80

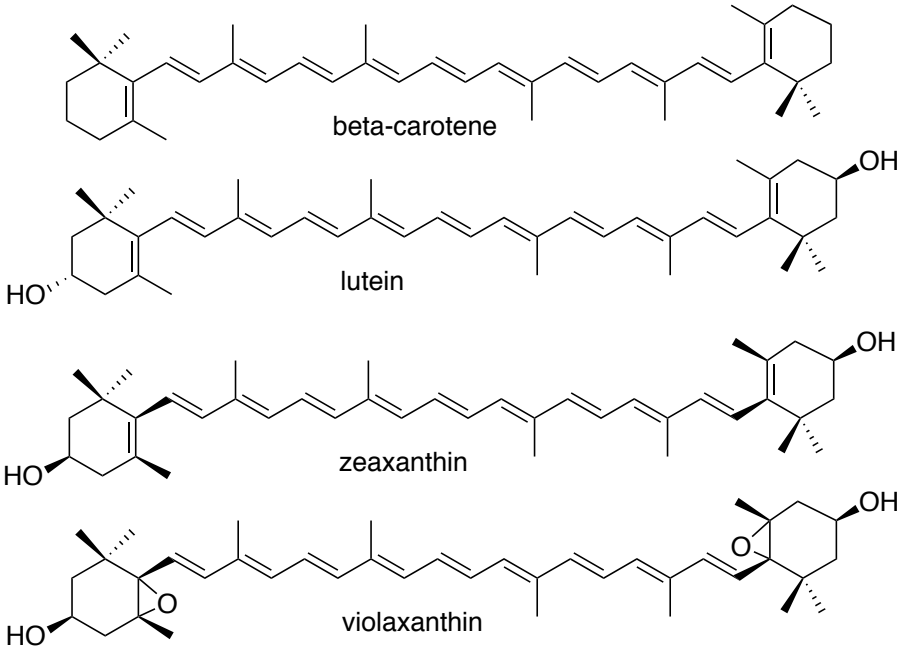
Caffeine can be extracted from tea using hot water; however various tannins will also dissolve into the aqueous solution. Alternatively, an organic solvent like dichloromethane can dissolve caffeine while the weakly acidic polyphenols remain in the aqueous phase. Industrial decaffeination of tea leaves and coffee beans undergo a similar process typically using chloroform as an extracting solvent.

In this experiment, caffeine will be extracted from a strong cup of tea using dichloromethane. The caffeine will subsequently be purified by sublimation, and its purity verified by TLC and melting point.

PART B – EXTRACTION OF CAROTENOIDS AND CHLOROPHYLLS FROM SPINACH

Plant leaves are composed of cellulose and pigments, where a number of pigments can generally be classified into carotenoids and chlorophylls. Carotenoids belong to a family of terpenes composed of repeating isoprene, or 2-methyl-1,3-butadiene units. Lycopene, found in tomatoes and watermelon, is the carotenoid responsible for the red colour. β-carotene is the pigment that provides the yellow-orange colour in carrots and apricots. Other common carotenoids include lutein, zeaxanthin, and violaxanthin; xanthophylls responsible for the yellow pigmentation in egg yolks and in the leaves of most green plants. The chemical structures of a few carotenoids are illustrated in figure 1.6.

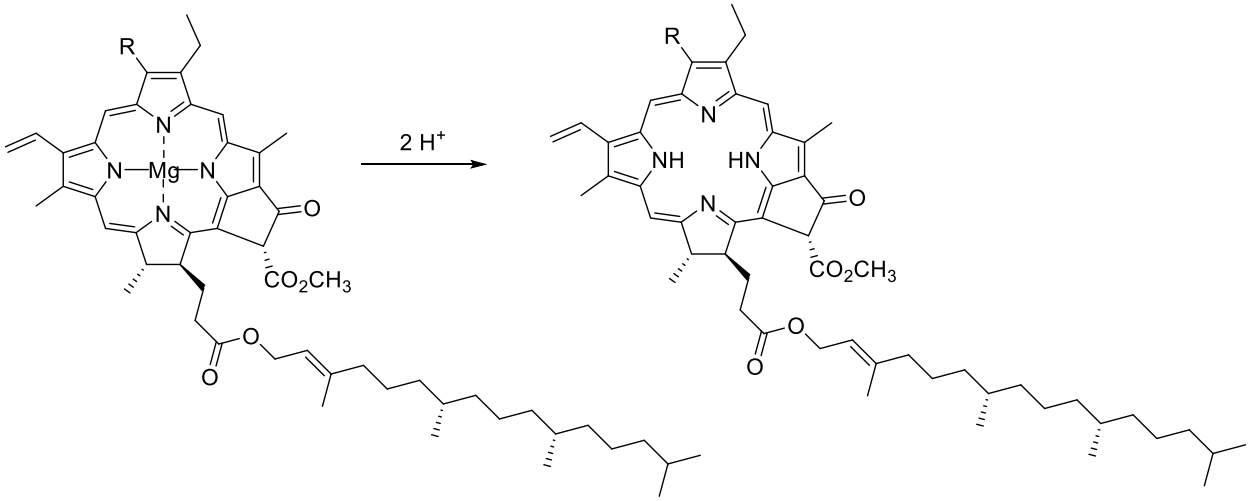
Figure 1.6 - Structures of various carotenoids found in spinach leaves



Chlorophylls such as chlorophyll *a* (blue-green) and chlorophyll *b* (green) are the compounds responsible for the green colour in plant material. These highly conjugated molecules play an active role in photosynthesis by capturing light *via* its porphyrin ring. Solar energy is trapped by chlorophylls and converted to chemical energy, namely electrons shuttled to other parts of the plant to be stored as sugars and starches. Chlorophylls, as presented in Figure 1.7, are sensitive to strong acids, which displace the magnesium of the porphyrin ring to form pheophytins. Pheophytins are chlorophylls

responsible for the olive-coloured pigments present in vegetables once they begin to rot, or have been cooked for too long.

Figure 1.7: Conversion of chlorophylls to pheophytins



Chlorophyll

R = CH_3 Chlorophyll a
R = CHO Chlorophyll b

Pheophytin

R = CH_3 Pheophytin a
R = CHO Pheophytin b

The carotenoids and chlorophylls will be isolated from fresh spinach leaves by liquid-liquid mini-extraction, and the chromophores will be subsequently separated and identified using thin layer chromatography by exploiting the differences in polarity of the described molecules. A number of pigments of various colours may be isolated. Identify each of the pigments on your TLC plate.

MANIPULATION

All transfers of liquids and solids should be as quantitative as possible. For example, in the transfer of the original solution to the separatory funnel some of it invariably remains in the small Erlenmeyer flask. To recover most of this, rinse the flask with a small amount of solvent and add this extra volume to the separatory funnel.

Use the right size of equipment! In this laboratory course, this aspect is not always possible because you do not have all sizes of each type of equipment. Nevertheless, do not attempt to re-crystallize a sample from 10–20 mL of solvent using a 500 mL flask; a 50 mL or 25 mL flask would be much more appropriate. An Erlenmeyer flask is the appropriate vessel for re-crystallization (not a beaker).

SAFETY

- Evaporation of all organic solvents **must be done in the fume hood**.

- Read the instructions in the appendix on the proper use of the extraction funnel before starting the experiment. Do not hesitate to ask your TA for help.

- Before the experiment, wash your glassware with water and then acetone in order to avoid contamination.

*** You should always wash your hands before leaving the lab ***

Work carefully!

It is recommended that each partner take the lead on one part of the experiment, communicating and sharing all observations and results ***before leaving the lab****. You are each responsible for submitting a report describing both parts A and B of this experiment.*

EXPERIMENTAL PROCEDURE

Part A: Extraction and recrystallization of caffeine from black tea

1. Pour 60 mL of distilled water into a beaker and place it on a hot plate. Place a watch glass over the beaker and bring the water to a boil.
2. Obtain two (2) tea bags and record their mass together, assuming that the average mass of an empty tea bag is 0.120 g. DO NOT REMOVE THE TEA FROM THE BAGS.
3. Place the tea bags into the boiling water and turn off the heat on the hot plate. Stir the bags occasionally for the next 10 minutes as the hot plate cools to room temperature.
4. Remove the beaker from the hotplate, and discard the tea bags. While the tea is cooling, add 2.0 g Na_2CO_3 to the steeped tea. Stir with a Teflon-coated stirring rod until the solid has dissolved.
5. Transfer the solution into a 125 mL separatory funnel supported on a wooden support rack, and add 10 mL of dichloromethane to the separatory funnel, noting which layer is the aqueous and which is the organic. Never fill an extraction funnel more than ¾ full!
6. Rinse the beaker with an additional 5 mL of dichloromethane to maximize transfer and add this to the separatory funnel.
7. Shake the funnel ***gently*** and vent it frequently. Return it to the support rack and allow the layers to separate. One brown, turbid phase may form. This is an undesired emulsion. If an emulsion is present, add 10 mL dichloromethane and continue the extraction.
8. If you still have an emulsion, pour the organic phases back into the clean separatory funnel and extract once more with 15 mL saturated NaCl solution (brine).
9. Draw off the organic layer into a 125 mL Erlenmeyer flask. With the aqueous layer still in the separatory funnel, repeat the extraction with 2x10 mL dichloromethane. Combine the extracted organic phases in an Erlenmeyer.
10. Carefully remove the aqueous phase into a beaker and leave to the side (do not discard any phases, washings until you are sure of what you need and what you do not), and clean the separatory funnel. Due to the likelihood of solid tea residues present in the mixture, it is

recommended to pour the aqueous phase out by the top (wide) opening of the separatory funnel.

11. Add a scoop of Na_2SO_4 to the organic phase. The Na_2SO_4 is a drying agent and will remove any trace amounts of water present in the organic phase. You will know that your drying agent has worked by the presence of clumps of hydrated salt in the beaker.
12. Gravity filter the organic solution into the beaker using filter paper and a funnel. Which funnel should you use?
13. **Set 1 – 2 mL of solution aside for TLC analysis.**
14. Using a steam bath in the fumehood, gently boil off the solvent until a few drops of liquid remain in the beaker. A solid will form (the crude product) once the droplets of solvent evaporate upon cooling.
15. Scrape out the crude caffeine product and obtain the mass.

Sublimation

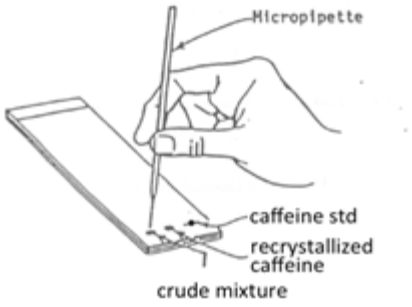
16. Heat a hotplate on medium (level 4 - 5).
17. Place the crude caffeine into a 100 mL side-arm flask.
18. Ensure that the cold finger (test tube + stopper) is clean and dry on the outside.
19. Place the cold finger (test tube + rubber stopper) into the mouth of the side-arm flask, ensuring that there is a tight seal and ~ 1 cm between the flask bottom and the cold finger.
20. Connect a rubber hose from the vacuum port on the water line. Gently turn on the water and feel the suction created at the other end of the rubber tube. If the suction is too strong, you could lose your crude product! Connect this hose to the side arm of the Erlenmeyer flask.
21. Place the side-arm flask on the hot plate and secure it with a clamp. *(Be sure to perform steps **21 – 22 quickly** so as to prevent the crude product from burning on the hot plate)*
22. Fill the cold finger with dry ice. You will likely need to refill the cold finger trap to maintain the condensation.
23. Allow the caffeine to sublime onto the cold finger until there is negligible product remaining in the base of the flask.
24. When sublimation is complete, break the vacuum by gently and carefully removing the hose from the side-arm, then turn off the vacuum by turning off the water tap.
25. Scrape the purified caffeine crystals onto a pre-weighed watch glass, and determine their mass.
26. Determine the melting point of the purified crystals. Consult Appendix I for instructions on using a melting point apparatus.

In your report, indicate the mass of the isolated product and determine the percent yield of caffeine. You can assume that the tea contained 4% caffeine by weight as a theoretical value.

Thin Layer Chromatography

Take the time to read the appendix on chromatography before starting this part. Before spotting on TLC plates, practice your technique using a piece of brown paper towel. Your TA will demonstrate proper technique.

27. Obtain a TLC plate and lightly pencil a line ~1 cm from the bottom of the plate.
28. Dissolve a few crystals of sublimed caffeine in about ~2 mL of dichloromethane in a test tube.
29. Repeat the above step with the crude product and the authentic caffeine sample in 2 separate test tubes.
30. Spot a small amount of crude caffeine in the left most lane, a small amount of sublimed caffeine in the centre lane, and a small amount of authentic caffeine in the right most lane of the TLC plate (see diagram). Label each lane appropriately. Check if good spotting occurred with a UV lamp before putting the plate in the developing chamber. **NEVER LOOK DIRECTLY AT A UV LAMP.**



31. Pour 10 mL of 99:1 acetone:acetic acid mixture in a developing jar to use as the developing solvent system. Make sure that the solvent level is below the starting line of your plate.
32. Carefully place the TLC plate into the jar, avoiding any splashing, and ensuring that the plate is as upright as possible. Cap the jar.
33. Allow the solvent to develop to about 1 cm from the top of the plate and mark the finish line with a pencil.
34. Allow the solvent to evaporate off the TLC plate and then visualize the developed plate with a UV lamp. Using a pencil, mark the spots on the plate.
35. If your spots are streaky or blotchy, then you over-spotted your TLC plate. If you have time, repeat this section spotting *less* on your next TLC plate. If your spots are faint, repeat the TLC by precisely double spotting for each necessary spot.
36. Record the results in your lab book. Including a digital picture of your TLC in your report is recommended. Never include your TLC plates with your report.

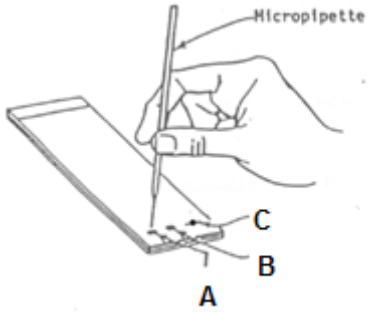
Part B: Extraction of Carotenoids and Chlorophylls from spinach

1. Obtain 1 – 2 spinach leaves and remove the large stems and veins. Tear the leaves into small pieces. Weigh out 1.0 g of the leaves and place them in a 15 mL centrifuge tube.
2. Add 1.0 g of anhydrous Na_2SO_4 and 1.0 g of sand to the tube. Carefully grind the leaves using a Teflon-coated stirring rod until you obtain a fine pulp.
3. Add 2.0 mL of acetone to the tube and then cap it and shake it. Allow the solids to settle for 5 minutes, then stir (or shake) and let settle for another 5 minutes. Note: A more concentrated spinach sample will result in better TLC results.

4. Using a pipette, carefully transfer the liquid into a clean test tube **labelled A**. To maximize the transfer of compounds, wash the centrifuge tube with an additional 2 mL acetone and pipette-transfer this liquid to the same test tube A. Finally, rinse out the centrifuge tube with soap and water and return it to your TA.
5. Transfer ~2 mL (half) of solution in test tube A to a new test tube **labelled B.** Add 3.0 mL of hexanes to test tube B, followed by 3.0 mL of water. Place a rubber stopper on test tube B and shake gently to perform a mini-extraction. Identify which layer is aqueous and which layer is organic. Take note of any changes you observe.
6. Pipette ~1 mL (half) of the organic phase of test tube B into a new, clean test tube, **labelled C**. Add 1.0 mL of 1.0 M HCl solution to test tube C. Stopper the test tube and shake gently, venting occasionally. Take note of any changes you observe.

Pigment separation by TLC

7. Take a picture of your TLC plate as soon as possible, for the pigments are sensitive to light and will degrade over time. Include this photo in your report. Obtain a TLC plate and, using a pencil, draw a line ~1 cm from the bottom of each plate. Obtain a capillary spotter from your TA and spot a small amount from test tube A in the left lane, B in the centre lane, and C in the right lane (see diagram) of each plate. Label each lane and each plate appropriately. Depending on the brightness (or lack thereof) your solutions, you may want to double-spot.



8. Obtain 10 mL of the mobile phase 50:25:25 cyclohexane:hexane:acetone and transfer it to a clean developing jar. Carefully place the TLC plate in the jar and seal the jar. Develop the plate until the solvent is ~1 cm from the top of the plate. If the solvent reaches the top of the plate, you must redo your TLC.

Using tweezers, remove the plate from the developing jar and trace a line where the solvent stops. This is the solvent front. Mark down all observations. Note the Rf values and colour of each spot. Label your initials on your TLC plate.

9.

The following table may help you organize your results. You may need more rows than are present. It is recommended to organize data based on similar colours and R_f values.

Rf value	TLC colour/description		
	A	**B**	**C**

Identify the spots on the TLC plate with the natural products found in spinach leaves (B-carotene, lutein, violaxanthin, chlorophyll a and b, pheophytin a and b). Justify your assignment of each spot using the principles of chromatography. You are encouraged to speculate on the assignment of additional spots. Your TLC should show between 4 – 7 different pigments in each lane, and may show more than those listed in the table above.

REPORT (38 POINTS)

Your report must include the following sections. See also the "Report Outline" section in the Laboratory Manual for guidelines on how to properly complete your report (an electronic copy is also available on Blackboard). Make sure to clearly organize the report between Part A and Part B. The report must NOT exceed 6 pages in length (not including the answers to the assigned questions).

- Title page (1 point)
- Introduction (6 points)
- Experimental procedure (1 point)
- Observations and Results (4 points)
- TLC plates (4 points)
- Flow chart (part A only) (10 points)
- Discussion (12 points)

EXPERIMENT 2

THE KINETIC STUDY OF NUCLEOPHILIC SUBSTITUTION

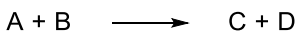
SUGGESTED READING: The sections covering NUCLEOPHILIC SUBSTITUTION, KINETICS OF SUBSTITUTION REACTIONS, in the Organic Chemistry textbook of your choice.

INTRODUCTION

In order to truly understand the mechanism for a chemical reaction, the behaviour of the molecules through the transition from reactants to products must be studied. Determining how favourably a reaction goes is accomplished by studying the energetics of the reactants and products. It is important to know if the reaction can produce the desired products at a reasonable rate. This can be accomplished by investigating the kinetics of the reaction under different reaction conditions. While a reaction deemed too slow could be industrially useless, a reaction occurring too fast could be violent or explosive.

Studying the kinetics of a reaction provides us with evidence to postulate a mechanism. With this insight, the reaction conditions can be tuned to give optimal yield – or even inhibit a reaction completely. Without kinetics, chemistry remains a 'black box' where reactants go in and products come out. The rate of the reaction is measured by monitoring the disappearance of reactants or the formation of products over time. The rate of formation/degradation of an intermediate species can also be monitored if these species are stable on the timescale of detection.

The rate of a reaction is dependent on a variety of parameters, including the concentration of its reagent(s). The greater the number of particles in a medium, the greater the likelihood for collisions leading to bond breaking and bond formation. The rate of a reaction is expressed with a rate equation generalized as follows:



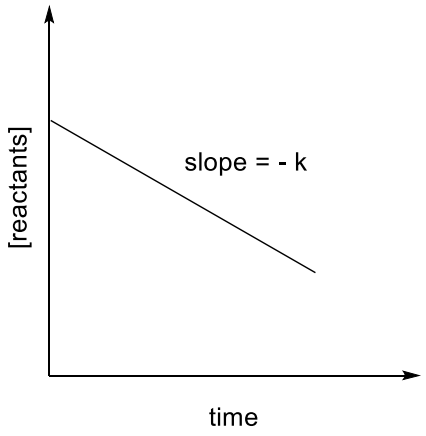
The rate of the reaction, *r*, is given by:

$$r = k[A]^x[B]^y$$

The rate equation is given as a function of the concentration of the reactants [A] and [B] multiplied by *k*, the rate constant of the reaction. This value, *k*, depends upon experimental conditions. The **order of the reaction** with respect to each reactant, A and B, is denoted by the exponents *x* and *y*. These orders are determined experimentally and the sum of these powers defines the overall order of the reaction.

Zero-order reactions are uncommon. Most often, a reaction is zero-order with respect to one of the reactants, and of higher order with respect to the other reactants. For example, if the formation of C and D is independent of the concentration of B, yet is dependent of the concentration of A, we can say the reaction is zero order with respect to B and first order with respect to A. To ascertain whether a reaction is zero-order with respect to one reactant, a graphical analysis of the concentration of the reactant or product in question with respect to time can be performed. A linear slope of the data reveals its zero-order dependence to the reactant, as shown in figure 2.1.

Figure 2.1: A plot of the concentration of reactants with respect to time of a zero order reaction.

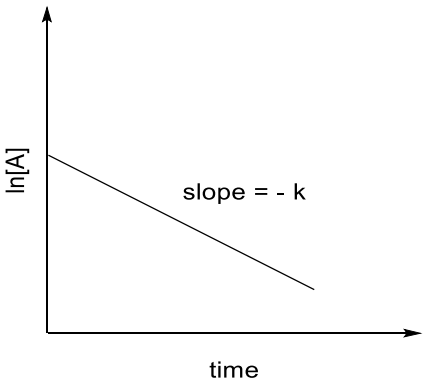


When a reaction is first-order overall the rate of the reaction can be obtained by solving the following first-order differential equation, and thus giving the first order rate law:

$$\frac{d[A]}{dt} = -k[A] \rightarrow \ln([A]_t) = -kt + \ln([A]_o)$$

where $[A]_0$ represents the initial concentration and $[A]_t$ represents the concentration of A at a particular time, *t*. If a reaction is first order with respect to reactant A, a plot of $\ln[A]_t$ vs. time will give a straight line with slope of *-k*, as shown below in Figure 2.2.

Figure 2.2: A plot of ln[A] vs. time of a first-order reaction.



A reaction is **second-order** in one of two cases: the formation of products depends on the concentration of *both* reactants (the reaction is first order with respect to [A] and [B]) or the formation of products depends on the square of one of the reactants (second order with respect to [A] or [B], but not both). The rate of the equation can be solved by solving this differential equation.

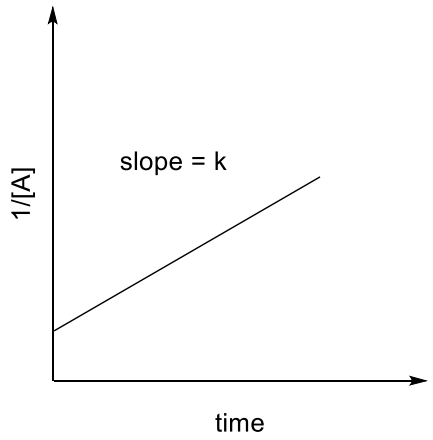
$$\frac{d[A]}{dt} = -k[A][B]$$

When a reaction is first order with respect to both [A] and [B], solving the differential equation can be difficult. By studying the reaction and equal concentrations of reagents, [A] = [B], this equation can be simplified and solved giving the second order rate law as follows:

$$\frac{d[A]}{dt} = -k[A]^2 \rightarrow \frac{1}{[A]_t} = kt + \frac{1}{[A]_o}$$

A plot of $\frac{1}{[A]_t}$ vs. time will produce a straight line with a slope of k in units of L mol^{-1} s^{-1} shown in figure 2.3. Note that, unlike the previous two examples, in this graph the slope is +k rather than –k for the disappearance of a reactant. The opposite is observed if monitoring the formation of products.

Figure 2.3: A plot of $\frac{1}{[A]_t}$ vs. time for a second order reaction in A.



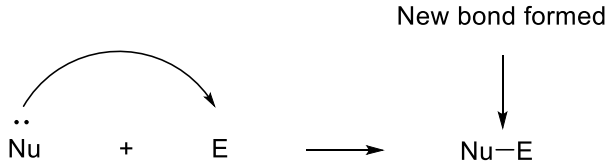
The various rate equations are summarized in the following table

	Zero Order	**First Order**	**Second Order**
Rate law	-k	-k[A]	$-k[A]^2$
Rate equation	$[A] = [A]_0 - kt$	$[A] = [A]_0 e^{-kt}$	$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$
Units of k	$M \cdot s^{-1}$	s^{-1}	$M^{-1} \cdot s^{-1}$
Linear plot to determine k	[A] vs. t	ln[A] vs. t	$\frac{1}{[A]}$ vs. t

NUCLEOPHILIC SUBSTITUTION

Many reactions in organic chemistry involve two key components – a *nucleophile* and an *electrophile*. In general, the nucleophile bears a lone pair of electrons which attacks an empty orbital of an electrophile to create a new bond. A generic nucleophilic attack is shown in scheme 2.4

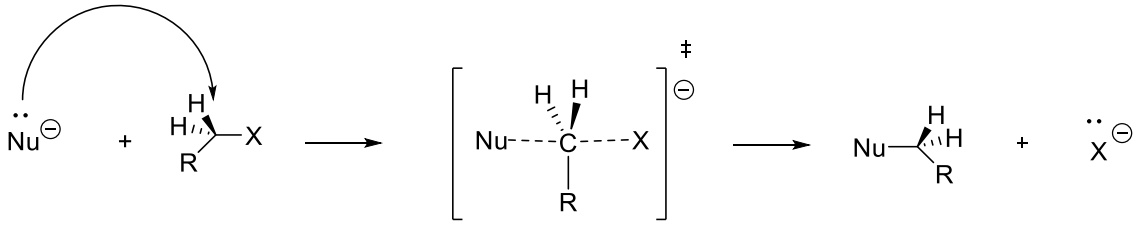
Scheme 2.4: Nucleophilic attack of an electrophile.



In a nucleophilic substitution reaction, the electrophile bears two more key groups: the *α-carbon,* which is the site of nucleophilic attack, and the *leaving group*, an atom or molecule that can stabilize the additional electrons from the reaction. In this experiment, we will examine two classes of nucleophilic substitution reactions, each of which proceeds through a different mechanism.

In a bimolecular substitution reaction, **S_N2**, the nucleophile attacks the electrophile before the departure of the leaving group. This reaction is concerted meaning that the attack of the nucleophile and the departure of the leaving group occur simultaneously, shown in scheme 2.5.

Scheme 2.5: The mechanism and transition state of a generic S_N2 reaction.

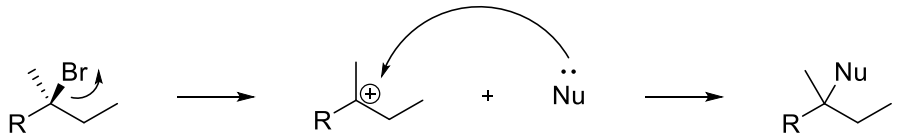


The nucleophile must approach the electrophile at an angle of attack of 180° with respect to the leaving group, resulting in an inversion of stereochemistry at the carbon centre. If the nucleophile were to approach from the same side as the leaving group, the transition state would be too crowded and no reaction would be possible. This backside approach is characteristic of S_N2 reactions and leads to stereospecific product, making the reaction synthetically useful for organic chemists.

The formation of products is dependent on both the nucleophile and the electrophile. **What order does that make this reaction? What is the rate equation of an S_N2 reaction?**

Unimolecular substitution reactions, **S_N1**, take place when the nucleophile attacks *after* the departure of the leaving group. This reaction proceeds through a carbocation intermediate and is not concerted. The mechanism of this two-step reaction is shown in scheme 2.6.

Scheme 2.6: The mechanism of a sample S_N1 reaction. Note the racemization of the products



This reaction is step-wise and will produce a racemic mixture of enantiomers, since the carbocation intermediate is planar. The nucleophile can attack the trigonal planar carbocation either from the top or the bottom of the intermediate, resulting in a 50:50 mixture of the two possible enantiomers. The formation of products in an S_N1 reaction is independent of the concentration of the nucleophile and only dependent on the concentration of the electrophile. **What order does that make this reaction? What is the rate equation of an S_N1 reaction?**

The mechanism of a substitution reaction is dependent on multiple factors inherent to the electrophile, nucleophile, and solvent of the reaction. The simplest way to predict the mechanism is to examine the α-carbon: the only substitution reaction possible on a primary α-carbon will be an S_N2 mechanism, since the carbocation intermediate is too unstable. Similarly, direct nucleophilic attack on a tertiary α-carbon is not possible, since the transition state is too crowded, making S_N1 the only possible mechanism. The reactivity of secondary α-carbons is influenced by many factors and nucleophilic attack may proceed by either mechanism.

Changes to the nucleophile can have drastic effects on the rate of the reaction of an S_N2 reaction. A molecule makes a better nucleophile when the nucleophilic atom has less hold on its electrons, either through increased atomic radius or decreased electronegativity. Modifications to the nucleophile have no effect on an S_N1 reaction because the rate of the reaction is zero order with respect to the nucleophile.

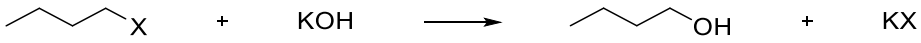
Good leaving groups increase the polarization of the carbon–leaving group bond, decreasing the activation energy, and thus increasing the overall rate of the reaction. Leaving groups which are large molecules, resonance stabilized, or possess strong electron-withdrawing groups are faster to depart, as they can more easily stabilize their negative charge.

THE EXPERIMENT

You will perform two substitution reactions and use quantitative measurements to examine the rate of the reaction under different conditions. You must determine the order of each reaction.

It is strongly recommended to divide the workload between each partner, with one partner completing Part A and the other partner completing Part B.

PART A: THE EFFECT OF LEAVING GROUP ON A SUBSTITUTION REACTION



The goal of this experiment is to examine the effect of the leaving group on the rate of a reaction. You will perform the hydrolysis of either 1-chlorobutane or 1-bromobutane with KOH. Kinetic analysis of the concentration of nucleophile consumed with respect to time will allow you to determine the overall order of the reaction. By titrating a small amount of the reaction mixture, you can determine how much KOH has been consumed, at each time point. **You will submit your [KOH] data to your TA at the end of the experiment.**

PART B: THE SOLVENT EFFECT ON A SUBSTITUTION REACTION

The goal of this experiment is to examine how a solvent will affect the rate of a reaction. You will examine the hydrolysis of 2-chloro-2-methylpropane (*tert*-butyl chloride, abbreviated as *t*BuCl). You will analyze the kinetics of the reaction by determining the amount of time required to consume a known amount of reactants under different solvent conditions. A by-product of the reaction is HCl, allowing you to monitor the progress of the reaction using an acid-base indicator. **You will submit your time data to your TA at the end of the experiment.**

At the end of the experiment and before leaving the laboratory, you will submit your data for part A and part B to your demonstrator. A graphical analysis is required to determine the overall order of the reaction. Your TA will tabulate the data from your lab section, and **post it on Brightspace within 24 h** of the experiment, allowing you to determine the rate constant of the reaction based on a group average of results with both electrophiles from part A, and a group average of the solvent effect from part B. You will compare your personal data to your group data, as well as to a set of rate constants representative of 'Global Data' for the week. **Based on these data, you will be able to determine whether these reactions follow an S_N1 or S_N2 reaction mechanism.**

EXPERIMENTAL PROCEDURE

<u>Part A:</u> Hydrolysis of 1-chlorobutane or 1-bromobutane.

Your demonstrator will assign you either 1-chlorobutane or 1-bromobutane. Please take note of which compound you are given.

1. Obtain a 25 mL burette and fill it with 0.100 M HCl in preparation for the titrations. Keep an additional 50 mL 0.100 M HCl in a beaker.

2. While working in the fume hood, clamp a 100 mL round bottom flask above a magnetic stirrer and a heating mantle. Make sure that you are using a stirrer and *not a hot plate*, as the hot plate will damage the heating mantle.
3. Using a graduated cylinder, measure out 40 mL of 0.5 M 1-bromobutane or 1-chlorobutane. Pour the solution into the round bottom flask in the fume hood and place a magnetic stir bar into the flask.
4. Attach a large reflux condenser to the round bottom flask. Attach the cooling tubes with water in at the bottom and out at the top (**why?**) and start the flow of cold water.
5. Once the solution has started to reflux, add 10 mL of 2.0 M KOH in EtOH through the top of the reflux condenser. Rinse the condenser with an additional 5 mL EtOH. **IT IS AT THIS POINT THAT YOU START YOUR STOP WATCH!**
6. After **two minutes**, carefully remove the heat source from underneath the flask and wait for bubbling to stop. **DO NOT TURN OFF THE HEAT SOURCE,** just remove the reflux condenser and pipette out 5.0 mL of solution into a 10 mL graduated cylinder. Take note of the exact volume of your aliquot.
7. Reset your condenser and continue the reflux of the reaction mixture.
8. Pour the aliquot into a 50 mL Erlenmeyer flask. Add 2 – 3 drops of phenolphthalein indicator to the solution
9. Begin titrating the aliquot with 0.100 M HCl from the burette, noting accurately how much HCl is required to reach the end point.
10. Repeat steps 6 – 10 after 15 minutes, 30 minutes, 45 minutes, and 60 minutes. Record your data in the table below

Reaction Time	2 minutes	15 minutes	30 minutes	45 minutes	60 minutes
V aliquot (mL)					
V_i HCl (mL)					
V_f HCl (mL)					
ΔV HCl (mL)					
Moles of HCl titrated (cΔV)					
Moles of KOH neutralized in the titration					
[KOH] neutralized $\frac{\boldsymbol{mol\ KOH\ neutralized}}{\boldsymbol{volume\ of\ aliquot\ (in\ Liters)}}$					

* Submit [KOH] values to your demonstrator before leaving the lab.

Hint: For Part A, your TA will send you the average [KOH] ***titrated*** *by their students, for both electrophiles. Think carefully about what this value represents. If a certain number of moles of KOH was titrated in your aliquot, what is the concentration of KOH that has actually been consumed during the reaction at a particular time point? How does the concentration of KOH consumed relate to the concentration of electrophile that has reacted? Remember that you are responsible for plotting the data and interpreting the kinetics for the* ***electrophiles****. These calculations need to be done for both 1-chlorobutane and 1-bromobutane, regardless of which one you used for the experiment.*

Data Analysis

Once the experiment is completed, provide your demonstrator with your [KOH] data. Do NOT rip this data out of your notebook, but simply copy it onto a second sheet of paper. **Within 24** hours, your demonstrator will post the average data for your lab group on BL. Using Excel (or other data plotting software), you and your partner are responsible for plotting the data and interpreting the kinetics for both **electrophiles.** You are responsible for producing **three graphs for part A,** each with the analysis of both electrophiles, with a zero, first and second order analysis. Display the linear equation, and the R^2 value (linear regression) for each data set. You must determine the rate constant and order of each reaction based on this analysis. Compare the rate constant of your group with the global average of all CHM2123 students, as posted on Brightspace. Your graphs must include a title, properly labeled axes, and identification of each data set.

Part B: Hydrolysis of *tert*-butyl chloride

You will determine the time required for the hydrolysis of tBuCl to go to 10 %, 20 %, 30 %, and 40 % completion in two different solvent systems. Before starting the experiment, prepare a burette with 0.10 M tBuCl in acetone. You may have to refill the burette. Note: it is possible to perform all three time trials per experiment at once if you can work diligently and without distraction.

1. Obtain a square water bath and fill it with 3 – 4 cm of water (**why?**) Do not overfill the bath.
2. Clean and dry three 50 mL or 125 mL Erlenmeyer flasks.
3. Load each flask with the volumes of distilled water and 0.02 M NaOH indicated in the table below for 10%. Add 2 – 3 drops of bromothymol blue indicator and place the flasks in the water bath.
4. Pour 3.0 mL of the 0.10 M *t*BuCl in acetone solution into each flask. **Start the timer as soon as the addition is complete.** Leave the flask in the water bath and swirl regularly. You will measure the time required until the colour of the indicator changes (the time required for 10 % conversion will be very quick!)
5. Repeat the above steps for 20, 30, and 40 % conversions, using the values indicated in the table below. You must clean and dry the Erlenmeyer flasks before each new experiment.

85:15 H_2O:Acetone

% Conversion	**10 %**	**20 %**	**30 %**	**40 %**
0.10 M *t*BuCl in acetone	3.0 mL	3.0 mL	3.0 mL	3.0 mL

0.02 M NaOH in water	1.5 mL	3.0 mL	4.5 mL	6.0 mL
Distilled water	15.5 mL	14.0 mL	12.5 mL	11.0 mL
Time to colour change #1 (s)				
Time to colour change #2 (s)				
Time to colour change #3 (s)				
Average time of trials 1 – 3 (s)				
[*t*BuCl] in solution (M) [*t*BuCl] remaining in solution (M) (initial [*t*BuCl] x % remaining)				
ln([*t*BuCl])				
$\frac{1}{[t\text{BuCl}]}$				

* Submit Average time of trials 1 – 3 values to your TA before you leave.

6. Repeat the above procedure using a 70:30 H_2O:Acetone solution and fill out the following table.

70:30 H_2O:Acetone

% Conversion	**10 %**	**20 %**	**30 %**	**40 %**
0.10 M *t*BuCl in acetone	3.0 mL	3.0 mL	3.0 mL	3.0 mL
0.02 M NaOH in water	1.5 mL	3.0 mL	4.5 mL	6.0 mL
Distilled water	12.5 mL	11.0 mL	9.5 mL	8.0 mL
Acetone	3.0 mL	3.0 mL	3.0 mL	3.0 mL
Time to colour change #1 (s)				
Time to colour change #2 (s)				
Time to colour change #3 (s)				
Average time of trials 1 – 3 (s)				
[*t*BuCl] remaining in solution (M) (initial [*t*BuCl] x % remaining)				

$\ln([t\text{BuCl}])$				
$\frac{1}{[t\text{BuCl}]}$				

*Submit Average time of trials 1 – 3 values to your demonstrator before you leave.

It is important to find the actual concentration of [tBuCl] at the point when the indicator turns from blue to yellow. This can be done by determining the initial concentration of [tBuCl] in the overall solution and multiplying it by the percent that is not consumed. (e.g. at 10 % completion, 90 % of the tBuCl remains in solution).

Data Analysis

Once the experiment is completed, provide your demonstrator with your average time trials for each solvent system (six experiments total). Do NOT rip the data sheet out of your notebook, but copy the data onto a second sheet of paper. **Within 24 hours**, your demonstrator will post the average data for your lab group on Brightspace. Using Excel (or other data plotting software), you and your partner are responsible for producing **two graphs for part B**, each comparing both solvent systems, for a zero and first order analysis. You must determine the rate constant, and the order of each reaction. Display the line equation and the R^2 value for each data set. Compare the rate constant of your group with the global average of all CHM2123 students as posted on Brightspace. Your graphs must include a title, properly labeled axes, and identification of each data set.

REPORT (60 POINTS)

Make sure to clearly organize Part A and Part B in your report. The maximum length is 3 pages for each part, not including the Title Page. The report must include the following sections:

- Title page (1 point)
- Introduction (5 points)
- Table of reactants (2 points)
- Experimental Procedure (2 points)
- Observations and Results (4 points)
- Calculations (8 points)
- Graphs (15 points)
- Mechanism of the reactions (8 points)
- Discussion (15 points)

  You need to be able to identify the type of substitution for both Part A and Part B based on the data. It is essential that they give the two reactions a CLEAR identification (S_N1 or S_N2). Explain your reasoning. For Part A, you also need to discuss the observed difference in reactivity between 1-chlorobutane and 1-bromobutane, and hence the effect of the leaving group (which one is better, and why?) For Part B, discuss the solvent effect, and why one combination is better than the other. For both parts, relate your discussion to the type of substitution.

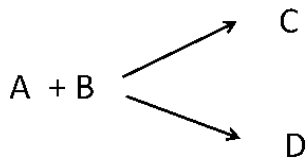
EXPERIMENT 3

COMPETITION STUDIES OF SUBSTITUTION AND ELIMINATION REACTIONS

SUGGESTED READING: The sections covering NUCLEOPHILIC SUBSTITUTION, ELIMINATION REACTIONS, and SUBSTITUTION VS. ELIMINATION, in the Organic Chemistry textbook of your choice.

INTRODUCTION

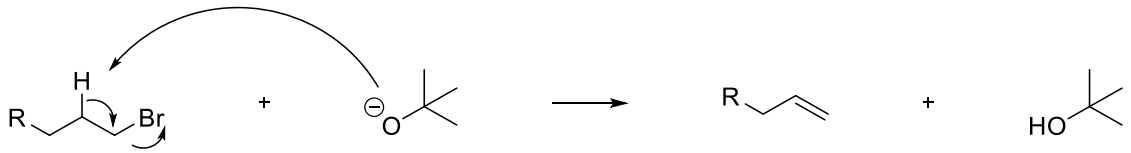
In an ideal laboratory setting, the mixing of two chemicals will produce one single product in quantitative yield in a reasonably short amount of time. Unfortunately, this is often not the case. There is frequently more than one possible reaction pathway that can be followed, leading to side products and a lower the overall yield of the target molecule. These side reaction pathways are called *competing reactions.* When multiple products are formed, a simple yield calculation lacks information. A process is characterized by the **conversion** of reactants to products, and then by the **selectivity** of a major product among two or more. Where conversion represents the ratio of all products produced to the total that could have been produced, selectivity represents the ratio of the desired product among the products formed.



$$\text{Conversion} = \frac{n_C + n_D}{n_{\text{limiting reagent}}} \times 100 \qquad \text{Selectivity} = \frac{n_C}{n_C + n_D} \times 100$$

In addition to substitution reactions, a mixture of alkyl halides with a base can also undergo *elimination reactions*. In an elimination reaction, a strong base deprotonates a hydrogen (called the β-hydrogen) adjacent to the α-carbon, allowing the electrons from the C–H bond to then attack the α-carbon. The leaving group is displaced and a new alkene bond is formed, as shown in scheme 3.1.

Scheme 3.1: An E2 elimination reaction



Much like substitution reactions, there are two main types of elimination reactions. An E1 reaction is step-wise and unimolecular. The first step of an E1 reaction involves the rate-determining formation of a carbocation upon departure of the leaving group, followed by β-hydride elimination to create the alkene. An E2 reaction, in comparison, is bimolecular and concerted, meaning that the whole reaction occurs is a single step. This experiment will examine the E2 reaction.

As most nucleophiles can also act as bases, substitution and elimination reactions are often in competition. There are many techniques available to favour one type of reaction over another. The choice of nucleophile or base is the most effective way to control the reaction pathway. Selecting a nucleophile/base of the right strength is also paramount to directing the chemistry. Employing a strong nucleophile that is not basic enough to deprotonate the β-hydrogen will selectively lead to a substitution reaction. By choosing a strong base with weak nucleophilicity, such as potassium *tert*-butoxide or sodium hydride, substitution reactions can be avoided all together.

Variations in the electrophile can also favour one reaction pathway over another. Methyl substrates react exclusively by an S_N2 pathway. Elimination outcomes on more complex substrates are possible provided that there is a β-hydrogen anti-periplanar to the leaving group. Primary α-carbons will favour the substitution reaction, though elimination is possible. Secondary substrates can undergo two of four different mechanisms and are thus rather interesting to study. In the case of secondary α-carbons, when the nucleophile is anionic (negatively charged) elimination reactions will dominate, though a small amount of substitution is possible. As S_N2 reactions are not possible on tertiary α-carbons, elimination is the only possible reaction pathway with anionic bases. Table 3.2 summarizes the expected outcome of a reaction of an alkyl halide with different types of nucleophiles. This table represents general outcomes, but there are exceptions.

R-X	Nucleophile/Base Strength			
	Strong/Strong	Strong/Weak	Weak/Strong	Weak/Weak
Methyl	S_N2	S_N2	-	-
Primary	S_N2	S_N2	E2	-
Secondary	E2	S_N2	E2	S_N1/E1
Tertiary	E2	-	E2	S_N1/E1

Strong/Strong	HO^-, RO^-(non-bulky), R_2N^- (non-bulky)
Strong/Weak	$RCOO^-$, I^-, N_3^-, CN^-, R-S^-, R-NH_2, R-SH
Weak/Strong	bulky RO^- , bulky R_2N^-, H^-
Weak/Weak	H_2O, ROH, RCOOH

Table 3.2: The expected outcome of the reaction of an alkyl halide with nucleophiles/bases

The choice of solvent can also play a role in influencing the reaction outcome. In practical terms, the solvent is only considered for secondary substrates once the choice of nucleophile has been determined. Working with a secondary substrate with all else being equal, substitution reactions are generally favoured over elimination in the presence of a polar aprotic solvent. Increasing the temperature of the reaction can further favour the formation of the elimination product. The activation energy of an elimination reaction is often higher than that of a substitution reaction, owing to entropy effects of the reaction.

THE EXPERIMENT

In this experiment, you will study the competition between S_N2 and E2 in the reaction of KOH with 1-bromobutane and 2-bromobutane. The elimination product of this reaction is a gas, 1-butene or 2-butene respectively, under ambient conditions. The gas will be collected in a eudiometer tube and the ratio of S_N2:E2 products formed can be estimated by measuring the volume of gas collected. We will assume that the gas generated obeys the Ideal Gas Law.

In order to determine the pressure of the gas inside the eudiometer, you will need to recall the laws of partial pressure:

$$P = P_{atm} - P_{water} - P^*$$

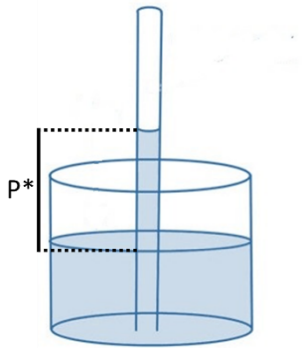
Where P represents the pressure of the gas in the enclosed system, P_{atm} is the atmospheric pressure, P_{water} signifies the vapour pressure of water at a given temperature (Table 3.3 below), and P^* *is the pressure exerted by the height of the water column above the level of the water in the beaker* (see also Figure 3.4). P^* is converted to mmHg with the simple formula:

$$13.6 \text{ mm } H_2O = 1 \text{ mmHg}$$

Table 3.3: The pressure of water (P_{water}) at different temperatures.

T (K)	P_{water} (mmHg)	T (K)	P_{water} (mmHg)	T (K)	P_{water} (mmHg)
290	14.7	294	18.8	298	24.0
291	15.8	295	19.5	299	25.5
292	16.5	296	21.0	300	27.0
293	17.3	297	22.5	301	28.5

Figure 3.4: P* is the pressure exerted by the height of the water column above the level of the water in the beaker.



The temperature and atmospheric pressure will be measured from the mercury thermometer and barometer located in the lab. By applying the ideal gas law equation, PV = nRT, the number of moles of gas can be determined. This corresponds to the amount of E2 product formed.

Since both E2 and S_N2 reactions produce KBr as a side product, measuring the amount of KBr produced will allow you to determine the amount of bromobutane consumed. This information will allow you to calculate the amount of butanol formed, which corresponds to the amount of S_N2 product formed.

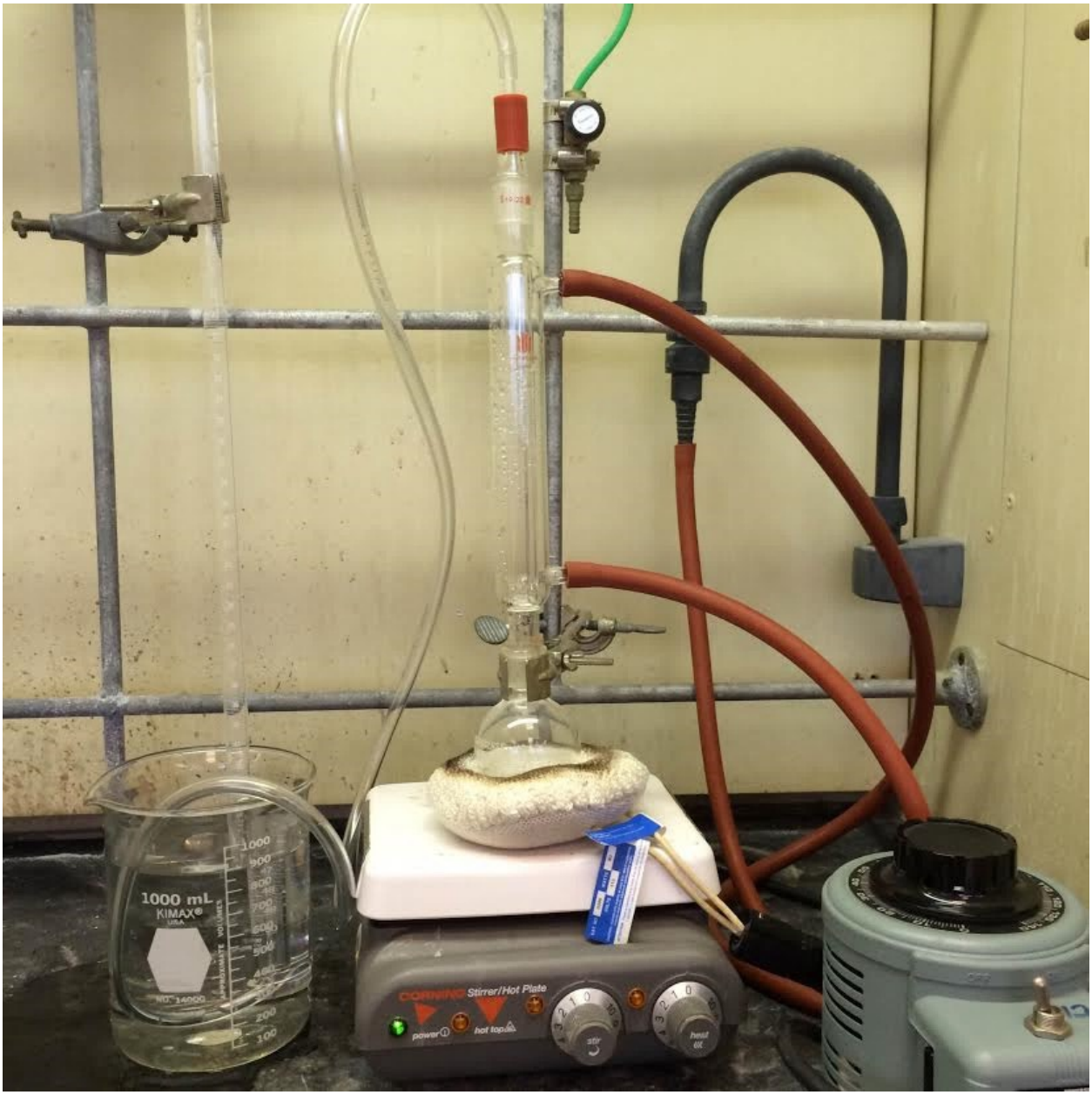
$$n_{KBr} = n_{gas} + n_{butanol} = n_{E2} + n_{S_N2}$$

The ratio of E2:S_N2 products can then be determined.

EXPERIMENTAL PROCEDURE

See the diagram below for the proper set up of the apparatus and consult with your demonstrator before adding the alkyl halide. Before beginning the experiment, note the room temperature and atmospheric pressure in the lab.

Figure 3.5: The set-up of the experiment.



1. Measure out 8 g of KOH into a 100 mL round bottom flask.
2. While working in the fume hood, clamp the round bottom flask above a magnetic stirrer and a heating mantle. Connect the heating mantle to a Variac transformer.
3. Using a graduated cylinder, dissolve the KOH in 35 mL of 95 % ethanol and place a magnetic stirrer into the flask.
4. Attach a large reflux condenser to the round bottom flask. Attach the cooling tubes to the condenser and start the flow of water (does water go in the bottom or the top of the condenser?)

5. Place a thermometer adapter into top of the reflux condenser, connecting the ground glass joints.
6. Begin to reflux the KOH solution.
7. Fill a 1 L beaker with water and place it in the fume hood beside your set up.
8. Fill a eudiometer tube with water (to the brim), place your thumb over the brim, flip the tube upside down, and submerge the brim down into the 1L beaker. Clamp the eudiometer tube to the frame.
9. Obtain a Tygon Tube from your demonstrator. Place one end onto the thermometer adapter and the other end into eudiometer.
10. As the system reaches equilibrium, bubbles will form in the eudiometer.
11. Dissolve 1.5 mL of 1-bromobutane in 5 mL 95 % ethanol in a graduated cylinder.
12. Mark down the volume of gas in the Eudiometer tube. This will be starting point.

THE NEXT STEP MUST BE DONE AS QUICKLY AS POSSIBLE. MAKE SURE YOU HAVE EVERYTHING PREPARED BEFORE PROCEDING.

13. Once the bubbles have stopped forming (or slowed down), break the seal at the top of the condenser and quickly add the 1-bromobutane solution into the top of the condenser. Replace the thermometer adaptor quickly to prevent gas from escaping.
14. As gas is formed during the reaction, water will be displaced from the eudiometer tube. Continue the reaction until there is no significant change in the volume of water displaced from the eudiometer, approximately 15 minutes. Watch the gas evolution closely, for you may need to use a **second eudiometer tube**.
15. Note the room temperature and the atmospheric pressure on the barometer in the lab.
16. Note the final volume of water displaced.
17. Remove the heat source from underneath the manifold, and **immediately** disconnect the tygon tube from the top of the reflux condenser. Failing to remove the connection will result in water backfilling into the reaction flask due to the drop in pressure.
18. Allow the solution to cool and filter the KBr by suction filtration. Rinse your flask with 5 – 10 mL cool EtOH, and use this to rinse the filter cake.
19. Allow the KBr to dry and determine the mass.
20. Repeat the experiment using 1.5 mL of 1-bromobutane by heating the flask in a metal water bath at 50˚C.
21. Repeat the experiment using 0.7 mL of 2-bromobutane under reflux conditions.

Results

The table below will help you record your results.

Reaction conditions	Pressure of gas (atm)	Volume of gas (mL)	Moles of Butene (mol)	Mass of KBr (g)	Moles of KBr (mol)	Moles of butanol (mol)	Yield
1-bromobutane at reflux							
1-bromobutane at 50 °C							
2-bromobutane at reflux							

Report

Determine the yield of the products in each of the three reactions. Calculate the yield of KBr, and use this value to report the **% conversion** of your reaction. Note that gaseous butene products are indistinguishable using the present method. **Which isomer do you suppose is in highest yield, and why?** Using ideal gas conditions, calculate the moles of any alkene products evolved as well as moles of alcohol formed. Report the **selectivity** of each set of reaction conditions towards S_N2 and E2 products.

REPORT (40 POINTS)

Your report must include the following sections

- Title page (1 point)
- Introduction (6 points)
- Experimental procedure (2 points)
- Table of Reagents (2 points)
- Observations and Results (4 points)
- Calculations (10 points)
    - P_{gas}
    - Moles of butene
    - Moles of butanol
    - Conversion
    - Selectivity
- Discussion (15 points)

EXPERIMENT 4

BENZYLIDENE ACETALS AS A PROTECTING GROUPS

SUGGESTED READING: The sections covering **ACETALS, ADDITION OF OXYGEN NUCLEOPHILES TO A CARBONYL, and PROTECTING GROUPS** in the Organic Chemistry textbook of your choice.

INTRODUCTION

Carbohydrates are polyhydroxy aldehydes and ketones, often referred to as sugars and starches, which constitute an abundant class of biomolecules. Carbohydrates are a vital source of energy in the form of glucose for animals and starch for plants, but they also mediate a number of biological processes. Complex carbohydrates such as oligosaccharides and glycoconjugates contribute to the shape of cells and organs. They provide structure and function via the building blocks in DNA, RNA, ATP, and glycoproteins. Additionally, oligosaccharides are widely present on cell surfaces in the form of glycans, and play a central role in cellular recognition and signaling. Glycans on the cell surface become targets for carbohydrate-binding proteins, and changes to the glycan structure signal disease progression such as inflammation or the onset of cancer. Tumor cells produce unique carbohydrate sequences on their surfaces relative to healthy cells. In recent years, carbohydrates have demonstrated strong potential in diagnostics, vaccines, and therapeutics. A carbohydrate-based vaccine is in development for the HIV virus, where antibodies are directed to specific oligosaccharides and glycoproteins on the protein's outer shell. An example of this research is illustrated in Figure 4.1.

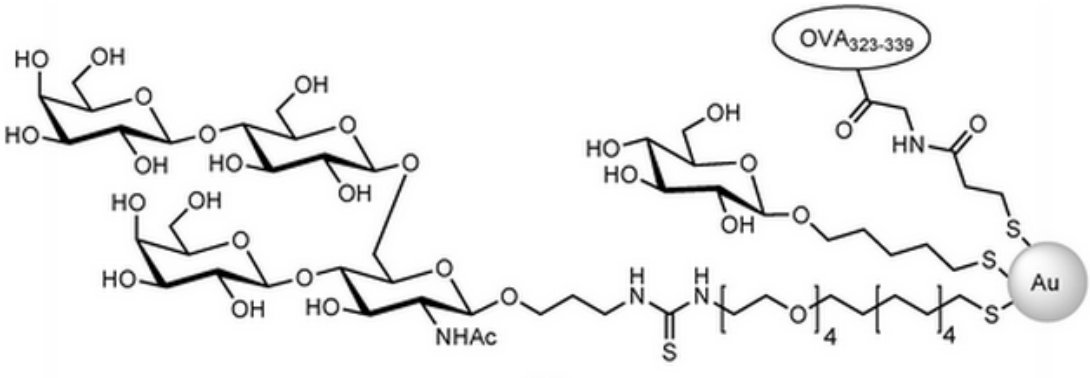
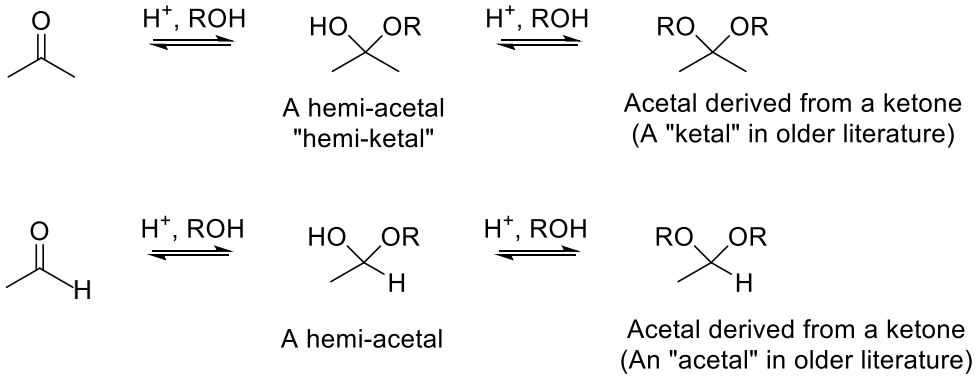


Figure 4.1 – Gold nanoparticles functionalized with oligosaccharides and ovalbumin (OVA) peptide to deliver IgG antibodies to the HIV virus. D.Safari et. al., *Nanomedicine*, **2012**, *7*, 651-662.

The multifunctional and multi-chiral nature of carbohydrates requires efficient techniques for their modification and purification. Just dissolving a monosaccharide in aqueous base gives rise to keto-enol tautomerization leading to isomerization. Employing a protecting group can prevent such side reactions. One of the most common protecting groups in carbohydrate chemistry is an acetal.

Acetals as Protecting Groups

Aldehydes and ketones react reversibly with alcohols in the presence of acid catalysts to give acetals. The key structural feature of these compounds is a carbon bonded to two alkoxy groups (OR). In older nomenclature, compounds formed from aldehydes and ketones were termed acetals and ketals, respectively; however, the term "acetal" will be used in this document. A "hemiacetal" has a carbon bonded to one alkoxy (OR) and one hydroxyl (OH) group.

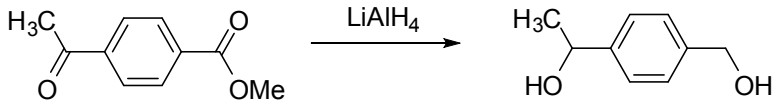


Scheme 4.1 – Acetal and hemi-acetal formation.

The acetal functionality is important in organic chemistry and in biochemistry, serving as a protecting group for alcohols, aldehydes, and ketones. For example, a ketone or an aldehyde is susceptible to reducing agents and to nucleophilic additions; it is also reactive under conditions that are sufficiently basic to allow enolate formation. If that ketone or aldehyde function is first converted to ("protected as") an acetal, the resulting molecule can now be subjected to strongly reducing conditions since acetals are stable to reducing conditions. When that reduction is complete, the aldehyde or ketone group can be regenerated simply by the addition of excess water and acid. An example is illustrated below.

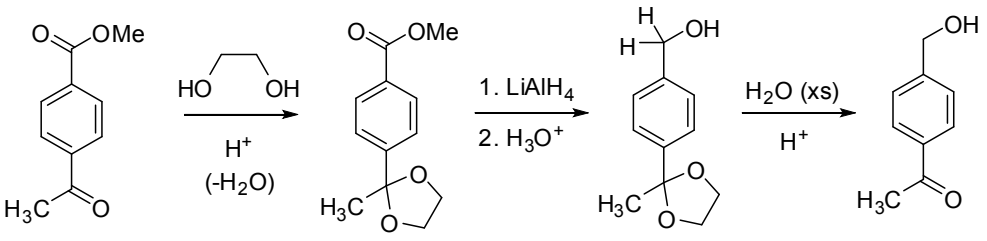
Example: The selective conversion of a keto-ester into a keto-alcohol

How can we reduce an ester but leave the ketone moiety untouched? Reducing agents for esters, like $LiAlH_4$, will also reduce the ketone which is more reactive, giving a diol.

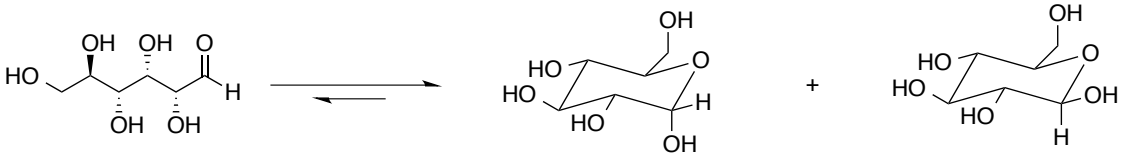


To prevent the reduction of the ketone, and selectively reduce the ester functionality, we must use a protecting group. The ketone can be protected as an acetal using 1,2-ethandiol under acidic

conditions. The ester is then reduced using lithium aluminum hydride after workup. The acetal can then be hydrolyzed under acidic conditions to give the desired product, the keto-alcohol.



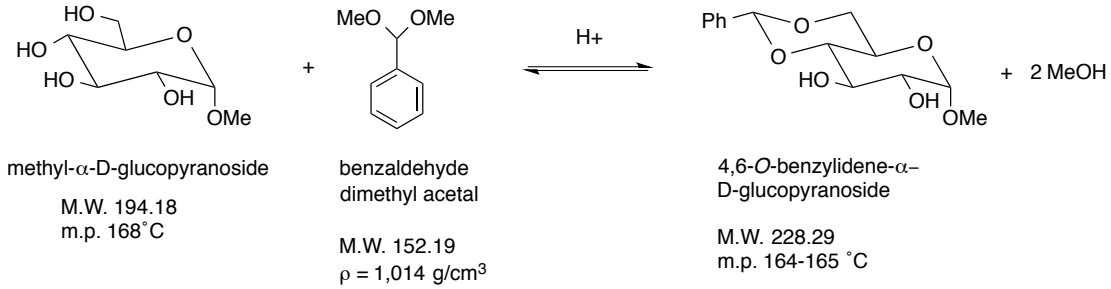
Many important naturally occurring compounds exist as hemiacetals, acetals or ketals. Glucose is a hemiacetal that can exist in both cyclic and acyclic form. Because cyclization forms a new stereogenic centre, the sugar exists in two anomeric forms; α-D-glucose and β-D-glucose. When the anomeric alcohol is axial, it is said to be in the α position; whereas when it is equatorial, it is said to be in the β position. In solution, the acyclic aldehyde and two cyclic hemi-acetals exist in equilibrium as described in Scheme 4.2, where the cyclic stereoisomers can be separated, isolated, and crystallized.



Scheme 4.2 - The interconversion of cyclic to acyclic glucose.

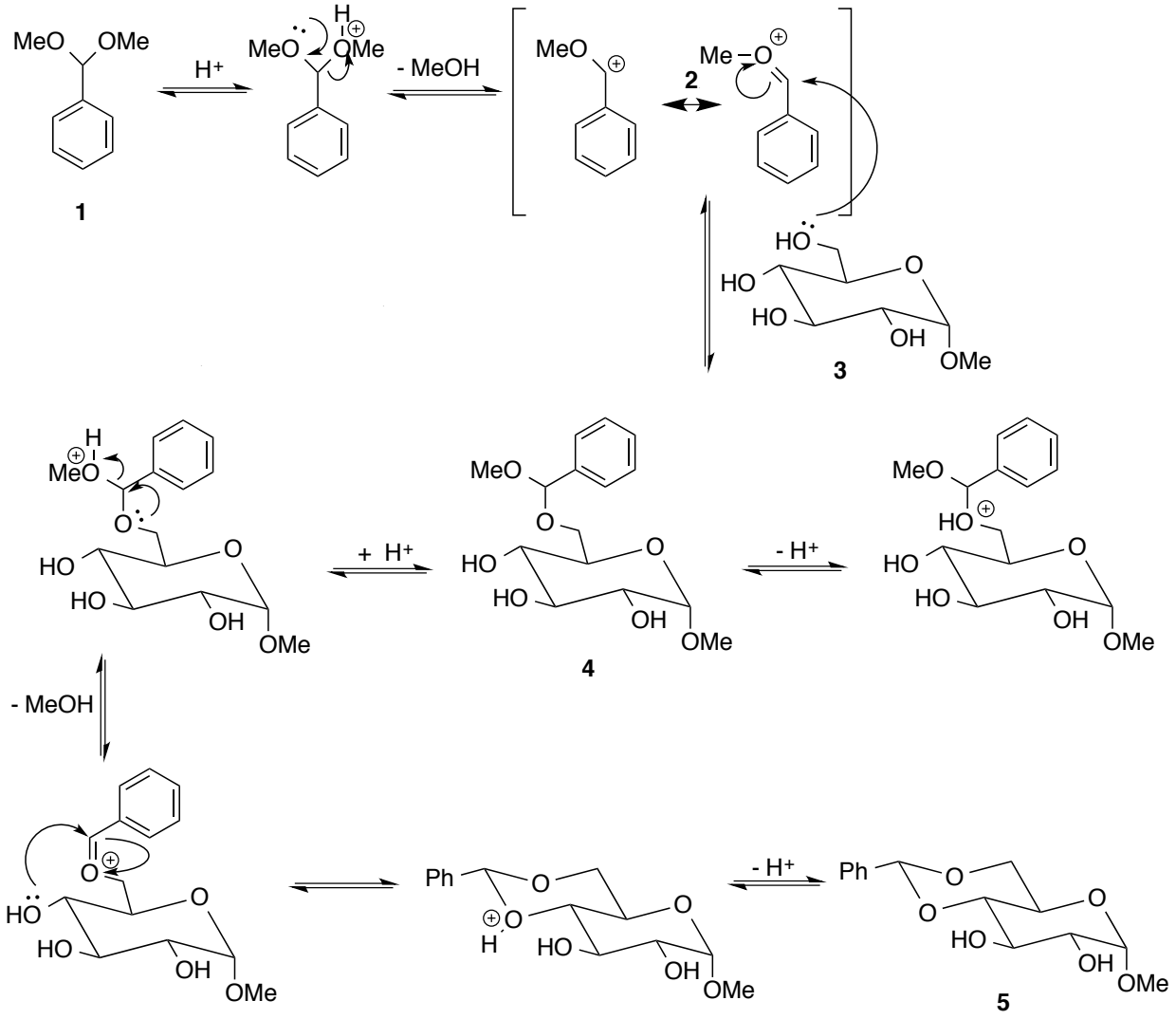
Preparation of a cyclic acetal

In this experiment, you will create a benzylidene acetal at the C4 and C6 alcohols of glucose, creating a "protected" glucose via transglycosylation (Scheme 4.3). These protecting groups are important in synthesis since each alcohol has reactivity. By creating an acetal at a specific position, the reactivity of these two alcohols is blocked, allowing further reactivity at other positions.



Scheme 4.3: Protection of a glucose at the C4 and C6 positions.

The reaction is carried out under anhydrous conditions with tetrahydrofuran, THF, an easily removable solvent, and 10-camphorsulfonic acid as catalyst. In the presence of a strong acid, the dimethyl acetal **1** is protonated to generate the oxonium **2**, as shown in Scheme 5.4. The oxonium is then trapped by one of the hydroxyl groups of the sugar **3** to give the mixed acetal **4**. Methanol is then eliminated *via* acid catalysis and the resultant oxonium ion is trapped internally by another hydroxyl group, giving the final product, **5**. Note that all steps in this acid-catalyzed process are reversible, and the thermodynamically-favoured product with the equatorial phenyl substituent is exclusively formed.



Scheme 4.4 – Acid-catalyzed benzylidene acetal formation.

Reducing Sugars

Sucrose, or common table sugar, is a disaccharide made by combining glucose and fructose. It has both an acetal and a ketal functional group. Lactose is another disaccharide composed of glucose

and galactose with an acetal and a hemiacetal functional group. Cellulose**,** a polysaccharide of glucose, contains repeating sugar units linked through an acetal group. These natural sugars are illustrated in Figure 4.2, below.

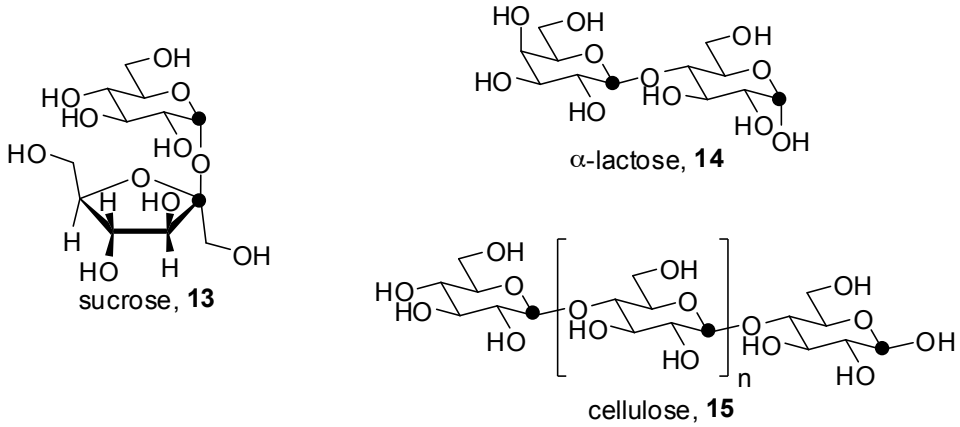
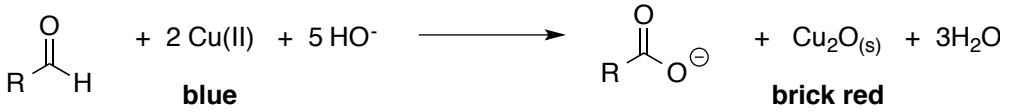


Figure 4.2 - Other natural products containing acetal functional groups.

Sugars containing free aldehyde or alpha-hydroxy-ketones (including those that occur in certain ketoses) groups are known as reducing sugars. Any sugar containing a hemi-acetal will be a reducing sugar, thus all monosaccharides, some disaccharides, oligosaccharides and polysaccharides are reducing sugars. A colourimetric assay can be used to determine the amount of reducing sugar in a sample using Benedict's reagent – an alkaline cupric citrate solution.



Under thermal conditions, reducing sugars are converted to enediols in the presence of base. Enediols then reduce Cu(II) to Cu_2O. Commonly used for qualitative analysis of monosaccharides and reducing disaccharides in food, a sample is dissolved in water, followed by addition of a few drops of Benedict's solution and heated for a few minutes in a steam bath. Benedict's reagent converts the bright blue solution to green, to yellow, to orange, to brick red in the presence of increasing concentration of reducing sugar. In this experiment, you will test for the presence of reducing sugars in samples of milk and fruit samples that you may choose to bring into the lab. (Fruit juice, fruit 'drink', small pieces of cut fruit in a sealed plastic bag).

EXPERIMENTAL PROCEDURE *While waiting for the reaction to heat for 30-60 minutes, proceed to part B.*

Part A: Protection of a monosaccharide

1. Obtain a 25 mL round bottom flask and place 0.5 g of methyl-α-D-glucopyranoside into the flask. Clamp the flask in the fume hood above a magnetic stirrer and place a magnetic stir bar into the flask.
2. Dissolve the solid in 10 mL of tetrahydrofuran and added 0.70 mL of benzaldehyde dimethyl acetal and 0.10 g of 10-camphorsulfonic acid.
3. Equip the flask with a reflux condenser and heat at reflux for 30 minutes.
4. After 30 minutes, take a TLC (3:7 hexanes:ethyl acetate) to monitor the progress of your reaction.
5. Visualize your TLC using a methanolic solution of sulfuric acid (10% H_2SO_4 in methanol). Using tweezers, dip the TLC plate into the solution, allow it to dry, and heat the plate. Due to the equilibrium of this reaction, there will always be a small amount (5%) of starting material remaining in solution. If the reaction is complete, continue with the experimental procedure. If not, allow the reaction to reflux for another 30 minutes.
6. Cool the reaction to room temperature and add a few drops of trimethylamine (**why?**). Transfer the contents of the flask into a separatory funnel and dilute with 50 mL of ethyl acetate.
7. Wash the organic layer with 3x15 mL distilled water.
8. Separate off the aqueous phases and dry the organic phase with Na_2SO_4. Gravity filter the organic phase into a 125 mL Erlenmeyer flask.
9. Carefully evaporate the ethyl acetate in a steam bath. (b.p. ethyl acetate 77˚C) Remove the flask when there is ~1 mL of solvent remaining. You should have a thick syrup.
10. Dissolve the contents of the flask in 10 – 15 mL of dichloromethane, and then *slowly* added hexanes (70 – 80 mL) to force the precipitation of the product. (Reserve a few mL of the mother liquor for TLC analysis.) Isolate the crystals by vacuum filtration. Consult Appendix II for assistance in recrystallization.
11. Run a TLC to assess the efficiency of your reaction and purification. Dissolve a few mg of crystals in acetone and take a TLC (3:7 hexanes:ethyl acetate). The glucose starting material can be dissolved in water. Include a lane for the mother liquor on your TLC plate.

Part B: Benedict's test for reducing sugars

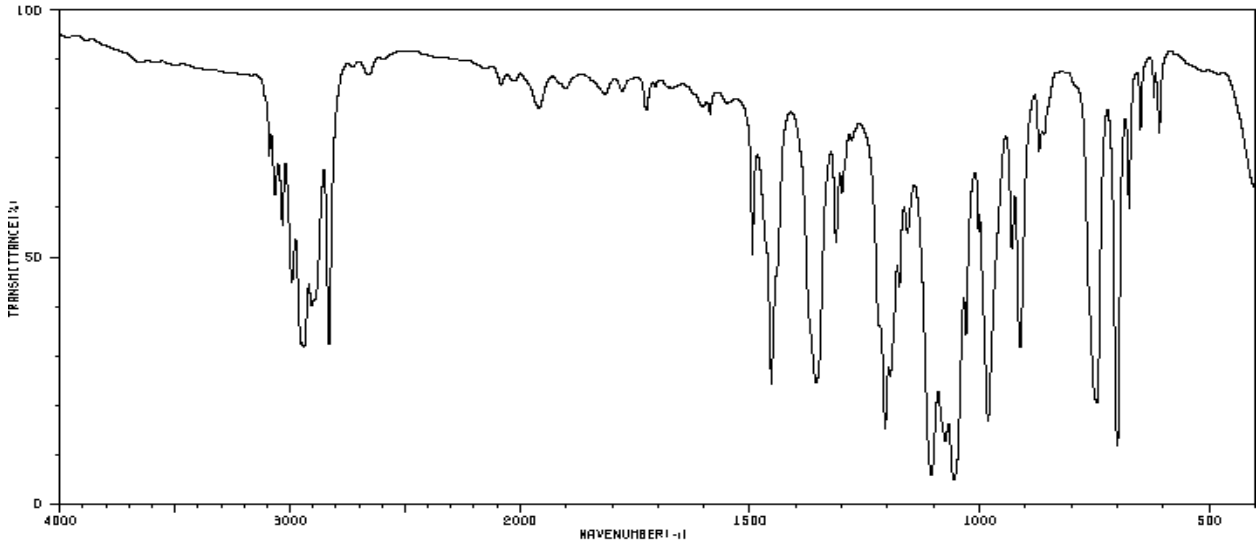
1. In a test tube labeled S, dissolve 0.1 g of sucrose in 1 mL of water.
2. In a test tube labeled G, dissolve 0.1 g of glucose in 1 mL of water.
3. In a test tube labeled M, add 1 mL of milk.
4. In a test tube labeled F, add 1 mL of your dissolved fruit or juice of choice.
5. Add 3 mL of Benedict's solution to each of the test tubes.
6. Place test tubes in a beaker in a steam bath and heat for about five minutes. Note your observations.

7. Add a few drops of concentrated HCl to test tube S, and note your observations.
8. Based on the results of Benedict's test, identify which test tubes contain reducing sugars and non-reducing sugars. What chemistry is at play when HCl is added to sucrose?

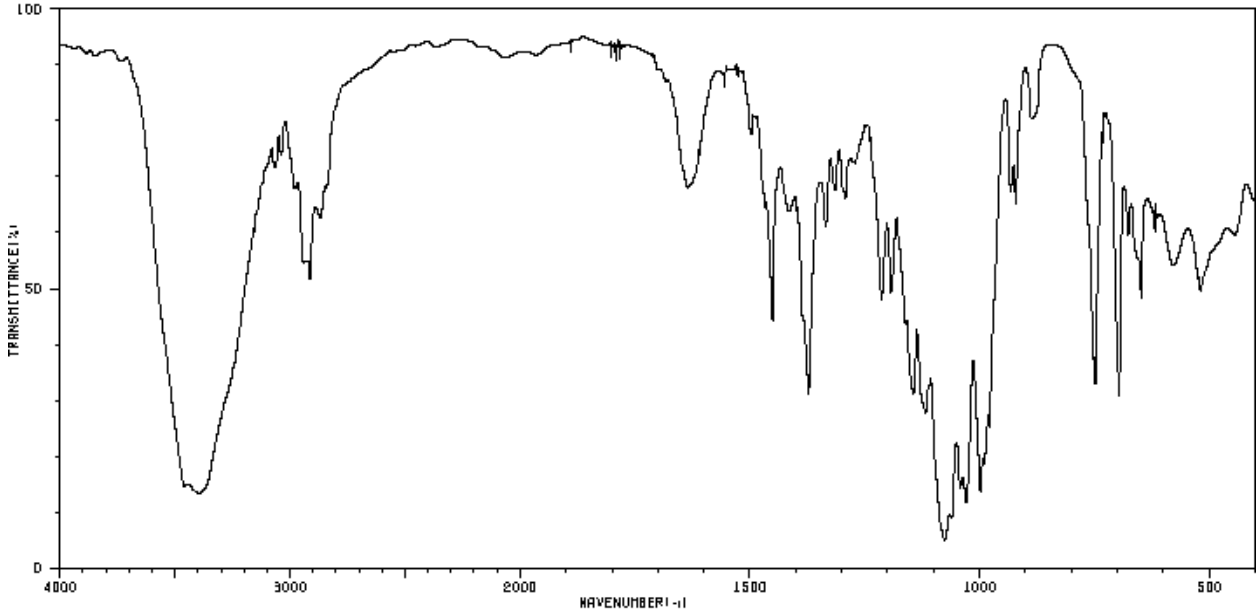
Infrared Analysis:

The infrared spectra of two compounds chosen from Scheme 4.3 are provided below. Determine which compound is best represented by spectrum A and spectrum B in your lab report, and justify your choice.

Spectrum A:



Spectrum B:



REPORT (56 POINTS)

Your report must include the following sections:

- Title Page (1 point)
- Introduction (6 points)
- Mechanism (3 points)
- Table of reagents (3 points)
- Experimental Procedure (1 point)
- Observations and Results (6 points)
- Flowchart (10 points)
- TLC plates (6 points)
- Discussion (14 points)
- Infrared analysis (6 points)

EXPERIMENT 5

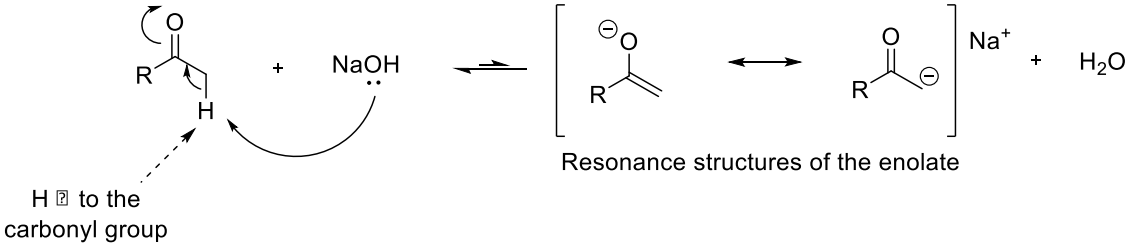
REACTIONS OF CARBONYLS UNDER BASIC CONDITIONS

SUGGESTED READING: The sections covering **REACTIONS AT AN α CARBON, ENOLATES, ALDOL REACTION, HALOFORM REACTION, and α-HALOGENATION OF A KETONE** in the Organic Chemistry textbook of your choice.

INTRODUCTION

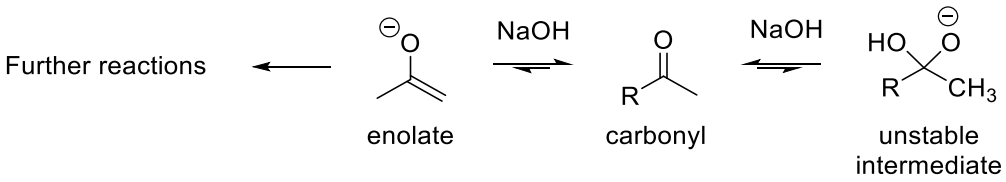
The α-hydrogens of ketones and aldehydes are slightly acidic. The pK_a of these protons is generally given as ~20 (in H_2O), whereas the pK_a of aliphatic C–H protons is ~ 40 – 50. This increased acidity can be attributed to the delocalization of the negative charge upon deprotonation of the α-carbon via resonance. The species formed upon deprotonation of the α-hydrogen of a ketone or aldehyde is called an enolate, shown in scheme 5.1.

Scheme 5.1: The formation of an enolate in basic conditions.



Since the pK_a of the protons α to the carbonyl in water is ~20, the hydroxide anion is basic enough to generate a small concentration of the enolate ion when an aldehyde or ketone is dissolved in water or an alcohol. Kinetically, however, the most favourable reaction between NaOH and a ketone or aldehyde is the nucleophilic addition of hydroxide to the carbonyl group. The product of this reaction is not stable and reverts to the starting material. The alternate, slower reaction of the removal of a α-hydrogen leads to the formation of an enolate anion, which can undergo further reactions. These competing reactions are shown in Scheme 5.2.

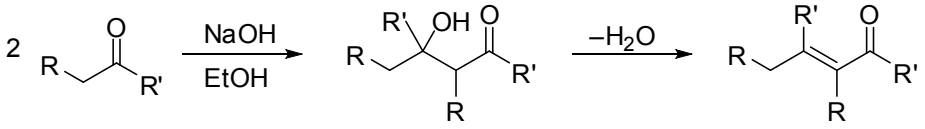
Scheme 5.2: Possible reactions of a carbonyl with NaOH.



ALDOL CONDENSATION

An important property of aldehydes and ketones is their tendency to dimerize in basic medium forming a new carbon-carbon bond. The initial product is a β-hydroxyaldehyde or β-hydroxyketone, an "**aldol**" (from the **ald**ehyde and alcoh**ol** functional groups present in the product). The reaction is illustrated in scheme 5.3 below. The aldol product can then eliminate the hydroxyl group in protic solvents to form a α,β-unsaturated ketone or aldehyde. The unsaturated product is also easily obtained when there are aromatic groups adjacent to the carbonyl.

Scheme 5.3: The dimerization of two ketones.

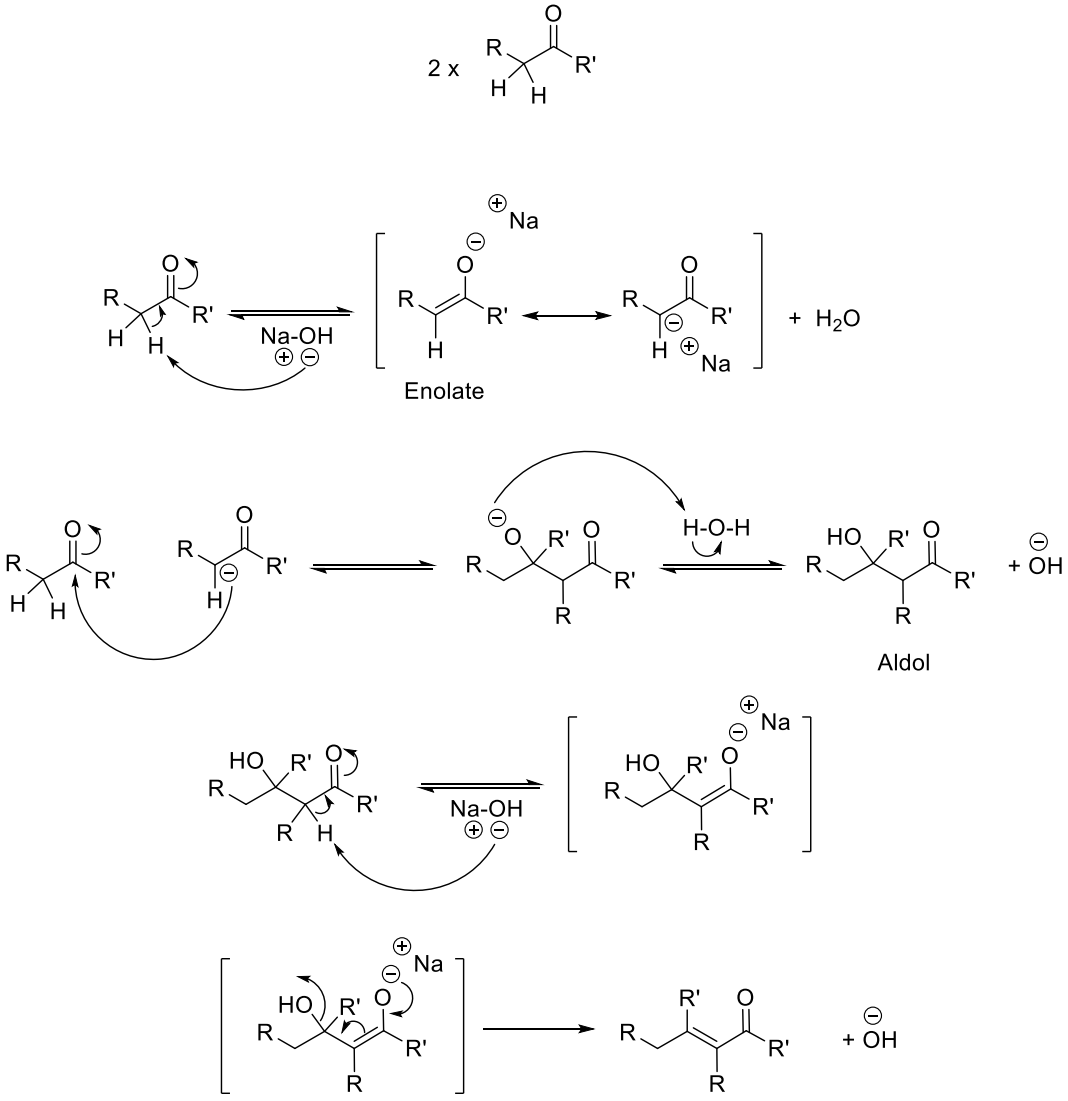


aldol

The unsaturated product is easily obtained when R and/or R' are aromatic groups.

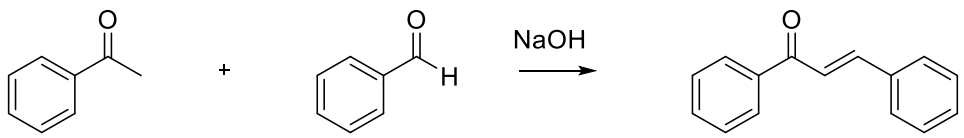
The mechanism for the aldol reaction is shown in Scheme 5.4 below. At least one α-proton must be present for the aldol reaction to take place, and another must be available for elimination to occur, which gives the α,β-unsaturated product. The first α-proton is removed by the base to generate the enolate. The carbonyl of the second molecule, acting as an electrophile, is subsequently attacked by the newly formed enolate. Abstraction of a second α-proton leads to elimination of the hydroxyl group and formation of the α,β-unsaturated product.

Scheme 5.4: The mechanism of the self-aldol reaction.



"Crossed condensation" may be achieved with two different carbonyl compounds reacting with each other; one reacts an enolate (a nucleophile) while the other reacts as an electrophile. Generally, a mixture of self and "crossed" products can be expected. In order to make the crossed condensation synthetically useful, the compounds must be chosen carefully so that only one is capable of forming an enolate (the other not having an α-hydrogen, for example), and this molecule does not self-condense too rapidly (ketones self-condense much more slowly than aldehydes). Such a pair is found in the compounds to be used in this experiment, acetophenone (1-phenylethanone) and benzaldehyde, shown

below. The only possible cross-condensation leads, after dehydration, to benzalacetophenone (1,3-diphenyl-2-propen-1-one).



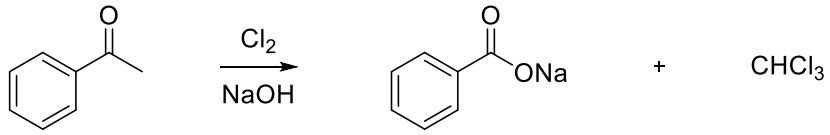
Acetophenone	Benzaldehyde	Benzalacetophenone
M.W. 120.16 g/mol	M.W. 106.13 g/mol	M.W. 208.26 g/mol
b.p. 202 ^{o}C	b.p. 178 ^{o}C	m.p. 58 - 59 ^{o}C

Sodium hydroxide is used as a base in this reaction. Few secondary reaction products form since the self-condensation of acetophenone is very slow. In addition, benzaldehyde cannot self-condense because it does not possess any α-protons.

THE HALOFORM REACTION

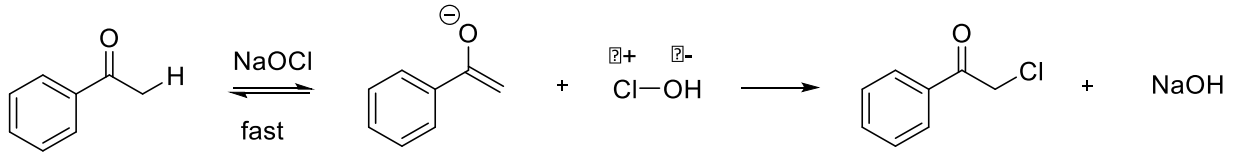
The overall haloform reaction is shown in scheme 5.5. The classic transformation involves a methyl ketone reacting with a halogen X_2 (Cl_2, Br_2, or I_2) in the presence of NaOH to yield a haloform and a carboxylate.

Scheme 5.5: The overall haloform reaction.



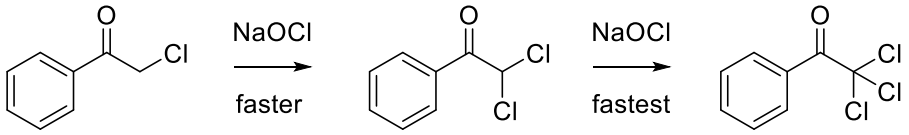
Since halides are often very toxic, this reaction can also be performed using sodium hypobromite (NaOBr) or sodium hypochlorite (NaOCl). Sodium hypochlorite is the chemical name for commercial bleach. These reactants provide an excellent source of electrophilic halogens because oxygen is more electronegative than both chlorine and bromine. Sodium hypochlorite can also act as a base, eliminating the need of NaOH as a reagent. The first step of the reaction sequence is shown in Scheme 5.6.

Scheme 5.6: The initial step of the haloform reaction with bleach.



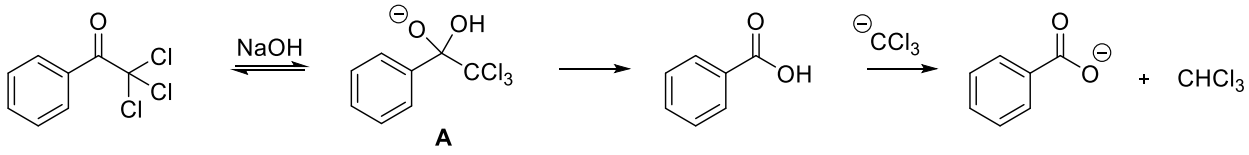
Enolate formation followed by reaction with the hypochloride (Cl–OH) at the α-carbon of the enolate gives a α-haloketone. Once the α-haloketone has formed, the α-protons become increasingly acidic due to inductive effects. Thus, they are more easily deprotonated by the NaOCl, and the reaction sequence repeats itself until an α,α,α-trihaloketone has formed, as shown in scheme 5.7.

Scheme 5.7: The formation of the trihaloketone.



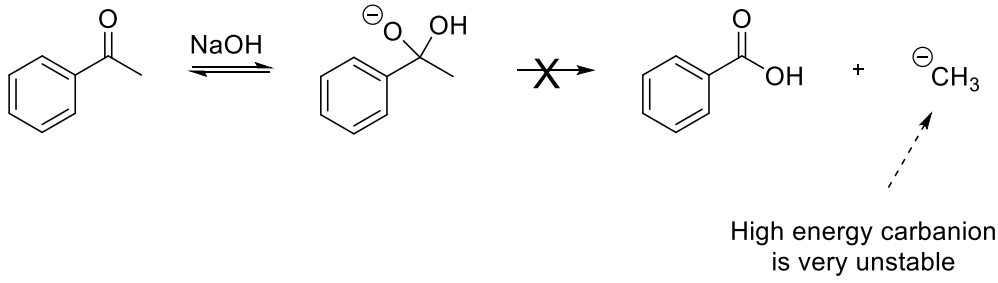
The NaOH produced reacts in a different manner with the trihaloketone. There are no longer any α-protons available for abstraction and the carbonyl has become highly electrophilic due to the inductive effects of the halogens. Nucleophilic attack by the hydroxide on the carbon of the carbonyl takes place easily, forming tetrahedral intermediate **A**. This will collapse through the expulsion of the relatively stable $^{-}CX_3$ anion. This anion is rapidly protonated to give the haloform CHX_3, which transforms the carboxylic acid into a carboxylate through the acid/base reaction.

Scheme 5.8: Nucleophilic attack of the hydroxide anion on the trihaloketone.



This mechanism is an example of an addition-elimination process: the nucleophilic attack onto the carbonyl forming a tetrahedral intermediate is followed by departure of the leaving group ($^{-}CX_3$). This can't occur in the case of the methyl ketone starting material because it is too difficult to break the C-C bond (the highly unstable $^{-}CH_3$ is a poor leaving group).

Scheme 5.9: Nucleophilic attack of a hydroxyl on a carbonyl does not produce CH_3^- as a leaving group.



EXPERIMENTAL PROCEDURE

The benzalacetophenone product of Part A is a mild skin irritant. Avoid direct contact and wash off any accidental spills with soap and water immediately upon contact. Be sure to wash your hands with soap and water after finishing the experiment.

Part A: Aldol Reaction

Preparation of benzalacetophenone

1. Working in the fume hood, combine 4.0 mL of 10% NaOH solution and 4 mL EtOH in a 50 mL round bottom flask.
2. Dissolve 1.0 mL (8.6 mmol) of acetophenone into your solution.
3. Add 1.0 mL (9.8 mmol) of benzaldehyde and a magnetic stir bar to your flask. Clamp the flask above a stir plate.
4. Monitor the reaction for 45 minutes to observe the formation of a yellow product. If no reactivity is apparent, heat the sample. Avoid the formation of a red oil from excess heating.
5. Using a filter funnel, pour the reaction mixture into an Erlenmeyer flask. Rinse the flask with a small amount of ethanol to ensure complete transfer of the reaction mixture.
6. Cool the reaction in an ice bath. Crystallization should be complete within 30 minutes. Scratch the sides of the flask to induce crystallization if necessary. You may also add a small volume of cold water to induce precipitation.
7. Filter the crystals by suction filtration using a Buchner funnel and wash the crystals with 20 – 30 mL of cold water and then with 5 mL cold EtOH. Dry the crystals by aspiration
8. Determine the melting point and yield of the crude product

Recrystallization

1. Dissolve the crude product in a *minimum* amount of boiling ethanol.
2. Allow the solution to cool to room temperature and allow crystallization to proceed for 10 minutes at room temperature.
3. Cool the solution in an ice bath for up to 5 minutes to complete the process.
4. Filter and dry the crystals by aspiration using a Buchner funnel.
5. Dissolve a small amount of your pure product in ethanol and take another TLC to verify the purity.
6. Determine the melting point and yield of your pure product. The product should appear as pale yellow needles. The literature melting point for benzalacetophenone is 57 – 58 °C.

Part B: Haloform Reaction

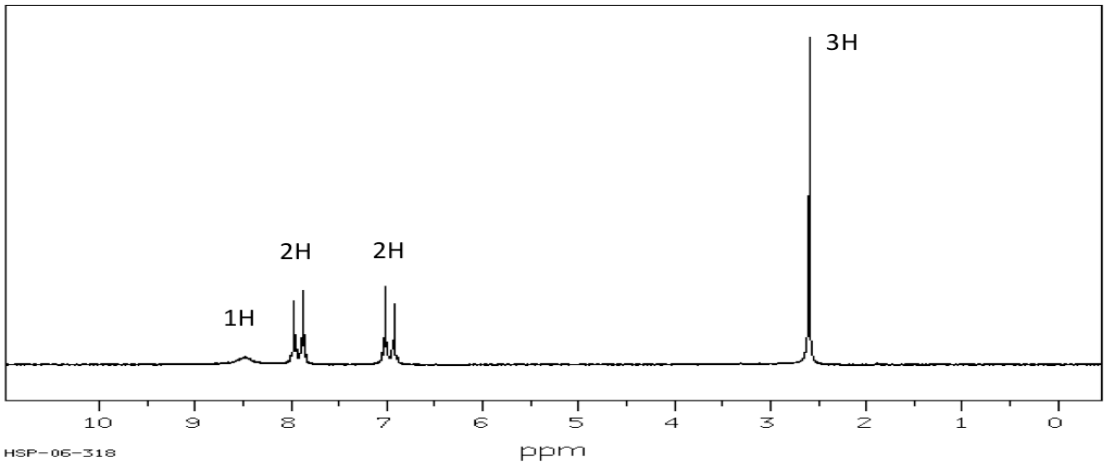
1. Add about 1.0 g (measured accurately) of acetophenone to 40 mL of bleach solution (5% NaOCl in water – this reactant should be included in your table of reagents!) in a 100 mL round bottom flask equipped with a magnetic stirrer. Clamp the flask in the fume hood above a stir plate.

2. After the initial reaction has subsided, boil the reaction mixture gently for 5 – 10 minutes using a heating mantle.
3. After 15 minutes, take a TLC of your reaction mixture.
4. Add 0.5 mL of acetone to destroy any unreacted hypochlorite (how?) and boil gently for another 15 minutes to remove the chloroform and unreacted acetone.
5. If your solution is very dark, add a small amount of decolorizing charcoal and filter the solution while it is still hot.
6. Pour the mixture into a 125 mL Erlenmeyer flask using a filter funnel and place the flask in an ice bath. Acidify the solution by adding concentrated HCl drop-wise until the pH is near 1 and no more precipitate forms. Keep the mixture in the ice bath for an additional 5 minutes to complete the precipitation.
7. Filter the product by suction filtration using a Buchner funnel. Wash the crystals with about 5 – 10 mL of cold water and let them dry.
8. Determine the melting point and the yield of your product once the crystals have dried at the end of the lab period.

^{1}H NMR Analysis:

1. Explain how you could use ^{1}H NMR to tell whether you formed the aldol product or the condensation product in your synthesis of part A. Be sure to include the chemical shift, multiplicity, and integration of the peaks involved in your answer. What peaks would you anticipate for each product? Construct a table containing chemical shifts, integration, and multiplicity for anticipated peaks with a clear description of what peak corresponds to what proton. It is recommended to label protons A-Z on a drawing of the molecule, and include a column for the label in your table.

2. The ^{1}H NMR spectrum of an unknown compound with the molecular formula $C_8H_8O_2$ is provided below. The IR spectrum for this compound shows a large, broad peak at 3147 cm^{-1} and a strong, sharp peak at 1645 cm^{-1}. Provide a structure for this unknown.



REPORT (65 POINTS)

Your report must include the following sections.

- Title Page (1 point)
- Introduction (6 points)
- Mechanism
    - Aldol Reaction in Part A (6 points)
    - Haloform Reaction in Part B (8 points)
- Table of reagents (3 points)
- Experimental procedure (1 point)
- Observations and Results (4 points)
- Melting points (2 points)
- Calculations (3 points)
- TLC plates (4 points)
- Discussion (12 points)
- ^{1}H NMR analysis (15 points)

EXPERIMENT 6

REDUCTIVE AMINATION OF VANILLIN WITH p-TOLUIDINE

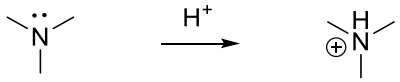
SUGGESTED READING: The sections covering **IMINES, ADDITION OF NITROGEN NUCLEOPHILES TO A CARBONYL, and REDUCTIVE AMINATION** in the Organic Chemistry textbook of your choice.

INTRODUCTION

Amines are a common class of organic compounds, which contain a nitrogen atom with a lone pair of electrons. Amines are found in a wide variety of organic molecules such as drugs, synthetic dyes, fertilizers, pesticides, and cosmetics. Biologically, they are an important component of proteins, amino acids, nucleic acids, and other natural products. Naturally-occurring compounds that contain basic nitrogen atoms are known as *alkaloids* and have been used by various civilizations for thousands of years for their medicinal, euphoric, and/or toxic effects. Opiates, such as morphine, were initially produced and sold to the public by pharmaceutical companies as potent analgesics. It was only after their dangerous effects were recognized did they become controlled substances. Today, more than 90 per cent of drugs on the market contain at least one nitrogen atom, making transformations involving amines very important for synthetic and medicinal chemistry.

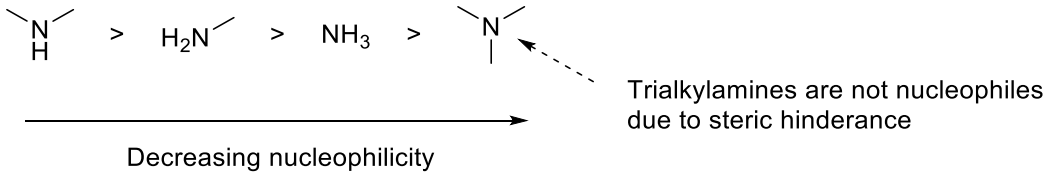
Nitrogen can form three covalent bonds with other elements and stay neutral. This leaves it with an available lone pair, making amines basic – they react easily with acids to form a positively-charged ammonium ion after accepting a proton, as shown in scheme 6.1.

Scheme 6.1: The protonation of trimethylamine.



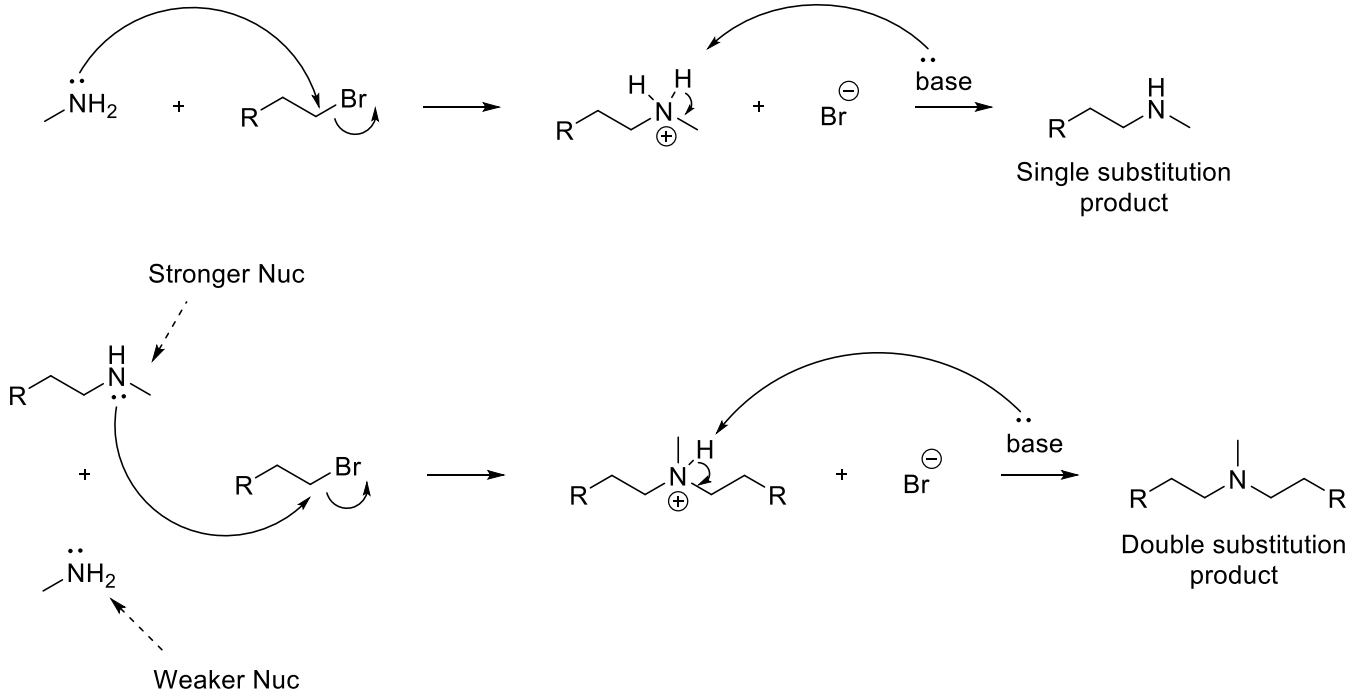
Any primary or secondary amine can also act as a nucleophile – reacting with any electrophile to form new covalent bonds. An amine with more carbon substituents can be more nucleophilic than an amine with hydrogen substituents, due to the electron-donating ability of the alkyl groups. However, bulky alkyl groups decrease nucleophilicity due to increased steric interactions, as shown in scheme 6.2.

Scheme 6.2: Nucleophilicity of amines increases with alkyl substituents up to dialkylamines. However, trialkylamines are not nucleophiles due to steric hindrance.



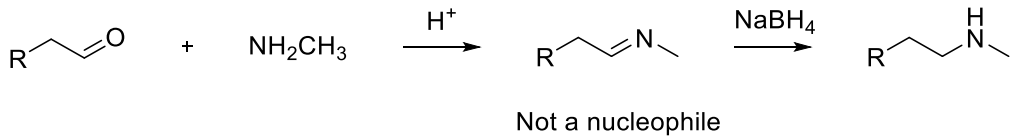
It can be difficult to selectively add amines into organic compounds due to the increasing strength of the amine as a nucleophile with respect to the addition of aliphatic carbon chains. Once one alkyl group has been added, that product is now a stronger nucleophile than the original amine and can react with the electrophile. This leads to the formation of multiple cross-alkylated products, making single substitutions difficult to achieve via traditional nucleophilic substitution, as shown in scheme 6.3.

Scheme 6.3: The difficulty in achieving a single substitution with alkylated amines.



The increased nucleophilicity of the amine can be mitigated by instead reacting it with a carbonyl (ketone or aldehyde) to form an *imine,* then reducing the product to the corresponding amine, shown in scheme 6.4. This is known as *reductive amination*.

Scheme 6.4: A reductive amination allows the addition of a single amine.



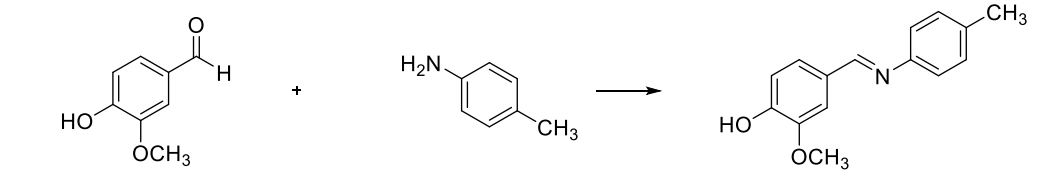
It is no longer possible for the nitrogen to act as a nucleophile once the imine has formed because the imine cannot attack the carbonyl group of the starting material. The nitrogen is now locked into place and no side reactions are possible.

The imine intermediate is easily converted to an amine using a simple reduction. Reductive amination avoids the formation of undesirable side products and allows for regioselective control to form the desired product.

Even if a selective monoamination can be achieved, amine-based products remain inherently reactive species and often decompose when stored for long periods of time. Chemists usually choose to store these compounds in other forms to minimize degradation. A very common technique is to react the final product with acid to generate an ammonium salt (i.e. $NR_3H^+Cl^-$), which renders the amine no longer a nucleophile. A second option is to add a protecting group to the amine nitrogen. This is typical for complex syntheses in which the reactivity of the amine is undesirable so that another functional group on the molecule can be manipulated.

In this experiment, you will carry out a reductive amination between *para*-toluidine, an aromatic amine, and vanillin, the active ingredient in vanilla. The reaction between these two compounds is done under solvent-free conditions, an emerging trend in organic chemistry due to its environmental benefits (this is sometimes referred to as *green chemistry*). The reaction scheme is shown below in scheme 7.5.

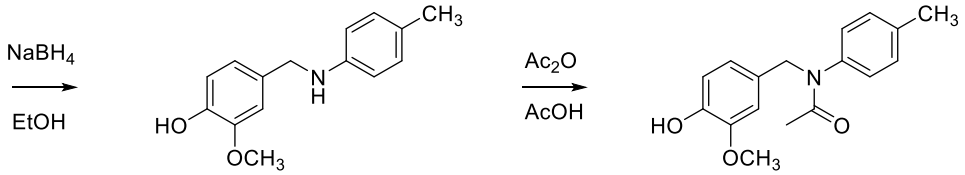
Scheme 6.5: The overall reaction scheme.



vanillin
M.W. 152.15 g/mol
m.p. 40 - 42 °C

p-toluidine
M.W. 107.15 g/mol
m.p. 41 - 46 °C

imine intermediate, **1**
m.p. 102-103°C



amine product, **2**

amide product, **3**
M.W. 285.34 g/mol
m.p. 102 - 108 °C

The imine intermediate, **1**, is formed quickly (5 – 10 min) and is easily observable through colour change. Although imines are often unstable, the product of this reaction is relatively inert and can therefore be characterized before the reduction step. You will reduce the imine with sodium borohydride and subsequently acetylate the newly-formed amine, **2**, with acetic anhydride in glacial acetic acid to form the more stable amide, **3**. All of these steps can be carried out rather quickly and in one beaker, highlighting the convenience of this ''one-pot synthesis''. The final product is easily isolated by precipitation with cold water after the reaction is complete. You will evaluate the progress and purity of each reaction by TLC and melting point analysis.

EXPERIMENTAL PROCEDURE

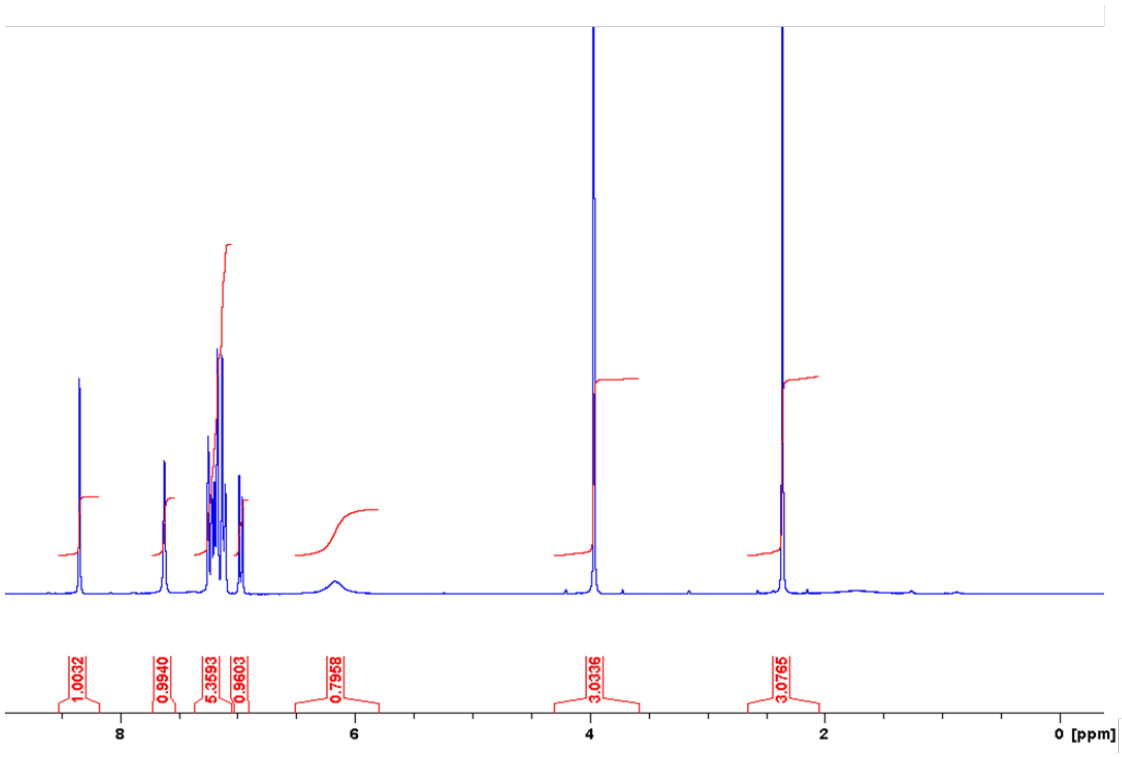
The following experimental procedure has been provided from a peer-reviewed chemical research journal. You are asked to replicate this procedure, submitting your amide product to your TA. Your product will be evaluated based on colour, composition, TLC, melting point, and yield. If time remains in your lab period, you may choose to repeat the synthesis for optimization, submitting the ***best*** *of two samples to your TA. (DO NOT combine samples)*

''Vanillin (0.58 g, 3.8 mmol) and para-toluidine (0.40 g, 3.8 mmol) are combined in a 250 mL beaker, and the solids are mixed with a Teflon stir rod to afford a dry, bright orange powder. (crude 90-95%, m.p. 102-103˚C). The imine is subsequently reduced and then converted to the more stable amide by acetylation. The reduction is accomplished by the addition of 15 mL 95% ethanol to the beaker containing the imine, followed by the addition of $NaBH_4$ (0.1g, 2.7 mmol) in small increments to the stirred reaction mixture. The imine is only partially soluble in ethanol, but the product is quite soluble. Within 10 minutes, a colourless solution is an indication that the reaction is complete. Glacial acetic acid (2.0 mL) is added to the ethanoic solution followed by 2.0 mL acetic anhydride, and the reaction mixture is warmed on a steam bath for 5-10 minutes. The amide product precipitates out when 75mL of cold water is added slowly. White crystals are collected by vacuum filtration and analyzed by TLC and melting point. (crude 80-90%, m.p. 127-128˚C.)''

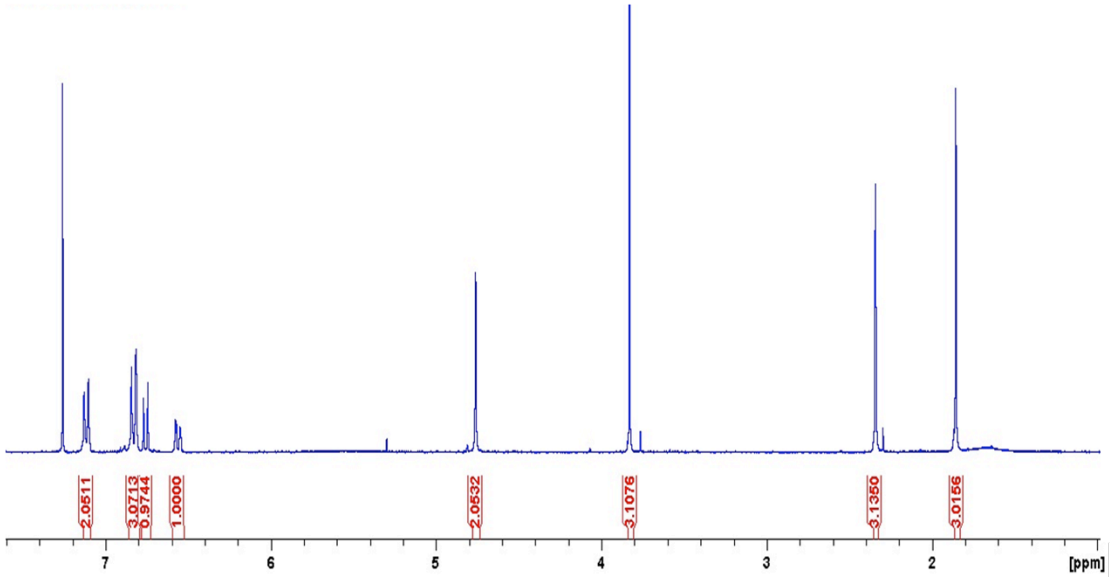
Using hexanes and ethyl acetate, develop a co-solvent that will provide optimal resolution of your starting materials. (Target a system in which the slower compound has an R_f value of 0.3) Use this co-solvent system to determine R_f values for your starting materials, two intermediates, and final product.

Below are the ^{1}H NMR spectra of two compounds (the imine and the amine) that you have synthesized in this experiment. For each NMR, assign each peak to the corresponding hydrogen(s). Identify which spectrum corresponds to which compound.

NMR Spectrum A:



NMR Spectrum B:



REPORT (60 POINTS)

Your report must include the following sections, including a **detailed experimental procedure**.

- Title page (1 point)
- Introduction (6 points)
- Mechanism (6 points)
- Table of reagents (2 points)
- Experimental procedure (6 points)
- Observations and Results (5 points)
- TLC plates (4 points)
- Yield Calculations (5 points)
- Discussion (15 points)
- NMR assignment (10 points)

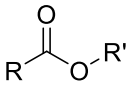
EXPERIMENT 7

FISCHER ESTERIFICATION

SUGGESTED READING: The sections covering **ESTERIFICATION, and NUCLEOPHILIC ACYL SUBSTITUTION** in the Organic Chemistry textbook of your choice.

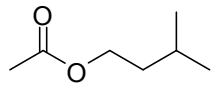
INTRODUCTION

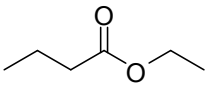
Esters are a class of compounds widely distributed in nature. They have the general formula shown below and are typically formed from a carboxylic acid (RCO_2H) and an alcohol (R'OH).

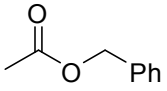


Simple esters tend to have pleasant odours. In many cases the characteristic fragrances associated with flowers and fruits are due mainly to simple compounds containing an ester as the only functional group. Small structural changes give distinctly different fragrances. Examples of esters which are associated with common fruits are shown in figure 7.1 in below.

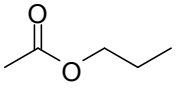
Figure 7.1: A variety of esters and their associated scents.

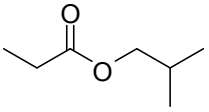

isoamyl acetate
banana

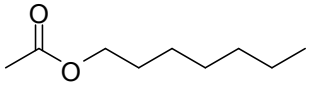

ethyl butyrate
pineapple

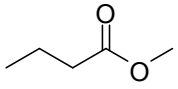


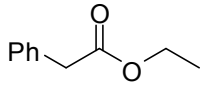
benzyl acetate
peach


n-propyl acetate
pear

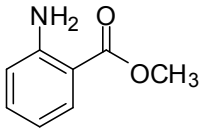

isobutyl propionate
rum


n-octyl acetate
oranges

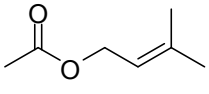

methyl butyrate
apple



ethyl phenylacetate
honey



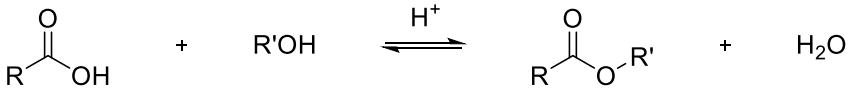
methyl anthranilate
grape


isopentenyl acetate
"juicy fruit"

Natural fragrances and flavours are due to complex mixture of compounds. Food and beverages manufacturers use compounds prepared in the laboratory as food additives to enhance the appeal of their products. Good quality imitations or synthetic flavours are generally a combination of many compounds which attempt to mimic the natural fragrance.

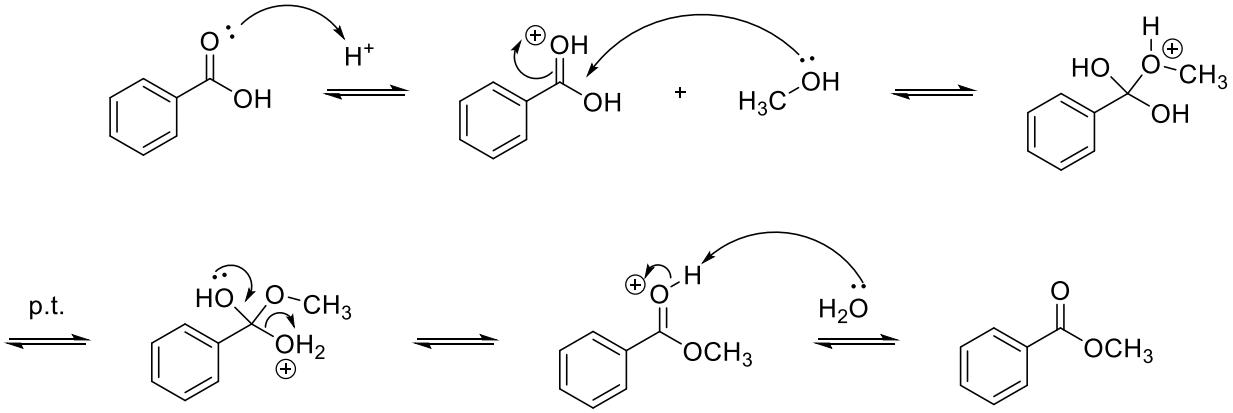
Simple esters are usually made by "direct" esterification of an acid by an alcohol in the presence of an acidic catalyst according to the reaction shown in scheme 7.2:

Scheme 7.2: Esterification of a carboxylic acid with an alcohol



The Fischer esterification reaction is initiated by protonation of the carboxylic group, followed by the addition of the alcohol to give the key tetrahedral intermediate, proton transfer, elimination of water and finally deprotonation. The mechanism of the esterification of benzoic acid with methanol is shown in scheme 7.3.

Scheme 7.3: Acid catalyzed esterification of benzoic acid with methanol.



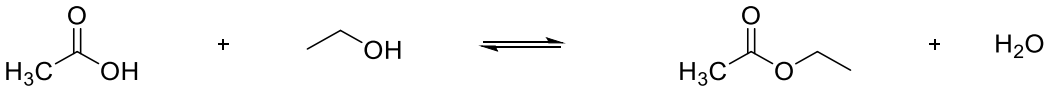
As indicated above, esterification is a reversible reaction. The reaction has obtained its maximum yield of product once equilibrium is achieved between the four components of the chemical equation. The composition at equilibrium is given by the law of mass action:

$$K = \frac{[ester][H_2O]}{[acid][alcohol]}$$

The yield of the reaction is governed by the concentration of the reactants and products. The yield can be manipulated by increasing the relative concentration of one reactant over the other (for example, using an excess of alcohol, as in this experiment) to force the equilibrium to favour the products.

It has been determined, for example, that the reaction between ethanol and acetic acid to give ethyl acetate and water has an equilibrium constant K=4. If the reaction is performed using equimolar amounts of alcohol and acetic acid, the concentrations of the reagents at equilibrium can be summarized as in table 7.3.

Table 7.3: An example equilibrium calculation for the esterification of acetic acid with ethanol.



Initial	1 mol	1 mol	0	0
Change	- x	- x	+ x	+ x
Equilibrium	1 - x	1 - x	x	x

Solving for x in the equilibrium above gives x = 0.67, and thus a theoretical yield of ethyl acetate of 67%. If one were to increase the amount of alcohol relative to the acid, the overall yield of the ester would increase, based on conversion of the limiting reagent, the carboxylic acid. An example starting with 2 moles of ethanol and 1 mole of acetic acid would give x = 3.16 and 0.845 with only the latter value being physically possible. Under these conditions, the yield based on the limiting reagent, acetic acid, is 85%. If a 5-fold excess of ethanol were used, the yield of ethyl acetate at equilibrium based on acetic acid would approach 95%.

The percent yield of equilibrium reactions is calculated under the assumption that the reaction went to completion. The theoretical yield is calculated using the stoichiometric equation, considering that the limiting reagent was completely converted to product.

There are generally no side reactions in esterification, if properly performed. Improper mixing and spot-overheating, particularly when sulfuric acid is used as catalyst may cause charring, ether or alkene formation, oxidation and, in specific cases, decarboxylation. The product is, however, always contaminated with the starting materials remaining in the mixture due to the nature of the reaction. Depending on the structure, the contaminants are removed by washing, drying or distillation.

In this experiment, you will prepare methyl benzoate from methanol and benzoic acid. You will also determine the equilibrium constant K based on the recovery of unreacted benzoic acid.

EXPERIMENTAL PROCEDURE

Fischer Esterification

1. Weigh a dry 50 mL round bottom flask and dissolve 5 g of benzoic acid in 15 mL of methanol in the flask. Place a magnetic stir bar into the flask and clamp the flask above a stirrer in the fume hood.
2. Very slowly, add 1.0 mL concentrated sulfuric acid to the reaction flask while stirring.
3. Fit the flask with a reflux condenser and, using a heating mantle, reflux the solution gently for 60 minutes.
4. Cool the solution to room temperature and transfer the contents to a separatory funnel containing 25 mL water.
5. Rinse the reaction flask with about 25 mL of dichloromethane and add this to the separatory funnel. Draw off the aqueous phase and set aside.
6. Wash the organic phase with 25 mL water, then 25 mL 5% $NaHCO_3$ solution, and finally with 20 mL saturated NaCl solution.
7. Combine aqueous phases and acidify with dropwise addition of concentrated hydrochloric acid. When sufficient acidity is established, unreacted benzoic acid will precipitate out.
8. Isolate unreacted benzoic acid by suction filtration, washing the filter cake with cold H_2O. Leave the crystals to dry until the end of the lab period, and record their mass. You will need this value for K_{eq} calculations.
9. Drain the organic layer into a clean 125 mL Erlenmeyer flask and dry it over anhydrous Na_2SO_4 for a few minutes.
10. Filter the organic layer with a filter funnel into a pre-weighed 50 or 125 mL round bottom flask.
11. Attach a 3-way adapter and condenser with thermometer, and distill off the solvent.
12. Record the mass of the remaining residue, and determine the yield methyl benzoate. Note the odor of the ester.
13. Determine the K value of the ester using both (a) the mass of residual benzoic acid plugged into the K_{eq} equation, and (b) the yield of methyl benzoate. Compare your observed K value with the theoretical K value. What does your K value signify? Is it much greater than 1? Much less than 1? Approximately equal to 1?

Spectroscopic characterization:

Using ^1H NMR spectroscopy, explain how you would establish a successful synthesis and isolation of your methyl benzoate product. What peaks would you anticipate if your product was impure? Construct a table containing chemical shifts, integration, and multiplicity for anticipated peaks with a clear description of what peak corresponds to what proton. It is recommended to label protons A-Z on a drawing of the molecule, and include a column for the label in your table.

Using IR spectroscopy, explain how you would establish a successful synthesis and isolation of your methyl benzoate product. What bands would you anticipate if your product was impure? Construct a table containing wavenumber, intensity, and type of IR vibration for important parts of the molecules.

REPORT (56 POINTS)

Your report must include the following sections:

- Title page (1 point)
- Introduction (6 points)
- Table of reagents (3 points)
- Experimental procedure (1 point)
- Observations and Results (4 points)
- Calculations (6 points)
    - Theoretical K value
    - Observed K value
    - Yield
- Flow chart (10 points)
- Discussion (15 points)
- Spectroscopic analysis (10 points)

EXPERIMENT 8

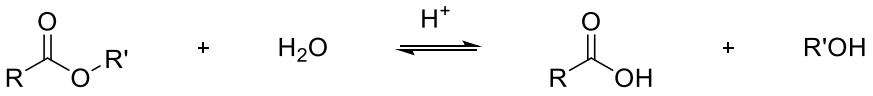
THE HYDROLYSIS OF AN UNKNOWN ESTER AND THE STUDY OF FATS AND DETERGENTS

SUGGESTED READING:

INTRODUCTION

Ester hydrolysis can be accomplished under acid or base catalysis. The general acid-hydrolysis reaction is shown in scheme 8.1 below.

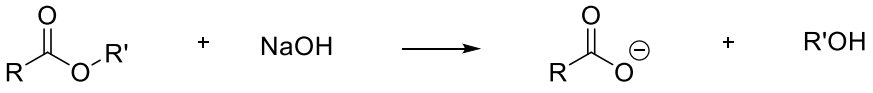
Scheme 8.1: The acid catalyzed hydrolysis of an ester.



Under acidic conditions, the reaction is in equilibrium, and a mixture of reagents and products is obtained. The distribution depends on the equilibrium constant and on the initial reactant quantities. Unless an excess of water is added, a large quantity of the ester remains at equilibrium. Since the majority of esters are insoluble in water, this synthetic approach can be impractical and inefficient.

Saponification is instead the name given to base-promoted ester hydrolysis. The general reaction is shown in scheme 8.2 below:

Scheme 8.2: The base promoted hydrolysis of an ester.



It is most advantageous to use a base (rather than an acid) to effect ester hydrolysis because the reaction is essentially irreversible. Once the carboxylic acid has formed, it immediately reacts with the base in the reaction mixture to give the initial products of the reaction, the carboxylate and the alcohol.

Consequently, the acid is continually eliminated from the reaction mixture. Looking at the stoichiometry of the reaction, a full equivalent of base, rather than a catalytic amount, is required for the reaction.

The **neutralization equivalent** is the quantity of ester, in grams, that reacts with an equivalent of base (in moles) in a saponification reaction. If a mono-ester is being hydrolyzed/saponified, the neutralization equivalent corresponds to the molar mass of the ester. The formula for calculating the neutralization equivalent is as follows:

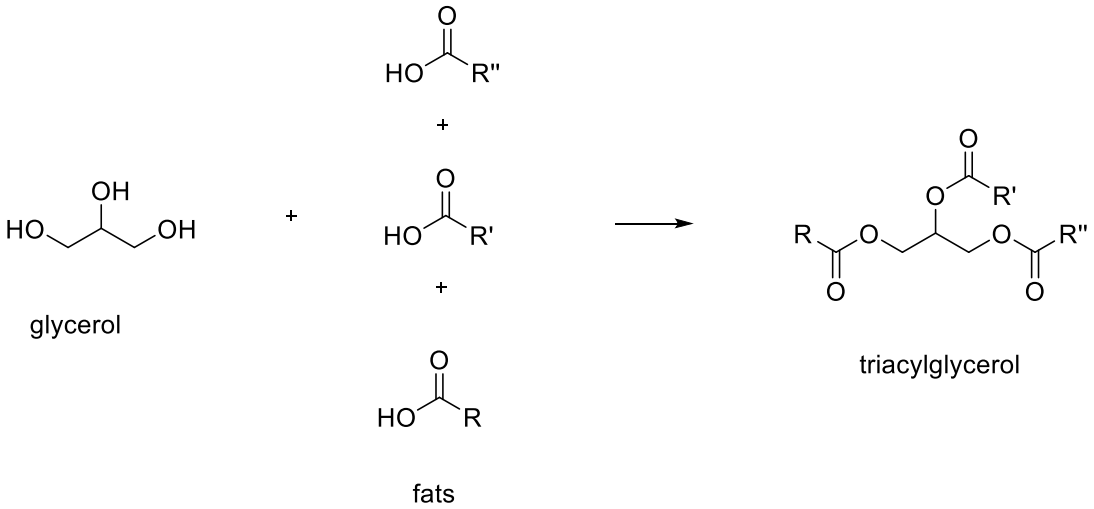
$$Neutralization\ equivalent\ \left(\frac{g}{mol}\right) = \frac{mass\ of\ ester\ (g)}{moles\ of\ base\ required\ for\ neutralization\ (mol)}$$

In this reaction, a known mass of the ester reacts with an **excess** of a base whose molarity is known. Once the reaction is complete, the quantity of base remaining in solution is determined by back titration with a solution of acid of known concentration. The number of moles of base that reacted with the ester can then be calculated. In the case of a mono-ester, this value also represents the number of moles of ester. Once the mass and number of moles of ester has been determined, the molar mass of the ester can be calculated. Your goal is to calculate the molar mass of an unknown ester.

Fats and detergents

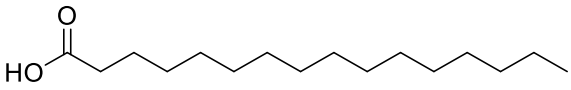
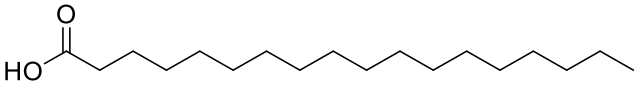
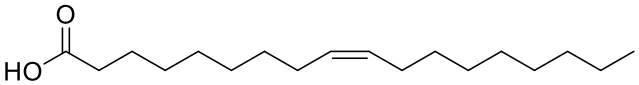
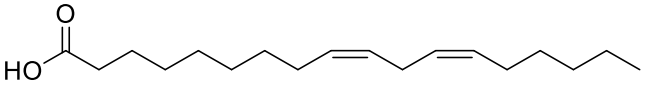
Most carboxylic acids of biological origins are derived from esters of glycerol. Glycerol reacts with fats to form triacylglycerols, as shown in scheme 8.3 below:

Scheme 8.3: Synthesis of a triacylglycerol.



A variety of long chain carboxylic acids are attached to glycerol in typical fats. The diversity of structure of these acids gives rise to different physical properties. If the attached acids are mainly saturated acids, such as palmitic ($C_{16}H_{32}O_2$) or stearic ($C_{18}H_{36}O_2$) acid, then the glycerides are solid at room temperature. Common examples are lard and hydrogenated vegetable oils. Soft or liquid fats contain instead significant amounts of unsaturated acids, such as the monounsaturated oleic acid ($C_{18}H_{34}O_2$) and the di-unsaturated linoleic acid ($C_{18}H_{32}O_2$). Notice that the double bonds in these compounds have the *cis* geometry which hinders stacking, decreasing Van der Waals interactions, and therefore lowering the melting point.

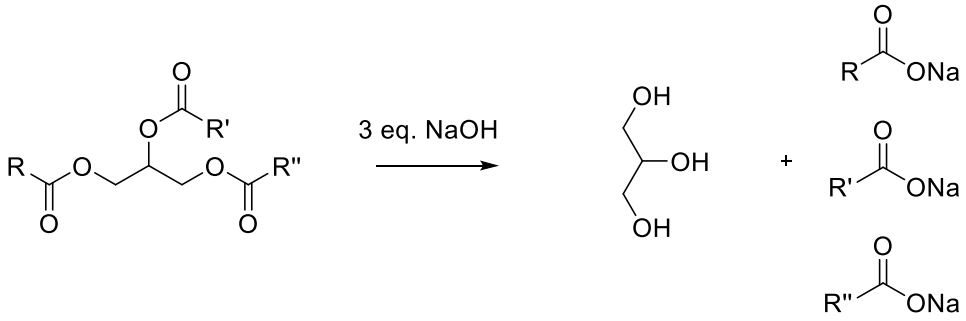
Scheme 8.4: The structure of some common fats.

Fat	Number of carbons	Structure
Palmitic Acid	16	
Stearic Acid	18	
Oleic Acid	18	
Linoleic Acid	18	

Saponification of triacylglycerols

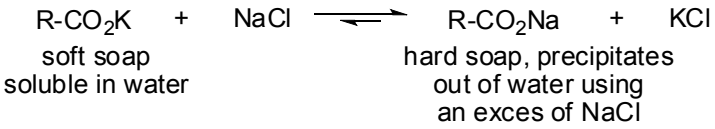
Saponification, or hydrolysis, occurs when glycerides are heated with potassium or sodium hydroxide, the ester functional groups are cleaved to produce glycerol and the potassium or sodium salts of the carboxylic acids as shown in scheme 8.5 below.

Scheme 8.5: The hydrolysis of triacylglycerol.

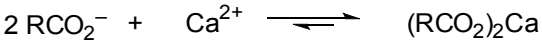


The mixture of the salts obtain are known as soaps. The sodium salts are generally solids or flakes and can be pressed into soap bars or other convenient shapes. In the soap industry, manufacturers add other key ingredients such as perfumes and colouring dyes. These additives make the soap more appealing to consumers, but do not affect its cleansing action.

The potassium salts are quite soluble in water, and are typically syrupy liquids, called soft soaps or liquid soaps. The sodium salts, on the contrary, have only partial solubility in water. An increase in the concentration of sodium ions in the solution, for example by addition of sodium chloride, causes the 'sodium soap' to precipitate because its solubility product is exceeded. This process, called 'salting out' is a useful process for obtaining hard soap from a soft soap solution.

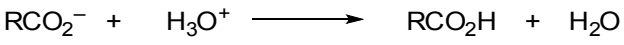


Calcium and magnesium salts of soap-forming carboxylic acids have very low solubility in water. This precipitation is observed when soap in used in 'hard water', and it greatly reduces the effectiveness of soap under these conditions.

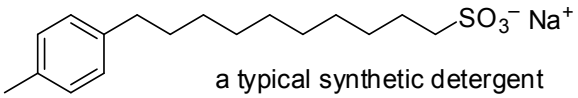


In order to act as desired, soaps must be partly soluble in water. As pointed out above, hard water, which contains higher concentrations of calcium and magnesium ions, causes the detergency to be greatly reduced or even lost. The detergency can be restored by addition of phosphates or carbonates which bind more strongly to these metal ions than carboxylates.

Soaps are also ineffective in strongly acidic conditions. An acid-base reaction occurs to produce the long chain carboxylic acid which is insoluble in water. Since soaps are mixtures of carboxylate salts, the mixture of acids formed is usually a semi-solid or an oily mass, and it has no significant detergency.



The salts of alkylbenzenesulfonic acids are known as synthetic detergents. The advantage of these detergents over common soaps is that they are equally effective in the presence of both hard and soft water. The Mg^{2+} and Ca^{2+} salts of these sulfonic acids have good solubility on water.



a typical synthetic detergent

The important property of soaps is their detergency, their ability to decrease the surface tension between water and water insoluble materials (greasy dirt). The soap molecules are equipped with both hydrophilic (the carboxylate ion) and hydrophobic (the long hydrocarbon chain) parts. The hydrophobic chain entraps the grease, while the hydrophilic carboxylate ion is soluble in the water. The result is an emulsion that lifts the dirt off a surface and allows it to be washed away. A typical compound capable of forming an emulsion in water, and thus acting as a soap, has a polar head, generally ionic, and a carbon chain of at least 10 – 12 carbons.

In this experiment, you will prepare a soft soap solution through the saponification of a fat and a solution of hard soap by the process of salting out the soft soap produced. You will also perform detergency tests to compare your soaps to that of a commercial detergent.

EXPERIMENTAL PROCEDURE

It is essential that you organize your time in order to finish the experiment within 3 hours. We suggest doing the experiments in the following order:

a) *Prepare the soft soap (30 minutes of reflux, followed by a distillation).*
b) *Prepare and normalize the alcoholic NaOH solution during the saponification of the fat. Set up the apparatus for the ester hydrolysis.*
c) *Once (a) is complete, rinse the condenser with cold, distilled water and attach the flask containing the unknown ester and alcoholic NaOH. Reflux this mixture.*
d) *While reflux (c) is proceeding, perform the detergency tests.*
e) *Complete the determination of the neutralization equivalent.*

Part A: Base promoted ester hydrolysis.

1. Mix 12 mL of 9 M NaOH stock solution with 100 mL of 95 % EtOH in a 250 mL Erlenmeyer flask; Cover the flask to avoid evaporation.
2. Determine the exact molarity of the NaOH solution by titrating exactly 10.00 mL of this solution against an available standard solution of HCl (0.500 M) using phenolphthalein as indicator. Do this titration at least twice. If your trials differ by more than 1%, repeat the titration a third time.

3. Accurately weigh 1.0 – 1.3 g of the unknown ester into a clean 100 mL round bottom flask.
4. Add exactly 20.0 mL of the NaOH solution from step 2 (a graduated cylinder is not the appropriate way to measure this volume).
5. Clamp the round bottom flask above a magnetic stirrer in the fume hood and place a magnetic stir bar in the flask. Attach a reflux condenser to the flask and reflux the solution for 30 minutes using a heating mantle.
6. After 30 minutes, remove the flask from the heat and allow the mixture to cool to room temperature. Add 20 mL of distilled water through the top of the condenser.
7. Add a few drops of phenolphthalein indicator into the mixture and titrate the reaction mixture against the HCl solution (0.500 M).
8. Calculate the neutralization equivalent.
9. Propose a few plausible structures for the ester, known that the compound contains only one ester group and that it is saturated. Give the molecular formula.

Part B: Fats and detergents

Preparation of a soft soap

1. Dissolve 5 mL of 50 % KOH solution (**USE CAUTION**) in 40 mL EtOH in a 100 mL round bottom flask. Clamp the flask in the fume hood above a magnetic stirrer and place a magnetic stir bar into the flask.
2. Add 5 g of fat to the round bottom flask and attach a reflux condenser to the flask. Heat the flask under reflux for 30 minutes.
3. Replace the condenser with a distillation set-up and distill the reaction mixture until about 20 mL of thick residue remains in the flask. Discard the distillate, mainly ethanol, in the organic waste.
4. Dissolve the thick residue in distilled water to make about 80 mL of soft soap.

Preparation of a hard soap

1. Add an equal volume of saturated NaCl solution to about 10 mL of your soft soap solution in a small beaker. Let the mixture sit for a few minutes and a precipitate of hard soap should form. Carefully decant off the liquid so that only solid remains.
2. Use a spatula to remove this solid onto a paper towel. This will absorb most of the remaining water.
3. Test the properties of the soap by rubbing some between your fingers and by placing a small amount in a test tube and shaking it with some distilled water. Record your observations.

Detergency tests

1. Prepare or obtain about 10 mL of a 1% solution of the commercial synthetic detergent.
2. Conduct the tests in steps 3 and 4 to solutions (a) and (b) listed below and note your observations in a table after each step:

a. 5 to 10 mL of soft soap solution in a test tube
b. 5 to 10 mL of detergent solution in a second test tube

3. Add about 1 mL of calcium chloride to each test tube and shake. Note your observations.
4. Add about 2 mL of 0.5 M trisodium phosphate solution to each test tube and shake. Note your observations.

Acid/Base properties of soaps

1. Add about 5 mL of your soft soap solution to a test tube.
2. Add 10 % sulfuric acid solution until the solution becomes acidic. Verify with pH paper.
3. Note your observations (compare foaming action before and after acidification)
4. Heat the test tube to about 80 °C. Note your observations.

REPORT

You must hand in the following work sheet to your TA at the end of the lab period.

CHM2123: EXPERIMENT 8 – HYDROLYSIS OF ESTERS, AND FATS AND DETERGENTS

(38 points)

Name: _____ TA: _____

Student #: _____ Date: _____

Introduction (8 points)

Part A- Ester hydrolysis

(objective(s), theory, methodology)

Part B – Fats and Detergents

(objective(s), theory, methodology) - save mechanisms of part B for discussion

Tables of Reagents (4 points)

Part A

Compound	MW (g/mol)	Quantity	Density (g/mL)	mmol	Equivalents

Procedure (2 points)

Observations (4 points)

Part A – Hydrolysis of an Unknown Ester

Important Steps	Observations

Part B – Fats and Detergents

Important Steps	Observations
Preparation of a Hard and Soft Soap	
Detergency and acid-base properties of soaps	

Results (4 points)

Part A:

Concentration of HCl = _____ M

Standardization of NaOH	**trial 1**	**trial 2**	**trial 3**	**Average**
V_{NaOH} (mL)				
$V_{HCl\ init.}$ (mL)				
$V_{HCl\ final}$ (mL)				
ΔV_{HCl} (L)				
moles H^+ (mol.)				
[NaOH] (M)				

Saponification of an Unknown Ester

m_{ester} (g)	
$V_{NaOH\ soln}$ (mL)	
reflux time (min.)	

Back Titration to Determine Remaining NaOH

V_{H2O} (L)	
Drops of phenolphthalein	
$V_{HCl\ init.}$ (mL)	
$V_{HCl\ final.}$ (mL)	
ΔV_{HCl} (L)	
moles H^+ (mol.)	
Equivalents ^-OH consumed	
Neutralization equivalent (g/mol)	

Possible structure(s) of unknown ester (**2 points**)

Part B:

Preparation of Soft and Hard Soaps

m_{fat} (g)	
$V_{KOH\ soln}$ (mL)	
$[KOH]_{soln.}$ (M)	
reflux time (min.)	
$V_{NaCl\ soln}$ (mL)	
$V_{soft\ soap}$ (mL)	

Calculations (4 points)

Part A: Standardization of NaOH

Neutralization Equivalents of Unknown Ester

Discussion (10 points)

Part A

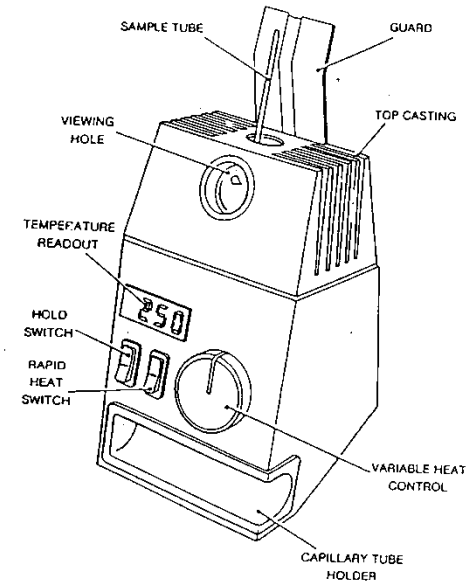
Part B

(reactions and mechanisms for soap tests)

This template document is to be submitted to your TA at the end of your experiment.

This report will be corrected and available for pick-up from your TA in MRN301 from ***12:30-1:00pm on Friday, December 8th****.*

APPENDIX I



GENERAL VIEW

Controls

1. **Variable Heat Control**. This is the large yellow knob on the front of the case. It incorporates the ON / OFF switch, and when rotated to the "ON" position, both the temperature display and the sample illumination lamp should light up. Turned past the "ON" position, the knob controls the amount of energy delivered to the block and has a range suitable for operation from room temperature up to 350 °C.

   NOTE: Control position 0 does not equate to zero power input to the heater. Left at 0, the block temperature will in time rise to about 50 °C, so always switch off when not in use.

2. **Rapid Heat Switch**. Do not use this control.

3. **Hold Switch**. This is at the left below the temperature display. When this switch is set to the OFF position, the temperature display shows the actual thermometer temperature. When it is set to the ON position the temperature displayed is held at the temperature of the thermometer at the time of switching. A lamp in the switch bezel lights up to remind the user that the display is held. For safety reasons, the switch should always be returned to the OFF position as soon as the hold temperature has been recorded.

4. **Background Screen Control**. The background color is controlled by the small yellow button on the rear of the casing. It slides from side to side, giving a light or dark background, as required to give optimum contrast for the sample.

Procedure. Melting point determination is in principle straightforward, but optimum results will only be obtained through the exercise of care and technique.

1. Load sample crystals into a capillary tube to a depth of no more than 1 mm by pushing the inverted tube onto the crystals. Better results will be obtained with a **small** sample. Take just enough so you can see the sample in the tube. Turn the tube right-side up (opening is up) and tap the bottom of the tube on the bench until the sample crystals are at the bottom of the tube (sealed end).
2. Insert the sample tube(s) into the top of the heating block. The block will hold up to three tubes. Be sure to keep track of the sample(s).
3. Turn on the instrument and watch the sample through the magnifying glass. Record the temperatures at which melting starts and stops. This is the melting range. If the approximate melting point of the sample is not known, it will be quickest to approximately find it by heating the block rapidly (turn the control to 6 or 7 initially) and noting where the sample melts. Turn off the instrument; allow it to cool until about 30 °C below the melting temperature. Place a fresh sample in the block and determine the melting point, this time allowing the temperature to increase slowly. An ideal rate is about 2 °C per minute. Remember that at high heating rates a difference will exist between the thermometer reading and the sample temperature of up to as much as 15 °C when rapid heat is in use. The apparent melting temperature is usually a little high.

APPENDIX II

RECRYSTALLIZATION

Recrystallization is perhaps the most important method of purifying organic compounds. Its use in the research lab has declined somewhat since flash chromatography was developed, however recrystallization is still the best way to obtain ultra-pure samples of organic materials, and to purify large quantities. Recrystallization is used extensively in industry because it is scalable – it works with 10 mg or 10 tons of material. Most pharmaceuticals are manufactured using recrystallizations to purify intermediates and final products.

Procedure

Recrystallization makes use of the differential solubility of the compound to be purified in hot and cold solvents. The process consists of dissolving the impure material in hot solvent, filtering the solution to remove impurities, allowing the desired compound to crystallize by cooling, and finally recovering the crystals. In practice, recrystallization requires that the substance has undergone some kind of preliminary purification. The technique will not work well if it is done with very impure material.

Solvent

The most important factor in recrystallization is the solvent. Choosing a solvent requires experience, but there are guidelines that can be followed to help make a reasonable choice.

The most important consideration is solubility. The substance to be purified should have high solubility in the solvent at elevated temperatures and low solubility at low temperature. Solvents can be screened by placing a small sample of the compound to be crystallized (~50 mg) in a test tube and shaking it with about 0.5 mL of the solvent. If the compound dissolves it is too soluble in that solvent for a good crystallization. If the sample does not dissolve, heat the solvent to the boiling point (**never** use a flame for this). If necessary add small portions of solvent, maintaining boiling, until the sample dissolves. If more than 3 mL of solvent is required to do this, the substance is probably not soluble enough in that solvent. If the sample dissolves using less than 3 mL of solvent, then the solvent is a good candidate and you can proceed.

The solvating power towards impurities must also be considered. The solvent should readily dissolve the impurities, or not dissolve them at all. If the impurities are soluble, they will remain in solution as the crystals of the desired compound are forming. Filtration will give the desired compound and leave the impurities behind in solution. If the impurities are insoluble, once the desired compound has dissolved in the hot solvent the solution can be filtered while still hot thus removing those impurities.

A chemical substance can crystallize in more than one form depending on conditions. These different crystal forms are called polymorphs, and are a huge issue in the pharmaceutical industry. Crystal form affects purity (see below), and so ideally a solvent should be chosen to produce a “well formed” crystal. Judging crystal form requires experience. In general, crystals with a well-defined shape are often of higher purity than amorphous lumps or grains.

Finally, the solvent should be easy to remove from the crystal. Small amounts of solvent stick to crystals after filtering. This normally is removed by "drying" the crystals, usually under reduced pressure. Basically this means that either the solvent must be volatile, or it must be easily washed away with a second, more volatile solvent.

The choice of solvent can be made based on the principle of "like dissolves like". For example, if you are recrystallizing a polar compound, you will most likely need a polar solvent. Common solvents for crystallization are listed below in order of decreasing polarity.[2] The boiling point of the solvent must also be considered. If the boiling point of the solvent is higher than the melting point of your compound, you will likely have problems with "oiling out". As the hot solution cools, the compound becomes insoluble in the solvent. If this happens above the melting point of the compound, the material will separate as a liquid (called an oil) and not as a solid. The oil will sometimes solidify on standing, but because this solid is not formed from a solution the purity will usually be low (impurities are often soluble in the oil and are trapped when it solidifies).

The ideal solvent satisfies the following criteria:

- Non-reactive
- Boiling point lower than the melting point of the crystal
- Dissolves a large quantity of product when hot
- Dissolves a small quantity of product when cold
- Easily removed by evaporation
- Non-toxic, non-flammable, and cheap
- Either readily dissolves impurities or does not dissolve them at all
- When a mixture of solvents is used, they must be **miscible**

[2] Ethyl ether is sometimes used for recrystallization, but it is a very poor choice. Ether is very flammable, has a low boiling point and tends to creep up the walls of glassware and deposit materials after evaporation. Some of the older literature recommends the use of isopropyl ether. This is a very dangerous solvent that has no place in a modern lab.

Solvent	Boiling Point (°C)
Water	100
Methanol	65
Ethanol	78
Acetone	56
Ethyl Acetate	78
Chloroform	61
Methylene Chloride	40
Benzene	80
Toluene	110
Carbon Tetrachloride	77
Petroleum ether (ligroin)	Varies

Crystal Formation

Besides the nature of the solvent, cooling rate has the highest impact on the success of the technique. Rapid cooling tends to produce impure material as the crystals simply grow around pockets of solvent and impurities. Slow cooling is best. As the solution cools crystals begin to form. Once these small crystals are available, molecules move out of solution and onto the surface of the crystals joining the lattice in an ordered way. Occasionally a molecule will pack imperfectly onto the crystal lattice. Equilibrium conditions (slow cooling) allow these imperfectly packed molecules to re-dissolve thus "fixing" the crystal defects and preventing the formation of solvent pockets.

This mechanism "selects" the proper molecules. As the whole process is random, occasionally an impurity molecule becomes incorporated into the crystal. These impurity molecules usually will not "fit" the crystal lattice and so will readily re-dissolve, provided that cooling is slow enough so that the defect does not become covered in additional molecules. In other words, defects correct themselves if the crystal growth is in equilibrium with the solution. Slow crystallization, from an undisturbed solution, will provide the purest crystals.

Oiling out

Oiling out can be avoided by choosing a solvent with a lower boiling point. If that is not possible, try "scratching" or "seeding" the mixture as it cools. Scratching the walls of the flask with a glass rod produces small defects in the glass around which crystals can grow. Seeding involves adding a small crystal of the desired compound. This serves as a nucleus or "seed" around which larger crystals can form. Seed crystals can be obtained by evaporating a small amount of your solution on a watch glass.

If oiling out cannot be prevented, you can allow the oil to solidify then filter this solid from the solution. Sometimes this removes enough impurities so that a second recrystallization will be successful.

Removing impurities

Steps must be taken to remove impurities before inducing crystal formation. In the case of insoluble materials and dust, the solution is filtered just after the product has dissolved. When doing this, it is important to keep the solution hot as it is filtered. Add a small amount of extra solvent just before filtering and heat the funnel you will use. You can pre-heat a funnel by placing it on top of your boiling flask. The hot solvent vapors will often do the job. Choose a short stem funnel (a powder funnel is a good choice) or use vacuum filtration. Hot filtering usually works best with porous filter paper (Whatman #1). If your material crystallizes in the funnel, try heating the receiving flask or washing the funnel with some hot solvent.

Crude materials often contain colored impurities. The vast majority of organic solids are white, but most reactions generate small amounts of aromatic, colored impurities as side products. These compounds can be removed by using decolorizing carbon (also called Norit or charcoal). The decolorizing carbon adsorbs these impurities and removes them from solution. The decolorizing carbon is added to the hot solution and is then filtered off while the solution is still hot. It is important to use a small amount of decolorizing carbon as using too much may remove some of the desired product.

Mixed Solvent Recrystallizations

Sometimes a single solvent will not give an effective recrystallization. In cases like these, you can use a mixed solvent or solvent pair. A solvent pair consists of two *miscible* liquids, one in which the compound readily dissolves and one in which it does not. The compound is dissolved in the minimum amount of hot solvent in which it is soluble. This is usually done by adding some of the "good" solvent (in which the compound is soluble), heating to the boiling point and then adding additional solvent slowly until the compound just dissolves. The second solvent (in which the compound is insoluble) is then slowly added until the solution becomes turbid (cloudy). This addition is done while the mixture is boiling. A few drops of the first solvent are then added until the solution again becomes clear. The flask is then removed from heat and allowed to cool.

Additional crops

Recrystallization does not remove the entire compound from solution. The liquid that remains after filtration may still contain a significant amount of material. This liquid is called the *mother liquor* (because it is the solution that gave birth to the crystals). In many cases additional material can be recovered from the mother liquor by evaporating some or all of the solvent and performing a second recrystallization. The crystals that are obtained in this manner are called the *second crop*. The first crop of crystals normally has the highest purity and so crops are usually not combined unless both crops are of similar purity.

APPENDIX III

EXTRACTION

A separatory funnel is expensive and easily broken, especially near the stopcock. When filled with solvent, it is not well balanced and is top heavy. Separatory funnels must be supported by a ring or clamp. NEVER PLACE A SEPARATORY FUNNEL IN A BEAKER OR FLASK. Your equipment locker contains a wooden rack that is specifically designed to hold separatory funnels.

Here is a summary of the extraction method:

- Technique employed to separate components of a mixture.
- Based on differences in solubility of the compounds in two **immiscible** solvents.
- **Extraction** generally implies movement of a desired compound from one phase into another, while **washing** generally means keeping the desired compound in one phase and forcing the impurities into the other.
- Generally used at the end of a reaction to separate organic compounds, which are typically quite soluble in organic solvents, from inorganic compounds, which are typically much more soluble in water than in organic solvents.
- We can increase the quantity of a compound transferred from solvent A to solvent B by using more of solvent B. It is, however, move efficient to perform multiple extractions with a smaller volume of solvent B than to do a single extraction with a larger volume of B (3 × 10 mL of solvent B transfers more of the desired compound into B than 1 × 30 mL).
- Acid-base properties of a compound are frequently used to render a compound more/less soluble in a given solvent.

Fill the separatory funnel no more than three-quarters full so that mixing is possible. Before filling, check that the stopcock can be rotated (make sure it is not stuck) and that it is closed. Check that the stopper fits well. All of this can be done using a small amount of water.

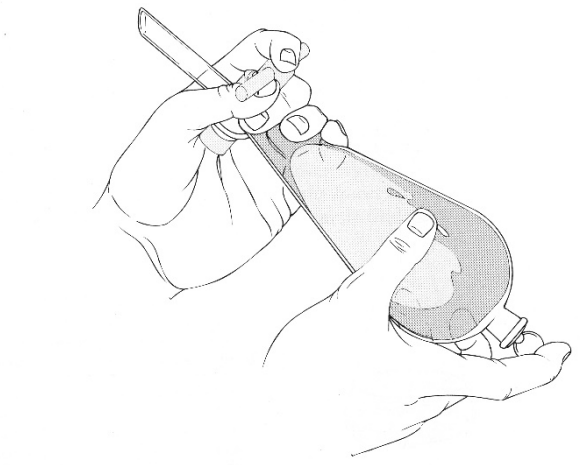
To mix, hold the separatory funnel with both hands. Place one hand over the stopper. The other hand should be placed AROUND the stopcock (see illustration).

Invert the separatory funnel so that the stopcock is pointing up, away from you, and is *not pointed at anyone else*. Open the stopcock briefly, you may hear some gas being vented, this is normal. Close the stopcock. Shake gently for a few seconds then invert the funnel as before and vent it, by opening the stopcock, to release any built-up pressure.

Repeat this shaking/venting sequence three or four times. Always be careful not to point the stopcock in the direction of anyone, since built-up pressure may also force out liquid and thus spray him or her.

Place the funnel in the ring clamp or wooden rack, remove the stopper from the top and allow the layers to separate. Never leave a stopper in a separatory funnel when it is not being shaken. The separation of phases normally takes a few minutes.

Carefully draw off the lower layer by opening the stopcock. Before discarding any layer, make sure you know that you are keeping the correct one. It is good practice to save all phases until you are sure you have the correct product.



A common problem during extraction is the formation of **emulsions**. Emulsions are suspensions of droplets of one phase in the other. They occur in the presence of impurities that act as surfactants. If emulsion formation seems to be a problem, try swirling the contents of the funnel, rather than shaking it (shaken, not stirred actually does affect mixing when emulsions are possible). If emulsions have formed during shaking (and do not disappear on their own) they can sometimes be dispersed by the

addition of a small amount of solid sodium chloride or saturated sodium chloride solution (brine). The resulting increase in the ionic strength of the aqueous phase favors the breakup of emulsions.

Selection of Solvents

The choice of solvents used in extractions is dictated by several factors. The two solvents must be immiscible. The compound to be extracted should have higher solubility in the solvent into which it is being extracted than in the other solvent. Keep in mind that "like dissolves like", polar compounds tend to be more soluble in polar solvents (salts in water), and non-polar compounds in non-polar solvents (hydrocarbons in hexane or diethyl ether). In the vast majority of cases (99.99 %), water is used as one of the phases.

When selecting a solvent for extraction from water, it is important to keep in mind other characteristics such as toxicity, flammability, odor and boiling point. Usually relatively low-boiling solvents are used so that the solvent may be easily removed from the product by evaporation.

Commonly used solvent pairs are ether/water; ethyl acetate/water or dichloromethane/water. Other organic solvents may be used with water, but they are employed less commonly.

When extracting, it is important to know which layer is on top and which is on the bottom. In general, non-chlorinated solvents (ether, ethyl acetate) are less dense than water and will be on top. Chlorinated solvents (dichloromethane) are denser than water and will be on the bottom. The water layer is frequently called the aqueous phase. If you are not sure which phase is organic and which is aqueous, you can add a few drops of water to the mixture. The phase that the water drops mix with will be the aqueous phase.

Ethyl acetate is the solvent of choice for most extractions, especially when working with polar organic compounds. It is cheap, safe and easily removed. This solvent is very flammable and so care must be taken to avoid sources of ignition. Dichloromethane is chosen either for solubility reasons or when it is desired that the organic phase is on the bottom (when doing repeated extractions from water for example). Dichloromethane has the added benefit of not being flammable. Ether is convenient when it is necessary to remove polar organic impurities that have small distribution coefficients with water, or when solvent volatility is important. Ether is also very useful in removing organic compounds from certain dipolar aprotic solvents like DMF. Ether is extremely flammable and is used less commonly than ethyl acetate or dichloromethane. Ether also tends to creep up the walls of glassware and deposit materials by evaporation. Flasks containing ether should be covered.

If an extraction is inefficient because the desired organic compound is partly soluble in water, improved recoveries can be achieved by **salting out**. In this technique, salt (sodium chloride) is added to the aqueous phase. This increases the ionic strength of the water and will often “force” the organic compound into the organic layer by decreasing its solubility in the salty water. Salting out is also useful in removing small amounts of organic solvents from water, and helps to “dry” organic solvents by making the water less soluble in the organic solvent. The salt may be added as crystals, but it is more

common to use a saturated solution in water (called brine). The efficiency of the salting out technique can be improved by using ether as the organic phase.

Drying Agents

After any extraction process, the organic solution inevitably contains some residual water. This is partly due to the physical separation process and to the partial solubility of water in organic solvents. Water is considered to be an impurity and must be removed from the organic solution. This is normally done by adding a drying agent – a salt that readily forms hydrated crystals. Drying agents are added as solids to the solution to be dried, and the hydrated crystals are later filtered off thus removing the water. A few commonly used drying agents are shown below.

$MgSO_4$	Very common. It has a high drying capacity, is relatively neutral (very slightly acidic) and works quickly. This compound is also relatively safe (it is a common construction material).
Na_2SO_4	Very common. It has a high drying capacity and is neutral. The formation of the hydrate is slow however and this salt must be held in contact with the solution longer than $MgSO_4$.
K_2CO_3	Uncommon and is used for basic compounds. It has intermediate water capacity and works slowly.
NaOH	Used for basic compounds. It has low water capacity and works slowly.
$CaCl_2$	Used for acidic compounds. It has low capacity and works slowly.

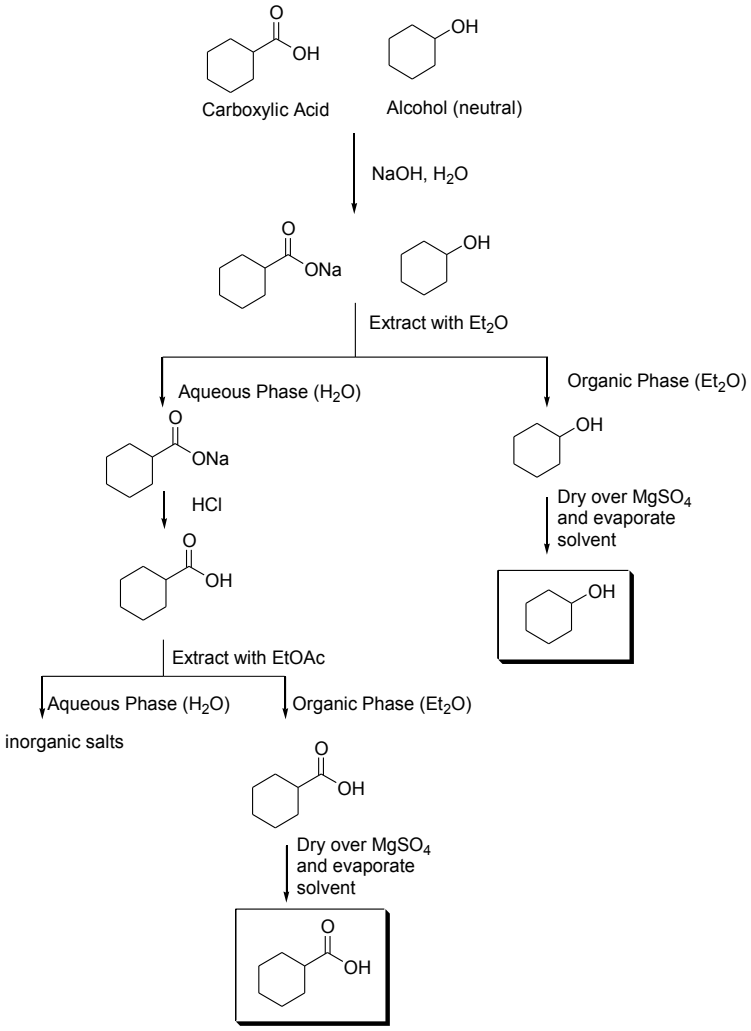
When drying a solution, it is important to keep the drying agent in contact with the solution long enough to be effective. For $MgSO_4$ and Na_2SO_4, a few minutes (5 minutes) is normally sufficient. When drying neat liquids (no solvent used) the drying process is considerably slower.

Organic solvents containing water appear “hazy” or turbid. When the organic phase becomes clear, it is a good indication that sufficient drying has taken place. When $MgSO_4$ is used, the appearance of the $MgSO_4$ can be used to estimate when sufficient drying has taken place. Wet $MgSO_4$ forms clumps whereas dry $MgSO_4$ appears “fluffy”. If you swirl a solution containing $MgSO_4$, the appearance of a “fluffy” suspension in a clear liquid is a good indication that the solution is dry.

Glass wool can also be used as a drying agent for reactive compounds or very small volumes. Filtering the solution through glass wool removes water by surface tension effects. This method does not remove dissolved water.

Reactive separations

Acid-base reactivity can sometimes be exploited to enhance the separation of acidic, basic and neutral compounds. When an organic acid (carboxylic acid or phenol) is reacted with a base a salt is produced that is charged and therefore water soluble. Similarly, when an organic base (amines) is reacted with acid a charges species is also produced. The formation of charged salts can be combined with extraction to separate various components in a mixture. This type of process is most easily understood by using a flow chart such as that shown below.



To separate a neutral compound (alcohol) from a carboxylic acid for example, the original mixture could be treated with an aqueous solution of base (NaOH) then extracted with an organic solvent like ether (Et_2O). The acid is converted to the carboxylate salt and remains in the aqueous layer. The neutral compound is in the organic phase. After the phases are separated, the neutral compound is recovered by drying and then removing the solvent. The acid is recovered by acidifying the aqueous

layer. This converts the carboxylate salt into the carboxylic acid which is no longer charged and is therefore organic soluble. An extraction from the aqueous layer into an organic solvent (EtOAc) recovers the carboxylic acid. This can be isolated in pure form by drying and removing the solvent.

An effective reactive extraction relies on knowing how a functional group will react in the presence of an acid or base. The reactivity of a group can be determined by knowing its pK_a. An acid/base reaction is an equilibrium process favoured by the reaction of a strong acid and a strong base to yield a weaker conjugate acid and weaker conjugate base. The direction of equilibrium will favour the formation of the more stable (and weaker) conjugate acid with a thus higher pK_a.

Acidity constant = $K_a = [H_2O] \times K_{eq} = \frac{[H_3O^+][A:^-]}{[H-A]}$ and $pK_a = -\log K_a$

Thus, the lower the pK_a, the more acidic the proton. The acidity of a given proton is related to the stability of the conjugate base formed once the proton has been removed. The more stable the conjugate base, the more acidic the given proton. The stability of a conjugate base can be determined using the acronym A.R.I.O. for factors such as

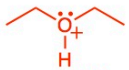
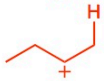
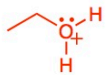
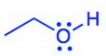
- **A**tom size and electronegativity of the atom bearing the negative charge
    - the larger the atomic radius and the more electronegative the atom, the more stable the charge on the conjugate base
- **R**esonance effects
    - the greater the delocalization of electrons, the more stable the conjugate base
- **I**nductive effects
    - the greater the proximity of electron withdrawing groups, the greater the pull of electron density through sigma bonds and greater stabilization of the conjugate base
- **O**rbital hybridization
    - The greater the percentage of s-character on the atom bearing the negative charge, the more stable the conjugate base

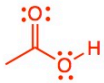
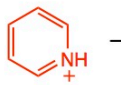
A strong acid such as HCl can acidify both an amine and an alkoxide, but a weak acid such as acetic acid will only protonate the amine. Similarly, a strong base such as NaOH can deprotonate both a carboxylic acid and an aromatic alcohol, but a weak acid such as bicarbonate can only deprotonate a carboxylic acid.

The selection of appropriate reagents for reactive extractions can be determined with the help of pKa values for select functions groups are presented in the tables on the following pages.

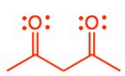
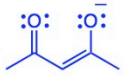
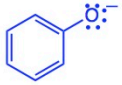
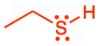
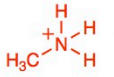
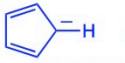
pKa Chart

strongest acids ... **weakest bases**

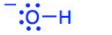
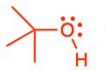
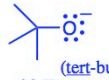
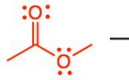
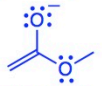
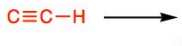
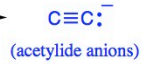
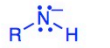
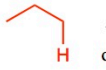
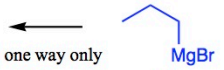
	conjugate acid	conjugate base	
sulfuric acid	H_2SO_4	HSO_4^-	-10
hydroiodic acid	HI	I^-	-9
hydrobromic acid	HBr	Br^-	-8
protonated ketone			-7.3
hydrochloric acid	HCl	Cl^-	-7
protonated ether			-3.6
carbocations			-3
protonated alcohol			-2.4
hydronium ion			-1.7

	conjugate acid	conjugate base	
nitric acid	HNO_3	NO_3^-	-1.3
tosic acid	TsOH	TsO^-	-0.6
hydrofluoric acid	HF	F^-	3.2
hydrogen nitride	HN_3	N_3^-	4.7
carboxylic acids			4.8
protonated pyridine			5.2
carbonic acid	H_2CO_3	HCO_3^- (bicarbonate)	6.37
hydrogen sulfide	H_2S	SH^-	7

3

	conjugate acid	conjugate base	
1,3-diketones			**9**
hydrogen cyanide		(cyanide)	**9.1**
ammonium ions			**9.4**
phenols			**10**
bicarbonate	HCO_3^-	CO_3^{-2}	**10.25**
thiols			**10.5**
protonated amines			**10.6**
cyclopentadiene		 (aromatic)	**15**
amides			**15**

4

	conjugate acid	conjugate base	
water		 (hydroxide)	**15.7**
primary alcohols		 (alkoxides)	**16**
tertiary alcohols		 (tert-butoxide)	**18**
ketones		 (enolates)	**18-21**
esters		 (ester enolates)	**25**
alkynes		 (acetylide anions)	**26**
hydrogen		 (hydride) one way only	**35**
ammonia/amines		 (amide bases)	**36**
alkanes		 one way only (Grignards/ organolithium reagents)	**~60**

weakest acids

strongest bases

APPENDIX IV

CHROMATOGRAPHY

Chromatography is the name given to the group of techniques used to separate and purify the constituent components of a mixture. While it was initially introduced to separate coloured compounds (the word "chroma" is Greek for colour), chromatography is now used to separate all sorts of chemical and biological substances, both coloured and non-coloured.

In chromatography, different compounds move at different rates, depending on their polarity. The rate of migration depends on the interaction of each compound with two phases: the stationary phase, silica gel in this case (the compound is adsorbed[3] onto the silica gel), and the mobile phase (an organic solvent or mixture of solvents), which passes over/through the stationary phase. Generally, more polar compounds interact most strongly with the polar silica gel and therefore migrate more slowly than less polar compounds. Less polar compounds generally migrate faster than more polar compounds.

In summary:

1) The stationary phase (silica gel) is polar: more polar compounds interact more strongly with this phase than less polar compounds.

2) Increasing the polarity of the mobile phase (solvent) will cause all compounds to migrate further on the plate.

Types of chromatography	**Stationary phase**	**Mobile phase**
a) Gas chromatography (gas-liquid)	liquid adsorbed on solid support	gas (ex. He, N_2)
b) Column chromatography	Alumina or silica gel	liquid ex. hexanes, ether, acetone, methanol
c) Thin layer chromatography (TLC)	Alumina or silica gel	liquid ex. hexanes, ether, acetone, methanol

The choice of adsorbent and mobile phase depends on the nature of the products being separated and on the preference and experience of the chemist. The overall polarity of the adsorbent

[3] **Adsorb:** superficial accumulation of a compound onto a surface

will depend on its activity that in turn depends on its composition. Several common adsorbents are listed in Table 1. By far the most common adsorbent is silica gel. This material is readily available in a variety of particle sizes. In general you will get better separation with smaller particles, but the separation will also take longer. Although you can optimize separations by changing adsorbents, in practice silica gel is used as the adsorbent and separations are tuned by varying the mobile phase.

Table 1. Common adsorbents for liquid-solid chromatography.

Cellulose	Least active
Starch	↓
Silica gel	↓
Florisil®	↓
Alumina	Most active

The surface of silica gel is very polar, consisting of Si-O-Si and Si-OH bonds. Organic compounds containing polar functional groups will be held stronger than those without polar groups through dipole-dipole interactions and hydrogen bonding. In other words, polar molecules capable of forming hydrogen bonds with the silica gel will be retained more strongly at the base of the silica plate and will migrate slower than compounds that are less polar. Alcohols, for example, which are great acceptors and donors of hydrogen bonds, are better retained on silica gel than ketones, esters, or ethers (these latter groups can only function as acceptors).

The mobile phase is typically an organic solvent. Table 2 shows a list of solvents that are commonly used. The solvents are listed in order of polarity. Commonly used solvents are hexanes, petroleum ether, CH_2Cl_2, ethyl acetate, and methanol. Separations are optimized by altering the polarity of the solvent. This may be done by simply changing solvents, but a much better method is to use mixtures. To do this, one mixes a polar solvent with a non-polar one. By varying the amounts of each, a mixture of the right overall polarity can be found. Common mixtures are ethyl acetate/hexanes; ethyl acetate/petroleum ether; ethyl acetate/benzene and CH_2Cl_2/EtOH.

Table 2. Common solvents for silica gel chromatography

Petroleum ether or hexanes	
CCl_4	
Benzene	
CH_2Cl_2	
$CHCl_3$	
Ethyl ether	*Increasing Polarity*
Ethyl acetate	
Pyridine	
Acetone	
Ethanol or methanol	
Water	
Acetic acid	

When optimizing a separation, the polarity of the compound being purified must also be considered. This can be estimated by considering the functional groups on the molecule. In general, the polarity of a compound will increase as its ability to participate in hydrogen bonding increases. Table 3 shows a list of common functional groups and their approximate elution sequence. More polar compounds will interact more with the most polar phase (the stationary one in the case of silica gel) and so will be retained on the column longer. These compounds with therefore elute later.

Table 3. Approximate adsorption order on silica gel of common functional groups.

Saturated hydrocarbons	Elute fastest
Alkenes	
Aromatics	
Halogen	
Ethers	
Nitro groups	Elute slowest
Aldehydes and ketones	
Alcohols	
Amides	
Carboxylic acids	

Thin layer chromatography (TLC)

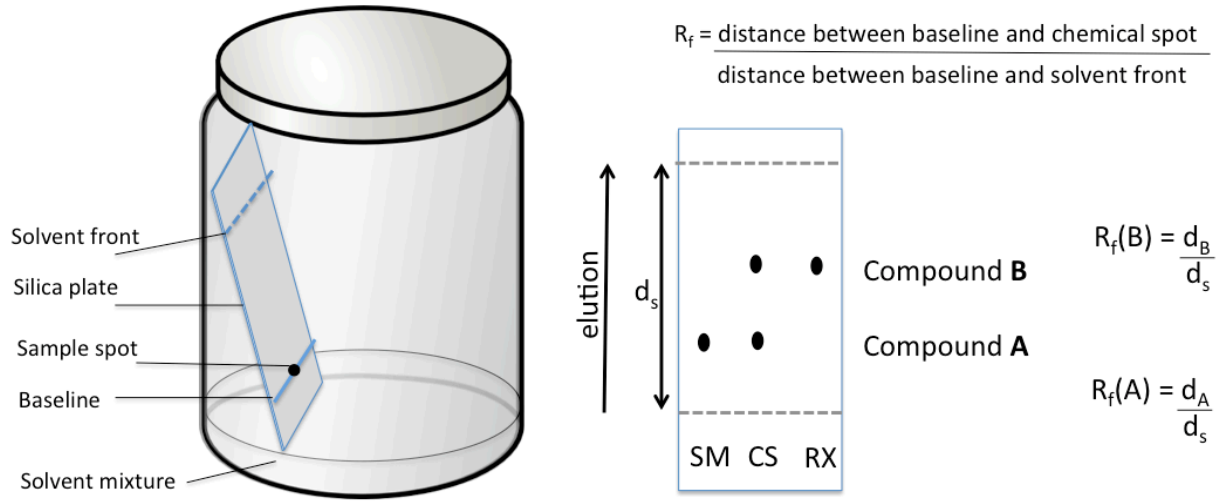
To prepare a TLC plate, first draw a line approximately 1 cm from the bottom of the plate. Apply the sample to the plate on the line using a glass capillary. Simply dip the capillary into the dilute sample solution (for neat samples first dissolve the sample in a volatile solvent). Touch the capillary to the plate on the line as shown in Figure 1a. You do not need a lot of sample. Smaller sample spots will generally give better results.

Place the plate in the developing jar as shown. The silica should face away from the wall of the tank (Figure 1b). The solvent level should be below the sample spot. Allow the solvent to move up the plate until the solvent front is almost at the top then remove, dry and develop it.

The chromatogram is developed to locate the position of the components on the plate. This is most commonly done using ultraviolet light and by staining. Typically the plate will be visualized under UV light, and then stained. In this way compounds with different properties may be visualized.

The movement of components is described by the R_f value. This is defined as the displacement of the component (d_1) divided by the displacement of the solvent front (d_s, figure 2a).

Figure 1. TLC in development jar, left, and R_f determination, right.



When TLC is used for reaction monitoring or to demonstrate the purity of a sample, a solvent system is chosen so that the R_f of the target component is approximately 0.5 to 0.6. In this way components can be visualized that are more and less polar that the targeted component. Analytical TLC works best when the components move relatively high on the plate as this gives the best resolution between zones. When using TLC to follow a reaction or to verify the identity of compounds, three lanes should be spotted (figure 1, left). The first lane contains the starting material (SM) or reference compound. The last lane contains the reaction mixture (Rx) or compound being evaluated. The middle lane contains a co-spot of both the reference compound and the reaction mixture. The co-spot identifies the expected R_f of the starting material in the reaction mixture lane - this is necessary since components of the reaction mixture (acid, solvents, etc.) can sometimes change the R_f value of a compound.

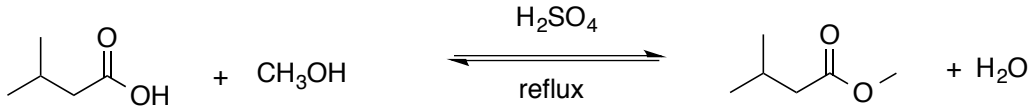
Usually, only a small amount of compound is necessary to be visible on the TLC (<1 mg/mL). Spotting a solution which is too concentrated may result in streaking/blotching on the plate, which makes interpreting the TLC very difficult. Streaking may also occur if a compound exists in equilibrium between a charged and uncharged state; in this case, neutralization or extraction is required before spotting the compound.

A convenient way to alter solvent polarity is to use mixtures of two solvents. The solvents are chosen so that there is one polar solvent and one non-polar solvent. The easiest way to systematically vary polarity is to use ratios of solvents that add up to 10. For example, EtOAc : hexanes 4 : 6 or EtOAc : hexanes 5 : 5 or EtOAc : hexanes 9 : 1. Using such mixtures makes it easy to keep track of relative polarity.

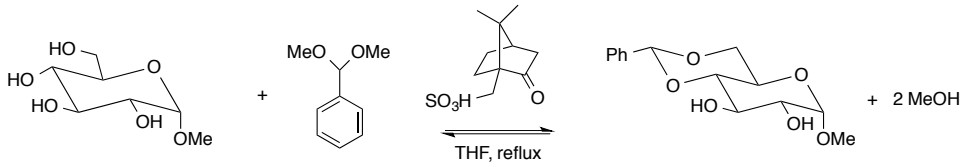
APPENDIX V

Selection of Questions from CHM2123 Final Exam 2015

1. (**10 points**) **Separation and extraction**. A student performs the following esterification reaction for one hour and determines by TLC that the reaction did not go to completion. The student wishes to try again, and recovers the leftover starting materials as well as the ester from their reaction mixture. Starting with the reaction mixture diluted with dichloromethane at room temperature, use a **flowchart** and any necessary reagents from the **reagents box on p.15** and/or work-up techniques to provide a method for the separation of the following mixture of compounds. Isolate each of the three compounds as individual crude products. (Recrystallization not necessary).

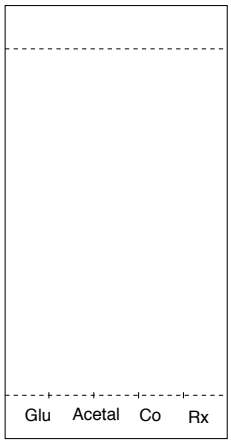


2. (**10 points**) In the preparation of a benzylidene acetal, a student arrived late in the lab. They quickly rinsed and dried their glassware with water and acetone, placed it in the oven for two minutes, and proceeded to start the reaction as illustrated below.



a) 10-camphorsulfonic acid was used as an acid for this reaction. Why did the student use this instead of a more conventional acid such as HCl? (**1 point**)

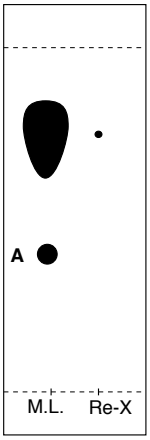
b) After allowing the reaction to reflux for 45 minutes, they performed a TLC using 3:7 hexanes : ethyl acetate. They then visualized the plate using a UV lamp, followed by dipping the plate in a methanolic solution of 10% H_2SO_4 and heating the plate. Complete the anticipated TLC plate for this step of the experiment. (**4 points**)



c) Why was the methanolic H_2SO_4 treatment plus heating necessary in this reaction protocol? (**1 point**)

d) The reaction mixture was cooled and a few drops of triethylamine were added. The contents of the reaction flask was transferred to a separatory funnel, diluted with 50 mL ethyl acetate, and washed three times with water. What purpose does the addition of triethylamine serve in this step of the experiment? (**1 point**)

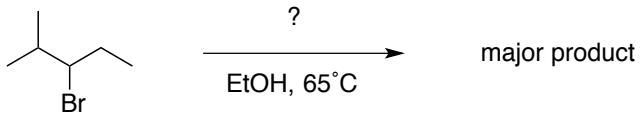
e) Following recrystallization, the student performed a TLC to access the purity of their crystals, and obtained the plate illustrated below, where M.L. and Re-X represent a spot of the mother liquor and the isolated crystals, respectively. A large streak appeared in the M.L. lane. What likely caused this, and what can you do to improve the resolution? (**2 points**)



f) There is an additional spot A whose R_f value does not correspond to any of the reagents in solution. This spot required visualization using the methanolic H_2SO_4 solution and heat. Propose a structure for this side-product. (**1 point**)

Structure of A:

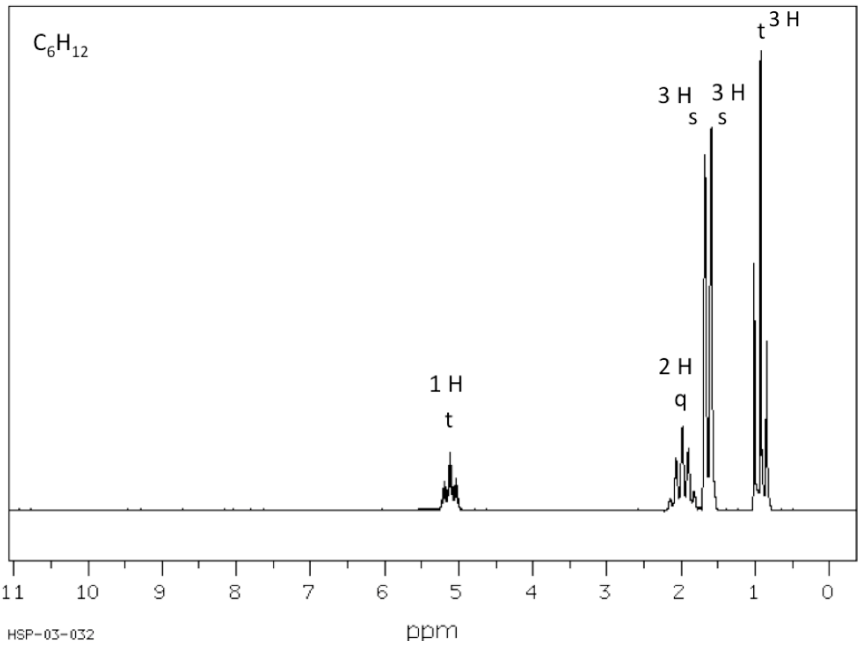
3. (**10 points**) A student carried out a synthesis in the undergrad lab, following the lab manual that instructed them to combine 0.5g of 3-bromo-2-methlypentane with 20 mL of ethanol, followed by the addition of NaOEt, and allowing the reaction to reflux for 45 minutes. Arriving 20 minutes late, the student rushed to set up the experiment. About 30 minutes into the reflux, the student panicked and questioned whether they may have accidentally added $KOC(CH_3)_3$ instead of NaOEt . Their TA told them to continue on with the experiment, and to characterize their final product by ^1H NMR.



a) Identify the possible products that could form in the case of each reagent. (**2 points**)

NaOEt: $KOC(CH_3)_3$:

b) After workup and recrystallization, the ^1H NMR spectrum of the student's major product is below. Identify the compound, and indicate which peak corresponds to which proton. (**5 points**)

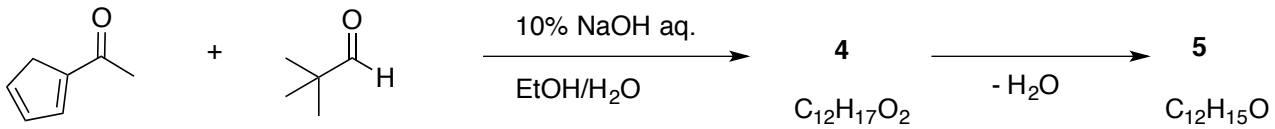


C_6H_{12}

c) Did the student successfully add NaOEt to their reaction mixture? Explain your reasoning. (**3 points**)

4. ***Reactions of carbonyl compounds*.** (**10 points**)

a) Provide a mechanism for the following reaction. Be sure to include key resonance structures. (**5 points**)



b) Provide a name for the reaction to yield product **5**. (**1 point**)

c) How does an aqueous solution of bleach, NaOCl, serve as a reagent for the haloform reaction? Demonstrate with a mechanism showing a single mono-halogenation step using the methyl ketone in part a). (**4 points**)

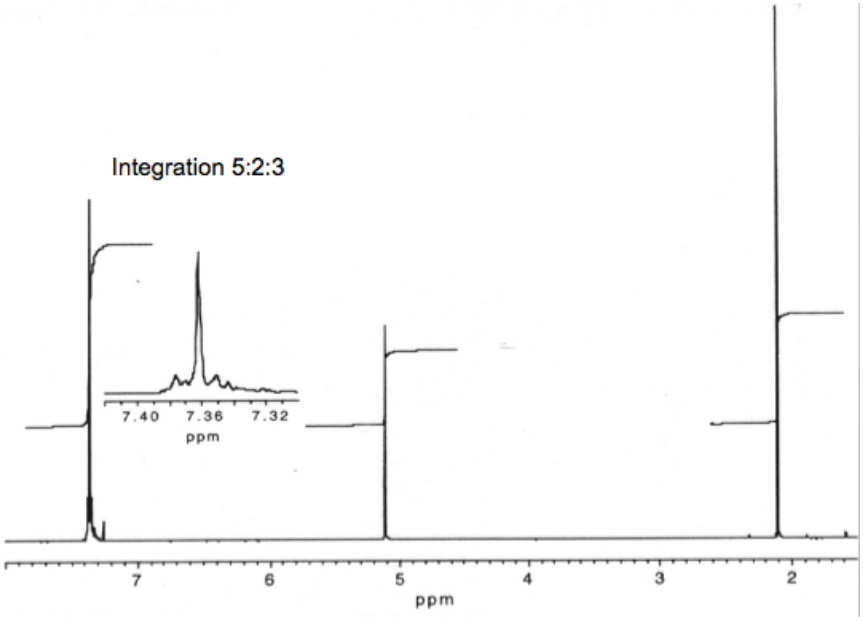
5. Ester hydrolysis **(8 points)** In an experiment to determine the molar mass of an unknown ester, a student dissolved 400 mg of the unknown ester into 35 mL of alcoholic NaOH. After the hydrolysis reaction was complete, 20.9 mL of 0.25 M H_2SO_4 was required to be neutralize the reaction mixture. In determining the concentration of the starting material, 20 mL of the NaOH alcoholic solution was neutralized with exactly 15.0 mL 0.25 M H_2SO_4.

a) Determine the concentration of NaOH used in this saponification reaction. (**2 points**).

b) Determine the moles of unreacted NaOH after ester hydrolysis. (**3 points**)

c) Determine the molar mass of the ester. (**2 points**)

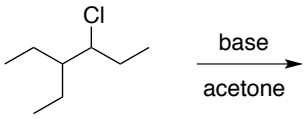
d) Based on the ^1H spectrum below, identify the structure of the unknown ester. (**1 point**)

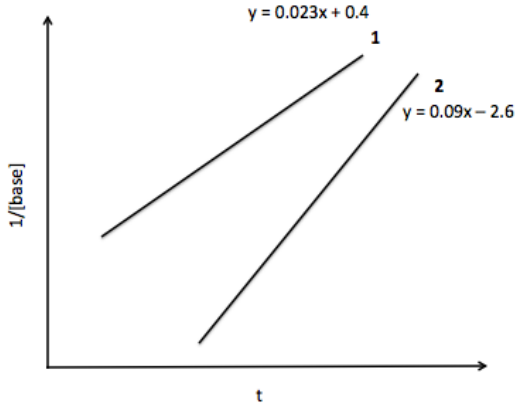


unknown ester:

You have the CHOICE to complete either question 6 OR 7. Clearly indicate your choice.

6. (**8 points**) ***Kinetic Analysis***. A student was performing the following substitution reaction, where triethylamine and sodium acetate were employed to investigate the effect of base on the reaction rate under identical reaction conditions. The rate of the reaction was obtained by titrating aliquots of the reaction mixture against HCl to determine the concentration of base remaining in solution at different time points, and performing a linear analysis as shown below. The student shared their data with their lab partner, but unfortunately failed to label their trials.





a) Based on the trials 1 and 2 above, calculate the rate constants corresponding to each trial. What is the order of each reaction? (**4 points**)

1	**2**

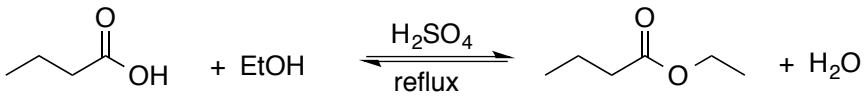
b) Based on the analysis above, help the lab partner assign triethylamine and sodium acetate to trials 1 and 2. (**2 points**)

1	**2**

d) Explain your rationale in part b). (**2 points**)

You have the CHOICE to complete question 6 OR 7. Clearly indicate your choice.

7. (**8 points**) *Esterification*. A student was interested in synthesizing ethyl butyrate to add a pineapple scent to a soft soap that they had previously prepared. In this second experiment, the student added 3.0 mL of butyric acid (88.11 g/mol, 0.95 g/mL) to a 50 mL round bottom flask and followed by 15 mL ethanol (46.07 g/mol, 0.79 g/mL). Slowly, 1.0 mL H_2SO_4 (98.08 g/mol, 1.84 g/mL) was added while stirring, and the reaction was allowed to progress for 90 minutes under reflux conditions. Following workup, 0.7g of ethyl butyrate (116.16 g/mol, 0.88 g/mL) was isolated.

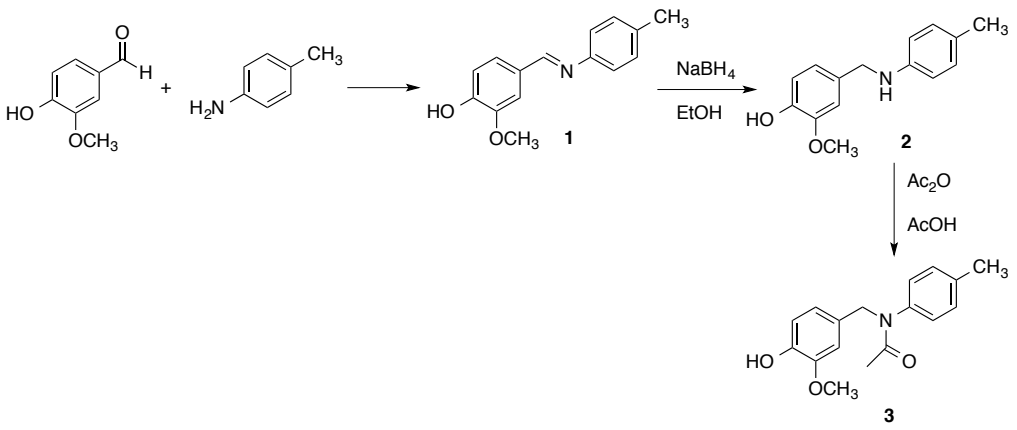


a) Determine the number of equivalents of each reactant in this reaction. *A table of reagents is not necessary.* (**3 points**)

b) Knowing that the reaction has an equilibrium constant of 4.0, determine the theoretical yield of ethyl butyrate. (**4 points**)

c) Calculate the percent yield of this reaction. (**1 point**)

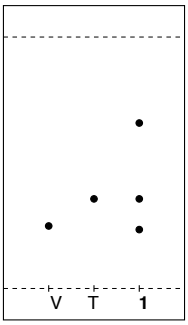
8. (**12 points**) ***Reductive Amination***. You are asked to repeat the following experiment, as detailed from a research journal, for the preparation of an amide from an amine.



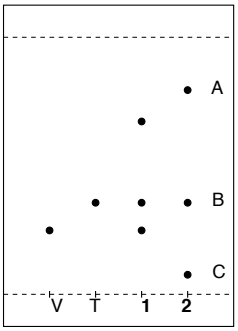
''Vanillin (0.58 g, 3.8 mmol) and para-toluidine (0.40 g, 3.8 mmol) are combined in a 250 mL beaker, and the solids are mixed with a Teflon stir rod to afford a dry, bright orange powder. (crude 90-95%, mp 102-103˚C). The imine is subsequently reduced and then converted to the more stable amide by acetylation. The reduction is accomplished by the addition of 15 mL 95% ethanol to the beaker containing the imine, followed by the addition of $NaBH_4$ (0.1g, 2.7 mmol) in small increments to the stirred reaction mixture. The imine is only partially soluble in ethanol, but the product is quite soluble. Within 10 minutes, a colourless solution is an indication that the reaction is complete. Glacial acetic acid (2.0 mL) is added to the ethanoic solution followed by 2.0 mL acetic anhydride, and the reaction mixture is warmed on a steam bath for 5-10 minutes. The amide product precipitates out when 75mL of cold water is added slowly. White crystals are collected by vacuum filtration and analyzed by TLC and melting point. (crude 80-90%, mp 127-128˚C.)''

a) Show a detailed mechanism for the formation of **1**, and be sure to show any proton transfer. (**4 points**)

b) After mixing the two starting materials for 15 minutes, you take the following TLC. What do these results indicate, and what should you do next? (**2 points**)



c) Your lab partner doesn't agree, and insists you proceed to the reduction step of the experiment. Your ethanoic solution goes colourless after about 15 minutes following addition of $NaBH_4$ in ethanol, but you're worried that something went wrong. You take a TLC, as shown below.



Unfortunately your instincts were right, and your reaction mixture shows impurities. Propose structures for the compounds responsible for spots A, B, and C. (**3 points**)

A	**B**	**C**

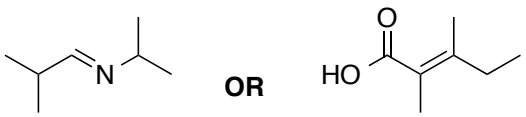
d) Clearly you need to deviate from the protocol before you can proceed with the amination step. You decide to purify your desired product by recrystallization. What are three key criteria to use when selecting a solvent system for recrystallization? (**3 points**)

i.

ii.

iii.

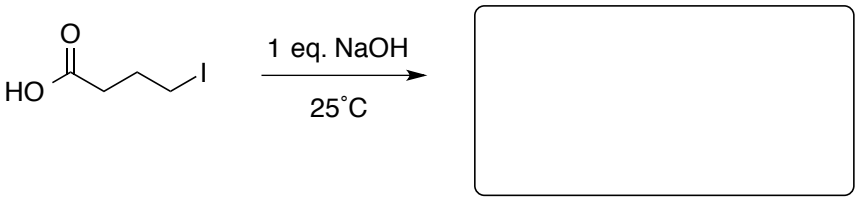
9. (**9 points**) With access to ***only*** the chemicals listed in the **reagents box on p.15**, outline a synthetic approach (ie. reaction scheme) for ***one*** of the following two compounds. A mechanism is not necessary, but all reagents, solvents, intermediates and co-products, as well as reaction conditions, must be shown. Use reactions that you have see in this course this semester.



10. ***Short answer questions.*** (**15 points**)

a) To what phase is Na_2SO_4 added during an extraction? (1 point)

b) Determine the product of the following reaction. (1 point)



c) How can you convert a soft soap into a hard soap? Use an equation to illustrate the change that is occurring. (**2 points**)

d) Why is saponification with NaOH considered an irreversible reaction? Show with an equation.(2 points)

e) Briefly explain how sublimation with a cold finger works to purify a compound. (2 points)

f) After dissolving your crude product in hot ethanol and allowing it to cool slowly at room temperature, no crystals have formed. Suggest three techniques to induce crystallization. (3 points)

i.

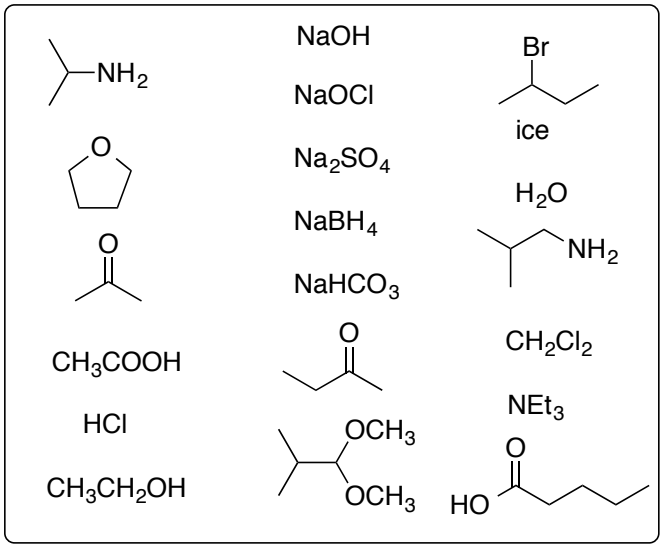
ii.

iii.

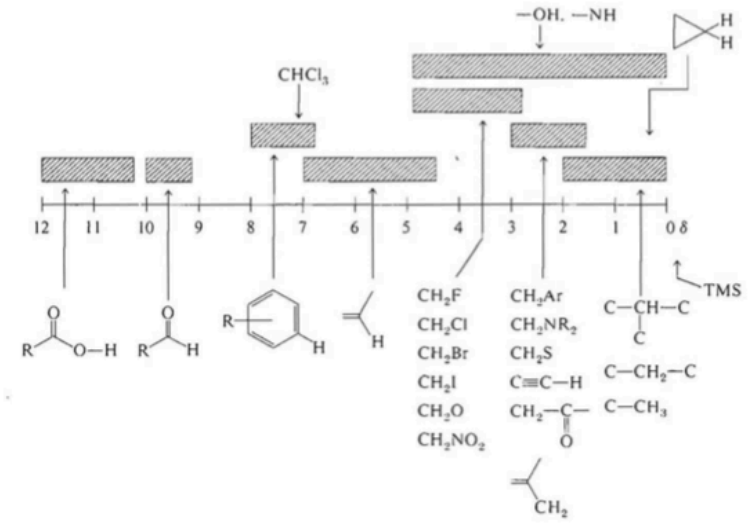
g) Between Br^- and I^-, which ion performs as a better nucleophile in ethanol? Why? (1 point)

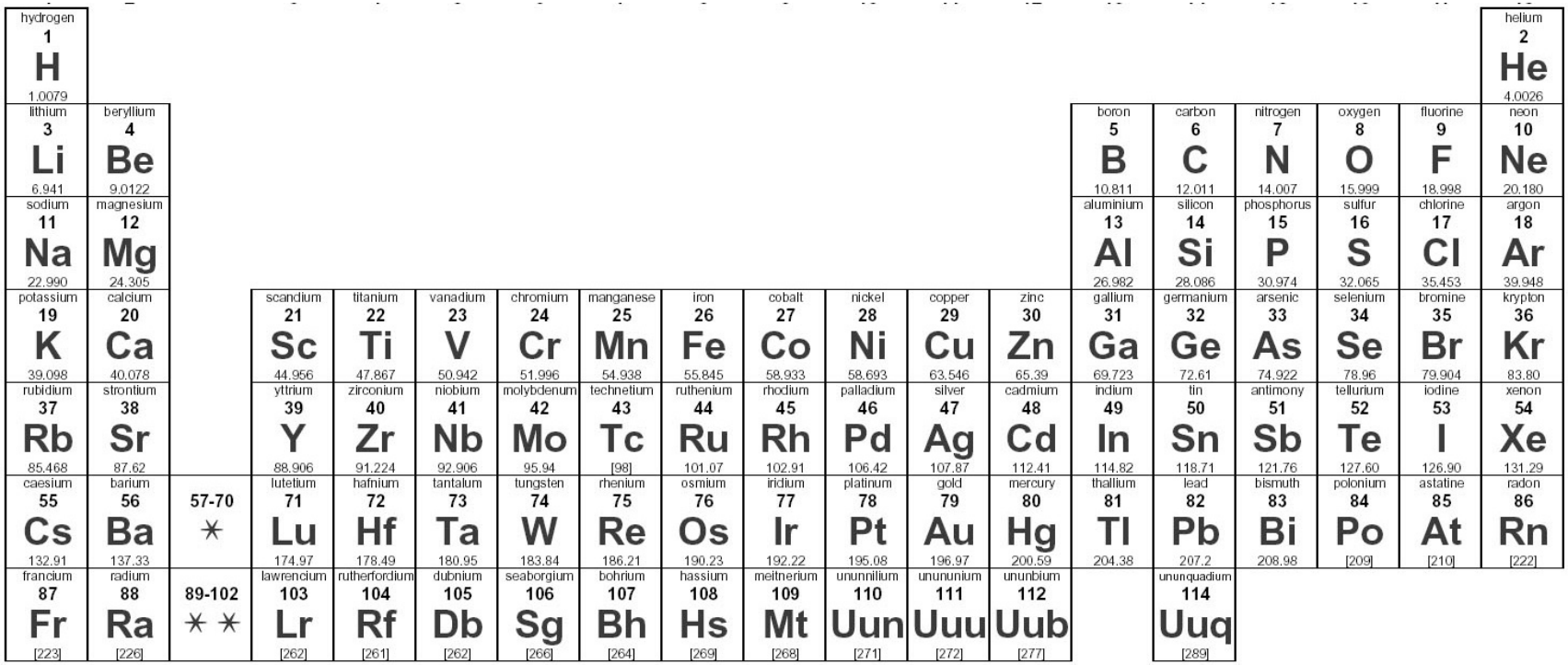
h) What does it mean when Benedict's reagent turns red? Give one example of a reactant that would cause this observation. (2 points)

Reagent Box



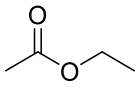
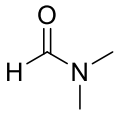
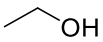
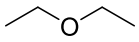
^{1}H NMR




hydrogen 1 H 1.0079
helium 2 He 4.0026
lithium 3 Li 6.941
beryllium 4 Be 9.0122
boron 5 B 10.811
carbon 6 C 12.011
nitrogen 7 N 14.007
oxygen 8 O 15.999
fluorine 9 F 18.998
neon 10 Ne 20.180
sodium 11 Na 22.990
magnesium 12 Mg 24.305
aluminium 13 Al 26.982
silicon 14 Si 28.086
phosphorus 15 P 30.974
sulfur 16 S 32.065
chlorine 17 Cl 35.453
argon 18 Ar 39.948
potassium 19 K 39.098
calcium 20 Ca 40.078
scandium 21 Sc 44.956
titanium 22 Ti 47.867
vanadium 23 V 50.942
chromium 24 Cr 51.996
manganese 25 Mn 54.938
iron 26 Fe 55.845
cobalt 27 Co 58.933
nickel 28 Ni 58.693
copper 29 Cu 63.546
zinc 30 Zn 65.39
gallium 31 Ga 69.723
germanium 32 Ge 72.61
arsenic 33 As 74.922
selenium 34 Se 78.96
bromine 35 Br 79.904
krypton 36 Kr 83.80
rubidium 37 Rb 85.468
strontium 38 Sr 87.62
yttrium 39 Y 88.906
zirconium 40 Zr 91.224
niobium 41 Nb 92.906
molybdenum 42 Mo 95.94
technetium 43 Tc [98]
ruthenium 44 Ru 101.07
rhodium 45 Rh 102.91
palladium 46 Pd 106.42
silver 47 Ag 107.87
cadmium 48 Cd 112.41
indium 49 In 114.82
tin 50 Sn 118.71
antimony 51 Sb 121.76
tellurium 52 Te 127.60
iodine 53 I 126.90
xenon 54 Xe 131.29
caesium 55 Cs 132.91
barium 56 Ba 137.33
57-70 ✶
lutetium 71 Lu 174.97
hafnium 72 Hf 178.49
tantalum 73 Ta 180.95
tungsten 74 W 183.84
rhenium 75 Re 186.21
osmium 76 Os 190.23
iridium 77 Ir 192.22
platinum 78 Pt 195.08
gold 79 Au 196.97
mercury 80 Hg 200.59
thallium 81 Tl 204.38
lead 82 Pb 207.2
bismuth 83 Bi 208.98
polonium 84 Po [209]
astatine 85 At [210]
radon 86 Rn [222]
francium 87 Fr [223]
radium 88 Ra [226]
89-102 ✶ ✶
lawrencium 103 Lr [262]
rutherfordium 104 Rf [261]
dubnium 105 Db [262]
seaborgium 106 Sg [266]
bohrium 107 Bh [264]
hassium 108 Hs [269]
meitnerium 109 Mt [268]
ununnilium 110 Uun [271]
unununium 111 Uuu [272]
ununbium 112 Uub [277]
ununquadium 114 Uuq [289]
*Lanthanide series
lanthanum 57 La 138.91
cerium 58 Ce 140.12
praseodymium 59 Pr 140.91
neodymium 60 Nd 144.24
promethium 61 Pm [145]
samarium 62 Sm 150.36
europium 63 Eu 151.96
gadolinium 64 Gd 157.25
terbium 65 Tb 158.93
dysprosium 66 Dy 162.50
holmium 67 Ho 164.93
erbium 68 Er 167.26
thulium 69 Tm 168.93
ytterbium 70 Yb 173.04
**Actinide series
actinium 89 Ac [227]
thorium 90 Th 232.04
protactinium 91 Pa 231.04
uranium 92 U 238.03
neptunium 93 Np [237]
plutonium 94 Pu [244]
americium 95 Am [243]
curium 96 Cm [247]
berkelium 97 Bk [247]
californium 98 Cf [251]
einsteinium 99 Es [252]
fermium 100 Fm [257]
mendelevium 101 Md [258]
nobelium 102 No [259]

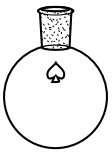
APPENDIX VI

PHYSICAL PROPERTIES OF COMMON ORGANIC SOLVENTS

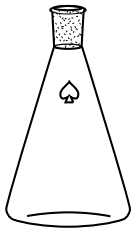
Solvent	***Structure***	***b.p. (°C)***	***Density (g/mL)***	***Dielectric constant***
Ethyl acetate		77	0.902	6.0
Acetone		56	0.791	20.7
Chloroform	$CHCl_3$	61	1.484	4.7
Dichloromethane (methylene chloride)	CH_2Cl_2	40	1.336	8.9
N,N-dimethylformamide		153	0.944	36.7
Water	H_2O	100	1.00	78.5
Ethanol		78	0.789	24.6
Ether (diethyl ether)		35	0.713	4.2
Methanol	CH_3OH	65	0.792	32.7

APPENDIX VII

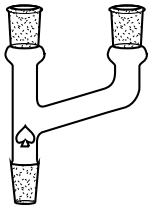
COMMON GLASSWARE USED IN ORGANIC CHEMISTRY



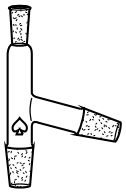
Round bottom flask



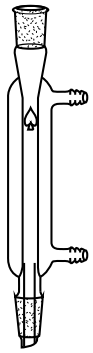
Erlenmeyer flask



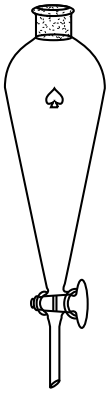
Claisen head



Distillation head



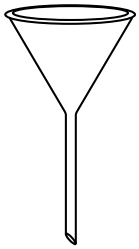
Condenser



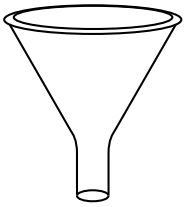
Extraction funnel

Column for chromatography

Thermometer with ground glass joint



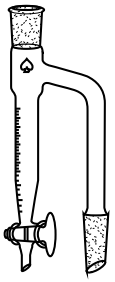
Funnel for liquid



Funnel for solids

Distillation joint

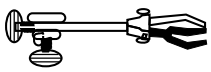
Vacuum take-off adaptor



Dean-Stark apparatus

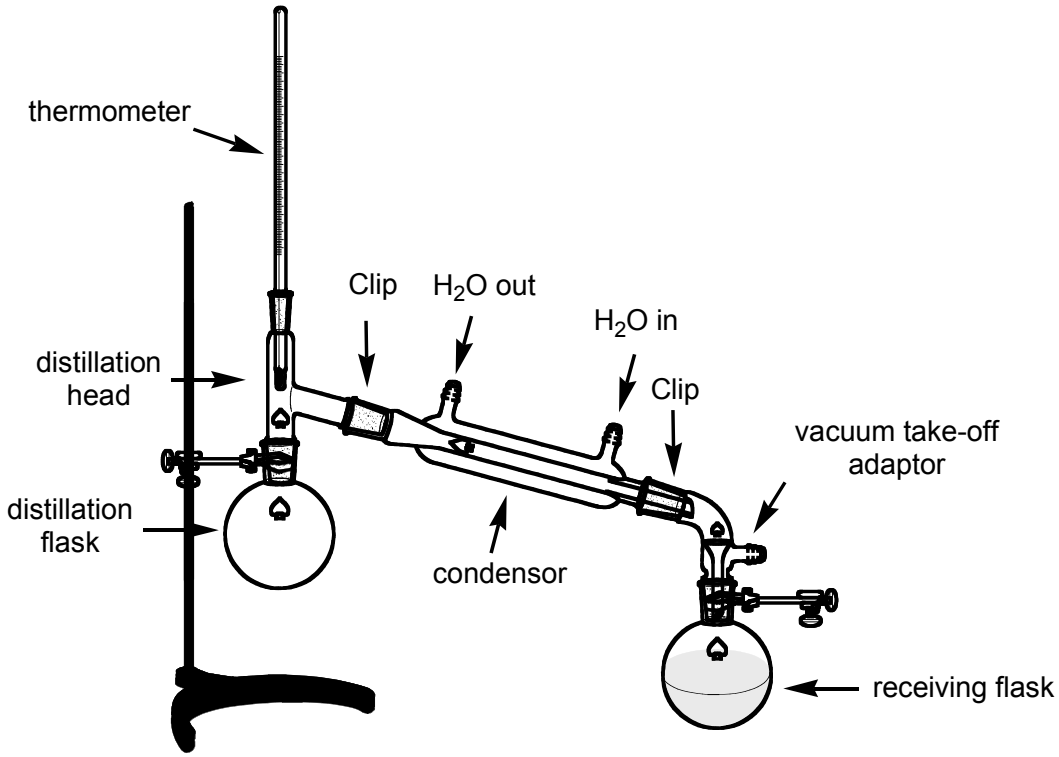
Beaker

Glass stopper

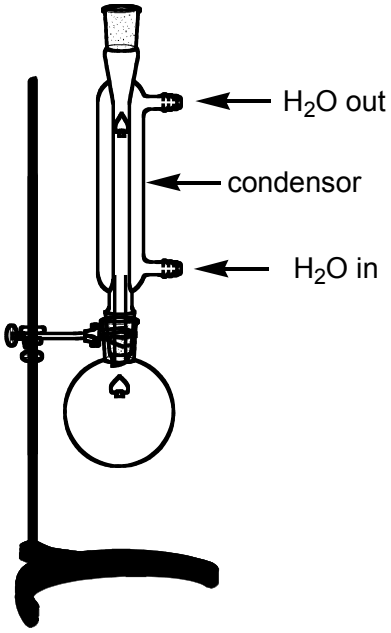


Universal clamp

Setup for a simple distillation either under vacuum or at atmospheric pressure



Setup for reflux



Locker Check-Out

Print your name and student number

Student (1) _____

Student (2) _____

Locker #

TA

TOP SHELF	IN	OUT
RUBBER TUBING	2	
WASH BOTTLE 250 ml	1	
UNIVERSAL CLAMP	4	
EXTENSION CLAMP	3	
CLAMP HOLDER	7	
TEST TUBE HOLDER	1	
CRUCIBLE TONGS	1	
SUPPORT RING 3"	1	
WIRE GAUZE	1	
CORK RING MEDIUM.	2	
CORK RING SMALL	2	

BOTTOM SHELF	IN	OUT	STORAGE BOX (ORANGE)	IN	OUT
BEAKER 50 ml	2		FILTRATION FUNNEL	1	
BEAKER 150 ml	1		POWDER FUNNEL	1	
BEAKER 250 ml	2		WATCH GLASS 90mm	2	
BEAKER 400 ml	1		ERLENMEYER 50 ml	3	
BEAKER 600 ml	1		TEFLON STIRRER	2	
BEAKER 1000 ml	1		DRYING TUBE (plastic)	1	
TEST TUBE 16 x 150	12		SCOOPULA	1	
TEST TUBE RACK	2		SPATULA	1	
DEVELOPMENT JARS 8 oz.	2		GRADUATED CYLINDER 10 ml	2	
ERLENMEYER 125 ml	2		GRADUATED CYLINDER 50 ml	1	
ERLENMEYER 250 ml	1		GRADUATED CYLINDER 100 ml	1	
SUCTION FLASK 500 ml	2		« C » CLAMP (NALGEN JAR)	5	
BUCHNER FUNNEL	1				
SUCTION ADAPTER	1				
FUNNEL SUPPORT (WOOD)	1				