IMPORTANT NOTICE TO ALL STUDENTS

SAFETY GLASSES OR GOGGLES AND LAB COATS ARE MANDATORY IN ALL CHEMISTRY LABORATORIES.

STUDENTS CAN PURCHASE THESE SAFETY ITEMS FROM THE STOREROOM MRN 308. ONLY THE UOTTAWA (STUDENT) CARD WILL BE ACCEPTED FOR PAYMENT.

NO STUDENTS WILL BE ALLOWED TO WORK IN THE LABORATORY WITHOUT THEIR SAFETY GLASSES.

AVIS IMPORTANT A TOUS LES ETUDIANTS

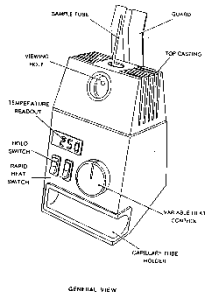
LES LUNETTES PROTECTRICES ET LES BLOUSES DE LABORATOIRE SONT OBLIGATOIRES DANS TOUS LES LABORATOIRES DE CHIMIE.

LES ÉTUDIANTS PEUVENT ACHETER CES ARTICLES SÉCURITAIRES DANS NOTRE MAGASIN MRN 0308. PAIMENT SERA ACCEPTE SEULEMENT PAR LA CARTE UOTTAWA (CARTE D'ETUDIANT)

AUCUN ÉTUDIANT NE SERA ADMIS DANS LE LABORATOIRE SANS SES LUNETTES.

CHM 1321 Organic Chemistry Laboratory

Contents

7 **Stereochemistry**	A stereochemistry assignment will be provided. This is to be done outside of the laboratory and is due for ALL groups on **January 30, 2015, No later than 5 pm**
Appendix I – Melting Points	
Appendix II – Example of a Good Lab Report	
Appendix III – Instructions on using ImageJ software for TLC analysis	

CHM 1321 Introductory Organic Chemistry

Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, Ontario, K1N 6N5.

June 2013

NOTICE

Safety glasses or goggles and lab coats are mandatory and must be worn when you are in the laboratory. **These items may be purchased at the science stores in the basement or from the chemistry stores in the 3rd floor lab. ONLY the uOttawa card (student card) will be accepted for payment.**

The experiments chosen for this course illustrate techniques and reactions that are widely used in organic synthesis, and include methods for the isolation and purification of organic compounds.

READ THE BACKGROUND AND THE PROCEDURE FOR EACH EXPERIMENT PRIOR TO COMING TO THE LABORATORY. THE DEMONSTRATORS WILL DISMISS ANYONE WHOSE LACK OF PREPARATION MAY BE PRESENTING A HAZARD.

Experiments are done every other week. Experiment reports must be handed in one week after YOU complete the experiment at the beginning of the laboratory period.

YOU MUST PASS A PRE-LAB QUIZ (80% OR BETTER) AT LEAST 30 MINUTES BEFORE EACH SESSION BEGINS TO BE ALLOWED TO ENTER THE LAB.

The quiz may be taken using Blackboard Learn. You may try the quiz as many times as you like, but only the **last** mark is recorded. The grades for these quizzes are not counted towards your final mark. The quiz is simply a "permit" for entry into the lab.

You will be working in pairs so make it a true partnership and a cooperative effort. If you let your partner do all of the work, you will learn very little and will have trouble on the lab exam.

Organize your time and divide the labor. If one partner is weighing out chemicals the other should begin to assemble the equipment. Similarly, while one partner keeps an eye on the reaction, the other can begin to set up for the next part of the experiment.

If an experiment does not work as advertised, do not panic. Consult your demonstrator for advice; in most cases the experiment can be rescued.

Safety

Make it a habit to be safety-conscious at all times. Understand what you are doing and pay attention to details. If you are uncertain about a procedure ask your demonstrator.

NO FOOD OR DRINK IN THE LABORATORY

CELL PHONES AND iPODS ARE NOT PERMITTED IN THE LABORATORY

APPROVED EYE PROTECTION (SAFETY GLASSES OR GOGGLES) MUST BE WORN AT ALL TIMES.

Glasses with side shields are available at nominal cost at the stores counter. Those persons wearing prescription lenses must wear goggles or safety glasses over their prescription glasses. Prescription safety glasses equipped with side shields are permitted.

Students caught without proper eye protection will be given one warning. Subsequent violations will result in immediate ejection from the laboratory and a mark of zero for the experiment and accompanying evaluation.

Accidents occur unexpectedly, and eye tissue is sensitive. PROTECT YOUR EYES. YOU ONLY GET TWO AND THEY CANNOT BE REPLACED. If chemicals are splashed into your eye(s), wash the open eye immediately for at least 5 - 10 minutes with a gentle spray of water. All eye injuries should be checked without delay by a physician.

Chemical Spills and Gloves: You should always be careful handling reagents and solvents to avoid exposing your hands to caustic and/or toxic materials. If you spill something on your hands, IMMEDIATELY wash your hands with cool water and consult your demonstrator.

Disposable gloves provide limited and temporary protection and are NOT permitted in the organic laboratory. Organic solvents and many organic reagents easily pass through disposable gloves, therefore only a few seconds protection is afforded. Worse, many people continue to work with dirty gloves, transmitting the chemical to their skin, thus providing LESS protection than simply using bare hands. Persons wearing dirty gloves will contaminate themselves, equipment and co-workers (people who spill things on their hands tend to wash them).

Better protection is afforded by **neoprene dishwashing gloves**, which are readily available from supermarkets. These gloves are more cumbersome than disposables, but are considerably less permeable and provide excellent protection. They are also reusable and provide better grip on glassware than disposable gloves.

Students wearing neoprene gloves are expected to show courtesy to others in the lab by removing their gloves before handling group equipment or instruments. Do not open doors with gloves on. Do not write with gloves on.

A guide to gloves in the laboratory can be found on the Faculty of Science safety web page at **www.science.uottawa.ca/securite-safety/welcome.html**

Safety Equipment. Note the location of all the emergency showers, eye-washers, and fire extinguishers. Do not hesitate to use them in case of an accident.

In Case of Fire: Do not run! Small fires, if contained in a localized area, often burn themselves out, or can easily be put out. Try to smother a small fire by covering it to cut off the supply of oxygen. Turn off all open gas outlets and remove inflammable liquids from the vicinity. If clothing is on fire, try to smother the flames with a towel or lab coat. Stop, Drop and Roll. Call your demonstrator immediately!

Chemical Burns. Inhalation of fumes is best treated by exhaling at once, as deeply as possible, and immediately going to a fresh-air area. In case of skin burns, wash the affected area with *cold* water for several minutes. Do not use ointments. Get professional medical attention, especially if the burn is severe.

Cuts. Stop the bleeding with gentle pressure, using a sterile bandage if possible. Get medical attention if the cut is at all serious.

Fume Hoods. Whenever possible, use fume hoods to carry out chemical transformations. Ideally, everything should be done in the fume hood. Unfortunately, only a limited number of hoods are available and thus a considerable amount of the lab work must be done outside the hoods, on the bench top.

Accidents. All accidents, including minor and seemingly inconsequential ones, must immediately be reported to the demonstrator.

There will be absolutely no one allowed in the laboratory at a time other than the designated period.

Equipment

Take care of all equipment and clean it immediately after use. The proper apparatus necessary for each experiment is well described in the experimental instructions. For glassware, do not use a different size from that described unless you are sure the experiment allows it. ALWAYS MAKE SURE THAT YOUR APPARATUS IS SECURELY FASTENED WITH CLAMPS.

When setting up apparatus, work from the bottom up. For example, when setting up a reflux, start with the stir plate.

WHEN MAKING ANY ORGANIC SET UP, FLASKS THAT WILL CONTAIN CHEMICALS MUST ALWAYS BE CLAMPED.

The equipment in your locker is being used by other groups of students. It is common courtesy that you leave the equipment in good shape (clean, dry, not broken) for the next group.

Develop the neatness habit. Neatness impresses the demonstrators favorably and should result in better laboratory grades. Most importantly, neatness promotes safety.

An inventory list is provided for your locker. During the first lab period, perform an inventory of all your equipment and replace and broken or missing items.

Molecular models are required for this lab. Students are required to provide their own molecular models, as they will not be provided. Darling® molecular model kits are best for the purposes of this course. They are large, rugged, easily assembled and inexpensive. Molecular models may be purchased from the undergraduate laboratory on the third floor of Marion hall. If you have another set of models and wish to use them, you must obtain permission from the course instructor **before** entering the lab.

Reports

Reports must be handed one week after you do each experiment at the beginning of the period (10:00 for morning labs and 2:30 for afternoon labs). Reports do not need to be extensive, a few pages will suffice. Reports may be typed or hand written. Neatness counts, if we can't read it we can't mark it. If you prepare your report with another person, be sure to write your report in your own words. Plagiarized reports will result in disciplinary action.[1]

A report presents your results and shows that you understood what you did. It is important that you write your reports in your own words.

Each experiment should have a title, experiment number and date. For experiments involving chemical

1. Regulations on academic fraud can be found at web5.uottawa.ca/mcs-smc/academicintegrity/regulation.php Detailed information about plagiarism can be found in the following document www.uottawa.ca/plagiarism.pdf.

transformations, the balanced equations and reaction mechanisms must be provided for **all reactions**. For most experiments, this section of the report should be less than one page. If you draw large molecular structures, it may fill two pages.

Procedure and Observations. This section should describe what YOU actually did and observed - not necessarily what is stated in this manual. A table of reagents must be included listing the reagent used, the molecular weight, the amount used, the density (if applicable) and the number of moles or millimoles. A sample table is shown below. This table should only include those materials used in your reaction, not in the work-up phase (see below). It is not necessary to calculate the number of moles of the reaction solvent. Report all observations as they occurred - not as described for the average experiment in this manual. If you have heated a solution for 35 minutes instead of the 30 minutes suggested, record 35 minutes. The procedure should be described in point form (repeat the lab manual including any changes made). You should include quantities, the visual appearance (e.g., pale yellow liquid, colorless crystals, white powder), images of TLC plates and yields obtained. Indicate the method of purification used, the amount obtained, and physical constants (m.p. or b.p.) of the purified product.

Table 1. Sample reagent table for procedures.

Compound	Mol. Wt (g/mol)	Amount	density (g/mL)	mmol
Benzoic acid	106.54	250 mg		2.05
MeOH	32.05	20.5 mL	0.791	
DCC	206.32	633 mg		3.07

Organic reactions are normally followed by a **workup**. During the reaction phase, the molecules undergo the reaction you are trying to accomplish. During the workup phase you will destroy any excess chemical reagent and purify the product of your reaction. If you are not sure what the workup phase of your reaction is, ask your demonstrator. Note that the materials used in the work-up are not normally listed in the table of reagents.

Reporting numerical data. When reporting weights and volumes be as accurate as your equipment allows you to measure. Don't give meaningless decimal places that pretend an accuracy that does not exist. Decimal fractions smaller than unity must be rendered with a zero preceding the decimal point, for example, 0.25 g, not .25 g. This is how numerical data is generally presented in the chemical literature.

Discussion. Show any required calculations such as % yield. You should explain briefly what is happening in your experiment.

Explain the steps of the procedure to outline why each operation is done. One or two sentences per step are sufficient. You may refer to your chemical equations or mechanisms for this. Each procedural step is done for a reason, and your goal in this lab is to understand how and why you do things. The use of a chemical mechanism is better than a half-page explanation. Be sure to briefly explain each step of the workup.

Labeling products. In some experiments your products are to be handed in for inspection and marking. The products should be submitted in well-stoppered vials, properly labeled with the following information: your name and your partner's name, the date, tare weight of the container (weight of the empty container including the stopper and label), the name of compound, the weight of material present and the melting or boiling point of your sample (not the literature value).

Questions. You must answer ALL of the questions at the end of each experiment.

You will frequently be given hints as to how to organize your lab write-ups in the worksheets provided. Every now and then come back to the introduction section and re-read the "Reports" part.

A lab report should take no more than 2 to 4 hours to prepare.

Marking

Reports. Reports will be marked for quality not quantity. We are looking for an understanding of the material and procedures. Neatness counts. If we can't read it we can't mark it. Make sure to get your reports done on time. Reports that are handed in up to 24 hours late will be penalized 20%. Late reports MUST be EMAILED in FULL to your TA. The hard copy must still be deposited in your TA's box. Reports handed in more than 24 hours late will receive a grade of 0.

You must attend labs and hand in reports for at least 5 experiments to pass the course. Absence from an experiment without an approved exemption will result in a grade of 0 for the evaluation and report for that experiment.

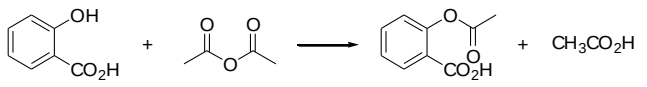
Evaluation. A demonstrator's evaluation will be a significant part of your mark. This evaluation will be based on your performance in the lab: how well you are prepared, how efficiently you use your time, how neat you are etc.

Final Exam. The lab exam will be part of the final exam for the course. This exam will test your understanding of the experiments you did.

Calculating Yields

In order to carry out the interconversion of one substance into another it is necessary to know the correct quantities of reagents required for the transformation. This information can be obtained from the balanced equation of the interconversion.

For example, aspirin (acetylsalicylic acid) is obtained by heating salicylic acid with acetic anhydride in the presence of a small amount of sulfuric acid as catalyst. The balanced equation is:



salicylic acid	acetic anhydride	aspirin	acetic acid
MW = 138	MW = 102	MW = 180	MW = 60

Thus, 1 mole (138 g) of salicylic acid reacts with 1 mole (102 g) of acetic anhydride to give 1 mole (180 g) of acetylsalicylic acid and 1 mole (60 g) of acetic acid. To get complete conversion of salicylic acid to the desired product, one mole of acetic anhydride must be used for each mole of salicylic acid. The reagents in a synthetic experiment should always be chosen in their proper molar ratio (stoichiometry) unless there are valid reasons to do otherwise.

Calculation of yield. In an organic experiment one rarely isolates the theoretical amount of desired product. There are several reasons for this: (1) The reaction may not be 100% efficient. Some of the reagent may be consumed in a reaction which is different from the desired one – this is referred to as a side reaction. (2) The starting material(s) and product(s) may be in equilibrium under the reaction conditions and so complete conversion to products is not achieved. (3) The desired product cannot be isolated with 100% efficiency because of the physical properties of the compound. There are inevitable losses during any purification step.

The efficiency of a reaction is reported as the percent yield, defined as follows:

$$\%\ \text{yield} = \frac{\text{amount obtained (g)}}{\text{theoretical amount possible (g)}} \times 100\ \%$$

Example. A student carrying out the synthesis of acetylsalicylic acid as described above starts with 13.8 g (0.1 mole) of salicylic acid and 10.2 g (0.1 mole) of acetic anhydride and eventually isolates l4.0 g of purified acetylsalicylic acid. Using the balanced equation, it is calculated that the theoretical yield is 0.1 mole or 18.0 g.

The % yield would be (14.0 g / 18.0 g) x 100 % = 77.8 %. Yields are commonly reported using only 2 significant figures. Thus the student would report a yield of 78 %.

Another, less commonly used method of calculating yields involves the conversion of all weights to moles. From the balanced equation it is obvious that 1 mole of salicylic acid and 1 mole of acetic anhydride will give 1 mole of acetylsalicylic acid. Starting with 0.1 mole of each reagent, the theoretical yield would be 0.1 mole of product. The amount of product obtained is 14.0 g/ 180 $g \cdot mol^{-1}$ which equals 0.0778 moles of product isolated. The percent yield is: % yield = (0.0778 moles / 0.10 moles) x 100 % = 78 %.

Non-stoichiometric amounts. There are times when the amounts of reagents used in a synthetic experiment are not equal to those indicated by the balanced equation. Various reasons exist for using some reagent in excess.

(1) The starting materials and the desired product are in equilibrium.

$$A + B \rightleftharpoons C \qquad [C] = K[A][B]$$

The yield of C, as calculated on the basis of A, can be improved considerably by increasing the concentration of reagent B (to drive the equilibrium to completion). Usually the cheaper reagent, or the one which is easier to separate from the product, is used in excess. The yield in cases like this is always calculated on the basis of the limiting reagent (the reagent present in smaller equivalent amount).

(2) One reagent may be harder to separate from the product than the other. In these cases, the limiting reagent will be the material that is more difficult to remove. This is done to ensure that the problematic substance is completely consumed during the reaction.

(3) One of the starting reagents is unpleasant or dangerous. Making this reagent limiting will ensure it is completely consumed.

(4) One reagent is unstable under the reaction conditions. This unstable component is used in excess to ensure a sufficient amount of it is always available.

(5) Occasionally, one of the reagents also acts as the solvent, and is thus is present in excess.

Calculation of yield with limiting reagents. Suppose that, in the preparation of acetylsalicylic acid described previously, some of the acetic anhydride was hydrolyzed by water present in the reaction mixture before it had time to react with the salicylic acid. In order to circumvent this problem, it was decided to repeat the experiment using 13.8 g (0.1 moles) of salicylic acid and an excess of acetic anhydride (15.3 g, 0.15 moles). The yield in this case turned out to be 16.0 g of acetylsalicylic acid.

In this experiment the limiting reagent is the salicylic acid. According to the equation, only 1 mole of acetylsalicylic acid can be obtained from 1 mole of salicylic acid. Hence, the theoretical yield in the experiment is l8.0 g (0.1 mole of acetylsalicylic acid), and

the percentage yield is: % yield = (16.0 g / 18.0 g) x 100 = 89 %

The theoretical yield must be calculated on the basis of the limiting reagent.

Thin Layer Chromatography

For all labs that use TLC (starting in experiment 1), you must include a drawing of all TLCs in your report. They should be labeled as shown in the next figure. Do not include the real TLCs.

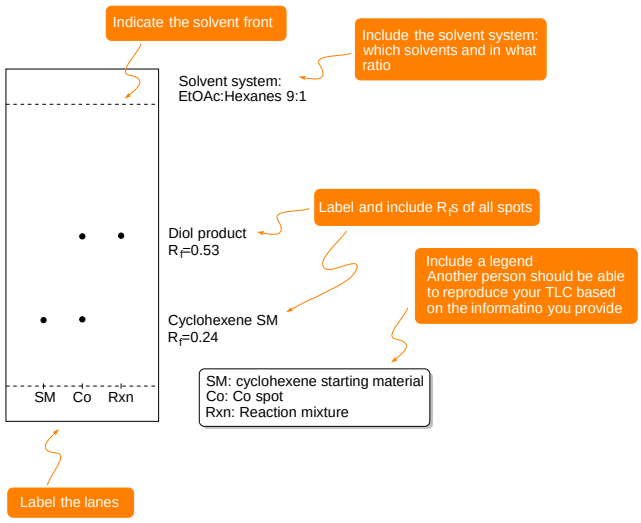


Figure. How to label a TLC plate for a lab report

Locker Check-Out

Please circle your course number. (2123 : 2523 : 1321 : 1721)
Print your name.
Student (1) _____
Student (2) _____
Locker # _____
Demonstrator _____

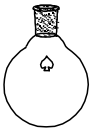
TOP SHELF	IN	OUT
RUBBER TUBING	4	
WASH BOTTLE 250 ml	1	
UNIVERSAL CLAMP	4	
EXTENSION CLAMP	3	
CLAMP HOLDER	7	
TEST TUBE HOLDER	1	
CRUCIBLE TONGS	1	
SUPPORT RING 3"	1	
WIRE GAUZE	1	
CORK RING MEDIUM.	2	
CORK RING SMALL	2	

BOTTOM SHELF	IN	OUT	STORAGE BOX	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				

Note : Each locker contains a full organic glassware kit on the bottom shelf. You must show your locker to the demonstrator before leaving.

Glassware

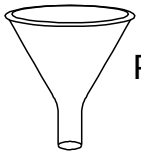
25 or 50 mL Round Bottom Flask



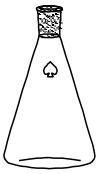
100 mL and Larger Round Bottom Flask

West Condenser

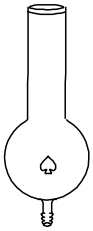
Liebig Condenser



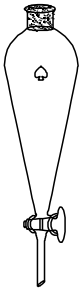
Powder Funnel



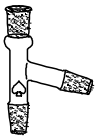
Erlenmeyer Flask



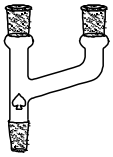
Drying Tube



Separatory Funnel

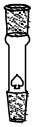


Distillation Head

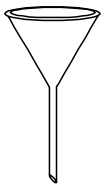


Claisen Adapter

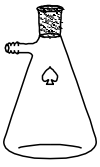
Vacuum Take Off Adapter



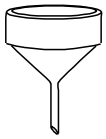
Thermometer Adapter



Filtering Funnel

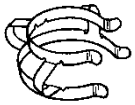


Suction Flask

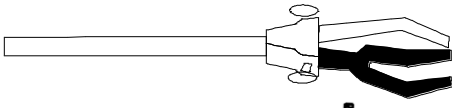


Büchner Funnel

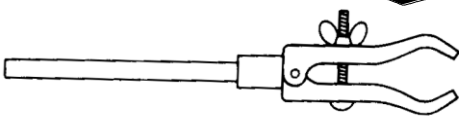
Equipment



Keck Clip

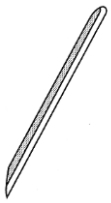


Universal Clamp (Three-finger Clamp)



Extension Clamp

Clamp Holder



Scoopula

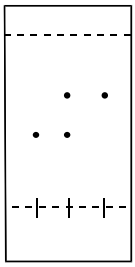
Spatula

Experiment 1: Thin Layer Chromatography

Department of Chemistry, University of Ottawa, 10 Marie Curie Priv, Ottawa, ON, K1N6N5

September 2014

ABSTRACT



This lab will introduce the technique of thin layer chromatography, used to monitor the progress of a reaction

Thin Layer Chromatography

The most difficult and time consuming part of a modern organic preparation is the purification of the final product. There are many ways to do this, but perhaps the most rapid and reliable method is chromatography. Chromatography is also a good way to monitor reactions. It can be used to determine when a reaction is complete, and to verify that the correct product is being produced.

In chromatography, mixtures are separated according to differences in their solubilites between two phases (solvents). One of these phases is held stationary, while the other phase is mobile. The mixture to be separated is dissolved in the mobile phase, and this solution is then passed through the stationary phase. Since one phase moves, even a small difference in affinity for the two phases will become magnified as the interactions are multiplied over time. The net result is the separation of the components.

Extraction and chromatography are related in that they each involve the distribution of chemicals between two phases. In fact, chromatography can be considered as a continuous extraction that happens because of the movement of phases relative to one another.

Thin Layer Chromatography (TLC). In this experiment you will perform a specialized type of adsorption[2] chromatography called thin layer chromatography (TLC). In this method, a thin layer of adsorbant (stationary phase) is attached

[2] **Adsorb:** accumulation of a compound onto a surface.

to a plate (usually made of glass or aluminum) and the mobile phase passes over the plate and through the stationary phase. TLC is used for reaction monitoring, for sample purification, and to determine the purity of samples.

The choice of adsorbent and mobile phase depends on the materials being separated and on the preference and the experience of the chemist. By far the most common adsorbent is silica gel. Silica is a polar material, and the mobile phase is an organic solvent. More polar compounds are attracted more strongly to the silica than to the solvent, and so move more slowly up the TLC plate. Less polar compounds are attracted less strongly to the silica and so move more quickly up the TLC plate. Increasing the polarity of the solvent makes a polar compound more attracted to that solvent, making it move up the plate faster (but still not as quickly as a non-polar compound). Separations are optimized by changing the polarity of the mobile phase.

Table 2 shows a list of laboratory solvents listed in order of polarity. Separations are optimized by altering the polarity of the solvent to achieve the desired level of purity. This may be done by simply changing solvents, but a much better method is to use mixtures of a polar solvent and 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; ether/petroleum ether; and dichloromethane/methanol.

Table 2. Common solvents used for silica gel chromatography.

Petroleum Ether	Least Polar
Benzene	↓
Hexanes	↓
Dichloromethane	↓
Chloroform	↓
Diethyl ether	↓
Ethyl acetate	↓
Acetone	↓
Methanol	↓
Water	↓
Acetic Acid	Most Polar

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.

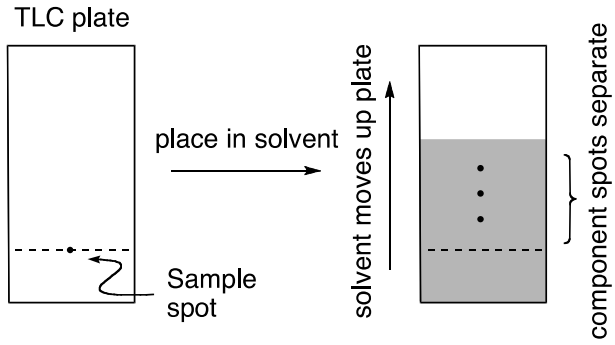
Table 3. Relative mobility of common solvents for chromatography.

Saturated hydrocarbons	Fastest
Alkenes	↓
Aromatics	↓
Halogens	↓
Ethers	↓
Aldehydes and Ketones	↓
Alcohols	↓
Carboxylic Acids and Amides	Slowest

In a TLC experiment, the sample to be separated is applied to the silica in a small spot (or zone) near the bottom. The plate is placed in a developing tank that contains the mobile phase such that that the bottom of the plate is immersed in this mobile phase solvent. Capillary action draws the mobile phase up through the silica gel layer, moving the mobile phase through the silica gel (stationary) phase. The mobile phase draws the sample with it, and in the process the components separate according to their affinity for each phase. When this happens a series of spots appears on the plate, each one corresponding to a different compound in the mixture.

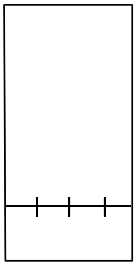
During this process, it is important that the solvent not be allowed to evaporate from the surface of the plate. A cover is therefore placed over the developing tank to prevent evaporation of the mobile phase.

Figure 8. Separations on a TLC plate



To prepare a TLC plate, **gently** use a pencil to draw a line across the plate *approximately* 1 cm from the bottom of the plate. Draw small tic marks on this line at locations where samples will be applied. These marks should be located at least 5 mm from the edges of the plate, and should be spaced no closer than 3 mm from each other.

Figure 9. Preparing a TLC plate

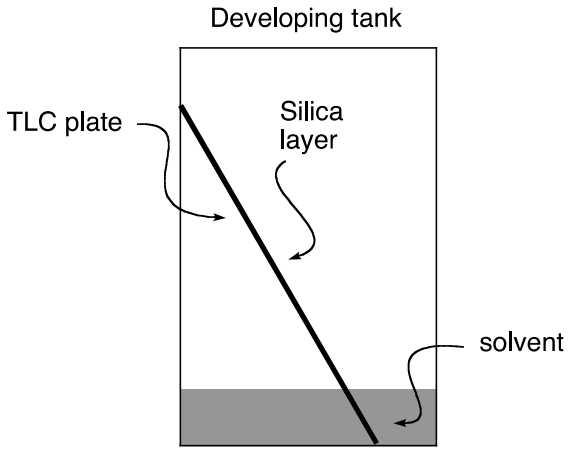


The samples are then applied to the plate as small spots at the appropriate intersection along the line. This is done using a small capillary tube called a spotter. The spotter is dipped into the sample solution (all samples should be dissolved in a small volume of a volatile solvent such as dichloromethane). The tube will fill by capilliary action (1 or 2 seconds). Once filled, touch the spotter to the plate on the line at the appropriate location. The silica gel will draw the liquid from the tube and you will see a small spot formed on the plate. **You do not need to apply a lot of sample; smaller sample spots will give better results**. Before proceeding to the next step, you should allow enough time for the solvent to evaporate from the spot (about 30 seconds). As the solvent evaporates, the spot will disappear.

The mobile (or eluting) solvent mixture (called a solvent system) should be prepared a few minutes before spotting the plate. Pour the solvent into the developing tank and close the cover. Only a small amount of solvent is needed, it should be less than 1 cm deep in the tank (5 mm is ideal). Wait for a minute or two to allow a little of the solvent to evaporate and fill the chamber with vapor. The plate can then be placed in the tank and eluted.

When placing the plate in the developing tank (jar), the layer of silica should face away from the wall of the tank as shown in Figure 2, It is important that the surface the solvent is **below** the line on the silica showing the position of the sample spot(s). If the solvent level is too high, the sample will dissolve in the liquid and ruin the plate. Once the plate is in the tank, place the cover over the top of the chamber. Allow the solvent to move up the plate (elute) until the solvent front is almost at the top (approx 5 mm from the top). Remove the plate from the tank and allow the solvent to evaporate (this usually only takes about 30 seconds). Once the plate is dry, the spots are visualized.

Figure 10. Eluting a TLC plate

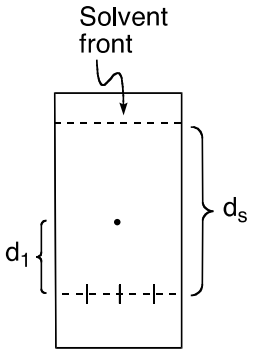


The position of the components on the plate is determined by visualization and development. Typically, a TLC plate will be visualized by placing it under UV light. The silica gel will fluoresce and dark spots will appear on the plate. *Lightly* draw the outlines of the spots with a pencil.

After it is visualized, a plate is then developed using a chemical reagent or dye. Only one type of stain can be applied to each plate. Because development (staining) alters the chemical makeup of each spot, it is important to visualize using UV light *before* the plate is developed.

The movement of compounds up a TLC plate is described by the R_f value. This is defined as the displacement of the compound (d_1) divided by the displacement of the solvent front (d_s) as shown in Figure 3a. The R_f value is a measure of the polarity of each component (spot). Molecules that are non-polar will move quickly through the silica gel and therefore will have large R_f values. These spots appear near the top of a TLC plate. Polar molecules travel slowly through the silica gel and appear at the bottom of the TLC plate. These compounds have a small R_f value.

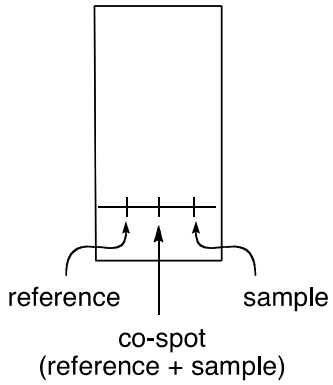
Figure 11. Determining R_f values



When the TLC is used for monitoring a reaction or to demonstrate the purity of a sample, a reference compound is normally added to the plate. For reaction monitoring, this reference compound is often one of the starting materials used in the reaction. TLC plates are run at regular intervals during the reaction, and the reaction is considered to be complete when this spot disappears from the reaction mixture. The solvent system should be chosen so that the R_f of the reference compound is approximately 0.5 to 0.6. In this way, compounds in the mixture can be visualized that are either more or less polar than the reference compound.

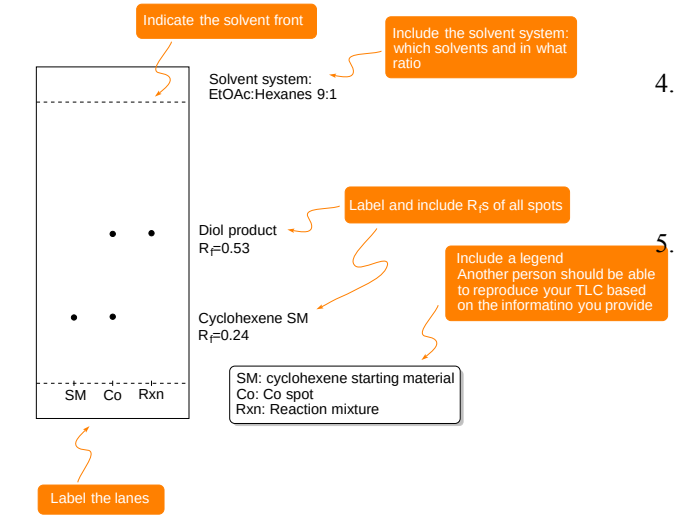
Three lanes should be spotted on all TLC plates (NO MORE, NO LESS). Typically, the first lane contains the reference compound. The third lane contains the sample being analyzed. The middle lane contains a co-spot consisting of both the reference compound and the analyte sample. The co-spot allows for the differentiation of compounds with very similar R_f values. To create a co-spot, simply apply the reference spot to the left location (lane) and then to the centre location (co-spot). Allow the solvent to evaporate. Then apply the reaction mixture to the right most lane and then to the centre lane (co-spot). It is important to apply sample to the co-spot area last (why?).

Figure 12. Reference, sample and co-spot lanes



Proper TLC analysis requires reference samples in order to be able to directly compare R_f values. In case the mobility of materials is very similar, the co-spot ensures that closely spaced spots can be compared. You must include a co-spot. Do not use more than 3 lanes on a single TLC. For your lab report, redraw your TLCs as shown in the next figure. Do not take your TLCs home.

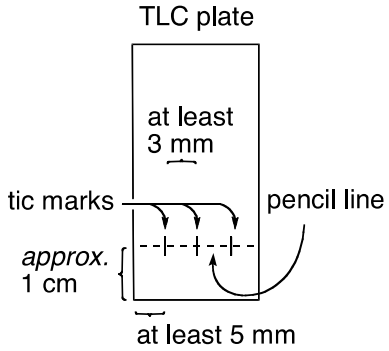
Figure 13. Reference, sample and co-spot lanes



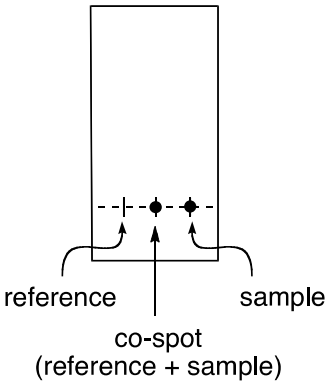
The Experiment

Part A: Identifying the components of an unknown mixture using TLC.

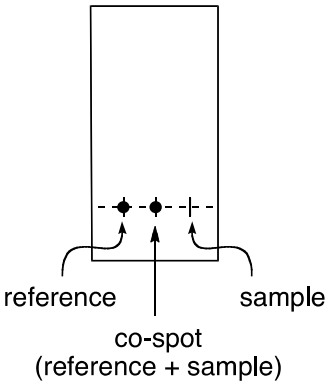
1. Add approximately 10 mL of a 2:8 mixture of ethyl acetate (EtOAc) and hexanes to your developing jar and place the lid on the jar.
2. Prepare two TLC plates by lightly drawing a pencil line *approximately* 1 cm from the bottom of each plates (be careful not to touch the silica with your fingers). Draw three equally spaced tic marks on this line. The outside lanes should be at least 5 mm from each edge. Make sure to keep track of what will be spotted in each lane (you may wish to draw identifying marks on each lane).



3. Obtain a sample containing an unknown compound from your demonstrator. **Record the identification number of your unknown in your lab report.**
4. In a small test tube, dissolve *approximately* 10 mg of your sample in 1 to 2 mL of dichloromethane. Be sure to label the test tube so that you know it contains your sample.
5. Spot this sample solution onto both of the plates you prepared in step 2 on the sample lane and on the co-spot lane. To apply a spot, dip a capillary in the solution. Touch the end of the capillary to the TLC plate at the appropriate lane. You will see the solution expand on the plate into a small spot (about 2 mm wide). Wait a few seconds for the solvent to evaporate. **Apply SMALL amounts to the plate. Small spots give better results.**



6. Place *approximately* 2 mL of the reference solution of benzophenone in a separate test tube. Spot this sample solution onto ONE of the plates you prepared in step 2, on the reference lane and on the co-spot lane. Ensure to apply the solution to the co-spot lane second. Use a fresh capillary for each sample.



7. Place *approximately* 2 mL of the reference solution of biphenyl in a separate test tube. Spot this sample

solution onto the other plate you prepared in step 2, on the reference lane and on the co-spot lane.

8. Carefully place the TLC plates into the developing jar, ensuring that the pencil line is above the top of the solvent level. Make sure that the silica layer faces towards the center of the jar. Place the cover on the developing jar. Allow the solvent to elute until the solvent front is approximately 1 cm from the top of the plate (3–5 min).
9. Remove the plates from the jar and mark the solvent finish line with a pencil. After allowing the solvent to evaporate from the plate (30 seconds), visualize your TLC plate by illuminating it under a UV light. (**NEVER LOOK DIRECTLY AT A UV LAMP)**. Gently circle the visible spots with a pencil. Measure the R_f of each spot on the plate (remember to measure to the center of each spot) and draw an exact replica of the TLC plate in your lab report. Using the R_f values, identify the compounds that are in your mixture.

Part B: Effect of solvent on TLC.

1. Empty the solvent from your developing jar into the solvent waste. Add approximately 10 mL of ethyl acetate to your developing jar and place the lid on the jar.
2. Prepare two new TLC plates, using the procedures described above (part **A** steps 2–7).
3. Develop these plates in the jar containing EtOAc as the eluant. Visualize your TLC plates with UV light, and mark the spots with a pencil. Draw a replica of the TLC plates in your lab report. Be sure to make a note of the R_f of each compound in your lab discussion.
4. Empty the solvent from your developing tank into the solvent waste. Add approximately 10 mL of hexanes to your developing jar and place the lid on the jar.
5. Prepare two new TLC plates, using the procedures described above (part **A** steps 2- 7).
6. Develop these plates in the jar containing hexanes as the eluant. Visualize your TLC plates with UV light, and mark the spots with a pencil. Draw and label a replica of the TLC plates in your lab report. Be sure to make a note of the R_f of each compound in your lab discussion.
7. Describe the effects of each solvent system on the R_f of the compounds, and account for the appearance of your plate taking into consideration the polarity of the solvent used.

Part C: Ratio of compounds

1. Take a vial labeled XX, YY, OR ZZ, which contains a mixture of compounds. Record which vial you have taken.
2. Determine the identity of each component of your mixture, through TLC comparison with reference compounds: bromobenzene, *o*-bromonitrobenzene, *m*-bromonitrobenzene, and *p*-bromonitrobenzene. DO NOT circle the spots on your TLC plates or discard them yet. You can label them at the bottom or top to remind yourself of what each one represents.
3. Once the lab is completed and you have cleaned up your area, turn off the light in the fumehood and take a photo of each TLC plate from this part under UV.

4. Use ImageJ and the appropriate calibration curves to determine the ratio of compounds in your mixture. Instructions can be found in Appendix III. ImageJ can be downloaded from Blackboard.

Questions

1. How does increasing the polarity of the solvent system affect the results of a TLC?
2. In the following sets of compounds, which would have the smallest R_f on silica gel? Use a sentence to explain your reasoning. Draw Line structures of each compound
   a. Benzyl alcohol, benzaldehyde, benzyl acetate
   b. Aniline, *N,N*-dimethylaniline, naphthalene
   c. Benzophenone, Biphenyl, Benzoic acid

Report Notes

Refer to the instructions early in the lab manual regarding the preparation of reports. Note that you do not need to provide a table of reagents for this report.

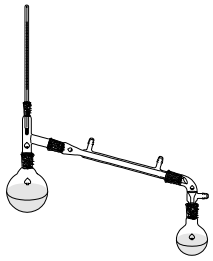
You should have accurate drawing of all TLCs, as shown in a figure above. Do not take your TLCs home. In your discussion, interpret the results of each TLC. For example, explain how you identified each spot and how the R_f values relate to the compounds' relative polarities.

Experiment 2: Purifying Chemicals by Distillation

Department of Chemistry, University of Ottawa, 10 Marie Curie Priv, Ottawa, ON, K1N6N5

September 2013

ABSTRACT



In this experiment you will carry out and compare simple and fractional distillations.

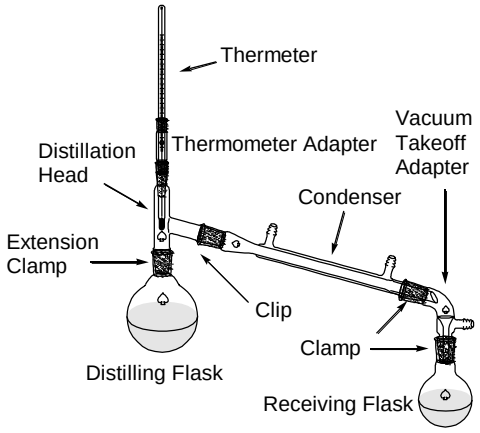
Distillation

Distillation is a method of separating two or more compounds on the basis of differences in their boiling points. It is a process in which liquids are vaporized and the vapors are condensed and collected. Evaporation refers to a distillation in which the vapors are allowed to escape. A reflux is a process of distillation with a return of the condensate to the original flask. Reflux is used as a method of maintaining a specific temperature in a solution, evaporation is used as a way of removing one or more components (usually solvent), whereas distillation is used as a means of purification.

The boiling point of a pure liquid is defined as the temperature at which the vapor pressure of the liquid is equal to the pressure applied to the solution. In most cases, this pressure refers to atmospheric pressure. At lower pressures, liquids boil at lower temperatures. Because the boiling point is sensitive to pressure, it is necessary when reporting a boiling point to state the pressure at which boiling was observed. In most cases this is simply atmospheric pressure, but organic chemists sometimes reduce drastically the pressure of the apparatus as a way of decreasing boiling points below decomposition temperatures.

A pure substance will show a narrow boiling range spanning one or two degrees Celsius. The boiling point range of a liquid can be used as an indication of purity.

Figure 1. A simple distillation apparatus



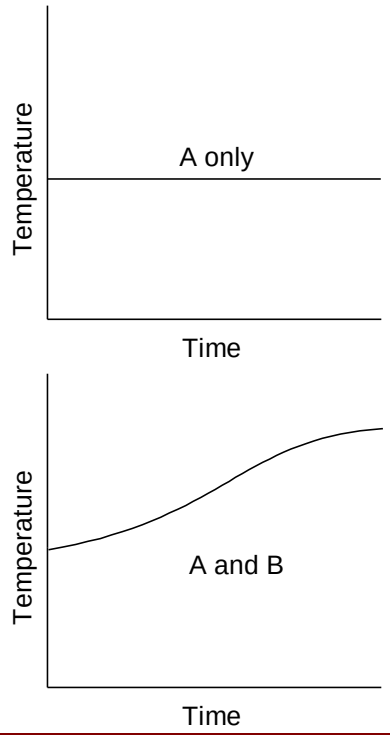
The characteristic boiling point of a substance reflects the molecular structure and type of forces holding the molecules together in the liquid state. Polar compounds, especially those that are hydrogen bonded, tend to have higher boiling points than non-polar compounds, which are attracted to each other only by relatively weak Van der Waals forces. Generally, an increase in molecular weight leads to an increase in boiling point if the polarities of the compounds being compared are roughly equivalent.

Simple distillation. A simple distillation apparatus is shown below. The liquid to be distilled is placed in the distilling flask and heated. The heated liquid vaporizes and is carried upwards past the thermometer bulb, through the distillation head (still head) and into the condenser. The condenser is cooled by a *slow* water flow through its water jacket. The vapor cools on contact with the condenser and returns back into liquid form. The drops in the condenser run down the slope of the glass and are collected in the receiving flask. The vacuum takeoff adapter connects the condenser and receiving flask and provides a way of controlling the pressure (the takeoff adapter is sometimes called an elbow). At all times the pressure inside the apparatus must be equal to atmospheric pressure or the glass will fracture. This is simply done by leaving the tubing connector of the elbow open. In advanced distillations under vacuum, the vacuum takeoff adapter provides a way to *lower* the pressure of the distillation by providing a connection to a vacuum pump through the tubing connector.

At the beginning of the distillation, the component with the lowest boiling point will be removed, and so the distillate that is collected in the receiving flask will be enriched in that component. As the distillation proceeds, compounds with higher boiling points will begin to be removed from the distilling flask and the distillate collected in the receiving flask will be enriched in these components. By changing the receiving flask from time to time, one obtains several fractions, each containing a different mix of the components of the original sample. Ideally these fractions will contain pure compounds, but in practice mixtures may be obtained. The degree of separation depends on the difference in boiling point between the components, the technique used and the skill of the operator.

The temperature of the distillation of a pure substance will remain constant as long as both vapor and liquid are present (top curve below). As a mixture is heated, the temperature does not often remain constant, but increases throughout the distillation. This occurs because the composition of the vapor varies continuously as the distillation proceeds. The resulting temperature profile is shown in the bottom curve, in which a steady increase in temperature is noted as the mixture is distilled.

Figure 2. Temperature changes during a distillation



Consider a mixture of two liquids, A and B, and suppose A has the lower boiling point. In other words, A is more volatile, and consequently will have a higher vapor pressure than B. The behavior of each liquid, and the corresponding vapor, is governed by Raoult's Law, which states that the partial pressure (P_A) of liquid A in the mixture of vapors is equal to the partial pressure of the pure liquid (P_{A°) times the mole fraction of that compound (N_A) in the mixture. The partial pressure of liquid B (P_B) in the mixture is calculated similarly. The total pressure (P_{total}) exerted by the mixture is equal to the sum of the partial pressures of the components. Raoult's law tells us that the vapor will contain more of liquid A (which has a higher vapor pressure) than is present in the liquid.

Figure 3. Raoult's law

$$P_A = (P_{A^\circ}) \cdot (N_A)$$

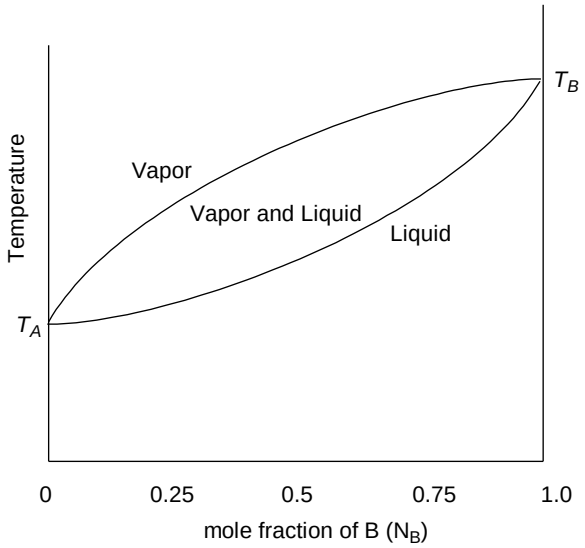
$$P_B = (P_{B^\circ}) \cdot (N_B)$$

$$P_{total} = P_A + P_B$$

$$P_{total} = (P_{A^\circ}) \cdot (N_A) + (P_{B^\circ}) \cdot (N_B)$$

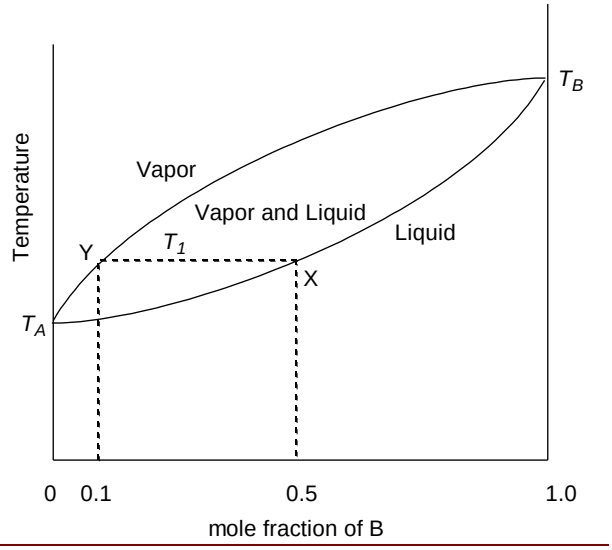
The behavior of the system can be described by a phase diagram such as shown below. The boiling points of pure liquid A and pure liquid B are given by T_A and T_B respectively. The lower curve represents a boundary below which there is only liquid, and corresponds to the boiling points of mixtures of A and B (given by the mole fraction of B in the mixture – remember that $N_A+N_B=1$). The upper curve is a limit above which there is only vapor. In between the two curves we have vapor and liquid. At the extreme left of the phase diagram there is only A (mole fraction of B is 0) and at the extreme right of the diagram there is only B (mole fraction of B is 1). As we move left to right along the lower curve, one can see that the temperature of the boiling liquid increases steadily as the amount of B in the *liquid* increases.

Figure 4. Phase diagram for mixture of two liquids



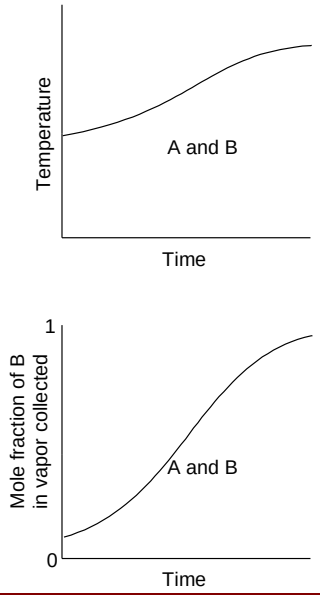
The upper curve represents the composition of the *vapor* at any given temperature. We can determine the composition of the vapor by drawing a horizontal line between the curves, then extrapolating down. Suppose we have a 50:50 liquid mixture of A and B, in other words the mole fraction of B in the liquid mixture is 0.5. Extrapolating up to the lower curve, we see that this corresponds to point X, which gives a boiling point of T_1 for that mixture. Reading horizontally, we see that temperature T_1 crosses the vapor line (upper curve) at point Y. This corresponds to a vapor composition of approximately 10 % B and 90 % A (mole fraction of B = 0.1). The vapor contains more of the lower boiling point liquid (A). *The boiling **liquid** contains **50 %** A whereas the **vapor** produced by this boiling liquid is composed of **90 %** A.*

Figure 5. Boiling point of a 50:50 mixture



As we perform the distillation, we continuously remove the vapor that is produced. As illustrated above, this vapor always contains more of the lower-boiling component (A), and so more A than B is removed from the liquid. The composition of the *liquid* therefore shifts to the right of the curve as the distillation proceeds and A is removed (liquid has a higher mole fraction of B). The overall result is that the temperature recorded during the distillation increases steadily as the distillation proceeds. If we plot the temperature of the vapor as the reaction proceeds, we get a smooth curve. Similarly the composition of the distillate collected will vary as a curve.

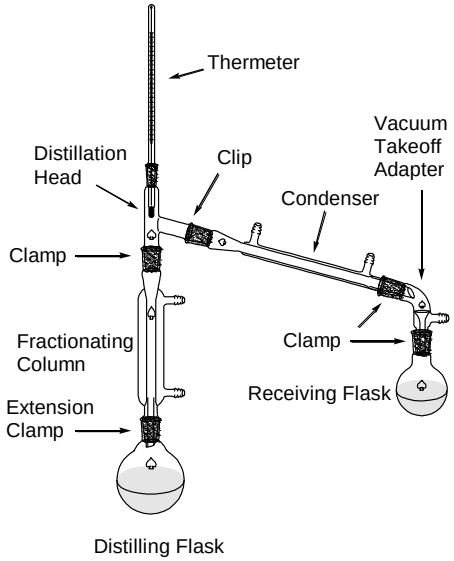
Figure 6. Temperature changes and composition changes during distillation



Although we do get enrichment of A at the beginning of the distillation, and enrichment of B at the end, the efficiency of the separation will not be good because the composition of the vapor varies continuously. Simple distillation therefore is not a good method to choose for complex mixtures, or when the boiling points of the components are similar. Simple distillation is very effective if the starting liquid contains mainly one component, or if the difference in boiling point between the components is large ($\Delta T > 100$ °C).

Fractional distillation. Improved separations can be achieved by using fractional distillation. The apparatus is that of a basic distillation, with the addition of a fractionating column.

Figure 7. Fractional distillation apparatus

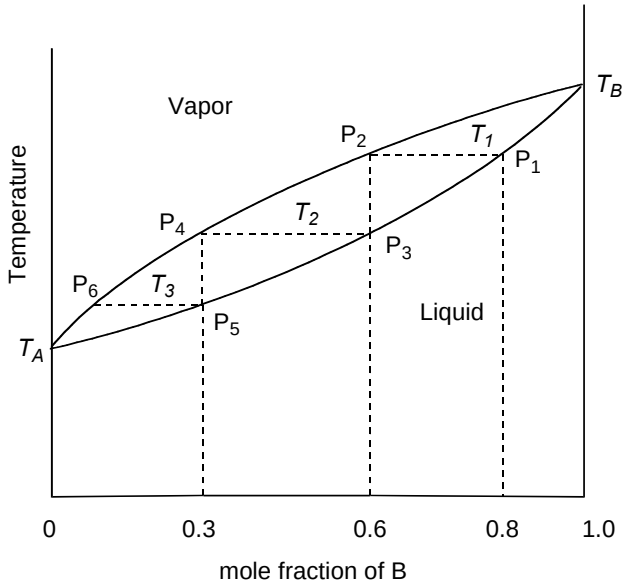


In a typical distillation using a fractionating column, a series of condensations and vaporizations take place that enrich the vapor in the lower boiling component. The fractionating column provides a surface for the vapor to condense on as the distillation proceeds. The greater the surface area of this column, the better the separation. The surface area of the column may be increased by adjusting the shape of the column (Vigreaux column), or by the introduction of *packing* into a simple straight column. Packing is an inert material with a high surface area. Glass rings, metal sponge (usually copper-brass) or glass wool are commonly used. In addition to the packing, a fractionating column is also normally insulated to provide a smooth temperature gradient.

As vapor rises in the fractionating column it condenses on the packing. Heat from the rising vapor below causes some of this condensate to re-vaporize, and in doing so more of the lower boiling component (A) is vaporized. As the vapor rises, it becomes enriched in the lower boiling component, while the liquid that condenses, and drips back into the distilling flask, becomes enriched in the higher boiling component (B). Each condensation-vaporization cycle enriches the vapor in (A) and returns some condensate, enriched in B, to the distilling flask. If enough cycles take place, effective separation of the vapors takes place.

This process can be understood once again by using a phase diagram. Suppose we are distilling a liquid mixture composed of 20 % A and 80 % B (mole fraction B = 0.8). From the phase diagram below it is apparent that the mixture boils at temperature T_1 (obtained from point P_1). At this temperature, the vapor has a composition of about 40 % A and 60 % B (obtained by reading across the phase diagram to get to point P_2 and extrapolating down). As this vapor comes in contact with the packing in the column, it condenses to a liquid composed of 40 % A and 60 %B (**the same composition as the vapor at point P_2**). Most of this liquid will then drip back down into the distillation flask. Heat rising through the column will re-vaporize ***a small portion*** of this liquid. Because the liquid in the column is composed of 40 % A and 60 % B, it boils at a new, lower temperature T_2 (read from point P_3). At this temperature, the vapor will have a composition of about 70 % A and 30 % B (point P_4). The remaining liquid, which is not re-vaporized, is enriched in B and now has a higher boiling point than the temperature of the column at that point (T_2). This liquid drips down the column and back into the distilling flask. The vapor in the column (at temperature T_2) now travels up the column until it again condenses. The condensed liquid at this point has the same composition as the vapor (70 % A and 30 % B, point P_4). Once again, heat rising through the column vaporizes *a portion* of this liquid. Because the liquid at this point is composed of 70 % A and 30 % B, it boils at a new temperature T_3 (read from point P_5). At this temperature, the vapor will have a composition of about 90 % A and 10 % B (point P_6). The droplet left behind will therefore have more of component B, have a higher boiling point than T_3, and drip back down the column.

Figure 8. Phase diagram for fractional distillation



The process in effect is a series of mini-distillations taking place in the fractionating column. Each of these mini-distillations, referred to as theoretical plates, enriches the mixture in the lower boiling component. The number of theoretical plates in a column is a function of the surface area available. This can be adjusted by lengthening the column or by increasing the surface area inside the column. This can be done by adding indentations or packing material. More plates (more surface area) equals better separation.

There is a catch however. For the fractionating column to work, condensing liquid must drip down the column as vapor is flowing up. Increasing the packing density makes it more difficult for liquid to drip down. If a fractionating still is heated too strongly, liquid will be prevented from flowing downwards by the motion of the vapor moving upwards. This generates a condition known as "flooding" in which a pocket of liquid forms in the column. If this happens, the efficiency of the column is lost because the distillation is effectively re-started at the point of the flood. Increasing the number of theoretical plates therefore means that the distillation must be run more slowly. Column packing must therefore be adjusted to balance resolution with the time required, and care must be taken by the experimentalist to ensure that the rate of distillation is balanced.

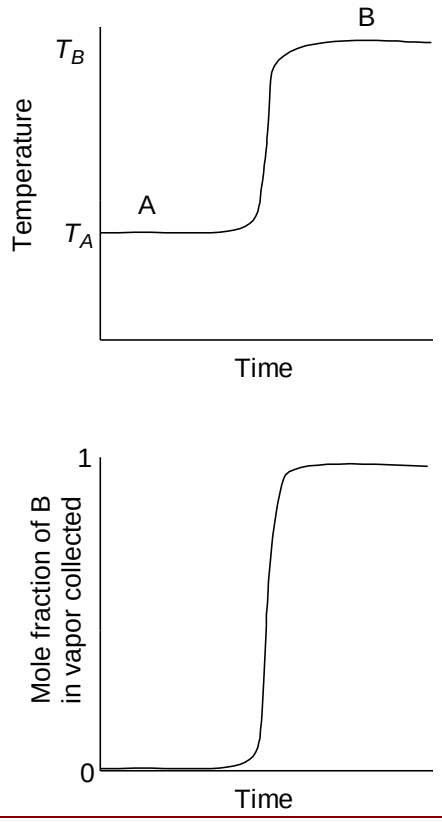


Figure 9. Temperature and composition changes during fractional distillation

An ideal fractional distillation shows a temperature profile like the one depicted above. Initially the vapor collected consists only of A and reaches the still head at a temperature of T_A (the boiling point of A). Once all of component A has been removed from the solution, the temperature rises to T_B and B is collected. It is common to collect a small mixed fraction just as the removal of A is completed and B begins to be collected. It is also very common to see a small *drop* in temperature when A has been completely removed and just before B is collected. A skilled chemist will recognize this and can often achieve excellent separations. There are three main factors that control the quality of the separation. (1) The difference in boiling point between A and B – the larger the better. (2) The number of theoretical plates, more plates give better separation (resolution). (3) The speed of the distillation – fast enough to be efficient but not too fast so as to avoid flooding the column. Distillation requires patience.

Positive Deviations. Many liquid mixtures do not behave ideally (they do not follow Raoult's Law). Mixtures that show a greater than expected vapor pressure are said to show a positive deviation. Other mixtures have a smaller than expected vapor pressure and show a negative deviation.

In mixtures of liquids that show a positive deviation, the two liquids seem to "dislike" each other and have a tendency to escape from each other's presence. This leads to a higher combined vapor pressure and a lower boiling point than expected. These mixtures are called azeotropes.

Ethanol - water is perhaps the best known example of

an azeotrope. A mixture of 95.6 % ethanol and 4.4 % of water boils at 78.1 °C, lower than the boiling point of both pure ethanol (78.3 °C) and pure water (100 °C). Although the difference is small, it has an important implication. Any ethanol-water mixture that contains less than 95.6 % ethanol will produce a distillate that is richer in the more volatile, lower-boiling component (ethanol). However, a mixture containing *more* than 95.6 % ethanol gives a distillate that is richer in the *less* volatile component (H_2O). This means that if 95.6 % aqueous ethanol is distilled, the entire distillate will have the same, unchanged composition; no change in the boiling temperature (78.1 °C) nor in the proportion of components in the distillate [as well in the residue in the distilling flask] will occur during the whole operation. A mixture which behaves in this manner is called an azeotropic mixture or simply an "azeotrope". Mixtures consisting of two components (as in this case), are called binary mixtures.

A consequence of this is that 100 % pure ethanol cannot be produced by the distillation of an ethanol-water mixture. To prepare anhydrous (absolute) ethanol, the water (at least the last 4.4% of it) must be removed by another means. This can be done chemically or by distillation with an added, third substance that will forms an even lower-boiling, ternary azeotrope together with water and ethanol. Benzene is used for this purpose in the commercial production of absolute alcohol. Benzene is an extremely useful material in a lab because it forms azeotropes with a number of solvents, including water. Azeotropic distillation with benzene is a very effective way of removing water from compounds that are otherwise difficult to dry and is used very commonly in synthetic organic laboratories.

Steam Distillation. When two liquids do not mix they form separate layers. The two liquids behave independently of one another and each exert the vapor pressure of the corresponding pure liquid. In other words, there is no correction for composition and $P_{total} = P_{A^\circ} + P_{B^\circ}$. The boiling point of such a liquid mixture is reached as soon as the combined vapor pressures reach atmospheric pressure. This will happen at a temperature much less than that of the most volatile component (with the lowest boiling point). The composition of the distillate is given by the ratio of the vapor pressures at this boiling point. This can be exploited for the separation of organic compounds from other substances. Usually water is employed as the immiscible phase. Steam is passed through the mixture and the slightly volatile organic compound is carried with the steam and separated from non-volatile impurities. The desired materials can be recovered from the resulting mixture by an extraction. The major advantage of this technique is that it allows one to "distill" an organic compound at temperatures significantly below 100 °C, and thus avoid thermal decomposition.

How to set-up the apparatus

Glassware for organic chemistry is designed to be modular, interchangeable and to give an airtight seal between parts. This is accomplished by the use of *ground glass joints*. To work properly, the joints must be **clean**. Always inspect them for dirt and dust before assembly. If they are dirty, wipe with a paper towel. Clean ground glass joints appear white. Transparency or color on such joints indicates that the part is dirty.

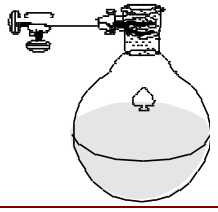
Some experiments require the use of grease to prevent the ground glass joints from locking (called seizing or freezing). This will be indicated in your manual, or by your demonstrator. In these cases, use a *small* amount of grease, *lightly applied* to the top of the male joints before assembly.

Grease can be removed from glassware using hexanes. The best method is to wipe away the grease using a Kleenex soaked in hexanes.

Organic apparatus are easiest to set up if you **work from the bottom up**. Start with your magnetic stir plate. Gently slide a stir bar into your round bottom distillation flask by tilting the flask 45 ° and *sliding the bar down the inside of the flask*. The distilling flask is then clamped a few inches above the magnetic stirrer. Use an extension clamp on the neck of the flask in the position indicated in the diagram below. **ALWAYS CLAMP FLASKS THAT WILL CONTAIN CHEMICALS**. Use solid extension clamps for flasks, they hold better than three-fingered universal clamps. Clamp your flask so that the clamp holds just below the top lip of the female joint.

Place the appropriate heating mantle on top of the stir plate, loosen the clamp and lower the flask until it rests inside the mantle, then retighten the clamp. Test to make sure everything is solid.

Figure 10. Proper clamp location for flasks



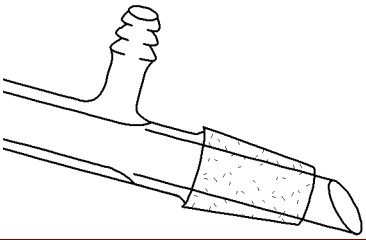
Once your distilling flask is in position, it is easy to add the other glassware. Work from what you have already set up, and add pieces one at a time. Add clamps or clips as necessary to hold the apparatus solidly. **NEVER** use clips and clamps on the same joint, use one or the other. Proper locations for each are indicated in the various drawings. Once your set-up is complete, inspect it before proceeding. The joints should be fully seated and the apparatus should be solidly clamped.

The condenser serves to cool the distillate which is then collected, via the adapter, in the receiving flask.

Cooling is achieved by passing water through the jacket of the condenser. Connect rubber hoses so that water enters the bottom of the condenser and exits the top. The condenser should be mounted so that the bevel at the bottom of the condenser faces downward as shown below.

Why is it important to fill the water jacket from the bottom? To answer this, connect the inlet and outlet hoses in two different ways and see what happens.

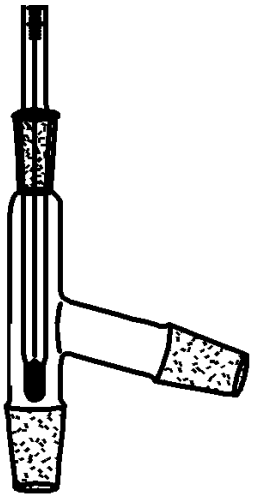
Figure 11. Condenser bottom bevel



The thermometer should be placed so that the top of the bulb is just below the exit arm of the still head. In this way you will be measuring the temperature of the vapor as it exits the still. When using a metal probe, the bottom of the probe should be in the same position as a regular thermometer.

For a pure liquid, this temperature of the vapor as it exits the still head is the boiling point. If the thermometer bulb is placed too high, the observed temperature will be too low since the hot vapors will not completely surround the thermometer. A high value will be observed if the bulb is too low in the still head because it will be in contact with vapor that contains more of the higher boiling components.

Figure 12. Proper thermometer location in still head



When liquids are boiled, bubbles of vapor form in the bulk liquid. These bubbles form around imperfections in the vessel or solids suspended in the liquid. This process is called nucleation. Laboratory glassware will usually not contain enough "defects" to ensure proper nucleation, and so heated liquids can sometimes superheat (heat above the boiling point). Vapor bubbles can then form violently forcing the liquid out of the apparatus. This potentially dangerous event is called "bumping". Bumping is easily controlled by stirring the solution while heating. When magnetic stirring is not available, the addition of several boiling chips or boiling sticks will suffice. These promote even boiling by providing a source of small bubbles.

NEVER REMOVE YOUR GLASSES IN ORDER TO READ THE THERMOMETER.

The Experiment

Simple distillation. Use the apparatus in your locker to assemble a simple distillation apparatus (see diagram and discussion above). After you clamp your distillation flask, fill it with 50 mL of a 50:50 mixture of 2-propanol and 1-butanol using a long stem funnel. Continue your set up by adding the distillation head, thermometer, condenser and takeoff adapter. Be sure to clamp the apparatus properly at the points indicated. Use a 100 mL graduated cylinder (make sure it is clamped properly) as the receiving flask. Have your apparatus checked by your demonstrator before beginning the distillation.

Stir the solution using the magnetic stirrer. You do not need to use maximum speed, the distillation will work better if you stir just fast enough to generate a gentle vortex in the liquid. Slowly distill the solution by using minimum heat, on the setting your demonstrator tells you to use. Record the temperature on the thermometer after each 2 mL portion of distillate is collected until the distillation is complete. Return the distillate to the proper waste bottle.

Fractional distillation Disassemble your still, but leave the stirrer, mantle and distilling flask in place. Charge the distilling flask with 50 mL of a 50:50 mixture of 2-propanol and 1-butanol. Use the apparatus in your locker to assemble a fractional distillation apparatus (see diagram and discussion above). The fractionating column is available from your demonstrator. This is a special insulated glass column that has been packed with metal sponge. Be sure to clamp the apparatus properly at the points indicated. When you are finished with your experiment, be sure to return the CLEAN, fractionating column to your demonstrator.

Distill the mixture slowly being careful that flooding does not occur in the column. If your column does flood, remove the heat until the pool of liquid disperses (this takes 15 seconds or so) and resume your distillation. Record the temperature after each 2 mL portion of distillate has been collected. When the distillation is complete, return the distilled material to the proper waste bottle.

Plot graphs of temperature vs. volume of distillate for each of the three distillations. In your report discussion, compare the distillation of the mixture by simple and fractional methods. As discussion points you should consider the equipment required, the difficulty of the technique, the purity of the products and the nature of the starting mixture.

Questions

1) Explain why you must have liquid flowing back through the fractionating column in order to get separation of the components during a fractional distillation.
2) Fractionating columns normally work better if they are insulated in order to maintain a smooth temperature gradient in the column. Why is it important to maintain a uniform temperature gradient in a fractionating column?
3) The boiling point of benzene is 81 °C. What is the vapor pressure of benzene at this temperature?
4) What effect does an increase in atmospheric pressure have on the boiling point of a liquid?
5) Why is it important to have cooling water enter the bottom of the condenser and not the top?
6) Compound A has a vapor pressure of 350 mm Hg at 95 °C whereas compound B has a vapor pressure of 150 mm Hg at the same temperature. If A and B are miscible, what is the vapor pressure of a 3:1 mixture of A and B at 95 °C?

Report Notes

Refer to the instructions early in the lab manual regarding the preparation of reports. Note that you do not need to provide a table of reagents for this report.

In the discussion section, explain briefly the shape of each temperature/volume curve you obtained during your distillations. Describe what you think each curve should look like and why. Account for any differences between your results and what you think you should have obtained.

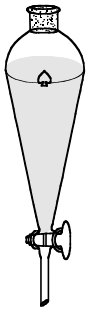
Distillation curves may be prepared electronically (excel) or by hand using metric graph paper.

Experiment 3: Extraction (WILL NOT BE DONE IN 2015!!)

Department of Chemistry, University of Ottawa, 10 Marie Curie Priv, Ottawa, ON, K1N6N5

September 2013

ABSTRACT



This lab will introduce the technique of thin layer chromatography, used to monitor the progress of a reaction and the technique of extraction, a method used to separate compounds from a mixture.

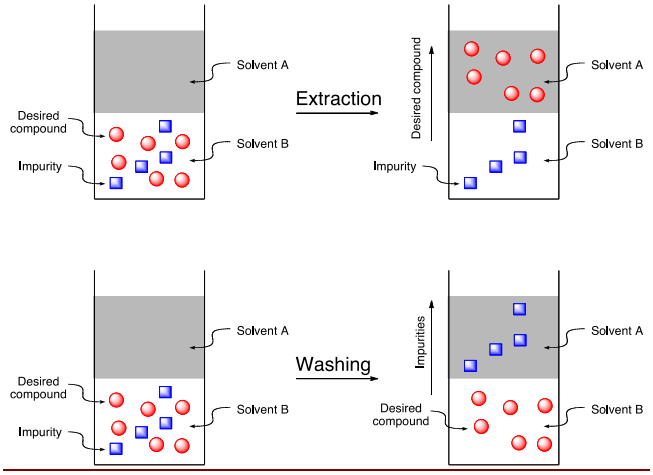
Extraction

Extraction is an essential technique in chemistry that is used to separate different chemical compounds in a mixture based upon the differences in solubility of the compounds in two **immiscible**[3] solvents, called phases. In the vast majority of extractions, one phase is water (called the aqueous phase) while the other phase is an organic solvent. Depending on the movement of components between the two solvents, the technique may sometimes be called washing. *Extracting* involves the movement of the desired compound from one phase to the other while leaving impurities behind in the first phase. *Washing* keeps the desired compound in one phase, while impurities are transferred to the other phase. Both techniques are routinely used at the end of a reaction to separate the organic products (generally soluble in organic solvents) from the inorganic reagents or byproducts (generally more soluble in water). Extraction is often the first step in the **work-up**[4] of an experiment.

Figure 1. Extraction and washing

[3] **Immiscible:** Two liquids that do not mix.

[4] **Work-up** refers to the procedures used to isolate and purify a compound at the end of a reaction.



An extraction uses a special piece of glassware called a separatory funnel. The funnel is tapered, and has an opening at the top and a stopcock at the bottom. The solution to be extracted is placed in the funnel and shaken with an immiscible solvent. Compounds that were dissolved in the original solution become distributed between the two solvents (phases) in proportion to their solubilities in each solvent. After the funnel is shaken, it is placed in a special stand and the two phases are allowed to separate. The stopcock at the bottom of the funnel is used to remove each phase separately, and in the process complete the extraction.

The ratio of the solubility of the compound in each of the phases is called the distribution coefficient or partition coefficient, K_D.

$$K_D = \frac{[A]_{solvent\ 1}}{[A]_{solvent\ 2}}$$

$[A]_{solvent\ 1}$ = solubility of compound **A** in solvent 1

$[A]_{solvent\ 2}$ = solubility of the *same compound* (**A**) in solvent 2

This formula can be rearranged to give a more applicable version:

$$K_D = \frac{W_1 / V_1}{W_2 / V_2}$$

W_1 and W_2 are the masses of the compound dissolved in volumes V_1 and V_2 of the solvents 1 and 2 respectively.

K_D is an equilibrium constant and has a characteristic value for any compound in a pair of solvents at a given temperature. It is important when defining K_D to have the solvent order clearly stated, since the value becomes reciprocal when the order is reversed. The above relationship holds well at low concentration, which is what will generally be encountered in this laboratory. At high concentration, molecular associations can give considerable variations in K_D.

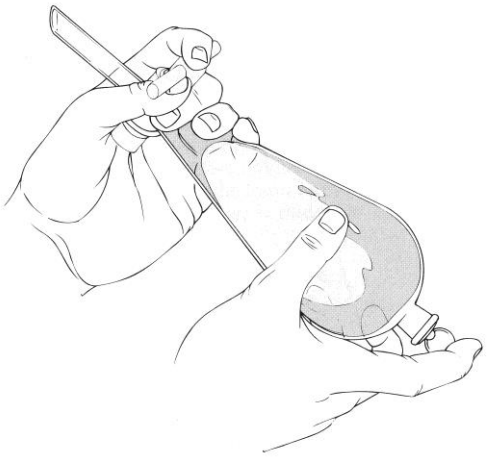
A single extraction will not efficiently transfer a compound from one solvent to another unless the distribution coefficient between the solvents is very large (or very small). The efficiency of the transfer from one solvent to another solvent can be improved by using larger volumes of the solvent that you want the compound to be in. It is more efficient, however, to repeat such an extraction several times, with small volumes of this solvent, rather than to extract once with a large volume.

Figure 2. Separatory funnel

Separatory Funnel. A separatory funnel is expensive and easily broken, especially near the stopcock. **NEVER PLACE A SEPARATORY FUNNEL IN A BEAKER OR A FLASK.** Your equipment locker contains a wooden rack that is specifically designed to hold separatory funnels.

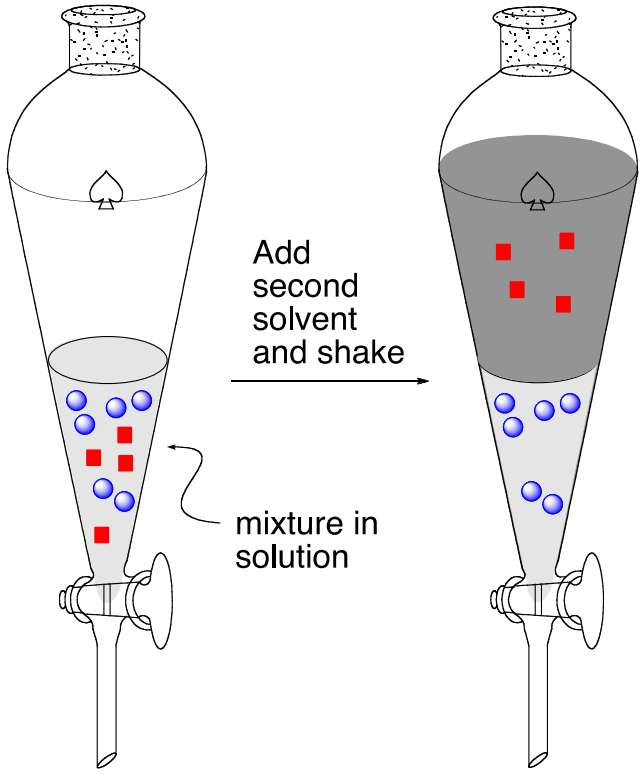
Fill the separatory funnel no more than three-quarters full so that mixing is possible. Before filling, check that the stopcock can be rotated and that it is closed. Ensure that the stopper fits well. Teflon stoppers are the safest and most effective, although glass stoppers are occasionally used (**NEVER USE RUBBER STOPPERS**). The fit of the stopper can be verified by adding a small amount of water to the funnel, closing the stopper and inverting the funnel.

Figure3. Proper way to hold a separatory funnel.



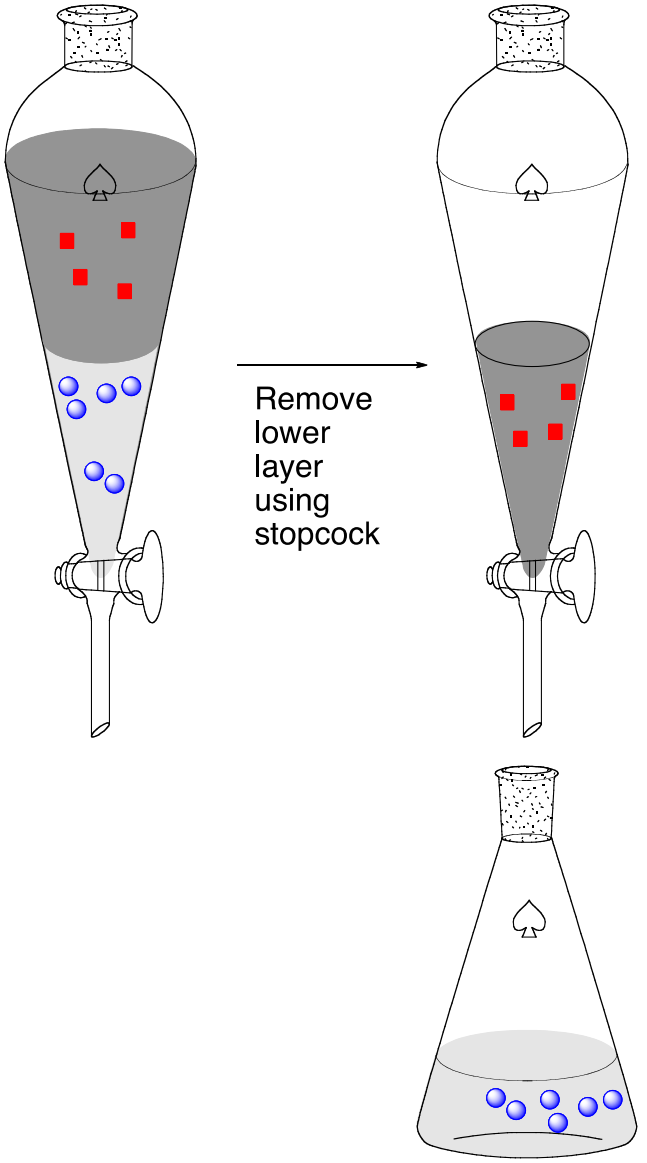
To mix, hold the separatory funnel with both hands, place one hand over the stopper and the other hand around the stopcock as shown above. Invert the separatory funnel so that the stopcock is pointing up and away from you and **not pointed at anyone else**. Open the stopcock briefly. You may hear some gas being vented – this is normal. Close the stopcock, and shake gently for a few seconds, then invert the funnel as before and open the stopcock to vent it. It is important to do this frequently to release any built-up pressure that develops as the solvents are mixed. Repeat this shaking and venting sequence three or four times, always ensuring that the stopcock is not pointing at anyone before you open it, since the built-up pressure may also force liquid out.

Figure 4. Purification by extraction



Make sure the stopcock is closed and place the funnel in the ring clamp or wood rack. Remove the stopper from the top and allow the layers to separate**. <u>Never leave a stopper in a separatory funnel when it is not being shaken</u>**. The separation of phases normally takes a few seconds. While the layers are separating you will see three layers in the funnel. The "cloudy" layer in the middle is called an emulsion and consists of a mixture of blobs from the top and bottom phases. This layer will disappear as the blobs separate. Once the separation of phases has finished, this emulsion will disappear and the remaining two layers will appear clear. Carefully draw off the lower layer by opening the stopcock. When the bottom of the upper layer gets to the stopcock, close the stopcock. It is a good practice to save both phases until you are sure that you have isolated the correct product.

Figure 5. Drawing off the bottom layeer



A common problem encountered during extraction is the formation of **emulsions**[5] that occur in the presence of impurities that act as surfactants. Emulsions will often clarify in a few minutes. If emulsion formation lasts longer than this, try swirling the contents of the funnel, rather than shaking it (shaken, not stirred actually does make a difference). If emulsions form during shaking (and do not disappear), they can sometimes be dispersed by the addition of a small amount of solid sodium chloride or brine (a saturated sodium chloride solution) to the funnel.

The selection of solvents used in extractions is dictated by several factors. The two solvents must be immiscible. The compound to be extracted should have a much higher solubility in one solvent relative to the other. When choosing solvents, keep in mind that "like dissolves like" polar compounds tend to be soluble in polar solvents and non-polar compounds in non-polar solvents. In the vast majority of cases, water is used as one of the phases, and this should be considered to be the polar phase. The water layer is often called the aqueous phase and the organic solvent layer is called the organic phase.

When extracting, it is important to know which layer is the organic solvent and which layer is the aqueous solvent. In general, most organic solvents (ether, ethyl acetate, hexanes) are less dense than water and will form the layer on top. Chlorinated solvents (dichloromethane, chloroform) are denser than water and will be on the bottom. 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 mixes with is the aqueous phase.

Ethyl acetate is the solvent of choice for most extractions. It is cheap, safe and easily removed. This solvent is very flammable, so care must be taken to ensure there are no sources of ignition nearby. You will also be using dichloromethane. Dichloromethane is not flammable, but care should be taken when handling it. Always handle organic materials as if they are flammable.

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 (or a solution of salt in water called brine) is added to the aqueous phase. This increases the ionic strength of the water and will "push" the organic compound out of the aqueous layer.

Drying agents. After any extraction process, the organic solution inevitably contains some residual water. This is partly due to the physical separation process and partly due to the 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 – to the isolated organic phase. Drying agents are added as solids to the solution to be dried, and the hydrated crystals are later filtered off. A few commonly used drying agents are shown in table 4.

Table 1. Common drying agents.

$MgSO_4$	Most common. High capacity, neutral, fast, very safe.
Na_2SO_4	Common. High capacity, neutral, safe. Hydrate formation is slow.
K_2CO_3	Uncommon. Used for basic compounds. Intermediate capacity, slow.
$CaCl_2$	Uncommon. Used for acidic compounds. Low water capacity, very slow.

When drying a solution, it is important to keep the drying agent in contact with the solution long enough to be

[5] **Emulsions:** Suspensions of droplets of one phase in the other.

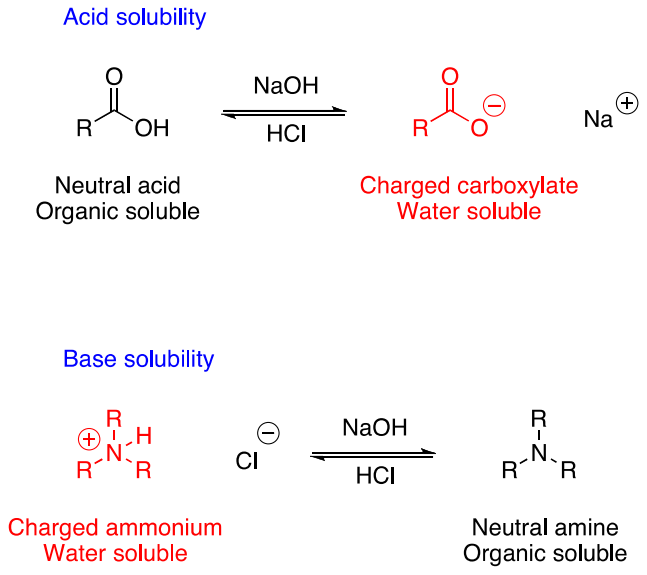
effective. For $MgSO_4$ and Na_2SO_4, a few minutes is sufficient. When drying neat liquids (no solvent used), the drying process is considerably slower and the drying agent should be left in place for at least 5 minutes.

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 salt can be used to estimate when sufficient drying has taken place. Wet $MgSO_4$ clumps together while 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.

Reactive separations. Acid-base reactivity can sometimes be exploited to enhance the separation of acidic, basic and neutral compounds. The reaction of an organic acid (carboxylic acid) with a base will produce an ionic salt that is water soluble. Similarly, reacting an organic base (amines) with an acid will produce a charged species that is soluble in the aqueous phase. Charged materials will be soluble in water, whereas neutral organic compounds normally remain in the organic solvent.

The formation of charge can be reversed using acid-base chemistry. Charged organic materials can be recovered from water by adding acid (to carboxylic acids) or base (to amines) to regenerate the *neutral* organic forms and performing another extraction.

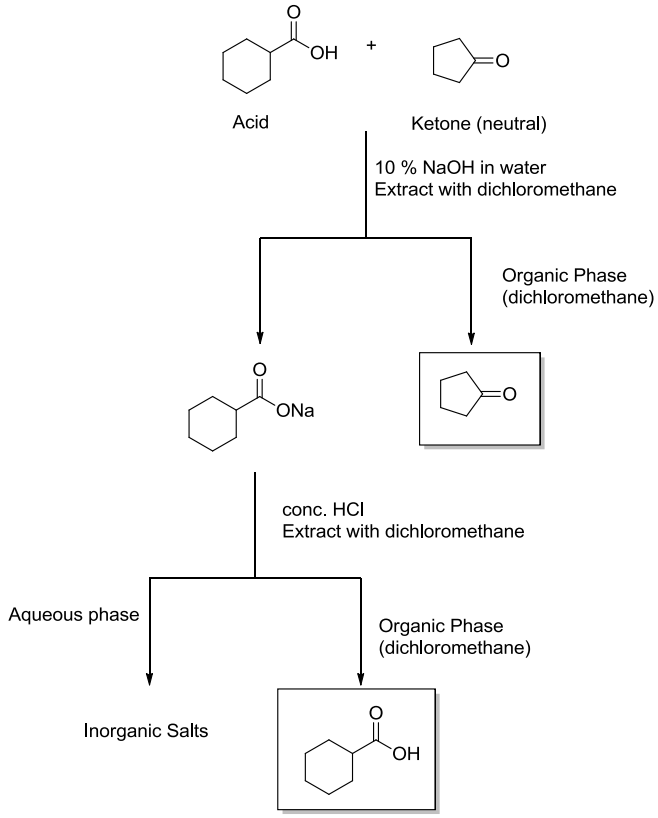
Figure 6. Acids and bases in reactive separations



For example, to separate a neutral compound (a ketone) from a carboxylic acid (an acid), the mixture is dissolved in an organic solvent, like dichloromethane, and an aqueous solution of base (NaOH) is added. The base will convert the carboxylic acid to its corresponding conjugate base, that is charged and soluble in water. The ketone will not react and remains in a neutral form. The mixture can then be separated using a separatory funnel. The neutral compound is recovered by drying and then evaporating off the solvent.

The acid is still in the water, in the charged salt form. This material is recovered by acidifying the aqueous phase with concentrated HCl. This converts the carboxylate salt into the carboxylic acid, which is no longer charged, making it soluble in organic solvents. Extraction from the aqueous layer using an organic solvent, like dichloromethane, places the carboxylic acid in the organic phase. This can be isolated in pure form by drying and then evaporating the solvent.

Figure 7. A reactive extraction.



The Experiment

Part A: Extraction of water soluble dyes (One partner should do step 1, the other partner step 2).

1. Place ~2 mL of ether, ~2 mL of distilled water and 1 drop of a 0.006 M methylene blue solution in a test tube and mix the contents by shaking vigorously for 10 seconds (be sure to cap the tube). Allow the layers to settle and determine if the dye is in the aqueous, organic or both layers.
2. Place ~2 mL of ether, ~2 mL of distilled water and 1 drop of a 0.006 M methyl red solution in a test tube and mix the contents by shaking vigorously for 10

seconds (be sure to cap the tube). Allow the layers to settle and determine if the dye is in the aqueous, organic or both layers.

3. Would an extraction between ether and water be a good way to separate a mixture of methylene blue and methyl red? Test your answer by mixing the contents of the tubes from steps 1 and 2 together and then shaking vigorously for 10 seconds. Allow the layers to settle and determine the color of each layer. Explain your observations.

The salting out effect.

1. Prepare two test tubes, each containing 5 mL of distilled water, 1 drop of 0.003 M aqueous crystal violet and 0.5 mL of 1-butanol. Shake the two tubes until the color of the crystal violet is distributed throughout both layers.
2. Add some solid NaCl to <u>one</u> of the tubes until the aqueous layer is saturated (some solid at the bottom). Shake this tube to dissolve the salt. Compare the distribution of the dye between the two tubes. Explain what is happening.

Part B: Separating a mixture with reactive extraction.

8. Dissolve an unknown sample (record which one you have) in ~10 mL dichloromethane in a 50 mL Erlenmeyer flask. Keep a small amount aside as a TLC reference. Pour the solution into a separatory funnel (**ensure that the stopcock at the bottom is closed first**). Rinse the contents of the flask with ~5 mL of dichloromethane and transfer the rinsings to the separatory funnel. Save all extracts until the end!
9. Add 10 mL of a 2M solution of NaOH in water to the separatory funnel. Place a stopper on the separatory funnel and invert the funnel. Being sure that the tip is pointed away from you and is not pointed at anyone else, open the stopcock to vent the funnel. You will hear some gas escape. Close the stopcock and shake for 15 to 30 seconds. Be careful to vent the funnel occasionally as the dichloromethane will partly evaporate and generate pressure. Remember to close the stopcock each time.
10. Place the separatory funnel on the wooden rack and allow the layers to separate.
11. Carefully drain the lower phase into a clean 50 mL Erlenmeyer flask. Transfer the upper phase into a second 125 mL Erlenmeyer flask and set it aside.
12. Pour the organic phase (the lower layer in the funnel) back into the separatory funnel and extract twice more with 2 M NaOH. Combine the basic aqueous phases and set aside.
13. Prepare two TLC plates. Spot your original sample (mixture) as the reference and co-spot on both plates. On one of the plates, spot the organic layer from your extraction as the sample and co-spot. Spot the aqueous layer as the sample and co-spot on the other plate.
14. Develop both plates using the 2:8 mixture of ethyl acetate (EtOAc) and hexanes as your eluant. Draw a picture of each plate and explain what you see. Poor quality TLCs should be repeated.

Questions

1. Why would it be difficult to perform an extraction using ethanol and water?
2. Would adding NaCl to a test tube containing water, ether and methylene blue increase or decrease the amount of dye in the aqueous layer?
3. Compound Y has a solubility of 2.0 g/100 mL in water and 20.0 g/100 mL in ether. What weight of compound Y would be removed from a solution of 1.8 g of Y in 100 mL of water by a single extraction with 100 mL of ether?
4. What weight of compound Y would be removed from the original water solution in question 6 by two extractions using 50 mL of ether each time?
5. During an extraction a student loses track of which layer is the organic layer. How could she determine which layer is the aqueous phase?
6. Describe how you would separate a mixture of benzyl amine (an organic base) and naphthalene. Both compounds are insoluble in water and soluble in ether.

Report notes

Be sure to provide brief explanations for your observations (1-2 sentences each) of the extractions you performed using using dyes. You should have accurate drawing of all TLCs, as shown in the introduction of the lab manual. Do not take your TLCs home.

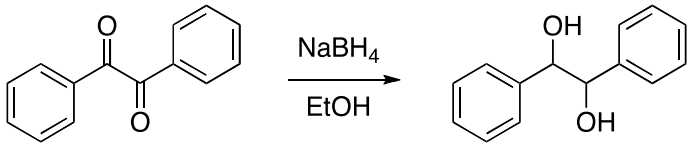
Make sure to explain the steps of the reactive separation (a flow chart + text) and precipitateion.

Experiment 4: Stereochemical Analysis of the Reduction of Benzyl

Department of Chemistry, University of Ottawa, 10 Marie Curie Priv, Ottawa, Ontario, K1N6N5

January 2014

ABSTRACT



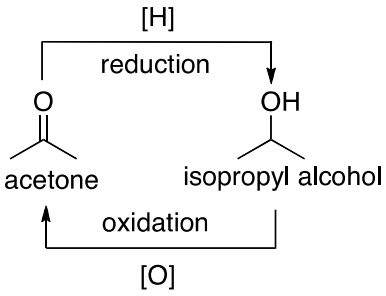
The carbonyl group of benzyl will be reduced in this experiment.

Introduction

Oxidation-reduction reactions play a pivotal role in chemical transformations. The controlled oxidation of glucose into carbon dioxide is the primary means of producing energy in living things and provides the key raw materials for life-sustaining processes. In plants, carbon dioxide and water are reduced into glucose using the energy provided by sunlight.

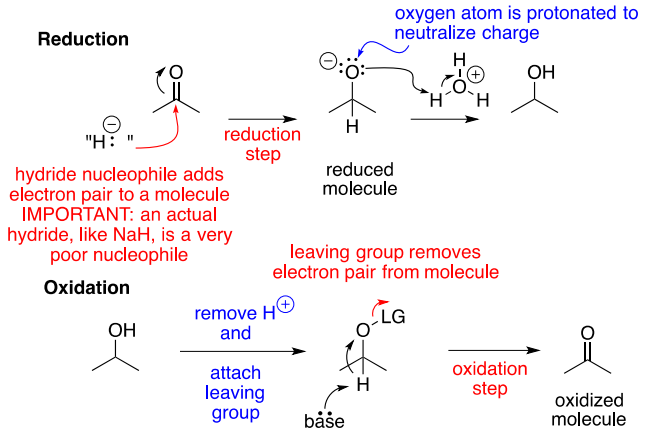
The basic definition of oxidation in chemical compounds is that the compound loses electrons. Reduction implies that a compound has gained electrons. In organic chemistry, oxidation and reduction are often defined in other terms, related to the gain or loss of hydrogen atoms by a molecule. During oxidation, the number of hydrogen atoms on a molecule normally decreases. Conversely, adding hydrogen atoms to a molecule constitutes a reduction. In the example shown below, acetone can be reduced to give isopropyl alcohol. Because this involves the addition of hydrogen atoms to the molecule, the symbol [H] is used to donate a reduction process. Isopropyl alcohol can be converted into acetone by removing hydrogens in an oxidation process. In this case the symbol [O] is used.

Figure 1. Oxidation and reduction



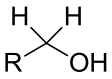
Mechanistically, many reductions involve the interaction of nucleophiles with electrophilic π bonds. Nucleophiles are electron pair donors, and so the addition of a nucleophile to a π bond will increase the number of electrons in a functional group thereby producing a reduction. Oxidation reactions normally occur when π bonds are formed by the loss of a leaving group through elimination. The leaving group removes a pair of electrons from the molecule thereby oxidizing the functional group.

Figure 2. Organic reduction and oxidation



Alkanes represent the lowest oxidation state that organic molecules can attain. The introduction of heteroatoms increases the oxidation state of a molecule (electrons are added), as does the removal of hydrogen atoms to make π bonds. As shown below, the oxidation state of a functional group increases systematically as this is done.

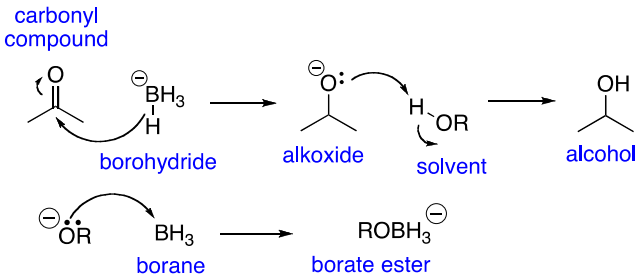
Table 1. Oxidation states.

Alkane		Least oxidized
Alcohol		
Aldehyde		
Carboxylic Acid		Most oxidized

Reagents that carry out oxidations or reductions are often called "agents". A reducing agent will reduce a molecule; an oxidizing agent will oxidize a molecule. A commonly used reducing agent is sodium borohydride ($NaBH_4$). This molecule behaves as both a nucleophile and reducing agent. It is a reducing agent because it is a good source of nucleophilic hydride (H^-) ions, and thus adds hydrogens and electrons to electrophiles. Because BH_4^- has four available hydride units, one $NaBH_4$ molecule is capable of reducing four functional groups. $NaBH_4$ is usually added in excess when performing small-scale reactions. When reducing on large scale, this stoichiometry is altered for safety reasons and to reduce cost and waste.

The widely used mechanism for the reduction of a carbonyl group using sodium borohydride is presented in scheme 1. You should be aware that this is an approximation of the actual mechanism (discussed later). The key step in this reaction is the transfer of a hydride unit from the borohydride to the carbonyl group. Following the nucleophilic addition of the hydride ion, the negatively charged oxygen in the alkoxide that is produced forms an O-H bond by removing a proton from a molecule of solvent. The alkoxide that results reacts will the borane byproduct forming a borate ester (more on this below).

Scheme 1. Simplified mechanism of the reduction of a ketone.

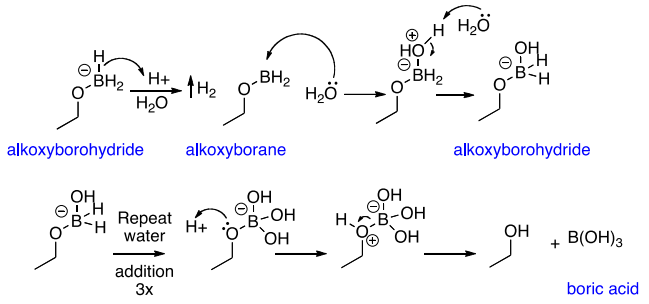


Once all of the carbonyl molecules have been reduced, an acid or base work-up is often performed. The workup is a process that is done to stop (or quench) a reaction, and to facilitate the purification of the desired product.

The first goal of a $NaBH_4$ workup is to destroy any excess $NaBH_4$ that may remain. This ensures that the subsequent steps will be safe. The second goal of the workup is to purify the desired product of the reaction.

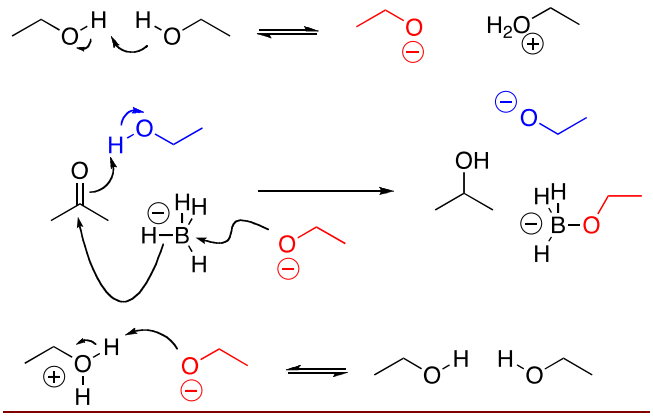
The purpose of acid or base in a $NaBH_4$ workup is to catalyze the hydrolysis of the borate ester into water-soluble boric acid or tetrahydroxyborate. This facilitates the isolation and purification of the product, which is not normally soluble in water. The mechanism of this reaction is shown in scheme 2 below. When acid and water are added, any remaining hydrides will react very quickly with the added acid producing hydrogen gas. This can sometimes be a violent reaction and so the addition of acid during the workup phase must be done carefully. Water will react with the resulting borane to fill its octet producing an intermediate that loses a proton to form a mixed borate ester. The loss of H^+ from this intermediate implies that acid is a catalyst in the process – it is not consumed in the reaction. Once the hydrides have been replaced by water, the resulting borate ester will be hydrolyzed by water to form boric acid and release the solvent alcohol.

Scheme 2. Acidic Mechanism of Borate Ester Hydrolysis



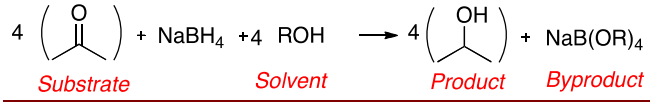
Sodium borohydride is soluble in many organic solvents, however reductions with this reagent require the presence of a protic solvent such as water or alcohol. The reason for this is that the protic solvent actually participates in the reaction mechanism,[6] which is shown in scheme 3 below. In this mechanism, two molecules of solvent participate in each hydride transfer. Just like in the simplified mechanism above, borohydride provides a nucleophilic hydride to reduce the carbonyl-containing compound. However, the presence of a protic solvent increases the activity of BH_4^- significantly. Protic solvents are in equilibrium with their alkoxide forms, and the reaction begins when a small amount of alkoxide forms as part of the normal acid/base equilibrium that exists in the bulk solvent (top equation). The alkoxide is nucleophilic and donates electrons to a molecule of BH_4^- to form an oxygen-boron bond This process increases the nucleophilicity of the borohydride, causing it to donate a hydride nucleophile to the carbonyl group. While this happens, the oxygen of the carbonyl gains electrons from the fracturing π bond, and uses this electron excess to form a bond with an H^+ taken from another molecule of solvent. All of these bond-forming and bond-breaking events happen simultaneously, and produce the product of the reaction along with a molecule of borate ester (middle equation). The alkoxide formed by this sequence then reforms a molecule of alcohol through the normal acid/base equilibrium.

Scheme 3. Full Mechanism of Sodium Borohydride Reduction in Protic Solvent



The borate ester that is formed possesses three active hydrides that can reduce additional carbonyls. When all of the hydrides are consumed following four reductions, a full borate ester and four molecules of the reduced alcohol are produced. The overall equation for this process is shown in scheme 4.

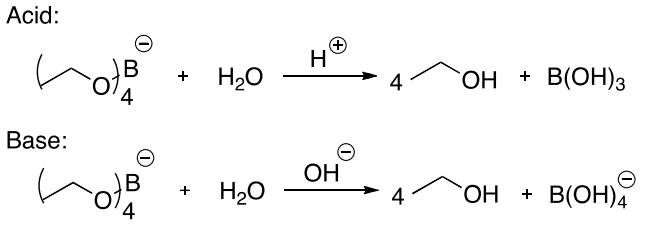
Scheme 4. Overall stoichiometry for borohydride reduction.



The borate ester byproduct of this reaction is a jelly-like solid that is sometimes difficult to remove from the desired product. For this reason, acid or base is often added at the end of a $NaBH_4$ reaction to convert this byproduct into boric acid. Boric acid and tetrahydroborate dissolve easily in water, making them much easier to separate from the organic materials present. This makes the desired product much easier to purify. The overall equations for the hydrolysis of borate ester into boric acid are shown below in scheme 5.

6. (a) Brown, H.C.; Mead, E.J.; Rao, B.G.S. *J. Am. Chem. Soc.* **1955**, *77*, 6209. (b) Wigfield, D.C.; Gowland, F.W. *J. Org. Chem.* **1977**, *42*, 1108. (c) Wigfield, D.C.; Gowland, F.W. *Can. J. Chem.* **1978**, *56*, 786

Scheme 5. Equations for the conversion of borate esters to boric acid and tetrahydroxyborate.



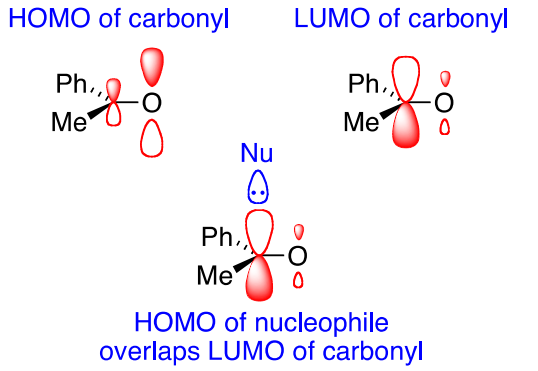
The nature of the alcohol solvent has a strong influence on the rate of $NaBH_4$ reactions. $NaBH_4$ reacts very quickly in water or methanol, but reacts much more slowly in isopropanol or tert-butanol (why?).

Stereochemistry

Reducing a carbonyl group to an alcohol converts an sp^2 hybridized carbon into an sp^3 hybridized one. If the two groups on the carbonyl carbon are different, the newly formed sp^3 carbon will be stereogenic.

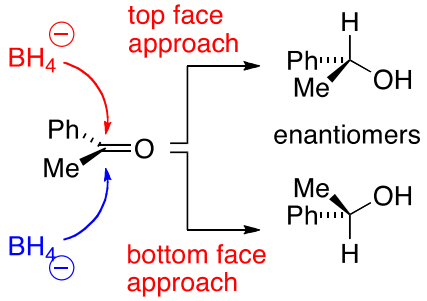
Reducing an asymmetric ketone such as acetophenone with $NaBH_4$ produces a racemic mixture of products. When $NaBH_4$ reacts with the carbonyl group, the filled σ orbital of the B-H bond (HOMO) interacts with an empty orbital on the carbonyl (LUMO). The LUMO (lowest energy unoccupied orbital) on a carbonyl group is the π* orbital. This orbital is located above and below the plane that is defined by the atoms of the carbonyl group (scheme 6).

Scheme 6. Orbital interactions control the stereochemistry of carbonyl reduction.



The only way that the HOMO of the nucleophile can interact with the LUMO of the electrophile is if the nucleophile approaches perpendicular to the plane of the carbonyl atoms. This approach is toward one face of the carbonyl. Approach of the nucleophile to the top of the carbonyl produces one enantiomer, approach to the bottom face generates the other enantiomer. The π* orbital is located both above and below the plane of these atoms, and therefore there is an equal probablility that the nucleophile can approach from either the top face or the bottom face of the carbonyl (scheme 7).

Scheme 7. Top and bottom face approach produces equal mixture of enantiomers.

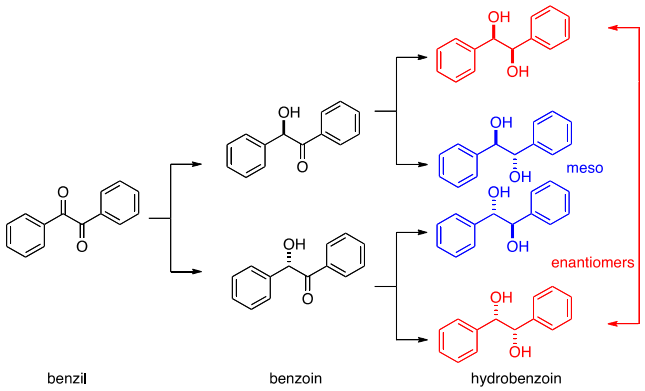


The reaction is this experiment converts a starting material (benzil) that has no stereocenters, into a product (benzoin) containing two stereogenic carbons. This means that the complete reaction can generate a maximum of four stereoisomers.

The BH4- nucleophile reduces one carbonyl at a time. When this nucleophile approaches the first carbonyl, there is an equal probability of top face and bottom face approach. This results in an equal mixture of both possible configurations of the alcohol in the hydrobenzoin intermediate, making this material racemic.

The two enantiomers have identical physical properties and behave like one compound. When the second carbonyl is reduced, the reaction produces diastereomers.

Scheme 8. Sequential reductions produce diastereomers.



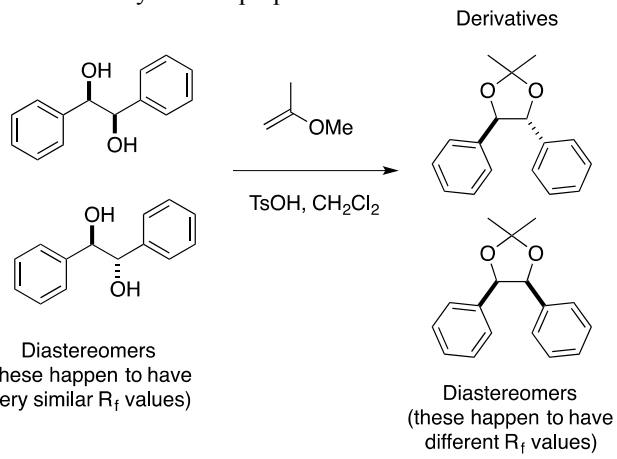
The final benzoin products in which the two OH groups are *syn* to each other are enantiomers. These two products are formed in equal amounts (racemic mixture). Because this diastereomer is present as an equal mixture of *d* and *l* forms, the product is referred to as the *dl* isomer.

The two benzoin products with *anti* stereochemistry are actually the same and so represent a *meso* compound. The *meso* and *dl* compounds are diastereomers of each other, and therefore have different physical and chemical properties. For example they have different R_f values on TLC.

The transition states of the reductions of hydrobenzoin into the diastereomeric benzoins are themselves diastereomers and have different energies. This means that the rates of conversion of hydrobenzoin into *syn* or *anti* diastereomers are different. This will produce a mixture that will be enriched in one of these isomers. In this experiment, you will use TLC to identify the stereoisomers present in your reduction process.

To more easily identify the stereoisomers produced in the reduction of benzil, you will convert the diol product(s) to acetonide(s). The mechanism of this reaction will be studied in detail in Organic Chemistry II.

The two diastereomeric products that you will make in this experiment do not separate well by TLC. In order to see the two isomers it is necessary to convert your product into a *derivative*. Reacting the diols produced in this experiment with 2-methoxypropene and acid generates acetonides. The cis and trans isomers of these derivatives have different R_f values on TLC, allowing you to identify the stereoisomer you have prepared.



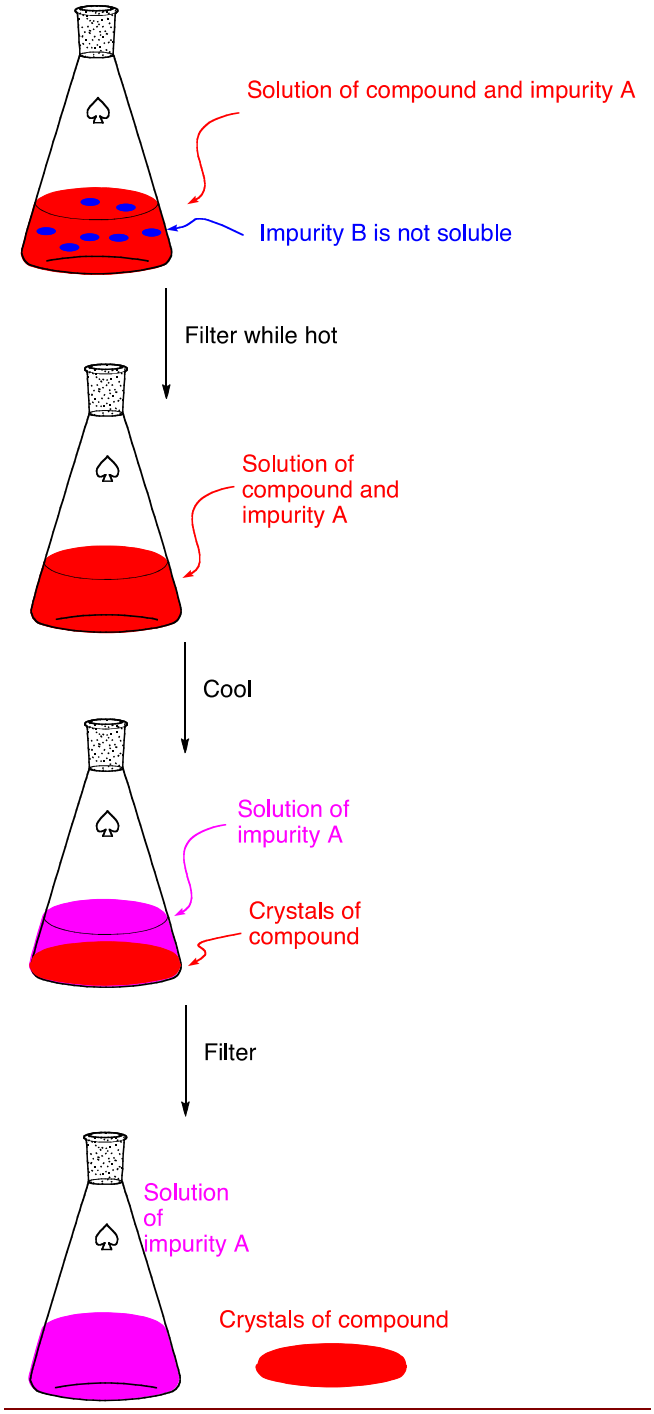
Once you have performed the reduction of benzil, you will convert your product diol into an acetonide derivative. You can determine the relative stereochemistry of your product by running TLC plates using samples of the known isomer derivatives as references. If you know the stereochemistry of the derivatives, you know the stereochemistry of the starting diols.

Recrystallization

Recrystallization is an important method of purifying organic compounds. Its use in the research lab has declined somewhat, 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 tones of material. Most pharmaceuticals are manufactured using recrystallizations to purify intermediates and final products.

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.

Scheme 9. General strategy for recrystallization



In recrystallization (scheme 4), the material to be purified is dissolved in a minimum of hot solvent. This will dissolve some impurities and not others. Those impurities that do not dissolve (dust for example) can be filtered off while the solution is hot. It is common, however, to skip this initial filtration.

The hot solution is then allowed to cool causing the desired compound to crystallize while leaving impurities behind in solution. The crystals can be recovered by filtration.

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. 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.

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 recrystalizing a polar compound, you will most likely need a polar solvent. Common solvents for crystallization are listed below in order of decreasing polarity.[7] 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).

[7] Ethyl ether is sometimes used for recrystallization, but it is generally a 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. Sometimes compounds separate from solution as liquids. When this happens, a two-phase mixture is formed. The process is called oiling out, and happens when the compound has a low melting point or when the molecular structure makes crystal fromation difficult. Oiling out can sometimes be avoided by choosing a solvent with a low boiling point, or by cooling the solution very slowly. If this 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.

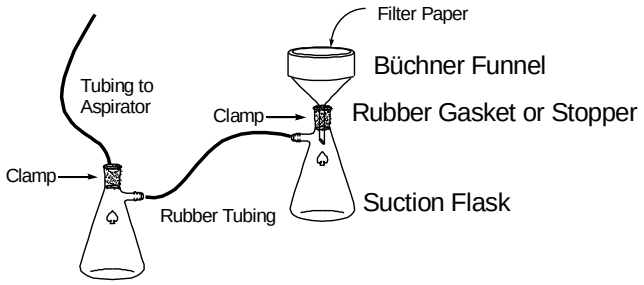
Mixed Solvent Recrystallizations. Sometimes a single solvent will not give an effective recrystallization. In cases like these, you can use a mixed solvent or solvent pair. A solvent pair consists of two *miscible* liquids, one in which the compound readily dissolves and one in which it does not. The 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 all of the 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.

Suction Filtration

Filtration in an organic chemistry lab is normally performed using a vacuum to accelerate the process and ensure complete removal of the solvent (see diagram below). The trap is included in the system to prevent any water from being "sucked back" into your solution from the aspirator (this frequently happens if the water pressure changes). Place a piece of the proper size filter paper on the bottom of the Büchner funnel. Apply vacuum by opening the water valve fully. Make sure that the filter paper is firmly set on the bottom of the Büchner funnel before adding your solution. A quick way to do this is to run your finger around the edge of the filter paper to fully seat the edges. Wet your filter paper with a *small* amount of solvent to complete the seal. The paper should now be tightly held on the bottom of the funnel. After filtering, "break the vacuum" by detaching the hose from the vacuum flask containing your solution. Never turn off the water to the aspirator before you have broken the vacuum.

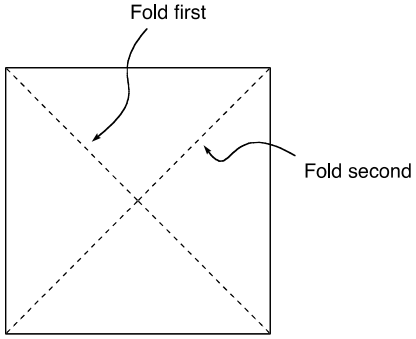
Figure 3. Suction filtration setup



The Experiment

Part A

1. Accurately measure 1 g of benzil using a folded piece of weighing paper and place the compound in a 50 mL Erlenmeyer flask.



To fold weighing paper, fold along one diagonal. Open the crease and fold along the second diagonal. Opening this crease produces a bowl shaped weighing paper.

2. **Carefully** place a magnetic stir bar in the flask. To do this tilt the flask to one side and **gently** slide the stir bar into the flask.
3. Add approx. 10 mL of ethanol. Clamp the flask over a magnetic stir plate. Place an empty ice bath underneath the flask. Add ice and then water to the ice bath. Stir for 5 minutes.
4. Add 0.2 g of sodium borohydride in three equal portions to the reaction flask, waiting approximately 2 minutes between additions. Continue stirring for 10 minutes. The mixture should lose its original yellow colour.
5. Remove the ice bath and stir your solution while allowing it to warm to room temperature (10 minutes).
6. Take a TLC of the reaction mixture to determine if the reaction is complete. The TLC plate should have three lanes: reference (benzil), co-spot, and the reaction mixture. Use 2:8 EtOAc:Hexanes as the eluent. If the reaction is not complete, add an additional 0.1 g $NaBH_4$ and stir for 5 min.
7. While your reaction is running (step 5), warm 50 mL of water. To do this, place the water in a second Erlenmeyer flask along with a stir bar. Place the flask on a hotplate stirrer, and heat to 80 °C while stirring.
8. Once your TLC shows that the reaction is complete, pour approximately 10 mL of hot water into your reaction mixture. Transfer the *reaction* flask to the hotplate, and stir while heating. The solution should clear and turn pale yellow. Continue stirring until the intense bubbling stops (5 minutes).
9. Add an additional 20 mL of hot water to the reaction mixture. Stir while heating for approximately 10 minutes. The mixture should gently boil during this time.
10. Remove the flask from heat and allow it to cool undisturbed until it reaches room temperature.
11. Collect the crystals that form using suction filtration. Wash the crystals with a small amount of cold water. Maintain suction for approximately 5 minutes to dry the crystals.
12. Determine the mass of the product and your percent yield. Perform a TLC of the final product (2:8 EtOAc:Hexanes). To spot your plate, place a few crystals in a small vial and dissolve by adding a few drops of acetone.

Part B

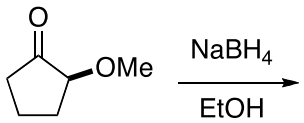
13. Place approximately 600 mg of your diol product in a 25 mL round bottom flask. Add 25 mL of CH_2Cl_2 to make a solution. Once your compound has dissolved, carefully add a stirring bar to your flask.
14. Add 1.0 mL of 2-methoxypropene and 50 mg of *p*-toluenesulfonic acid. Stir the mixture at room

temperature for ~30 minutes.

15. After 30 minutes, take a TLC of your reaction mixture using your diol product as the reference. You should use 2:8 EtOAc:Hexanes as the eluent.
16. If your TLC shows that the reaction is complete, prepare two TLC plates. Your reaction mixture should be spotted on each plate. For references, one plate should be spotted with the reference sample of the *syn* acetonide, the other plate should be spotted with the reference sample of the *anti* acetonide.
17. Elute both plates using 2:8 EtOAc:Hexanes as the eluent and determine which stereoisomer(s) of hydrobenzoin are present in your product.

Questions

1. You are given a mixture containing two compounds, A and B. Both compounds have a solubility of 1 g/ 100 mL of solvent at 20 °C and 16 g/ 100 mL of solvent at 100 °C. The sample is composed of 3.5 g of A and 10 g of B. At 100 °C the entire sample just dissolves in a minimum amount of solvent. The solution is cooled to 20 °C and crystals are collected. Calculate the composition of the crystals and the yield of the process. What is the composition of the mother liquor?
2. If the crystals obtained in question (2) are recrystallized from 100 mL of solvent, what will be the yield and composition of the crystals obtained?
3. A student dissolves 80 mg of a crude product in 4.5 mL (the minimum required) of methanol at 25 °C. She cools the solution in an ice bath and obtains crystals. The crystals are recovered by filtration and rinsed with 0.5 mL of ice-cold methanol. After drying, the weight of the crystals is 5 mg. Why was the recovery so poor? What could she do to improve the process?
4. When butanoic acid reacts with sodium borohydride, 1-butanol is not obtained. However, bubbling is still observed and heat is produced.
    a. Why is 1-butanol not obtained?
    b. What is the product of this reaction?
5. Predict the configurations of each stereocentre in the product of the following and provide a justification for your choice.



Report Notes

Refer to the instructions early in the lab manual regarding the preparation of reports. Be sure to explain all the steps of your experiment, paying attention to the workup/purification. Add mechanisms as necessary; you do not need to include the mechanism of the acetalization (i.e., the reaction with 2-methoxypropene)

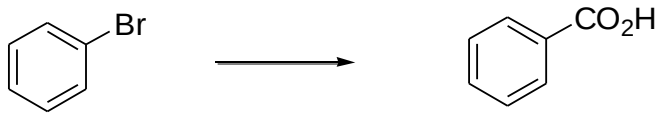
You should also explain how you determined the major product of the reaction.

Experiment 5: Preparation of Benzoic Acid using a Grignard Reagent

Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, Ontario, K1N 6N5.

June, 2008

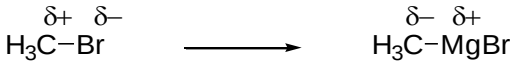
ABSTRACT



In this experiment you will synthesize benzoic acid using a Grignard reagent.

This experiment illustrates the preparation and use of a Grignard reagent. These compounds were first prepared in 1900 by Victor Grignard, a French chemist, by reaction of a haloalkane with magnesium in diethyl ether. This discovery was important enough that Grignard was awarded the Nobel Prize for Chemistry in 1912.

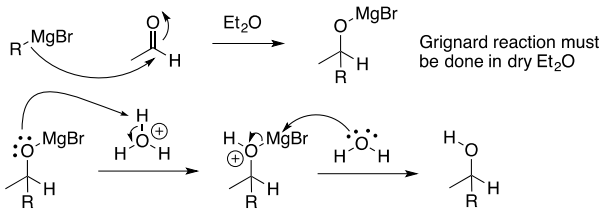
Grignard reagents represent one of the first organometallic reagents. Organometallic compounds consist of an organic component and a metal (often a transition element), and are important because they can be used to form bonds between carbon atoms. These types of bonds are the key aspect of organic compounds that make them interesting and useful.



Grignard reagents have the general structure RMgX where R can be an alkyl, vinyl, or aryl group; and X is a halogen (usually bromine or chlorine but sometimes iodine). These reagents are prepared by the reaction of an organo-halide compound, R-X, with magnesium metal. During the reaction, the metal becomes oxidized from Mg° to Mg^{+2}. The mechanism of this reaction is not well understood, even after almost 100 years of research, however it is clear that the reaction involves an initial interaction between magnesium and the halogen. During the process of Grignard formation, the α carbon of the Grignard reagent becomes formally negative and is now a powerful nucleophile (in the original alkyl halide, this carbon was electrophilic).

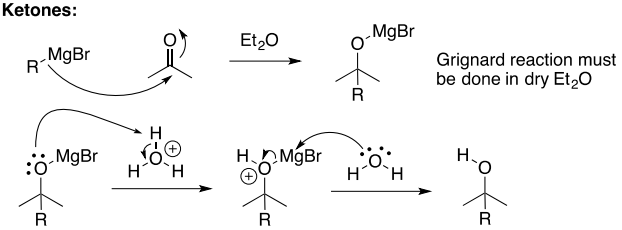
The structure of the Grignard reagent is usually depicted as R-Mg-X. It is known however that an equilibrium exists between R-Mg-X, R_2Mg and MgX_2, and that Grignard reagents prepared in the normal manner and those obtained by mixing R_2Mg and MgX_2 have essentially identical properties. This interconversion is called a Schlenk equilibrium.

Aldehydes:



Once the Grignard addition is complete, acid and water are added to break O-Mg bond

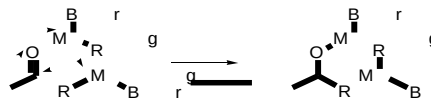
Ketones:



Once the Grignard addition is complete, acid and water are added to break O-Mg bond

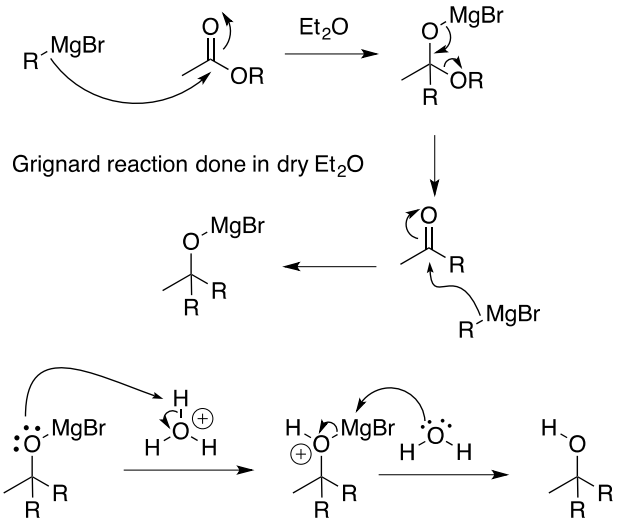
The most important reactions of Grignard reagents are nucleophilic displacements with compounds that contain polarized double bonds. The best examples of these types of bonds are aldehydes, ketones, and esters, which lead to the formation of secondary alcohols from aldehydes and tertiary alcohols from ketones and esters respectively. The mechanisms are similar for each case.

The reactions for aldehydes and ketones are shown schematically above. Each involves a nucleophilic displacement of the π-bond of the carbonyl group by the α-carbon of the Grignard. This process formally produces an alkoxide that can be considered to react with the magnesium counter ion to produce a magnesium alkoxide. The actual process is not completely understood, but is initiated by the association of the magnesium atom of a Grignard with the oxygen atom of the carbonyl component. There is evidence to suggest that the process actually involves two molecules of Grignard reagent for each carbonyl addition as shown below.



The reactions produce magnesium alkoxides that are present at the end of the reaction. The magnesium-oxygen bond is strong enough that a further step must be done to hydrolyzed these bonds during the workup phase. This is normally done by treatment with mild acid to provide the free hydroxyls and magnesium salts.

Esters:



After the Grignard addition is complete, acid and water are added to break O-Mg bond

The addition of Grignards to esters creates an initial magnesium hemiacetal that collapses to form a ketone. This ketone then reacts with a second molecule of Grignard reagent to form the alcohol product. Because the ketone is usually more reactive than the ester, it is difficult to stop Grignard additions to esters after the first step. The magnesium hemiacetal is unstable because the α carbon of the ester possesses both a nucleophile and reasonably good leaving group. The collapse of this intermediate can be considered a type of nucleophilic displacement.

In all of the mechanisms shown above, the actual Grignard reaction is considered to be the portion in which the organo-magnesium reagent adds to the carbonyl component forming the magnesium alkoxide R_1R_2COMgX. The latter part of the process, in which H^+ and H_2O are used, is considered to be part of the workup, because it involves the isolation of the desired product.

When carbon dioxide is used as the carbonyl component, the product after appropriate work-up is a carboxylic acid having one more carbon than the starting Grignard reagent. The intermediate magnesium carboxylate is an extremely poor electrophile (one of the oxygens of the carboxylate has a formal negative charge) and so a second addition of Grignard to the carbonyl group does not happen.

Grignards will only react with fairly active electrophiles like carbonyl groups. Other important electrophiles for Grignards are epoxides, that usually react at the least substituted position. Why? It is important to remember that Grignards do not normally react with alkyl halides (there are exceptions, but this type of coupling is not synthetically useful).

Another important class of reactions that Grignards undergo are acid-base reactions. A typical Grignard will have a pK_a greater than 30, and so these reagents are very strong bases. During a Grignard reaction, any acidic functionalities, such as alcohols, acids or phenols, will react with your Grignard reagent in acid-base processes. For this reason, these functionalities are normally "protected" by converting them to un-reactive, related functional groups.

The most important experimental consideration, however, is the reaction with water. The presence of water in your reaction mixture will "kill" your Grignard reaction. Water will protonate any Grignard reagent, and will strongly interfere with the formation of the magnesium intermediate. It is therefore very important to remove all traces of water from your reaction before attempting to generate the Grignard reagent. At room temperature, *everything* in the lab, including your glassware, is coated with a microscopic layer of water. This water layer must be removed from the inside of your glassware before you can attempt a Grignard reaction. The easiest way to do this is to heat your glassware to remove the surface water. You must then assemble your glassware quickly, while it is still hot, being sure to use a drying tube. As the glass

cools, it adsorbs water from the atmosphere. The drying tube removes water from the air entering your setup before it comes in contact with the inside of your apparatus, thus keeping the glass dry (in research labs and industrial reactors, air is replaced by inert gases such as argon or nitrogen). If you allow the apparatus to cool before assembly with the drying tube, your glass will adsorb water from the air and the drying operation will have been for nothing. If your reaction fails, it is most likely because your glassware or reagents (ether) were not dry.

Many organic solvents absorb small amounts of water from the air. Ether is one of these solvents. When you perform your reaction, there will be two grades of ether available. It is very important that you use the *dry* ether for your reaction (the other grade of ether is to be used during your workup), and that you add materials to your set up immediately after measuring them out.

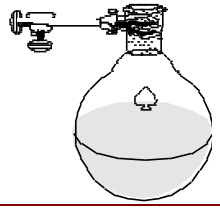
In all Grignard reactions, the initial product that is formed is a magnesium salt that has an oxygen-magnesium bond. This bond must be hydrolyzed to "free up" the desired alcohol and remove the magnesium salts. This hydrolysis is normally done with mild acid treatment. This last step is important because it affects the workup portion of the lab. Pay special attention to this phase of the experiment, and be sure you understand the processes involved.

How to set-up the apparatus

Glassware for organic chemistry is designed to be modular, interchangeable and to give an airtight seal between parts. This is accomplished by the use of ground glass joints. To work properly, the joints must be clean. Always inspect them for dirt and dust before assembly. If they are dirty, wipe with a paper towel.

Some experiments require the use of grease to prevent the ground glass joints from locking together (this is called seizing or freezing). This will be indicated in your manual, or by your demonstrator. In these cases, use a *small* amount of grease, *lightly applied* to the top of the male joints before assembly.

Organic apparatus are easiest to set up if you **work from the bottom up**. Start with your magnetic stirrer. Place the appropriate sized heating mantle on top of the stirrer and connect the heating mantle to the Variac. CAREFULLY place a magnetic stir bar in your distilling flask by tipping the flask on it's side and GENTLY sliding the bar along the inside of the flask. You should never drop a stir bar into a flask. The distilling flask is then clamped so that it rests in the heating mantle. Use an extension clamp on the neck of the flask in the position indicated in the diagram below. **ALWAYS CLAMP FLASKS THAT WILL CONTAIN CHEMICALS**. Use solid extension clamps for flasks, they hold better than three-fingered universal clamps. Clamp your flask so that the clamp holds just below the top lip of the female joint.



Once your refluxing flask is set, it is easy to add the other glassware. Work from what you have already set up, and add pieces one at a time. Add clamps as necessary to hold the apparatus solidly. The best place to clamp apparatus is at the joints. Proper locations are indicated in the various drawings. Once your set-up is complete, inspect it before proceeding. The joints should be fully sealed and the apparatus should be solidly clamped.

The condenser serves to cool the vapours which is then collected, via the adapter, in the receiving flask. Cooling is achieved by passing water through the jacket of the condenser. Connect rubber hoses so that water enters the bottom of the condenser and exits the top. In this way, gravity will ensure that the water completely fills the cooling jacket. This is important because the water must always be in contact with the inner glass surface in order to keep this surface cool.

When liquids are boiled, bubbles of vapor form in the bulk liquid. These bubbles form around imperfections in the vessel or solids suspended in the liquid. This process is called nucleation. Laboratory glassware will usually not contain enough "defects" to ensure proper nucleation, and so heated liquids can sometimes superheat (heat above the boiling point). Vapor bubbles can then form violently forcing the liquid out of the apparatus. This potentially dangerous event is called "bumping". Bumping is easily controlled by stirring the solution using a magnetic stir bar and stirrer. When stirring solutions, the speed should be controlled so that a small vortex is formed in the flask. Do not stir too fast as this will result in a loss of stirring or will splash the contents of the flask onto the flask walls resulting in loss.

The Experiment

THE SOLVENT THAT IS USED IN THIS EXPERIMENT (DIETHYL ETHER) IS **EXTREMELY** FLAMMABLE. ABSOLUTELY NO FLAMES DURING THIS LABORATORY SESSION.

1. The grades for this laboratory session will be determined to a considerable extent by the yield and quality of benzoic acid you produce. You will be supplied with 3.0 mL of bromobenzene. The demonstrators will be required to verify the weight and yield of your preparation.
2. In order to have a chance at a good yield it is necessary for you to work with CLEAN, DRY equipment and use good quality ANHYDROUS DIETHYL ETHER for the reaction.
3. When you first get to the lab, check your glassware. If dirty, clean and dry it before starting your reaction. Once the glass is dry, place the glassware in an oven for 15 to 20 minutes to remove the surface water layer. **DO NOT place glassware upside down. CAUTION: hot glassware is hot**.
4. When you remove the glassware from the oven, assemble it immediately and quickly fill the drying tube before the glass cools. The drying tube is filled by adding cotton (or glass wool), then $CaCl_2$ and finally another piece of cotton (see image above).
5. **Preparation of the Grignard Reagent**. Place 0.8 g of magnesium turnings and one or two **small** crystals of iodine in a 50 mL round bottom flask. Prepare a solution of 3.0 mL of bromobenzene in about 20 mL of ANHYDROUS diethyl ether. Be sure to keep the flask covered to avoid water contamination. Add one-half of this solution to the flask, and quickly re-assemble you reflux apparatus.
6. Swirl gently until the reddish I_2 color disappears (indicating that the reaction has begun). Watch for the formation of bubbles rising from the Mg. An exothermic reaction should start within a few minutes that causes the diethyl ether to boil (diethyl ether has a boiling point of 37 °C). If the reaction does not commence within 5 min, carefully break some of the magnesium turnings with a Teflon rod under the surface of the solvent to expose fresh magnesium surface which should help to start the reaction.
7. After the reaction has started, add the remaining bromobenzene solution slowly through the top of the condenser at such a rate that the solvent keeps refluxing gently. SEE FIGURE below to decide whether you should proceed or restart.
8. When the addition is complete, allow the mixture to boil on its own to ensure that the reaction is indeed taking place. As the boiling subsides, use a hot water bath (hot tap water is sufficient) to continue the reflux (boiling) for 20 to 30 minutes. **It is very important that the reaction start boiling on its own. Do not warm the flask until this exotherm has subsided**.
9. During this period most, but not all, of the magnesium should disappear and your solution will turn brown but remain clear or slightly turbid (cloudy). If your reaction is bright milky-white, it may have been contaminated with some water. If time permits, you may want to start over (ask your demonstrator to verify your observations).
10. Occasional swirling should help speed up the reaction. At the end of the reflux you should have a dark but clear solution of phenylmagnesium bromide in diethyl ether.
11. **Reaction with Carbon Dioxide.** Remove the flask from the hot water bath and allow any undissolved residue to settle.
12. Fill a DRY 100 mL beaker approximately 2/3 full of DRY ICE. This ensures you have an excess of dry ice relative to the limiting reagent. Decant the reaction solution carefully with stirring (use a Teflon rod), over a period of 15-30 seconds.
13. **CAUTION**: dry ice is very cold (–78 °C) and causes burns if touched for more than a second or two. **Never** touch dry ice if your fingers are wet, or if there is solvent on the dry ice.
14. **Isolation of the Benzoic Acid.** To the resulting sticky mass, add approximately 20 – 30 g of water ice, 20 mL of water, 5 mL conc. HCl, and finally 15 mL ordinary diethyl ether (do not use the anhydrous ether for this).
15. Transfer the mixture to a separatory funnel. Rinse the beaker with 15 mL of ordinary

diethyl ether and add the rinsings to the separatory funnel. Shake the mixture and separate the layers, storing them in separate containers. Re-extract the aqueous phase with another 15 mL of ether, and combine the organic extracts.

16. Take a TLC of the organic layer, using the starting material as a reference. Solvent system: EtOAc:Hexanes 1:9
17. Extract the combined organic phases twice, each time with 20 mL of aqueous 10 % NaOH solution.
18. Acidify the aqueous extracts by the drop wise addition of conc. HCl until the solution is strongly acidic (litmus) and no more precipitate is formed (adding too much HCl, a huge amount, will eventually cause NaCl to precipitate out.)
19. Cool the mixture in an ice bath and collect the solid product by suction filtration.
20. Disperse your product on a watch glass or in a clean dry beaker, and allow it to air-dry until the end of the laboratory period. Take the melting point and do a final TLC.
21. Once you have secured your product, please discard your remaining organic extracts in the containers provided throughout the laboratory.

Grignard reactions: TOP: good, carry on; MIDDLE: some water contamination but carry on; BOTTOM: a white reaction means too much water contamination for a successful reaction—**restart**.

Questions

1. 5-Methyl-1-hexanol (A) was reacted with NaBr / H_2SO_4 to give (B), which upon treatment with magnesium in diethyl ether formed an intermediate (C), which was not isolated. Reaction of (C) with Dry Ice gave, after the appropriate work-up, (D).
   a. Write the equations which describe the above sequence, including the structures for compounds (A) to (D).
   b. If the yield in the conversion of (A) to (B) was 65 % and that of (B) to (D) via (C) was 75 % what is the overall yield of (D) from (A)?
   c. Assuming the above yields, how much of (A) in grams is required in order to product 0.75 mol of (D)
2. Why is anhydrous ether used in this reaction?
3. Why is a water bath used to heat the raction?
4. What is the mechanism of the reaction of phenylmagnesium bromide and carbon dioxide? Include the work-up steps.
5. What is dry ice?
6. In this experiment, why is it important to use freshly obtained dry ice?

Report Notes

Refer to the instructions early in the lab manual regarding the preparation of reports. Be sure to explain all the steps of your experiment, paying attention to the workup/purification. This phase can be most easily explained by using a flow chart. Add mechanisms as necessary.

In your discussion you should briefly describe all of the steps in your procedure and give mechanisms for all transformations taking place. Interpret your results (e.g., interpret your TLCs and compre your m.p. to the literature value (be sure to cite your source).

Refer to the instructions early in the lab manual regarding the preparation of reports. Be sure to explain all the steps of your experiment, paying attention to the workup/purification. This phase can be most easily explained by using a flow chart. Add mechanisms as necessary.

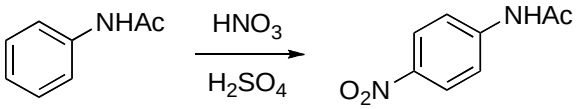
You should include references/citations for any data or other information that you looked up.

Experiment 6: Regioselective Nitration of Acetanilide

Department of Chemistry, University of Ottawa, 10 Marie Curie Private, Ottawa, Ontario, K1N6N5

September 2013

ABSTRACT

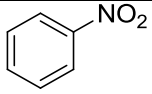
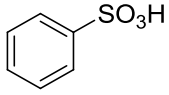
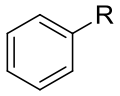
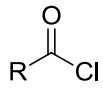
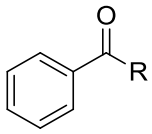
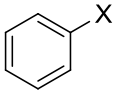


The nitration of acetanilide will be used to demonstrate electrophilic aromatic substitution.

Introduction

Electrophilic aromatic substitutions enable the installation of many substituent types into an aromatic ring. Like an alkene, benzene possesses π electrons that allow it to act as a nucleophile with strong electrophiles. Because the benzene ring is aromatic, it has a greater stability compared to an alkene. Benzene therefore reacts with only strong electrophiles. A few examples of electrophilic substitutions on aromatic rings are shown in scheme 1.

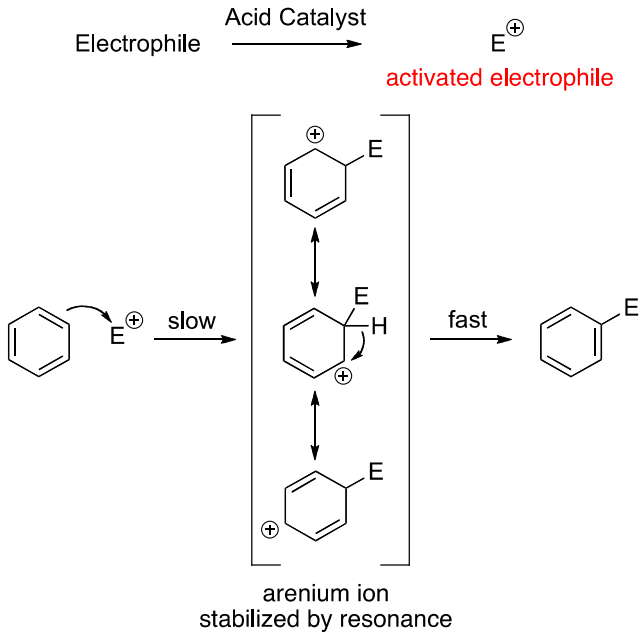
Table 1. Electrophilic substitution of benzene.

Reagent	Product
HNO_3, H_2SO_4	
SO_3, H_2SO_4	
RCl, $AlCl_3$	
 , $AlCl_3$	
X_2, FeX_2 (X = Cl, Br)	

Although there are many reagents that can be used in electrophilic aromatic substitutions with benzene, they all react by similar mechanisms (depicted in scheme 1 below). The substitution reaction begins with the formation of a highly reactive, positively charged electrophile (E^+). This

electrophile is most commonly formed by the action of strong protic or Lewis acids. Following the activation of the electrophile, a nucleophilic π bond in the aromatic ring attacks the electrophile. The electrophile must be very strong, because aromaticity is temporarily lost when the C=C bond is broken and a positive charge forms on the ring.

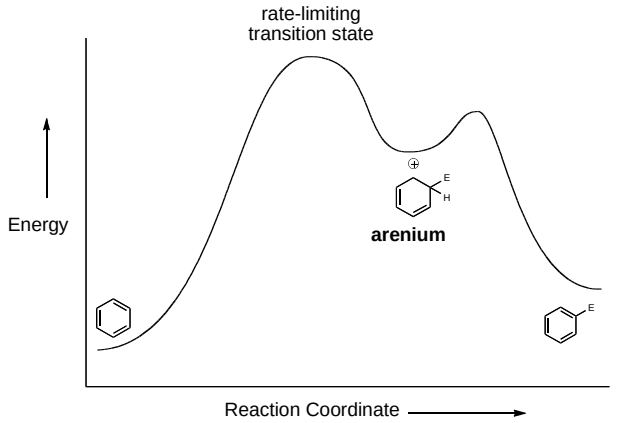
Scheme 1. General mechanism for electrophilic aromatic substitution.



The positive charge is resonance stabilized, and the stabilized ion that is formed is called an arenium ion. The addition of the electrophile to the ring to form the arenium ion has a high activation energy and so this step is the rate determining step of the reaction. The final step in the process is to restore aromaticity through the loss of a proton. When this happens, a carbon-hydrogen bond on the carbon that the electrophile becomes attached to is broken, releasing a proton and restoring the aromaticity of the ring.

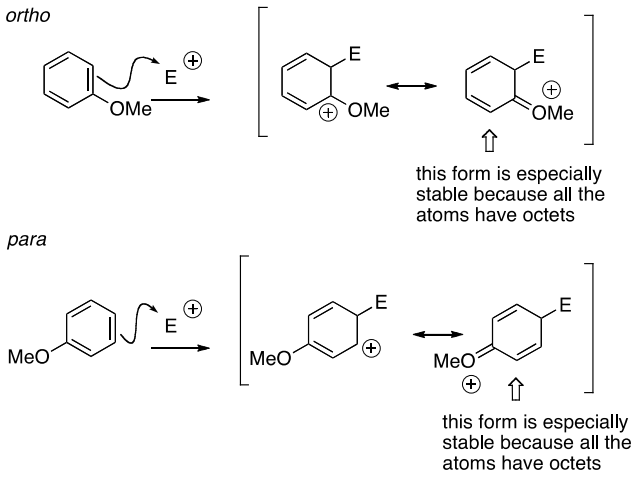
Preexisting functional groups on an aromatic ring will affect the regiochemistry of an electrophilic aromatic substitution reaction. The preference to bond at a particular position on the ring is called regioselectivity. The selectivity of the reaction is determined by the ability of existing functional groups on the benzene ring to stabilize the high-energy arenium intermediate in the reaction as depicted in scheme 2 below.

Scheme 2. Energy changes during electrophilic aromatic substitution.



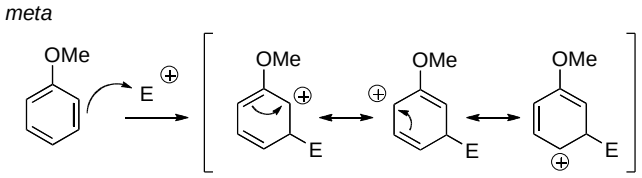
Electron donating groups stabilize arenium ions by dispersing the positive charge in the arenium and usually give faster reactions. These groups stabilize resonance forms containing adjacent positive charges, and may even contribute extra resonance forms if they contain lone pairs. These extra resonance forms are particularly stable because all the atoms have octets. This makes a significant contribution to the arenium structure and significantly stabilizes the arenium ions that are formed from *ortho* or *para* attack.

Scheme 3. *Ortho* and *para* directing groups stabilize adjacent charges in resonance forms.



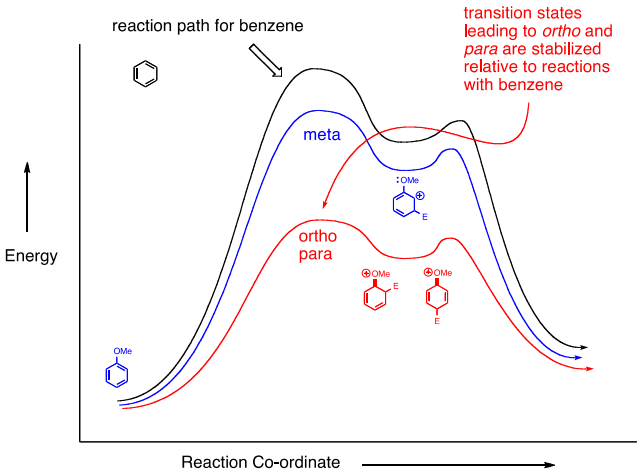
The arenium ions formed from *meta* attack do not benefit from much stabilization from the directing group. The overall ions are **slightly** stabilized (relative to benzene) because the charge is slightly reduced by the electron-donating character of the group.

Scheme 4. Meta substitution does not benefit from resonance stbilization from a directing group.



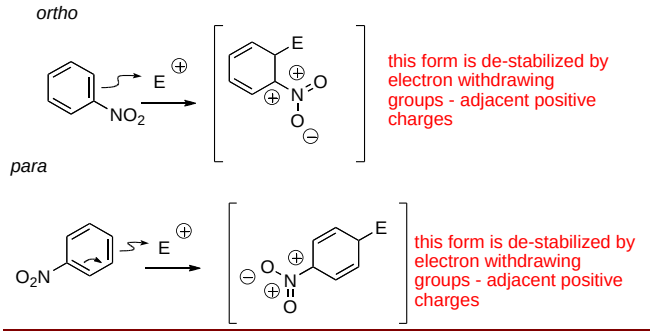
Aromatic rings with electron donating groups therefore favor reaction at the *ortho* and *para* positions. This is because the stabilization of the arenium ions accelerates the reactions leading to these intermediates by lowering the transition state energy leading to the arenium's formation. The *meta* arenium ion (and the transition state leading to it) are not significantly stabilized.

Scheme 5. Energy effects of electron donating groups on the reaction.



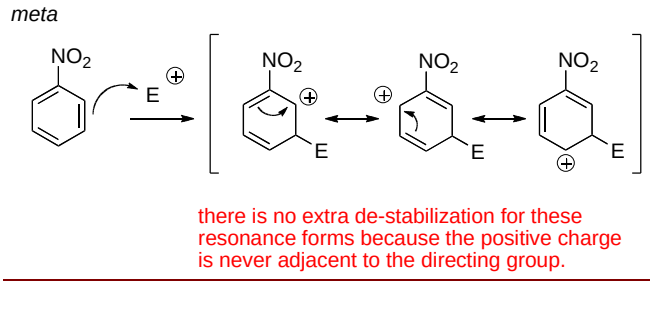
The situation is reversed for electron withdrawing groups. These substituents produce an increased effective positive charge on the arenium ions associated with electrophilic aromatic substitution. The arenium ions resulting from *ortho* and *para* substitution have resonance forms in which positive charge is located directly adjacent to the electron withdrawing group. These arenium ions, and the transition states leading to them, therefore have significantly higher energies that those of unsubstituted aromatic rings.

Scheme 6. *Meta* directors often have positive charges (partial or formal or actual) on the atom directly connected to the aromatic ring.



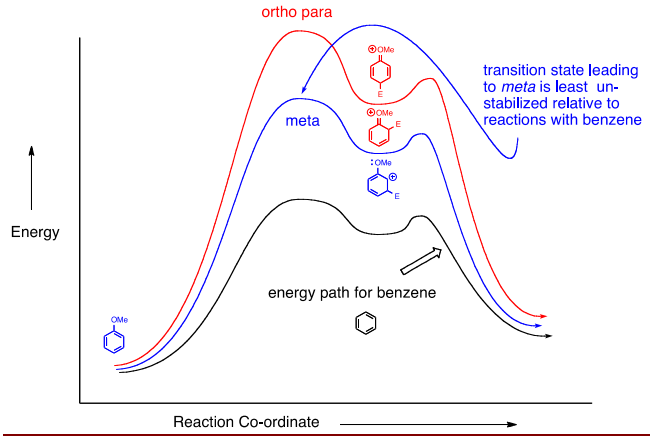
Because the *meta* pathway does not result in a positive charge next to the electron withdrawing group (Scheme 7), the energy of this ion is not significantly affected (it is raised slightly by the electron withdrawing nature of the group).

Scheme 7. The arenium ion in *meta* substitution is not greatly destabilized by the presence of nitro groups.



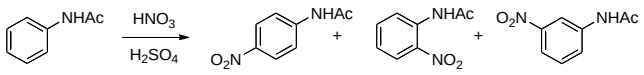
The net result is to slow the reaction relative to the reaction of benzene. However the *meta* pathway is affected much less strongly that either the *ortho* or *para* pathway. The molecule will "choose" the lowest energy route, which is the one leading to the meta product.

Scheme 8. Energy effects of electron withdrawing groups on the reaction.



In this experiment you will study the nitration of acetanilide. It is necessary to react nitric acid with sulfuric acid to form the strong electrophile needed for the reaction. **If you spill any acid on yourself, wash immediately with cold water.** Although both are strong acids, the nitric acid will act as a Brønsted base. Why is HNO_3 acting as a base and not H_2SO_4? The nitric acid is protonated by the stronger sulfuric acid, allowing water to leave and forming a nitronium ion. Once the nitronium ion, a highly reactive elctrophile, has formed, the π electrons of the aromatic ring can react, leading to the formation of three product isomers, shown in Scheme 9. Your main objective in this experiment is to identify the products of the reaction, and find the ratio of the major product to any minor products that are formed. You should then be able to justify the formation of the products and the relative amounts they are formed in.

Scheme 9. Possible nitration products formed.

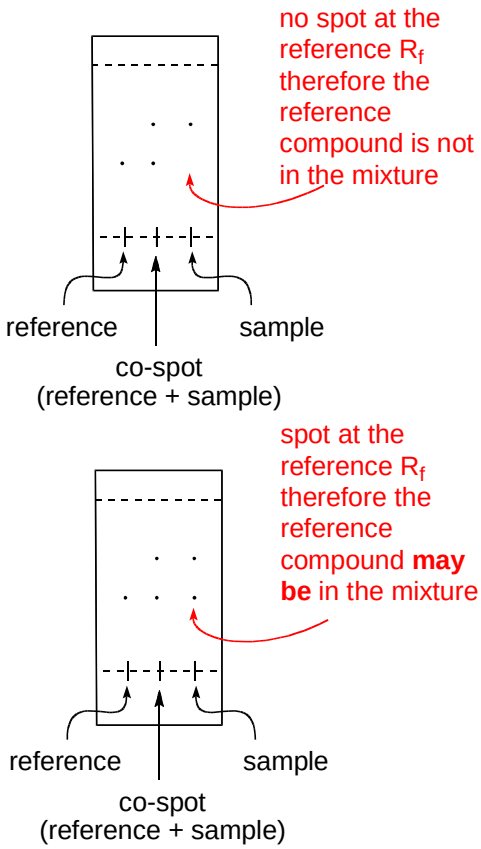


Once the nitroacetanilide has formed, it can undergo a second nitration. This reaction is much slower than the first and it is possible to minimize it by controlling the reaction temperature and relative reagent quantities.

Quantitative TLC

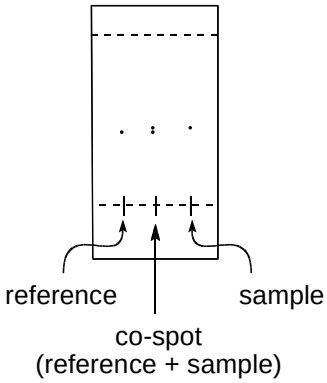
Thin layer chromatography (TLC) can be used in a variety of ways in an organic chemistry laboratory. **Qualitative** TLC is often used to determine purity and is very commonly employed to monitor chemical reactions. In qualitative TLC, the presence or absence of spots tells you if a component is present or absent. The plate below clearly shows that the reference compound is not in the sample, because no spot is noted at the same R_f value as the reference.

Scheme 10. Qualitative TLC shows the absence or possible presence of components .



In some cases, components can have similar R_f values (i.e., their spots overlap on the TLC). This may make it difficult or impossible to tell if a component is absent or present. These situations are the reason that co-spots are used, to be able to differentiate between spots with very similar R_f values. Qualitative TLC can provide an approximate indicator of the relative amounts of compound present. This is done by estimating the size (big, small) and intensity (dark, light) of the spots. The information provided, however, is not very accurate. At best, one can say that there appears to be a lot or a little of each component. In the plate below, the reference compound and sample have very similar R_f values. The co-spot shows a "snowman" shaped spot that demonstrates that the spots are different.

Scheme 11. Co-spots can help to distinguish similar components.



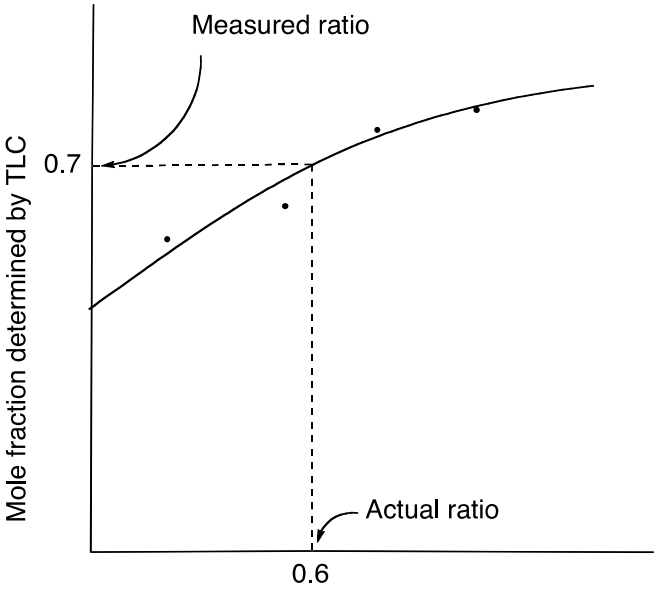
When components have the same R_f values, it may be necessary to use special visualization methods (such as other stains) to establish the identity of spots (same or different). Alternatively, variation in the solvent system can sometimes separate overlapping spots.

Quantitative TLC can be employed to determine how much of each component is present in a mixture. The TLC plate is prepared and developed in the normal way, then the amount of material in each spot is accurately measured. One way to do this is to scrape off the silica gel at each spot, chemically extract the compound from the silica, and weigh the amount obtained. Although this method works, it is difficult and time consuming because it must be done separately for each spot.

A more efficient method involves measuring the amount of ultraviolet light that is absorbed by each spot. Ultraviolet light is used because the energy of UV light is comparable with the energy of π bonds and is readily absorbed by organic compounds containing π bonds. The amount of UV light absorbed by each spot is proportional to the amount of compound present and to the ability of that compound to absorb UV light. The challenge with this method is that different compounds absorb light with differing efficiencies, and so a direct measurement cannot be made. A small amount of compound that strongly absorbs light will produce a dark spot, while a large amount of a compound that does not absorb light well will produce a light spot.

The way to deal with this issue is to prepare a calibration curve (calibration curves are used in almost every analytical chemistry method). Calibration curves are created by comparing the results of an analytical test (how large and dark a TLC spot is) to a known quantity (the actual weight of compound present). For this experiment, calibration curves were prepared by making accurately weighed mixtures of the products of your reaction. The ratio of the products was then measured by TLC, and the mole fraction of one of the components in each mixture was plotted against the mole fractions of the same component in accurately weighed ratios. The resulting graph is a calibration curve that relates the actual mole fraction to the measured mole fraction.

Scheme 12. Typical calibration curve.



To use the calibration curve, you determine the intersection point corresponding to the ratio that you measure. The corresponding intersection on the x axis then tells you the actual ratio of products.

The Experiment

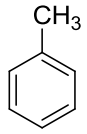
1. Refer to the section on designing a lab experiment to determine the amounts of each reagent to use. Complete the pre-lab quiz and bring the printout of your completed pre-lab quiz to your experiment.
2. Place acetanilide (approximately 1 g, accurately weighed)[8] into a clean 25 or 50 mL round bottom flask. Add a small stir bar and clamp the flask over a magnetic stir plate.
3. Add H_2SO_4 and stir **GENTLY** to dissolve the acetanilide. It is not necessary that the acetanilide dissolve completely. Stir for one or two minutes then proceed to step 3.
4. Cool the acetanilide solution in an ice bath by placing the empty ice bath container on the stir plate under your flask. Add ice to the bath and then water. Stir for 5 to 10 minutes to complete cooling.
5. In a 50 mL Erlenmeyer flask, carefully combine concentrated nitric acid and concentrated sulfuric acid. During your pre-lab exercise you have calculated the amounts you should use for each of these. Slowly add this mixture to your reaction flask. **CAUTION!** BOTH ACIDS ARE VERY CORROSIVE. WASH THOROUGHLY WITH WATER IF EXPOSED.

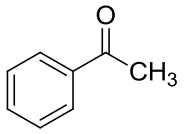
[8] What does this mean and why is it important?

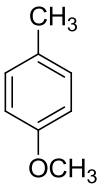
6. After 10 minutes, take a TLC (EtOAc:hexanes 5:5) of the reaction to verify that all the reaction is complete. How will you know if the starting materials have been consumed?
7. Continue stirring until TLC analysis shows that the reaction is complete. Remove the flask from stirring and remove the stir bar from your flask using a magnetic extractor. Clean the stir bar and extractor before proceeding.
8. **Isolation of crude product.** Place 3 or 4 small ice cubes in a 125 mL Erlenmeyer flask along with ~20 mL of cold water. Add a stir bar by sliding it down the side of your tilted flask. Place the flask on the stir plate, and gently stir the suspension. Pour the contents of your reaction flask into this flask, rinsing with a small volume of water. Stir the contents of the Erlenmeyer until all the ice has melted.
9. Collect the solid that forms by suction filtration using a Buchner funnel, washing with <u>cold</u> water.
10. Prepare a TLC sample of your product. To do this, place a few small crumbs of your crude product in a small vial, and add ~ 2 mL CH_2Cl_2 to dissolve it.
11. Prepare TLC plates that compare your crude product to reference samples of the *ortho*, *meta* and *para* and 2,4-dinitro product isomers.
12. Elute your TLC plates using the solvent system EtOAc:hexanes 5:5.
13. Write your initials at the top of your TLC plate; do not circle the spots before taking photos. Visualize each plate using the UV chamber. Once you are able to identify which spots in your crude are which isomers, take a photo of EACH of your plates.
14. Using the ImageJ software (available for download from the course website), determine the ratios of all compounds in your crude product.
15. **Recrystallization.** Dissolve the remaining crude product in a <u>minimum</u> amount of boiling ethanol in a 50 mL Erlenmeyer flask containing a stir bar. Refer to the discussion earlier in the manual for details on recrystallization.
16. Once dissolved, remove the flask from the heat and allow it to cool to room temperature **undisturbed** until crystals form.
17. Once crystallization is complete, isolate the crystals by suction filtration. Weigh the pure product and analyze the product by TLC (EtOAc:hexanes 5:5), comparing your purified product to your crude product. On a separate TLC plate, compare your purified product to your mother liquor. Be sure to write your initials at the top of your TLC plate. Visualize each plate using the UV chamber and photograph each plate.

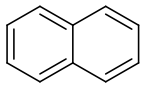
Questions

1. Explain the difference in polarity of the *ortho* and *para* isomers of nitroacetanilide with respect to the TLC.
2. Explain the difference in solubility between the *ortho* and *para* nitroacetanilide isomers in ethanol.
3. Why is the addition of a second nitro group in this reaction much slower than the first nitration of acetanilide?
4. Why are *para* isomers favored over *ortho* isomers in electrophilic aromatic substitution.
5. What is the preferred position of nitration for the following?

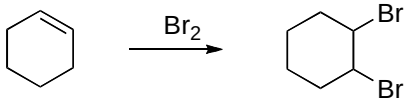
a. 

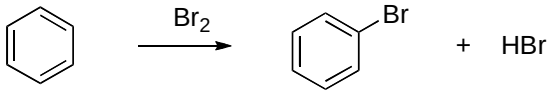
b. 

c. 

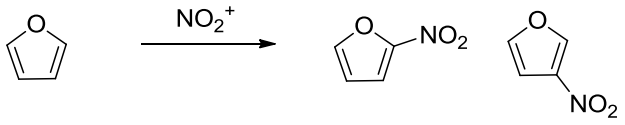
d. 

6. Explain the following observation





7. When 780 g of benzene (Molar mass = 78 g/mol) reacts with 750 mL of concentrated HNO_3 (16 M) and 750 mL of H_2SO_4 (18 M), 1000 g of nitrobenzene (molar mass = 123 g/mol) and 250 g of an unknown compound with a high boiling point are obtained.
   a. Identify the limiting reagent.
   b. Calculate the percent yield of nitrobenzene.
   c. Give the name of the most probable side product with the high melting point.
   d. Explain, with the aid of a mechanism, the role of H_2SO_4 in the reaction.
   e. Supposing the crystalline compound (250 g) has the formula $C_6H_4N_2O_4$, calculate the yield of this side product.
8. The nitration of furan leads preferentially to 1-nitrofuran rather than the 2-nitrofuran isomer. Explain by considering carefully the stability of the σ-complex leading to each isomer.



Report Notes

Refer to the instructions early in the lab manual regarding the preparation of reports. Be sure to explain all the steps of your experiment, paying attention to the workup/purification. This phase can be most easily explained by using a flow chart. Add mechanisms as necessary.

Using your photos of your TLC plates, calculate the ratio of all the isomers in your mixture. For this purpose, you should download and use the ImageJ visualization software from the course web site. Once you have the spot ratios from the visualization program, use the calibration curves from the website to determine the exact ratio of all isomers in all your products (crude, recrystallized, mother liquor). Include your photos (black and white is fine) in your report and show your calculations.

Part of the evaluation of your report will be in the organization of your data so that it can be easily read and interpreted.

Appendix I – Melting Point Apparatus

Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, Ontario, K1N 6N5.

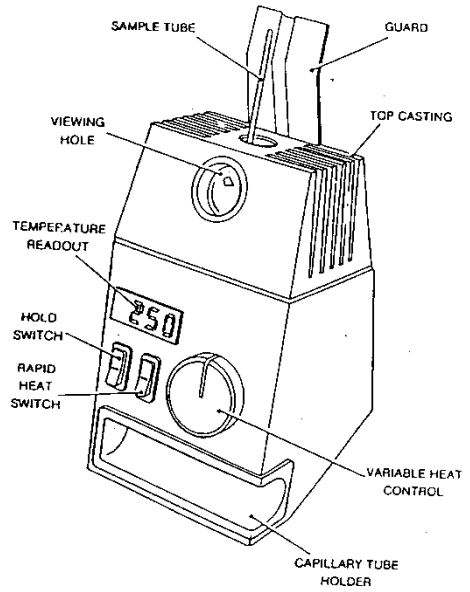
June, 2008

Melting Point

A pure organic substance usually possesses a sharp melting point - it melts over a short temperature range, less than 2 C°. The melting point range may vary due to the rate of heating, the crystal form and size, the sample size, and most importantly; the purity of the compound. Very rapid heating usually gives rise to large melting point ranges. Ideally, the rate of heating near the melting point should be about 2 C° per minute.

The presence of small amounts of impurities usually causes large increases in the melting point range, and lowers the temperature at which melting begins. This behavior is useful in establishing the identity of organic compounds by a method known as mixed melting point determination. Using this method, two substances that melt at the same temperature, say, 124 -126 °C may be shown to be identical if a mixture of the two substances continues to melt over this range. If the range of melting widens and drops, say to 105 – 115 °C, then one may conclude that the two substances are different. Occasionally, mixtures of two different substances may continue to give a sharp melting point. (The molecules of one compound fit into the crystal lattice of the other and form a "solid solution"). For this reason, this melting point test can only tell you if two compounds are different, not with confidence that they are the same.

Gallenkamp Instrument.



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.

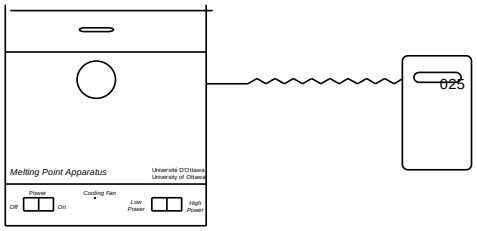
(1) Load sample crystals into a capillary tube to a depth of no more than 1 mm by pushing the inverted tube (the open end is facing down) onto the crystals. Better results will be obtained with a **small** sample (1 mm). Take just enough so you can see the sample in the tube. Turn the tube right-side 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 ° 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.

uOttawa Instrument.

The uOttawa melting point apparatus is equipped with a digital portable thermometer and an auxiliary fan unit to cool the apparatus when the melting point has been recorded.



Procedure

(1) Insert the portable thermometer into the hole on the right side of the melting point apparatus as shown. Make sure the thermometer passes through the inner sleeve of the heating core.

(2) Load sample crystals into a capillary tube to a depth of no more than 1 mm by pushing the inverted tube (the open end is facing down) onto the crystals. Better results will be obtained with a **small** sample (1 mm). 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). Place the capillary in the instrument.

(3) Turn on the instrument using the on/off switch. When the apparatus is turned on, the light will be lit and the apparatus will also begin to warm up.

(4) If the melting point of a given sample lies between 30 and 50 °C the instrument should be set at low power and the fan should be working in continuous intervals. This will slow the rate at which the instrument warms and allow for the precise measurement of the melting point. For samples that melt at higher temperatures (> 100 °C), the apparatus should be heated with high power until the temperature reaches either 100 °C or 20 °C below the actual melting point of the sample, which ever is *more*. The power switch should then be set to low power. This will allow for a gradual rise in temperature for accurate melting point determinations.

(5) When the sample starts to melt, the hold button should be pressed on the thermometer to record the temperature at which melting occurred.

(6) Withdraw the capillary tube and press the fan button to cool the unit. Once it has cooled, you may analyze a second sample. **The instrument should be cooled with the fan before turning off the power.**

Appendix II – Sample Lab Report

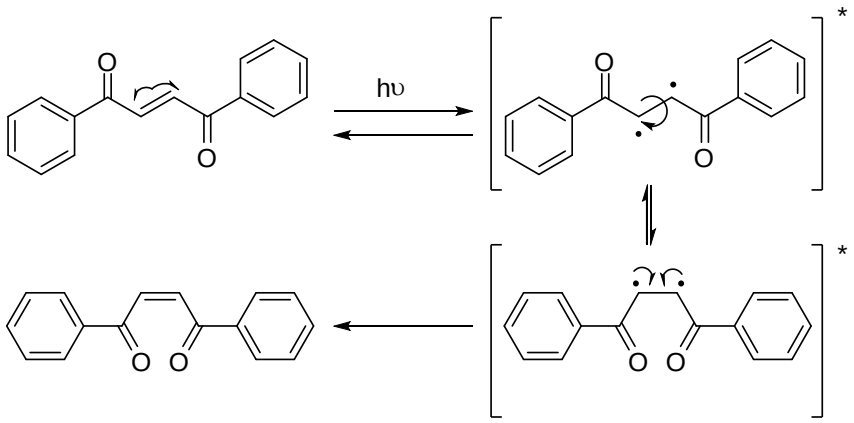
Lab # 9
Cis-Trans Isomerization of 1,2-dibenzoylethylene

Joan Smith

February 29, 2016

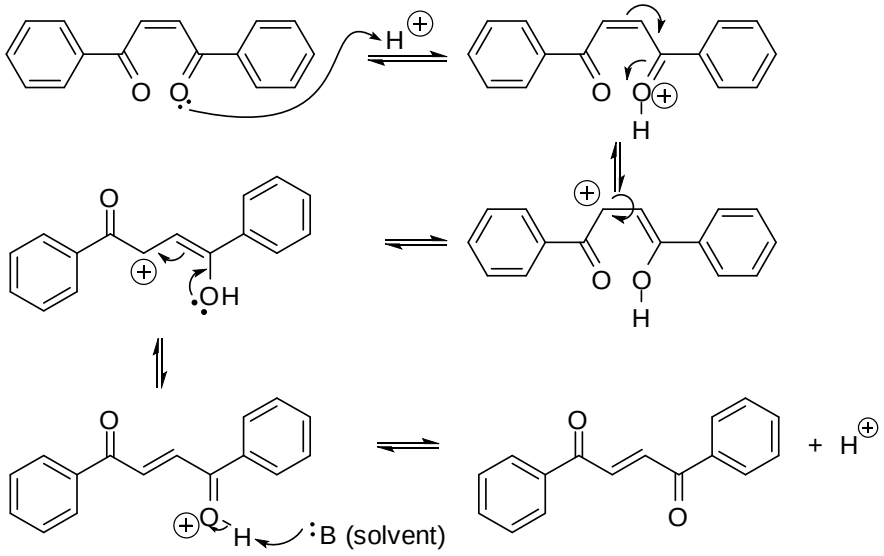
Note: Refer to the section entitled "Reports" at the start of the lab manual for complete instructions for completing a lab report. There are serious consequences for plagiarized lab reports. See the University policy for details.[9]

Mechanism for the *trans* to *cis* isomerization:



After isolating the *cis* compound, it will be converted back into the *trans* form by brief exposure to acid according to the process shown. Acid is a catalyst for this process.

The mechanism is shown below:



9 Plagiarism: www.uottawa.ca/plagiarism.pdf. Regulation on academic fraud: web5.uottawa.ca/mcs-smc/academicintegrity/regulation.php

Procedure and observations:

Part A: Isomerization of *trans* 1,2-dibenzoylethylene to *cis* 1,2-dibenzoylethylene.

Table of reagents

Compound	Mol. Wt (g/mol)	Amount	density (g/mL)	mmol
trans 1,2-dibenzoylethylene	236.27	1.01 g		4.275
EtOH	46.07	43 mL	0.790	

Procedure:

- the *trans* 1,2-dibenzoylethylene was dissolved in the ethanol. A bright yellow solution was produced.
- the resulting solution was placed in the photochemical apparatus and irradiated at 280 nm for 2 ½ hours. The solution became a very pale yellow (almost colorless) but remained clear. TLC showed that all of the starting material had been consumed, and a single spot was observed.

- The solution was cooled to 0 °C using an ice bath. Crystals formed after 30 minutes. These were filtered and washed with cold ethanol. Drying under vacuum gave 0.49 g of white needles (49 %). Melting point = 130-131 °C. Literature value (Lab manual) = 132 °C

Part B: Isomerization of *cis* 1,2-dibenzoylethylene to *trans* 1,2-dibenzoylethylene.

Table of reagents

Compound	Mol. Wt (g/mol)	Amount	density (g/mL)	mmol
cis 1,2-dibenzoylethylene	236.27	0.29 g		1.23
HCl (12 N)	36.5	1 drop		
EtOH	46.07	21 mL	0.790	

Procedure:

- the *cis* 1,2-dibenzoylethylene was suspended in the ethanol giving a colorless solution.
- while stirring, the solution was heated to reflux. The HCl was then added through the condenser. This initiated the isomerization as shown in the mechanism above
- the solution immediately turned bright yellow. TLC taken immediately showed no starting material remained

- flask removed from heat and allowed to cool to room temperature
- no crystals formed, cool in an ice bath 20 minutes, yellow crystals formed. Filtered and washed with cold ethanol. Gave 0.22 g bright yellow needles (76 %). Melting point 107 °C. Literature value (lab manual) = 109 °C.

Discussion:

Part A. In photoisomerization, the starting alkene absorbs UV light and undergoes a π to π^* transition. The π bond is no longer present and the molecule is free to rotate around the remaining σ bond. When the molecule relaxes, the π bond re-forms. Depending on the conformation of the molecule at the time that relaxation occurs, the resulting product will have either a *cis* or *trans* configuration.

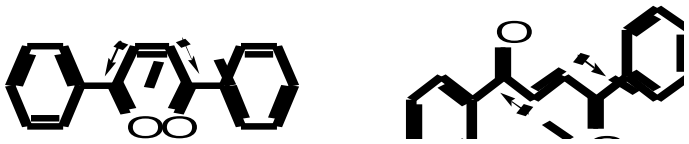
The wavelength of the light used to excite the alkene is chosen so that only the *trans* isomer absorbs. This is selected by examining the absorption spectra of the *cis* and *trans* forms. The *trans* form shows a λ_{max} of 280 nm whereas the *cis* form has a λ_{max} of 240 nm. By irradiating at 280 nm, only the *trans* form will be excited. Any *cis* product that forms will be unaffected as no π to π^* conversion takes place from this compound. As the reaction proceeds, the excited species relax to produce both *cis* and *trans* products. The *trans* molecules produced will be re-excited while the *cis* forms remain unaffected. Over time, all of the *trans* molecules will be converted to *cis*.

Part B. Reconversion to the *trans* form is done by treatment with acid. This process occurs by the mechanism shown in the introduction above. This is an equilibrium process. Because the *trans* form is more stable than the *cis* form, the *trans* compound forms preferentially.

The stability of the *trans* compound arises from a lack of crowding. The *cis* isomer is more sterically crowded, and this increases the energy of the *cis* form relative to the *trans*. The crowding in the *cis* form also forces the carbonyls out of alignment with the alkene thus decreasing conjugation since the overlap of the π orbitals will not be complete. This decreases the extent of conjugation for the *cis* form relative to the *trans*, thus further destabilizing the cis form relative to the *trans*.

Questions.

1) The *cis* isomer has a higher melting point because it has a larger net dipole moment. The trans compound has no net dipole because the individual dipoles of the carbonyl groups cancel each other out. The *cis* isomer has a larger dipole moment, and therefore the molecules in the solid will be held together by stronger dipole-dipole interactions than the *trans* isomer is resulting in a higher melting point for the *cis* compound.

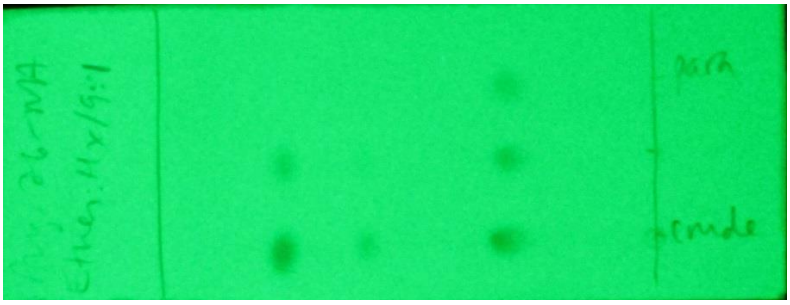


Appendix III – Instructions on using ImageJ software for TLC analysis

Using ImageJ to calculate ratio of compounds in a mixture

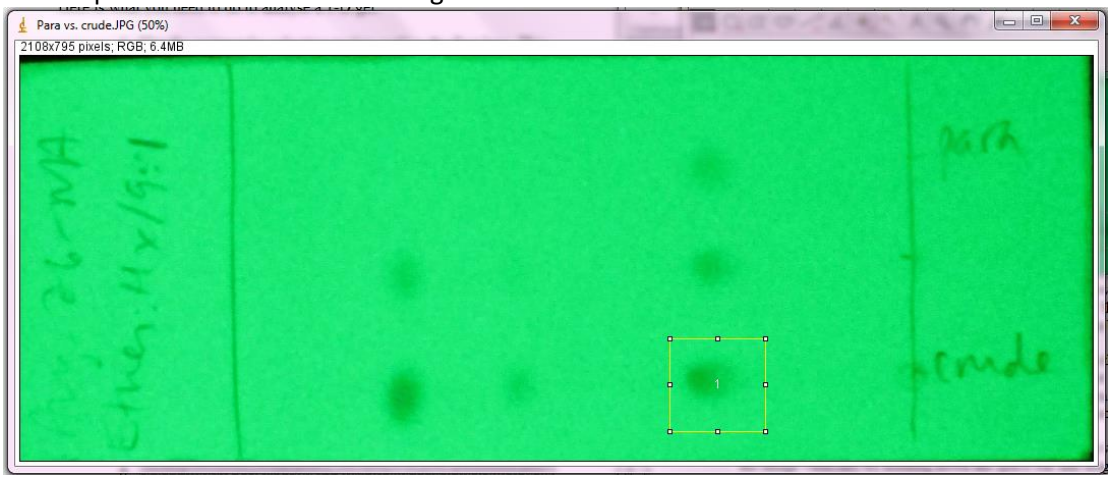
IN THE LAB

1. Identify each spot in your TLCs using reference samples. Do not circle the spots
2. Write your name/initials at the top of your TLC plate.
3. Ask your teaching assistant to take a picture of one of your TLC plates under UV light.

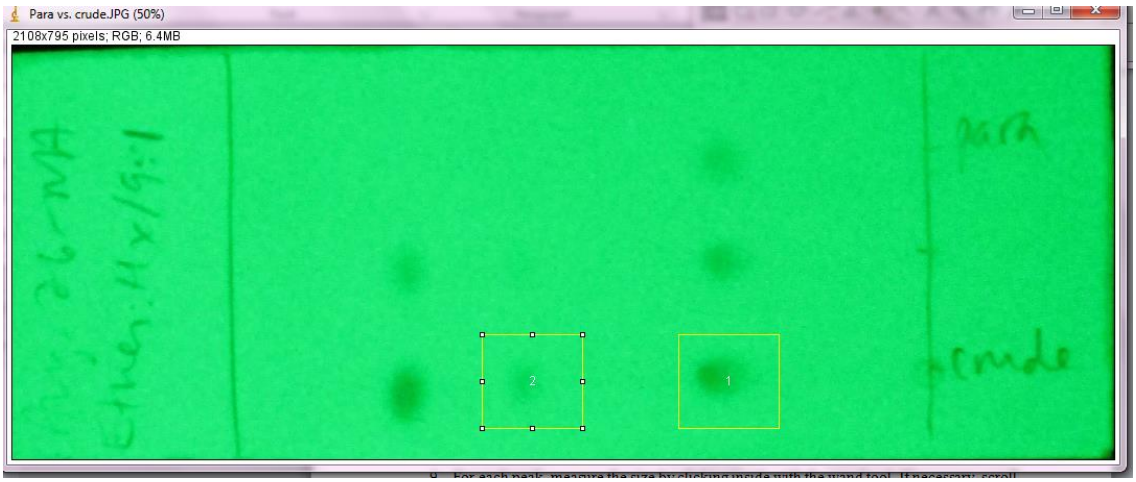


AT HOME or IN THE COMPUTER LAB

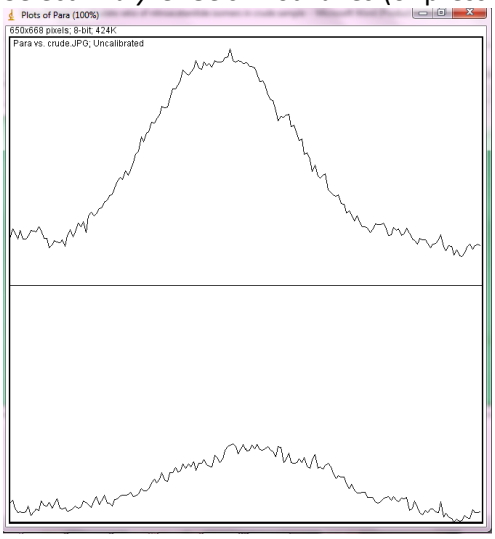
4. Download ImageJ: http://imagej.nih.gov/ij/
5. Open the ImageJ program and open your uploaded photo.
6. Use the rectangular selection tool to outline the first spot. Select *Analyze>Gels>Select First Lane* (or press "1") and the spot will be outlined and "Lane 1 selected" displayed in the status bar. Make sure the entire spot is enclosed in the rectangle.



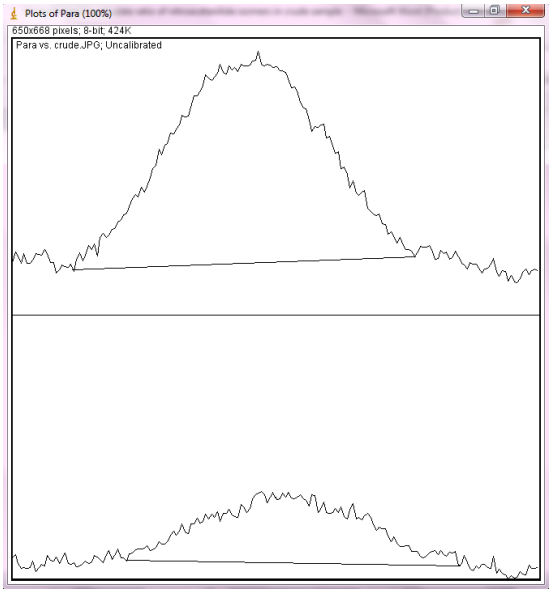
7. Move the rectangular selection over to the next spot to be analyzed and select *Analyze>Gels>Select Next Lane* (or press "2"). Make sure you are only selecting and analyzing spots that are **known compounds determined via all your TLCs**. The selected spot is outlined and labeled, and "Lane n selected" is displayed in the status bar.



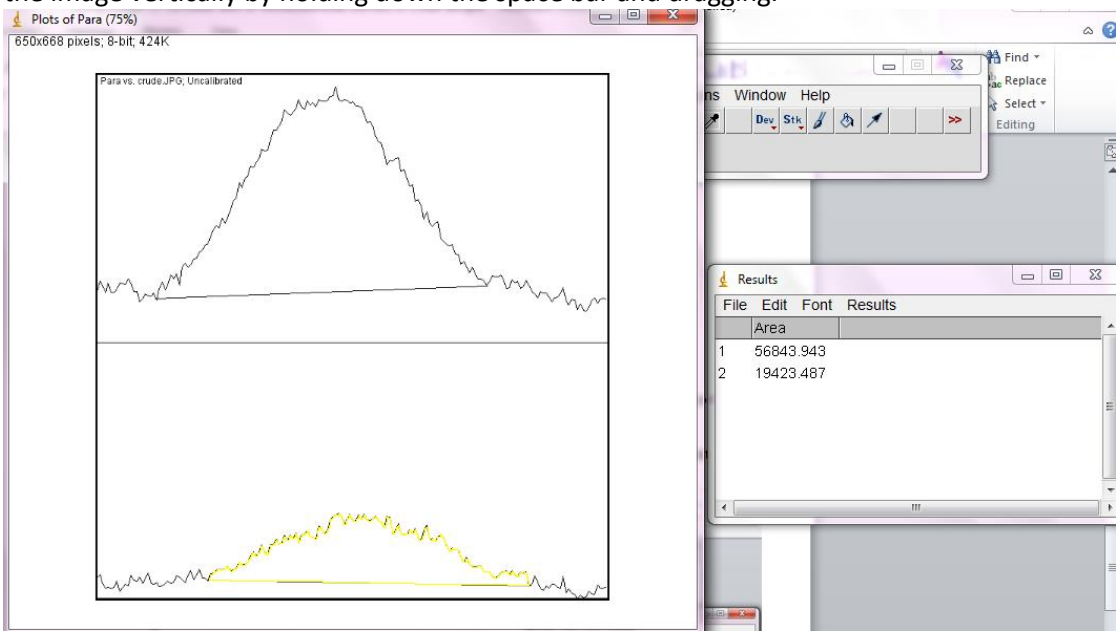
8. Repeat the previous step for each remaining known compound spot in your mixture. Compare only two compounds (spots) at a time.
9. Select *Analyze>Gels>Plot Lanes* (or press "3") to generate the lane profile plots.



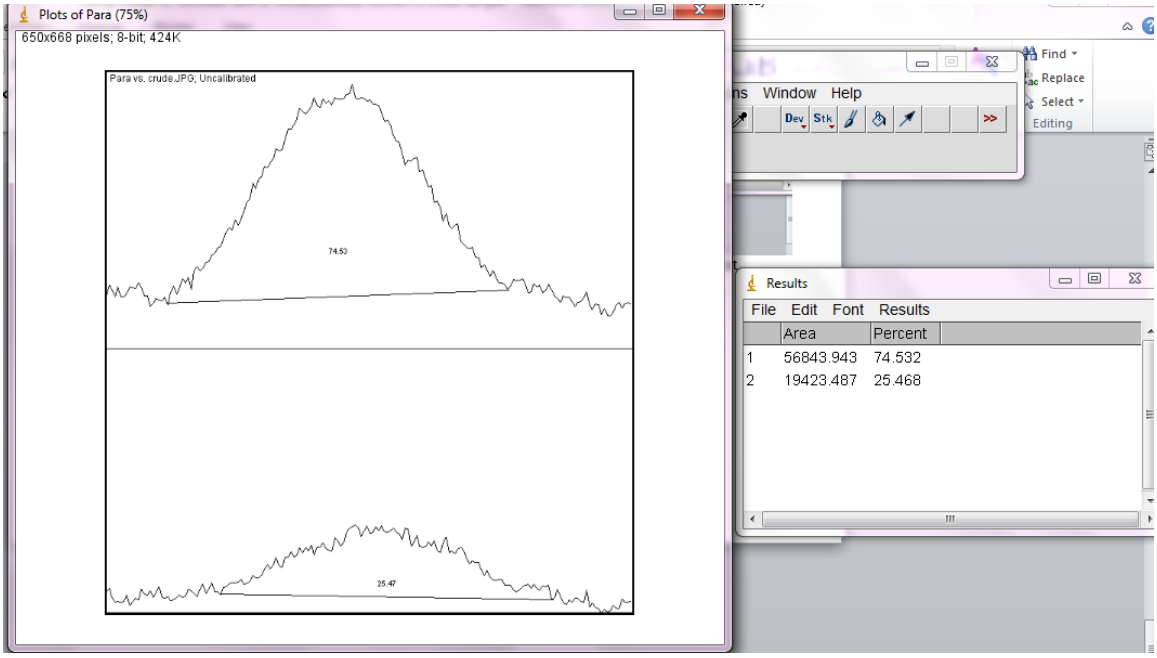
10. Use the straight line selection tool to draw base lines and/or drop lines so that each peak of interest defines a closed area. To get to all the lanes, it may be necessary to scroll the image vertically using the "Hand" tool. (Hold down the space bar to temporarily switch to this tool). Make sure that the line you draw actually touches the graph to form an **enclosed** peak area. The line does not have to be perfectly horizontal.



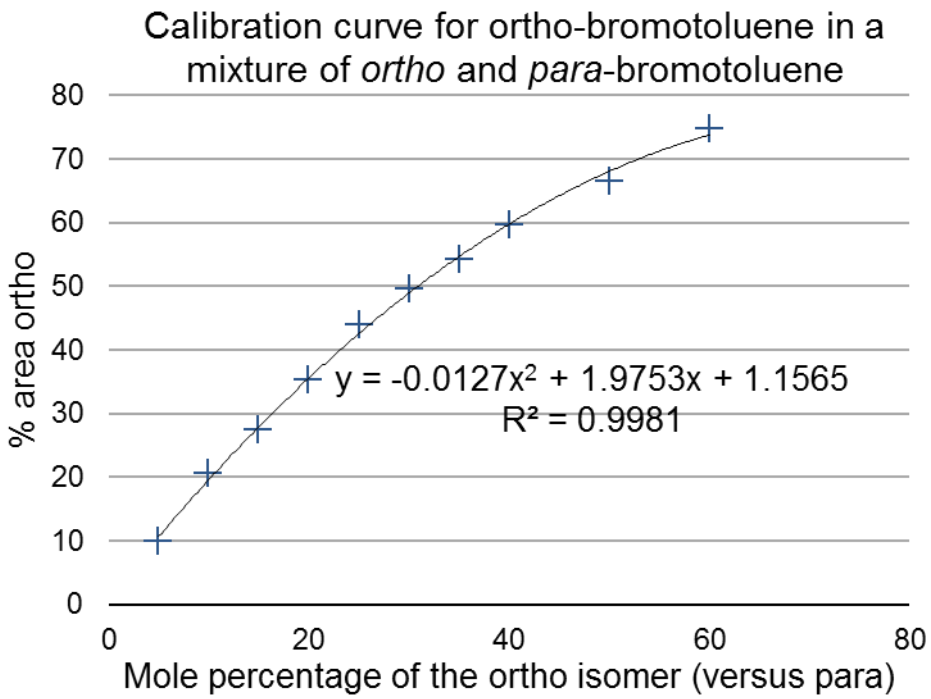
11. For each peak, measure the size by clicking inside each peak with the wand tool  . If necessary, scroll the image vertically by holding down the space bar and dragging.



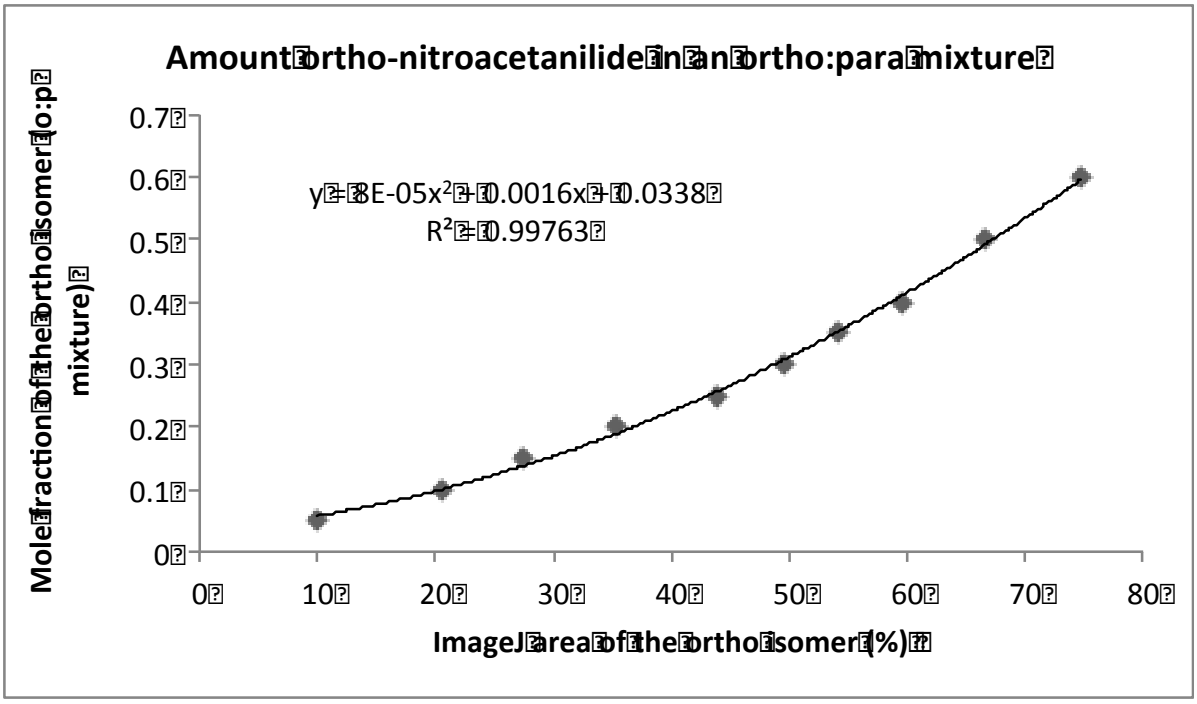
12. Select *Analyze>Gels>Label Peaks* to label each measured peak with its size as a percent of the total size of the measured peaks.

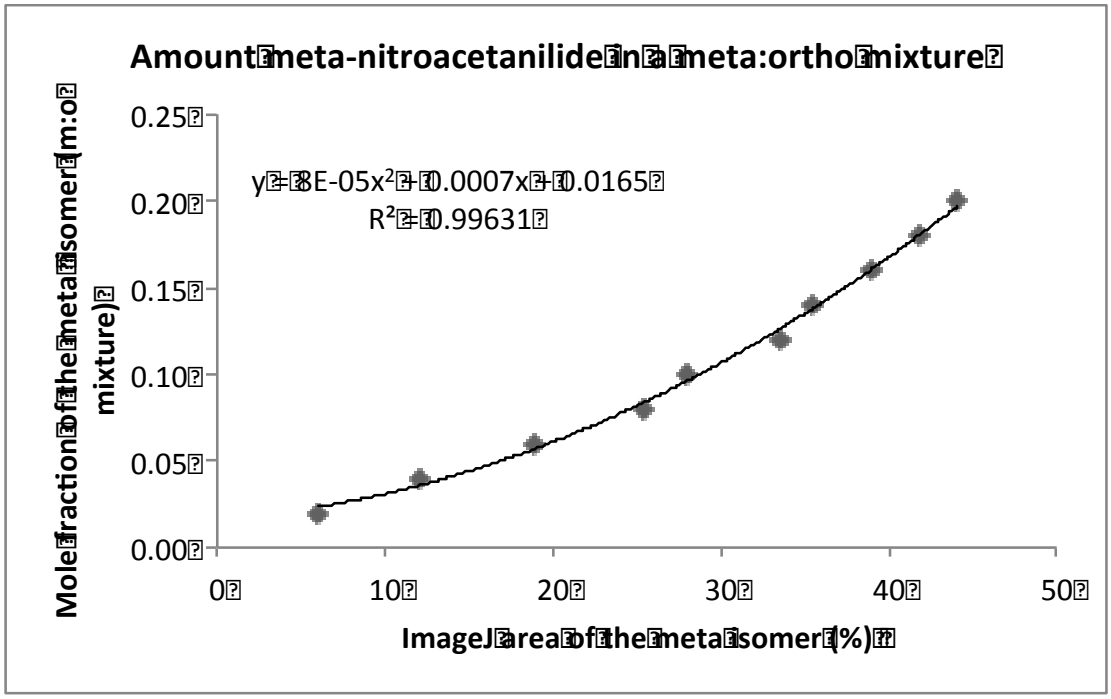


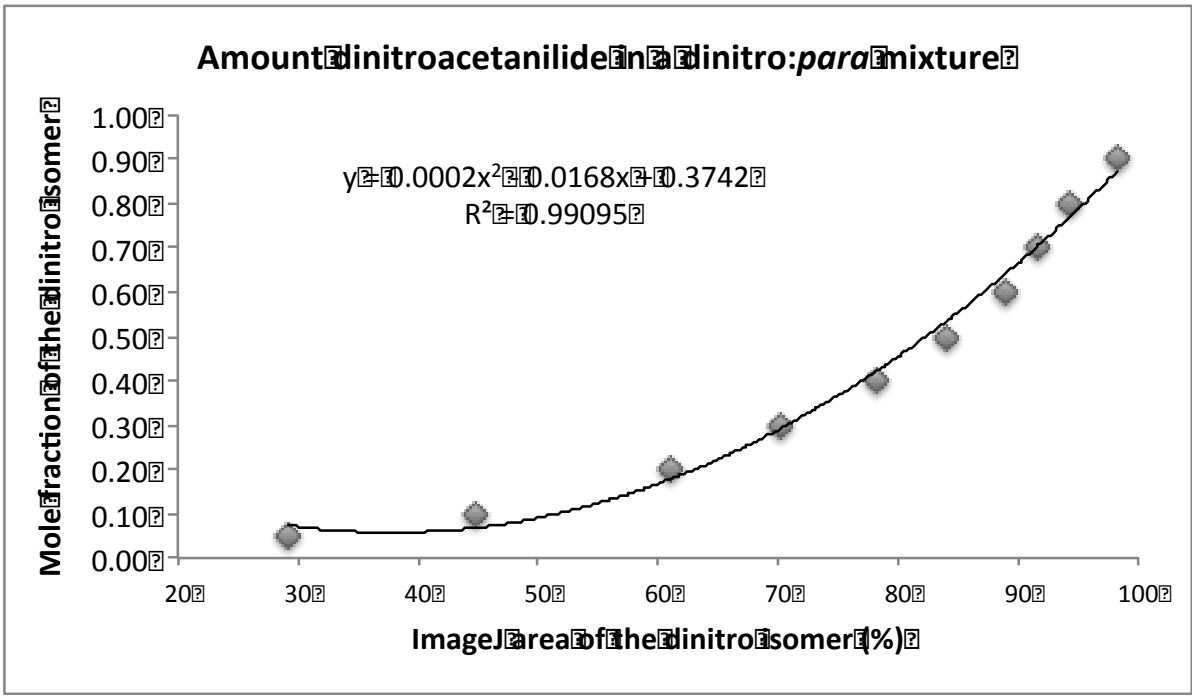
13. Compare your ratio to the appropriate calibration curve on the course website. For example, given an area of 25.468% *ortho* in an *ortho*:*para* bromotoluene mixture:
    a. Compare that percentage to the *ortho*:*para* calibration curve (see below)—you have x (the % area of the *ortho* isomer); solve for y (the mole percentage of the *ortho* isomer). You should get 43.23% mole % of the *ortho* isomer. So the *ortho*:*para* ratio is 43:57, or 0.75:1. Notice that there is not much difference in the amount of each product formed, even though the spots look very different on the TLC!



Nitroacetanilide calibration curves




Amount meta-nitroacetanilide in a meta:ortho mixture
Mole fraction of the meta isomer (m:o mixture)
y = 8E-05x² + 0.0007x + 0.0165
R² = 0.99631
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
ImageJ area of the meta isomer (%)


Amount dinitroacetanilide in a dinitro:*para* mixture
Mole fraction of the dinitro isomer
y = 0.0002x² + 0.0168x + 0.3742
R² = 0.99095
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
20
30
40
50
60
70
80
90
100
ImageJ area of the dinitro isomer (%)