Kingdom of Saudi Arabia
Ministry of higher Education
Umm Al- Qura University
College of applied Science
Chemistry

Practical Aliphatic Organic Chemistry (402131-4)

Department of Chemistry

1438

Contents

Lab Safety

The responsibility for lab safety rests with each student in the laboratory. You must use common sense and work carefully to avoid chemical spills, broken glassware, and fires. This ensures not only your own safety, but that of your lab mates. Know the hazards of each chemical you use so that you will know what level of caution to use when handling it. If you do this, you will not be exposed to a harmful amount of any chemical during your year in organic chemistry lab.

General Guide for Handling Chemicals in the Laboratory

One of our goals is to teach each student how to safely handle organic chemicals. Not only is this necessary for you to have a safe experience in organic lab, it is useful knowledge for almost any job you have after college, as well as for handling cleaning solvents and other chemicals in your home.

Flammable Chemicals

The method for proper handling of these flammable chemicals (such as diethyl ether, acetone, hexanes, ethanol, methanol) depends on their flammability rating, as given by a number 4-0 in the red area of a NFPA/HMIG label. The NFPA/HMIG rating for diethyl ether is "4" while acetone, methanol, ethanol, and hexanes are "3". Ether is extremely flammable and any spark or simply heat can ignite it.

In case of fire:

- If your clothing catches fire, immediately drop to the floor and roll to smother the flames and call for help.
- If a compound or solvent catch on fire, *if you can*, quickly cover the flames with a piece of glassware

- If it is feasible, use a fire extinguisher to put the fire out.
- Do not put water on an organic chemical fire because it will only spread the fire.
- If the fire is large, do not take chances: evacuate the lab and the building immediately and tell your TA or the Coordinator what has happened.
- If no one in authority is available, pull the fire alarm in the hallway and call 911 from a safe phone.

If the fire alarm sounds for any reason, leave the room immediately and exit the building. The types and use of fire extinguishers is covered in a separate orgchem web page: Fire Extinguishers.

Volatile Chemicals

. Volatile chemicals (such as hexanes, acetone, methylene chloride, diethyl ether) are ones that evaporate very easily. Diethyl ether and methylene chloride are the most volatile of the chemicals that you will use in the organic chem teaching labs. If they are accidentally inhaled, they can cause irritation of the respiratory tract. In large concentrations, symptoms such as intoxication, drowsiness, nausea, or central nervous system depression may occur. Note that diethyl ether presents a special problem because it is not only volatile, it is also extremely flammable.

Work in your student hood whenever possible, especially when you are handling volatile chemicals. If you need to carry volatile chemicals through the lab, carry them in a covered container. Everyone in the lab must work together to reduce the amount of volatile chemicals released into the lab room!

A student hood. (Note the flaps on the front face). The main hood. (Note that it is nearly closed).

If you inhale vapors:

Leave the area immediately - at least into the hallway. Tell your TA or the Coordinator; they will take you outside into the fresh air, and if necessary, provide first aid or take you to get medical attention.

Contact Hazards

The health hazard of a chemical (such as methanol, ethanol, hexanes, acetone, methylene chloride, diethyl ether) is designated by a number 4-0 in the blue area of a NFPA/HMIG label. None of the chemicals you will use has a "4" rating; most are 1 or 2. If you had a one-time overexposure to the above chemicals, you might suffer a minor or a serious injury. If you protect yourself by wearing proper protective equipment (gloves, lab coat, goggles, and closed-toed shoes), and if you are careful not to spill chemicals, you are not likely to come into contact with these chemicals. In the past, chemicals have been spilled by students and left where they were in the lab, especially by the balances.

If you spill a contact hazard on yourself:

Immediately rinse the affected area with lots of water. Use soap if you wish, but never try to "treat" the spill with another solvent or chemical unless directed to

do so by your TA. If the affected area remains more than slightly red after the rinsing period, seek medical attention.

Corrosives

Strong acids (hydrochloric acid, sulfuric acid, phosphoric acid, nitric acid) and bases (as sodium hydroxide) are used frequently in the organic chemistry teaching labs. If spilled on your skin, they cause a chemical burn. They are very harmful to your eyes. If you breath in a big whiff of vapors, you will feel a burning in your nasal and respiratory passages.

Handle corrosives with great care so as not to spill them or inhale their vapors. Always wear protective gear. The heavier style of Playtex gloves are recommended for use when handling corrosives.

If you spill a corrosive on yourself:

Immediately rinse the affected area with lots of water. Use soap if you wish, but never try to "treat" the spill with another solvent or chemical unless directed to do so by your TA. If the affected area remains more than slightly red after the rinsing period, seek medical attention.

Glassware Safety

Use common sense when handling glassware. Keep glassware away from the edge of the benchtop. Always clamp your reaction flask and the suction flask securely to a ring stand to prevent them from falling over. Check each piece of glassware for hairline or star cracks before using it. When doing a distillation, clamp each piece of glassware securely. If you do break a piece of glassware, do not leave it in the sink or on the benchtop because someone may inadvertently get cut.

If you cut or burn yourself:

If you cut yourself, wash the wound immediately with large amounts of cool water. If it is your neighbor who has been hurt, be prepared to help them if they are

unable to help themselves. Thermal burns are treated by covering the affected area with cool water or ice. After a while, you can apply a pain-relieving cream. If the burn looks like it is more than just a reddening of the skin, seek medical attention.

Equipment and Electrical Safety

Use electrical equipment (heating mantles, variances, stir motors, hot plates) properly to prevent electrical shock. Check the cord or plug to make sure that it is not damaged or frayed; if it is, tell your TA. Always disconnect the plug from the socket by pulling firmly on the plug: Do not yank it out by the cord! Keep water away from all electrical equipment.

Fire Extinguishers

If there is a fire in the lab room, you might need to use a fire extinguisher. Remember, it is more important to get to safety than it is to put the fire out. If you do decide that it is feasible, you must know where they are, what types they are, and how to use them. There are two fire extinguishers in each lab: one at the front of the lab near the blackboard, and at the back near the main hood.

Each of these fire extinguishers is safe for use on chemical fires, since the only fire extinguishers in the labs are "Dry Chemical" (the two on the left in the picture below) or "CO_2" (the one on the right). CO_2 extinguishers can be identified

by their large nozzles. In almost all cases, the best choice is a CO_2 extinguisher, since it does not leave a residue on the equipment when the fire is out. But do not hesitate: grab the closest fire extinguisher in an emergency. Never use a water fire extinguisher in the organic chemistry labs (there is one in the hallway for fireman use only).

Reaction Glassware

Round-bottom flask

Y-adaptor

Vacuum adaptor

Condenser

Thermometer adaptor

Claisen adaptor

Bench Glassware

Beaker

Erlenmeyer flask

Side-arm flask

Buchner funnels

Thermometer

Stemmed funnels

Separatory funnel

Watch glass

Stir rod

Vial

Graduated cylinder

Pasteur Pipet

Bench Equipment

Versatile clamp

3-pronged clamp

Ring clamp

Spatula

Scoopula

Forceps

Keck clip

Stir bar

Heating mantle

Stir motor

Variac

Stirring Hotplate

Ring stand

Tubing

List of common equipment in student's locker

- Beaker, 50,100, 250, 400, 600 mL
- Clamp, test tube
- Cylinder, graduated by 0.1, 10, 100 mL
- Dropper, medicine with rubber bulb
- Evaporating dish - Flask, Erlenmeyer, 125, 250, 500 mL
- File, triangular
- Forceps
- Funnel, short stem
- Gauze, wire
- Spatula, stainless steel
- Sponge
- Striker (or box of matches)
- Test tubes, approximately 15 - 150 mm
- Test tube brush
- Thermometer, 150°C
- Tongs, crucible
- Wash bottle, plastic
- Watch glass.
- Filter paper

Purification and separation of organic compounds

Organic compounds, whether solids, liquids or gaseous, when are separated from a natural source or organic reactions are seldom pure. They may be contaminated with other compounds, which are formed as a byproduct, owing to a side reaction. These organic compounds have to be purified before using them in other chemical reaction. Various methods used for the purification of organic compounds are based on the nature of the compound and the impurity present in it. These methods are listed below: -

1- Recrystallization.
2- Sublimation.
3- Extraction.
4- Distillation.
   a) Simple distillation.
   b) Fractional distillation.
   c) Vacuum distillation.
   d) Steam distillation.
5- Chromatographic Methods.

The method employed depends upon the nature of the material to be separated and purified.

Purification may often be successfully accomplished by re-crystallization or sublimation for solids; extraction for solid dissolved in liquid or mixture of liquids; fractional distillation under atmospheric or reduced pressure for liquids or low melting solids; molecular distillation for high-boiling liquids. In those cases where the use of these traditional methods does not yield product of adequate purity, resort must be made to preparative chromatographic procedures

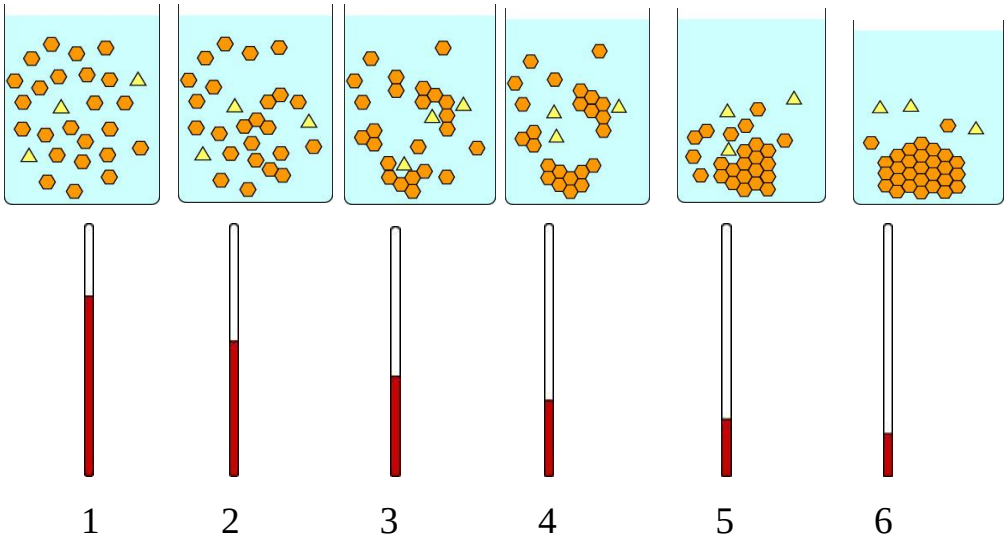
Crystallization

Crystallization is a technique which chemists use to purify solid compounds. It is one of the fundamental procedures each chemist must master to become proficient in the laboratory. Crystallization is based on the principles of solubility: compounds (solutes) tend to be more soluble in hot liquids (solvents) than they are in cold liquids. If a saturated hot solution is allowed to cool, the solute is no longer soluble in the solvent and forms crystals of pure compound. Impurities are excluded from the growing crystals and the pure solid crystals can be separated from the dissolved impurities by filtration. This simplified scientific description of crystallization does not give a realistic picture of how the process is accomplished in the laboratory.

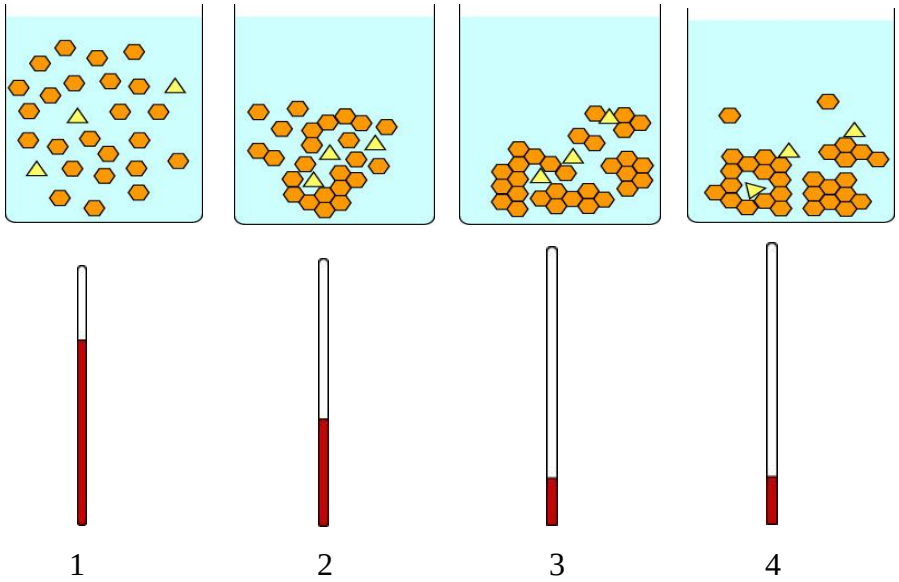
What Happens During a Crystallization?

To crystallize an impure, solid compound, add just enough hot solvent to it to completely dissolve it. The flask then contains a hot solution, in which solute molecules - both the desired compound and impurities - move freely among the hot solvent molecules. As the solution cools, the solvent can no longer hold all of the solute molecules, and they begin to leave the solution and form solid crystals. The chilled solution is then filtered to isolate the pure crystals and the crystals are rinsed with chilled solvent. This first series of diagrams shows what happens if you let a crystallization proceed **slowly**: first by setting the flask at room temperature undisturbed until crystals form, and then carefully on ice. The red bar to the right of each image is a thermometer, to indicate the temperature. The yellow triangles are an impurity in the hot solution of orange hexagons. If the solution is allowed to cool slowly, the impurities may attach briefly to the growing crystal lattice, but they soon leave as a compound with a more suitable geometry comes in to take their place.

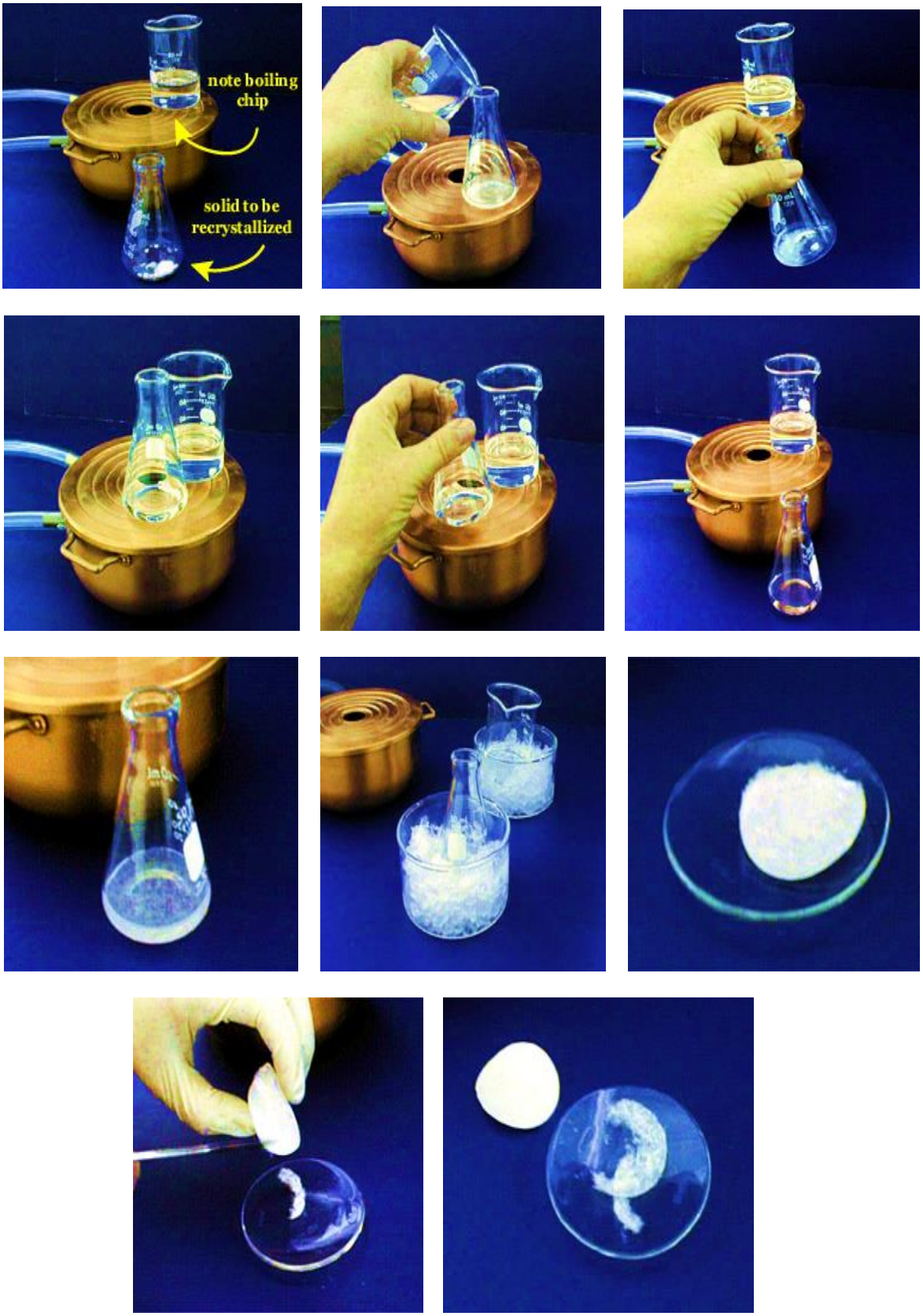
Suitable hexagons stay more readily in the growing lattice, and eventually pure crystals of orange hexagons are formed.



This second series of diagrams shows what happens if you cool the solution too **quickly**. The yellow triangle impurities are trapped inside the crystals being formed by the orange hexagons, thus, the crystals isolated are impure. Note that slow crystallization gives larger crystals than fast crystallization. Small crystals have a large surface area to volume ratio and impurities are located on the surface of the crystals as well as trapped inside the matrix.



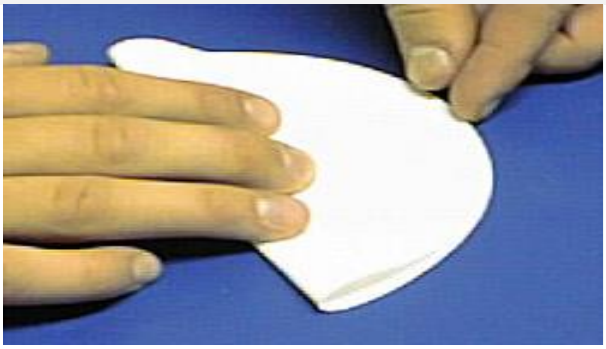
How to do a Crystallization

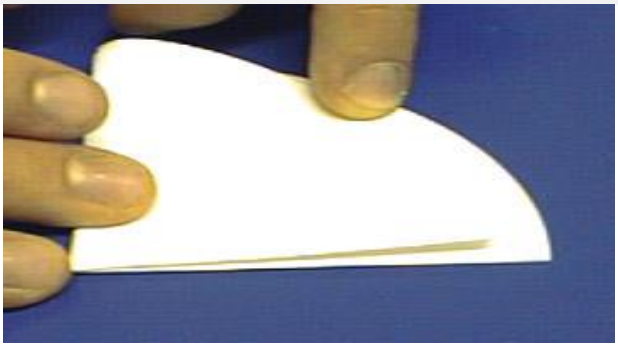


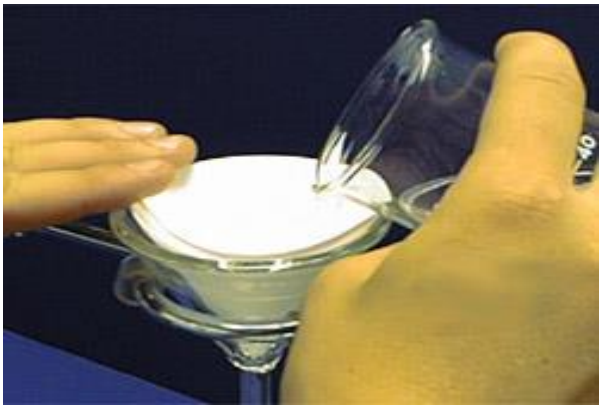
Filtration

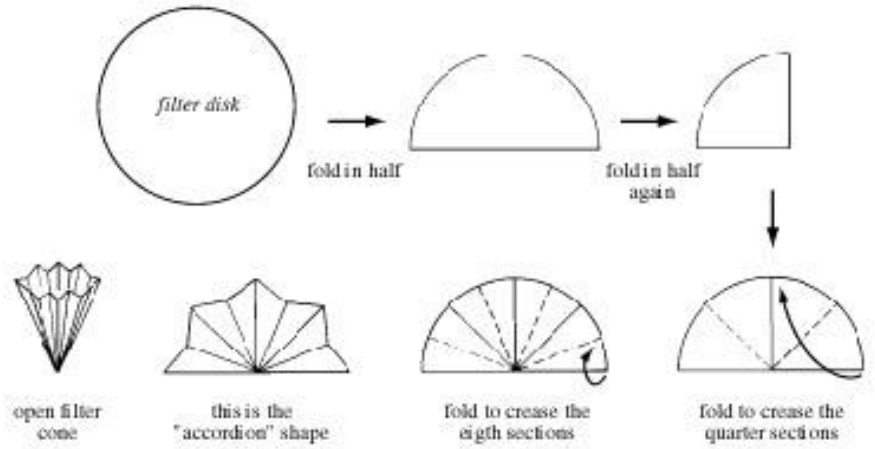
Filtration is a technique used either to remove solid impurities from an organic solution or to isolate an organic solid. The two types of filtration commonly used in organic chemistry laboratories are gravity filtration and vacuum or suction filtration.

Gravity Filtration



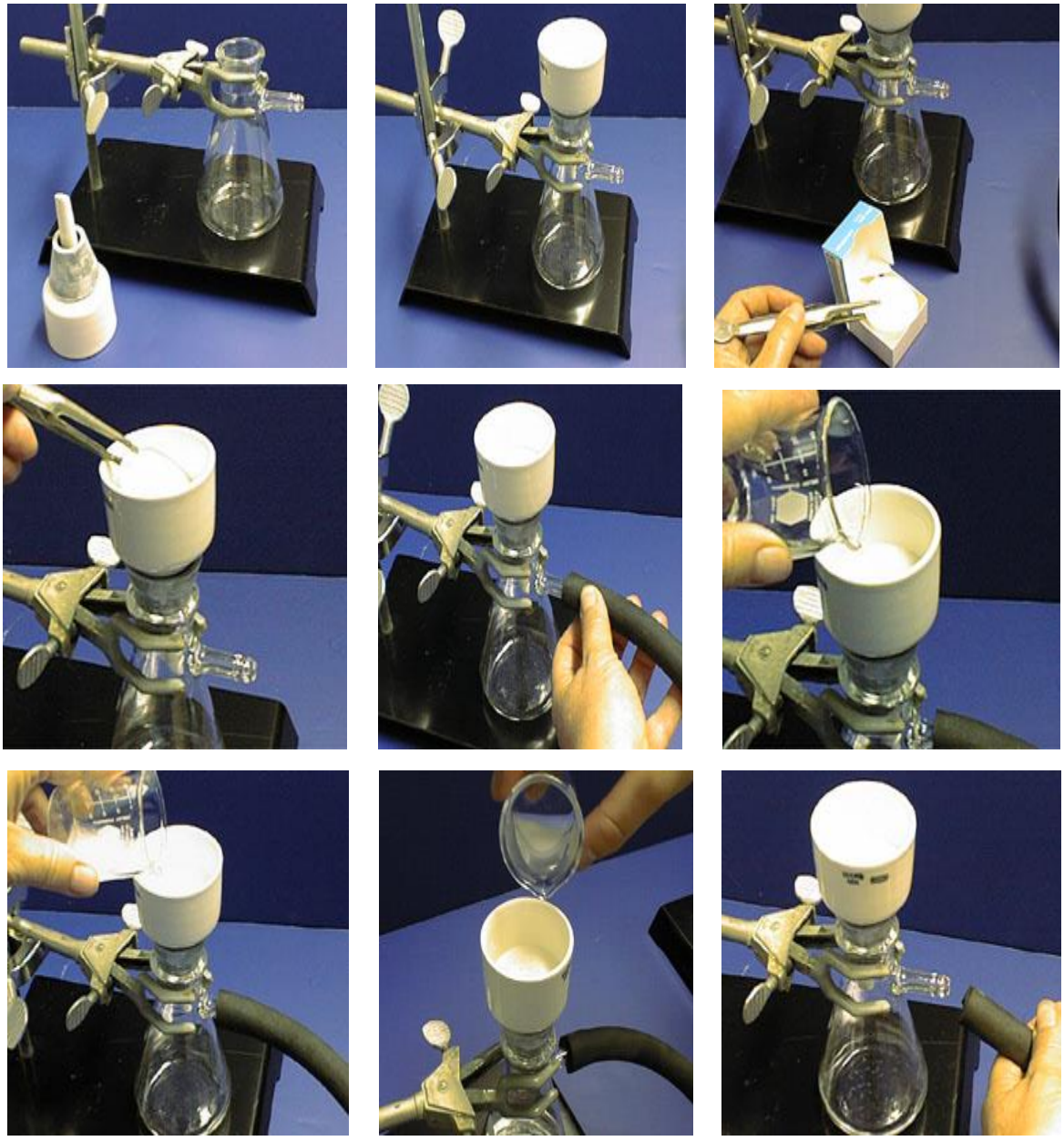




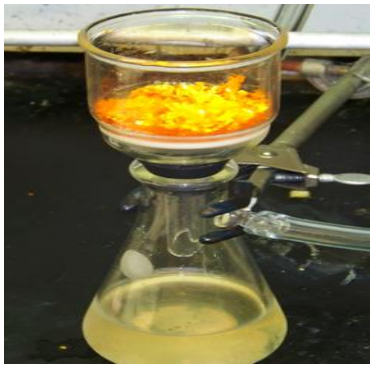


Vacuum Filtration

Vacuum filtration is used primarily to collect a desired solid, for instance, the collection of crystals in a recrystallization procedure. Vacuum filtration uses a Buchner funnel and a side-arm flask.







It is often necessary to remove solvent from a solution to recover either a solid or a high-boiling liquid. There are several ways to do this.

Sublimation

Sublimation is the transition of a substance directly from the solid to the gas phase without passing through an intermediate liquid phase. Sublimation is an endothermic phase transition that occurs at temperatures and pressures below a substance's triple point in its phase diagram. The reverse process of sublimation is de-sublimation, or deposition.

Sublimation (phase transition)

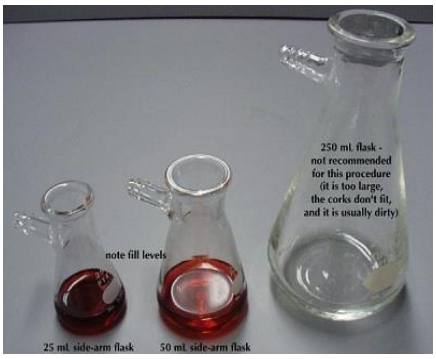
Dark green crystals of nickelocene, freshly sublimed on a cold finger

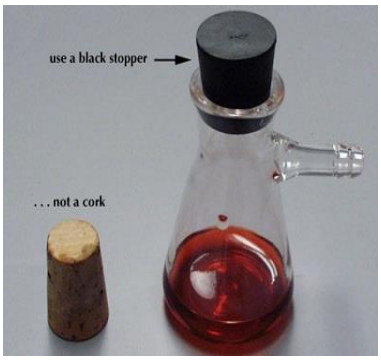
Distillation

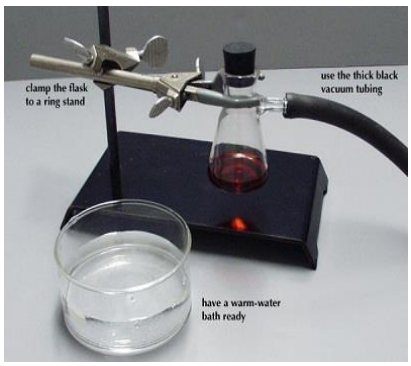
Simple distillation can be used to remove solvent. Distillation works well if the solution is composed of a solid and a low-boiling solvent, or if the solution is composed of a high-boiling liquid and a low-boiling solvent (with boiling point differences greater than 100°). Advantages of distillation are that the solvent can be collected and recycled and that no vapors are released into the atmosphere.

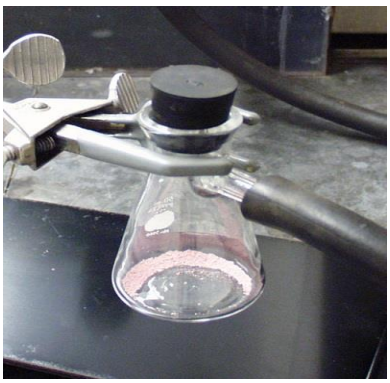
Reduced-Pressure Evaporation

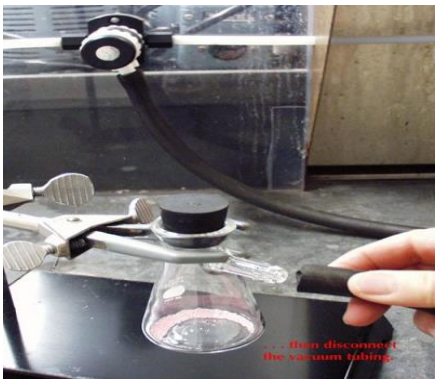
You can accomplish evaporation from a solution quickly by placing it in a side-arm flask, sealing the flask, and then applying vacuum. Under vacuum (reduced pressure) liquids vaporize and boil off at lower temperatures; effectively, the solvents come off a lot faster when under vacuum than at atmospheric pressure.





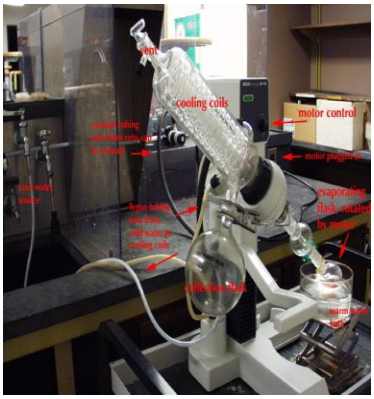


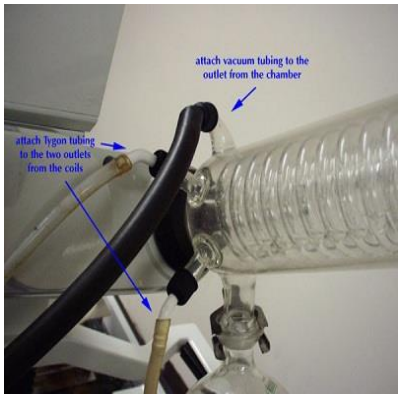


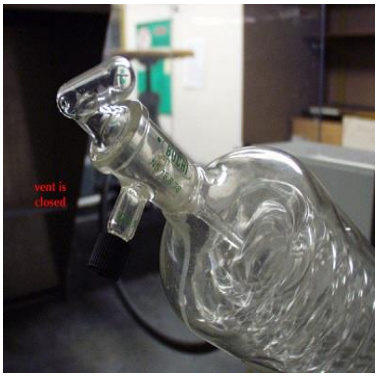


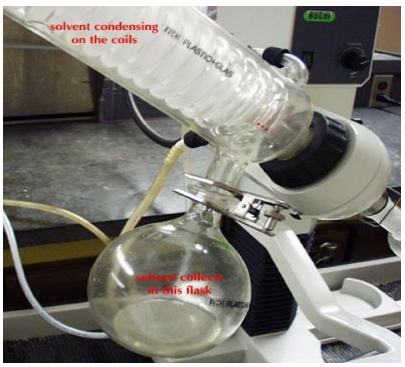
Rotary Evaporators

Rotary evaporators, or rotovaps, are standard equipment in most organic chemistry research labs. These evaporators are designed to remove solvent rapidly from solutions.









Distillation for Boiling Point Determination

The boiling point of a compound is one of the physical properties used to identify it. Distillation is used to **purify a compound** by separating it from a non-volatile or less-volatile material. When different compounds in a mixture have different boiling points, they separate into individual components when the mixture is carefully distilled.

The boiling point is the temperature at which the vapor pressure of the liquid phase of a compound equals the external pressure acting on the surface of the liquid. The external pressure is usually the atmospheric pressure. For instance, consider a liquid heated in an open flask. Different compounds boil at different temperatures because each has a different, characteristic vapor pressure: compounds with higher vapor pressures will boil at lower temperatures. Boiling points are usually measured by recording the boiling point (or boiling range) on a thermometer while performing

a distillation. This method is used whenever there is enough of the compound to perform a distillation.

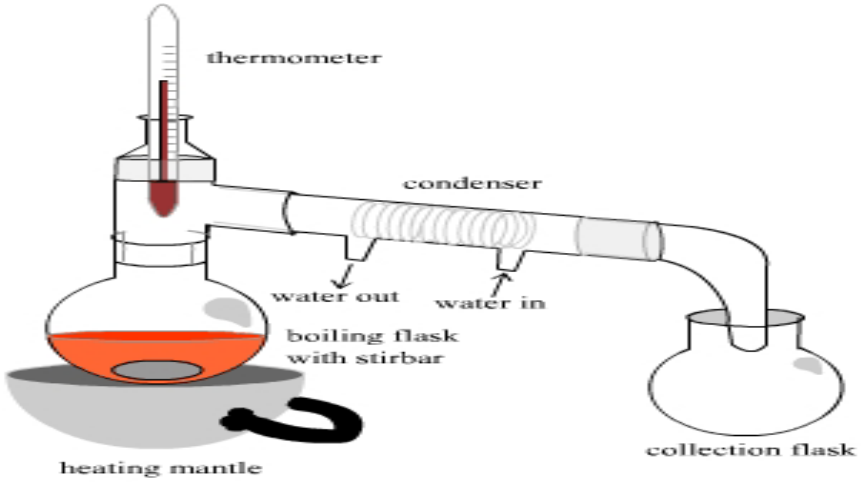
Simple Distillation

Simple distillations are used frequently in the organic chemistry teaching labs. They are useful in the following circumstances:

- The liquid is relatively pure to begin with (e.g., no more than 10% liquid contaminants)
- The liquid has a non-volatile component, for example, a solid contaminant
- The liquid is contaminated by a liquid with a boiling point that differs by at least 70°C

What is simple distillation?

The setup for a simple distillation is shown in Figure 1. A simple distillation apparatus consists of a boiling flask (round-bottom flask) attached to an adapter holding a thermometer (to determine the boiling temperature of the liquid). The adapter connects to a condenser into which cold water is constantly passed through. The condenser leads into a collection flask for the purified liquid.



- **Figure 1**. *Simple distillation.*

Fractional Distillations

Mixtures of liquids whose boiling points are similar (separated by less than 70°C) cannot be separated by a single simple distillation. In these situations, a fractional distillation is used. You can see photos of a fractional distillation set-up here.

What is fractional distillation?

Fractional distillation is essentially the same as simple distillation except that a fractionating column is placed between the boiling flask and the condenser. The fractionating column is usually filled with glass or plastic beads. These beads improve the separation between the liquids being distilled. The reason that fractional distillation gives better separation between the liquids is because the glass beads in the fractionating column provide "theoretical plates" on which the refluxing liquid can condense, re-evaporate, and condense again, essentially distilling the compound over and over.

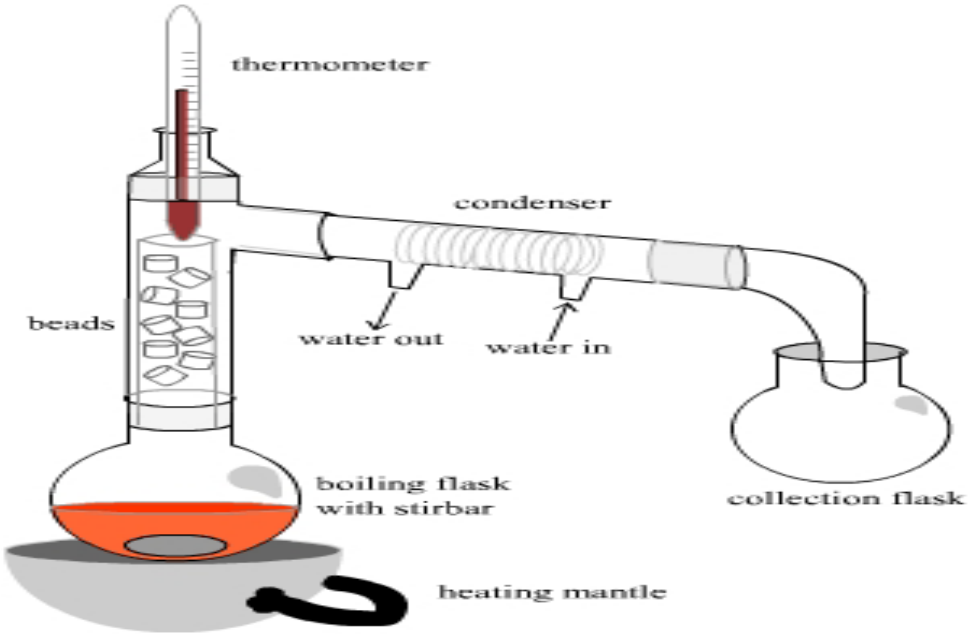


Figure 2. *Fractional distillation.*

Vacuum Distillations

Vacuum distillation is distillation at a reduced pressure. Since the boiling point of a compound is lower at a lower external pressure, the compound will not have to be heated to as high a temperature in order for it to boil. Vacuum distillation is used to distill compounds that have a high boiling point or any compound which might undergo decomposition on heating at atmospheric pressure. The vacuum is provided either by a water aspirator or by a mechanical pump. You can see photos of a fractional distillation set-up here. Always check for star cracks in the flasks before beginning a vacuum distillation.

Distillation Guide

What's distillation used for?

Distillation is a laboratory technique used for separating and purifying liquids.
How does distillation work?

To separate two or more liquids by distillation, you first heat them in a flask. The more volatile liquid (the liquid with the lower boiling point) will typically evaporate first and the vapor will pass into a condensing column, where it can revert into a liquid (condense) on the cool glass where it trickles into a collection flask. Heating further will cause the less volatile liquids to evaporate and distill at higher temperatures. The two main kinds of distillation are *simple distillation* and *fractional distillation*, and both are used widely.

So, simple or fractional?

The choice of whether to use fractional distillation or simple distillation depends on the two liquids being separated. Typically, using simple distillation is preferable because the apparatus is, well, simpler, and a simple distillation typically goes faster than a fractional distillation (and requires less energy). On the other hand,

fractional distillation gives better separation between the liquids. The choice of whether to use simple or fractional distillation, then, depends usually on the difference in boiling temperatures between the two liquids. If there is a large difference in the boiling points (>70°C) between the two liquids then simple distillation is probably the best option. On the other hand, if there is only a small temperature difference between the two liquids a fractional distillation is the preferable option.

	Simple distillation	Fractional distillation
Advantages	• simpler setup than fractional • faster distillation times • consumes less energy than fractional distillation	• much better separation between liquids than simple distillation • can more readily purify complex mixtures than simple distillation
Disadvantages	• requires the liquids to have large boiling point differences (>70°C) • gives poorer separation than fractional distillation • only works well with relatively pure liquids	• more complicated setup than simple distillation • takes longer for liquids to distill • consumes more energy than simple distillation
Best used for:	separating relatively pure liquids with large boiling differences or liquids with solid impurities	Separating complex mixtures of liquids with smaller boiling point separations.

Melting Point Determination

An organic compound's melting point is one of several physical properties by which it is identified. A physical property is a property that is intrinsic to a compound when it is pure. Since melting points are relatively easy and inexpensive to determine, they are handy identification tools to the organic chemist. If you want to use the melting point to identify a solid compound which you have isolated in the lab, you will need to compare its melting point with that of the true compound. If the

compounds are sold slightly impure, the melting point range will reflect this fact. The melting point listed in the CRC, Merck Index, or on the MSDS is the melting point of the pure compound. While theoretically these should be constant from source to source, in reality the reported melting points sometimes vary. Therefore, always reference the source of the physical data which you write in your lab report.

Descriptions and Use of Melting Point Apparatus

Three types of melting point apparatus are available in the Organic Chemistry teaching labs, the Fisher-Johns, the Mel-Temp, and the DigiMelt. Explanations (including pictures) of how to take a melting point on each type of apparatus are linked on this website.

Melting Point Apparatus

The picture below shows a melting point apparatus as an example. This type of melting point apparatus uses small round, glass coverslips.

Technique for Taking a Melting Point

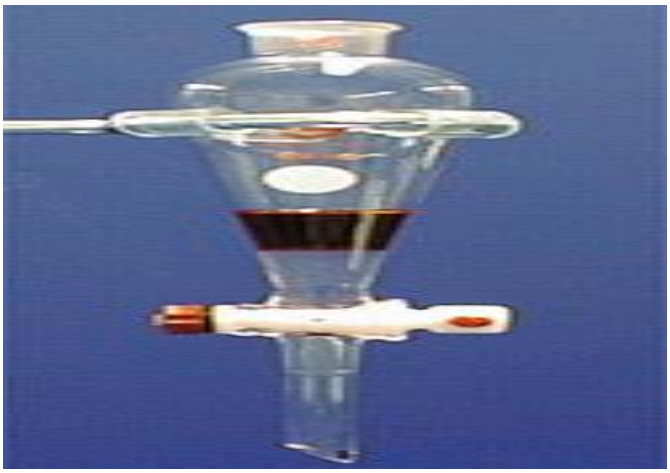




The rate of temperature increase in the vicinity of the melting point must be small, about 2 degrees C per min. This insures that the temperature of the hot plate, thermometer, and sample will be in thermal equilibrium. Increase the temperature rapidly at first and then slowly as the melting point is approached in the following manner:

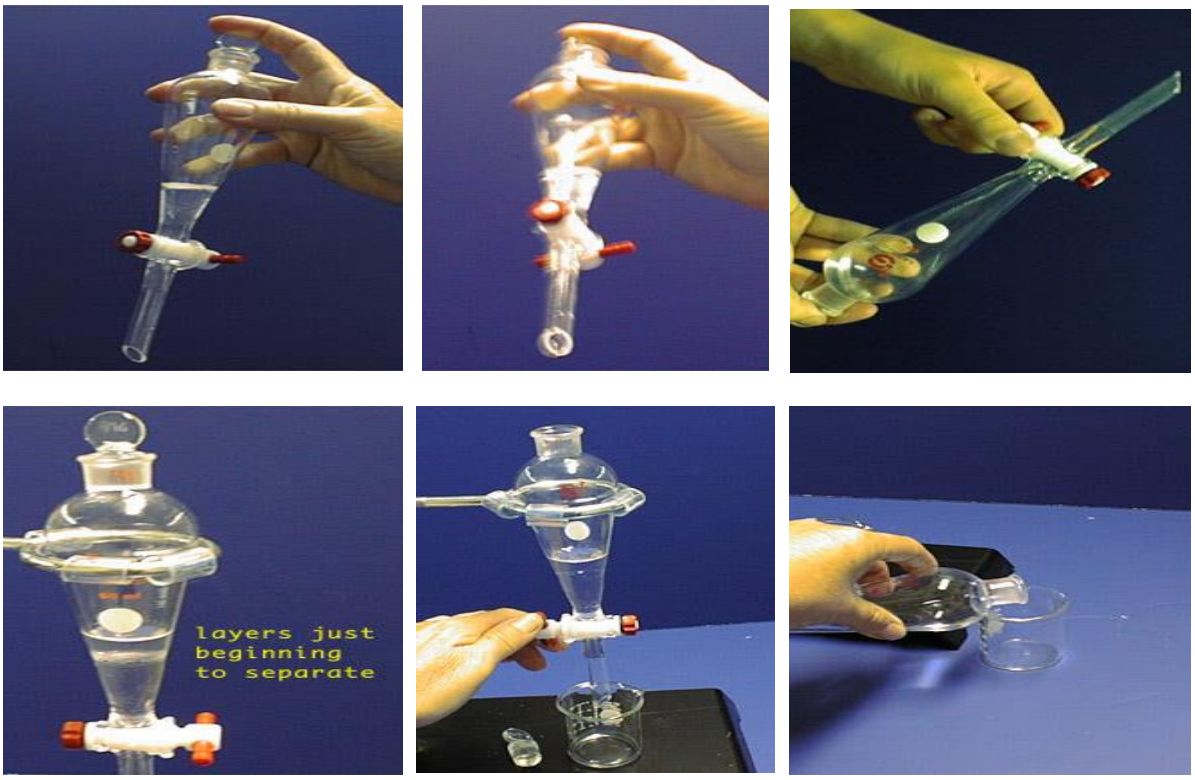
Extraction

Liquid-liquid extractions using a separatory funnel are essentially the only kind of extraction performed in the organic teaching labs. Liquid-liquid means that two liquids are used in the extraction procedure. The liquids must be immiscible: this means that they will form two layers when added together, like oil and water. Some compounds are more soluble in the organic layer (the "oil") and some compounds are more soluble in the aqueous layer (the "water").



In a particular experiment in simple extraction or in chemically active extraction, you will be able to figure out which layer, aqueous or organic, will contain the compound you want to isolate. (Please read the theory section in the *Handbook for Organic Chemistry Lab*.) You will also need to know which layer will be on top in the separatory funnel. This is determined by the *density* of the two solvents. Densities are listed in various sources of scientific data, as referenced on the Chemical Information page on this site.

How to do an Extraction



Chromatography

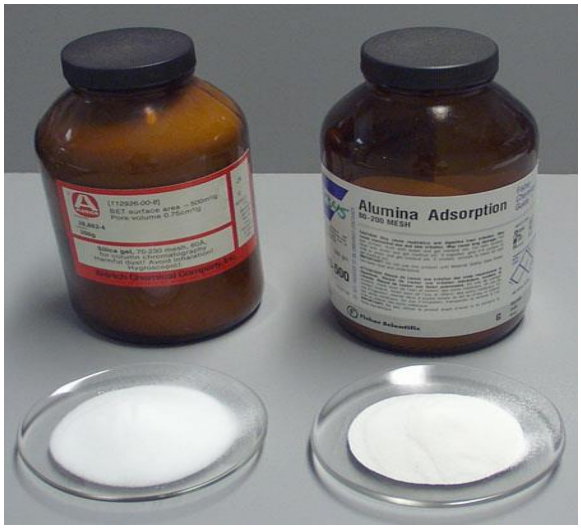
Column Chromatography

In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical glass (usually) column. The mobile phase, a liquid, is added to the top and flows down through the column by either gravity or external pressure. Column chromatography is generally used as a purification technique: it isolates desired compounds from a mixture. The mixture to be analyzed by column chromatrography is placed inside the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure.

Column chromatography is separated into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called **gravity column chromatography**. If

the solvent is forced down the column by positive air pressure, it is called **flash chromatography**, a "state of the art" method currently used in organic chemistry research laboratories.

Silica gel (SiO_2) and alumina (Al_2O_3) are two adsorbents commonly used by the organic chemist for column chromatography. An example of each of these adsorbents is shown below.



Alumina is used more frequently in column chromatography than it is in TLC. Alumina is quite sensitive to the amount of water which is bound to it: the higher its water content, the less polar sites it has to bind organic compounds, and thus the less "sticky" it is. This stickiness or activity is designated as I, II, or III, with I being the most active. Alumina is usually purchased as activity I and deactivated with water before use according to specific procedures. Alumina comes in three forms: acidic, neutral, and basic. The neutral form of activity II or III, 150 mesh, is most commonly employed.

The Solvent

The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column. Polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvate the polar constituents. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds will elute from the column. Proper choice of an eluting solvent is thus crucial to the successful application of column chromatography as a separation technique. Thin-Layer Chromatography (TLC) is generally used to determine the system for a column chromatography separation.

Often a series of increasingly polar solvent systems are used to elute a column. A less-polar solvent is first used to elute a less-polar compound. Once the less-polar compound is off the column, a more-polar solvent is added to the column to elute the more-polar compound.

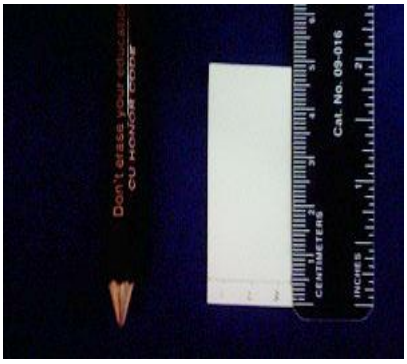
Analysis of Column Eluents

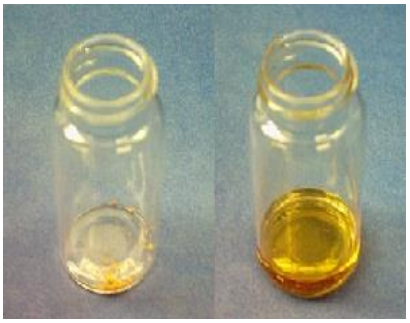
If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually. More commonly, the compounds to be isolated from column chromatography are colorless. In this case, small fractions of the eluent are collected sequentially in labeled tubes and the composition of each fraction is analyzed by TLC. (Other methods of analysis are available; this is the most common method and the one used in the organic chemistry teaching labs.)

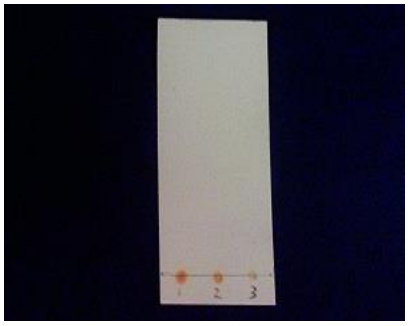
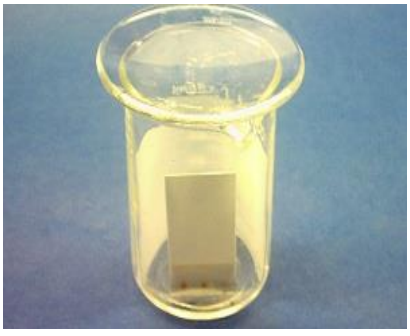
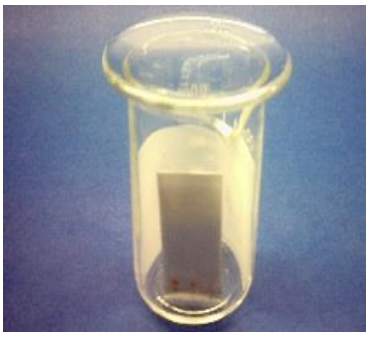
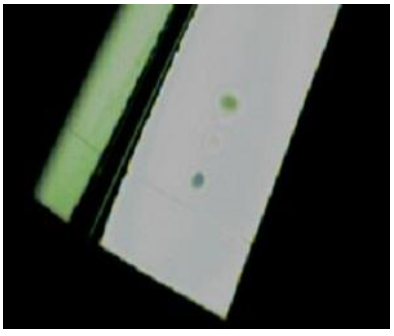
Thin Layer Chromatography (TLC)

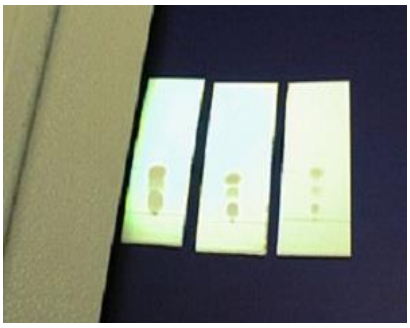
TLC is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate). A TLC plate is a sheet of glass, metal, or plastic, which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate.

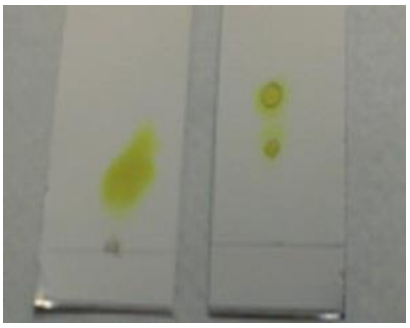
How To Run a TLC Plate







Step 1: Prepare the developing container

The developing container for TLC can be a specially designed chamber, a jar with a lid, or a beaker with a watch glass on the top (the latter is used in the undergrad labs at CU). Pour solvent into the chamber to a depth of just less than 0.5 cm. To aid in the saturation of the TLC chamber with solvent vapors, you can line part of the inside of the beaker with filter paper. Cover the beaker with a watch glass, swirl it gently, and allow it to stand while you prepare your TLC plate.

Step 2: Prepare the TLC plate

TLC plates used in the organic chem teaching labs are purchased as 5 cm x 20 cm sheets. Each large sheet is cut horizontally into plates which are 5 cm tall by various widths; the more samples you plan to run on a plate, the wider it needs to be. Shown in the photo to the left is a box of TLC plates, a large un-cut TLC sheet, and a small TLC plate which has been cut to a convenient size. Handle the plates carefully so that you do not disturb the coating of adsorbent or get them dirty. Measure 0.5 cm from the bottom of the plate. Using a pencil, draw a line across the plate at the 0.5 cm mark. This is the origin: the line on which you will spot the plate. Take care not to press so hard with the pencil that you disturb the adsorbent. Under the line, mark lightly the name of the samples you will spot on the plate, or mark numbers for time points. Leave enough space between the samples so that they do not run together; about 4 samples on a 5 cm wide plate is advised.

Step 3: Spot the TLC plate

If the sample is not already in solution, dissolve about 1 mg in 1 mL of a volatile solvent such as hexanes, ethyl acetate, or methylene chloride. As a rule of thumb, a concentration of 1% usually works well for TLC analysis. If the sample is too concentrated, it will run as a smear or streak (see troubleshooting section below); if it is not concentrated enough, you will see nothing on the plate. Sometimes you will need to use trial and error to get well-sized, easy to read spots. Obtain a microcapillary. In the organic teaching labs, we use 10µL microcaps - they are easier to handle than the smaller ones used in research labs. Dip the microcap into the solution and then gently touch the end of it onto the proper location on the TLC plate.

Step 4: Develop the plate

Place the prepared TLC plate in the developing beaker, cover the beaker with the watch glass, and leave it undisturbed on your bench top. The solvent will rise up the TLC plate by capillary action. Make sure the solvent does not cover the spot. Allow the plate to develop until the solvent is about half a centimeter below the top of the plate. Remove the plate from the beaker and immediately mark the solvent front with a pencil. Allow the plate to dry.

Step 5: Visualize the spots

If there are any colored spots, circle them lightly with a pencil. Most samples are not colored and need to be visualized with a UV lamp. Hold a UV lamp over the plate and circle any spots you see. Beware! UV light is damaging both to your eyes and to your skin! Make sure you are wearing your goggles and do not look directly into the lamp. Protect your skin by wearing gloves. If the TLC plate runs samples which are too concentrated, the spots will be streaked and/or run together. If this happens, you will have to start over with a more dilute sample to spot and run on a TLC plate. Here's what overloaded plates look like compared to well-spotted plates.

TLC Solvents Choice

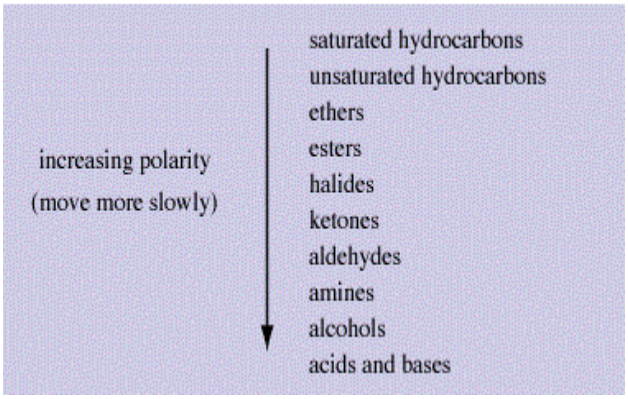
When you need to determine the best solvent or mixture of solvents (a "solvent system") to develop a TLC plate or chromatography column loaded with an unknown mixture, vary the polarity of the solvent in several trial runs: a process of trial and error. Carefully observe and record the results of the chromatography in each solvent system. You will find that as you increase the polarity of the solvent system, all the components of the mixture move faster (and vice versa with lowering the polarity). The ideal solvent system is simply the system that gives the best separation. TLC elution patterns usually carry over to column chromatography elution patterns.

Since TLC is a much faster procedure than column chromatography, TLC is often used to determine the best solvent system for column chromatography. For instance, in determining the solvent system for a flash chromatography procedure, the ideal system is the one that moves the desired component of the mixture to a TLC R_f of 0.25-0.35 and will separate this component from its nearest neighbor by difference in TLC R_f values of at least 0.20. Therefore, a mixture is analyzed by TLC to determine the ideal solvent(s) for a flash chromatography procedure.

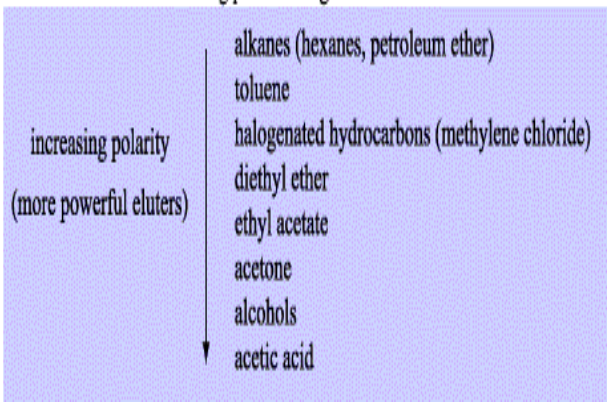
Beginners often do not know where to start: What solvents should they pull off the shelf to use to elute a TLC plate? Because of toxicity, cost, and flammability concerns, the common solvents are hexanes (or petroleum ethers/ligroin) and ethyl acetate (an ester). Diethyl ether can be used, but it is very flammable and volatile. Alcohols (methanol, ethanol) can be used. Acetic acid (a carboxylic acid) can be used, usually as a small percentage component of the system, since it is corrosive, non-volatile, very polar, and has irritating vapors. Acetone (a ketone) can be used. Methylene chloride or and chloroform (halogenated hydrocarbons) are good solvents, but are toxic and should be avoided whenever possible. If two solvents are

equal in performance and toxicity, the more volatile solvent is preferred in chromatography because it will be easier to remove from the desired compound after isolation from a column chromatography procedure. Ask the lab instructor what solvents are available and advisable. Then, mix a non-polar solvent (hexanes, a mixture of 6-carbon alkanes) with a polar solvent (ethyl acetate or acetone) in varying percent combinations to make solvent systems of greater and lesser polarity. The charts below should help you in your solvent selection. You can also download this pdf chart of elution order.

The expected elution order of organic classes.



Eluting power of organic solvents.



Interactions between the compound and the adsorbent

The strength with which an organic compound binds to an adsorbent depends on the strength of the following types of interactions: ion-dipole, dipole-dipole, hydrogen bonding, dipole induced dipole, and van der Waals forces. With silica gel, the dominant interactive forces between the adsorbent and the materials to be separated are of the dipole-dipole type. Highly polar molecules interact fairly strongly with the polar SiOH groups at the surface of these adsorbents and will tend to stick or adsorb onto the fine particles of the adsorbent while weakly polar molecules are held less tightly. Weakly polar molecules generally tend to move through the adsorbent more

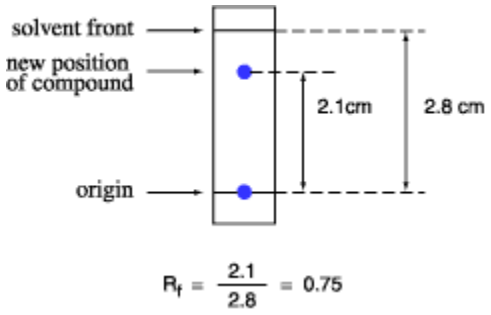
rapidly than the polar species. Roughly, the compounds follow the elution order given above.

The R_f value

The retention factor, or R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

For example, if a compound travels 2.1 cm and the solvent front travels 2.8 cm, the R_f is 0.75:



The R_f for a compound is a constant from one experiment to the next only if the chromatography conditions below are also constant:

- solvent system
- adsorbent
- thickness of the adsorbent
- amount of material spotted
- temperature

Since these factors are difficult to keep constant from experiment to experiment, relative R_f values are generally considered. "Relative R_f" means that the values are

reported relative to a standard, or it means that you compare the R_f values of compounds run on the same plate at the same time.

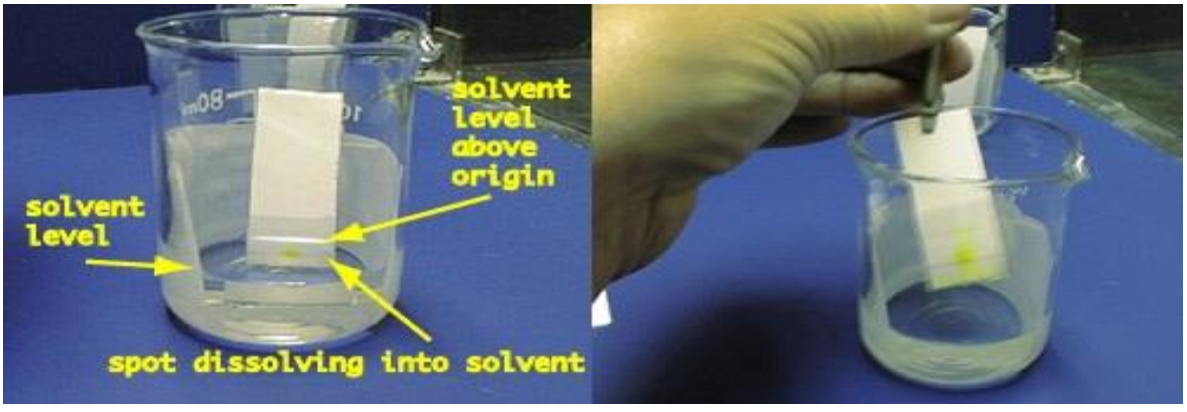
The larger an R_f of a compound, the larger the distance it travels on the TLC plate. When comparing two different compounds run under identical chromatography conditions, the compound with the larger R_f is less polar because it interacts less strongly with the polar adsorbent on the TLC plate. Conversely, if you know the structures of the compounds in a mixture, you can predict that a compound of low polarity will have a larger R_f value than a polar compound run on the same plate.

Troubleshooting TLC

All of the above (including the procedure page) might sound like TLC is quite an easy procedure. As with any technique, with practice you get better. Examples of common problems encountered in TLC:

- **The compound runs as a streak rather than a spot:** The sample was overloaded. Run the TLC again after diluting your sample. Or, your sample might just contain many components, creating many spots which run together and appear as a streak. Perhaps, the experiment did not go as well as expected.
- **The sample runs as a smear or a upward crescent:** Compounds which possess strongly acidic or basic groups (amines or carboxylic acids) sometimes show up on a TLC plate with this behavior. Add a few drops of ammonium hydroxide (amines) or acetic acid (carboxylic acids) to the eluting solvent to obtain clearer plates.
- **The sample runs as a downward crescent:** Likely, the adsorbent was disturbed during the spotting, causing the crescent shape.

- **The plate solvent front runs crookedly:** Either the adsorbent has flaked off the sides of the plate or the sides of the plate are touching the sides of the container (or the paper used to saturate the container) as the plate develops. Crooked plates make it harder to measure R_f values accurately.
- **Many random spots are seen on the plate:** Make sure that you do not accidentally drop any organic compound on the plate. If get a TLC plate and leave it laying on your workbench as you do the experiment, you might drop or splash an organic compound on the plate.
- **You see a blur of blue spots on the plate as it develops:** Perhaps you used an ink pen instead of a pencil to mark the origin.
- **No spots are seen on the plate:** You might not have spotted enough compound, perhaps because the solution of the compound is too dilute. Some compounds do not show up under UV light; try another method of visualizing the plate (such as staining or exposing to iodine vapor), or perhaps you do not have any compound because your experiment did not go as well as planned. These photos show how the yellow compound is running into the solvent when lifted from the developing jar.



Strategy for identifying an unknown

The following steps should be taken to identify an unknown compound:

1. Perform a solubility classification test to determine the possible functional group classes to which the unknown may belong.
2. Narrow the choices of possible functional groups to one group by performing appropriate functional group tests.
3. Make one or more derivatives to finally determine the exact identity of the unknown.

The above approach should lead to a successful identification of an unknown about 80 % of the time. In other cases, the unknown may pose difficulties that would require imagination and careful analysis of the data to be successful in its identification.

FUNCTIONAL GROUP ANALYSIS:

Below are listed 24 chemical tests that could be used to help identify an unknown. The tests are listed in numerical/alphabetical order.

1. Introduction to qualitative tests
2. 2,4-Dinitrophenylhydrazine (for aldehydes and ketones)
3. Acetyl chloride (for acidic hydrogen compounds such as alcohols)
4. Basic hydrolysis (for amides, esters and nitriles)
5. Beilstein test (for halogenated compounds)
6. Benedict test (for aldehydes and reducing sugars)
7. Bromine in carbon tetrachloride (for alkenes and alkynes)
8. Ceric nitrate (for alcohols and phenols)
9. Chromic acid (for aldehydes, primary and secondary alcohols)
10. Combustion test (for flammable or combustible compounds)

11. Ferric chloride (for phenols)
12. Ferric hydroxamate (for esters, acid chlorides and acid anhydrides)
13. Ferrous hydroxide (for nitro compounds)
14. Hinsberg test (to distinguish primary, secondary and tertiary amines)
15. Hydroxylamine hydrochloride (for aldehydes and ketones)
16. Iodoform test (for methyl carbonyl compounds)
17. Lucas test (to distinguish primary, secondary and tertiary alcohols of six carbons or less)
18. Nitrous acid (to distinguish primary, secondary and tertiary amines)
19. pH in ethanol/water (to distinguish low molecular weight acidic or basic compounds)
20. Potassium permanganate (for compounds that can be oxidized)
21. Silver nitrate in ethanol (for Sn1 reactions of alkyl halides)
22. Sodium fusion (for compounds containing halogen, nitrogen or sulfur)
23. Sodium iodide in acetone (for Sn2 reactions of alkyl chlorides or bromides)
24. Solubility classification (for general classification or organic compounds)
25. Tollens test (for aldehydes and reducing sugars)

Procedure for Determining Solubility of Organic Compounds

1) Water Solubility

Place approximately 0.1 g or 0.2 mL (2-3 drops) of compound in a small test tube and add about 1 mL of water in small portions. Shake test tube vigorously after the addition of each portion of solvent. Check the pH of the water to determine if your unknown is partially or completely soluble in water and whether your compound has changed the pH of the water.

- Litmus turns red: water soluble acidic compound

- Litmus turns blue: water soluble basic compound
- Litmus neutral: water soluble general compound or insoluble compound

2) 5% NaOH Solubility

Place approximately 0.1 g or 0.2 mL (2-3 drops) of compound in a small test tube and add about 1 mL of NaOH solution in small portions. Shake test tube vigorously after the addition of each portion of solvent. If soluble, then your unknown is behaving as an organic acid. The most common organic acids are carboxylic acids and phenols. Carboxylic acids are usually considered stronger acids than phenols, but both of these acids will react with NaOH (a strong base).

3) 5% $NaHCO_3$ Solubility

Place approximately 0.1 g or 0.2 mL (2-3 drops) of compound in a small test tube, and add about 1 mL of $NaHCO_3$ solution in small portions. Shake test tube vigorously after the addition of each portion of solvent. If soluble, then it is a strong organic acid. If not, then it is a weak organic acid, if it dissolves in NaOH. The most common weak organic acid are phenols. Typically, only a carboxylic acid will react with $NaHCO_3$

4) 5% HCl Solubility

Place approximately 0.1 g or 0.2 mL (2-3 drops) of compound in a small test tube, and add about 1 mL of HCl solution in small portions. Shake test tube vigorously after the addition of each portion of solvent. If HCl soluble, then it is an organic base. Amines are the most common organic base.

If insoluble in all solutions, then your unknown is not an acidic or basic organic compound.

Identifications of Alcohols

1- Methyl alcohol CH_3OH

Physical properties:

Colorless liquid, M.F is CH_4O, boiling at 65 °C, miscible with water and toxic material as it causes blindness before death.

Reactions of Methanol

1- Ester formation

In dry test tube put 1 ml of methyl alcohol then add o.5 ml of conc. H_2SO_4 and o.5 gm. of salicylic acid or its derivatives. Heat the mixture for 3mintsin water bath, then cool and pour the contents in a beaker containing about 30 ml of sodium carbonate solution. Note the characteristic odor of methyl salicylate.

2- Oxidation reaction

In dry test tube, place 0.5 ml of $K_2Cr_2O_7$ and 0.5 ml of conc. H_2SO_4, then cool. Add 0.5 ml of methanol and boil gently (on water bath) notice the pungent odor of formaldehyde and change of color to green.

$$C H_3 O H \xrightarrow[H_2SO_4]{K_2Cr_2O_7} H C H O + H_2O + CrSO_4 + K_2SO_4$$

2- Ethyl alcohol CH_3CH_2OH

Physical properties:

Colorless liquid, miscible with water, M. F is C_2H_6O, b. p 78 °C.

Reaction s of ethanol

1- Oxidation reaction

Place 1 ml of $K_2Cr_2O_7$ and 0.5 ml of conc. H_2SO_4, then cool. Add 0.5 ml of ethanol and boil gently (on water bath) notes the odor of acetaldehyde and change the color solution to green.

$$CH_3CH_2OH \xrightarrow{(O)} CH_3CHO$$

2- Ester formation

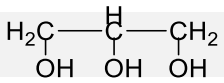
Place 1 ml of ethanol in dry test tube, then add 0.5 ml of conc. H_2SO_4 and 1 ml of acetic acid. Heat the mixture gently for 3 mints in water bath, cool and pour the tube into baker containing sodium carbonate solution. Not the characteristic odor of ester.

$$CH_3CH_2OH + CH_3COOH \xrightarrow{H_2SO_4} CH_3CH_2COOCH_3 + H_2O$$

3- Iodoform test

Add 4 ml of iodine solution to 1 ml of ethyl alcohol then add NaOH solution drop wise until the color of solution becomes straw yellow. Heat the solution in water bath for 5 mints then leaves it to cool gradually, a yellow ppt. of iodoform is appearing.

$$CH_3CH_2OH + 3\,I_2 \xrightarrow{NaOH} CHI_3 + HCOONa + 3HI$$

3- Glycerol 

Physical properties:

Colorless liquid, viscous liquid, odorless, has sweet taste, miscible with water, alcohol in all proportions.

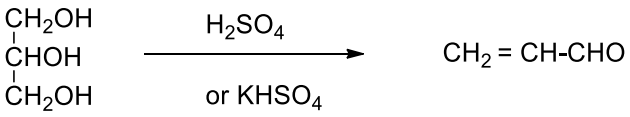
Reactions of glycerol

1- Oxidation reaction

Glycerol oxidized to give several products but it is ultimately transformed into CO_2 and H_2O. Add 2 ml of $K_2Cr_2O_7$ and 2 ml of conc. H_2SO_4, then cool .Add 0.5 ml of glycerol and boil gently (on water bath) notice the effervescence due to the evolution of CO_2.

2- Acroline test

Heat of 0.5 gm of glycrine with 1gm of hydrogen potassium sulphate $KHSO_4$ or 2 ml of conc. sulphuric acid in dry test tube and notice odor of acroline.



3- Borax test

Add one drop of ph.ph to 1 ml of dil. Borax solution red color appears. Add 1 ml of glycerol and note that the color disappears, heat gently and observe the appearance of the red color once more, which disappear on cooling the solution.

Data and Results

Name:

Sec.No.

Date:

Physical properties

Solubility: ……

Color: ……

Shape: ……

Chemical properties

Exp.	Obs.	Res.

Unknown is:

Identifications and reactions of Aldehydes and ketones.

Purpose:

To identify aldehydes and ketones by using properties and reactions.

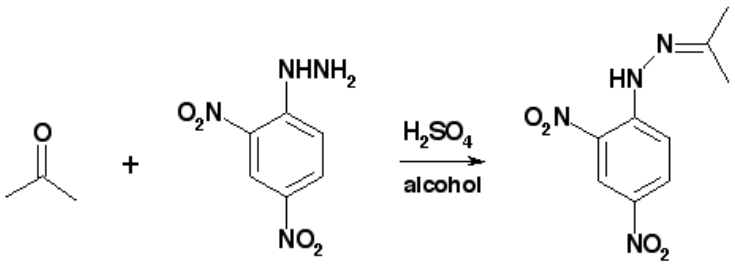
Discussion

Aldehydes and ketones share the carbonyl functional group which features carbon doubly bonded to oxygen. In the case of ketones there are two carbon atoms bonded to the carbonyl carbon and no hydrogen. In the case of aldehydes there is at least one hydrogen atom bonded to the carbonyl carbon, the other attachment may be a carbon or hydrogen. In all cases the carbon(s) that are attached to the carbonyl group may be aliphatic (not part of an aromatic ring) or aromatic (part of an aromatic ring). Since they share the carbonyl group, aldehydes and ketones share much of their chemistry, but they are different enough to be considered different classes of compounds.

General tests for Aldehydes and Ketones

2, 4-Dinitrophenyl hydrazine test

2,4-Dinitrophenylhydrazine can be used to qualitatively detect the carbonyl group functionality of an aldehyde or ketone functional group. A positive test is signaled by a yellow/red precipitate, known as a dinitrophenylhydrazone.



This reaction can be described as a condensation reaction, with two molecules joining together with loss of water. It is also called addition-elimination reaction: nucleophilic addition of $–NH_2$ group to C=O carbonyl group, followed by removal of H_2O molecule.

Procedure

Add a solution of 1 or 2 drops or 30 mg of unknown in 2 mL of 95% ethanol to 3 mL of 2,4-dinitrophenylhydrazine reagent. Shake vigorously, and, if no precipitate forms immediately, allow the solution to stand for 15 minutes. The 2,4-dinitrophenylhydrazine reagent will already be prepared for you.

Positive test

Formation of a precipitate is a positive test.

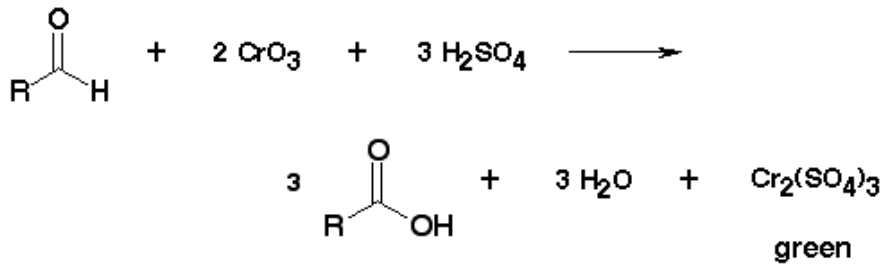
Complications

- Some ketones give oils, which will not solidify.
- Some allylic alcohols are oxidized by the reagent to aldehydes and give a positive test.
- Some alcohols, if not purified, may contain aldehyde or ketone impurities.

<u>To differentiate between aldehydes and ketones</u>

1-Chromic Acid Test

Regardless of which mechanism actually operates, these reactions are usually referred to as nucleophilic additions. Aldehydes are oxidized by chromic acid, ketones are not. When an aldehyde is oxidized by orange brown chromic acid the chromic acid is reduced to Cr^{3+}, which is green. Consequently, chromic acid can distinguish between aldehydes and ketones. It is also true those other functional groups; primary and secondary alcohols for example, can be oxidized by chromic acid, causing the formation of a green color.



Standards: Cyclohexanone

Procedure

Dissolve 10 mg or 2 drops of the unknown in 1 mL of pure acetone in a test tube and add to the solution 1 small drop of Jones reagent (chronic acid in sulfuric acid). A positive test is marked by the formation of a green color within 5 seconds upon addition of the orange-yellow reagent to a primary or secondary alcohol. Aldehydes also give a positive test, but tertiary alcohols do not.

The Jones reagent will already be prepared for you.

Positive Test

A positive test for aldehydes and primary or secondary alcohols consists in the production of an opaque suspension with a green to blue color. Tertiary alcohols give no visible reaction within 2 seconds, the solution remaining orange in color. Disregard any changes after 15 seconds.

Complications

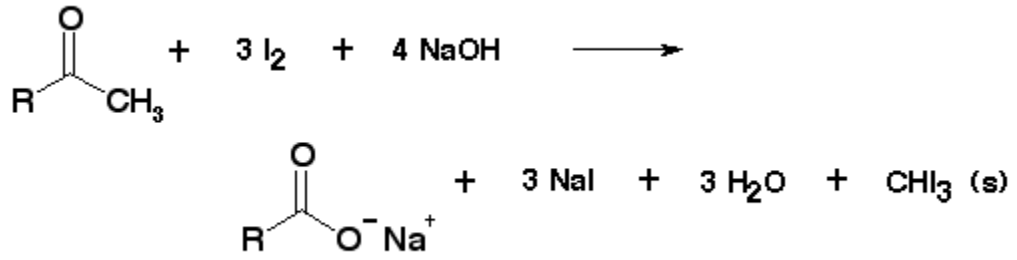
- Aldehydes are better characterized in other ways. The color usually develops in 5-15 seconds.

3- Tollens' Test

Aldehydes are also oxidized by Tollens' reagent, a substance that contains Ag^{+1}. The silver ion is concomitantly, reduced to metallic silver. Silver ion is a weak

oxidizing agent; aldehydes are very easily oxidized and are essentially unique in being able to reduce silver ion to silver metal.

4-Iodoform Test for Methyl Ketones



Standard: Acetone

Procedure

If the substance to be tested is water soluble, dissolve 4 drops of a liquid or an estimated 50 mg of a solid in 2 mL of water in a large test tube. Add 2 mL of 3 M sodium hydroxide and then slowly add 3 mL of the iodine solution. Stopper the test tube and shake vigorously. A positive test will result in the brown color of the reagent disappearing and the yellow iodoform solid precipitating out of solution. If the substance to be tested is insoluble in water, dissolve it in 2 mL of 1,2-dimethoxyethane, proceed as above, and at the end dilute with 10 mL of water.

Positive Test

Formation of solid iodoform (yellow) is a positive test. (Iodoform can be recognized by its odor and yellow color and, more securely, from the melting point 119°-123°C).

Complications

Test will not be positive if the R group is a di-ortho substituted aryl group

1-Formaldehyde HCHO

Physical properties:

Colorless liquid, with characteristic pungent odor.

Reactions of formaldehyde

1- Schiff's reagent test. Add 2 ml of Schiff's reagent to 2 ml cold aldehyde solution shake vigorously and allow standing for two minutes – a deep – violet color indicates the presence of aldehydic group.

2- In dry test tube put 2 ml of formaldehyde with few crystals of resorcinol then add 2 ml conc. H_2SO_4 carefully from the side of the tube. The red ring formed and white ppt. in aqueous layer turns to violet red.

3- It is reduced by Fehling's reagent.

Add 1ml of formaldehyde solution to Fehling's solution (1ml of Fehling A +1ml of Fehling B) and heating the solution notice the blue color convert to red color

$$HCHO + 2CuO \longrightarrow HCOOH + CU_2O$$

4- Add 2 ml of formaldehyde + 1% phenyl hydrazine + few drops of sodium nitro prusside solution in excess of NaOH. Blue color will appear then turns to green then red then brown.

5- Add diluted formaldehyde solution + 1% phenyl + 5% 2 ml pot.ferricyanide +conc. HCl it gives a rose red color.

6- It gives 2, 4-dinitrophenyl hydrazine m.p 166 °C.

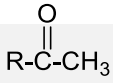
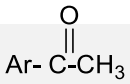
2-Acetaldehyde CH_3CHO

Physical properties:

Colorless liquid Pungent, fruity odor, b.p 20 °C; miscible with water, alcohol and ether, M. F is C_2H_4O.

Reactions of acetaldehyde

1- Give violet color with Schiff's reagent.
2- 2 ml of acetaldehyde with 2 ml aqueous sodium nitroprusside and 5 drops of NaOH gives a deep win red color.
3- Responds to iodoform test
4- Boiling of 2 ml of solution with 2 ml 20% KOH give yellow ppt.
5- It is reduces Fehling's solution and Tollen's reagents.
6- Formed white crystals by reaction with sodium bisulphate.
7- It is give 2,4- dinitrophenyl hydrazone, m.p 168 °C.

KETONES  

Physical properties:

Molecular formula (M.F.) is C_3H_6O, Colorless liquid with characteristic pleasant smell, miscible with water, alcohol, and ether.

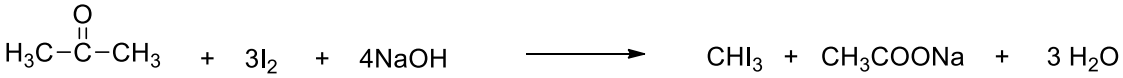
Reactions of acetone

1- **Colors test**.

- Add 1 ml of acetone to 1 ml of sodium nitroprussid with 0.5 ml of NaOH and notice appearance red color
- Add 1 ml of acetone to 1 ml of sodium nitroprussid with 0.5 ml of pyridine and notice appearance blue color

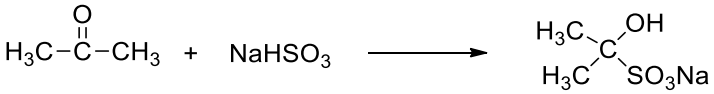
- Add 0.5 gm of m-dinitrobenzen to 1 ml of acetone with 0.5 ml of NaOH and notice appearance red color
- Add 0.5 ml of Schiff's reagent to 0.5 ml of acetone and notice do not appearance violet color

2- **Iodoform test**. On addition of 3-4 drops of iodine solution and then NaOH solution drop by drop to the substance and warming the brown color of iodine disappear and a yellow ppt. is formed.

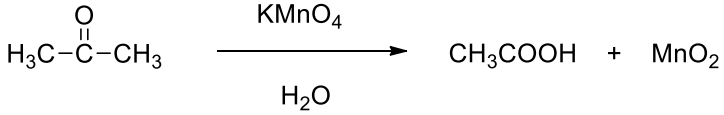


3- **Ding`s test**. Add 2 ml of acetone to 2 ml of acid solution of mercury sulphate and heating on water produce heavy white precipitate.

4- To 2 ml of standard sodium bisulphite add few drops of acetone. White crystals are formed.



5- **Oxidation test**. Add 1 ml of acetone to 2 ml of solution of $KMnO_4$ and heating lead to disappear of violet color of permanganate



6- **2,4- Dinitrophenylhydrazine test.**

Add 1 ml of acetone to 3 ml of alcoholic solution of 2,4-dinitrophenyl-hydrazine and heating the mixture in water bath and notice formation yellow precipitate

EXPERIMENT No.

Identifications and reactions of aldehydes and ketones.

Data and Results

Name:

Sec.No.

Date:

Physical properties

Solubility: ……..

Color: …….

Shape: ……

Chemical properties

Experiment	observation	Result

Unknown is:

Identifications and reactions of carboxylic acids and acid salts

Purpose:

To study properties and reactions of carboxylic acids and esters

Discussion

The functional group of a carboxylic acid is a carboxyl group. The general formula for an aliphatic carboxylic acid is RCOOH and for an aromatic carboxylic acid is ArCOOH. Carboxylic acids have significantly higher boiling points than other types of organic compounds of comparable molecular weight. They are polar compounds and form very strong intermolecular hydrogen bonds. Carboxylic acids are more soluble in water than alcohols, ethers, aldehydes, and ketones of comparable molecular weight. They form hydrogen bonds with water molecules through both their C=O and OH groups. They are dissolving in Na_2CO_3 with evaluation of CO_2; they are also dissolving in NaOH. Carboxylic acids are divided into two categories, aliphatic and aromatic carboxylic acids.

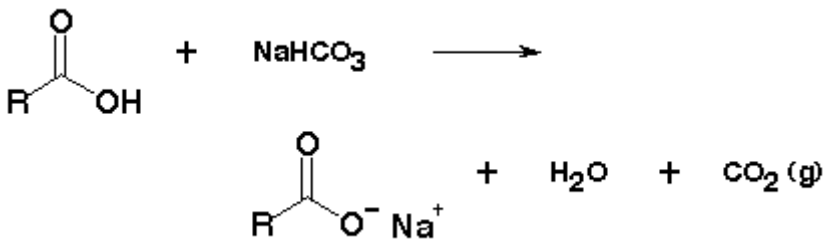
To use carboxylic acids neutral solution (must be prepared).

To the solid, add aqueous solution of ammonia (excess). Boil the solution until all ammonia odor evolved. Cool.

$$RCOOH + NH_4OH \longrightarrow RCOONH_4 + NH_3$$

Tests for Carboxylic Acids

Acidity test



Procedure

A few drops or a few crystals of the unknown sample are dissolved in 1mL of methanol and slowly added to 1 mL of a saturated solution of sodium bicarbonate.

Positive Test

Evolution of a carbon dioxide gas is a positive test for the presence of the carboxylic acid and certain phenols listed in the Complications section.

Complications

Negatively substituted phenols such as nitrophenols, aldehydrophenols, and polyhalophenols are sufficiently acidic to dissolve in 5% sodium bicarbonate.

1- Formic acid HCOOH

Physical properties:

It is colorless liquid, miscible with water, alcohol and ether, boiling point 100 °C.

Reactions of formic acid

1- It is reduces Fehling's solution and Tollen's reagent.
2- It is decolorized $KMnO_4$
3- With $FeCl_3$: n. solution of acid gives red color which converted to brown by boiling.
4- Ester formation: to 1 ml of acid add 1 ml of ethyl alcohol and 1 ml of conc. H_2SO_4 in attest tube, heat in water bath, and then pour to Na_2CO_3 solution .the characteristic odor of ethyl format is evolved.

$$HCOOH + C_2H_5OH \longrightarrow HCOOC_2H_5 + H_2O$$

2-Acetic acid CH_3COOH

Physical properties:

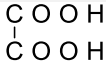
It is colorless viscous liquid, miscible with water, alcohol and ether, b.p 122 °C.

Reactions of acetic acid

1- It does not reduce Fehling's solution and Tollen's reagent.
2- With $FeCl_3$: n. solution of acid gives red color which converted to brown by boiling.
3- Ester formation: to 1 ml of acid add 1 ml of ethyl alcohol and 1 ml of conc. H_2SO_4 in attest tube, heat in water bath, and then pour to Na_2CO_3 solution .the characteristic odor of ethyl acetate was evolved.

$$CH_3COOH + C_2H_5OH \longrightarrow CH_3COOC_2H_5 + H_2O$$

3-Oxalic acid



Physical properties:

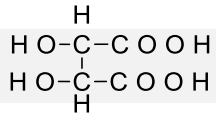
It is colorless crystalline solid m.p. 100 °C. It is readily soluble in water and alcohol.

Reactions of oxalic acid

1- Flaming test. When the acid or its salt is heated on a piece of porcelain it is decomposed with little or no charring.
2- When the acid is heated with conc. H_2SO_4 it is decomposed into CO and CO_2 with no charring.
3- n. Solution + $CaCl_2$: A white ppt. of Ca oxalate is separated immediately on cold which is soluble in mineral acids.
4- n. Solution + $AgNO_3$: gives white ppt. of Ag oxalate.

5- When heated a few drops of dil. $KMnO_4$ sol .and the acidified sol. Of oxalate the color is discharged.

4-Tartaric acid



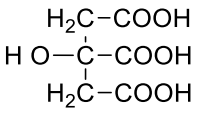
Physical properties:

It is colorless crystalline solid, m.p. 167 °C and readily soluble in cold water and alcohol.

Reactions of tartaric acid

1- It gives Acidity test +ve.
2- With conc. H_2SO_4: when the solid is heated with conc. H_2SO_4 charring is occur with the evaluation of odor of burnet sugar.
3- n. Solution + $CaCl_2$: it gives white ppt. after shaking from calcium tartrate, which is soluble in mineral acids.
4- n. Solution + $AgNO_3$: it gives Ag mirror after heating in w.b.
5- $KMnO_4$ + n. solution: by heating in presence of dil. H_2SO_4 a de colorization of color is occurring.

5- Citric acid



Physical properties:

It is colorless crystalline solid, m.p 100 °C, soluble in cold water

Reactions of citric acid

1- Heating the solid with conc. H_2SO_4 gives yellow color.
2- It gives Acidity test +ve.

3- With $CaCl_2$ solution: it gives white ppt. after boiling

4- Deng's test: I ml of $HgSO_4$solution is added to 5 ml of neutral solution. Then heat to boiling and then add 1-2 drops of 2 % $KMnO_4$ where de colorization occurs and a heavy whit ppt appear.

Identifications and reactions of acid salts

A) Ammonium salts: $RCOONH_4$

Ammonium salts of aliphatic acids,

- All colorless solids,
- Soluble in cold water
- Giving the neutral solution of the corresponding acid.

General reactions:-

1- They give off ammonia when treated with aqueous NaOH sol. in the cold (distinction from amides, imides)

2- Ammonia is liberated when the solid salt is mixed with solid Na_2CO_3, moistened with one drop of water and grind between the fingers.

3- Aqueous solution (neutral solution of the corresponding acid) gives with $FeCl_3$ or $CaCl_2$ the characteristic reactions of the corresponding acid.

4- Identify the carboxylic acid by the test given before.

B) Metallic Salts

They are solid of acid (metallic salt) have the general formula RCOOM

1- Effect of the heat:

$$RCOOM \xrightarrow{\triangle} CO_2 + H_2O + M$$

$$M + \text{dil.HCl} \longrightarrow MCl + H_2\uparrow \text{ (effervesces)}$$

2- **Nitration** -ve aliphatic salt

3- **Effect $FeCl_3$**

Soln. + $FeCl_3$ Buff Succinate

Red $\xrightarrow{\triangle}$ **Brown**

4- Effect of $CaCl_2$

Soln. + $CaCl_2$ White ppt. (at once) Oxalate

White (after shaking) Tartarate

White (after heating) Citrate

Then carry out the confirmatory test for each salt of acid

EXPERIMENT NO.

Identifications and reactions of carboxylic acids and esters

Data and Results

Name:

Sec.No.

Date:

Physical properties

Solubility: ……

Color: ……

Shape: ……

Chemical properties

Exp.	Obs.	Res.

Unknown is

Identification of Carbohydrates

Carbohydrates consist of simple sugars (e.g. glucose, fructose and sucrose) and complex carbohydrates (large molecules called polymers made of hundreds of simple sugars). There are a large number of carbohydrates in living organisms, varying from small sugar molecules such as the simple sugar glucose, which provides all cells with the fuel needed to do cell work, to polymers such as cellulose (structural molecules of plants) and glycogen (a storage carbohydrate in animals). Because there are so many different types of carbohydrates, one chemical test cannot identify all of them. Different chemical reagents, or testing agents, are used to test for simple sugars and carbohydrate polymers. A reducing sugar is a type of sugar that can cause a specific chemical reaction, called a reduction. A reduction is a very common type of chemical reaction where a substance "gains an electron".

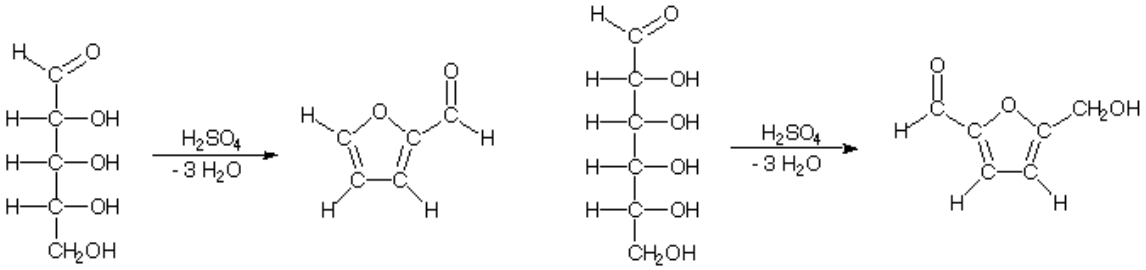
Reducing sugars can cause certain types of molecules to get reduced (gain an electron). Not all sugars have the chemical structure to be reducing sugars. A reduction is always coupled to a chemical reaction called an oxidation, where a substance loses an electron. Reducing sugars lose electrons when they cause a reduction of some other chemical. Oxidation-reduction, or Redox, reactions are very important in the energy transfer chemical reactions of living organisms. A Benedict's test can be used to identify reducing sugars. The Benedict's solution (a chemical reagent) contains a blue soluble form of copper ions (Cu^{++}) that can undergo a reduction (that is the copper ions gain electrons) when heated in the presence of a reducing sugar. When the blue copper ions are reduced, they change from the soluble blue color to reddish color copper ions (Cu+) that are insoluble. The color of the test solution changes from blue ---> green ---> orange ---> red-brown or rust color as more reduced copper ions are formed. You will use the Benedict's solution to test for the presence of reducing sugar in various substances

The Molisch Test

Shows positive test for: All carbohydrates. Monosaccharides give a rapid positive test. Disaccharides and polysaccharides react slower.

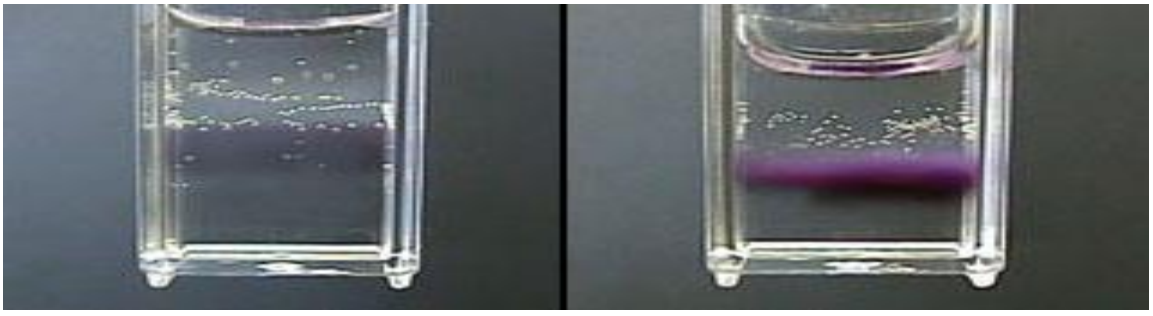
Reactions:

The test reagent dehydrates pentoses to form furfural (top reaction) and dehydrates hexoses to form 5-hydroxymethyl furfural (bottom reaction). The furfurals further react with ∝-naphthol present in the test reagent to produce a purple product (reaction not shown).



How to perform the test:

Two ml of a sample solution is placed in a test tube. Two drops of the Molisch reagent (a solution of ∝-napthol in 95% ethanol) is added. The solution is then poured slowly into a tube containing two ml of concentrated sulfuric acid so that two layers form. The formation of a purple product at the interface of the two layers.



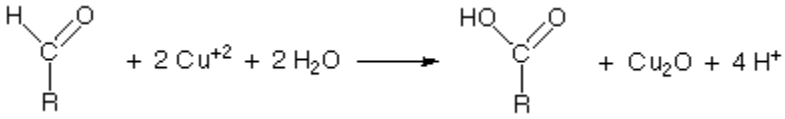
A negative test (left) and a positive test (right)

1) Barfoed's Test

Shows positive test for: Reducing monosaccharides

Reactions:

Reducing monosaccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide within three minutes. Reducing disaccharides undergo the same reaction, but do so at a slower rate.



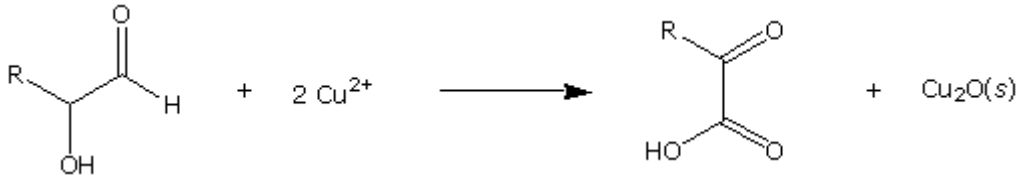
How to perform the test:

One ml of a sample solution is placed in a test tube. Three ml of Barfoed's reagent (a solution of cupric acetate and acetic acid) is added. The solution is then heated in a boiling water bath for three minutes.

A positive test is indicated by: The formation of a reddish precipitate within three minutes.

a negative test (left) and a positive test (right)

2) Fehling's Solution



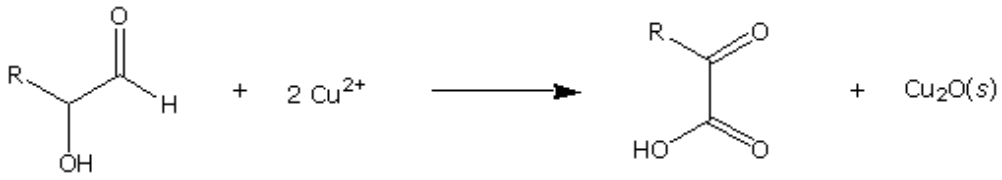
Procedure

To a solution or suspension of 0.2 g of unknown in 5 mL of water, add 5 mL of Fehling's solution and heat the mixture to boiling. Cool the solution. Fehling's

solution: Dissolve 17.32 g of hydrated copper sulfate in 200 mL of water and dilute to 250 mL. Dissolve 86.5 g of sodium potassium tartrate and 35 g of sodium hydroxide in 100 mL of water and dilute to 250 mL. Mix 2.5 mL of each solution immediately prior to use.

Positive Test: Precipitation of copper (II) oxide as a red, yellow, or yellowish-green solid is a positive test.

3) Benedict's Solution



Procedure

To a solution or suspension of 0.2 g of unknown in 5 mL of water, add 5 mL of Benedict's solution. If no precipitate is formed, heat the mixture to boiling and cool. Benedict's solution: A solution of 17.3 g of sodium citrate and 10.0 g of anhydrous sodium carbonate in 80.0 mL of water is heated until the salts are dissolved. Additional water is added to bring the volume up to 85.0 mL. A solution of 1.73 g of hydrated copper sulfate in 10.0 mL of water is poured slowly with stirring into the solution of the citrate and the carbonate. Add water to make a final volume of 100 mL.

Positive Test: Precipitation of copper (II) oxide as a red, yellow, or yellowish-green solid is a positive test.

4) Osazone Test:

This test is used to differentiate the maltose and lactose

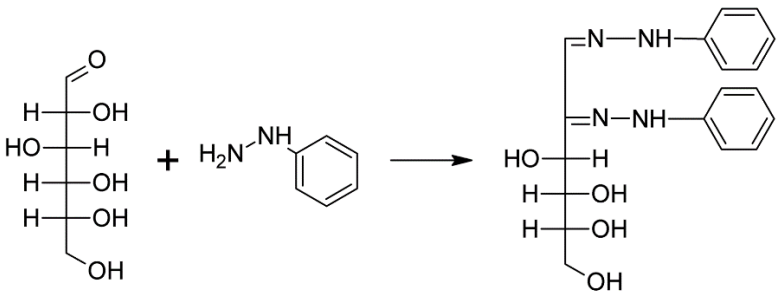
Principle:

An organic compound phenylhydrazine reacts with carbonyl carbon of sugar to form the osazones. These osazone crystals have yellow colour characteristics shapes and melting point, time of formation and solubility. The characterstics features of osazone are given in the following table: -

	Time of formation (Minutes)	**Solubility in boiling water**	**Crystalline structure**
Fructosazone	2	Insoluble	Needle shape
Glucosazone	5	Insoluble	Needle shape
Galactosazone	20	Insoluble	Thorny ball shape
Maltosazone	30-45	Soluble	Sunflower/Star shape
Lactosazone	30-45	Soluble	Cotton ball/Powder puff shape

Procedure:

Dissolve of 0.2g of carbohydrate, 0.4g of phenyl hydrazine hydrochloride and 0.6 g of sodium acetate in 4 ml of water and dip the tube in a beaker containing boiling water and leave it as such for 20 minutes then place it aside to cool slowly. The ozazone separates as a yellow crystalline precipitate.



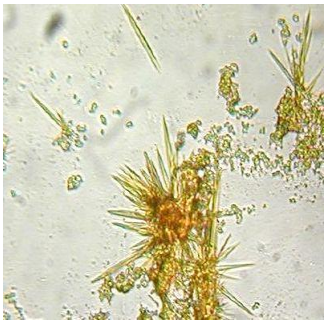
Observations and inference:

- Lactose is soluble in hot water and will only separate on cooling of the solution and forms powder puff shape crystals,

- Galactose, and Maltose are soluble in hot water and will only separate on cooling of the solution and forms sunflower shaped or star shaped crystals,
- Glucose and fructose are insoluble in hot water and separates readily from the solution before cooling and forms needle shaped crystals.

Yellow on heating
(Needle shape)

Yellow after cooling
(Powder puff shape)



Yellow after cooling
(Cotton ball)

EXPERIMENT NO.

Identifications and reactions of amines.

Data and Results

Name:

Sec.No.

Date:

Physical properties

Solubility: ……

Color: ……

Shape: ……

Chemical properties

Exp.	Obs.	Res.

Unknown is: