Isfahan University of Technology

Department of Chemistry

The Systematic Identification of Organic Compounds

Laboratory Manual

Contents: **page**

LAB SAFETY GUIDELINES

In the organic laboratory, we will work with a wide range of solvents, organicmolecules, acids, and bases which could be harmful if you were to come intodirect contact with them. **The following safety rules must be followed withoutexception:**

(a) Be aware of the location of exits from the building, and the location of eyewash stations, the fire extinguishers, and safety shower. The emergencyphone number is **115.**

(b) You must **always wear safety goggles** in the lab.

(c) **Do not wear sandals** in the lab. Anyone wearing any form of open–toedshoes will be sent back to their room to change.

(d) **Do not wear shorts, skirts, or dresses in the lab.** Anyone wearing anyform of clothing that leaves the legs uncovered will be sent back to theirroom to change.

(e) **No food or drink is allowed** in the lab.

(f) **Never leave a reaction unattended.**

(g) No work can be done unless an instructor or TA is present.

(h) **Do not touch common items with your gloved hand(s).** This includes

doorknobs, computer keyboards, the hallway, etc.

(i) You should **never** pour solvents in the sink, or put solid chemicals into atrash can. All non–halogenated waste must be poured into the nonhalogenatedwaste container, and halogenated waste must be poured intothe halogenated waste container. Solid organic materials are discarded inthe solid waste container. There is also a container for broken glassware inthe laboratory.

(j) Be alert to hazards and prepared for emergencies. If you are ever unsureof whether something is safe, immediately consult with your instructor or TA.

LABORATORY REPORTS

Lab Preparation Write-ups (Pre-Labs): It is necessary that you study the experiment that you are to do prior to arriving to the laboratory. You should read the lab manual carefully and try to understand the overall content and purpose. Your performance in the laboratory will benefit enormously with proper advance work, and so will your grade!

Report Format: After the identification of an unknown has been completed, the results should be reported on special forms supplied by the instructor. The following report is an example illustrating the information to be reported. Each report should be accompanied by a vial containing the derivative. A separate report should be written up for each component in a mixture.

EXAMPLE OF A REPORT FORM

Compound: Unknown Number:

Name: Date March:

1. Physical Examination:

(a) Physical state: - (b) Color:

(c) Odor: (d) Ignition test:

(e) TLC:

(f) GC:

2. Physical Constants:

(a) mp: observed: corrected:

(b)bp: observed: corrected:

3.Elemental Analysis:

F: 'CI: Br: I: N: S: Metals:

4.Solubility Tests:

H_2O	Ether	NaOH	$NaHCO_3$	HCl	H_2SO_4

Reaction to litmus:

Reaction to phenolphthalein: _

Solubility class:

Possible compounds:

5.Molecular Weight Determination:

6.Preliminary Classification Tests:

Reagent	Results	Inferense

Functional group indicated by these tests:

7.Further Classification and Special Tests:

Reagent	Results	Inferense

8.Probable Compounds:

	Useful Derivatives and Their mp, NE, etc.		
Name	1…	2…	3…
1…			

9. Preparation of Derivatives:

Name of Derivative	Observed mp ("C)	Reported mp("C)

10. Special Comments:

Qualitative Analysis of Organic Compounds

The analysis and identification of unknown organic compounds constitutes a very important aspect of experimental organic chemistry. There is no definite set procedure that can be applied overall to organic qualitative analysis. Sundry books have different approaches, but a systematic approach based on the scheme given below will give satisfactory results. Qualitative tests that require substantial quantities of a number of (often hazardous) chemicals to be stocked in the lab for experimental use are frequently being phased out of organic chemistry in favor of modern spectroscopic techniques. In order to deduce the identity of your two unknowns, you will combine one qualitative test, that for the classification of halides, with modern analytical techniques of infrared spectroscopy (IR) and mass spectrometry (MS).

General Scheme of Analysis

A. Preliminary Test

Note physical characteristics: solid, liquid, color, and odor. Compounds that are yellow to red in color are often highly conjugated. Amines often have a fish-like odor, while esters usually have a pleasant fruity or floral smell. Acids have a sharp, biting odor. Odors can illicit information about your unknown; it is wise to sniff them with caution. Some compounds can have corrosive vapors or make you feel nauseous.

B. Physical Constants

Determine the boiling point or melting point. Distillation is recommended in case of liquids. It serves the dual purpose of determining the boiling point as well as purifying the liquid for subsequent tests.

Experiment #1: Melting Point Determination

PURPOSE

To introduce the technique of melting point determination.

INTRODUCTION

The melting point of a solid can be easily and accurately determined with only small amounts of material and, in combination with other measurements, can provide rapid confirmation of identity. The most accurate method of determination is to record a cooling curve of temperature versus time. However, this approach requires quite large amounts of material and has been exclusively replaced by the **capillary method**.

Capillary Melting Point Determination

The method involves placing a little of the sample in the bottom of a narrow capillary tube that has been sealed at one end. The determination is made using a **Melting Point Apparatus** that simultaneously heats both the sample tube and a thermometer. The temperature **range**, over which the sample is observed to melt, is recorded. Some pure materials have a very narrow melting range, perhaps as little as 0.5–1.0 °C, while more typically a 2–3 °C range is observed. Data is typically recorded as, for example, mp 232–234 °C. Though formally denoting the melting range, this piece of data is almost universally referred to as a **melting point (mp)**.

The **rate** of heating, controlled by a dial, should be kept relatively low,especially for samples with a low melting point, to ensure that the thermometerreading represents, as accurately as possible, the true temperature experiencedby the sample tube (since the transfer of heat within the apparatus is relativelyslow). With this fact in mind, it is sensible when recording a melting point of anunknown material to perform a **trial run** where the temperature is increasedrelatively rapidly in order to ascertain a **rough** melting range. The determinationis then repeated by heating rapidly to within around twenty degrees of theexpected melting point and then very carefully (slowly) increasing thetemperature the remaining few degrees until the melting point is reached. Ofcourse, if the melting point of the material is known with some confidence, forinstance if the determination is being made to confirm identity, then the trial run isunnecessary.

Although a pure solid might be expected to have a single, sharp meltingpoint, most samples are observed to melt over a narrow range of a few degreesCelsius. The observation of a melting range may be a result of the heatingprocess involved in capillary measurements (mentioned above), may reveal thepresence

of in homogeneities in the macroscopic nature of the solid sample, ormay indicate the presence of other substances in the sample (contaminants orby–products of the method used to prepare the materials).

Melting Points as Criteria of Purity

Thermodynamics tells us that the freezing point of a pure material decreasesas the amount of an impurity is increased. The presence of an impurity in asample will both **lower** the observed melting point, and cause melting to occurover a **broader range** of temperatures. Generally, a melting temperature rangeof 0.5–1.0 °C is indicative of a relatively high level of purity. It follows that for amaterial whose identity is known an estimate of the degree of purity can be madeby comparing melting characteristics with those of a pure sample.

Melting Points as a Means of Identification and Characterization

For pure samples a clear difference of melting point between two materialsreveals that they must possess different arrangements of atoms, orconfigurations. If two materials are found to have the same melting point thenthey may, but not necessarily, have the same structure. Clearly, the recording ofa melting point is a desirable check of purity and identity but must be combinedwith measurements from other analytical techniques in order to unambiguouslyidentify a material and assess the purity. Part of the need for additionalverification derives from the subjective nature of capillary melting pointdetermination. Even when heating is very finely controlled, to ensureconsistency of sample and thermometer temperature, the human element ofvisual inspection of the melting point introduces significant variation.

Mixed Melting Points

Mixtures of different substances generally melt over a range of temperaturesthat concludes at a point below the melting point of either of the **pure**components—each component acts as an impurity in the other. Two puresubstances, with sharp melting points, can be shown to be different by mixingthem and recording the **lowered melting point range**. This type of experimentprovides a means by which to confirm a proposed identity for an unknownsample: if a **sharp** melting point is observed for a **mixture** of the unknown with agenuine sample then it is highly likely that the samples are **identical**.

Melting Points and Molecular Structure

Melting points are notoriously difficult to predict with any accuracy orconfidence. Systematic variations of melting point with variation in structure arenot as obvious or predictable as with boiling points. However, the sometimessurprising variations are often only highlighted in very closely related

moleculesand the obvious general rule can be applied: melting points do generally increasewith increasing molecular weight.

The difficulties involved in predicting melting points are a result of theproblems associated with predicting molecular packing in crystals. Many,potentially conflicting, factors play a role in determining melting points includingmolecular shape, interactions between groups within the molecule, and thedegrees of freedom the molecule possesses within the crystal.

PROCEDURE

For melting point determinations. many commercial melting point capillary tubes are available. These tubes are typically 1.1-1.8 mm wide and 90-100 mm long. One end is sealed. Use a new melting point capillary tube for each melting point. Place a small amount of the sample, approximately one-half of a spatula, on a hard, clean surface such as a watch glass. Tap the open end of the capillary tube into the sample until a few crystals are in the tube (Figure 3.1a). Hold the capillary tube vertically, open end up, and tap it gently on the counter so that the crystals pack to the bottom. If necessary, rub it with a file or a coin with a milled edge (Figure 3.1h) or drop it through a glass tube (Figure 3.1c) to move

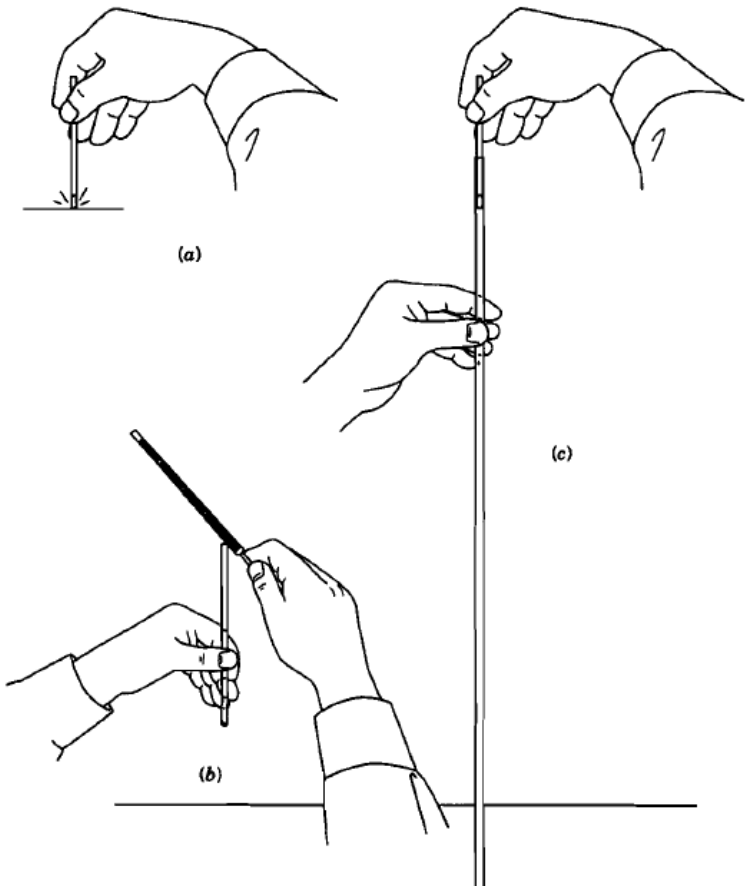


Figure1 Charging (a) and packing (b, c) capillary melting point tubes.

the crystals to the bottom. The capillary tube should contain 2-3 mm of sample. Use the capillary tube in melting point apparatuses such as the Thomas-Hoover melting point apparatus (Figure 3.2), a Thiele tube (Figure 3.3), (figure 1)

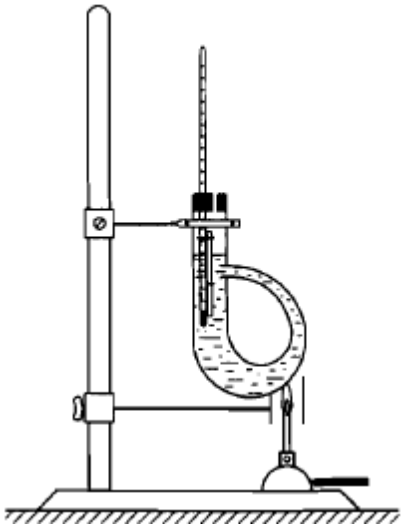


Figure2:Thiele tube melting point apparatus.

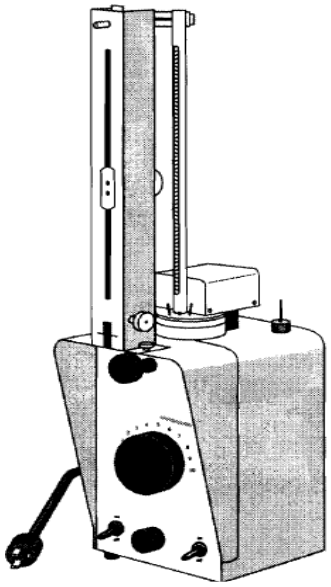


Figure3:Thomas-Hoover Uni-Melt melting point apparatus.

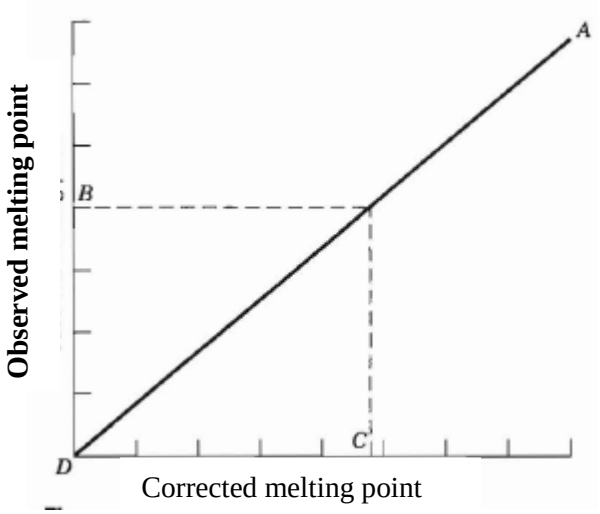
Corrected Melting Points

The thermometer should always be calibrated by observing the melting points of several pure compounds (Table 3.1). If care is taken to use the same apparatus and thermometer in all melting point determinations, it is convenient and timesaving to prepare a calibration curve such as that shown in Figure 3.6. The observed melting point of the standard compound is plotted against the corrected value, and a curve, DA, is drawnthrough these points. In subsequent determinations the observed value, B, is projected horizontally to the curve and then vertically down to give the corrected value, C. Such a calibration curve includes corrections for inaccuracies in the thermometer and stem correction. The thermometer should be calibrated by observing the melting points of several pure compounds.

It is important to record the melting point range of an unknown compound, because this is an important index of purity. A large majority of pure organic compounds melt within a range of 0.5°C or melt with decomposition over a narrow range of temperature (about l°C). If the melting point range or decomposition range is wide, the compound should be recrystallized from a suitable solvent and the melting or decomposition point determined again. Some organic compounds, such as amino acids, salts of acids or amines, and carbohydrates, melt with decomposition over a considerable range of temperature.

Table 1-1. Melting Point Standards

Standards	mp (corr.) (^{O}C)	Standards	mp (corr.) (^{O}C)
Ice	o	HippuriC acid	187
p-Dichlorobenzene	53	Isatin	200
m-Dinitrobenzene	90	Anthracene	216
Acetanilide	114	1,3-Diphenylurea	238
Benzoic acid	121	Oxanilide	257
Urea	132	Anthraquinone	286
Salicylic acid	157	N,N' -Diacetylbenzidine	332



Part 2

Working **individually**, determine the melting point of an unknown sampleprovided by your TA or instructor (**reminder:** a crude melting point run will benecessary to determine the approximate melting temperature). Confirm theproposed identity of your unknown sample by performing a mixed melting pointdetermination with an authentic sample of the suspected material. Recall thatthe melting point **will not be lowered or broadened** if the materials are identical,i.e. **if you have correctly identified your unknown.** Your sample will be oneof the compounds listed in Table 1-1.

CLEAN UP

Put all used tubes in the solid waste container marked for this purpose.

REPORT

Record all of the melting point ranges for the three parts of the experiment inthe results section of the report. In the discussion section describe what isrevealed by each measurement about the sample in question (refer to thediscussion in this script for some reminders).

QUESTIONS

1. Why should samples for melting point determination be finely powdered?

2. Why is it important that the heating rate be carefully controlled once near thesuspected melting point of a sample?

3. Describe and analyze the trend lines for the initial and final meltingtemperatures versus composition revealed by the graph you generated inPart 2 of this experiment. To what extent do you think it would be possible toemploy this graph to determine the composition of an unknownurea/cinnamic acid sample?

Experiment #2: Boiling Point Determination

PURPOSE

To introduce the technique of boiling point determination.

INTRODUCTION

The boiling point of a substance is the temperature at which the vapor pressure of the liquid equals the pressure surrounding the liquid[1][2] and the liquid changes into a vapor.

The boiling point of a liquid varies depending upon the surrounding environmental pressure. A liquid in a partial vacuum has a lower boiling point than when that liquid is at atmospheric pressure. A liquid at high pressure has a higher boiling point than when that liquid is at atmospheric pressure. For a given pressure, different liquids boil at different temperatures.

The normal boiling point (also called the atmospheric boiling point or the atmospheric pressure boiling point) of a liquid is the special case in which the vapor pressure of the liquid equals the defined atmospheric pressure at sea level, 1 atmosphere. At that temperature, the vapor pressure of the liquid becomes sufficient to overcome atmospheric pressure and allow bubbles of vapor to form inside the bulk of the liquid. The standard boiling point has been defined by IUPAC since 1982 as the temperature at which boiling occurs under a pressure of 1 bar.

The heat of vaporization is the energy required to transform a given quantity (a mol, kg, pound, etc.) of a substance from a liquid into a gas at a given pressure (often atmospheric pressure).

Liquids may change to a vapor at temperatures below their boiling points through the process of evaporation. Evaporation is a surface phenomenon in which molecules located near the liquid's edge, not contained by enough liquid pressure on that side, escape into the surroundings as vapor. On the other hand, boiling is a process in which molecules anywhere in the liquid escape, resulting in the formation of vapor bubbles within the liquid.

Procedure A

Set up a simple distillation as illustrated in Figure 3.8. Add a few bOiling chips and 10 mL of the unknown liquid. Insert the thermometer so that the top of the mercury bulb is just below the side arm. If necessary, wrap the diStilling head in glass wool to prevent heat loss. Heat the liquid to bailing using a sand bath (illustrated in Figure 3.8),

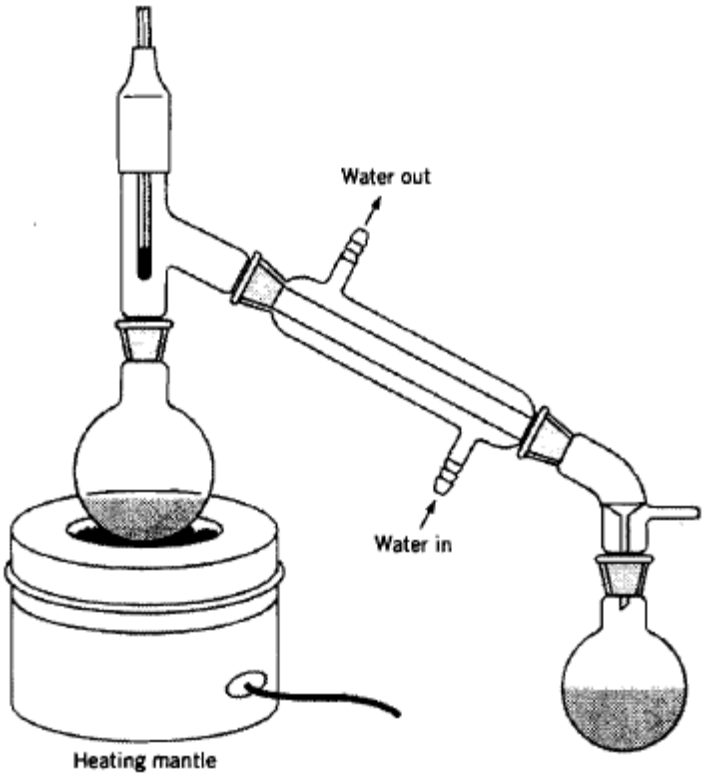


Figure 3.8 A small-scale simple distillation apparatus. Sand has been used to fill in the well.

a heating mantle, a heating block, or a Bunsen burner. For liquids with low boiling points, use steam or a hot-water bath. Distill the liquid at as uniform a rate as possible. Change the receiver, without interruption of the distillation, after the first 2-3 mL of liquid has been collected. Collect the next 5-6 mL in a dry receiver. Record the boiling point range during the distillation of the second portion of the liquid.

Great care should be exercised against overrelying upon boiling point as a criterion of purity or a basis for identity. Atmospheric pressure variations have a significant effect upon boiling point. Many organic liquids are hygroscopic, and some decompose on standing. Generally the first few milliliters of distillate will contain any water or more volatile impurities, and the second fraction will consist of the pure substance. If the boiling point range is large, the liquid should be refractionated through a suitable column (see Chapter 4 pp. 68-70).

The boiling point determined by the distillation of a small amount of liquid as described above is frequently in error. Unless special care is taken, the vapor may be superheated; also, the boiling points observed for high-boiling liquids may be too low because of the time required for the mercury in the thermometer bulb to reach the temperature of the vapor. The second fraction collected above should be used for a more accurate boiling point determination by Procedure B

below. Portions of the main fraction should also be used for the determination, as far as possible, of all subsequent chemical, spectral, and physical tests.

Procedure B

Place a thermometer 1-1.5 cm above approximately 0.5 mL of the liquid in a test tube (Figure 3.9). Slowly heat the liquid to boiling so that the thermometer bulb is immersed in the vapor. Allow the temperature to remain at a constant value for 30 sec. This value is the boiling point of the liquid. This technique is also useful for determining the boiling point of some low-melting solids provided they are thermally stable.

Procedure C

Set up a micro boiling point tube (Figure 3.10). Use a 5-cm test tube for the outer tube. Add two drops of the unknown liquid. Place an inverted sealed capillary tube inside thetest tube. Place the micro boiling point tube in a Thiele tube (Figure 3.3). Raise the temperature until a rapid and continuous stream of bubbles comes out of the small capillary tube and passes through the liquid. Remove the heat and allow the Thiele tube to cool. Note the temperature at the instant bubbles ceases to come out of the capillary and immediately before the liquid enters it. Record this temperature as the boiling point of the liquid.

Figure 3 Boiling point determination: Procedure B.

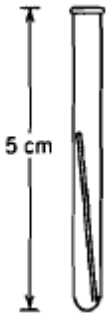


Figure 3 Micro boiling point tube.

Effect of Pressure on Boiling Point

At the time the boiling point is being determined, the atmospheric pressure should be recorded. Table 3.2 illustrates the magnitude of such barometric "corrections" of boiling point for pressures that do not differ from 760 mm by more than about 30 mm.

These corrections are applied in the following equation:

$$\text{corrbp} = \text{obsbp} + \frac{760 - \text{obspressure}}{10\text{mm}}\left\{\left(\frac{\Delta\ \text{factor}}{\Delta\text{T1}} * \Delta\text{T2}\right) + \text{originalfactor}\right\}$$

TABLE 3.2 Boiling Point Changes per Slight Pressure Change

bp (°C)	bp (K)	Correction in °C for 10-mm Difference in Pressure	
		No associated· Liquids	Associated· Liquids
50	323	0.38	0.32
100	373	0.44	0.37
150	423	0.50	0.42
200	473	0.56	0.46
300	573	0.68	0.56
400	673	0.79	0.66
500	773	0.91	0.76

QUESTIONS

1.Calculate the corrected boiling point for an aromatic halide that had an observedboiling point of 167°C at 650 mm Hg. Give the name and structure of the compound. (Hint: Use Appendix 11).

2.Calculate the corrected boiling point for an alcohol that contained halogen and that had an observed boiling point of 180°C at 725 mm Hg. Give the name and structure of the compound.

3. Using the pressure-temperature nomograph in Appendix I, give the corrected bp ·of a compound that has a bp of 120°C at 10 mm.

4. Give the bp of a compound at 25 mm that has a corrected bp of 250°C.

Experiment #3: An Introduction to Chromatography

PURPOSE

To introduce four chromatography techniques and to learn how to manipulatethese techniques to both separate mixtures of compounds and to analyzematerials.

INTRODUCTION

Chromatographic analysis is used to separate complex mixtures ofcompounds. First used in the early 1900s chromatography got its name becauseit was used to separate different mixtures of colored compounds. By the 1930sthe popularity of chromatography had increased as chemists realized that thisexperimental technique could also be used to separate mixtures of colorlesscompounds. All chromatographic systems have two phases, a mobile phase anda stationary phase. The **mobile phase** is a liquid or a gas that carries a samplethrough a solid **stationary phase**. As the sample in the mobile phase passesthrough the stationary phase the compounds in the mixture will separate becauseof differences in their affinities for the stationary phase, and differences in theirsolubilities in the mobile phase.

In the following three-week experiment you will examine four different types ofchromatography: thin layer chromatography (TLC), liquid (column)chromatography (LC), high-pressure liquid chromatography (HPLC), and gas liquid chromatography (GC). In the first week of the experiment you willcomplete part A, thin layer chromatography. In weeks 2 and 3 you will completeparts B, C, and D (in any order) where you will gain experience with the otherthree chromatographic techniques.

In **thin layer chromatography (TLC)**, glass, metal, or plastic plates arecoated with a thin layer of a stationary phase, usually a polar adsorbent such assilica gel or aluminum oxide. The mobile phase consists of a developing solventor mixture of solvents, carefully chosen based on an assessment of the polaritiesof the sample compounds. Generally, less polar developing solvents are usedfor less polar compounds and more polar solvents are used for more polarcompounds. As the solvent travels up the plate, the components in the samplemove with it and separate from each other as they reversibly adsorb to, anddesorb from the plate according to their differences in polarity.

Liquid or column chromatography (LC) is used to separate mixtures ofcompounds of low volatility. The stationary phase is packed into a hollow verticalglass column: as the sample travels down the column, compounds adsorbreversibly onto the stationary phase and the components of the mixture areseparated. More polar compounds will bind more tightly to the stationary

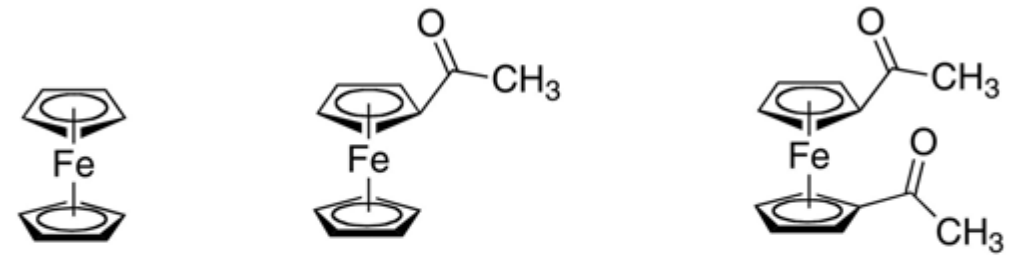
phaseand will stay on the column longer; the non-polar compounds will **elute** (exit)from the column first. Fractions of solvent are collected as the compounds areeluted from the bottom of the column. Unlike TLC, which is a micro scale andpurely qualitative technique, column chromatography can be used to separateand purify large amounts of sample.

In **high performance liquid chromatography (HPLC)** the basic principlesare the same as those for liquid chromatography. A column of small, tightlypacked particles serves as the stationary phase. The small particles have alarge surface area, allowing for better separation of the components of a mixture.High pressure is used to force the mobile phase through the column because thecolumn is so tightly packed. A detector and computer are used for sampledetection and monitoring so that each eluent (fraction) can be collected such thatit is free of the other components in the mixture.

Finally, **gas chromatography (GC)** is used to analyze small amounts ofmixtures of volatile organic compounds. The stationary phase is a nonvolatileliquid with a high boiling point and the mobile phase is an inert gas such ashelium or nitrogen. Once more, mixtures separate based on how theircomponents interact with the mobile gas phase and the liquid stationary phase.Generally, compounds with lower boiling points will elute from the GC columnmore quickly than compounds with higher boiling points.

The target compounds

You will work with two sets of samples as you learn about the techniquesdescribed above. The first set of compounds is a series of closely relatedorganometallic compounds called ferrocenes, the second set are organicmolecules that constitute the active ingredients in a series of modern, over thecounter, painkillers.



Ferrocene Acetylferrocene 1,1'-Diacetylferrocene

Figure 3-1. Set 1: Ferrocenes.

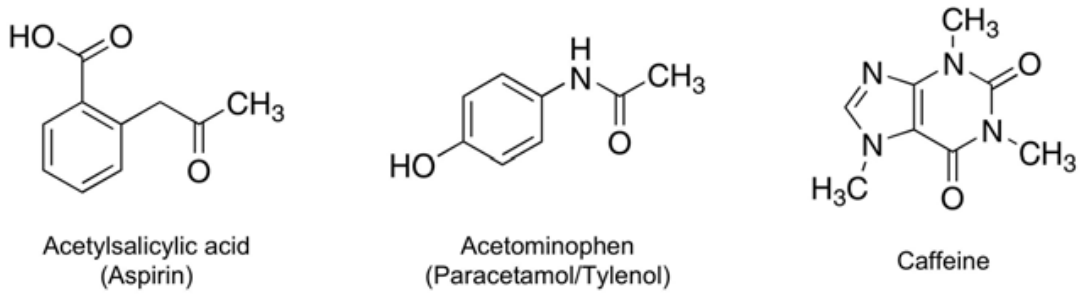


Figure 3-2. Set 2: Painkillers.

Part A: Thin Layer Chromatography (TLC)

INTRODUCTION TO TLC

Though organic chemistry looks neat and tidy on paper, in practice this is rarely the case. Most reactions, conversions of one substance into another,usually generate more than one product, and often many. **Thin layerchromatography (TLC)** is a useful method for quickly assessing how manydifferent products have been formed, and for identifying a set of conditions bywhich a large scale separation of a set of materials may be achieved.

The theoretical and practical basis for thin layer chromatography is the sameas for "full scale" preparative chromatography. A stationary solid phase**adsorbent**, spread as a thin layer (0.25 mm depth) on a plastic or glass sheet, istreated with the subject material or mixture of materials, the **adsorbate**. Theadsorbent has different affinities for different materials. As the plate is developed(solvent is eluted through the solid–phase) those molecules that adsorb moststrongly to the adsorbent spend comparatively little time in solution and do notmove very far as the solvent front advances. In contrast, those molecules that donot adsorb so strongly spend comparatively little time stuck on the solid phaseand most of their time in solution, as a consequence they move farther. This isthe principle behind all forms of chromatography.

A range of **stationary phases** (adsorbents) are available but, by far the mostwidely used in organic chemistry is silica gel (SiO2). However, the **mobile phase**(solvent or solvent mixtures) varies widely, and must be matched to the systemunder investigation in order to obtain good results. Silica gel contains manyrelatively polar silicon–oxygen bonds and therefore, has high affinity for polarmolecules: **the more polar the molecule the shorter the distance it will travelduring TLC development**.

In practice

Plastic TLC plates will be available in the laboratory, pre–cut into small rectangles. The sample is applied to the layer of adsorbent as a solution in an appropriate solvent using a small glass applicator called a **spotter**. Spotters are available in the lab and are made by stretching an open-ended capillary tube that has been heated in a flame. The aim is to deposit a small concentrated spot a few millimeters from the bottom of the plate. Once the application solvent has evaporated from this spot the material is left adsorbed on the solid phase and the plate is ready for development. A few mLs of the chosen solvent, or solvent mixture, are poured into the bottom of a beaker. The prepared TLC plate is then placed so that it stands up in the beaker and its bottom edge is in the puddle of solvent (see Figure 3-3). The solvent is drawn up the layer of adsorbent—if the placing of the plate is executed carefully a perfectly horizontal solvent front should result. The solvent passes over the spot of applied material and causes a separation of the components of the spot according to their relative polarities. When the solvent front has reached around 1 cm from the top of the plate it is removed from the beaker and the point of the furthest extent of the solvent is marked as a faint line with a pencil. **Do not** use a pen when making any marks on TLC plates: the dyes in the ink will adsorb to the plate, separate and interfere with the results obtained. Graphite from a pencil marking will not dissolve in the developing solvent or in any other manner affect the results of TLC. Also, be careful to mark gently; the layer of adsorbent is easily scratched and will flake from the surface.

Because the distance traveled by a substance relative to the distancetraveled by the solvent front depends upon the molecular structure of thatsubstance, TLC can be used to separate and identify substances. The distancerelationship is expressed as an Rf value given in Equation 3-1.

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

Due to the small size of the TLC plates we use a small ruler to make these distance measurements. When measuring the distance traveled by the spot be sure to measure from the middle of the spot, especially if the spot has smeared as it traveled up the plate (see Figure 3). The **ratio to the front** (R_f) value for a given substance depends on both the nature of the adsorbent and the solvent used to develop the plate. Though consistency should be obtainable if boththese factors are replicated, in practice there is not sufficient accuracy in thesemeasurements to allow for the unique identification of substances.

However,TLC is a very useful **comparative** technique when the identity of a substance issuspected and a genuine sample is available for comparison.

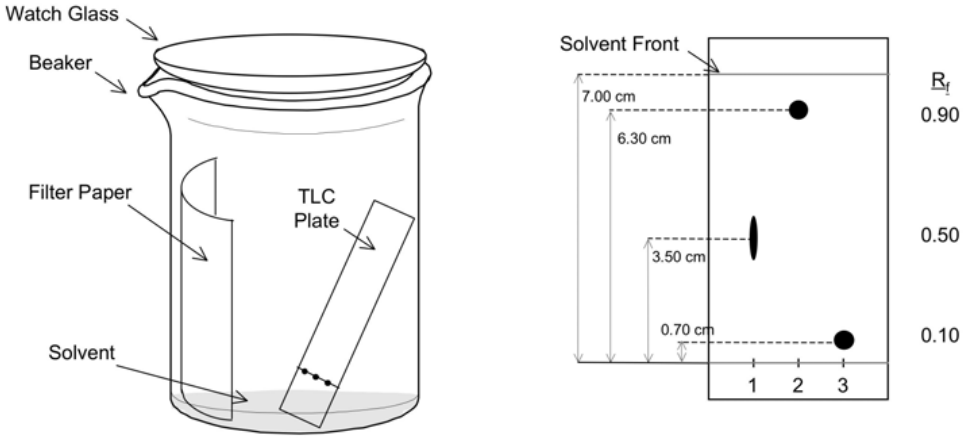


Figure 3-3. Example TLC chamber (left panel) and TLC plate (right panel). The distances traveled by each spot and by the solvent front are shown along with the R_f value for each spot.

The practice of TLC can be summarized in three discrete steps:

1. Sample Application

A small amount of sample is dissolved in a small volume of a solvent in which it is readily soluble to provide a spotting solution. A glass spotter is dipped in this solution and then applied to the plate to form a small concentrated spot at a marked position around 1 cm from the bottom of a TLC plate. The spot is made as small as possible to ensure, in as far as this is possible, that all of the applied material starts the journey up the TLC plate from the same point. The amount of material deposited on the plate is also very important: if the sample spot is too concentrated then the adsorbent can become overloaded and the features in the TLC will streak and spread (making it hard to measure their position, see Figure 3-3), if the spot is rather weak it may be hard to see the features on the plate after it has been developed. Preparing a good TLC plate requires practice and experience.

2. Development

The container used for development is most often a simple beaker covered with a watch glass. Sometimes a piece of filter paper is placed along the wall of the beaker to ensure saturation of the atmosphere with solvent vapor. Once a suitable solvent system for plate development has been found, a few mLs are added to the beaker to provide a depth of a few millimeters. The plate is placed in the beaker so that the bottom edge (the edge nearest the applied spot of

material) is immersed in the puddle of solvent. Care should be taken to ensure that the **solvent level is below that of the spot itself**. A watch glass, which serves as a crude lid, is placed on top. The solvent is allowed to run up the plat until the solvent front has reached around 1 cm from the top edge of the plate. At this point the plate should be removed and the furthest extent of the solvent front marked.

3. Visualization

The solvent is allowed to evaporate from the adsorbent on the plate. If the substances under investigation are colored, i.e. they absorb light in the visible region of the spectrum, then both during and after development of the plate you will be able to see where the spots are and how they have moved relative to one another and the solvent front. Direct measurement can then be made of the $R_f$values of the components. If the components of the sample are not colored, and this is most often the case for typical organic materials, you will not see much happening during the development of the plate. In these cases an Ultra–violet (UV) lamp may reveal the location of the spots and their positions may then be marked with a pencil. Molecules containing multiple bonds are typically revealed by use of a UV lamp. Alternatively, the staining of a TLC plate with iodine vapor is among the oldest methods for the visualization of organic compounds. This technique exploits the fact that iodine has a high affinity for both unsaturated and aromatic compounds. There are also chemical methods for revealing the positions of materials on plates that cannot be located by eye, UV lamp, or the iodine staining technique.

PROCEDURE

Perform all the experiments in the hood.

Part 1: TLC Analysis of Mixtures of Ferrocene Derivatives

You will gain some familiarity with the technique of TLC by examining the behavior of some colored compounds on silica TLC plates using a set of solvents and solvent mixtures that differ in polarity. The compounds are called ferrocene, acetylferrocene, and 1,1'–diacetylferrocene (See Figure 3-1). Don't worry about the ferrocene part, just note that the acetyl group contains a relatively polar carbon–oxygen bond. Therefore the compounds **increase** in polarity from ferrocene (no acetyl group) to acetyl ferrocene (1 acetyl group) to diacetylferrocene (2 acetyl groups). Each material is provided as a solid. You should take small samples of each (a small spatula tip of material is sufficient) and dissolve each material in around 1 mL of methylene chloride (dichloromethane) in three small, readily identified, sample vials.

Draw a faint pencil line around 1 cm from the bottom edge of the plate. Using a fresh spotter for each sample, apply each of the three solutions to evenly spaced points on this line aiming to prepare a colored spot of material no more than 3 mm in diameter. Allow the application solvent to evaporate for a few moments, leaving three small colored spots. Develop the plate in neat methylene chloride.

Remove the plate and mark the maximum extent reached by the solvent front. Measure the R_f values of each material.

Repeat the experiment using some of the mixtures of methanol and methylene chloride available in the lab, measure the Rf values on each occasion.

Keep trying mixtures until you have identified that which you think gives the bestresults, i.e. the one which gives the best separation (resolution) of the threematerials. Keep in mind that if the three components are bunched together at thetop or bottom of the plate then they haven't really separated—you are looking fora situation where the spots are spread about a median line about halfway up theplate.

Now, as before, take small amounts of each of the solid compounds and addthem to a **single** sample vial. Dissolve the mixture in around 1–2 mL ofmethylene chloride. Spot the mixture at one side of a TLC plate and thenalongside spot the three "pure" materials from before. Develop the plate in yourchosen solvent system (from above). Note the results.

Sketch all of the TLC plates in your notebook, and label them carefully withthe solvent mixture, the material in question, and the Rf values of all of thecomponents.

Part 2: TLC Analysis of a Pharmaceutical

In the previous experiment identity of the compounds were known along withtheir relative polarity. The intention was to gain some experience in running andanalyzing the data from TLC experiments in a well behaved and readilyvisualized system. The next experiment provides a more interesting applicationof TLC and involves probing the constituents of some commercialpharmaceuticals, mostly painkillers.

Spot a plate with each of the three standard solutions: caffeine,acetaminophen, and aspirin (available at the front of the lab). Develop the platewith neat ethyl acetate. Examine the plate under UV light and mark the positionsof the spots you observe. Now place the plate in an iodine chamber for about 10minutes and record and note any differences in the nature of the spots. **Be sureto keep the iodine stained plates in the hood.** Calculate the R_f values foreach of the three components examined.

Prepare a second plate exactly as before with each of the three standardsolutions. Develop the plate with a mixture of 3 mL of ethyl acetate plus onedrop of glacial acetic acid. Examine the plate under UV light and mark thepositions of the spots you observe. As before, now place the plate in an iodinechamber for about 10 minutes and record and note any differences in the natureof the spots. Calculate the Rf values for each of the three componentsexamined. Compare the results of the two runs and decide which experimentgave the better results.

Next, obtain a tablet of an unknown painkiller from your instructor or TA.Place the tablet in a 10 mL Erlenmeyer (conical) flask, add around 5 mL ofmethanol and crush the tablet with the end of a glass rod. Allow the undissolvedmaterial to settle out (this is mostly starch). Spot some of the resulting solutionon a TLC plate and let the solvent evaporate (you will need room for four spots intotal so make sure to leave room). Check that the concentration of sample onthe plate is appropriate by looking at the plate under the UV light. Rememberthat if the sample is too concentrated then poor results will be obtained, but toolittle sample will make it hard, or impossible, to measure the R_f values of thecomponents. If the concentration appears low, i.e. you can't see much materialwhere you spotted, then spot several times more in exactly the same place. Ifthe concentration appears too high then dilute a couple of drops of the solutionwith some more methanol (about, 1 drop of solution and 10 drops of methanol)and re–spot on a fresh plate. Spot the three standard solutions you looked atearlier in the remaining places and develop the plate under the conditions youfound to work best for the first part of the experiment. Measure the R_f values ofthe components and conclude which of the three components were present inyour unknown tablet. Check your conclusions with your TA or instructor.

CLEAN UP

All of the liquid samples should be poured into the halogenated solvent wastecontainer. The different solvents and solvent mixtures used for TLC should bepoured into the appropriate solvent waste container, either halogenated or non–halogenated. Dispose of all vials and spotters in the glass waste container.

REPORT

In the results section include sketches of all of your plates, and label allfeatures with their Rf values and identities. Describe the behavior of theferrocene compounds when the plates are developed with different solvents, andhow you arrived at your decision of the optimal solvent system for separation ofthese materials. Use the TLC evidence you obtain in Part 2 to indicate whichmaterials

were present in your pharmaceutical. Discus/rationalize the presenceof any other TLC features that you observed during this second experiment.

QUESTIONS

1. Does the solvent in which the samples are dissolved for plate preparation(i.e. spotting) have any bearing on the observed R_f values? Explain.

2. Why is it not possible to uniquely identify a material by its R_f value? (Hint:think about the maximum accuracy with which an R_f value might berecorded).

Part B: Column Chromatography: Separating Ferrocene

Compounds

INTRODUCTION TO COLUMN CHROMATOGRAPHY (LC)

Column chromatography is one of the most common and useful ways to separate mixtures of materials and isolate large amounts of the individual components in pure form. You will isolate three ferrocene derivatives from a mixture and determine the effectiveness of this technique in terms of the purity of the products.

Thin Layer Chromatography (TLC) was used last week to analyze the components of a mixture. In the TLC analyses, the stationary phase was silica (SiO_2, affixed to a plastic plate) and the mobile phase was a solvent or mixture of solvents, chosen to optimize the separation of compounds in a given mixture.

Silica gel contains many relatively polar silicon–oxygen bonds and therefore has a high affinity for polar molecules. As a result, it was observed that the more polar the molecule the shorter the distance it traveled up the TLC plate.

In this experiment **liquid chromatography** will be employed to separate the components of a mixture. Much like TLC, the stationary phase will be silica gel and the mobile phase will be a solvent, or mixture of solvents. In contrast to TLC, where the components of a mixture were separated from top to bottom using capillary forces to draw solvent onto the bottom of a TLC plate, column chromatography is performed using gravity to draw solvent downward through a glass tube containing the silica gel. Column chromatography allows separation of relatively large amounts of a product mixture into pure individual compounds, each of which can be isolated, characterized, and perhaps used for other experiments or syntheses. This type of purification and isolation is crucial to all synthetic organic chemistry and the modern pharmaceutical industry could simply not exist without it. Various types of liquid chromatography are also widely used in separations and preparations of biological macromolecules such as proteins, carbohydrates, and DNA.

In practice

Using column chromatography, you will separate a mixture of ferrocenederivatives (ferrocene, acetylferrocene, and 1,1’–diacetylferrocene), and thenconfirm their identities using TLC.

To begin, you must first determine the most effective solvent system forseparating the three compounds. The solvent, or solvent mixture you select,should allow good separation of the three compounds from one another,

and alsocause all three materials to elute from (that is, exit) the column in a reasonabletime. Additionally, the desired solvent, or solvent system must be **non–halogenated**, in order to minimize exposure to large quantities of (nasty)chlorinated solvents, and to reduce the volume of toxic and expensive waste.

Please note that this restriction means you **cannot** use the solvent conditionsyou carefully worked out in the previous experiment, although those conditionswould likely work very well.

To determine the best solvent, you could run a bunch of trial columns, but thatwould be expensive, wasteful, and very time–consuming. Instead, you will testseveral solvent mixtures by TLC, just as you did in the previous experiment.

Since the stationary phase, silica gel, is the same for both TLC and columnchromatography, the separations observed by TLC will accurately reflect theseparations seen on the column.

Once you have determined which solvent, or solvent mixture, you will use inthis separation, you will set up your column by neatly packing it with silica andsolvent. The mixed sample will be added to the column and its components willmove through the column at rates determined by their polarities and that of themobile phase (the solvent) that you have selected. Since the three compoundsare colored, you will be able to see at all times where they are on the column,how well they are separating, and when they are about to elute from the bottom.

Once you have collected the separate "fractions", each containing a pure sampleof one of the three compounds, you can confirm the identity of each one bycomparative TLC with an authentic sample.

PROCEDURE

Perform this entire experiment in the hood.

A. Determine the conditions for running the column using TLC

1. Using a spatula, transfer a tiny amount of the mixture of ferrocenecompounds to a small vial and dissolve in a few drops of methanol. Preparea TLC plate with a spot of this mixture exactly as you learned in the previousexperiment. Consult last week's experiment for additional details if you needthem.

2. Prepare 5 mL of a 1:1 mixture of hexanes and ethyl acetate and use this mixtureto develop the TLC plate. Once the solvent front nears the top, remove the TLCplate from the beaker and immediately mark the final position of the

solvent frontin pencil. You may need to use the UV lamp to confirm the location of the products (though they are colored the spots can be very faint). Sketch the TLCplate in your notebook and note the characteristics of the separation: which product, or products, moved most along the plate with the solvent? Whichadsorbed strongly to the stationary phase (that is, did not move very far)? Did the three products separate well from each other?

3. Repeat this experiment with different ratios of solvents, making careful noteof the results of each in your notebook. Think carefully about how you mightmodify the mixture based on your first run. For example, if a mixture causesall three components to run toward the top of the plate, your mixture is **toopolar**and you will want to reduce the proportion of the more polarcomponent in the solvent mixture (the ethyl acetate). Of course, the oppositewill hold if your components remain stuck to the bottom of the plate.

4. Based on these TLC runs, decide which solvent mixture, or mixtures, you willuse to achieve the best separation and elution of the three products. Keep in mind that you may change mobile phases during the course of running the column to optimize the separation. For example, you may need to elute thefirst two products in solvent mixture "A", **and then change** to solvent mixture "B"to elute the final product. You will want to use the least polar solvent mixturefirst, then switch to successively more polar mixtures to "chase" the productsfrom the polar surface of the silica.

B. Prepare the column

5. Clamp your column securely but gently to a ring stand, leaving enough space at the bottom to allow for easy switching of the flasks or beakers you will use to collect the eluted solvent. Try to clamp the column such that it is asstraight as possible so the compounds will run evenly down the column.Place a funnel on the top of the column and place a beaker underneath. Also have ready several clean, labeled sample vials for collection of the compound containing fractions.

6. You will use a crude dry–pack method to fill your column. Weigh 2.3 g of silica gel into a small beaker and pour into the column. Using 20 mL of your **initial** solvent mixture (as determined above) add portions of 1–2 mL at atime to the column, letting the solvent adsorb to the silica until the column issaturated. You may need to use a glass rod to poke down in the column and force out air bubbles, and use small batches of the solvent to wash any remaining silica gel from the inside surfaces of the column.

7. Very carefully open the stopcock and let some of the solvent drip through(both the column and stopcock assembly are quite fragile so please becareful here). As the solvent collects in the waste beaker below you

mayrecycle it (as needed) to aid in washing all of the silica gel into an even,tightly packed, cylinder, within the column. Please note that it is veryimportant that the column does not **ever run dry** during this process. Littlecracks and channels are formed when air is allowed to enter the column, andseparation is greatly compromised as a result.

8. Close the stopcock when the solvent level is just covering the top of thecolumn. If the top surface of the packed silica appears crooked, tap gentlyon the side of the column until it flattens out.

C. Run the column and identify the fractions

9. Dissolve around 20 mg (0.02 g) of the mixture of ferrocene derivatives in~0.5 mL of your starting solvent mixture. Also prepare 20 mL of fresh solventmixture to begin the column.

10. Using a glass pipette and rubber bulb, start to add your dissolved sample tothe top of the column. Try to add the solution gently, and evenly, to avoiddisplacing the silica gel, an ensuring that the sample starts running down thecolumn from an even level. Open the stopcock and allow the sample to runonto the column, adding drops of the dissolved sample slowly and gently tothe top via the pipette so that the column will not run dry and ensuring that afew millimeters depth of solvent is **always** maintained above the level of thesilica gel. Leave any undissolved material behind in the vial (for thepurposes of this experiment it is better to keep the volume of added solventsmall, rather than try to get every last bit of material onto the column).

11. Once the sample is loaded onto the column, you can (very) gently add ~5 mLof fresh solvent mixture to the column and start the separation. Add moresolvent as needed, at all times ensuring that there is no danger of the columnrunning dry. Try to run the column continuously without closing the stopcockvia regular additions of solvent. If you have elected to change solventsystems during the separation then at some point in your running of thecolumn you will need to switch solvent systems.

12. Collect the three colored fractions (ferrocene, acetylferrocene, 1,1'–diacetylferrocene) in clean glass vials, and label each clearly. All of thecolorless solvent fractions can be collected in a beaker to be disposed of asnon–halogenated waste.

13. Once you have collected all of the samples, empty the silica into a beaker byturning the column upside down and gently tapping. Dispose of the liquid inthe non–halogenated waste (by decanting), and the silica gel in the solidwaste container.

14. Confirm the identity and purity of each of the fractions by running TLCanalysis against pure samples of each material. Your fractions are relativelydilute so you may need to examine the developed plates under the UV lamp(the spots may be too faint to be directly visible). If your spots are difficult tosee, and analysis of the results is ambiguous, you should choose one (ormore if you wish) of your samples for evaporation: transfer to a small roundbottomed flask, remove the solvent using a rotary evaporator (consult yourinstructor or TA), and then redissolve the obtained solid material in a tinyamount of solvent to make a concentrated TLC sample. Repeat the TLCanalysis using this concentrated sample to achieve clearer results.

CLEAN UP

All of the solvents and samples left over from the TLC runs and liquidchromatography should be poured into the non–halogenated solvent wastecontainer. Silica gel should go into the solid waste container. Dispose of allspotters in the glass waste container. Vials should be thoroughly rinsed withacetone, the washings discarded in the non–halogenated waste container andleft in the hood to dry for the next lab group.

REPORT

In the report you should include sketches of all of your TLC plates, and labelall features with their Rf values and identities. You should describe the behaviorof the ferrocene compounds when the plates were developed with differentsolvents, and how you arrived at your decision of the optimal solvent system forseparation of these materials on a column. Discuss the purity of your isolatedfinal products, as determined by comparative TLC with authentic samples.

QUESTIONS

1. Last week we separated the same mixture of ferrocene compounds by TLCusing dichloromethane/methanol solvent mixtures. Why did we use ethylacetate and hexanes instead of dichloromethane/methanol for columnchromatographic separation? What property of these solvent mixtures mustbe similar?

2. Even if the components of the mixture had not been distinctly colored, youcould still have used liquid chromatography to separate and isolate them.Describe the practical measures you would need to employ in order to collectand identify the correct fractions?

Experiment #4: Solubility Tests

The solubility of the unknown in the following reagents provides very useful information. In general, about 1 mL of the solvent is used with approximately 0.1 g or 0.2 mL (2-3 drops) of the unknown compound. Assistance in analyzing the results from your solubility tests can be found in the solubility flowchart given in Fieser and Williamson on page 606. (Careful, this flowchart is much more complex than our lab requires and often can make it more difficult to form conclusions from your solubility data..)

Procedure for Determining Solubility of Organic Compounds

The amounts of material to use for a solubility test are somewhat flexible. Use 2-3 drops of a liquid or approximately 10 mg of a solid. Unless the solid is already a fine powder, crush a small amount of the solid on a watch glass with the back of a spatula. Do not weigh the solid; simply use enough to cover the tip of a small spatula. Your instructor will demonstrate how to estimate the correct amount. Place the appropriate amount of either your solid or liquid unknown in a small test tube and proceed with the following solubility tests.

1) **Water Solubility**

   Add approximately 1 mL of water to the test tube containing your organic compound. Shake the tube and/or stir with a glass stirring rod. A soluble organic compound will form a homogeneous solution with water, while an insoluble organic compound will remain as a separate phase. You may add additional water, up to 1.5 mL, if your compound does not completely dissolve with the smaller amount.

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

An organic compound which is soluble in water is typically a low molecular weight polar compound of up to 5-6 carbon atoms or less.

2) Acid-Base Solubility Tests

Please write a general chemical reaction(s) for any positive solubility test result(s) that you obtain for your unknown compound. Your reaction should demonstrate how any organic compound with a specific functional group can dissolve/react in an aqueous solution.

3) 5% NaOH Solubility

Add approximately 1 mL of 5% NaOH in small portions to the test tube containing your organic compound. Shake the test tube vigorously after the addition of each portion of the aqueous solution. Solubility will be indicated by the formation of a homogeneous solution, a color change, or the evolution of gas or heat. If soluble, then your organic compound 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 should react with 5% NaOH (a dilute strong base).

4) 5% $NaHCO_3$ Solubility

Add approximately 1 mL of 5% $NaHCO_3$ in small portions to the test tube containing your organic compound. Shake the test tube vigorously after the addition of each portion of the aqueous solution. Solubility will be indicated by the formation of a homogeneous solution, a color change, or the evolution of gas or heat. If soluble, then it is behaving as 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 $NaHCO_3$.

5) 5% HCl Solubility

Add approximately 1 mL of 5% HCl in small portions to the test tube containing your organic compound. Shake the test tube vigorously after the addition of each portion of the aqueous solution. Solubility will be indicated by the formation of a homogeneous solution, a color change, or the evolution of gas or heat. If your compound is HCl-soluble, then it is an organic base. Amines are the most common organic base. If insoluble in all solutions, then your unknown is a large (>5-6 carbon atoms) neutral compound that has none of the acidic or basic organic functional groups mentioned above.

6) Solubility in Cold, Concentrated Sulfuric Acid

Cold, concentrated sulfuric acid is used with neutral, water insoluble compounds containing no elements other than carbon, hydrogen, and oxygen. If the compound is unsaturated, is readily sulfonated, or possesses a functional group containing oxygen, it will dissolve in cold, concentrated sulfuric acid. This is frequently accompanied by a reaction such as sulfonation, polymerization, dehydration, or addition of the sulfuric acid to olefinic or acetylenic linkages. In many cases, however, the solute may be recovered by dilution with ice water.

SOLUBILITY TABLE

TABLE 5.1 Organic Compounds Comprising the Solubility Classes of Figure 5.1·

S_2	Salts oforganic acids (RC02Na, $RS0_3Na$); amine hydrochlorides (RNH_3Cl); amino acids(^+H_3N-R-CO_2^-); polyfunctional compounds with hydrophilic functional groups: carbohydrates (sugars), polyhydroxy compounds, polybasic acids, etc.
S_A	Mono functional carboxylic acids with five carbons or fewer; arylsulfonic acids.
S_B	Mono functional amines with six carbons or fewer.
S_1	Mono functional alcohols, aldehydes, ketones, esters, nitriles, and amides with five carbons or fewer.
A_1	Strong organic acids: carboxylic acids with more than six carbons; phenols with electron withdrawing groups in the ortho and/or para position(s); l3-diketones (l,3-diketones).
A_2	Weak organic acids: phenols, enols, oximes, imides, sulfonamides. thiophenols, all with more than five carbons; l3-diketones (l,3-diketones); nitro compounds with a-hydrogens.
B	Aliphatic amines with eight or more carbons; anilines (only one phenyl group attached to nitrogen); some ethers.
MN	Miscellaneous neutral compounds containing nitrogen or sulfur and having more than five carbon atoms.
N	Alcohols, aldehydes, ketones, esters with one functional group and more than five but fewer than nine carbons, ethers, epoxides, alkenes. alkynes, some aromatic compounds (especially those with activating groups).
I	Saturated hydrocarbons, haloalkanes, aryl halides, other deactivated aromatic compounds, diaryl ethers.

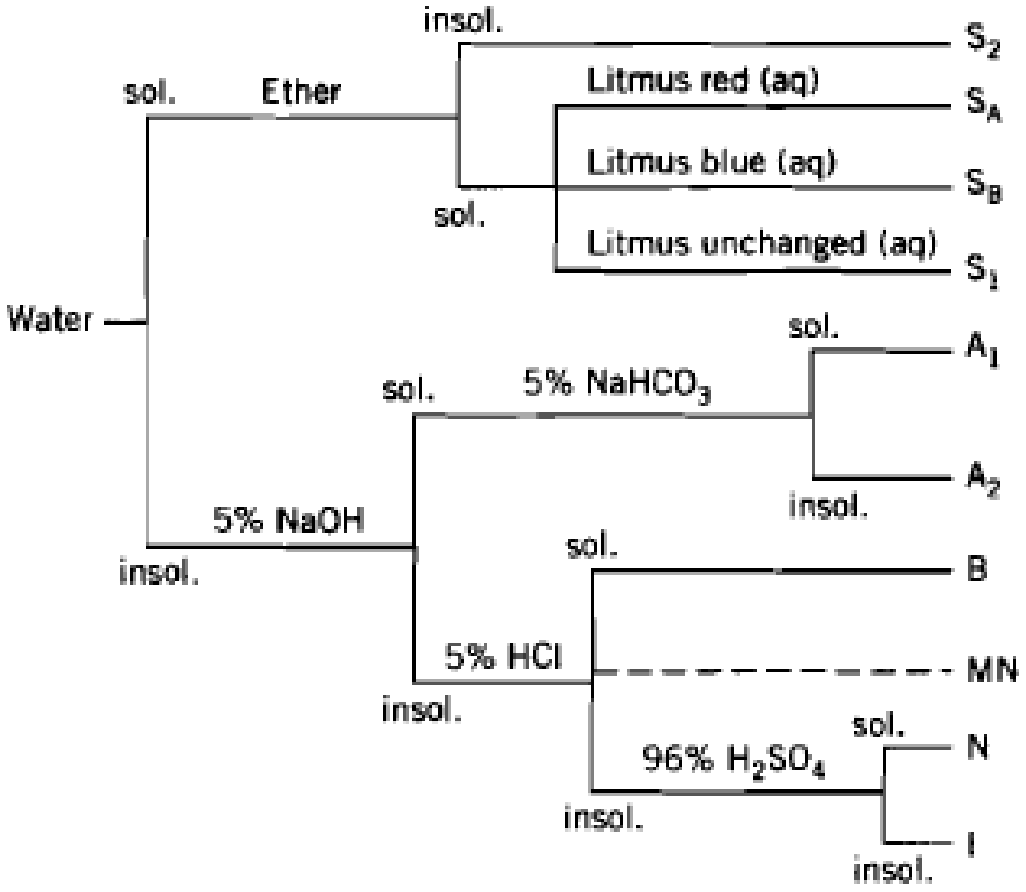


Figure 5.1 Classification of organic compounds by solubility: determination in water, acids, bases, and ethers. sol. = soluble, insol. = insoluble; litmus is red at pHs below 4.5 and blue above 8.3.(see Table 5.1 for compounds comprising each class).

QUESTIONS

1. Give the solubility class and the possible types of compounds for the solubilities listed below.

a. The unknown compound was insoluble in water, 5% NaOH, and 5% HCI, but soluble in 96% H2S04,
b. The unknown compound was soluble in water and ether and was unchanged with litmus.
c. The unknown compound was insoluble in water and 5% NaHC03 , but soluble in 5% NaOH.

2. Tabulate the structure, name, and solubility behavior of the follOwing compounds.

a.I-chlorobutane	b. 4-methylaniline
c.I-nitroethane	d. alanine
e.benwphenone	f. benzoic acid
g.hexane	h. 4-methylbenzyl alcohol
i.ethylmethylamine	j. propoxybenzene
k.propanal	l. 1,3-dibromobenzene
m.propanoic acid	n. benzenesulfonamide
o.I-butanol	p. methyl propanoate
q.4-methylcyclohexanone	r. 4-aminobiphenyl
s.4-methylacetophenone	t. naphthalene
u.phenylalanine	v. benzoin

Experiment #5:Qualitative Analysis for Elements

In organic compounds the elements commonly occurring along with carbon and hydrogen, are oxygen, nitrogen, sulphur, chlorine, bromine and iodine. The detection of these elements depends upon converting them to water-soluble ionic compounds and the application of specific tests.

Assignee's Sodium Fusion Test

C, H, O, N, S, X NaX
NaCN
-> Na2S
NaCNS

PROCEDURE

Place a piece of clean sodium metal, about the size of a pea into a fusion tube. Add a little of the compound (50 mg or 2 - 3 drops).* Heat the tube gently at first, allowing any distillate formed to drop back onto the molten sodium. When charring begins, heat the bottom of the tube to dull redness for about three minutes and finally plunge the tube, while still hot, into a clean dish containing cold distilled water (6 mL) and cover immediately with a clean wire gauze.**

***For liquids** it is better to first melt the sodium add the liquid drop by drop.

****CAUTION**: The tube shatters, and any residual sodium metal reacts with water. Stir the mixture, boil for 1 - 2 minutes, on a tripod and filter hot through a fluted paper.The 'fusion' filtrate which should be clear and colourless, is used for the SPECIFIC TESTS DESCRIBED BELOW:

1. NITROGEN

To a portion (2 mL) of the 'fusion' filtrate add 0.2 g of powdered ferrous sulphate crystals. Boil the mixture for a half a minute, cool and acidify by adding dilute sulphuric acid dropwise. Formation of a bluish-green precipitate (Prussian blue) or a blue solution indicates that the original substance contains nitrogen. If no precipitate appears, allow to stand for 15 minutes, filter and inspect filter paper.

2. SULPHUR (SULPHIDE)

To the cold 'fusion' filtrate (1 mL) add a few drops of cold, freshly prepared, dilute solution of sodium nitroprusside. The latter may be prepared by adding a small crystal of the solid to 2 mL of water. Production of a rich purple colour indicates that the original substance contains sulphur. This test is very sensitive. Only strong positive results are significant.

3. HALOGENS (HALIDES)

Acidify a portion (1 mL) of the 'fusion' filtrate with 2N nitric acid, and if nitrogen and/or sulphur are present, boil for 1 - 2 minutes.* Cool and add aqueous silver nitrate (1 mL), compare with a blank. Formation of a heavy, white or yellow precipitate of silver halide indicates halogen. If a positive result is obtained: acidify the remaining portion of the 'fusion' filtrate with dilute sulphuric acid, boil and cool. Add carbon tetrachloride (1 mL) and a few drops of freshly prepared chlorine water. Shake the mixture.

(a) If the carbon tetrachloride layer remains colourless - indicates chlorine.

(b) If the carbon tetrachloride layer is brown - indicates bromine.

(c) If the carbon tetrachloride layer is violet - indicates iodine.

*If nitrogen and/or sulphur are also present, the addition of silver nitrate to the acidified 'fusion' solution will precipitate silver cyanide and/or silver sulphide in addition to the silver halides. The removal of hydrogen cyanide and/or hydrogen sulphide is effected by boiling the 'fusion' solution.

QUESTIONS

10. 13.66 mg of a compound produced 10.71 mg of carbon dioxide and 3.28 g ofwater. Another 4.86 g of the same compound yielded 3.46 g of bromine. The molecular weight is 673.72 g/mole. Calculate the percentages of carbon, hydrogen, bromine, and oxygen in the sample. Determine the empirical formula and the molecular formula of the compound.

11. Calculate the unsaturation number and give the interpretation for the following formulas.

a. $C_6H_{12}O$

b. $C_5H_{10}Cl_2$

c. $C_7H_{13}N$

d. $C_{12}H_{10}$

Group Classification Tests

After analysis of the previous tests and the compound's IR spectrum, if needed, further information can be deduced by performing carefully selected functional group classification tests.

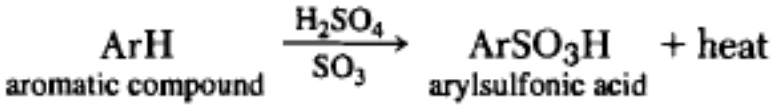
Experiment #6:Ignition Test for Aromaticity

If the results of preliminary chemical tests suggest aromatic character for an unknown, then a variety of tests can be used to chemically characterize this class of organic compound. Specifically, new substituents can be added onto the aromatic ring or existing substituents can be modified, such that the new compound may be more readily characterized. If the molecule already contains reactive chemical substituents (acids, amines, ethers, carbonyl compounds, etc.), the chemist is referred to other sections for that particular group.

The most vigorous test will be described first, and the tests that follow will be described in decreasing order of the severity of conditions. A few of the most inert aromatic compounds may remain unchanged after even the most vigorous test; characterization of those compounds may rely more on the spectral and physical tests than is usually the case.

Fuming Sulfuric Acid

Fuming sulfuric acid converts aromatic compounds to arylsulfonic acids . The aromatic compound dissolves completely with the evolution of heat.



Caution: Use this reagent with relatively inert compounds only, such as those compound3 that do not dissolve in the solubility tests with concentrated sulfuric acid. Compound3forwhich preliminary tests indicate highly activating groups (OH, NH2, etc.) may be decomposed violently by fuming sulfuric acid.

This test must be done in a hood. Place 0.5 mL of 20% fuming sulfuric acid (hazardous) in a clean, dry test tube, and add 0.25 mL or 0.25 g of the unknown. Shake the mixture vigorously, and allow it to stand for a few minutes. A positive test for the presence of an aromatic ring is complete dissolution of the unknown, evolution of heat, and minimal charring.

Standards
Benzene and bromobenzene will give a positive test. 1,2-Dibromoethane and cyclohexane will give a negative test.

Ignition Test

Procedure

Place a 1O-mg sample of the substance in a porcelain crucible lid (or any piece of porcelain) and bring the sample to the edge of a flame to determine flammability. Heat the sample gently over a low flame, behind a safety shield. Heat the sample until ignition has occurred. Note (1) the flammability and nature of the flame (is the compound explosive?); (2) whether the compound is a solid, whether it melts, and the manner of its melting; (3) the odor of the gases or vapors evolved *(caution!);* and (4) the residue left after ignition. Will it fuse? Ifa residue is left, allow the lid to cool. Add a drop of distilled water. Test the solution with litmus paper. Add a drop of 10% hydrochloric acid. Note whether a gas evolves. Perform a flame test, with a platinum wire, on the hydrochloric acid solution to determine the metal present.

Discussion

Many liquids burn with a characteristic flame that assists in determining the nature of the compound. Thus, an aromatic hydrocarbon (which has a relatively high carbon content) burns with a yellow, sooty flame. Aliphatic hydrocarbons burn with flames that are yellow but much less sooty. As the oxygen content of the compound increases, the flame becomes more and more clear (blue). If the substance is flammable, the usual precautions must be taken in subsequent manipulation of the compound. This test also shows whether the melting point of a solid should be taken and indicates whether the solid is explosive.

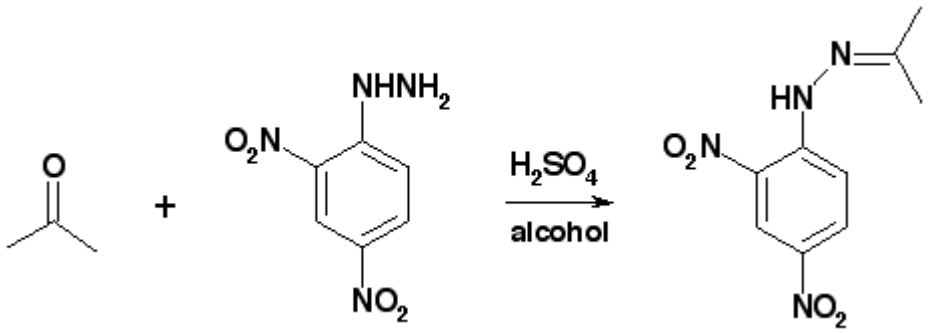
Ifan inorganic residueis left after ignition, it should be examined for metallic elements. A few simple tests will often determine the nature of the metal present.2 If the flame test indicates sodium, a sample of the compound should be ignited on a platinum foil instead of a porcelain crucible cover.

QUESTIONS

1. Note that this test is useful only for compounds insoluble in sulfuric acid. Why?

Experiment #7:Tests for Aldehydes and Ketones

I. 2,4-DNP Test for Aldehydes and Ketones



Standards
Cyclohexanone, Benzophenone, and Benzaldehyde

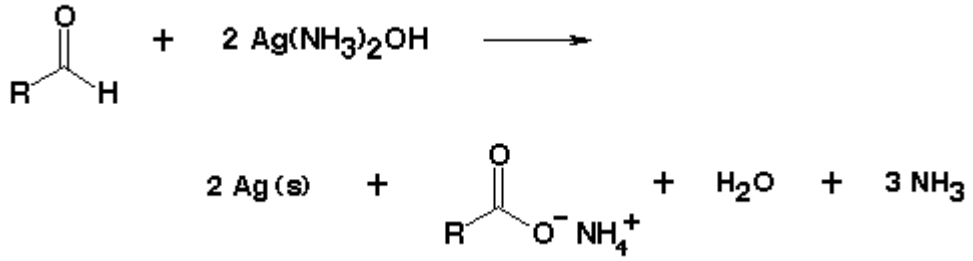
Procedure
Add a solution of 1 or 2 drops or 30 mg of unknown in 2 mL of 95% ethanol to 3 mL of 2,4-dinitrophenylhydrazine reagent. Shake vigorously, and, if no precipitate forms immediately, allow the solution to stand for 15 minutes. The 2,4-dinitrophenylhydrazine reagent will already be prepared for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

II. Tollen's Test for Aldehydes



Standards
Cyclohexanone and Benzaldehyde

Procedure
Add one drop or a few crystals of unknown to 1 mL of the freshly prepared Tollens reagent. Gentle heating can be employed if no reaction is immediately observed.

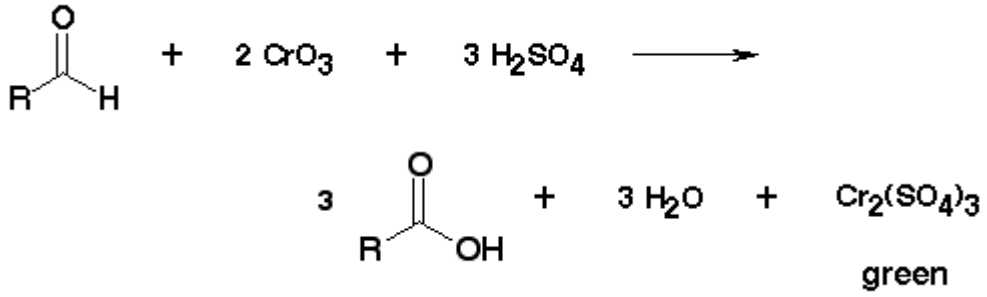
Tollen's reagent: Into a test tube which has been cleaned with 3M sodium hydroxide, place 2 mL of 0.2 M silver nitrate solution, and add a drop of 3M sodium hydroxide. Add 2.8% ammonia solution, drop by drop, with constant shaking, until almost all of the precipitate of silver oxide dissolves. Don't use more than 3 mL of ammonia. Then dilute the entire solution to a final volume of 10 mL with water.

Positive Test
Formation of silver mirror or a black precipitate is a positive test.

Complications

- The test tube must be clean and oil-free if a silver mirror is to be observed.
- Easily oxidized compounds give a positive test. For example: aromatic amine and some phenols.

III. Jones (Chromic Acid) Oxidation Test for Aldehydes



Standards
Cyclohexanone and Benzaldehyde

Preparation of Jones' reagent

Jonesreagent is prepared by dissolving 26.72 grams of chromium trioxide (CrO_3) in 23ml of concentrated sulfuric acid, and then diluting the mixture to 100ml with water.

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.

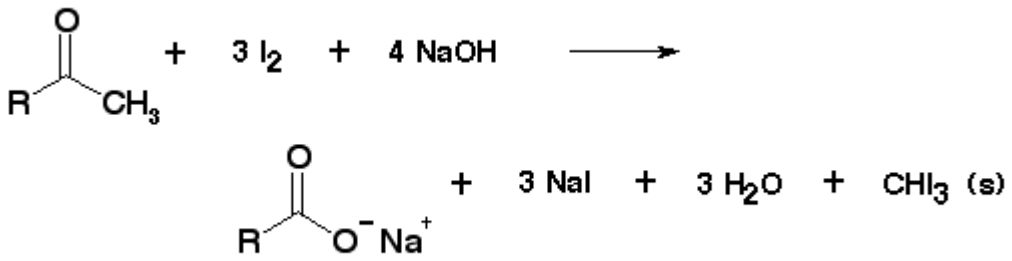
Positive Test
A positive test for aldehydes and primary or secondary alcohols consists in the production of an opaque suspension with a green to blue color. Tertiary alcohols give no visible reaction within 2 seconds, the solution remaining orange in color. Disregard any changes after 15 seconds.

Complications

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

IV. Iodoform Test for Methyl Ketones

Ketone



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.

Preparation of reagent

The iodine-potassium iodide solution is prepared from 10 g of iodine and 20 g of potassium iodide in 100 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

QUESTIONS

1. Suggest an explanation of the fact that cyclohexanone reacts with sodium bisulfite readily, whereas 3-pentanone does not.

2. What is the explanation of the failure of pinacolone to react? Compare the case with that of acetophenone.

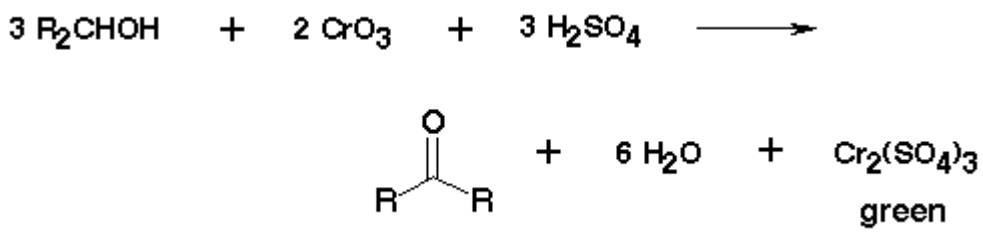
3. Explain the behavior of cinnamaldehyde.

4. Why is an alcoholic solution of sodium bisulfite used? *Try* the test on acetone, using an aqueous solution.

5. Would the presence of a reactive halogen atom interfere with this test?

Experiment #8:Tests for Alcohols

I. Jones Oxidation for Primary and Secondary Alcohols



Standards
1-Butanol, 2-Butanol, t-Butyl alcohol

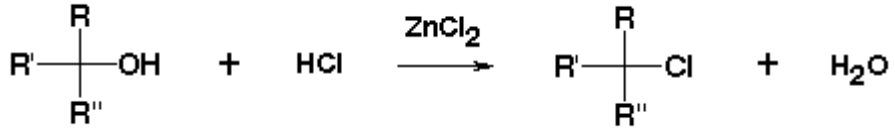
Procedure
Dissolve 10 mg or 2 drops of the unknown in 1 mL of pure acetone in a test tube and add to the solution 1 small drop of Jones reagent (chromic acid in sulfuric acid). A positive test is marked by the formation of a green color within 15 seconds upon addition of the orange-yellow reagent to 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

- Enols may give a positive test.
- Phenols give a dark colored solution which is not blue-green like a positive test.

II. Lucas Test for Secondary and Tertiary Alcohols



Standards
1-Butanol, 2-Butanol, t-Butyl alcohol.

Procedures
To 0.2 mL or 0.2 g of the unknown in a test tube add 2 mL of the Lucas reagent at room temperature. Stopper the tube and shake vigorously, then allow the mixture to stand. Note the time required for the formation of the alkyl chloride, which appears as an insoluble layer or emulsion.
The Lucas reagent is already prepared for you.

Positive test
Appearance of a cloudy second layer or emulsion

- 3^o alcohols: immediate to 2-3 minutes
- 2^o alcohols: 5 -10 minutes
- 1^o alcohols: no reaction

Complications
The test applies only to those alcohols soluble in the reagent (monofunctional alcohols lower than hexyl and some polyfunctional alcohols.) This often means that alcohols with more than six carbon atoms cannot be tested.

QUESTIONS

1. Explain the difference in reactivity of the primary, secondary, and tertiary alcohols with the acetyl chloride.

2. Write the structural formulas and names of the isomeric five-carbon saturated alcohols that were not used in this experiment. How would they react with this reagent?

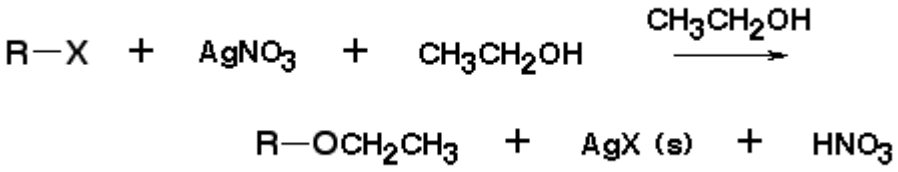
3. How would you account for the difference in the behavior of allyl alcohol and I-propanol? Benzyl alcohol and I-pentanol?

4. List two tests, with the equations, that will give a positive test for butanal and I-butanol.

5. List two tests, with the equations, that will distinguish between butanal and I-butanol.

Experiment #9:Tests for Halides

I. Silver Nitrate in Ethanol Test



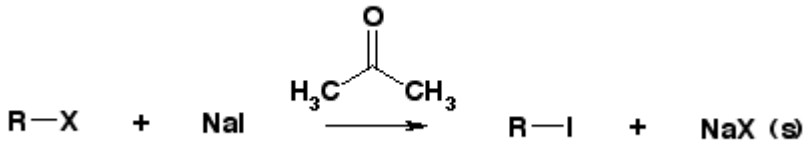
Standards, as done in the Classification Tests for Halides lab

1-chlorobutane	1-bromobutane	1-iodobutane
2-chlorobutane	2-bromobutane	2-iodobutane
2-chloro-2-methylpropane	2-bromo-2-methylpropane	
benzyl chloride	bromobenzene	

Procedure
Place approximately 0.25 mL of each compound into a test tube. Add 2 mL of a 1% ethanolic silver nitrate solution to the material in each test tube, noting the time of addition. After the addition, shake the test tube well to ensure adequate mixing of the compound and the solution. Record the time required for any precipitates to form. If no precipitates are seen after 5 minutes, heat the solution on the steam bath for approximately 5 minutes. Note whether a precipitate forms in the test tube. Continue slow reactions for up to 45 minutes at room temperature.

Complications
Carboxylic acids have been known to react in this test, giving false positives.

II. Sodium Iodide in Acetone Test



Standards
Reference tests done in Classification Tests for Halides Lab

Procedure
In a test tube place 0.25 mL or 0.2 g of your unknown. Add 2 mL of a 15% solution of sodium iodide in acetone, noting the time of addition. After the addition, shake the test tube well to ensure adequate mixing of the unknown and the solution. Record the time needed for any precipitate to form. After about 5 minutes, if no precipitate forms, place the test tube in a 50°C water bath. Be careful not to allow the temperature of the water bath to go above this temperature since the acetone will evaporate, giving a false positive result. After 6 minutes more in the bath, if no precipitates are visible, remove the test tube and let it cool to room temperature. Note any change that might indicate that a reaction has occurred. Continue slow reactions for up to 45 minutes at room temperature.

Positive Test
The formation of a white precipitate indicates the presence of halides.

Complications
When the sodium iodide solution is added to the unknown, a precipitate of sodium iodide might occur leading to a false positive test. Upon mixing, the precipitate of sodium iodide should dissolve.

III. Beilstein Test

Standards
Any halogenated compound as a positive standard, such as, 1-Bromobutane, and any non-halogenated compound, such as 1-Butanol, as a negative standard.

Procedure
Heat the tip of a copper wire in a burner flame until there is no further coloration of the flame. Let the wire cool slightly, then dip it into the unknown (solid or liquid" and again, heat it in the flame. A green flash is indicative of chlorine, bromine, and iodine; fluorine is not detected because copper fluoride is not volatile. The Beilstein test is very sensitive, thus halogen-containing impurities may give misleading results.

Positive Test
A green flash is indicative of chlorine, bromine, and iodine, but NOT fluorine

QUESTIONS

1.Suggest a reason why $CICH_2OR$ compounds should be reasonably reactive toward $AgNO_3$. *(Hint:* Resonance theory supports a stable, although primary, carbocation.)

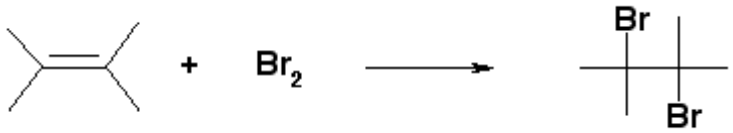
2.Suggest reasons why benzyl chloride reacts faster than cyclohexylmethyl chloride.

3.Suggest a reason why vinyl and aryl halides are quite inert toward ethanolic$AgNO_3$,

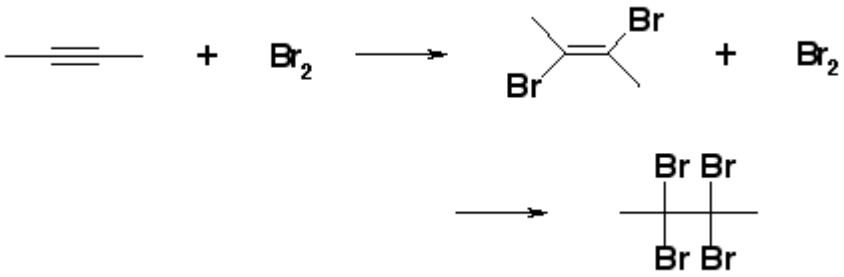
Experiment #10:Unsaturation

I. Bromine (in Methylene Chloride) Test for Multiple Bonds

Alkene



Alkyne



Standards
Cyclohexane, Cyclohexene, and Bromobenzene

Procedure
In a hood, 0.02 g or 1 drop of the unknown is added to 0.5 mL of methylene chloride. Add a dilute solution of bromine in methylene chloride dropwise, with shaking, until the bromine color persists. The bromine solution must be fresh.

Preparation of bromine water

Add 1 mL of bromine to 200 mL of DI water and stir. Keep in a tightly sealed bottle. The shelf life is poor due to evaporation of bromine. (polar/nonpolar solubility studies)

Positive Test
Discharging of the bromine color without the evolution of hydrogen bromide gas is a positive test.

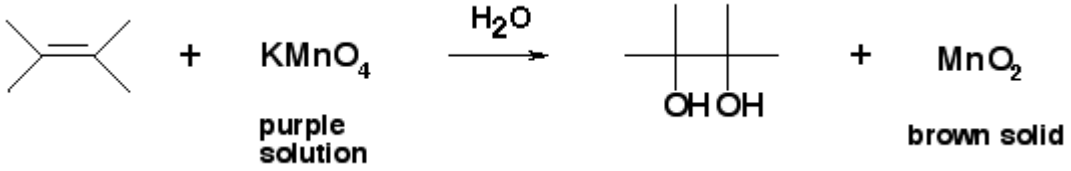
Complications

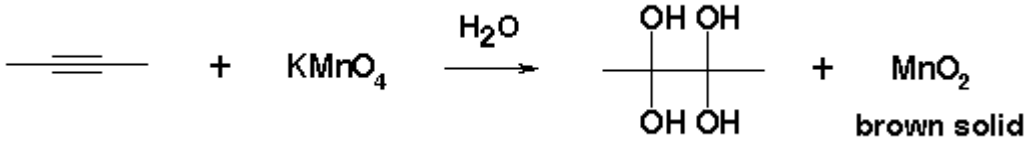
- Should be employed in conjunction with Baeyer test (dilute $KMnO_4$).
- Electron-withdrawing groups in the vinylic position can slow down bromine addition to the point that a negative test is erroneously produced.

- Tertiary amines (like pyridine) form perbromides upon treatment with bromine and lead to false positive tests.
- Aliphatic and aromatic amines can discharge the bromine color without the evolution of HBr gas.

II. Baeyer Test for Multiple Bonds (Potassium Permanganate Solution)

Alkene





Alkyne

Standards
Cyclohexane, Cyclohexene and Bromobenzene.

Procedure
Dissolve 1 drop or 0.02 grams of the unknown in 0.5 mL reagent grade acetone. Add a 1% aqueous solution of potassium permanganate dropwise with shaking. If more than one drop of reagent is required to give a purple color to the solution, unsaturation or an easily oxidized functional group is present. Run parallel tests on pure acetone and, as usual, the standards listed above.

Positive Test
The disappearance of the $KMnO_4$'s purple color and the appearance of a brown suspension of MnO_2 is a positive test.

Complications

- Water insoluble compounds should be dissolved in ethanol, methanol, or acetone.
- Often, the brown precipitate fails to form and the solution turns reddish-brown.
- Easily oxidized compounds give a positive test: a) Most aldehydes give a positive test. b) Formic acid and its esters give a positive test.
- Alcohols with trace impurities give a positive test.
- Phenols and aryl amines give a positive test.

- Carbonyl compounds which decolorize bromine/methylene chloride usually give a negative test.

III. Ignition Test for High Degrees of Unsaturation

Standards
Benzophenone, cyclohexane, and hexanes.

Procedure
Heat a small sample on a spatula. First, hold the sample near the side of a bunsen burner to see if it melts normally and then burns. Heat it in the flame. Aromatic compounds often burn with a smoky flame.

Positive Test
A sooty yellow flame is an indication of an aromatic ring or other centers of unsaturation.

QUESTIONS

1. What functional groups respond to both the bromine and the permanganate tests?

2. Which ofthesetests isbetterfordetectingthepresence ofmultiplebonds?Explain.

3. In what instances is it helpful to use both reagents?

4. Give the equations for a test that will distinguish between I-butyne and 2-butyne.

Experiment #11:Tests for Carboxylic Acids

I. pH of an Aqueous Solution for Carboxylic Acids

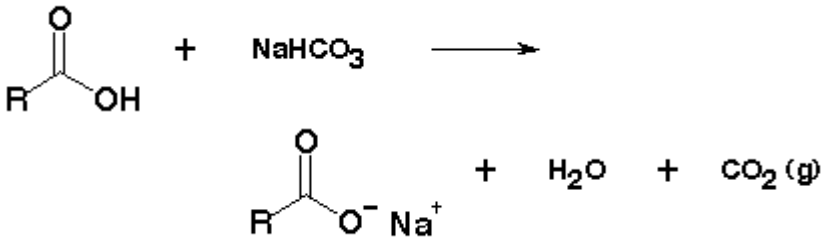
Standard
5% Acetic acid solution

Procedure

See procedure for solubility tests with water.

II. Sodium Bicarbonate Test for Carboxylic Acids

Carboxylic Acid



Standard
5% Acetic acid solution

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.

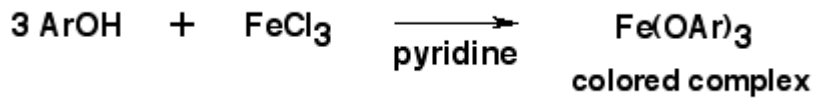
QUESTIONS

1. Volatility contributes greatly to odor. Briefly explain why ethyl esters are normally more volatile than the corresponding carboxylic acids.

Experiment #12:A Tests for Phenols and Nitro Groups

I. Iron (III) Chloride Test for Water-Soluble Phenols

Phenol



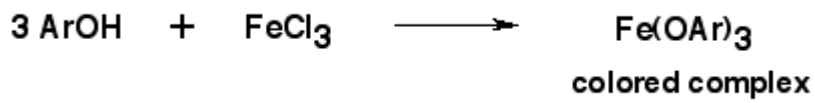
Standard
Phenol

Procedure (for water-soluble phenols)
The iron (III) chloride test for phenols is not completely reliable for acidic phenols, but can be administered by dissolving 15 mg of the unknown compound in 0.5 mL of water or water-alcohol mixture and add 1 to 2 drops of 1% aqueous iron (III) chloride solution.

Positive Test
A red, blue, green, or purple color is a positive test.

II. Iron(III) Chloride - Pyridine Test for Water-Insoluble Phenols

Phenol



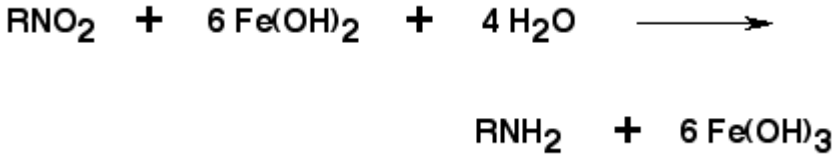
Standard
Phenol

Procedure (for water-insoluble phenols or less reactive phenols)
A more sensitive test for phenols consists of dissolving or suspending 15 mg of the unknown in 0.5 mL of methylene chloride and adding 3-5 drops of a 1% solution ferric chloride in methylene chloride. Add a drop of pyridine and stir.

Positive Test (b)
Addition of pyridine and stirring will produce a color if phenols or enols are present.

III. Iron (II) Hydroxide Test for Nitro Groups

Nitro Compounds



Standard
3-Nitrobenzaldehyde

Procedure
Add about 10 mg of the compound to 1 mL of the ferrous ammonium sulfate reagent in a test tube, and then add 0.7 mL of the 2N alcoholic potassium hydroxide reagent. Stopper the tube, and shake. Note the color of the precipitate after 1 minute.
Ferrous Ammonium Sulfate Reagent: To 50 mL of recently boiled, distilled water add 2.5 g of ferrous ammonium sulfate crystals and 0.2 mL of concentrated sulfuric acid.
The Alcoholic Potassium Hydroxide Reagent will already be prepared for you.

Positive Test
A positive test is the formation of the red-brown precipitate of iron(III) hydroxide.

Complications
The red-brown to brown precipitate of iron (III) hydroxide (ferric hydroxide) is formed by the oxidation of iron(II) hydroxide (ferrous hydroxide) by the nitro compound, which in turn is reduced to the primary amine. A negative test is indicated by a greenish precipitate. In some cases partial oxidation may cause a darkening of the ferrous hydroxide. Practically all nitro compounds give a positive test in 30 seconds. The speed with which the nitro compound is reduced depends on its solubility.

QUESTIONS

1. From 2,4-dichloro-l-nitrobenzene. give the equations for the formation of (a)N-(2,4-dichlorophenyl) acetamide, (b) N-(2,4-dichlorophenyl) benzamide, and (c)N-(2,4-dichlorophenyl) benzenesulfonamide.

2. Give the equations for the reaction of 4-nitrophenol with (a) phenyl isocyanate, (b) I-naphthylisocyanate, (c) benzoyl chloride, (d) 4-nitrobenzoyl chloride, (e) 3,5dinitrobenzoyl chloride, (f) acetic anhydride, (g) sodium hydroxide, followed by chloroacetic acid, and (h) bromine and potassium bromide.

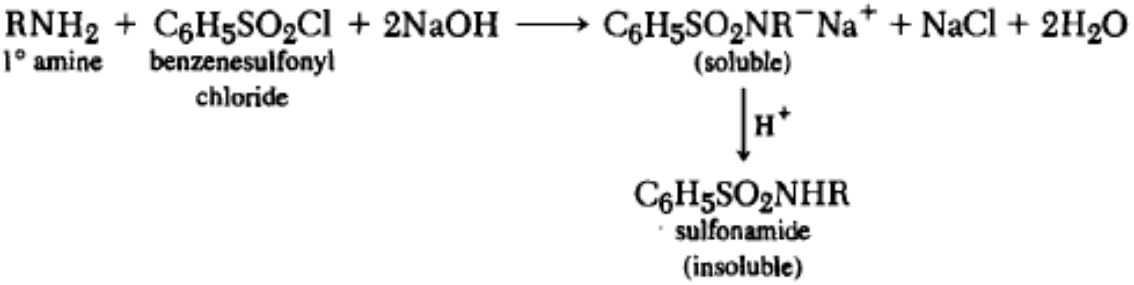
Experiment #13:Amines

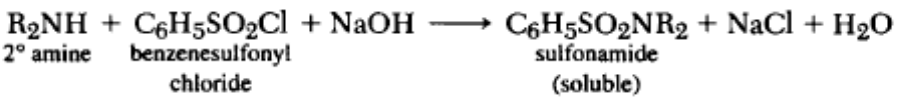
Standard

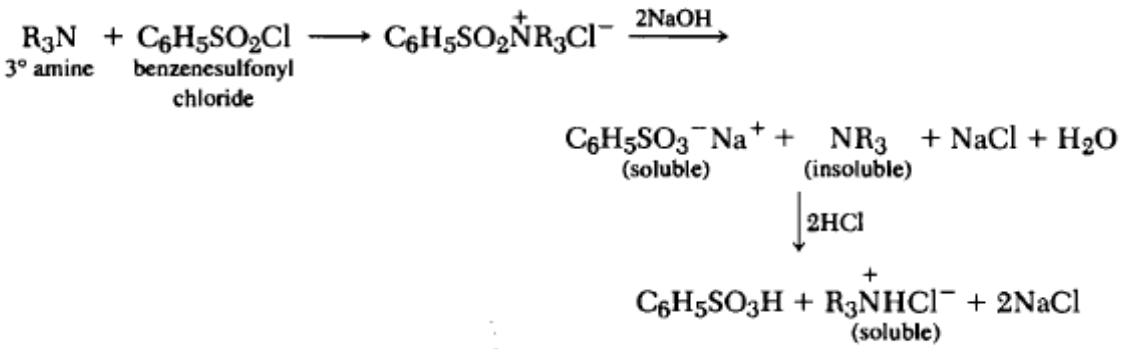
Use aniline, N-methylaniline and N,N-dimethylaniline for knowns.

A. Hinsburg Test for Amines.

If you have a basic compound which you believe to be an amine, you can corroborate your suspicion and determine if you have a primary, secondary, or tertiary amine using the Hinsberg test. You will react the amine with a sulfonyl chloride forming an insoluble sulfonamide of a primary or secondary amine or the soluble salt of a tertiary amine. The insoluble sulfonamide of a primary amine will be made soluble in base (via removal of the slightly acidic proton on N) but that of a secondary amine will not (no proton on N to remove).







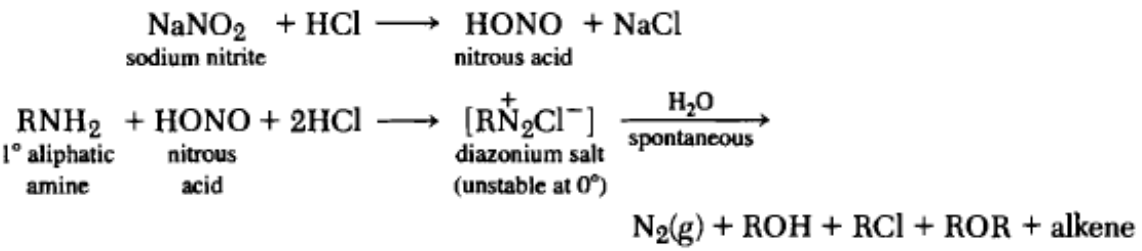
Procedure

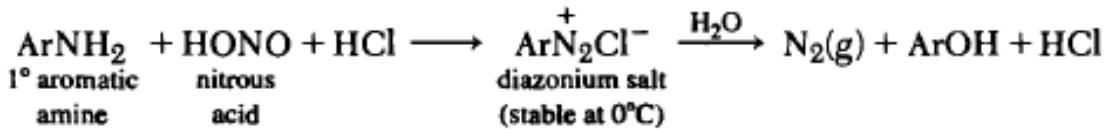
Add 100 mg of a solid or 0.1 mL of a liquid unknown, 200 mg of p-toleuenesulfonyl chloride, and 5 mL of 10% KOH solution to a clean test tube. Stopper the tube and shake it for several minutes. Remove the stopper and heat the mixture on a steam bath for 1 minute. Cool the solution and if it is not basic to pH paper, add additional KOH solution. If a precipitate has formed, add 5 mL of water and shake vigorously. If the precipitate does not redissolve in the basic solution, it is indicative of a sulfonamide of a secondary amine. If there is no precipitate, add 5% HCl until the solution is just acidic when tested by pH paper. Formation of a precipitate under acidic conditions suggests that the previously soluble sulfonamide was of a primary amine. If no precipitate has formed, the initial amine could have been tertiary.

Aniline, N-methylaniline, and N,N-dimethylaniline will give a positive test. Make note of all precipitate formation and dissolution and use melting points to check the identity of the compound as an amine or a sulfonamide.

I. Nitrous Acid

a) Diazotization





Procedure

Dissolve 0.5 mL or 0.5 g of the sample in 1.5 mL of concentrated hydrochloric acid diluted with 2.5 mL of water, and cool the solution to O°C in a beaker of ice. Dissolve

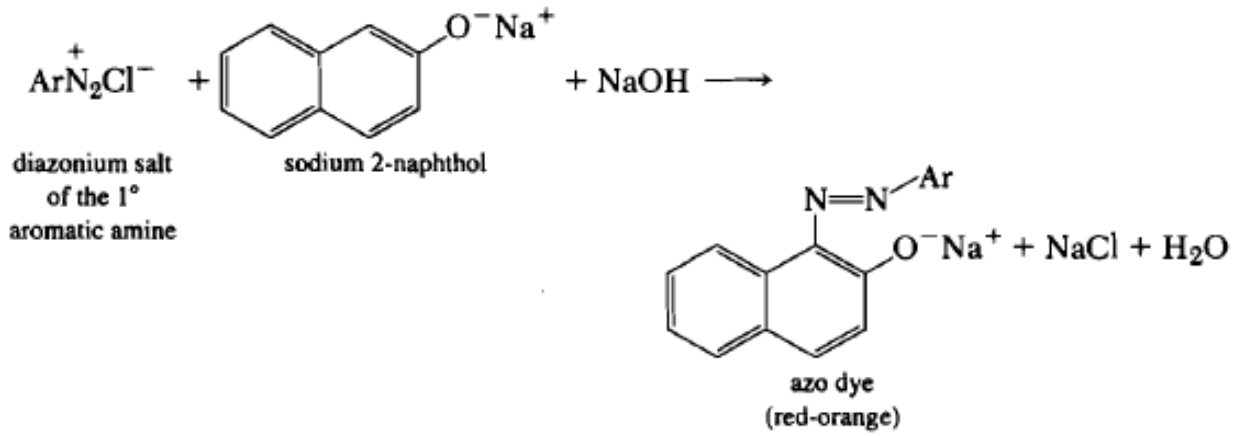
0.5 g of sodium nitrite in 2.5 mL of water, and add the solution dropwise, with shaking, to the cold solution of the amine hydrochloride. Continue the addition

until the mixture gives a positive test for nitrous acid. Perform the test by placing a drop of the solution on starch-iodide paper; a blue color indicates the presence of nitrous acid. If the test is positive, move 2 mL of the solution to another test tube, warm gently, and examine for evolution of gas.

The observation of rapid bubbling or foaming as the aqueous sodium nitrite solution is added at O°C indicates the presence of a primary aliphatic amine. The evolution of gas upon warming indicates that the amine is a primary aromatic amine, and the solution should be subjected to the coupling reaction (b).

If a pale yellow oil or low-melting solid, which is the N-nitrosoamine, is formed with no evolution of gas, the original amine is a secondary amine. The oil or solid is isolated and treated under conditions Qf the Liebermann nitroso reaction (c) to provide confirmation of the presence of the N-nitrosoamine.

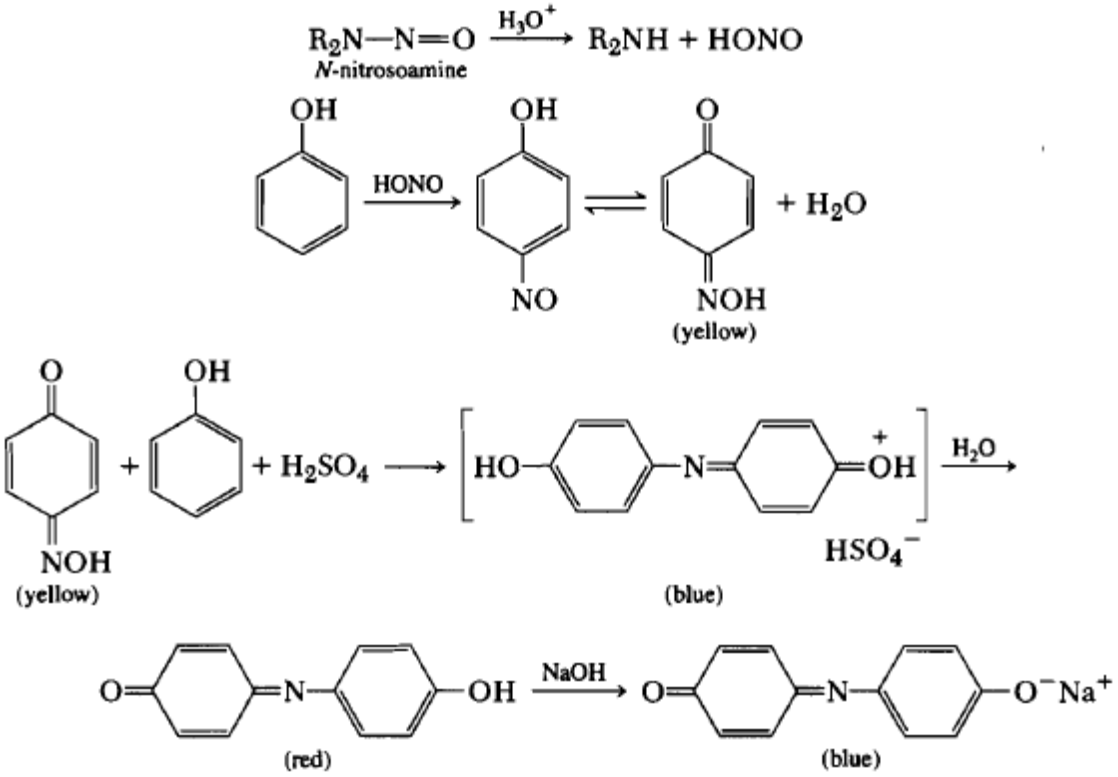
b) Coupling



Procedure

Add 2 mL of the cold diazonium solution to a solution of 0.1 g of 2-naphthol in 2 mL of 10% sodium hydroxide solution and 5 mL of water. The formation of the orange-red dye, with the evolution of gas only upon warming as noted in (a), indicates the original compound is a primary aromatic amine.

b. Liebennann'sNitroso Reaction



Procedure

Add 0.05 g of the N-nitrosoamine, 0.05 g of phenol, and 2 mL of concentrated sulfuricacid to a test tube and warm gently for 20 sec. Cool the solution slightly. A bluecolor should develop, which changes to red when the solution is poured into 20 mL ofice water. Add 10% sodium hydroxide until the mixture is alkaline, and the blue coloris produced again.

The N-nitrosoamine liberates nitrous acid in the presence of sulfuric acid. The nitrousacid then undergoes reaction with phenol to yield the yellow 4-nitrosophenol(quinonemonoxime). The blue color observed in this reaction is due to phenolindophenolformed from the reaction of the initially produced 4-nitrosophenol (quinine monoxime) with excess phenol. This reaction is characteristic of phenols in which an*ortho*or *para*position is unsubstituted.To run a comparative test in order to check the colors, the following procedure maybedone.Addacrystalofsodium nitriteto2mL ofconcentratedsulfuricacid,andshakeuntil dissolved. Add 0.1 g ofphenol, and a blue color will appear. The solution is pouredinto 20 mL of ice water, and the color of the solution changes to red. Addition of 10%sodium hydroxide, until the mixture is alkaline, results in the return of the blue color.

QUESTIONS

1. Using the Hinsberg test and equations, show how propyl amine, diethylamine, and triethylamine can be separated.

2. Give the equations for the reaction of nitrous acid with propylamine, diethylamine, triethylamine, aniline, N-methylaniline, and N,N-dimethylaniline.