M.Sc. CHEMISTRY LAB MANUAL

2nd Semester

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Chemistrty

MIDNAPORE CITY COLLEGE

PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for PG Chemistry second semester. Hope this edition will help in practical. This edition mainly tries to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers , and non-teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

Laboratory Practice Safety Rules

1. Use safety glass when dealing with fire and chemical.
2. Should use front cover clothes during biochemistry practical.
3. Always use hand wash after dissection and any type of chemical use.
4. Carefully handle needles , forceps, microscope and any other dissecting instrument.
5. Use dustbin always.

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CEM-295

Organic Chemistry Practical

Unit-I, IDENTIFICATION OF A LIQUID

(i) Qualitative Organic Analysis:

Identification and characterization of the structures of unknown substances are an important part of organic chemistry. It is often, of necessity, a micro process, for example, in drug analyses. It is sometimes possible to establish the structure of a compound on the basis of spectra alone (IR, UV, and NMR), but these spectra must usually be supplemented with other information about the unknown: physical state, elementary analysis, solubility, and confirmatory tests for functional groups. Conversion of the unknown to a solid derivative of known melting point will often provide final confirmation of structure.

However, before spectra are run, other information about the sample must be obtained. Is it homogeneous (test by thin-layer, gas, or liquid chromatography)? What are its physical properties (melting point, boiling point, color, solubility in various solvents)? It might also be necessary to determine which elements are present in the sample and its percentage elemental composition.

Nevertheless, an organic chemist can often identify a sample by performing solubility tests and some simple tests for functional groups, coupled with spectra that have not been compared to a database. Conversion of the unknown to a solid derivative of known melting point will often provide final confirmation of structure. It is necessary to provide information needed to carry out the type of qualitative analysis of an organic compound.

Procedures

Note the Color

Common colored compounds include nitro and nitroso compounds, diketones, quinones, azo compounds, and polyconjugated olefins and ketones. Phenols and amines are often colored because of traces of air oxidation products.

Note the Odor

Some liquid amines are recognizable by their fishy odors; esters are often pleasantly fragrant. Alcohols, ketones, aromatic hydrocarbons, and aliphatic olefins have characteristic odors.

Solubility Tests

Solubilty serves as a useful classification scheme for all organic molecules. The solubility measurements are done at room temperature with 1 drop of a liquid, (or 5 mg in case of a solid of finely crushed), and 0.2 mL of solvent. The mixture should be rubbed with a rounded stirring rod and shaken vigorously.

If a very small amount of the sample fails to dissolve when added to some of the solvent, it can be considered insoluble; and, conversely, if several portions dissolve readily in a small amount of the solvent, the substance is obviously soluble.

If an unknown seems to be more soluble in dilute acid or base than in water, the observation can be confirmed by neutralization of the solution; the original material will precipitate if it is less soluble in a neutral medium.

If both acidic and basic groups are present, the substance may be amphoteric and therefore soluble in both acid and base. Aromatic amino carboxylic acids are amphoteric, like aliphatic ones.

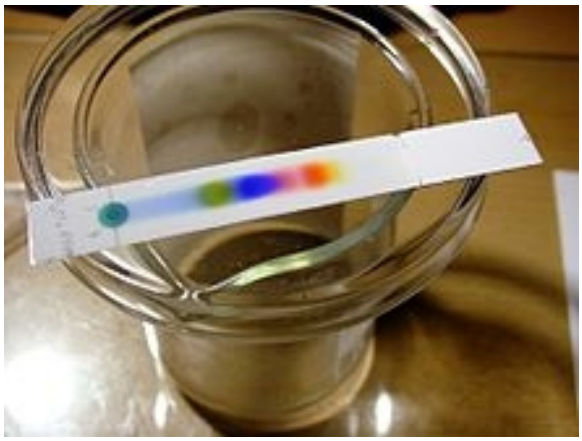
Notes to Solubility Tests:

1. Solubility in water.
   Test the solution with pH paper. If the compound is not easily soluble in cold water, treat it as water insoluble but test with indicator paper.
2. If the substance is insoluble in water but dissolves partially in sodium hydroxide, add more water; some sodium salts are less soluble in alkali than in water. If the unknown is colored, be careful to distinguish between the dissolving and the reacting of the sample.
3. Phenols are acidic to react with sodium bicarbonate.
4. Nitrogen-containing compounds form ammonium ions in concentrated H_2SO_4 and dissolve.
5. On reduction in the presence of HCl, these compounds form water-soluble amine hydrochlorides.
6. Most amides can be hydrolyzed by boiling with sodium hydroxide solution; the acid dissolves with evolution of ammonia to establish the presence of a nitrile or amide.

ii) Thin Layer Chromatography:

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase.

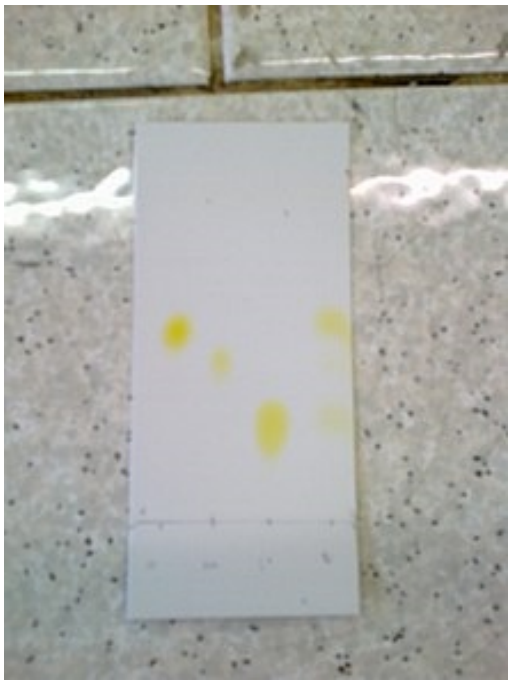
After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate. Because different analytes ascend the TLC plate at different rates, and thereby separation is achieved. The mobile phase has different properties from the stationary phase. For example, with silica gel, a very polar substance, non-polar mobile phases such as heptane are used. The mobile phase may be a mixture, allowing chemists to fine-tune the properties of the mobile phase.



Separation of black ink on a TLC plate

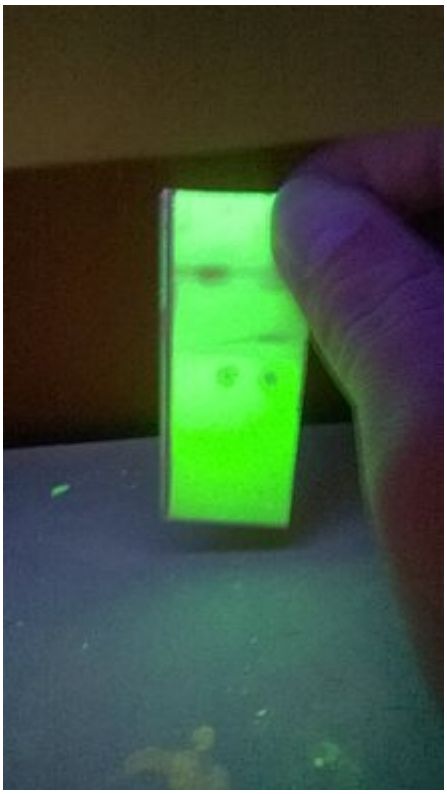
After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light onto the sheet; the sheets are treated with a phosphor (a substance that exhibits the phenomenon of luminescence), and dark spots appear on the sheet where compounds absorb the light impinging on a certain area. Chemical processes can also be used to visualize spots; for example, sulfuric acid will char most organic compounds, leaving a dark spot on the sheet.

To quantify the results, the distance traveled by the substance being considered is divided by the total distance traveled by the mobile phase. (The mobile phase must not be allowed to reach the end of the stationary phase.) This ratio is called the retardation factor (Rf). In general, a substance whose structure resembles the stationary phase will have low Rf, while one that has a similar structure to the mobile phase will have high retardation factor. Retardation factors are characteristic, but will change depending on the exact condition of the mobile and stationary phase. For this reason, chemists usually apply a sample of a known compound to the sheet before running the experiment.



TLC of three standards (ortho-, meta- and para-isomers) and a sample. This designates different retardation factor.

Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Specific examples of these applications include: analyzing organic molecules or identification of medicinal plants and their constituents.



Fluorescent TLC plate under an ultraviolet (UV) light.

A number of enhancements can be made to the original method to automate the different steps, to increase the resolution achieved with TLC and to allow more accurate quantitative analysis. This method is referred to as HPTLC, or "high-performance TLC". HPTLC typically uses thinner layers of stationary phase and smaller sample volumes, thus reducing the loss of resolution due to diffusion.

Preparation of TLC Plates

TLC plates are usually commercially available, with standard particle size ranges to improve reproducibility. They are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water. This mixture is spread as a thick slurry on an unreactive carrier sheet, usually glass, thick aluminum foil, or plastic. The resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the absorbent layer is typically around 0.1 – 0.25 mm for analytical purposes and around 0.5 – 2.0 mm for preparative TLC.

TLC Plates Analysis

i) Technique

TLC is often used for monitoring chemical reactions and for the qualitative analysis of reaction products. Plates can be labeled before or after the chromatography process using a pencil.

To run a thin layer chromatography plate, the following procedure should be carried out:

Using a capillary, a small spot of solution containing the sample is applied to a plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off to prevent it from interfering with sample's interactions with the mobile phase in the next step. If a non-volatile solvent was used to apply the sample, the plate needs to be dried in a vacuum chamber. This step is often repeated to ensure there is enough analyte at the starting spot on the plate to obtain a visible result. Different samples can be placed in a row of spots the same distance from the bottom edge, each of which will move in its own adjacent lane from its own starting point.

A small amount of an appropriate solvent (eluent) is poured into a glass beaker or any other suitable transparent container (separation chamber) to a depth of less than 1 centimeter. A strip of filter paper is put into the chamber so that its bottom touches the solvent and the paper lies on the chamber wall and reaches almost to the top of the container. The container is closed with a cover glass and is left for a few minutes to let the solvent vapors ascend the filter paper and saturate the air in the chamber.

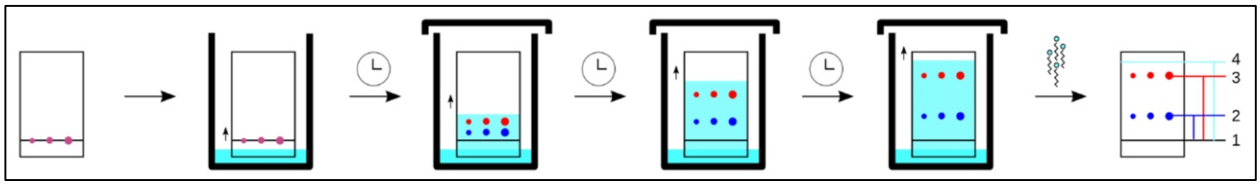
The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the eluent in the chamber, and the top is closed by glass. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate. The plate should be removed from the chamber before the solvent front reaches the top of the stationary phase (continuation of the elution will give a misleading result) and dried.

Without delay, the solvent front, the furthest extent of solvent up the plate, is marked.

The plate is visualized. As some plates are pre-coated with a phosphor such as zinc sulfide, allowing many compounds to be visualized by using ultraviolet light; dark spots appear where the compounds block the UV light from striking the plate. Alternatively, plates can be sprayed or immersed in chemicals after elution (such as by H_2SO_4).

ii) Separation Process and Principle

Different compounds in the sample mixture travel at different rates due to the differences in their attraction to the stationary phase and because of differences in solubility in the solvent. By changing the solvent, or perhaps using a mixture, the separation of components (measured by the R_f value) can be adjusted. Chemists often use TLC to develop a protocol for separation by column chromatography and use TLC to determine which fractions contain the desired compounds.



Development of a TLC plate:

Separation of compounds is based on the competition of the solute and the mobile phase for binding sites on the stationary phase. For instance, if normal-phase silica gel is used as the stationary phase, it can be considered polar. Given two compounds that differ in polarity, the more polar compound has a stronger interaction with the silica and is, therefore, better able to displace the mobile phase from the available binding sites. As a consequence, the less polar compound moves higher up the plate (resulting in a higher Rf value). If the mobile phase is changed to a more polar solvent or mixture of solvents, it becomes better at binding to the polar plate and therefore displacing solutes from it, so all compounds on the TLC plate will move higher up the plate. For silica gel-coated TLC plates, the eluent strength increases in the following order: perfluoroalkane (weakest), hexane, pentane, carbon tetrachloride, benzene/toluene, dichloromethane, diethyl ether, ethyl acetate, acetonitrile, acetone, 2-propanol/n-butanol, water, methanol, triethylamine, acetic acid, formic acid (strongest). However, if a mixture of ethyl acetate and hexane as the mobile phase is used, adding more ethyl acetate results in higher R_f values for all compounds on the TLC plate.

iii) Analysis

As the chemicals being separated may be colorless, several methods exist to visualize the spots. Fluorescent analytes like quinine may be detected under blacklight (366 nm UV light). Often a small amount of a fluorescent compound, usually manganese-activated zinc silicate, is added to the adsorbent that allows the visualization of spots under UV-C light (254 nm). The adsorbent layer will thus fluoresce light-green by itself, but spots of analyte quench this fluorescence.

Iodine vapor is a general unspecific color reagent. Specific color reagents (e.g. $KMnO_4$, H_2SO_4) into which the TLC plate is dipped or which are sprayed onto the plate exist.

Once visible, the R_f value, or retardation factor, of each spot can be determined by dividing the distance the product traveled by the distance the solvent front traveled using the initial spotting site as reference. These values depend on the solvent used and the type of TLC plate and are not physical constants.

R_f = distance traveled by the spot/distance traveled by the solvent.

c) Boiling point determination:

The boiling point of a liquid may be defined as the temperature at which the vapour pressure of the liquid is equal to the atmospheric pressure exerted upon the liquid surface. The boiling point of the liquid depends upon the pressure exerted upon the liquid surface. Since atmospheric pressure is different at different place, therefore a liquid has different boiling points at different places. For the sake of comparison we use normal boiling points. The normal boiling point of a liquid may be defined as the temperature at which vapour pressure of the liquid is equal to one standard atmospheric pressure (760 mm). The boiling point of a liquid increases if non-volatile impurities are present in it.

Materials

100 ml coming glass beaker, a small thin walled test tube, thermometer, a capillary tube, a tripod stand, wire gauze, stirrer, iron stand with clamp, liquid paraffin, and the given liquid.

Procedure:

1. Take a small test tube and fill it two-third with the given liquid whose boiling point is to be determined. Fix this tube to the thermometer with a rubber band. The rubber band should be fixed near the mouth of the tube so that it remains outside the liquid paraffin bath. Adjust the tube so that the bottom of the tube is somewhere at the middle of the thermometer bulb.
2. Clamp the thermometer carrying test tube in an iron stand through a cork. Lower the thermometer along with the tube into a liquid paraffin bath. Adjust the thermometer so that its bulb is well under the acid and open end of the tube with the rubber band is sufficiently outside the acid bath.
3. Take a capillary tube 5-6 cm in length and seal it at about one cm from one end by heating it in flame and giving a slight twist. Place this capillary in the test tube so that sealed part of it stands in the liquid.
4. Start heating the liquid paraffin bath slowly and stir the bath gently. Keep an eye on the liquid and the test tube and also on the thread of the mercury in the thermometer. At first a bubble or two will be seen escaping at the end of the capillary dipping in the liquid, but soon a rapid and continuous stream of air bubbles escapes from it. This is the stage when the vapour pressure of the liquid in the sealed capillary just exceeds the atmospheric pressure. Note the temperature when continuous stream of bubbles starts coming out. Remove the flame and note the temperature when the evolution of bubbles from the end of the capillary tube just stops. The mean of these two temperatures gives the boiling point of the liquid.
5. Allow the temperature fall by 10°C and repeat the heating and again note the boiling point.

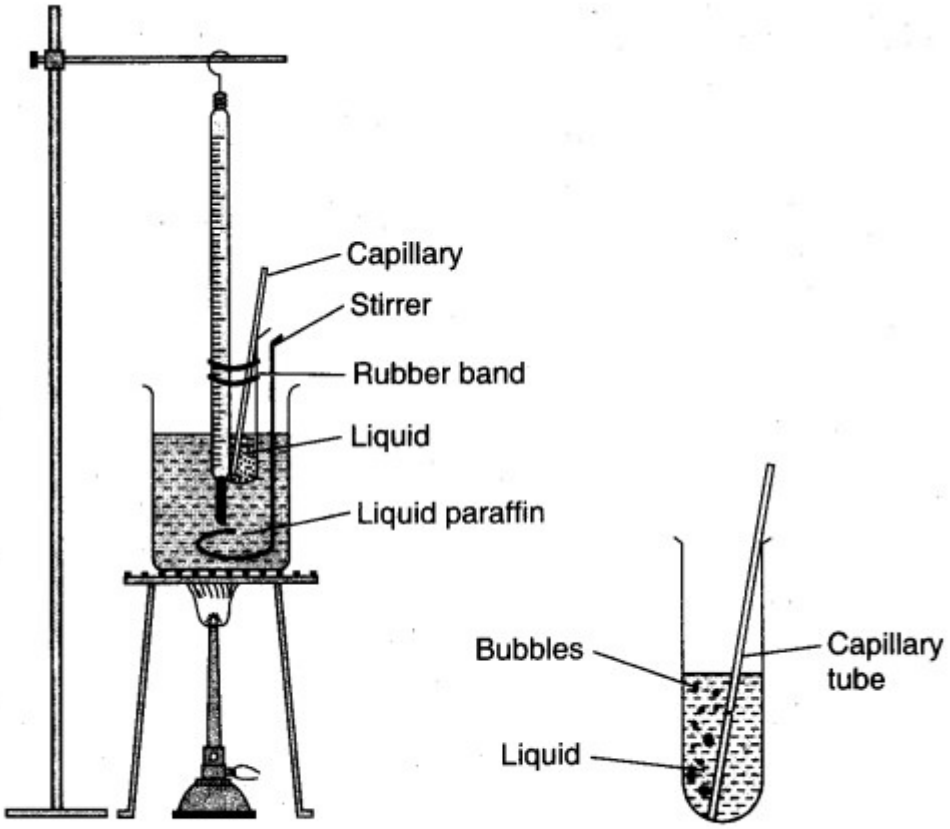


Fig. 4.1. Determination of the boiling point.

Precautions:

1. Keep the lower end of the ignition tube and the thermometer bulb at the same level.
2. Record the temperature as the boiling point at which brisk and continuous evolution of the bubbles starts from the lower end of the capillary dipped in the liquid organic compound.
3. If on placing the sealed capillary tube in the test tube, the liquid is seen rising in the capillary tube, it indicates that the capillary tube is not properly sealed. Reject this capillary tube and use a sealed new one.
4. The sealed point of the capillary tube should be well within the liquid.
5. The paraffin bath must be heated very slowly and the paraffin stirred to ensur e uniform heating.
6. Paraffin can be safely heated upto 220 °C while conc. H_2SO_4 can be heated upto 280°C.
7. For finding the melting points of solids, having lower melting points, liquid paraffin may be used while for solids having melting points greater than 200 °C conc. H_2SO_4 may be used.

Observations and comment:

Boiling point:

(i) t_1 °C

(ii) t_2 °C

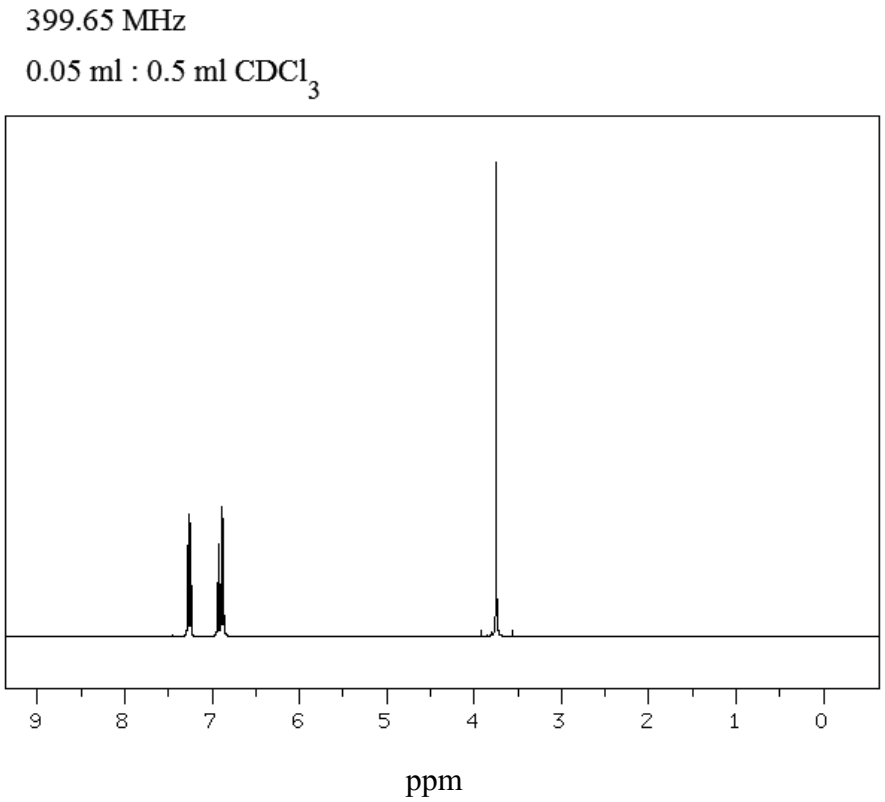
Mean = $(t_1+t_2)/2$ °C

iv) Assign of ^{1}H-NMR, ^{13}C-NMR spectra

In NMR, the nuclei of hydrogen, carbon, and other important elements undergo transitions in their magnetic states, leading to the absorbance of radiation in the radio frequency range of the electromagnetic spectrum. By analyzing the signals in NMR spectra (^{1}H-NMR, ^{13}C-NMR spectra) from these transitions, the chemical environment that each atom inhabits, including information about the presence of neighboring atoms can be known. This information from NMR depicts a possible structure of an organic molecule. Thus two most common forms of NMR spectroscopy, ^{1}H NMR and ^{13}C NMR, will be considered. Nuclear magnetic resonance spectroscopy is a very powerful tool, particularly when used in combination with other spectroscopic techniques for structure determination.

Example:

^{1}H NMR of C_6H_5-OCH_3



From the above spectrum, the following information can concluded:

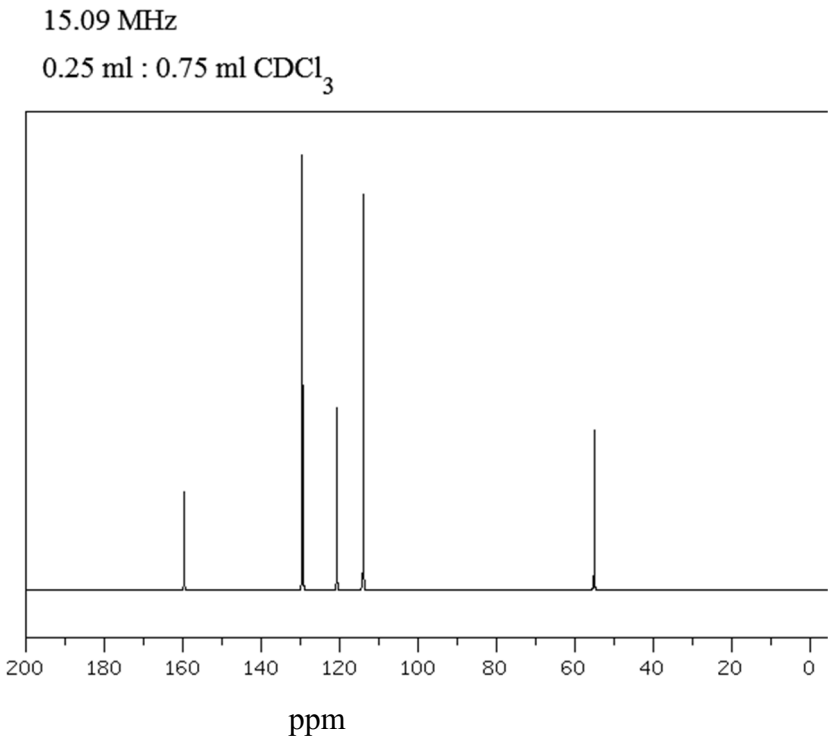
1) The peak at 3.74 ppm with integration area of 3 (approx.) is due to 3 H i.e. CH_3 group. As the group lies near 4.0 ppm, it may be present as OCH_3.

2) NMR range from 6.8-7.3 ppm signifies the presence of benzene group. Furthermore the integration area at 7.25 ppm is 2 (approx.) indicates two *meta*-CH of benzene ring.

3) Integration area at 6.91 ppm is 1 (approx.) indicates *para*-CH of benzene ring.

4) Integration area at 6.88 ppm is 2 (approx.) indicates two *ortho*-CH of benzene ring.

^{13}C NMR of C_6H_5-OCH_3



1) The peak at 55 ppm (integration of 3) is due to CH_3 which may be present as OCH_3.

2) Peak in the range of 110-160 ppm may be due to the aromatic carbons.

3) The peak at 160 ppm with integration area of 2.2 may be due to the carbon of benzene ring where OMe is attached.

4) The peak at 129 ppm (integration area 10) may be due to two *meta* carbons of benzene ring.

5) The peak at 120 ppm (integration area 4) is due to *para* carbon.

6) The peak at 113 ppm (integration area 9) is due to two *ortho* carbon atoms.

v) Identification of liquid substance

Procedure

First the chemical liquid substance should be qualitatively analyzed i.e. findings on the odour, color, solubility, etc. The thin layer chromatography illustrates the nature of the polarity of the liquid substance. Additionally, boiling point gives the indication about the substance. Finally, ^{1}H NMR and ^{13}C NMR help to depict the possible structure of an organic liquid substance.

References:

1. '*An Advanced Curse in Practical Chemistry'* book by Nad, Ghoshal, and Mahaparta.
2. Harry W. Lewis & Christopher J. Moody (13 Jun 1989). *Experimental Organic Chemistry: Principles and Practice* (Illustrated ed.). Wiley Blackwell. pp. 159–173. ISBN 978-0-632-02017-2.
2. A.I. Vogel; A.R. Tatchell; B.S. Furnis; A.J. Hannaford & P.W.G. Smith (1989). *Vogel's Textbook of Practical Organic Chemistry* (5th ed.). Longman Scientific & Technical, ISBN 978-0-582-46236-6.
3. *Spectrometric identification of organic compound* by R. Silverstein, F. Webster, and D. Kiemle. (7th Ed.) Wiley & Sons. ISBN 978-0-632-02017-2.

Unit-II
PREPARATION OF PURE ORGANIC COMPOUND

(i) Preparation of acetyl salicylic acid (aspirin)

Reaction:

Materials required:

Salicylic acid 5 g
Acetic anhydride 7.5 mL

Procedure:

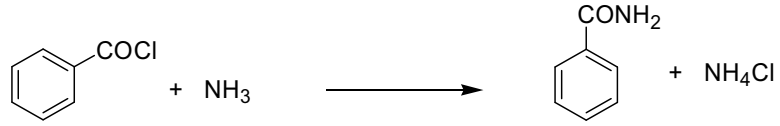
Salicylic acid (5 g) and acetic anhydride (7.5 mL) are taken in a 250 mL stoppered conical flask and mixed thoroughly with shaking. Conc. H_2SO_4 (0.5 mL) is added to that mixed solution and shaken well. The mixture is warmed at 50-60 °C on a water bath for 20 min. with constant stirring. The temperature of the solution should be checked by thermometer. The mixture is allowed to cool with occasional stirring. Then water (75 mL) is added to the cooled solution, stirred and filtered by suction. The product is recrystallized from 50% ethanol and dried.

Yield of the product: …..

M.P. of the product: ……

(ii) Preparation of benzamide

Reaction:



Materials required:

Benzoyl chloride 5 mL
Ammonia 25 mL

Procedure:

Liquid ammonia (25 mL) is taken in a 250 mL conical flask and cooled in an ice-bath. Benzoyl chloride (5 mL) is added drop wise to the flask with occasional stirring. Then the reaction mixture is heated for 5 min. Next, 50 mL water was added to that conical flask containing reaction mixture, the solid separated is filtered under suction, washed finely with cold water. Finally the product is recrystallized from hot water and dried.

Yield of the product: …..
M.P. of the product: ……

References:

1. '*An Advanced Curse in Practical Chemistry'* book by Nad, Ghoshal, and Mahaparta.