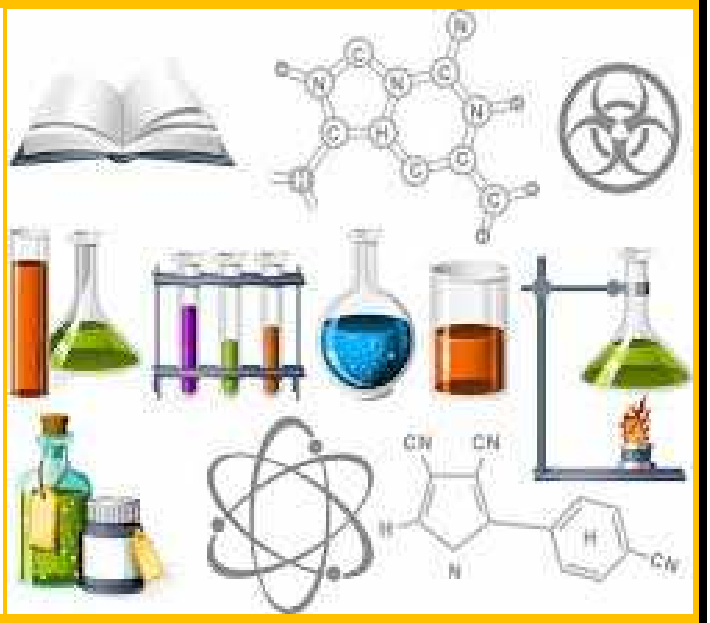
MSCCH-609

M. Sc. IV Semester

Lab Course IV(Organic)



SCHOOL OF SCIENCES

DEPARTMENT OF CHEMISTRY

UTTARAKHAND OPEN UNIVERSITY

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UNIT-1 SEPARATION AND IDENTIFICATION OF ORGANIC COMPOUNDS

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1.1 Introduction

1.2 Objectives

1.3 Choice of Extraction Solvent

- 1.3.1 Flow chart for separation of Ternary mixture by using DCM solvent
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- 1.3.3 Detection of Elements
- 1.3.4 Detection of Functional Group(s)
- 1.3.5 Confirmatory Tests of respective Functional group

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1.1 INTRODUCTION

- For the ternary mixture separation we have use physical method that depending on solubility of compounds in organic solvent (Ether or DCM).
- Ternary mixture also separated by using chemical method that can be describe above, but nature of all constituents in the mixture must different.
- If nature of two or three compounds is same then we can't separate by chemical method.

1.2 OBJECTIVES

- To impart knowledge of basics of separation of organic ternary mixtures.
- To make able to identify type and chemical nature of components of the mixture and separate solid, semi-solid and liquid organic mixtures.
- Identify the elements and functional groups presents in the organic molecule.

1.3 CHOICE OF EXTRACTION SOLVENT

Although water is almost always one of the liquids in the liquid-liquid extraction process, the choice of organic solvent is quite wide. A good extraction solvent needs five essential features:

(1) Has high solubility for the organic compound.

(2) Be immiscible with the other solvent (usually water).

(3) Has a relatively low boiling point so as to be easily removed from the compound after extraction.

(4) Extract little or none of the impurities and other compounds present in the mixture.

(5) Be nontoxic, nonreactive, readily available, and inexpensive.

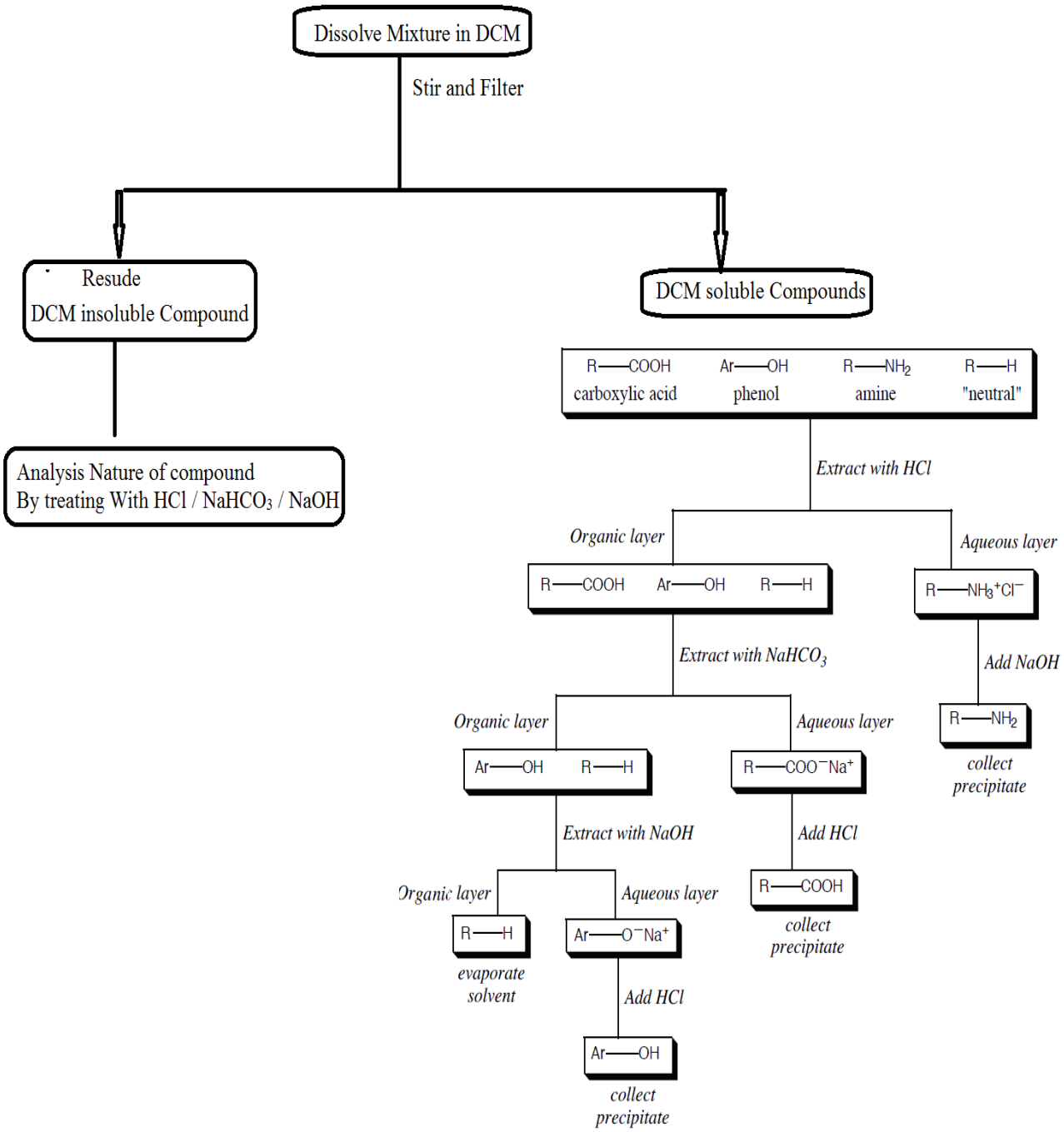
Solvent	Solubility in H_2O	Boiling point (°C)	Density (g/mL)	Safety information
Methylene chloride CH_2Cl_2	Very slightly soluble	40	1.3255	Narcotic in high concentrations; suspected carcinogen
Diethyl ether $(CH_3CH_2)_2O$	Slightly soluble	35	0.7134	Very flammable; forms peroxides
Ethyl acetate $CH_3CO_2CH_2CH_3$	Fairly soluble	77	0.902	Flammable
Hexane $CH_3(CH_2)_4CH_3$	Insoluble	69	0.660	Narcotic in high concentrations

Table 1: below shows some organic solvents used in extraction.

Thus DCM is the good solvent for ternary mixture separation, it has some advantages over other solvent like Diethyl ether these are:

1. It is inflammable, so safe for use in lab.
2. Very slight soluble in water, so avoid mixing in Aqua. layer
3. Somewhat cheaper than Diethyl ether.

1.3.1 Flow chart for separation of Ternary mixture by using DCM solvent



1.3.2 Individual Analysis:

Identification of Organic Compounds

Preliminary Tests-

Test	Observation	Inference
i. Physical State **ii. A. Colour (Solids)**	Solid White Cream	Acids, Phenols, Amines, Anilides, Hydrocarbons may be Preesent. Benzoic acid, Salicylic acid, Naphthalene, Acetanilide may be Present. Cinnamic acid may be Present.
	Light pink	α-Naphthol may be Present.
	Pink Brown White buff / Pinkish Light buff	β-Naphthol may be Present. p-Toluidine, Resorcinol may be Present. Diphenylamine may be Present.
	Yellow Light Yellow	p-Nitroaniline/Nitro Compounds may be Present. m-Dinitrobenzene may be Present.
	Orange Red	O-Nitro aniline May be Present. Azo compounds, β- Napthoquinone, Alizarine may be present.
B. Colour (Liquids)	Colourless (reddish-brown colour appears due to oxidation) Yellow Indistinct	Acetone, Acetophenone, Methyl Ethyl Ketone, Methyl Acetate, Ethyl Acetate, Chloroform, Chlorobenzene May be Present Aniline, Dimethylaniline, Nitro Benzene May be Present.
iii. Odour	Carbolic	

		Benzoic acid, Salicylic acid, p-Nitroaniline, Acetanilide m-Dinitrobenzene may be Present. α-Naphthol, β-Naphthol may be Present.
	Fishy	p-Toluidine, Aniline, Dimethyl Aniline may be Present.
	Pleasant (Alcoholic) Sweet,Pungent irritating, Mouse-like	Alcohols, Ketones, Chloroform May be Present. Aliphatic And aromatic Halogenated Compounds May be Present. Acetic acid, acetic anhydride, Lower acids, lower aldehydes, acid halides, thioacids may be present Acetamide, acetonitrile
	Cinnamon like Bitter Almond Pleasant-fruity Pleasant, sweet Garlic	Cinnamic acid may present Aromatic aldehyde like Benzaldehyde, Nitrobenzene, Nitrotoluenes May be Presents. Ester may be present Chloroform, diphenylamine, alcohols Thiophenols, Thioalcohols May be presents.
	Benzene Like Odour Fragrant Camphor-like	Benzene, Toluene, Xylenes May be Presents. Diphenylamine may be Present. Pinacol, hexachloroethane
	Pyridine-like	Heterocyclic bases

iv. Solubility in Water **v. Saturation Test-Action of KMnO4 (Baeyer Test)** Compound+KmnO4(dil.)	Naptha ball Like Odourless	Naphthalene may be Present. Carbohydrates, aromatic acid, glycerol, solid aliphatic acid May be Present. Acids, Phenols, Amines, Anilides, Hydrocarbons may be Preesent. Unsaturated compound or easily oxidisable compounds, Exception Hydroxy acids, may be present
	No Decolourisation	Saturated compound is Present.
vi. Litmus Test – A small amount of the sub is shaken with water and a drop of the solution is tested for litmus action **vii. Flame Test** Heat the compound on copper wire	a) Acidic b) Faintly Acidic c) Faintly Basic d) Neutral Sooty Flame Non sooty Flame	Carboxylic acids, Nitrophenols, Sulphonic acids, Acid chlorides, Acid anhydries May be presents. Phenols, Cresols may be presents. Amines May present. Alcohols, Aldehydes, Ketones, Hydrocarbons Carbohydrates May be Presents. Aromatic compound is Present. Aliphatic compound is Present.

1.3.3 Detection of Elements-

Preparation of Sodium Fusion Extract-

Lassaignes Test:

Heat a small freshly cut piece of sodium metal in a dry fusion tube till sodium melts. Cool and add given compound to the molten sodium, heat the fusion tube to red hot and then drop it in about 15 ml of distilled water taken in china dish. Carry out three more fusions in the similar way. Concentrate the solution of the dish to half its volume by boiling. Cool and filter the solution. The filtrate, known as sodium fusion extrat is used for the detection of Elements.

This test is aimed to identify the nitrogen, sulphur and halogen in organic compounds and it can be carried out by fusion of the organic substance with sodium metal in the presence of

excess soda lime (NaOH/CaO). The following equations express the reaction of element after reacted with sodium:-

Na + C + N →NaCN (nitrogen compounds)

2 Na + S → Na2S (sulfur compounds)

Na + X → NaX (halogen compounds, X = Cl, Br, ..)

Test	Observation	Inference
1. Test for Nitrogen A) Sodium Extract (2 ml) + Freshly Prepared Ferrous Sulphate solution Boil and Cool. Add dil. H_2SO_4. B) Sodium Extract (2 ml) + $FeCl_3$	Green or Blue Precipitate or Colour Blood red colour	Nitrogen is Present. S And N Present.
2. Test for sulphur Sodium Extract (2 ml) + Sodium Nitroprusside.	Purple or Violet colour	Sulphur is Present
3. Test for Halogens Sodium Extract (2 ml) + Conc. HNO_3, Boil and Cool. Add $AgNO_3$. **If Halogen present proceed as follows**	Precipitate	Halogens are Present
Sodium Extract (2 ml)+ Conc. HNO_3 + Chlorine Water + 1 ml Chloroform + Shake well & Observe the Colour of	A) Violet Colour B) Yellow Colour	Iodine Present. Bromine Present. Chlorine Present.

Chloroform	C) Colour less	

Conclusion: - The compounds contains C, H, (O) and ---- as the Elements.

1.3.4 Detection of Functional Group(s)-

Test	Observation	Inference
1. Comp. + 10% $NaHCO_3$ Soln	Effervescence of CO_2 PPT By Adding Conc. HCl	-COOH group is Present.
2. Comp. + Water + Neutral FeCl3 Solution.	Blue, Violet or Green Colour.	–OH group is Present.
3. Comp.+ NaOH	Soluble and PPt by adding Conc. HCl	Phenolic –OH group is Present.
4. Comp. (if liquid) + Na metal	Soluble with effervescence	Alcoholic –OH present
5. Comp.+ Dil HCl	Soluble And PPT by adding NaOH	Amino –NH2 group is present.
6.**a)** Comp. + Alcoho l+ 2,4 dinitrophenylhydrazine (Boil) **b)**Comp. + Schiff's Reagent	Yellow, Orange or Red PPT Violet colour develops Pink colour slosly develops	Aldehydes or Ketones Present. Aliphatic aldehyde present Aromatic aldehyde present
7. Comp. + NaOH + Sodium nitroprusside(freshly prepare)	Red colouration	Ketone present
8. a) Comp.+NaOH + Zn Dust (Boil) b) Comp. + Conc. HCl+ Zn dust + $NaNO_2$ freshly prepare β- Napthol in NaOH	Black or Gray PPT Orange dye stuff	NO2 group is present.
9. Comp.+ NaOH (Boil)	Ammonia gas evolves turns	Amide –CONH2 group

	moist turmeric paper brown	Present.
10. Comp.+Conc. HCl (Boil) Cool and add Cold $NaNO_2$+**β**–Naphthol in NaOH.	Red dyestuff	Anilide –NHCO group present
11. Comp.+1 ml H_2O+2,3 drops 10% ά–Naphthol in alcohol+1 ml Conc. H_2So_4	Reddish Violet Colouration at the Junction of two layer	Carbohydrates present.
12. If all above test fails		Hydro Carbons Present.

Conclusion: - The compound contains ---------- as the Functional Group(s).

1.4.5 Confirmatory Tests of respective Functional group:

Compounds containing C, H and (O) as the Elements-

(A) Confirmatory Tests for Acid

Test	Observation	Inference
10 mg substance + sat. $NaHCO_3$	Effervesces of CO_2	Acid is Confirmed.
Separation Of Acid Neutral Solution of Acid Substance + 2 drops of $FeCl_3$	a)Buff or Brown PPt Soluble in Dil. HCl b) Violet colour discharged by dil HCl c) Red colour disappear in dil HCl	1)Benzoic Acid (Mp. 122oC) 2) Cinnamic acid (Mp. 133oC) 3) Phthalic Acid (Mp. 133oC) 1) Salicylic Acid (Mp. 158oC) 2) Aspirin (Mp. 135oC) Acetic acid (Bp 118oC)

Neutral Solution: 0.1 gm of given acid substance + 1 ml NH4OH boil well till ammonia gets evolved (i.e. moist turmeric paper should not turn brown). Use this solution for separation of above acids.

Separation of acids

(B) Confirmatory Tests for Benzoic Acid –

Test	Observation	Inference
1. Comp. + Ethyl alcohol + 2-3 drops of conc. H_2SO_4 and Heat.	Pleasant smell of Ethyl benzoate	Benzoic Acid is Confirmed.
2. Comp. + Water + Neutral $FeCl_3$ Solution.	Buff ppt soluble in NH_4OH.	Benzoic Acid is Confirmed.

(C) Confirmatory Tests for Cinnamic Acid-

Test	Observation	Inference
1. Comp. + Ethyl alcohol + $CaCl_2$ Solution.	White ppt insoluble in acetic acid.	Cinnamic Acid is Confirmed.
2. Comp. + 2 drops of 1% $KMnO_4$ solution + 1 ml of Na_2CO_3 Solution.	Decolourisation of $KMnO_4$ solution.	Cinnamic Acid is Confirmed.

(D) Confirmatory Tests for Salicylic Acid-

Test	Observation	Inference
1. 0.5 gm of Compound + 5 drops of CH_3OH + 3 drops of conc. H_2SO_4 and Heat gently on Water bath. Cool and pour this into about 5 ml of water. Add solid Na_2CO_3.	Pleasant smell of methyl salicylate (Oil of Winter Green).	Salicylic acid is Confirmed

(E) Confirmatory Tests for Oxalic Acid:-

Test	Observation	Inference
1.Neutral Solution of Acid Substance + 2 drops of $CaCl_2$ Solution	White PPT insoluble in dil. Acetic acid But Soluble in dil. HCl	Oxalic Acid is Confirmed.

1.4 RESULTS

Steps	Component I	Component II	Component III
• Nature • Type • Elements • Functional group(s) • Physical Constant • Name of Compound • Molecular formula of Compound • Structure of Compound			

1.5 EXPERIMENT

Aim: To Separate and Identify the give Ternary mixture.

Requirement: Water, Glassware, Burner, Filter paper, Test tube etc.

Chemical required: Alkaline $KMnO_4$, Na_2CO_3, $FeSO_4$, NaOH, H_2SO_4, $Na_2[Fe(CN)_5NO]$, HNO_3, $AgNO_3$, NH_4OH, $FeCl_3$, $CHCl_3$,$CuSO_4$, HCl etc.

SEPARATION:

In given ternary mixture of Organic compounds 'A' is liquid while other two (B+C) are solid. So compound 'A' can be separated by filtration, while 'B' and 'C' are separated on the basis of their solubility in water. 'B' is soluble while 'C' is insoluble water; 'B' can be separated by Evaporation after solubility.

Compound 'A'-

1. Preliminary examination:

Physical state -Liquid

Colour -Pale Yellow

Odour -Smell like bitter Almond

2. Ignition Test: Small portion of compounds on a metallic spatula and ignite it over flame.

Observation- Burns with a sooty flame.

Inference: May be an aromatic compound.

3. Solubility behavior-

S. No.	Observation	Inference
1.	Compounds + cold water	Insoluble
2.	Compounds + Hot water	Insoluble
3.	Compounds + dil. $NaHCO_3$	Insoluble
4.	Compounds + dil. NaOH	Insoluble
5.	Compounds + Con. H_2SO_4	Insoluble
6.	Compounds + Con. HCl	Insoluble

4. Test for unsaturation-

Original solution + 1ml dil. Na_2CO_3 + few drop $KMnO_4$ ⟶ Colour of KMnO4 disappears.

Observation: *Given compounds may be unsaturated.*

5. Element detection: Nitrogen, sulphur and halogen are usually detected by Lassaigne's test.

PREPARATION OF SODIUM EXTRACT:

1. Take a small piece of dry sodium in a fusion tube.
2. Heat the tube slightly on a Bunsen burner so that the sodium melts to a shining globule.
3. Add a pinch of the organic compound.
4. Heat it slowly to start with so that the compound reacts with sodium metal.
5. Now heat the tube strongly till it becomes red hot.
6. Plunge the red hot tube into a china dish containing distilled water.
7. Crush the contents with a glass rod and heat to boiling point.
8. Stop heating and remove the insoluble matter by filtration.
9. The filtrate is called Lassaigne's Extract.

ELEMENTAL DETECTION

1. Test for Nitrogen		
Experiment	**Observation**	**Inference**
2-3 ml sodium extract + 0.2 gm $FeSO_4$	Dirty green ppt	Nitrogen is present
2. Test for Sulphur		
1ml sodium extract + 1ml $Na_2[Fe(CN)_5NO]$	No Purple colour	Sulphur absent
3. Test for Halogens		
1.5ml sodium extract + dil. HNO_3 + 2.5ml $AgNO_3$	No ppt Present	Halogen absent

6. Test for Functional Group:

As we know that in this compound Nitrogen is present, hence, the functional groups may be Amide, 1^0 Amine, Anilide and Nitro.

S. No.	**Experiment**	**Functional Group**	**Observation**

1.	0.5ml organic substance + 1ml NaOH and Heat	Amide	No smell of Ammonia (Absent)
2.	***Carbylamine Test:*** Compound + 4ml alc. KOH + $CHCl_3$ + heat	$(-NH_2)$ 1^0 *amine*	No offensive order of Carbylamine (Absent)
3.	***Carbylamine Test:*** 0.5 gm of organic substance + 4ml alc. KOH + Heat	Anilide (Ar. NHCOR)	No bad smell of carbylamine (Absent)
4.	***Mulliken- Barker Test:*** 0.2 gm /2-3 drops of O.S. + 5 ml alcohol + Zinc dust + 10 ml 10% $CaCl_2$ + boil than filter it in 2ml Tollens reagents + heat	Nitro $–NO_2$	Black ppt formed ($–NO_2$) group present

7. Specific Test:

Reduce compound to aniline with the help of 5n HCl and perform carbylamine test.

Compound + 5n + HCl ⟶ *Aniline*

8. Melting point and boiling point test:

=Boiling point~210.9^0

Result: The given organic compound 'A' is Nitrobenzene

Compound 'B': (Cold water soluble)

1. Preliminary Test:

- *Physical state: Crystalline solid*
- *Colour: White*
- *Odour: odorless*

2. Ignition test

Experiment	Observation	Inference
Small portion of compound on metallic spatula and ignite it over flame	Burn with a non sooty flame	May be an aliphatic compound

3. Solubility behavior:

Experiment	Observation
Compound + cold water	Soluble

4. Test for Unsaturation:

Experiment	Observation	Inference
O.S.(1m) + 1ml dil. Na_2CO_3 + few drop of $KMnO_4$	$KMnO_4$ color does not appear	Compound may be saturated

5. Element Detection:

1. Test for Nitrogen		
Experiment	**Observation**	**Inference**
2-3ml sodium extract + 0.2gm $FeSO_4$	Dirty green ppt obtained	Nitrogen is present
2. Test for Sulphur		
1ml sodium extract + $Na_2[Fe(CN)_5 NO]$	No purple color obtained	Sulphur is absent
3. Test for Halogen		
1.5ml sodium extract + dil. HNO_3 + 2.5ml $AgNO_3$	No ppt Present	Halogen absent

6. Test for functional group:

Due to the presence of Nitrogen functional group may be Amide, Anilide or nitro group.

Functional group	Experiment	Observation
Amide	0.5ml O.S. + 1ml NaOH and Heat	Smell of Ammnia (Amide Present)

7. Specific Test:

Experiment	Observation	Inference
Heated alone or with dil. NaOH	Ammonia smell appeared	Urea is present
Biuret test: 0.2 gm of compound and heat gradually	Ammonia evolved and liquid solidified and biuret formed	Urea is present

8. Melting point:

The melting point is = 132^{o}C

Result: The given Organic Compound 'B' is Urea

Compound 'C'

1. Preliminary examination:

- Physical state- Solid state
- Colour – Orange colour

2. Ignition test:

Experiment	Observation	Inference
Small portion of compound on metallic spatula and ignite it over flame	Burn with a sooty flame	May be an aromatic compound

3. Test for Unsaturation:

Experiment	Observation	Inference
O.S.(1m) + 1ml dil. Na_2CO_3 + few drop of $KMnO_4$	$KMnO_4$ colour disappeared	Compound may be unsaturated

4. Element Detection:

1. Test for Nitrogen		
Experiment	**Observation**	**Inference**
2-3ml sodium extract + 0.2gm $FeSO_4$	Dirty green ppt obtained	Nitrogen is present
2. Test for Sulphur		
1ml sodium extract + $Na_2[Fe(CN)_5NO]$,	No purple color obtained	Sulphur is absent
3. Test for Halogen		
1.5ml sodium extract + dil. HNO_3 + 2.5ml $AgNO_3$	No ppt Present	Halogen absent

5. Test for functional group:

S. No.	**Na_2CO_3**	**Observation**
1.	Carboxilic acid	**$NaHCO_3$ test:** 1gm of compound + 5 ml cold 50% solution of Na_2CO_3
2.	Phenols (Phenolic OH)	**$FeCl_3$ test:** 1 ml of O.S. + 2-3 drops of dilute $FeCl_3$

6. Specific Test:

S. No	**Experiment**	**Observation**
1.	Alcoholic solution of compound + $FeCl_3$	Green color appeared
2.	Compound + $CHCl_3$ + KOH	Blue color obtained

7. Melting point: The melting point is = 132^oC

Result: The given Organic Compound 'C' is β-Napthol

1.6 REFERENCE

https://iscnagpur.ac.in/study_material/dept_chemistry/3.1_MIS_and_NJS_Manual_for_Qrganic_Qualitative_Analysis.pdf

UNIT 2: EXTRACTION

CONTENTS

2.1 Introduction

2.2 Materials and Methods

2.3 Results

2.4 Discussion

2.5 Summary

2.6 Sample experiment

2.7 Reference

2.1 INTRODUCTION

Tea is one of the most commonly used caffeinated beverages in the world. The caffeine $C_8H_{10}N_4O_2$ found in tea is a bitter, white, crystalline methylxanthine and a member of a class of compounds known as alkaloids (Wang, 2011). Alkaloids are basic nitrogen containing compounds present in plants. The structure of caffeine affects the functions it performs.

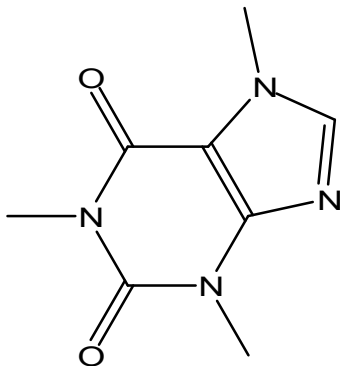


Fig. 1 Structure of caffeine

Alkaloids, such as caffeine, are often physiologically active in humans and are known central nervous system stimulants and diuretics (Wang, 2011). Caffeine also causes an increase in respiration and heart rate, as well as nervousness and insomnia. Though caffeine has demonstrated to have physical dependence, it is also capable of improving alertness, learning capacity, and exercise performance (NCBI, 2013). Tea leaves, in which caffeine is found, also contain acidic tannins, undecomposed chlorophyll, cellulose, and pigments. In order to extract caffeine from tea leaves, caffeine must be present as the free base (Amrita, 2013). In order to do so, the above-mentioned acidic substances must remain water-soluble. In order to extract caffeine from tea, several methods are used. First, a solid/liquid extraction must take place in order to get the solid natural product into the liquid solvent. This can be done by using a Soxhelet extractor, or by simply brewing a cup of tea. In order to isolate the desired reaction compounds from the natural product, liquid/liquid extractions are used.

Neutral and acid/base are two forms of liquid/liquid extractions (Williamson, 2011). Caffeine extraction from tea leaves involves an acid/base liquid/liquid extraction (Oneota, 2003). The reaction involves a homogenous mixture of an organic and aqueous layer. The ideal solvent

in the extraction should have a low boiling point, not react with the solute or other solvents, not be toxic or highly flammable, not miscible with water, be inexpensive, and should readily dissolve caffeine at room temperature. A common liquid/liquid solvent pair for the extraction of caffeine is water-dichloromethane (Williamson, 2011). Because water is present in the pairing, it possible to separate inorganic compounds from organic compounds due to the fact that organic substances are immiscible in water.

When mixing the liquid pairs, the density of the both solvents predict which solvent is the top and which the bottom layer is. Caffeine, which was present in the organic layer, was located below the aqueous layer. The product that is collected after extraction still has many impurities. Sublimation is one way to purify the sample, because caffeine has the ability to pass directly from the solid to vapor and reverse to form a solid all without undergoing the liquid phase. Caffeine has the ability to undergo sublimation under different conditions than the impurities, and can thus be isolated. A series of techniques were used to extract pure caffeine from tea leaves. The percent error and percent recovery were calculated to assess how much pure caffeine was obtained, and to account for errors that may have occurred that led to a loss of product.

2.2 MATERIALS AND METHODS

In order to determine the amount of caffeine in tea, a series of techniques were performed. First, a 50mL beaker was obtained and 2 grams of sodium carbonate were added in order to ensure that caffeine remains present as the free base. The solution was brought to a boil and one bag of Lipton tea was added. 10mL of distilled water was added and the bag was left so steep for 5 minutes. After the 5 minutes had passed, the tea bag was squeezed in order to remove excess water and second bag was added in its place. The second bag was left to steep for 5 minutes and then removed from the beaker after being carefully squeezed to remove excess water. These solid/liquid extractions were performed in order to separate caffeine into the solvent and isolate it from the natural product. The extract was then poured into a centrifuge tube, or separatory funnel, to ensure proper mixing and placed in an ice bath to cool. 3 portions of 2mL CH_2Cl_2 were then used to extract the caffeine from the solution. The tube was shaken gently after each portion was added in order to mix the solvents while avoiding emulsions. One portion was added, and a transfer pipet was used to remove the bottom layer that contained CH_2Cl_2 and

caffeine and place it in a 50mL beaker. This process was repeated two more times for the two remaining portions of CH_2Cl_2 (dichloromethane). There were only a few brown specks in the solution, so a quick swirling motion of the beaker caused them to stick to the sides. The dichloromethane organic layer that contained the caffeine was transferred to another 50mL beaker and a drying agent in the form of calcium chloride ($CaCl_2$) beads was added in order to remove excess water. Calcium chloride was the drying agent of choice because it is ideal for microscale experiments. After the excess water had evaporated, the caffeine was extracted and placed in a weighed filter flask. Evaporation of the solvent, dichloromethane would leave a crude version of caffeine. Sublimation would produce a more pure version of caffeine because it would go straight from the solid to vapor form, and then recrystallize again after bypassing the liquid form once more. The caffeine can also be extracted by using a vacuum filtration apparatus in which the organic solution is placed in a vacuum filter, and the caffeine is collected on the filter paper and let to dry (Williamson, 2011).

2.3 RESULTS

Table 1: Measured Weights of Flask, Solution, Flask/Caffeine, and Caffeine Product.

Weight of flask, g	29.5780 g
Weight of dichloromethyl/caffeine solution, g	4.960 g
Weight of flask and caffeine product, g	29.643 g
Weight of caffeine product, g	.065 g
Theoretical mass of Caffeine (1 bag)	.055 g (2 bags used)

The various weights of the flasks and solutions were obtained in order to calculate the final amount of caffeine product in grams. The final amount of caffeine extracted could then be used to calculate percent error and percent recovery.

Example

Percent Error = (expected-actual/ expected) x 100% (Eq. 1)

Percent Error = (.12-.063/.12) x 100%

Percent Error= 47.5%

Example
% Recovery= (actual/expected) x 100
% Recovery= .063 g/.12 g x 100
% Recovery=52.5 %
Table 2: Error and Percent Recovery

% Recovery	59.1 %
% Error	40.9%

The percent yield and percent recovery were calculated by using the final amount of caffeine obtained (.065 g) and by using the known value of caffeine in two tea bags (.11g total, .055 g per bag). The percent recovery makes it possible to understand how much pure product was recovered from the crude product. The percent error accounts for the mistakes that led to a loss of product.

2.4 DISCUSSION

The structure of the caffeine extracted from the tea leaves deeply impacts the functions it performs . Essentially, caffeine is a purine with three functional groups: an amine, amide, and an alkene. The basic property of caffeine comes from the lone pair of electrons found around the nitrogen(s) (NCBI, 2013). It is an achiral molecule and does not have any stereoisomers. Caffeine is also a polar molecule; this is evident because of the london dispersion forces, dipole dipole interactions, and hydrogen bonding present when it is in water. It also has a very hydrophobic region (NCBI, 2013). The nitrogen present in caffeine controls solubility. Caffeine is soluble in water at approximately 2.2 mg/ml at 25°C, 180 mg/ml at 80° C, and 670 mg/ml at 100°C (Williamson, 2011). It is an organic molecule that has the properties of an organic amine base (Tello, 2011) .When extracting caffeine, the water was kept at a high temperature in order to increase solubility of caffeine in water to about 670 mg/ml at 100°C. Boiling chips were added to the solution in order to prevent “bumping” and enable the smooth formation of bubbles when boiling occurs. The solution was later cooled to a lower temperature in order to impact the solubility once more and to minimize the attraction to the aqueous layer. While in the separatory

funnel. The solution was also cooled before the dichloromethane was added because dichloromethane has a boiling point of 40°C. If the cold water was not added to lower the temperature, the dichloromethane would have evaporated and caffeine would not be properly extracted. During the solid/liquid extraction the solid insoluble material such as cellulose is separated from caffeine and tannins, which are water soluble. In order to isolate caffeine a difference in solubility must occur to separate the tannins into the aqueous layer. Sodium carbonate is added to the extraction medium to ensure that the acidic components in the tea leaves remain water soluble and that caffeine is the free base. Sodium carbonate is basic. Tannins are acidic compounds with a high molecular weight that have an –OH directly bound to an aromatic ring. Because tannins are acidic and can be converted to phenolic salts by deprotonation of the –OH group when a base is added, it is possible to separate the tannins from caffeine. Sodium carbonate serves two main functions: to place caffeine in a more basic environment so that it has a higher affinity for dichloromethane and to cause the tannins to form phenolic salts in the aqueous solution. Adding something basic to caffeine will make it more neutral, and the "like dissolves like" idea can be applied. In this situation, the sodium carbonate acts as a nucleophile and the tannin is an electrophile.

Nucleophile attacks electrophile. It is basically an acid/base reaction. The aqueous layer (density of 1 g/ml) contained dissolved tannin salts and chlophyll. Dipole dipole interactions, london dispersion forces, hydrogen bonding, and ionic bonding with the salts took place.When dichloromethane was added to extract caffeine from the aqueous solution, two immiscible layers formed: an organic and aqueous layer. In this instance, caffeine is usually a polar substance, but it becomes significantly less polar when it is in a basic solution. Therefore, it is soluble in dichloromethane and suspends in the organic layer. Dichloromethane is an alkyl halide and is denser than water, so it is located at the bottom of the separatory funnel. It has a density of 1.325g/m. It had chloro functional groups that make it susceptible to both substitution and elimination reactions. The concentration of the solutes in the organic layer also contributes to the fact that it is located below the aqueous layer. There is a high concentration of caffeine, reactants (because the reaction does not go to 100% completion), and small amounts of water. The intermolecular forces in the organic layer are van der walls interactions, dipole dipole moments, and london forces. Caffeine was extracted with dichloromethane in order to "wash" it three separate times to obtain as much of the pure sample.

Emulsions are small droplets of the organic layer that are suspended in the aqueous that are a result of vigorous shaking of the separatory funnel (Williamson, 2011). There are numerous ways to remove emulsions, though the best form is prevention. However, they can the emulsions may break after a sufficient amount of time. The aqueous layer can also be made more ionic, and centrifugation works very well especially on a microscale level.

A drying agent was added to the organic layer because dichloromethane dissolved not only the caffeine, but water as well. The drying agent, anhydrous $CaCl_2$ was added to remove excess water so that a pure sample of caffeine could be obtained after the solvent evaporated at room temperature (Williamson, 2011). Anhydrous calcium chloride has a high affinity for water, and then reverses back to the hydrous form after it has absorbed the water. Calcium chloride is a preferred drying agent because the pellets form clumps when excess water is present that make it simple to identify how much drying agent is needed. The pellets stopped clumping together when excess water was removed. It is also very rapid, effective, and ideal for microscale experiments. In order to remove the dichloromethane, the beaker could be placed in a hot water bath so that the solvent would evaporate and leave a pure sample. Sublimation is a technique that may have been used to produce a purer caffeine sample, but it could have led to a higher loss of product. Liquid-liquid extractions were used to transfer a solute from one solvent to another and isolate desired product.

A total of 110 mg of caffeine could have possibly been extracted from the two bags of tea. The weight of the caffeine extract was .065 g. The calculated percent recovery was 59.1 %. This was the amount of caffeine extracted from the crude caffeine in the tea bags. This demonstrates that there was a significant amount of product lost throughout the procedure. It is also important to consider that the reaction cannot go to completion, so 100% yield is not possible. A loss of product could have occurred due to emulsions and due to not thoroughly "washing" with dichloromethane to extract as much caffeine as possible. There was a lot of transfer throughout the procedure, which presented many opportunities to lose product. It is also possible that the concentration of caffeine was not height enough because too much water was added. A systematic error with the scales was observed due to a lack of calibration, this could have affected the measurement of the final product. Another source of error could be the theoretical amount of caffeine in the tea bags, if it was more or less due to random error, the

percent recovery would be calculated differently. The overall percent error was about 40.9 %. This number could be skewed due to measurement errors of the crude product. In order to reduce sources of error in the future, two trials could be done. It is also possible to use a different source of caffeine, and to ensure all techniques are performed properly.

2.5 SUMMARY

Overall, a total of .065 g was obtained from a possible amount of .055 g per tea bag, or .11 g total. The total percent recovery was 59.1 %. This number reflects on how accurate the procedure was performed. It was not possible to obtain 100% recovery because the reaction never goes 100 % to completion and because of material loss through transfer during the procedure.

2.6 SAMPLE EXPERIMENT

Objective: Extraction of Lycopene from Tomato.

Requirement: Tomato puree, Petroleum ether, chloroform.

Procedure: At first dry the tomato slurry then add the dried paste of tomato puree in 1:1 mixing of petroleum ether and chloroform mixes it very well. Added only $MgSO_4$ to remove the remaining water present in tomato slurry. Now filter the mixture lycopene dissolve in chloroform so it comes in filtrate. Petroleum ether is highly volatile so it vaporized when mixture leaves for some time. The obtained dried compound is lycopene.

Result: λ_{max} value for lycopene is 476 nm.

2.7 REFERENCES

https://studylib.net/doc/25245243/isolation-of-caffeine-from-tea-leaves-via-acid-base-liqui.

UNIT 3: ISOLATION

CONTENTS

3.1 Objectives

3.2 Introduction

3.3 Experiments

- 3.3.1 To Isolate of casein from milk.
- 3.3.2 Isolation of Nicotine from Tobacco
- 3.3.3 Isolation of lactose from milk
- 3.3.4 Quinine from Cinchona bark
- 3.3.5 Isolation of piperine from black pepper.
- 3.3.6 Isolation of Lycopene and β-Carotene from Tomato
- 3.3.7 Isolation of limonene from citrus fruits

3.4 Reference

3.1 OBJECTIVE

In this chapter you will separate the casein and lactalbumin proteins from a milk sample. These numbers will be used to calculate the percentage of protein in milk compared to the stated amount.

3.2 INTRODUCTION

The main source of nutrition for young mammals is milk, a complex biological cocktail of molecules. Water, several vitamins, minerals, proteins, carbohydrates, and lipids are just a few of the biological substances found in milk. DNA, while essential for life, is produced by your body and is not required on a nutritional level. Although most mammals cease drinking milk once they reach adulthood, many (but not all) human cultures continue to do so, along with the use of dairy products like cheese, butter, and cream.

Because some of the compounds are only present in minute amounts, it would be incredibly difficult to separate every component of milk. But it's quite simple to separate the components that are most prevalent from one another. A typical illustration of this is the process of making skim milk by skimming the fat from cow's milk or lactose-free milk by skimming the sugar from milk. The average makeup of the milk that humans consume from various mammals is shown in Table 1 for reference. It is necessary to briefly explore the components of milk, which is what is done below.

Table 1. Milk components (% composition)

	Cow	**Human**	**Goat**
Water	87.8	87.4	87.0
Protein	3.0	1.4	3.3
Lipids	3.9	4.0	4.2
Sugars	4.6	7.0	4.8
Minerals	0.7	0.2	0.7

Lipids:

If you were asked if fat mixes with water, chances are you would say "no". However, milk contains around 4% fat in water. This means that in 100 mL of water, about 4 mL of fats would be present. This is possible because milk contains, in addition to fats, a number of lipids called phospholipids. These molecules are similar to the fatty acids you studied in class, but have a negatively charged phosphate group attached to one end.

The negatively charged phosphate group, like other charged, polar groups, is quite soluble in water. The non polar, uncharged hydrocarbon area is completely insoluble in water. The dua personality of the phospholipid (chemists would call it amphipathic) lets the milk fat be soluble in the milk water by aligning a number of its hydrophobic areas with the other fats, with the phosphates facing out toward the water (Figure 2). This makes a mixture called an emulsion, meaning a fat dissolved in water by means of an amphipathic molecule, called an emulsifying agent (soaps are emulsifying agents that work to dissolve grease). In this lab, you will be using powdered milk that has already had the fat removed.

Proteins:

There are three main proteins in milk; casein (case in), lactalbumin (lact al bumin), and lactoglobulin (lact o glob ulin). In this lab you will be isolating the casein, and a mixture of the lactalbumin and lactogobulin proteins (they isolate under the same conditions and are difficult to separate from each other). Chances are you have consumed casein without knowing it. Casein can precipitate from water when its normal structure is destroyed. We call this process protein denaturation. The curds in cottage cheese are precipitated casein protein (the enzyme rennin is used in this case to precipitate the protein) and the Indian cheese paneer is made by adding an acid to milk to precipitate the curds. Many cheeses are made by precipitating casein and removing it from the left over liquid, the whey. The lactalbumin proteins are easily precipitated by heating. After casein proteins are removed, heating the whey solution can provide the solid protein. Although a minor component, the lactoglobulin proteins are the immune proteins present in milk that protect a baby from illness until it can develop its own immune system.

Sugars:

The main carbohydrate present in milk is the sugar lactose. Lactose is a disaccharide containing the monosaccharides galactose and glucose. Mammals produce an enzyme called lactase that breaks the disaccharide into its monomers during digestion. Many mammals stop producing lactase after maturity leading them to be lactose intolerant—they lack the ability to digest the sugar in milk. Although most people can digest lactose upon maturity, there are many who can't. These people can buy lactose□free products, or buy the enzyme supplement lactase to aid in digestion of the sugar. On a practical note, when bacteria get into milk, they digest the lactose and form the acid lactic acid. This causes a precipitation of the protein casein. This is what happens when old milk "sours".

3.3 EXPERIMENTS

3.3.1. **To Isolate of casein from milk.**

Objectives:

- To carry out isoelectric precipitation of milk proteins.
- To purify the casein.
- To determine the casein content in milk.

Principle:

Most proteins show a minimum solubility at their isoelectric pH and this principle is used to isolate casein by adjusting the pH of the milk to 4.6, its isoelectric point. The main bulk of the precipitate is the casein. The entrapped residual fat can be removed by repeated washing with the solvents such as ethanol and ether. Casein is insoluble in these solvents and this property can be advantageously used to remove the unwanted fat from the preparation.

Requirements:

- Filter paper
- Thermometer
- Petroleum ether
- Acetate buffer (pH 4.6)
- Milk sample
- pH meter (digital)
- Muslin cloth
- Ethanol (95%)

Procedure:

1. Place 100 ml milk in 500 ml beaker and warm to 40□C

2. Warm 100 ml acetate buffer to 40□C
3. Add buffer slowly to the milk. Stir the contents during addition.
4. Check the pH with pH meter. Adjust the amount of buffer, if needed, to reach a final pH of 4.6
5. Cool the contents to room temperature and leave the whole for a further 5 min.
6. Filter the precipitate carefully through a piece of clean muslin cloth.
7. Wash the precipitate several times with the water.
8. Suspend the precipitate in 30 ml ethanol. Stir the contents.
9. Filter through filter paper.
10. Wash the precipitate with a 50 ml mixture of ethanol and ether (1:1).
11. Wash with 50 ml ether.
12. Collect the precipitate in the dry watch glass and allow ether to evaporate. (leave overnight for drying or apply mild heat for an hour)
13. Cool, weigh the casein by difference, and express the result as percentage casein (m/v) as follows:

Observations:

1. Initial pH of the milk.
2. Volume of acetate buffer needed to reach pH of 4.6
3. Appearance of the precipitate.
4. Weight of the precipitate.

3.3.2 Isolation of Nicotine from Tobacco

Objectives:

The experiment aims to measure the amount of nicotine present in a cigarette sampleby Soxhlet extraction.

Reagents:

Dichloromethane (methylene chloride, CH_2Cl_2) anhydrous alcohol (ethyl achohol, CH_3CH_2OH) methanol (methyl alcohol, CH_3OH) picric acid (2,4,6- trinitrophenol, $C_6H_3N_3O_7$)

Materials:

Cigarette sample, Soxhlet extractor, utility clamps, Bunsen burner, wire gauze, iron ring, iron stand, distilling flask, Buchner funnel, vacuum flask, condenser, rubber tubing, thread and filter paper

Procedure:

The cigarette sample will be weighed and 3 g will be placed on a filter paper. The filter paper will be rolled with the sample and the upper and lower flaps will be folded. A thread will be tied around the filter paper roll to prevent the sample from escaping. The roll should fit the mouth of the soxhlet extractor and its length should not exceed the length of the arm of thee xtractor. This should be observed to ensure that all leaves will be immersed in the solvent during extraction.

3.3.3 Aim: Isolation of lactose from milk.

Apparatus Required:

Beaker 100 mL	Thrompt
Beaker 25 mL	Hot plate
Erlenmeyer flask 50 mL	Thermometer
Graduated cylinder 10 mL	Dropper
Graduated cylinder 25 mL	Rubber tubing
Filtering Flask	Paper towels
Büchner Funnel	Glass rod

Chemicals Required:

Acetic Acid, CH_3COOH

Ethyl ether, $C_4H_{10}O$

Ethanol, C_2H_5OH

Milk

Procedure:

1. Measure 50 mL milk by a graduated cylinder and put it in a 100 mL beaker.
2. Heat the solution on a hot plate to bring the temperature to 55°C, control the temperature with thermometer.
3. Prepare 20 mL, 10 % acetic acid solution in a 25 mL beaker.
4. Add dropwise the 10% acetic acid solution while stirring. Do not add the acid too rapidly.
5. Keep the beaker on the hot plate, until the liquid becomes transparent and the casein no longer precipitates.

Isolation of Casein:

1. Collect the casein with suction filtration and place the casein in a 100 mL beaker.
2. Prepare 20 mL1:1 ethyl ether -ethanol solution in a 25 mL beaker, and then add this mixture to the casein precipitate. Stir the solution for a few minutes.
3. Weigh the filter paper and collect the casein by suction filtration.
4. Place the casein between several layers of paper towels to dry.

5. Keep the casein and allow it to dry for three days to determine its melting point and calculate the yield of our experiment.

Melting Point Determination:

1. Fill a melting point tube with the sample (a thin-walled capillary tube) sealed at one end.
2. Attach the capillary tube to a thermometer.
3. Place the capillary in the melting point stage with oil bath.
4. Turn on the power and allow the hot-stage temperature to rise fairly rapidly to within 15-20^{o}C below the expected melting point of the compound.
5. During the determination of the actual melting point range, heat the melting point hot-stage slowly at a uniform rate, about 2 degrees per minute.
1. Records the temperature at which the substance begins to liquefy and that at which it becomes completely liquefied.

Expected results and calculations:

Melting point of Casein: 280 °C

Melting point of casein: 342.3 °C

Percentage yield:

$$\text{Percentage of product} = \frac{\text{amount of product recovered}}{\text{Eight of milk}} \times 100\%W$$

Report objectives:

1. Calculate the percent yield of your product. Assume the density of milk 1.037 g/cm^3.
2. Why do we try to keep the temperature around 55°C?

3.3.4 Aim: Quinine from Cinchona bark

These alkaloids are derived from the dried barks of the stem and dried bark of the root of the plant Cinchona succirubra, Cinchona officinalis, Cinchona calysaya and Cinchona ledgeriana (F: Rubiaceae). They contain quinoline nucleus, a major alkaloids belong to this class are cinchona alkaloids, which has therapeutic activity.

Procedure: -

1. Moisten powdered cinchona (50gm) with ammonia water and allow it to stand for anhour, then hot water is added. To the mixture, after cooling, milk of lime is added and the whole evaporated to dryness.
2. Dry at room temperature or below 60°C.
3. Pack the material in soxhlet apparatus and extract with toluene for 6 hours.
4. Extract the toluene extract with dilute sulfuric acid under stirring condition.
5. Separate the acidic aqueous liquid, neutralize the acidulated layer and allow standing when neutral sulfates of the alkaloid (quinine, cinchonine, cinchonidine) are crystallized out.
6. Dissolve the crude quinine sulfate in water, decolorized with charcoal and recrystallized until the cinchonidine and cinchonine are reduced to the required percentage.
7. weigh and determine its melting point (177°C).
8. Report.

3.3.5 Aim: Isolation of piperine from black pepper.

Chemicals and materials:

Black pepper, dichloromethane, n-hexane and ethyl acetate.

Glassware and equipment:

Two Beakers (100 mL), a round bottom flask (100 mL), a pipette, a glass funnel, a magnetic stir bar, reflux condenser, filtration flask, a Buchner funnel, a heating mantle, filter papers, a tweezers, TLC plates, TLC tanks, capillary jets, an ice bath, a graduated cylinder and a UV lamp, etc.

Procedure:

Extraction: 12 g of pure ground pepper was taken in a 100 mL round bottom flask and 50 mL of CH2Cl2 added into it. A magnetic stir bar was placed in the round bottom flask (to ensure smooth heat distribution) and the reflux condenser assembly was set up. The reaction mixture was refluxed for about twenty minutes under constant magnetic stirring. Water was kept circulating through the condenser during reflux to ensure that all the reactants were being converted into products. After cooling the flask, vacuum filtration with a Buchner funnel and filter paper to was used to filter out the grounded pepper. Following pictures show the process:

Isolation and Purification of the product:

The filtrate was transferred to a 100 mL round bottom flask and CH2 Cl2 was evaporated using rotary evaporator until dark brown oil was left. The oil was placed in an ice bath for it to cool down. 6 mL of cold diethyl ether was added. After stirring for 5 min, the solvent was evaporated again by using rotary evaporator. The oil was placed in an ice bath for it to cool down. 6 mL of cold diethyl ether was added again. The flask was allowed to stand for 15 min in an ice bath with occasional stirring. Piperine was not precipitated out at this stage; and the entire process was repeated 4 times. After which the mixture with cold diethyl ether was left in the refrigerator for about 24 hours. After 24 hours, using Whatman filter paper, the yellowish piperine precipitates were collected. The precipitates were then was had with cold diethyl ether, dried in air and weighed.

Results:

3.3.6 Aim: Isolation of Lycopene and β-Carotene from Tomato

Introduction:

Lycopene ($C_{40}H_{56}$) is a bright red crotenoid pigment that found in tomato and other red fruits. Lycopene is a terpene assembled from 8 isoprene units. It is not water soluble and stains any porous material, including most plastics.

Extraction of Lycopene:

- Lycopene can be extracted by reflux condensation.
- The term reflux is very widely used in distillation columns. The laboratory reflux apparatus add energy to chemical reaction.
- A liquid reaction mixture is placed in a vessel open only at the top.
- The vessel is connected to Liebig condenser, such that any vapors given off are cooled back to liquid and fall back into the reaction vessel.
- The vessel is then heated vigorously for the course of reaction.
- The advantage of this technique is that it can be left for a long period of time without the need to add more solvent or fear of the reaction vessel boiling dry as any vapor is immediately condensed in the condenser.
- In addition, as a given solvent will always boil at a certain temperature, the reaction will proceed at the same temperature.
- This diagram shows a reflux apparatus for adding energy to chemical reactions.
- Determine each part of this apparatus??

Material:

- Tomato paste (0.12g/Kg).
- Ethanol (95%).
- Dichloromethane.
- Anhydrous sodium sulphate (Na2SO4).
- Saturated NaCl solution.
- Condenser.
- Buchner filter

Procedure:

1. Weigh 5g tomato paste in a flask

2. Add 10ml ethanol and heat for 5 minutes.

3. Filter with filter paper and press to take off all the filtrate

4. Keep the filtrate in conical flask.

5. Put the crude in round-bottom bottle and add 10ml DCM, start condensation

6. Boil the solution for 4min. then separate the supernatant and add it to the first filtrate.

7. Repeat this step 3 times.

8. Collect all the filtrate in separatory funnel

9. Add 10ml saturated NaCl solution, shake gently and allow separating into 2 layers.

10. Collect the lower layer.

11. Add 1 teaspoon anhydrous Na_2SO_4 and allow to stand for 5 minutes.

12. Filter with filter paper.

13. Keep the filtrate in dark bottle away from light otherwise the color of lycopene will disappear.

3.3.7 Aim: Isolation of limonene from citrus fruits

Equipment:

Apparatus:

- Eye protection
- Grater
- Bunsen burner
- Heat-resistant mat
- Tripod and gauze
- Orange x 2
- Thermometer up to 110°C
- Measuring cylinder, 100 cm^3
- Measuring cylinder, 50 cm^3
- Round bottomed flask, 250 cm^3
- Still head
- Thermometer pocket
- Condenser
- Receiver adapter
- Test tubes and bungs x 3
- Dropping pipette
- Anti-bumping granules

Chemicals:

- Bromine water, no more than 0.2% v/v
- Potassium manganate(VII), 0.001 M
- Cyclohexene

Procedure:

Stage 1

1. Grate the outer orange coloured rind of two oranges and add to 100 cm3 of distilled water in the 250 cm3 round bottomed flask. Add anti-bumping granules to the round bottomed flask.

2. Set up the distillation apparatus as shown above.
3. Heat the flask so that distillation proceeds at a steady rate, approximately one drop per second of distillate. (Note: Take care not to let the liquid in the round bottomed flask boil too strongly).
4. Collect approximately 50 cm^3 of distillate in the measuring cylinder. The oil layer will be on the surface.
5. Using a dropping pipette, carefully remove the oil layer into a test tube for the next stage.

Stage 2

Odour

Cautiously smell the extracted oil by wafting the fumes towards the nose. Do not breathe in directly from the test tube.

Action of bromine water

1. Measure out approximately 1 cm3 of bromine water into each of three test tubes.
2. Add a few drops of the limonene oil to one test tube, a few drops of cyclohexane to another, and a few drops of cyclohexene to the third. Place in the bungs and agitate. If the bromine water is decolorized the molecule contains double bonds.
3. 0.001 M potassium manganate (VII) can be substituted for the bromine water for class use. However, students need to know the action of bromine water.

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UNIT 4: STRUCTURE DETERMINATION OF ORGANIC COMPOUNDS BY SPECTROSCOPIC TECHNIQUES

CONTENTS:

4.1 Introduction
4.2 Objective
4.3 Spectroscopic techniques
4.4 Experiment
    4.4.1 Solved spectral problem
    4.4.2 Practical spectral problem
4.5 Summary
4.6 Biobliography

4.1 INTRODUCTION

The determination of structure of an organic compound requires specialized techniques. In the past, such techniques were laboriously time consuming and sometimes unreliable too. Modern technology has sorted out this difficulty with the advent of spectroscopic methods for determination of structure.

Spectroscopy is the study of interaction of electromagnetic radiation with matter. As the electromagnetic spectrum consists of radiations of different wavelengths, the responses of the molecules to these wavelengths differ. Thus, X-rays are scattered by the molecules, infrared radiation cause bonds in molecules to vibrate and radio waves make nuclei of atoms to resonate. Spectroscopy measures these interactions and a correlation is obtained between these interactions and molecular structure.

The elucidation of molecular structure by the spectroscopic techniques assumes a basic knowledge of chemistry and a familarity with various area of spectroscopy to be used. The infrared (IR) and nuclear magnetic resonance (NMR) spectra gives most of the information about

the molecular structure whereas the ultraviolet (UV) and mass spectra are used to reinforce the information gathered from the IR and NMR.

The most important characteristics of the spectroscopic methods are the following:

Spectroscopic technique	Information obtained
UV-visible spectroscopy	▪ It can provide information about the presence of unsaturation in the molecule as well as the presence of certain functional groups. ▪ Existence of chromophores and/or conjugation in the molecule from the observed absorptions.
Infrared spectroscopy	▪ It is used to determine the presence of certain functional groups in the molecule.
NMR spectroscopy	▪ It can reveal information about the carbon skeleton, the number of protons attached to each carbon as well the number and position of certain other elements. ▪ Functional groups, substructures, connectivities, stereochemistry, etc., from chemical shift data, peak areas and observed coupling constants
Mass spectrometry*	▪ This can give information about the molecular weight of the molecule and its composition in terms of elements. ▪ Molecular formula and substructures from the observed ions
**Mass spectrometry is not a spectroscopic technique in the sense that we are seeing because there is no electromagnetic irradiation of the substance and there is no absorption of such radiation*.	

4.2 OBJECTIVES

After studying this unit, you shall be able to

- Propose the expected spectroscopic features of organic molecules.
- Solve problem related with electronic transitions.
- Learn how to identify the molecular formula, functional group, carbon connectivities and position of substituents/ functional groups connected to carbon skeleton in molecule on the basis of spectroscopic techniques.

- Correlate spectra with structure of compound
- Interpret the spectroscopic data
- Determine the structure of organic compounds using different spectroscopic methods.

4.3 SPECTROSCOPIC TECHNIQUES

The characteristic and readily obtained structure information from spectrum/spectra of different spectroscopic techniques is described below:

4.3.1 UV-Visible Spectroscopy

UV-Visible spectroscopy employs the UV (400-200 nm) and visible (800-400 nm) region of the electromagnetic spectrum. This energy associated with this region is quite high and thus irradiation of a molecule with such energy causes excitation of electrons from their Highest Occupied Molecular Orbital (HOMO) to Lowest Unoccupied Molecular Orbital (LUMO). For this reason it is sometimes referred as electronic spectroscopy. The UV spectrometer measures the wavelength (λmax) at which the absorbance is maximum. The absorbance is defined the Beer-Lambert's law:

$$A = \varepsilon lc.$$

Where A is absorbance, l is the length of sample through which light traverses and c is the concentration of the sample. The ε is the constant of proportionality and has a particular value for a compound.

The following structural infromation can be obtained from a UV-spectrum:

- The nature of the congugated system and substituents, from the empirical rules for the calculation of λmax.
- There are five kinds of orbitals that need to be considered: the bonding σ and π orbitals and their corresponding antibonding σ* and π* orbitals and the non-bonding n orbitals. The σ to σ* orbital and π to π* orbitals are allowed while the transition from n to π* orbital is disallowed.
- Identification of the nature of the chromophore. n to σ* or n to π* transitions are identified by weak bonds in the absence of strong chromophores.

4.3.2 Infrared Spectroscopy

Infrared (IR) spectroscopy is an absorption method widely used in both qualitative and quantitative analyses. The infrared region of the spectrum includes electromagnetic radiation that can alter the vibrational and rotational states of covalent bonds in organic molecules. The major use of IR spectroscopy is in determining the functional group present in structures of organic compounds.The characteristics absorption frequencies of various functional group in the organic molecules occurs in the range of 400-4000 cm-1. The major functional group frequncies/wavelength is listd in the table3.1 and fig 3.1.

The absence of absorption in the IR spectrum is also important because if functional group present, it must abosrb at certain wavelength in the IR spectrum. Thus IR spectrum provides mostly information about the presence or absence of certain functional groups.

Table 4.1: IR absorption band of common functional groups

IR Absorptions of Common Functional Groups		
Functional Group	***Absorption Location (cm^{-1})***	***Absorption Intensity***
Alkane (C–H)	2,850–2,975	Medium to strong
Alcohol (O–H)	3,400–3,700	Strong, broad
Alkene (C=C) (C=C–H)	1,640–1,680 3,020–3,100	Weak to medium Medium
Alkyne (C≡C) (C≡C–H)	 2,100–2,250 3,300	 Medium Strong
Nitrile (C≡N)	2,200–2,250	Medium
Aromatics	1,650–2,000	Weak
Amines (N–H)	3,300–3,350	Medium
Carbonyls (C=O) Aldehyde (CHO) Ketone (RCOR) Ester (RCOOR) Acid (RCOOH)	 1,720–1,740 1,715 1,735–1,750 1,700–1,725	Strong

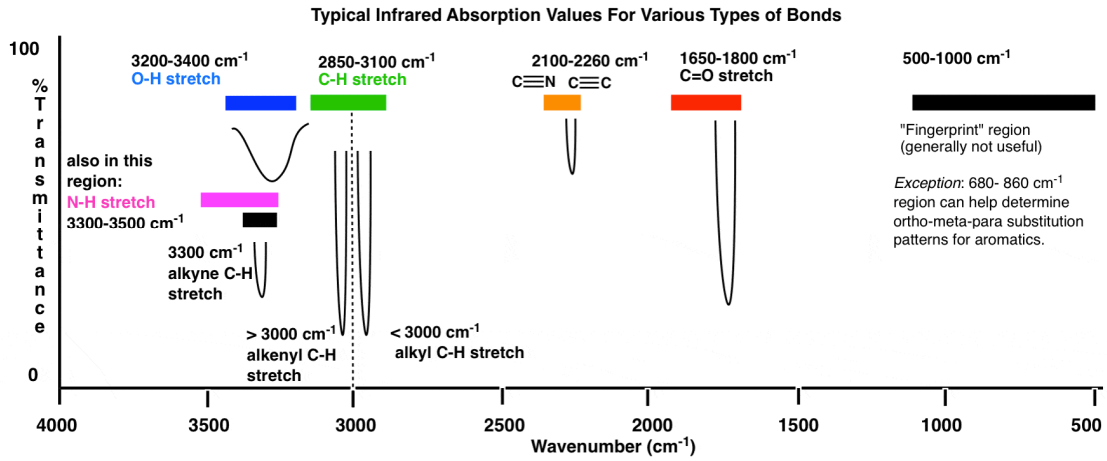


Fig.3.1 IR absorption band

4.3.3 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy, commonly referred to as NMR, has become the preeminent technique for determining the structure of organic compounds. In a broad sense, it still works by the same principle as other spectroscopies, and that is the interaction of the molecule with certain type of energy to produce different energy states and deduce information based on these differences. The nuclei of many elemental isotopes have a characteristic spin (**I**). Some nuclei have integral spins (e.g. I = 1, 2, 3 ....), some have fractional spins (e.g. I = 1/2, 3/2, 5/2 ....), and a few have no spin, I = 0 (e.g. ^{12}C, ^{16}O, ^{32}S, ...). Isotopes of particular interest and use to organic chemists are ^{1}H, ^{13}C, ^{19}F and ^{31}P, all of which have I = 1/2.

A. The following structural infromation can be obtained from ^{1}H-NMR-spectrum:

- The number of different types of protons present in the organic molecule structure.
- The number of groups of equivalent protons and intergration gives the number of protons in each group of equivalent protons.
- The position of signal represented in the form of chemical shift (Table3.2.)
- The value of coupling constant gives the information on the relative position of the coupled protons within the molecules.

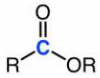
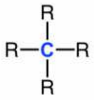
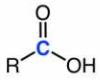
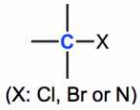
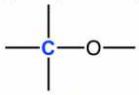
Table 4.2: Proton (^{1}H-NMR) chemical shift range

Type of Proton	Chemical Shift (ppm)	Type of Proton	Chemical Shift (ppm)
R—CH_3	0.9 – 1.2	X—CH_2R (X: Cl, Br, I)	3.1 – 3.8
R—CH_2	1.2 – 1.5	R—OH	variable, 1 – 5
R—CH	1.4 – 1.9	R—NH_2	variable, 1 – 5
C=C, CHR_2	1.5 – 2.5	C=C, H	4.5 – 6.0
C=O, CH_3	2.0 – 2.6	Ar—H	6.0 – 8.5
Ar—CH_3	2.2 – 2.5	C=O, H	9.5 – 10.5
R—C≡C—H	2.5 – 3.0	C=O, OH	10 – 13
(H)R—O—CH_3	3.3 – 4.0		

B. The following structural infromation can be obtained from ^{13}C-NMR-spectrum:

- The number of signals exhibits how many different carbons of different set of equivalent carbons present in the molecules.
- The splitting of signal indecates the number of hydrogen atom attached to the particular carbon atom.
- The value of chemical shift indicates the position of signal (Table3.3)

Table 4.3: Carbon (^{13}C-NMR) chemical shift

Type of Carbon	Chemical Shift (ppm)	Type of Carbon	Chemical Shift (ppm)
	0 – 35		80 – 150
	15 – 55		110 – 170
	25 – 55		165 – 175
	30 – 40		175 – 185
 (X: Cl, Br or N)	10 – 65		190 – 200
	50 – 90		200 – 220
	70 – 90		

4.3.4 Mass spectrometry:

Mass spectrometry is used to determine the molecular mass of an organic compound. A small sample of the compound is vaporised under very low pressure and high temperature and the vapour is irradiated with a beam of high energy electron. This causes electrons to be ejected from molecules in the sample, leaving them as positively charged cations, the molecular ion or parent ion. The molecular ions ($M^{+\cdot}$) may break down into fragment ions or daughter ions (m^+), the ions are characterised by their mass (m) to charge (z) ratio (m/z). The mass spectrum of a compound typically shows a number of signals and the peak at highest m/z (molecular ion) usually corresponds to the mass of the whole molecule. The signals with lower m/z are fragment ions and can provide some structural information **(Fig. 4.2)**

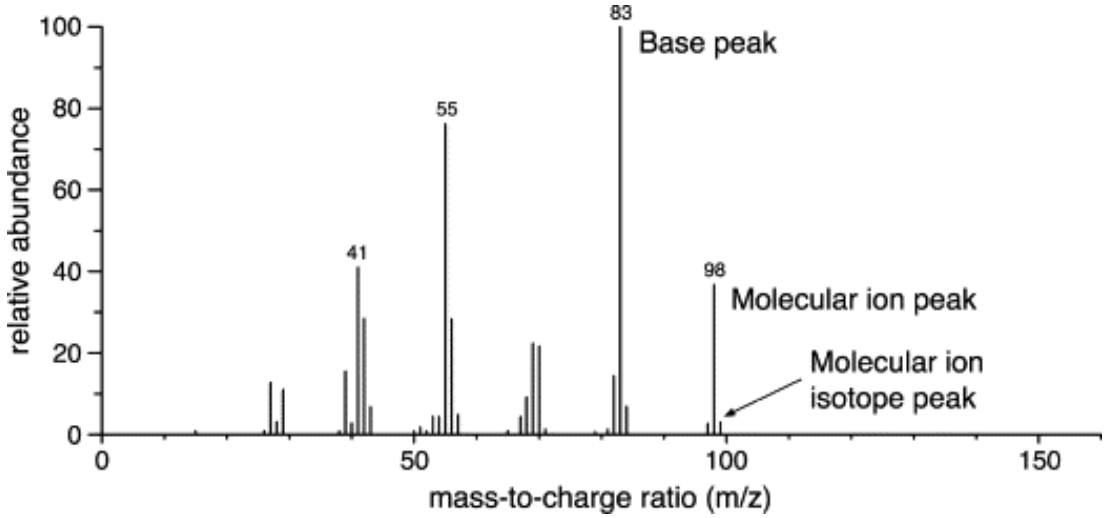


Fig 4.2 Mass-Spectrum

The following structural infromation can be obtained from Mass spectrum

- The most abundant ion in a mass spectrum is called the base peak.
- The ion with the same mass as the parent molecule is called the molecular ion.
- Isotopes of carbon and hydrogen lead to common M+1 peaks.
- The x-axis of a mass spectrum is m/z the mass to charge ratio, which in practice equals the mass of the ion.
- Accurate molecular mass/weight and molecular formula can be determined.
- The nature of carbon skeleton from the fragmentation pattern.
- Functional groups from the heavier m/z peaks and characteristic ion series.

4.4 EXPERIMENT

4.4.1 Solved spectral problem

The following sequence of the steps may be used for the structure determination/elucidation of organic compound.

- Determine the molecular formula from the molecular weight/mass.
- Calculate the number of Double Bond Equivalents (DBE) from the molecular formula.
- Examine/ Interpretation the λ_{max} value from the ultraviolet (UV) spectrum or claculate by Woodward Fiser rule.

- Examine/ Interpretation the infraed (IR) spectrum to determine the functional group.
- Examine/ Interpretation the ^{1}H-NMR spectrum for chemical shift value for chemical environment of proton and coupling constant value for the neighbouring protons.
- Examine/ Interpretation the ^{13}C-NMR spectrum for the types of carben skeleton present in the compound.
- Examine/Interpretation the Mass spectrum and fragmentation pattern for the molecular mass and the nature of carbon skeleton.
- Assemble/ compile all the spectral data to determine the complete structure of organic compound.

Experiment-1: Solved Problem

1. Aim: The aim of this study is to determination/identification of the structure of the organic compound by interpretation of given spectral data.

2. Requirment: UV, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data

An organic compound having molecular formula $C_6H_{12}O_2$ showed the following IR and NMR spectra data. Determine the structure of the compound.

IR: 2950 cm^{-1}, 2850 cm^{-1}, 1730 cm^{-1}

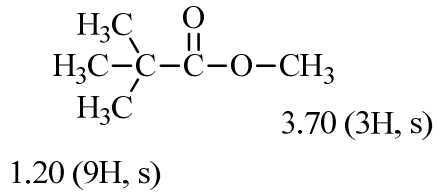
NMR: δ1.20 (9H, s), δ 3.70 (3H, s)

3. Interpretation of spectral data:

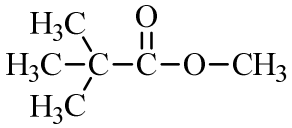
- Molecular formula of the compound is $C_6H_{12}O_2$.
- Double bond equivalent calculated by the using formula

  6+1-(12/2) = 1

  DBE is 1 thus molecule contains either on double bond (C=C) or (C=O) in the structure.
- The absorption band in the IR spectrum at 2950 cm^{-1} and 2850 cm^{-1} indicate the presence of methyl group (-CH_3) and absorption band at 1730 cm^{-1} indicate the presence of the cabonyl group in the structure.
- ^{1}H-NMR peaks at δ1.20 euivalent to 9H exhibit singlet peak indicate the presence of tert. butyl group attached with neighbouring carbon having no proton. While ^{1}H-NMR peaks at δ3.70 euivalent to 3H exhibit singlet peak indicate the presence of methyl group and no proton atom attached with the neighbouring carbon.
- Thus on the basis of the above spectral data the structure of the compound may be



4. Result: The structure of the compound is



Experiment-2: Solved Problem

1. Aim: The aim of this study is to determination/identification of the structure of the organic compound by interpretation of given spectral data.

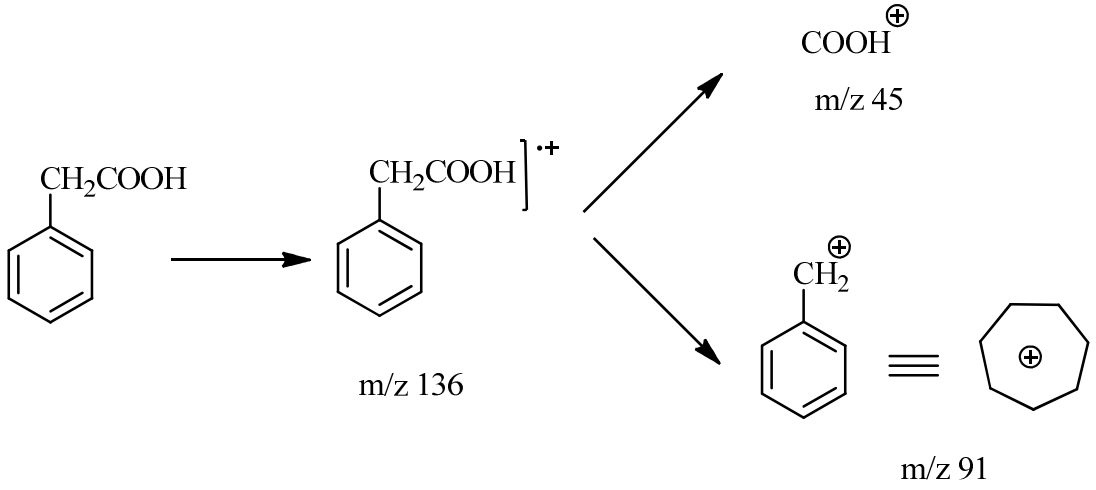
2. Requirment: UV, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data

An organic compound containing carbon, hydrogen and oxygen showed the abundant mass spectral peak at M^{+} (m/z 136), base peak (m/z 91) and fragment peak (m/z 45); other spectral data given below

UV: λ_{max} 229nm and λ_{max} 257nm; **IR:** 1710 cm^{-1}; **NMR:** δ7.2(5H, s), δ 3.5 (2H, s)

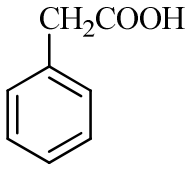
3. Interpretation of spectral data:

- UV absorption peak at λ_{max} 229nm (n-π*) and λ_{max} 257nm (π-π*) sugestted the presence of keto and aryl moiety in the compound.
- The absorption band in the IR spectrum at 1710 cm^{-1} indicates the presence of the cabonyl group in the structure.
- ^{1}H-NMR peaks at δ7.2 equivalent to 5H exhibit singlet peak indicate the presence of phenyl ring in the structure and singlet peak at δ3.5 of 2H suggeste that methyl group presnt and attached with the phenyl ring and the carboxylic group (-COOH).
- Most abundant peak (molecular ion peak) at m/z 136 indicates that the molecular mass of the compound is 136. the base peak at m/z 91 indicate the benzyl cation or tropylium ion formation during the mass fragmentation of the compound and fragment peak at m/z 45 due to the formation of –COOH group.

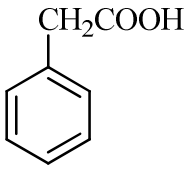


Mass fragmentation of proposed structure of compound

- Thus on the basis of the above spectral data the structure of the compound may be



4. Result: The structure of the compound is phenyl acetic acid.



4.4.2 Practical spectral problem

Experiment-1: Spectral Problem

Aim: The aim of this study is to determination/identification of the structure of the organic compound by interpretation of given spectral data.

2. Requirment: UV, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data

Determine the structure of the hydocarbon-A contain 85.7% carbon and 14.3% hydrogen, gave the following spectral data

UV: λ_{max} 210nm

IR: 3022 cm^{-1}, 1656 cm^{-1}, 965 cm^{-1}

NMR: δ1.60 (d), δ 5.55 (q)

3. Interpretation of spectral data:

4. Result: The structure of the compound is

Experiment-2: Spectral Problem

Aim: The aim of this study is to determination/identification of the structure of the organic compound by interpretation of given spectral data.

2. Requirment: UV, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data

Determine the structure of the organic compound having following spectra data. compound.

IR: 1730 cm^{-1}

NMR: δ 2.0(3H, s), δ 2.93 [2H, t (*J*=7Hz)], δ 4.30 [2H, t (*J*=7Hz)], δ 7.3 (5H, s)

Mass (m/z): 73, 91, 149 164

3. Interpretation of spectral data:

4. Result: The structure of the compound is

4.5 Summary:

The knowledge and concepts of UV-visible, IR, ^{1}H-NMR, ^{13}C-NMR and Mass help us in solving problems based on the experimental data. It will help us in analysing the experimental data to elucidate the structure of any organic compound.

While analyzing the data the following point must be kept in mind:

- In UV-visible spectroscopy; the types of bonds and electrons plays important role in understanding the electronic transitions.
- UV-visible spectroscopy gives information regarding the presence of conjugation, carbonyl group etc.
- The IR values gives information regarding the functional group present in the molecule.
- The ^{1}H-NMR tells us the number and environment of neighbouring hydrogens present.
- The ^{13}C-NMR helps in getting the information about the type of carbon atom(s) present in the molecule.
- Mass spectral data gives information about the total mass and fragmentation pattern of the molecule.
- By combining all the information one can find the structure of the molecule.

4.6 BIOBLIOGRAPHY/REFERENCES

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