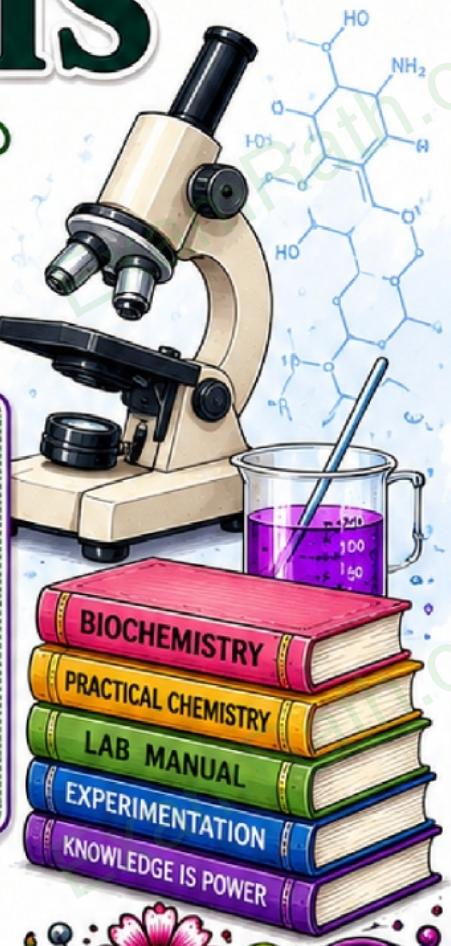
ATIBAL SINGH MAHAVIDYALAY

AFFLITE- Prof. Rajendra Singh (Rajju Bhaiya) University, Prayagraj

PRACTICAL FILE OF BIOCHEMICAL ANALYSIS

★ B.Sc. II SEMESTER ★

Name	:	.....
Class	:	B.Sc. II Semester
Roll No.	:	.....
Registration No.	:	.....
Session	:	2025–26
College Name	:	ATIBAL SINGH MAHAVIDYALAY
Department	:	Department of Chemistry



1. Biochemical Analysis Laboratory

Introduction :

The Biochemical Analysis Laboratory is concerned with the study of various biomolecules such as carbohydrates, proteins, amino acids, fats, enzymes and nucleic acids. It helps in understanding the chemical composition of living organisms and their biological functions.

Aim :

To study the biochemical constituents of living organisms and perform qualitative and quantitative analysis of biomolecules.

Instruments Used :

1. Test Tubes
2. Beakers
3. Pipettes
4. Burette
5. Measuring Cylinder
6. Centrifuge
7. Water Bath
8. pH Meter
9. Chromatography Chamber
10. Electronic Balance

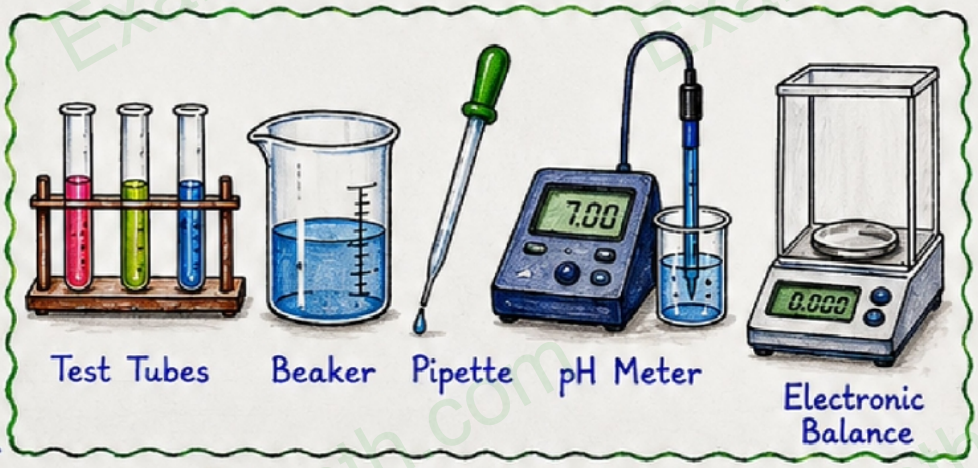


Figure 1 : Instruments Used in Biochemical Analysis Laboratory

Experiments Performed :

1. Separation of Sugars by Paper Chromatography
2. Identification of Reducing and Non-Reducing Sugars
3. Preparation of Osazone Crystals
4. Biuret Test for Proteins
5. TLC of Amino Acids
6. Action of Salivary Amylase on Starch
7. Estimation of Glycine
8. Determination of Saponification Value
9. Determination of Iodine Value
10. Extraction of DNA from Onion

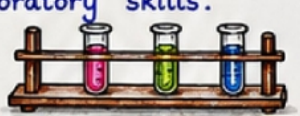
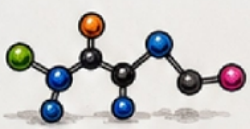
Significance :

- Helps in the identification of biomolecules.
- Useful in medical and pharmaceutical research.
- Assists in disease diagnosis.
- Provides knowledge of metabolic processes.
- Important in biotechnology and healthcare.

Conclusion :

Biochemical Analysis Laboratory plays an important role in studying the chemical constituents of living organisms. The experiments performed in this laboratory help students understand various biological and chemical processes and develop practical laboratory skills.




2. Separation of Sugars by Paper Chromatography

Aim :

To separate the mixture of sugars by paper chromatography.

Principle :

Paper chromatography is based on the principle of differential partition of the components of a mixture between the stationary phase (water held in the paper) and the mobile phase (solvent). Different sugars travel different distances depending on their solubility and affinity towards the solvent and paper.

Requirements :

1. Whatman filter paper
2. Sugar mixture (e.g., Glucose, Fructose, Sucrose)
3. Solvent system (n-Butanol : Acetic acid : Water in 4 : 1 : 5 ratio)
4. Capillary tubes / Fine brush
5. Pencil
6. Chromatography jar with lid
7. Ruler

Procedure :

1. A strip of Whatman filter paper is taken and a pencil line is drawn 2 cm from the lower edge.
2. Small spots of different sugars (Glucose, Fructose, Sucrose) are applied on the pencil line using capillary tubes.
3. The paper strip is suspended in a chromatography jar containing the solvent system, ensuring that the spots are above the solvent level.
4. The jar is closed with a lid and allowed to stand until the solvent front moves near the top.
5. The paper is removed and the solvent front is marked.
6. The paper is dried and the separated spots are observed.

Result :

The sugar mixture was successfully separated by paper chromatography. Different sugars moved different distances and were identified by their R_F values.

Diagram :

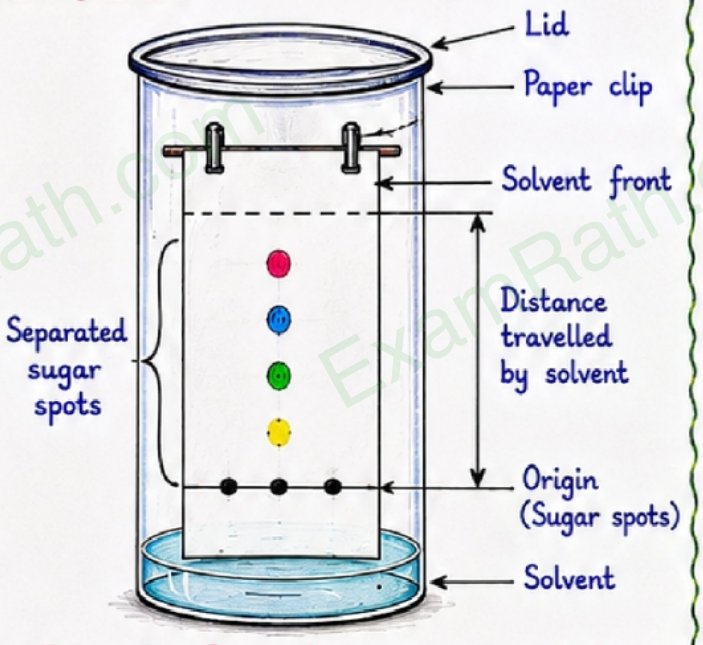


Figure 2 : Paper Chromatography of Sugars

Observation Table :

Sugar	Distance travelled by sugar (cm)	Distance travelled by solvent (cm)	R_F Value
Glucose			
Fructose			
Sucrose			

$$R_F \text{ Value} = \frac{\text{Distance travelled by sugar}}{\text{Distance travelled by solvent}}$$

Precautions :

1. Use a pencil to draw the baseline.
2. Do not dip the spots in the solvent.
3. Close the jar properly to saturate the atmosphere.
4. Do not touch the paper at the spot area.
5. Use fresh solvent for better separation.

3. Identification of Reducing and Non-Reducing Sugars

Aim :

To identify the given sugar as reducing sugar or non-reducing sugar.

Principle :

Reducing sugars have a free aldehyde or ketone group which reduces Fehling's solution (Cu^{2+}) to Cu_2O giving a brick red precipitate. Non-reducing sugars do not have free aldehyde or ketone group and do not reduce Fehling's solution.

Requirements :

1. Test tubes
2. Fehling's Solution A
3. Fehling's Solution B
4. Test sugar solution
5. Water bath
6. Dropper

Procedure :

1. Take 2 mL of Fehling's Solution A in a test tube.
2. Add 2 mL of Fehling's Solution B and mix well.
3. Add 1 mL of the test sugar solution.
4. Heat the mixture in a boiling water bath for 2–3 minutes.
5. Observe the colour change and note the result.

Result :

- Formation of brick red precipitate indicates the presence of reducing sugar.
- No precipitate (solution remains blue) indicates the presence of non-reducing sugar.

Diagram :

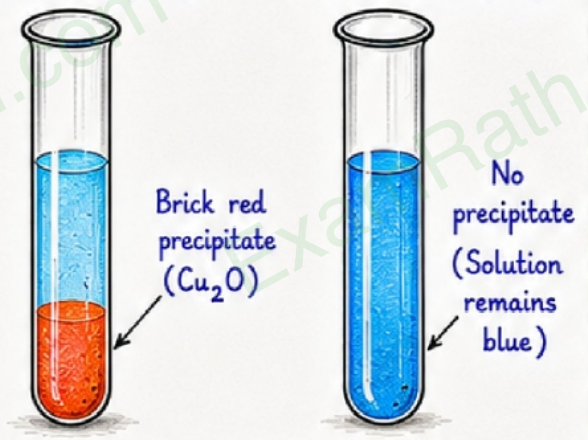


Figure 3 : Identification of Reducing and Non-Reducing Sugars

Observation Table :

Sugar	Observation		Result
	Colour before heating	Colour after heating	
Glucose (Reducing sugar)			
Sucrose (Non-Reducing sugar)			

Precautions :

1. Use fresh Fehling's solutions.
2. Heat the mixture gently in a water bath.
3. Do not boil directly on flame.
4. Clean the test tubes properly before use.
5. Use equal volumes of Fehling's A and B.

4. Preparation of Osazone Crystals

Aim :

To prepare osazone crystals from a given reducing sugar.

Principle :

Reducing sugars react with excess of phenylhydrazine to form osazones. In this reaction, the carbonyl group of the sugar (C-1) and the adjacent carbon (C-2) are converted into a characteristic osazone derivative which separates out as yellow crystalline precipitate.

Requirements :

1. Glucose solution
2. Phenylhydrazine hydrochloride
3. Sodium acetate solution
4. Acetic acid (glacial)
5. Test tubes
6. Water bath
7. Glass rod
8. Filter paper

Procedure :

1. Take 2 mL of glucose solution in a test tube.
2. Add 1 mL of phenylhydrazine hydrochloride solution.
3. Add 1 mL of sodium acetate solution.
4. Add 2-3 drops of glacial acetic acid and mix well.
5. Heat the mixture in a boiling water bath for 15-20 minutes.
6. Allow the test tube to cool.
7. Yellow crystals of osazone separate out.
8. Filter, wash with cold water and dry the crystals.

Result :

Yellow osazone crystals were successfully prepared from the given reducing sugar. This confirms the presence of a reducing sugar.

Diagram :

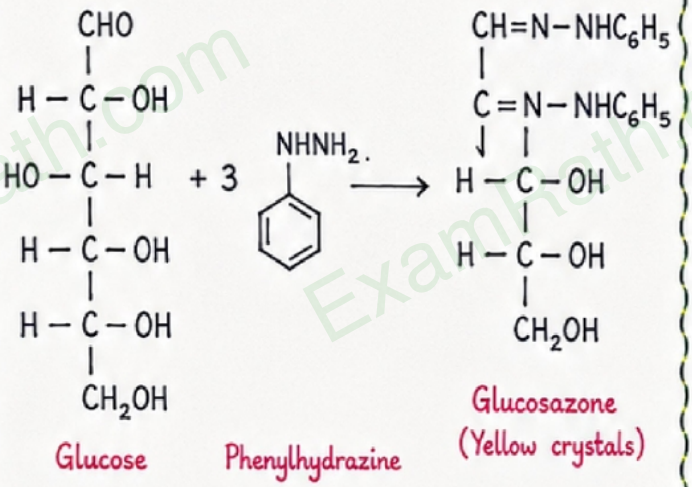


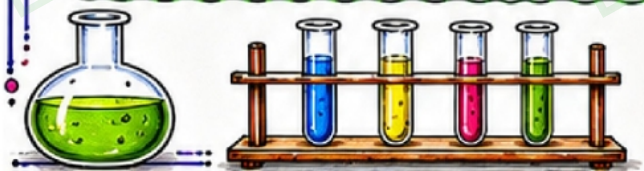
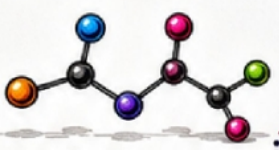
Figure 4 : Formation of Osazone

Observation Table :

Sugar	Appearance of osazone crystals	Colour of osazone
Glucose		
(Any other reducing sugar)		

Precautions :

1. Use excess phenylhydrazine.
2. Heat the mixture gently in a water bath.
3. Do not overheat.
4. Allow the mixture to cool before filtering.
5. Wash the crystals with cold water.


5. Isolation of Protein from Natural Sources

Aim : To isolate protein from a natural source such as egg white or pulse.

Principle :

Proteins are soluble in water and insoluble in their saturated solutions of neutral salts. When a salt solution like saturated $(NH_4)_2\ SO_4$ (Ammonium sulfate) is added to the protein solution, the protein precipitates out and can be separated by filtration.

Diagram :

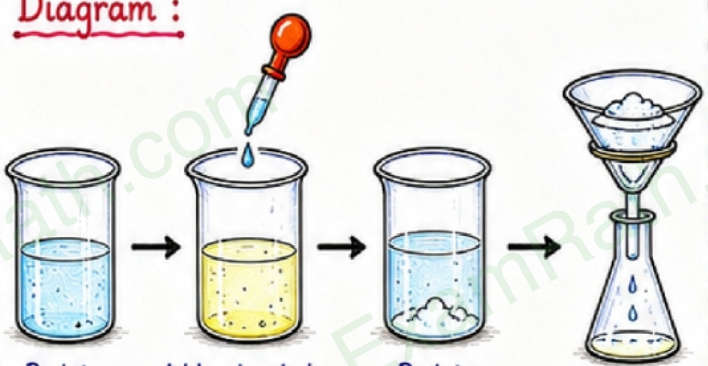


Figure 5 : Isolation of Protein from Natural Source

Requirements :

1. Egg white / Pulse (Gram dal)
2. Distilled water
3. Saturated $(NH_4)_2\ SO_4$ solution
4. Test tubes
5. Beakers
6. Glass rod
7. Filter paper and funnel

Observation Table :

S.No.	Step	Observation	Inference
1.	Protein solution (egg white)	.....	.....
2.	After adding saturated $(NH_4)_2\ SO_4$ solution	.....	.....
3.	After precipitation	.....	.....
4.	After filtration	.....	.....

Procedure :

1. Take 10 mL of egg white (protein solution) in a beaker.
2. Add an equal volume of distilled water and mix well.
3. Add saturated $(NH_4)_2\ SO_4$ solution drop by drop with constant stirring.
4. A white precipitate of protein appears.
5. Allow it to stand for some time.
6. Filter the mixture using filter paper and funnel.
7. Wash the precipitate with a small amount of saturated salt solution.
8. The precipitated protein is collected and dried.

Result :

Protein was successfully isolated from the given natural source in the form of a white precipitate.

Precautions :

1. Add $(NH_4)_2\ SO_4$ solution drop by drop.
2. Stir the solution continuously.
3. Do not heat the protein solution.
4. Use clean and dry glassware.
5. Filter the solution while it is cold.

6. Biuret Test For Proteins

Aim :

To detect the presence of proteins in a given sample by Biuret test.

Principle :

In alkaline medium, peptide bonds of proteins react with Cu^{2+} ions of copper sulfate to form a violet or purple coloured complex. The intensity of the violet colour is proportional to the number of peptide bonds present, thus confirming the presence of proteins.

Diagram :

Figure 6 : Biuret Test for Proteins

Requirements :

1. Protein solution (e.g., Egg albumin)
2. Biuret reagent (Alkaline $CuSO_4$ solution)
3. Test tubes
4. Pipette
5. Test tube stand

Procedure :

1. Take 2 mL of protein solution in a test tube.
2. Add 2 mL of Biuret reagent to it.
3. Mix the contents well.
4. Allow it to stand for 2–3 minutes.
5. Observe the colour change.
6. Note the result.

Observation Table :

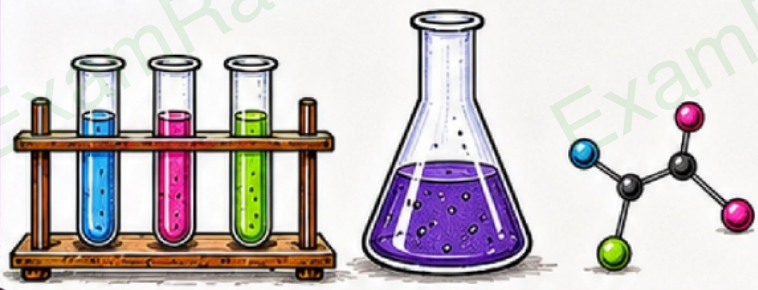
S.No.	Observation	Inference
1.	Blue colour of the solution remains unchanged.	Protein is absent.
2.	Violet or purple colour appears.	Protein is present.

Result :

Violet / purple colour appeared, therefore protein is present in the given sample.

Precautions :

1. Use fresh Biuret reagent.
2. Do not use dirty test tubes.
3. Do not take excess protein solution.
4. Add reagent slowly and mix properly.
5. Observe the colour in adequate light.



7. TLC OF AMINO ACIDS

Aim :

To separate and identify amino acids using Thin Layer Chromatography (TLC).

Principle :

Amino acids when spotted on a TLC plate and developed in a suitable solvent system, move at different rates depending on their polarity and affinity towards the stationary phase. This results in their separation. The separated spots are visualized using ninhydrin reagent.

Requirements :

1. Amino acid mixture (e.g., Glycine, Alanine, Valine)
2. TLC plates (Silica gel G)
3. Developing solvent (n-Butanol : Acetic acid : Water in 4 : 1 : 2 ratio)
4. Capillary tubes
5. Ninhydrin reagent
6. Developing chamber
7. Spray bottle / Dropper
8. Pencil, Ruler

Procedure :

1. Draw a pencil line 1.5 cm from the bottom of the TLC plate.
2. Spot small amounts of amino acid mixture on the line using capillary tubes.
3. Place the plate in the developing chamber containing the solvent.
4. Allow the solvent front to move up to about 1 cm from the top.
5. Remove the plate and mark the solvent front.
6. Dry the plate.
7. Spray evenly with ninhydrin reagent.
8. Heat gently to develop the spots.
9. Observe the separated spots and calculate R_f values.

Diagram :

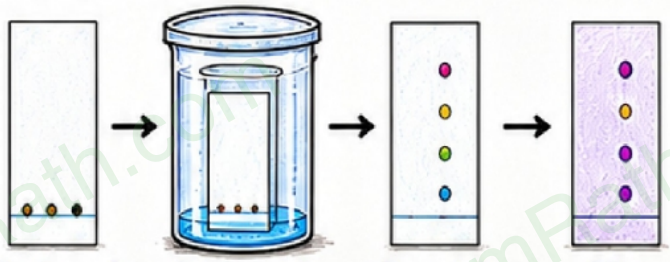


Figure 7 : TLC of Amino Acids

Observation Table :

Amino Acid	Distance travelled by the spot (cm)	Distance travelled by solvent front (cm)	R_f Value
Glycine	.....	.....	.....
Alanine	.....	.....	.....
Valine	.....	.....	.....
.....	.....	.....	.....
.....	.....	.....	.....

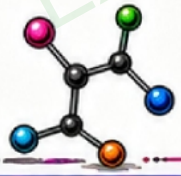
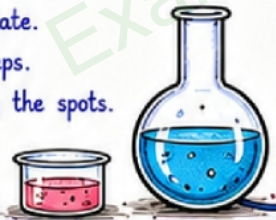
Result :

Amino acids were successfully separated by TLC. Different amino acids showed different R_f values.

Precautions :

1. Do not touch the silica surface of the TLC plate.
2. Use fresh and high quality solvent.
3. Do not over-develop the plate.
4. Handle the plate with forceps.
5. Heat gently while developing the spots.



8. Action of Salivary Amylase on Starch

Aim : To study the action of salivary amylase on starch.

Principle :

Salivary amylase (ptyalin) catalyzes the hydrolysis of starch into simpler sugars. Starch gives a blue colour with iodine. As the reaction proceeds, starch is broken down and the blue colour disappears. The amount of reducing sugar formed can be detected using Benedict's reagent.

Diagram :

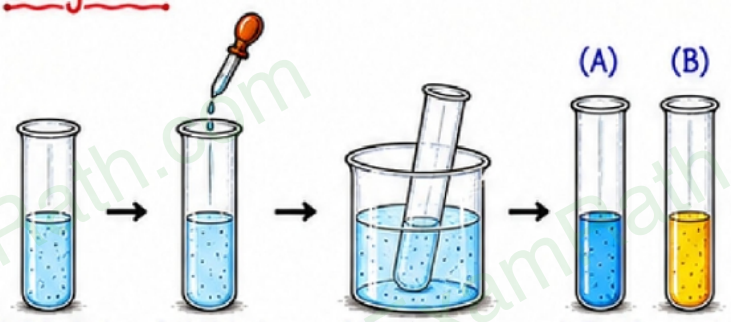


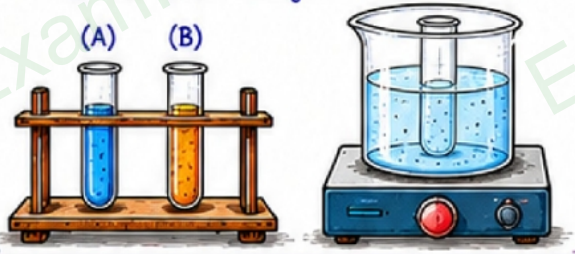
Figure 8 : Action of Salivary Amylase on Starch

Requirements :

1. Starch solution (1%)
2. Saliva (fresh)
3. Iodine solution
4. Benedict's reagent
5. Test tubes
6. Beaker
7. Water bath (37°C)
8. Pipette
9. Test tube stand

Procedure :

1. Take 2 mL of 1% starch solution in a test tube.
2. Add 1 mL of fresh saliva and mix well.
3. Incubate the mixture in a water bath at 37°C for 10 minutes.
4. After incubation, divide the mixture into two parts in two test tubes (A and B).
5. To test tube (A), add 2–3 drops of iodine solution. Note the colour.
6. To test tube (B), add 2 mL of Benedict's reagent and heat in a boiling water bath for 2 minutes. Note the colour change.



Observation Table :

Test	Test tube	Observation	Inference
Iodine Test for Starch	(A)	Before incubation : ..... After incubation :	Starch is .....
Benedict's Test for Reducing Sugar	(B)	Before incubation : ..... After incubation :	Reducing sugar is ..... formed.

Result :

Salivary amylase hydrolyzes starch into simpler sugars. The disappearance of blue colour with iodine and the formation of reducing sugar with Benedict's reagent confirm the action of amylase on starch.

Precautions :

1. Use fresh saliva.
2. Maintain the temperature at 37°C.
3. Do not boil the mixture during incubation.
4. Use clean and dry test tubes.
5. Note the observations immediately.

9. Estimation of Glycine

Aim :

To estimate the amount of glycine in a given sample by Folin's reagent method.

Principle :

Glycine reacts with Folin-Ciocalteu phenol reagent in alkaline medium to produce a blue colour. The intensity of the colour developed is directly proportional to the concentration of glycine present in the sample.

Requirements :

1. Glycine standard solution
2. Unknown sample solution
3. Folin-Ciocalteu reagent
4. 20% Sodium carbonate (Na_2CO_3) solution
5. Distilled water
6. Test tubes
7. Pipette
8. Colorimeter / Spectrophotometer
9. Measuring flask

Procedure :

1. Pipette 2 mL of standard glycine solution (or sample solution) into a test tube.
2. Add 2 mL of Folin's reagent and mix well.
3. Allow it to stand for 3 minutes.
4. Add 4 mL of 20% Na_2CO_3 solution.
5. Mix thoroughly and dilute to a suitable volume with distilled water.
6. After 30 minutes, measure the absorbance at 660 nm against reagent blank.
7. Prepare a standard curve using different concentrations of glycine.
8. Determine the concentration of glycine in the sample from the standard curve.

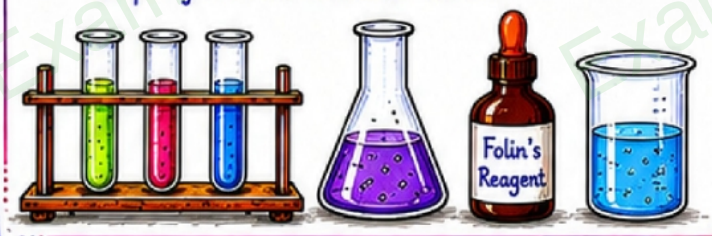
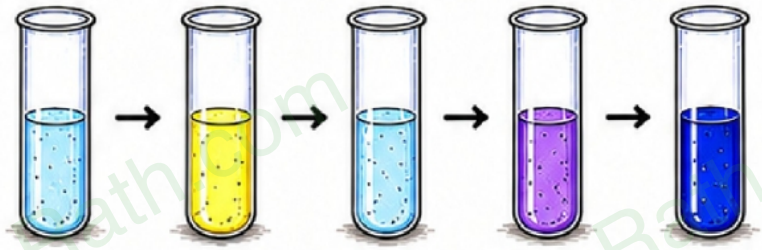


Diagram :



Glycine solution (2 mL) → Add 2 mL of Folin's reagent → Mix well and wait for 3 minutes → Add 4 mL of 20% Na_2CO_3 solution → Make up the volume and measure absorbance at 660 nm

Figure 9 : Estimation of Glycine

Observation Table :

S.No.	Standard Glycine (µg/mL)	Absorbance at 660 nm	Remarks
1.	.....	.....	.....
2.	.....	.....	.....
3.	.....	.....	.....
4.	.....	.....	.....
5.	.....	.....	.....

(Plot a standard curve of Absorbance vs Concentration)

Result :

The amount of glycine in the given sample is _____ µg/mL.

Precautions :

- Use freshly prepared reagents.
- Use clean and dry test tubes.
- Maintain proper time intervals.
- Do not mix the reagents in wrong order.
- Take absorbance after 30 minutes only.
- Use reagent blank for setting the instrument.

10. Determination of Saponification Value

Aim :

To determine the saponification value of a given fat or oil.

Principle :

Fats and oils (triglycerides) react with a known excess of alcoholic KOH. The unreacted KOH is then back titrated with standard HCl using phenolphthalein indicator. Saponification value is defined as the milligrams of KOH required to saponify 1 gram of fat or oil.

Diagram :

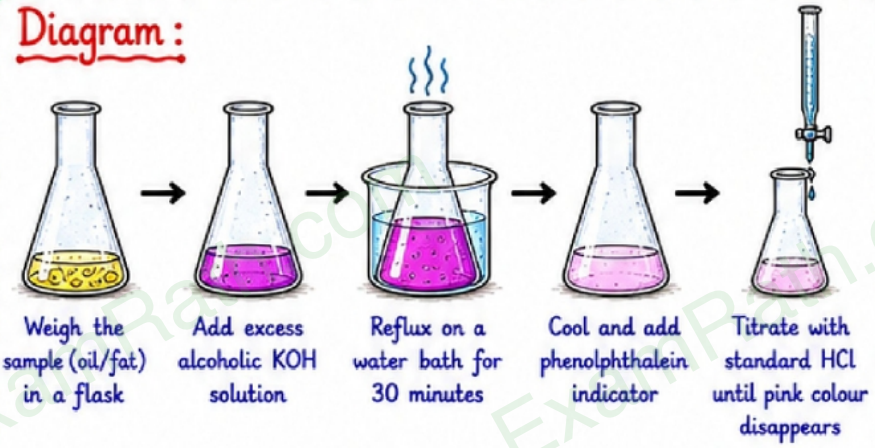


Figure 10 : Determination of Saponification Value

Requirements :

1. Sample (Oil / Fat)
2. Alcoholic KOH solution (0.5 N)
3. Standard HCl solution (0.5 N)
4. Phenolphthalein indicator
5. Conical flask (250 mL)
6. Reflux apparatus
7. Water bath
8. Burette, Pipette
9. Analytical balance

Procedure :

1. Weigh accurately about 2 g of the oil or fat in a conical flask.
2. Add 25 mL of alcoholic KOH solution (0.5 N).
3. Attach the reflux condenser and heat on a water bath for 30 minutes with occasional shaking.
4. Cool the flask and add 2–3 drops of phenolphthalein indicator.
5. Titrate the excess KOH with standard HCl solution (0.5 N) until the pink colour disappears.
6. Perform a blank titration without the sample.
7. Note down the volume of HCl used for sample (V) and blank (V_0).
8. Calculate the saponification value using the formula.

Observation Table :

S.No.	Weight of sample (g)	Volume of HCl used (mL)	Saponification Value (mg KOH / g of sample)
1.	.....	.....	.....
2.	.....	.....	.....
3.	.....	.....	.....

Result :

The saponification value of the given sample is _____ mg KOH per gram of sample.

Calculation :

$$\text{Saponification Value} = \frac{(V_0 - V) \times N \times 56.1}{\text{Weight of sample (g)}}$$

Where, V_0 = Volume of HCl used for blank (mL)
V = Volume of HCl used for sample (mL)
N = Normality of HCl
56.1 = Molecular weight of KOH (mg/meq)

Precautions :

- Use freshly prepared alcoholic KOH solution.
- Ensure complete reflux for 30 minutes.
- Do not lose any sample during transfer.
- Use phenolphthalein indicator (not methyl orange).
- Titrate carefully; end point is disappearance of pink colour.

11. Determination of Iodine Value

Aim : To determine the iodine value of a given oil or fat.

Principle :

Iodine reacts with the carbon–carbon double bonds present in unsaturated fats and oils (addition reaction). The unreacted iodine is then estimated by titrating with standard sodium thiosulphate solution using starch as indicator. Iodine value is the grams of iodine absorbed by 100 grams of fat or oil.

Requirements :

1. Sample (Oil / Fat)
2. Wijs solution (Iodine monochloride solution)
3. Potassium iodide (KI) solution (10%)
4. Sodium thiosulphate solution (0.1 N)
5. Starch indicator (1%)
6. Glacial acetic acid
7. Distilled water
8. Conical flask (250 mL)
9. Burette, Pipette
10. Analytical balance

Procedure :

1. Accurately weigh about 2–3 g of the oil or fat in a clean, dry conical flask.
2. Add 25 mL of Wijs solution using a pipette.
3. Stopper the flask and keep it in the dark for 30 minutes (shake occasionally).
4. Add 20 mL of 10% KI solution and 100 mL of distilled water to the flask.
5. Titrate the liberated iodine with 0.1 N sodium thiosulphate solution using starch indicator near the end point until the blue colour just disappears.
6. Perform a blank titration in the same way without oil/fat.
7. Note down the volume of $Na_2S_2O_3$ used for sample (V) and for blank (V_0).
8. Calculate the iodine value using the formula.

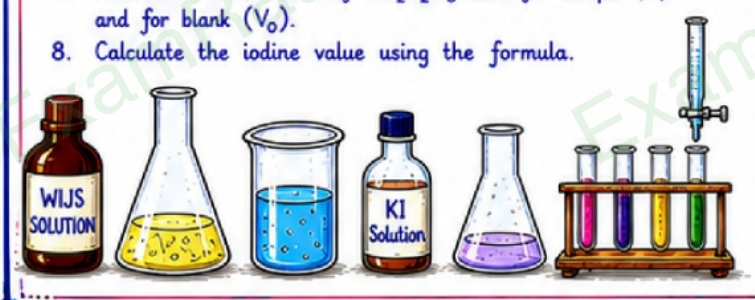
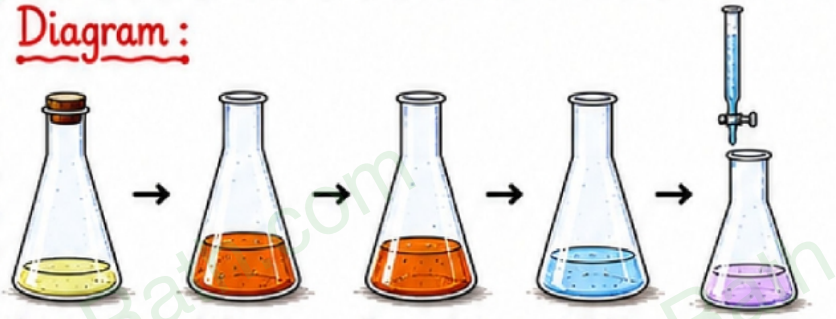


Diagram :



Weigh the oil/fat in a conical flask → Add excess Wijs solution → Keep in dark for 30 minutes → Add KI solution → Titrate with $Na_2S_2O_3$ using starch indicator

Figure 11 : Determination of Iodine Value

Observation Table :

S.No.	Weight of sample (g)	Volume of $Na_2S_2O_3$ used for blank (V_0) (mL)	Volume of $Na_2S_2O_3$ used for sample (V) (mL)	Iodine Value (grams of I_2 per 100 g of sample)
1.	.....	.....	.....	.....
2.	.....	.....	.....	.....
3.	.....	.....	.....	.....

Calculation :

$$\text{Iodine Value} = \frac{(V_0 - V) \times N \times 12.69}{\text{Weight of sample (g)}}$$

Where, V_0 = Volume of $Na_2S_2O_3$ used for blank (mL)
V = Volume of $Na_2S_2O_3$ used for sample (mL)
N = Normality of $Na_2S_2O_3$ solution
12.69 = Milliequivalent weight of iodine (mg/meq)

Result :

The iodine value of the given sample is _____ grams of iodine per 100 grams of sample.

Precautions :

- Use fresh Wijs solution.
- Keep the flask in dark for exactly 30 minutes.
- Add KI solution before titration.
- Use starch indicator carefully near the end point.
- Do not expose the sample to moisture.
- Perform blank under identical conditions.

12. Extraction of DNA from Onion / Cauliflower

Aim : To isolate and visualize DNA from onion / cauliflower.

Principle :

Cells are lysed using detergent and salt solution. This breaks the cell and nuclear membranes and releases DNA. Protein and other impurities are removed by the action of salt and detergent. DNA is insoluble in cold alcohol and separates out as white, stringy threads which can be spooled out.

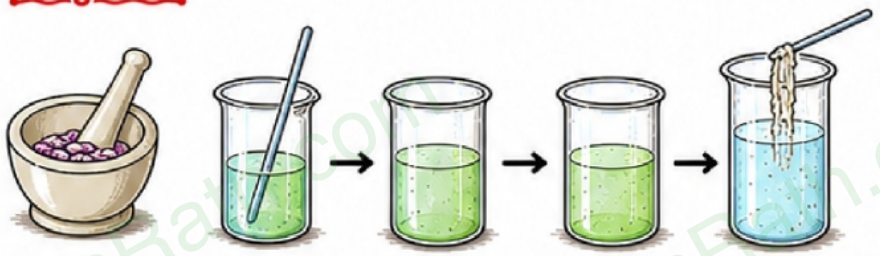
Requirements :

1. Onion / Cauliflower
2. Extraction buffer (Detergent solution)
3. Sodium chloride solution (10%)
4. Ice-cold ethanol (95%)
5. Muslin cloth
6. Beaker (100 mL)
7. Mortar and pestle
8. Glass rod
9. Test tubes
10. Funnel

Procedure :

1. Take a small piece of onion or cauliflower and chop it into small pieces.
2. Transfer the pieces to a mortar and add 10 mL of extraction buffer. Grind gently for 2–3 minutes.
3. Filter the mixture through muslin cloth into a beaker. Collect the filtrate.
4. Add 5 mL of 10% sodium chloride solution to the filtrate and mix gently.
5. Carefully add 10 mL of ice-cold ethanol along the inner wall of the beaker to form a separate layer.
6. After a few minutes, white, stringy DNA threads will appear at the interface.
7. Wind the DNA threads on a glass rod and transfer to a test tube containing alcohol.

Diagram :



1. Chop onion / cauliflower and crush in mortar with extraction buffer.
2. Filter the mixture through muslin cloth into a beaker.
3. Add salt solution and mix gently.
4. Carefully add cold alcohol along the inner wall.
5. DNA appears as white, stringy threads. Spool it out.

Figure 12 : Extraction of DNA

Observation Table :

S.No.	Observation	Inference
1.	Filtrate is clear or slightly cloudy.	Cell contents released.
2.	After adding salt solution, mixture becomes clear.	Proteins and impurities precipitate.
3.	After adding cold alcohol, white threads appear at the interface.	DNA precipitates out.
4.	Threads can be spooled out using a glass rod.	DNA is extracted.

Result :

DNA was successfully extracted from _____ (onion / cauliflower).

Precautions :

- Use ice-cold ethanol.
- Add alcohol slowly to avoid mixing with the filtrate.
- Do not shake the mixture after adding alcohol.
- Use clean glassware and fresh reagents.
- Handle the sample gently.

13. Determination of Nucleic Acids

Aim :

To determine the amount of nucleic acids (DNA/RNA) in a given sample.

Principle :

Nucleic acids contain phosphorus. On acid digestion, phosphorus is liberated as orthophosphate. Orthophosphate reacts with ammonium molybdate in acidic medium to form phosphomolybdic acid which is reduced by stannous chloride to give a blue colour ('molybdenum blue'). The intensity of blue colour is directly proportional to the amount of nucleic acids present.

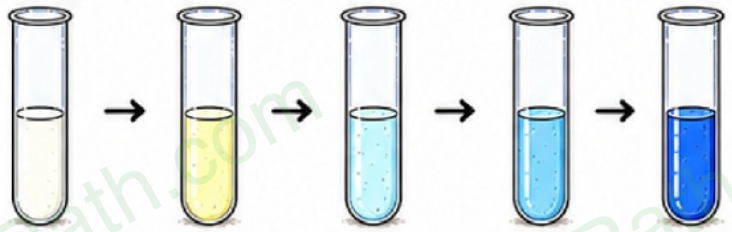
Requirements :

1. Sample (DNA/RNA solution)
2. Perchloric acid ($HClO_4$)
3. Ammonium molybdate solution (2.5%)
4. Stannous chloride solution
5. Standard phosphorus solution
6. Test tubes
7. Water bath
8. Pipette, Burette
9. Colorimeter / Spectrophotometer
10. Analytical balance

Procedure :

1. Take 2 mL of the sample in a clean dry test tube.
2. Add 4 mL of 10% perchloric acid carefully.
3. Heat the test tube in a boiling water bath for 15–20 minutes until a clear solution is obtained.
4. Cool the tube and make the volume up to 10 mL with distilled water.
5. Take 2 mL of this solution in a test tube.
6. Add 1 mL of ammonium molybdate solution (2.5%) and mix well.
7. Add 1 mL of stannous chloride solution. Mix gently and allow the blue colour to develop for 10 minutes.
8. Measure the absorbance at 660 nm against reagent blank.
9. Prepare a standard curve using standard phosphorus solutions and determine the amount of nucleic acids in the sample.

Diagram :



1. Take the sample in a test tube.
2. Add perchloric acid and digest.
3. Cool and add ammonium molybdate.
4. Add stannous chloride reagent.
5. Blue colour develops. Measure at 660 nm.

Figure 13 : Determination of Nucleic Acids

Observation Table :

S.No.	Standard Phosphorus (µg/mL)	Absorbance at 660 nm	Amount of Nucleic Acid in Sample (µg/mL)
1.	.....	.....	.....
2.	.....	.....	.....
3.	.....	.....	.....
4.	.....	.....	.....
5.	.....	.....	.....

(Plot a standard curve of Absorbance vs Concentration)

Result :

The amount of nucleic acids in the given sample is _____ µg/mL.

Precautions :

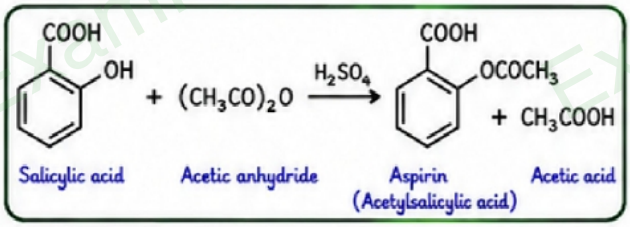
- Use clean and dry glassware.
- Handle perchloric acid carefully.
- Ensure complete digestion of the sample.
- Do not overheat during digestion.
- Allow proper time for colour development.
- Use reagent blank for zero setting.
- Read absorbance at 660 nm only.

14. Synthesis of Aspirin

Aim : To synthesize aspirin (acetylsalicylic acid) from salicylic acid.

Principle :

Salicylic acid reacts with acetic anhydride in presence of a few drops of concentrated sulphuric acid (as catalyst) to form aspirin (acetylsalicylic acid) and acetic acid.



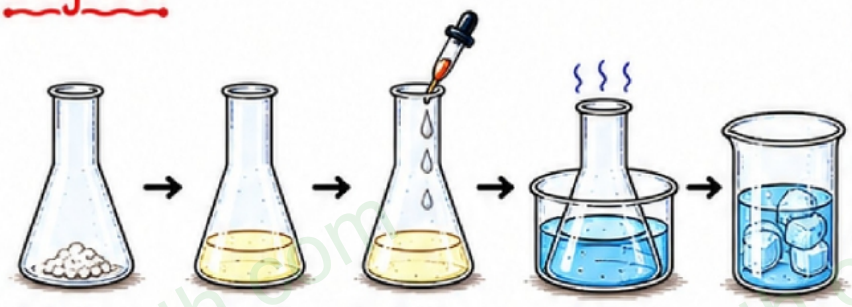
Requirements :

1. Salicylic acid
2. Acetic anhydride
3. Conc. Sulphuric acid
4. Distilled water
5. Sodium carbonate solution (5%)
6. Ice cubes
7. Beaker (100 mL)
8. Conical flask (250 mL)
9. Glass rod
10. Filter funnel and filter paper
11. Watch glass

Procedure :

1. Take 2.0 g of salicylic acid in a 250 mL conical flask.
2. Add 5 mL of acetic anhydride to it.
3. Add 2–3 drops of concentrated sulphuric acid carefully and swirl the flask gently.
4. Keep the flask in a water bath (60–70°C) for 10–15 minutes with occasional swirling.
5. Remove the flask and pour the reaction mixture into 50 mL of ice-cold water with stirring. Crystals of aspirin separate out.
6. Filter the crystals using a filter funnel.
7. Wash the crystals with a small amount of cold water.
8. Dissolve the crude aspirin in minimum amount of hot water.
9. Add 5% sodium carbonate solution dropwise until effervescence stops.
10. Cool the solution and filter the crystals.
11. Dry the crystals between filter papers and determine the melting point.

Diagram :



Observation Table :

S.No.	Observation	Inference
1.	White crystals of salicylic acid are taken.	Starting material.
2.	After adding acetic anhydride, a clear solution is formed.	Reactants mix properly.
3.	On heating, solution becomes clear with slight evolution of acetic acid.	Reaction takes place.
4.	On pouring into ice-cold water, white crystals separate.	Aspirin precipitates out.
5.	After recrystallization, white shiny crystals are obtained.	Pure aspirin is obtained.

Result :

Aspirin (acetylsalicylic acid) was synthesized successfully.

Melting point of the product = _____ °C

% Yield = _____

Precautions :

- Use dry glassware.
- Add conc. sulphuric acid dropwise.
- Do not overheat the reaction mixture.
- Pour the reaction mixture slowly into ice-cold water.
- Wash the crystals with minimum cold water.
- Dry the product completely before taking melting point.
- Wear gloves and avoid inhaling vapours.

15. Synthesis of Barbituric Acid

Aim : To synthesize barbituric acid from urea and malonic acid.

Principle :

Urea condenses with malonic acid in presence of concentrated sulphuric acid (as catalyst) on heating to form barbituric acid (2,4,6-trioxohexahydropyrimidine). On cooling, barbituric acid separates out as white crystals.

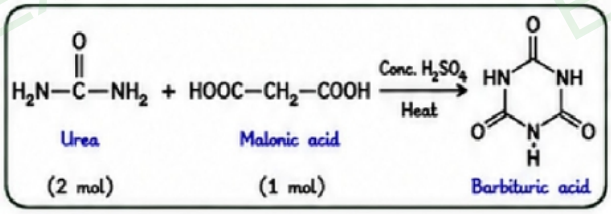


Diagram :

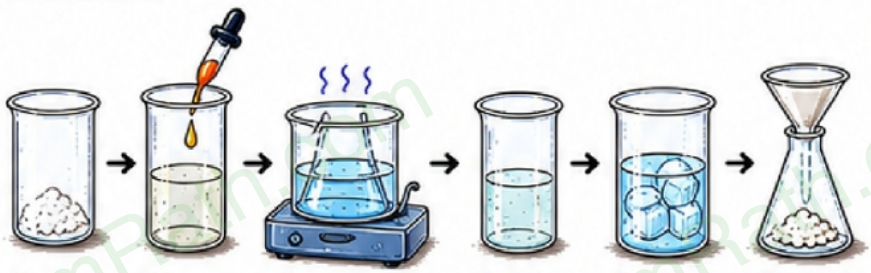


Figure 15 : Synthesis of Barbituric Acid

Requirements :

1. Urea
2. Malonic acid
3. Conc. Sulphuric acid
4. Distilled water
5. Ice cubes
6. Sodium carbonate solution (5%)
7. Beaker (100 mL)
8. Conical flask (250 mL)
9. Glass rod
10. Filter funnel and filter paper
11. Watch glass

Procedure :

1. Take 2.0 g of urea and 2.0 g of malonic acid in a 100 mL beaker.
2. Add 10 mL of concentrated sulphuric acid slowly with constant stirring.
3. Heat the mixture on a water bath for 20–25 minutes with occasional stirring.
4. A clear solution is obtained. Allow it to cool.
5. Pour the reaction mixture into 100 mL of ice-cold water with stirring. White crystals of barbituric acid separate out.
6. Filter the crystals using a filter funnel.
7. Wash the crystals with cold water.
8. Dissolve the crude product in minimum hot water and add 5% sodium carbonate solution dropwise until effervescence stops.
9. Refilter while hot if necessary.
10. Cool the solution and filter the crystals.
11. Wash the crystals with cold water and dry between filter papers.
12. Determine the melting point of the product.

Observation Table :

S.No.	Observation	Inference
1.	Mixture after adding conc. H_2SO_4 becomes warm.	Reaction starts.
2.	On heating, clear solution is obtained.	Condensation takes place.
3.	On pouring into ice-cold water, white crystals separate out.	Barbituric acid precipitates.
4.	On acidifying with sodium carbonate solution, solution becomes clear.	Impurities are removed.
5.	White crystals are obtained after recrystallization.	Pure barbituric acid is obtained.

Result :

Barbituric acid (2,4,6-trioxohexahydropyrimidine) was synthesized successfully.

Melting point of the product = _____ °C

% Yield = _____

Precautions :

- Add concentrated sulphuric acid slowly with stirring.
- Do not overheat the mixture.
- Pour the reaction mixture slowly into ice-cold water.
- Wash the crystals with cold water.
- Use dry glassware.
- Wear gloves and goggles while handling chemicals.
- Avoid inhaling vapours of conc. H_2SO_4.

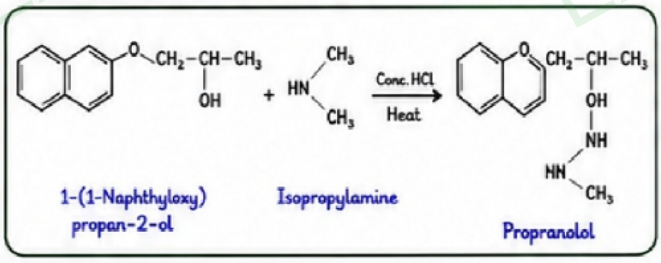
16. Synthesis of Propranolol

Aim :

To synthesize propranolol (1-(isopropylamino)-3-(1-naphthyloxy) propan-2-ol).

Principle :

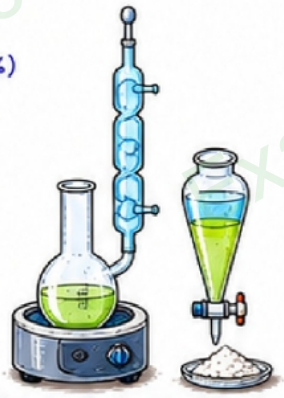
Propranolol is synthesized by the reaction of 1-(1-naphthyloxy)propan-2-ol with isopropylamine in the presence of a suitable solvent and acid catalyst. The reaction forms a secondary amine (propranolol). The product is isolated and purified by suitable method.



1-(1-Naphthyloxy) propan-2-ol + Isopropylamine → Propranolol

Requirements :

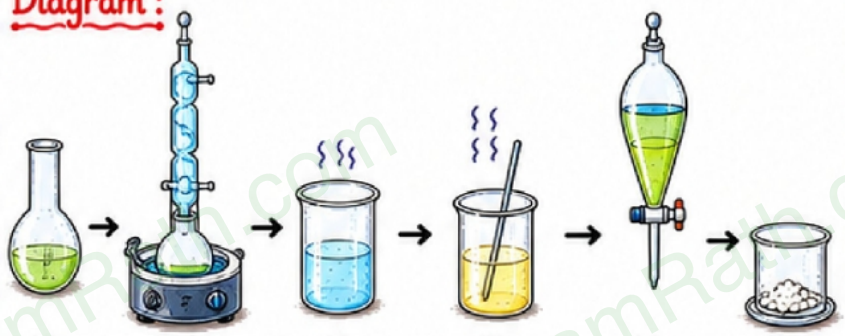
1. 1-(1-Naphthyloxy)propan-2-ol
2. Isopropylamine
3. Concentrated hydrochloric acid
4. Ethanol (95%)
5. Sodium hydroxide solution (10%)
6. Distilled water
7. Ice cubes
8. Beaker (250 mL)
9. Round bottom flask (250 mL)
10. Reflux condenser
11. Heating mantle / Water bath
12. Separating funnel
13. Filter funnel and filter paper
14. Watch glass



Procedure :

1. Take 10 g of 1-(1-naphthyloxy)propan-2-ol in a 250 mL round bottom flask.
2. Add 10 mL of isopropylamine and 10 mL of conc. HCl.
3. Attach a reflux condenser and heat the reaction mixture on a water bath for 3–4 hours with occasional stirring.
4. After completion, cool the mixture and pour into 100 mL of ice-cold water with stirring.
5. Make the solution alkaline with 10% sodium hydroxide solution (pH 8–9).
6. Extract the product with ethanol (3 × 25 mL).
7. Combine the alcoholic extracts and evaporate on a water bath to obtain crude propranolol.
8. Purify the crude product by recrystallization from ethanol–water.
9. Filter the crystals, wash with cold ethanol and dry.
10. Record the weight, melting point and % yield.

Diagram :



(a) Reactants + conc. HCl → (b) Heat under reflux for 3–4 hours → (c) Cool and pour into ice-cold water → (d) Make alkaline with NaOH solution → (e) Extract with ethanol → (f) Evaporate and recrystallize to obtain product

Figure 16 : Synthesis of Propranolol

Observation Table :

S.No.	Observation	Inference
1.	On heating, the solution becomes homogeneous.	Reaction proceeds.
2.	On pouring into ice-cold water, a cloudy mixture is obtained.	Product precipitates on basification.
3.	Two layers are formed after extraction with ethanol.	Product extracted in ethanol layer.
4.	On evaporation, a solid residue is obtained.	Crude product obtained.
5.	After recrystallization, white crystals are formed.	Pure propranolol crystals obtained.

Result :

Propranolol was synthesized successfully.

Melting point of the product = _____ °C

% Yield = _____

Precautions :

- Use pure and dry reagents.
- Add conc. HCl slowly with constant stirring.
- Maintain proper reflux to avoid loss of reactants.
- Cool the reaction mixture before basification.
- Do not make the solution highly alkaline.
- Use minimum amount of ethanol for extraction.
- Wash the crystals with cold ethanol.
- Dry the product completely before taking weight.
- Wear gloves and goggles while handling chemicals.

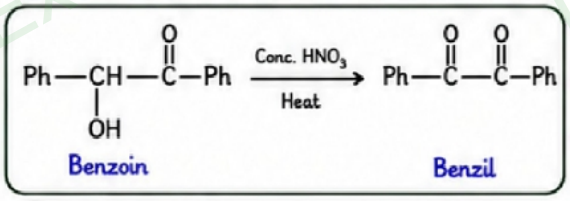
17. Synthesis of Benzil

Aim :

To synthesize benzil from benzoin by oxidation with concentrated nitric acid.

Principle :

Benzoin on oxidation with concentrated nitric acid gives benzil (1,2-diphenyl-1,2-ethanedione). The reaction proceeds through oxidation of the secondary alcohol group to a diketone.



Requirements :

1. Benzoin
2. Concentrated nitric acid
3. Distilled water
4. Crushed ice
5. Sodium bicarbonate solution (10%)
6. Ethanol (95%)
7. Beaker (250 mL)
8. Conical flask (250 mL)
9. Glass rod
10. Funnel
11. Filter paper
12. Watch glass

Procedure :

1. Take 5.0 g of benzoin in a 250 mL beaker.
2. Add 15 mL of concentrated nitric acid slowly with constant stirring.
3. Heat the mixture on a water bath for 15–20 minutes with occasional stirring.
4. Cool the reaction mixture and pour it into 100 mL of crushed ice with stirring.
5. Collect the solid product by filtration.
6. Wash the solid with water until washings are neutral to litmus.
7. Dissolve the crude product in hot ethanol (minimum quantity).
8. Cool the solution slowly, then in an ice bath to crystallize benzil.
9. Collect the crystals by filtration.
10. Wash with a small amount of cold ethanol.
11. Dry the product completely and record the weight, melting point and % yield.

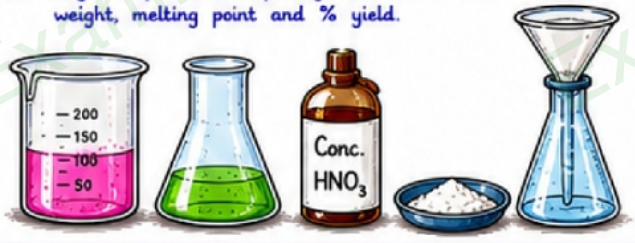


Diagram :

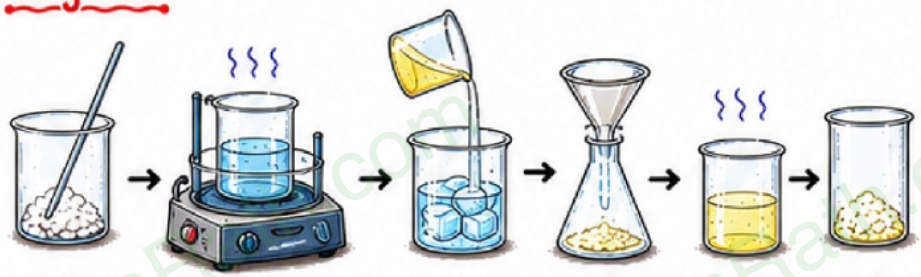


Figure 17 : Synthesis of Benzil

Observation Table :

S.No.	Observation	Inference
1.	On adding conc. HNO_3 to benzoin, yellow colour appears.	Oxidation starts.
2.	On heating, the yellow colour intensifies.	Reaction is in progress.
3.	On pouring into ice, a pale yellow precipitate separates.	Benzil precipitates out.
4.	On recrystallization, white to pale yellow crystals are obtained.	Pure benzil is obtained.

Result :

Benzil was synthesized successfully.

Melting point of the product = _____ °C

% Yield = _____

Precautions :

- Add concentrated nitric acid slowly with stirring.
- Do not overheat the reaction mixture.
- Pour the reaction mixture into ice, not vice versa.
- Wash the product thoroughly to remove acid.
- Use minimum quantity of hot ethanol for recrystallization.
- Dry the product completely before taking weight.
- Wear gloves and goggles while handling chemicals.

Thank You

I would like to express my sincere gratitude to my respected teacher _____ for their constant guidance, valuable suggestions and encouragement throughout the completion of this practical file.

I am also thankful to the laboratory staff for providing the necessary facilities and support during the practical sessions.

This practical file has been a great learning experience and has helped me to enhance my practical knowledge and skills.

Thank You All !

Name : _____

Class : _____

Roll No. : _____

Date : _____