Department of Chemistry/Biochemistry

III Semester B. Sc., Chemistry

Practical Manual

Name: ……

Register Number: ……

Section: ……

Department of Chemistry/Biochemistry

B. Sc., Semester-III
Chemistry – III Practical

Course Title: Chemistry-III Practical [24CHEP301]	**Course Credits: 2**
Total Contact Hours: 3 Hours/Week	**Duration of ESA: 03**
Formative Assessment Marks: 10	**Summative Assessment Marks: 40**

Course Objective:-To provide experimental practice of organic synthesis of involving single stage preparation

Course Outcome: Upon successful completion of these laboratory experiments, students will be able Develop proficiency in organic synthesis

List of Experiments:

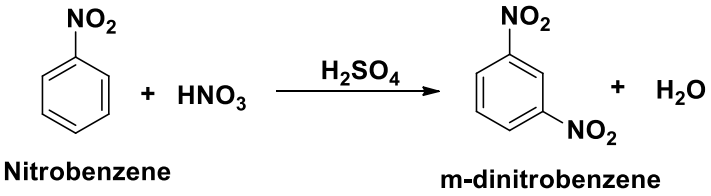
1. Preparation of m-dinitrobenzene from nitrobenzene.
2. Preparation of di-benzal acetone from benzaldehyde (using acetone and alcoholic NaOH).
3. Preparation of benzoic acid from toluene.
4. Preparation of tribromo aniline from aniline.
5. Preparation of coumarin.
6. Preparation of p-amino benzoic acid from p-nitrobenzoic acid.
7. Preparation of p-bromo aniline from acetanilide.
8. Preparation of p-nitro aniline from acetanilide.
9. Separation of a mixture of two organic compounds by TLC – ortho & para nitro-phenol.

Note:

a) Recrystallization and determination of melting point of above prepared compounds.

b) Determination of Boiling point using distillation technique.

Reaction:



Reaction Mechanism:

Observation and Calculations:

Specific gravity of nitrobenzene = 1.2 gcm^{-3}

Volume of nitrobenzene taken = 2 mL

Amount of nitrobenzene taken = Specific gravity x Volume of nitrobenzene = 2.4g

Theoritical Yield –

1 Mole of Nitrobenzene gives 1Mole of m-dinitrobenzene

123g of nitrobenzene gives 168g of m-dinitrobenzene

2.4g of nitrobenzene $= \frac{2.4 \times 168}{123}$ g of m-dinitrobenzene

= 3.278g of m-dinitrobenzene

Practical Yield –

Weight of empty weighing bottle (W_1) = ……g

Weight of weighing bottle + Crude Product (W_2) = ……g

Weight of Crude Product (W_2-W_1) = ……g

Percentage Yield –

% Yield = $\frac{\text{Practical Yield}}{\text{Theoritical Yield}} \times 100$ = ……

Melting point of the product was found to be = ……℃

Experiment No.1 **Date:**

Aim: To prepare m-dinitrobenzene from nitrobenzene.

Principle: The nitrobenzene undergoes nitration at meta position as the existing nitro group in nitrobenzene is an electron withdrawing group (EWG) and meta directing, thus forming meta di-nitro benzene. Nitration follows the general mechanism of an electrophilic aromatic substitution reaction. The nitration is carried out with a mixture of conc. nitric acid and conc. sulphuric acid.

Chemicals Required:

1. Nitrobenzene - 2mL
2. Conc. nitric Acid - 3mL
3. Conc. sulphuric Acid – 5mL

Procedure:

In a clean and dry 100mL conical flask 2mL of nitrobenzene is taken and 2mL of conc. sulphuric acid is added to it and placed in ice bath. Prepare nitrating mixture in a separate 100mL beaker placed in ice bath by carefully adding 3mL of conc. sulphuric acid to 3mL of conc. nitric acid. Now carefully add the nitrating mixture in dropwise manner to the flask containing nitrobenzene with constant stirring. Cool the reaction mixture and slowly transfer into a 250mL beaker containing 30mL of ice cold water. Separate the crude product using Buchner funnel at suction and wash with cold water. Dry and weigh the crude product.

Recrystallization – Take a pinch of the crude product in 1mL of ethanol, warm it slightly to dissolve the crystals and filter. The filtrate is cooled to room temperature slowly to allow the crystallisation to take place. The melting point of the recrystallized product is determined.

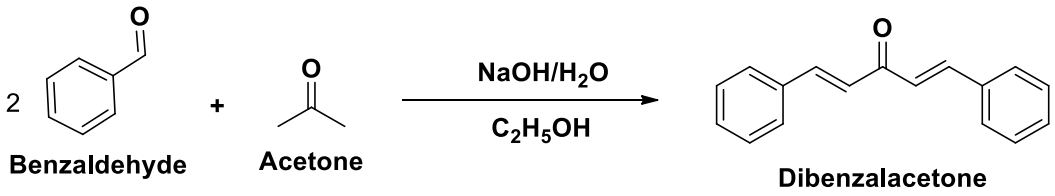
Result:

Practical Yield is ……g

% Yield is ……%

The melting point of the recrystallized product was found to be was found to be ……℃

Reaction:



Reaction Mechanism:

Observation and Calculations:

Specific gravity of Benzaldehyde = ……..gcm^{-3}

Volume of Benzaldehyde taken = ……..mL

Amount of Benzaldehyde taken (x) = Specific gravity x Volume of Benzaldehyde = ……..g

Theoritical Yield –

2 Mole of Benzaldehyde gives 1Mole of dibenzal acetone

212g of Benzaldehyde gives 234g of dibenzal acetone

xg of Benzaldehyde= $\frac{x \times 234}{212}$ g of dibenzal acetone

= ……..g of dibenzal acetone

Practical Yield –

Weight of empty weighing bottle (W_1) = ……g

Weight of weighing bottle + Crude Product (W_2) = ……g

Weight of Crude Product (W_2-W_1) = ……g

Percentage Yield –

% Yield = $\frac{\text{Practical Yield}}{\text{Theoritical Yield}} \times 100$ = ……

Melting point of the Product was found to be = ……℃

Experiment No.2 **Date:**

Aim: To prepare dibenzalacetone from benzaldehyde. (using acetone and NaOH)

Principle: Dibenzalacetone is synthesized from benzaldehyde and acetone through a Claisen-Schmidt condensation, a type of aldol condensation reaction, in the presence of a base like sodium hydroxide (NaOH). This reaction involves the condensation of two molecules of benzaldehyde with one molecule of acetone, resulting in the formation of the unsaturated ketone, dibenzalacetone.

Chemicals Required:

1. Benzaldehyde – 4mL
2. Acetone – 3mL
3. Ethanol – 25mL
4. Sodium Hydroxide – 4.5g

Procedure:

In a 250mL beaker, 4.5g of sodium hydroxide is dissolved in a mixture of 25mL ethanol and 30mL distilled water. Cool the above solution in an ice bath and maintain it at about 20℃. Now prepare a mixture of 4mL benzaldehyde and 3mL of acetone and slowly add half of this mixture to the ice cold NaOH solution with vigorous stirring. A fluffy precipitate is formed, stir the mixture gently for about 10 minutes. Now add the remaining mixture of benzaldehyde and acetone and allow it to sit for another 30 minute with occasional stirring. Filter the pale yellow solid thus obtained and wash with cold water, dry and weigh the crude product.

Recrystallization – Take a pinch of the crude product in 1mL of ethanol or ethyl acetate, warm it slightly to dissolve the crystals and filter. The filtrate is cooled to room temperature slowly to allow the crystallisation to take place. The melting point of the recrystallized product is determined.

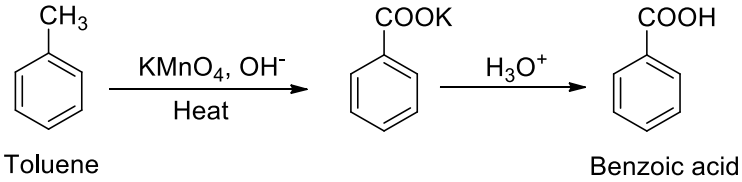
Result:

Practical Yield is ……g

% Yield is ……%

The melting point of the recrystallized product was found to be was found to be ……℃

Reaction:



Reaction Mechanism:

Observation and Calculations:

Specific gravity of Toluene = ……..gcm^{-3}

Volume of Toluene taken = ……..mL

Amount of Toluene taken (x) = Specific gravity x Volume of Toluene = ……..g

Theoritical Yield –

1 Mole of Toluene gives 1 Mole of benzoic acid

92g of Toluene gives 122g of benzoic acid

xg of Toluene $= \frac{x \times 122}{92}$ g of benzoic acid

= ……..g of benzoic acid

Practical Yield –

Weight of empty weighing bottle (W_1) = ……g

Weight of weighing bottle + Crude Product (W_2) = ……g

Weight of Crude Product (W_2-W_1) = ……g

Percentage Yield –

% Yield = $\frac{\text{Practical Yield}}{\text{Theoritical Yield}} \times 100$ = ……

Melting point of the product was found to be = ……℃

Experiment No.3 **Date:**

Aim: To prepare benzoic acid from toluene.

Principle:

The preparation of benzoic acid from toluene is based on the principle of oxidation of the methyl group attached to the benzene ring. In this reaction, the methyl group ($–CH_3$) of toluene is oxidized to a carboxylic acid group (–COOH) using strong oxidizing agents such as potassium permanganate ($KMnO_4$) or chromic acid (H_2CrO_4) under acidic or basic conditions. The oxidation proceeds through intermediate stages (e.g., benzyl alcohol and benzaldehyde), but under the reaction conditions, the entire side chain is fully oxidized to form benzoic acid, regardless of the length of the alkyl group. The aromatic ring remains unaffected, making this a selective oxidation of the side chain. This reaction demonstrates the stability of the benzene ring and the susceptibility of benzylic hydrogens to oxidation.

Chemicals Required:

1. Toluene – 1mL
2. $KMnO_4$ solution – 50mL
3. Anhydrous Na_2CO_3 – 2g
4. 25% Na_2SO_3 solution
5. Conc. HCl

Procedure:

Boil a mixture of 1mL of toluene, (5g $KMnO_4$ in 50ml water) 50mL of saturated aqueous $KMnO_4$ solution and 2g of anhydrous Na_2CO_3 under reflux for 30 minutes. Acidify with conc. HCl and then add 25% Na_2SO_3 solution until brown precipitate of MnO_2 has dissolved. On cooling benzoic acid crystallises out. Filter through small Buchner funnel, wash with water, dry and weigh the crude product. Recrystallize from water and measure the melting point.

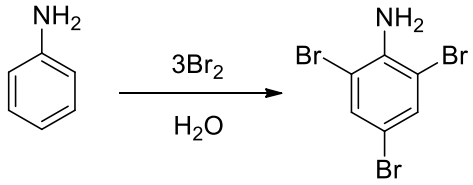
Result:

Practical Yield is ……g

% Yield is ……%

The melting point of the recrystallized product was found to be was found to be ……°C

Reaction:



Aniline 2,4,6-tribromoaniline

Reaction Mechanism:

Observation and Calculations:

Specific gravity of Aniline = ……..gcm^{-3}

Volume of Aniline taken = ……..mL

Amount of Aniline taken (x) = Specific gravity x Volume of Aniline = ……..g

Theoritical Yield –

1 Mole of Aniline gives 1Mole of Tribromoaniline

93g of Aniline gives 329.8g of Tribromoaniline

xg of Aniline $= \frac{x \times 329.8}{93}$ g of Tribromoaniline

= ……..g of Tribromoaniline

Practical Yield –

Weight of empty weighing bottle (W_1) = ……g

Weight of weighing bottle + Crude Product (W_2) = ……g

Weight of Crude Product (W_2-W_1) = ……g

Percentage Yield –

% Yield = $\frac{\text{Practical Yield}}{\text{Theoritical Yield}} \times 100$ = ……

Melting/Boiling Point of the Product was found to be = ……℃

Experiment No.4 **Date:**

Aim: To prepare tribromo aniline from aniline.

Principle:

The bromination of aniline operates on the principle of electrophilic aromatic substitution (EAS), a reaction in which an electrophile, such as bromine (Br_2), replaces a hydrogen atom on the benzene ring. The amino group ($–NH_2$) in aniline is a powerful electron-donating group that enhances the electron density of the ring, particularly at the ortho and para positions, through resonance and inductive effects. This increased reactivity causes the aromatic ring to readily undergo substitution with bromine, often resulting in multiple brominations and the formation of 2,4,6-tribromoaniline.

Chemicals Required:

1. Aniline – 1mL
2. Acetic acid – 13mL
3. Bromine – 1.7mL

Procedure:

1.7mL solution of bromine in 8mL of glacial acetic acid is added drop wise to a solution of distilled aniline and glacial acetic acid in a 100mL conical flask. During the addition continuously shake the flask and cool in ice bath. A yellow coloured pasty mass separates. Pour the reaction mixture in an excess of ice cooled water in a beaker. Filter the separated product, wash with water and crystallise from rectified spirit. The melting point of the recrystallized product is determined.

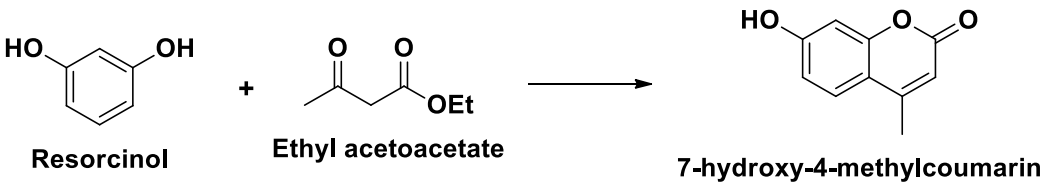
Result:

Practical Yield is ……g

% Yield is ……%

The melting point of the recrystallized product was found to be was found to be ……℃

Reaction:



Resorcinol + Ethyl acetoacetate → 7-hydroxy-4-methylcoumarin

Reaction Mechanism:

Observation and Calculations:

Amount of Resorcinol taken (x) = ……..g

Theoritical Yield –

1 Mole of Resorcinol gives 1Mole of 7-Hydroxy-4-methylcoumarin

110g of Resorcinol gives 176g of 7-Hydroxy-4-methylcoumarin

xg of Resorcinol $= \frac{x \times 176}{110}$ g of 7-Hydroxy-4-methylcoumarin

= ……..g of 7-Hydroxy-4-methylcoumarin

Practical Yield –

Weight of empty weighing bottle (W_1) = ……g

Weight of weighing bottle + Crude Product (W_2) = ……g

Weight of Crude Product (W_2-W_1) = ……g

Percentage Yield –

% Yield = $\frac{\text{Practical Yield}}{\text{Theoritical Yield}} \times 100$ = ……

Melting point of the Product was found to be = ……℃

Experiment No.5 **Date:**

Aim: To prepare coumarin from resorcinol and ethyl acetoacetate.

Principle:

The preparation of coumarin from resorcinol is based on the principle of Pechmann condensation, a type of acid-catalysed electrophilic substitution reaction between a phenol (like resorcinol) and a β-ketoester (commonly ethyl acetoacetate). In this reaction, under the influence of a strong acid catalyst such as concentrated sulphuric acid, the phenolic hydroxyl group of resorcinol attacks the carbonyl carbon of the β-ketoester, initiating a sequence of reactions including **e**sterification, electrophilic substitution, and intramolecular cyclization. This results in the formation of the lactone ring characteristic of coumarins. The process ultimately leads to the formation of coumarin, a fragrant compound with a benzopyrone structure, through the condensation and ring closure of the intermediates formed from resorcinol and ethyl acetoacetate.

Chemicals Required:

1. Resorcinol – 2g
2. Ethyl acetoacetate – 3mL
3. Conc. Sulphuric acid – 20mL

Procedure:

In a 250mL conical flask take 20mL of conc. sulphuric acid. Cool it to 0-5℃ in an ice bath. Then add a solution of 2g of resorcinol in 3mL of ethyl acetoacetate in a drop wise manner with constant stirring. Allow the contents to cool to room temperature and let it stand for 15 minutes. Now pour reaction mixture in ice cold water. The crude product of coumarin precipitates out, filter and wash with cold water for 2-3 times. The crude product is dissolved in 30mL of 5% NaOH. Acidify with 2N HCl with vigorous stirring until coumarin separates out. Collect the product by filtration, wash with 25mL cold water, dry and weigh.

Recrystallization – Take a pinch of the crude product in 1mL of ethanol or ethyl acetate, warm it slightly to dissolve the crystals and filter. The filtrate is cooled to room temperature slowly to allow the crystallisation to take place. The melting point of the recrystallized product is determined.

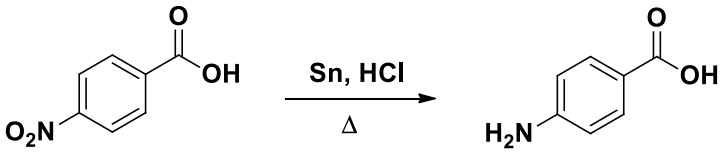
Result:

Practical Yield is ……g

% Yield is ……%

The melting point of the recrystallized product was found to be was found to be ……℃

Reaction:



p-nitrobenzoic acid p-aminobenzoic acid

Reaction Mechanism:

Observation and Calculations:

Amount of p-nitro benzoic acid taken (x) = ……..g

Theoritical Yield –

1 Mole of p-nitro benzoic acid gives 1Mole of p-amino benzoic acid

167g of p-nitro benzoic acid gives 137g of p-amino benzoic acid

xg of p-nitro benzoic acid $= \frac{x \times 137}{167}$ g of p-amino benzoic acid

= ……..g of p-amino benzoic acid

Practical Yield –

Weight of empty weighing bottle (W_1) = ……g

Weight of weighing bottle + Crude Product (W_2) = ……g

Weight of Crude Product (W_2-W_1) = ……g

Percentage Yield –

% Yield = $\frac{\text{Practical Yield}}{\text{Theoritical Yield}} \times 100$ = ……

Melting/Boiling Point of the Product was found to be = ……℃

Experiment No.6 **Date:**

Aim: To prepare p-amino benzoic acid from p-nitro benzoic acid.

Principle:

The preparation of p-amino benzoic acid from p-nitro benzoic acid is based on the principle of reduction of the nitro group ($–NO_2$) to an amino group ($–NH_2$). In this process, the nitro group attached to the benzene ring in p-nitro benzoic acid is chemically reduced, typically using reducing agents such as iron with hydrochloric acid, tin with hydrochloric acid, or catalytic hydrogenation. This reduction transforms the strongly electron-withdrawing nitro group into an electron-donating amino group, converting p-nitro benzoic acid into p-amino benzoic acid while retaining the carboxylic acid functional group. This reaction exemplifies the selective reduction of nitro groups on aromatic rings without affecting other sensitive functional groups.

Chemicals Required:

1. p-nitro benzoic acid – 3g
2. Tin granules – 10.6g
3. Hydrochloric acid – 15mL
4. Ammonia
5. Glacial acetic acid

Procedure:

Take 3g of p-nitro benzoic acid in a 100mL round bottom flask with reflux condenser containing 15mL of hydrochloric acid. To the above flask add 10.6g of tin granules. Now heat the resulting mixture on a water bath until tin dissolves at 100℃ with constant stirring. Cool the reaction mixture to lab temperature and decant the supernatant into a beaker and wash the residual tin with water. Add concentrated ammonia solution till solution is alkaline to red litmus paper and hydrated tin oxide separates out. Filter the content on filter paper and collect the filtrate. Concentrate the filtrate to reduce the volume, filter off any solids that separates. Acidify the concentrated liquid with glacial acetic acid until the solution is just acid to litmus and evaporate the content on water bath until crystals commence to separate, cool in ice, filter the crystals and dry it. Weigh and then the melting point of the recrystallized product is determined.

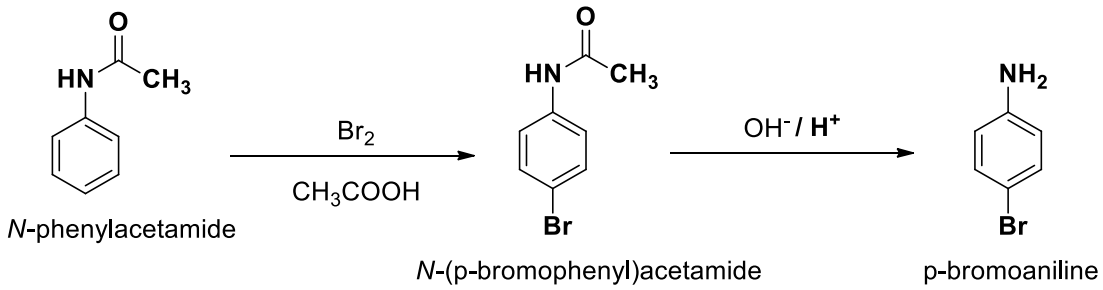
Result:

Practical Yield is ……g

% Yield is ……%

The melting point of the recrystallized product was found to be was found to be ……℃

Reaction:



Reaction Mechanism:

Observation and Calculations:

Amount of Acetanilide taken (x) = ……..g

Theoritical Yield –

1 Mole of Acetanilide gives 1Mole of p-bromo aniline

135g of Acetanilide gives 172g of p-bromo aniline

xg of Acetanilide $= \frac{x \times 172}{135}$ g of p-bromo aniline

= ……..g of p-bromo aniline

Practical Yield –

Weight of empty weighing bottle (W_1) = ……g

Weight of weighing bottle + Crude Product (W_2) = ……g

Weight of Crude Product (W_2-W_1) = ……g

Percentage Yield –

% Yield = $\frac{\text{Practical Yield}}{\text{Theoritical Yield}} \times 100$ = ……

Melting/Boiling Point of the Product was found to be = ……℃

Experiment No.7 **Date:**

Aim: To prepare p-bromo aniline from acetanilide.

Principle:

The preparation of p-bromoaniline from acetanilide is based on the principle of electrophilic aromatic substitution (EAS), where a bromine atom is selectively introduced onto the benzene ring. Acetanilide is used instead of aniline to temporarily protect the $–NH_2$ group by converting it into an amide ($–NHCOCH_3$), which is less activating than the free amino group. This moderates the reactivity of the ring and allows for controlled bromination, favouring substitution at the para position due to both electronic effects and reduced steric hindrance. After bromination with bromine in a suitable solvent (like glacial acetic acid), the product p-bromoacetanilide is formed. This is then hydrolysed under acidic or basic conditions to remove the acetyl group, regenerating the free $–NH_2$ group and yielding p-bromoaniline as the final product.

Materials Required:

1. Acetanilide – 4.5g
2. Glacial Acetic acid – 20mL
3. 20% Bromine in acetic acid
4. Saturated Sodium Bisulphite solution
5. Conc. hydrochloric acid solution – 10mL
6. 10% NaOH solution

Procedure:

STEP 1 - 4.5g of acetanilide is dissolved in 20mL of glacial acetic acid with stirring. To this add 20% solution of bromine in acetic acid is added in small portions with shaking till the solution becomes pale yellow. The reaction mixture is allowed to stand for 30minutes and then poured in cold water. The p-bromo acetanilide separates out. It is then filtered at pump using Buchner funnel washed with sodium bisulphite solution, then with water and dried.

STEP 2 – p-bromo acetanilide obtained is mixed with 10mL of conc. hydrochloric acid and the reaction mixture is refluxed for about 30 minutes. Then the reaction mixture is cooled under water diluted with water and10% solution of NaOH is added till the solution is basic. The crystals of p-bromoaniline separates out and are filtered at pump using Buchner funnel. It is then washed with water and dried. A portion is recrystallized from equal volume of alcohol and water. The melting point of the recrystallized product is determined.

Result:

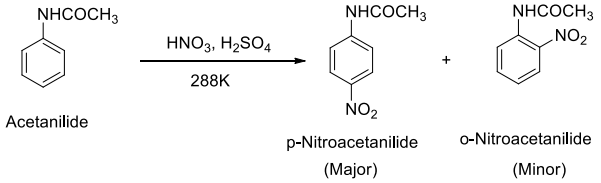
Practical Yield is ……g

% Yield is ……%

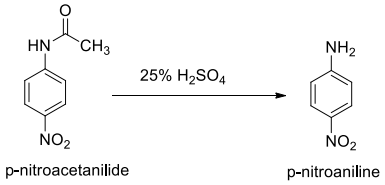
The melting point of the recrystallized product was found to be was found to be ……°C

Reaction:

STEP 1:



STEP 2:



Reaction Mechanism:

Observation and Calculations:

Amount of Acetanilide taken (x) = ……..g

Theoritical Yield –

1 Mole of Acetanilide gives 1Mole of p-nitro aniline

135g of Acetanilide gives 138g of p-nitro aniline

xg of Acetanilide $= \frac{x \times 138}{135}$ g of p-nitro aniline

= ……..g of p-nitro aniline

Practical Yield

Weight of empty weighing bottle (W_1) = ……g

Weight of weighing bottle + Crude Product (W_2) = ……g

Weight of Crude Product (W_2-W_1) = ……g

Percentage Yield –

% Yield = $\frac{\text{Practical Yield}}{\text{Theoritical Yield}} \times 100$ = ……

Melting/Boiling Point of the Product was found to be = ……℃

Experiment No.8 **Date:**

Aim: To p-nitro aniline from acetanilide.

Principle:

The preparation of p-nitroaniline from acetanilide is based on the principle of electrophilic aromatic substitution (EAS), specifically nitration, followed by hydrolysis. In this reaction, acetanilide is used instead of aniline to temporarily protect the amino group by converting it into an amide ($–NHCOCH_3$), which is less activating than the free $–NH_2$ group. This modification reduces the overall reactivity of the aromatic ring and directs the incoming nitro group ($–NO_2$) preferentially to the para position due to both electronic and steric effects. When acetanilide is treated with a nitrating mixture of concentrated nitric acid and sulfuric acid, p-nitroacetanilide is formed as the major product. This compound is then subjected to acidic or basic hydrolysis, which removes the acetyl protecting group and regenerates the free amino group, yielding p-nitroaniline.

Materials Required:

1. Acetanilide – 4.5g
2. Glacial acetic acid
3. Conc. Sulphuric acid
4. Conc. Nitric acid
5. 10% NaOH solution

Procedure:

STEP 1 - 4.5g of acetanilide is dissolved in glacial acetic acid, little conc. sulphuric acid is added and cooled in freezing mixture. To this 20mL of nitrating mixture is added carefully in small portions and temperature is maintained below 10℃ during addition. Then the reaction mixture is allowed to stand for one hour at room temperature. It is then poured into crushed ice. The precipitate is filtered at pump, washed with water, recrystallized with alcohol and dried.

STEP 2 - p-nitro acetanilide is refluxed with 70% sulphuric acid for about 30 minutes. p-nitro aniline obtained is then poured into water, made alkaline with 10% NaOH solution. The precipitate of p-nitro aniline is recrystallized from hot water, dried and weighed. The melting point of the recrystallized product is determined.

Result:

Practical Yield is ……g

% Yield is ……%

The melting point of the recrystallized product was found to be was found to be ……℃

Observation and Calculations:

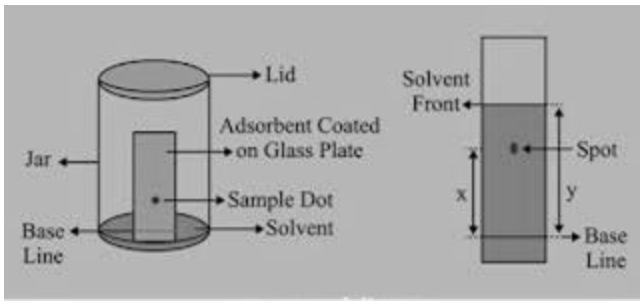


Figure 1: Schematic Representation of Thin Layer Chromatography (TLC)

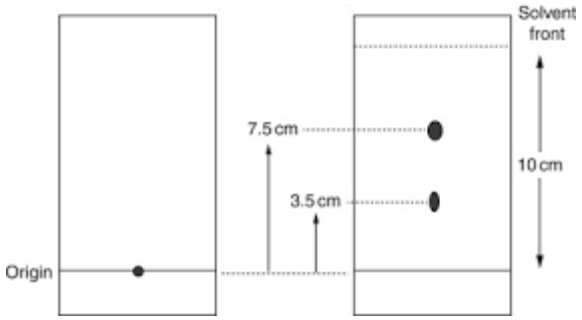


Figure 2: Separation of o - nitro phenol and p - nitro phenol

$$R_f = \frac{Distance\ travelled\ by\ o\text{-}nitro\ phenol}{Distance\ travelled\ by\ Solvent} =$$

$$R_f = \frac{Distance\ travelled\ by\ p\text{-}nitro\ phenol}{Distance\ travelled\ by\ Solvent} =$$

Experiment No.9 **Date:**

Aim: Separation of a mixture of two organic compounds by TLC – Ortho & Para nitro phenols.

Principle:

Thin Layer Chromatography (TLC) is an analytical technique used to separate and identify compounds in a mixture based on their different affinities toward a stationary phase (usually silica gel) and a mobile phase (solvent). In this experiment, a mixture of o-nitrophenol and p-nitrophenol is separated using a hexane and ethyl acetate solvent system. The two isomers differ in polarity and hydrogen bonding capability:

- o-Nitrophenol forms intramolecular hydrogen bonds, making it l**ess** polar, so it moves farther up the TLC plate.
- p-Nitrophenol forms intermolecular hydrogen bonds, making it more polar, so it sticks more to the polar stationary phase and moves **less**.

These polarity differences result in different R_f values, allowing clear separation on the TLC plate.

Materials Required:

1. TLC plates
2. Ethylacetate
3. Hexane
4. Ortho and para nito phenols
5. TLC Chamber
6. Capillary tubes

Procedure:

1. Preparation of TLC Plate:

- Take a pre-coated silica gel TLC plate.
- Draw a light pencil line about 1 cm from the bottom (baseline).

2. Spotting the Sample:

- Dissolve the mixture of o-nitrophenol and p-nitrophenol in a few drops of a suitable solvent (e.g., ethanol).
- Using a capillary tube, apply a small spot of the solution on the baseline.

3. Preparation of Mobile Phase (Eluent):

- Prepare a mixture of 80% hexane and 20% ethyl acetate.
- Pour the solvent into a TLC chamber (beaker with a lid) and allow it to saturate with vapor.

4. Running the TLC:

- Place the TLC plate vertically in the chamber without touching the solvent line.

- Allow the solvent to rise until it is about 3/4 up the plate.
- Remove the plate and mark the solvent front immediately.

6. Visualization:

- Dry the plate and observe under UV light or by spraying with a developing agent.
- Two distinct spots will appear:
  - The higher spot corresponds to o-nitrophenol.
  - The lower spot corresponds to p-nitrophenol.

7. Calculate R_f Values:

$$R_f = \frac{\text{Distance moved by compound}}{\text{Distance moved by solvent}}$$

Result:

1. Two separate spots confirm the successful separation.
2. R_f of o-nitrophenol =
3. R_f of p-nitrophenol =

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