BGS SCIENCE ACADEMY & RESEARCH CENTER
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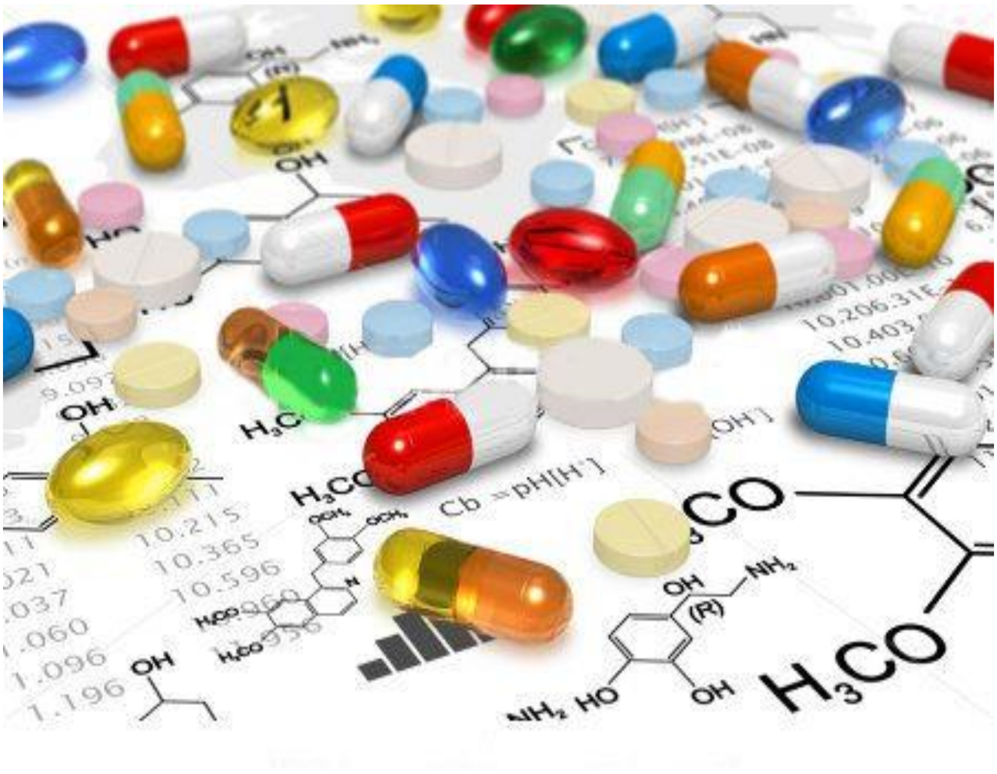
III Semester B.Sc.,
Chemistry Laboratory Manual



CONTENTS

Two stage preparations

EXPERIMENT NO. 01

PURIFICATION OF ORGANIC COMPOUNDS

AIM: RECRYSTALLISATION AND DETERMINATION OF MELTING POINT OF SOLIDS

PART A: PURIFICATION OF AN ORGANIC SOLID BY RE- CRYSTALLISATION

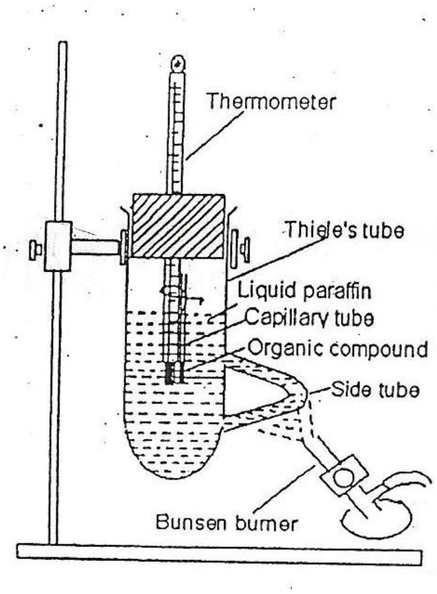
Principle: Recrystallisation is the most common method for the purification of crude organic solids. It is based on the fact that in a particular solvent, certain organic compounds dissolves less at room temperature but more at high temperatures compared to the impurities. The saturated solution of the compound is heated to evaporate the solvent and the recrystallised sample is the purified compound.

Procedure: The given sample of m-dinitrobenzene is transferred into a clean beaker. 10 ml of alcohol is added, heated on a water bath for 10-15 mins. till the volume of the solution reduces to 3-4 ml. Suddenly the saturated hot solution is filtered through a filter paper fixed on a stem less funnel. The filtrate is collected into a beaker containing 50 ml of ice water. The needle shaped pure product is filtered through a Buchner funnel and filtered.

PART-B: DETERMINATION OF MELTING POINT OF AN ORGANIC SOLID

Principle: Melting point of a substance is the temperatue at which the solid and liquid phases of a substance exist together. A pure organic solid melts sharply at a definite temperature.

Procedure: The apparatus used for the determination of melting points is a Thiel's tube which consists of liquid paraffin. A small amount of the finely powderedorganic solid is introduced into a small capillary tube sealed at one end.



The capillary tube is attached into a thermometer whose lower end is wetted by the liquid paraffin. The thermometer along with the tube is suspended in liquid paraffin bath. The bath is heated slowly by rotating the burner around the side tube. The temperature at which the solid becomes transparent that is just melts is noted. organic solid is introduced into a small capillary tube sealed at one end. The capillary tube is attached into a thermometer whose lower end is wetted by the liquid paraffin bath. The bath is heated slowly by rotating the burner around the side tube. The temperature at which the solid becomes transparent that is just melts is noted.

Result: The melting point of the given organic solid is………. °C

EXPERIMENT NO.02

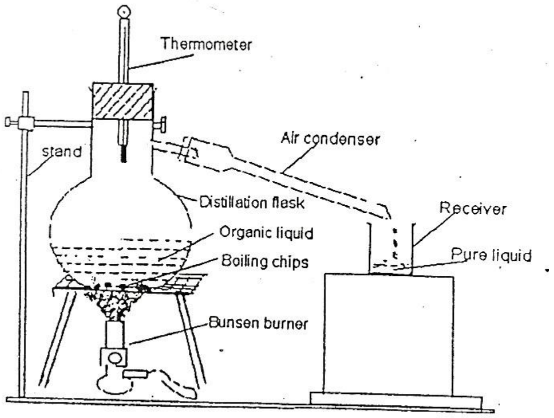
PURIFICATION OF ORGANIC LIQUIDS BY DISTILLATION

AIM: DETERMINATION OF BOILING POINT OF ORGANIC LIQUIDS (PURIFICATION OF AN ORGANIC SOLID BY DISTILLATION)

Principle: Boiling point of a liquid is the temperature at which vapour pressure of a liquid becomes equal to the atmospheric pressure. This principle is used for the purification of those liquids which boil without decomposition provided the impurities present are non-volatile.

Procedure: The given impure liquid is transferred into a round bottomed flask or a distillation flask fitted with a thermometer and an air cooled condenser as shown in the diagram. The flask is heated slowly, the liquid vapourises. The vapours pass through the condenser continuously, at this stage, the temperature is recorded. The vapours changes into liquid and this liquid is collected in the receiver flask which is the purified liquid. The constant temperature at which the liquid vapourises is recorded from the thermometer, which the observed boiling point of the liquid. To the observed boiling suitable correction is applied (3%) and it is added to the observed boiling point which gives the corrected boiling point of the liquid.

Diagram:



Recording the temperatures:

a. Observed Boiling point: …………………..^{o}C
b. 3% Correction to (a): ………………………… ^{o}C
c. Corrected Boiling point: (a+b) : ……………………^{o}C

Note: Boiling point of liquid decreases with increase in altitude, since Bengaluru region is 3000 ft. above the sea level, 3% correction is added to the observed boiling point.

Result: The boiling point of the given organic liquid is …………^{o}C

EXPERIMENT NO.3:

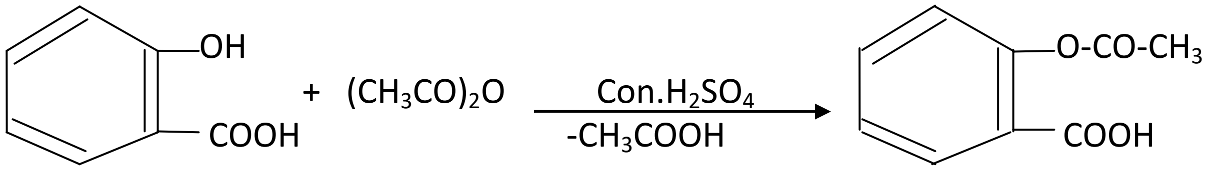
AIM: PURIFICATION OF SOLIDS BY SUBLIMATION

Principle: Sublimation method of purification is used for the solids which on heating readily converts directly from solids to vapor state with a subsequent and ready reversal of this process when the vapor is cooled.

Procedure: About 5g of the crude sample to be purified is placed in a small clean and dry china dish, a wire guage is placed above it, then the dish is covered with a filter paper in which small holes have been made by piercing it with a glass rod. An inverted glass funnel is kept on the filter paper. The tip of the funnel is plugged with cotton. It is gently heated using a Bunsen flame. The substance vaporizes and the vapor passes up through the holes into the funnel. The vapors are condensed as fine crystals on the upper surface of the filter paper and on the walls of the funnel. The heating is continued till no more vapors are evolved. The heating is continued till no more vapors are evolved, then the heating is stopped and the crystals are scrapped from the filter paper.

OBSERVATIONS

REACTION:



Salicylic acid Acetic anhydride Acetyl Salicylicacid (Aspirin)

Calculation of Theoretical Yield:

Molecular formula: $\mathbf{C_7H_6O_3} \longrightarrow \mathbf{C_9H_8O_4}$

Salicylic acid → Aspirin

Molecular weight: **(7x12) + (6x1) + (3x16)** **= 138 g**   **(9x12) + (8x1) + (4x16)** **= 180 g**

138 g of Salicylic acid on acetylation gives 180 g of acetyl salicylic acid

2.5 g of Salicylic acid on acetylation gives………..= $\frac{180x2.5}{138}$ = 3.26 g of aspirin

EXPERIMENT NO.4.

AIM: PREPARATION OF ACETYL SALICYLIC ACID (ASPIRIN) FROM SALICYLIC ACID

Principle: Salicylic acid when treated with acetic anhydride in presence of con.sulphuric acid as dehydrating agent undergoes acetylation reaction to give its acetylated product acetyl salicylic acid or aspirin.

Procedure: About 2.5g of salicylic acid crystals are transferred into a 100 cm^3 clean and dried conical flask. To this 3 cm^3 of acetic anhydride and one drop of con. sulphuric acid are added. The contents are shaken well and the conical flask is tightly stoppered. It is then heated in a hot water bath for about 15 minutes with constant stirring till homogeneous solution is got. Then the conical flask is cooled under the tap and the reaction mixture is poured into a beaker containing ice cold water with constant stirring. The Solid acetyl salicylic acid thus obtained is filtered through the Buchner funnel with suction, washed with excess of water, dried and weighed. The sample is kept for inspection.

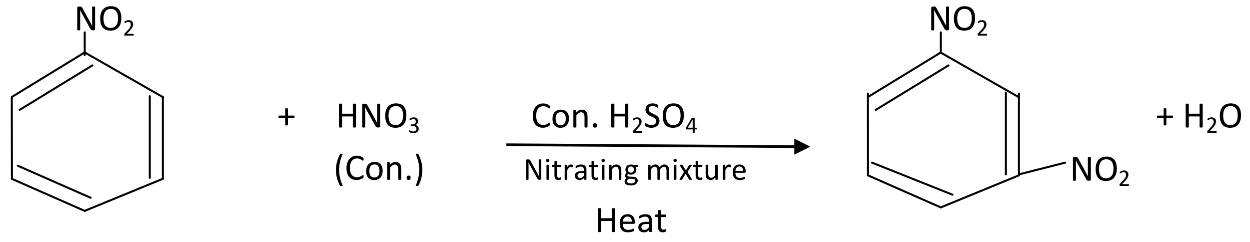
Recrystallisation: A pinch of aspirin obtained above is added to about 1 cm^3 of dilute acetic acid. The solution is warmed slightly to dissolve the crystals and filtered. The filtrate is cooled to room temperature slowly. Pure crystals of aspirin gets crystallized which are filtered, dried and kept for inspection. The melting point of the recrystallized sample is determined.

Report:

Theoretical Yield	Practical Yield
3.26 g	

OBSERVATIONS

REACTION:



Nitrobenzene m-dinitrobenzene

Calculation of Theoretical Yield:

i) Density of nitrobenzene = 1.2 g.cm^{-3}

ii) Volume of nitrobenzene = 2.0 cm^{3}

iii) Mass of nitrobenzene = 1.2 x 2 = 2.4 g

Molecular formula:

$$\mathbf{C_6H_5NO_2 \longrightarrow C_6H_4(NO_2)_2}$$

Nitrobenzene m-dinitrobenzene

Molecular wt. (6x12) + (5x1) + (1x14)+ (2x16)→ (6x12) + (4x1) + (2x14)+ (4x16)
= 123g = 168g

123 g of Nitrobenzene on nitration gives 168 g of m-dinitrobenzene

2.4 g of Nitrobenzene on nitration gives $\frac{168 \times 2.4}{123}$ = 3.278 g of m-dinitrobenzene

EXPERIMENT NO.5.

AIM: PREPARATION OF meta-DI-NITROBENZENE FROM NITROBENZENE

Principle: Nitrobenzene is heated with nitration mixture, it undergoes nitration to give meta-dinitrobenzene. This reaction is an electrophilic substitution reaction involving the electrophile nitronium ion.

Procedure: About 2 cm^3 of nitrobenzene is taken a 100 cm^3 clean and dry conical flask. 5 cm^3 of con.sulphuric acid is added to the flask, then 3 cm^3 of con.nitric- acid is added drop wise with constant stirring for 10 mins. The contents of the flask is heated in a boiling water bath till the reaction is completed (reddish- brown fumes of nitrogen peroxide ceases to evolve). The completion of the reaction is tested by testing a drop of the reaction mixture produces yellow precipite in cold water. The contents of the reaction mixture is cooled to the room temperature, then it is added to a beaker containing ice cold water with slow stirring of the contents. A yellow precipitate of meta dinitrobenzene obtained is filtered using a Buchner funnel at suction, it is washed with cold water, dried and weighed. The sample is kept for inspection(nitration can also be carried out using potassium nitrate and Con.H_2SO_4)

Recrystallization: A pinch of meta-dinitrobenzene is added to about 1 cm^3 of alcohol, the solution is warmed slightly to dissolve the crystals and filtered. The filtrate is cooled to the room temperature slowly. Pure crystals of meta dinitrobenzene gets crystallized which are filtered, dried and kept for inspection. The melting point of the recrystallised sample is determined.

Report:

Theoretical Yield	Practical Yield
3.278 g	

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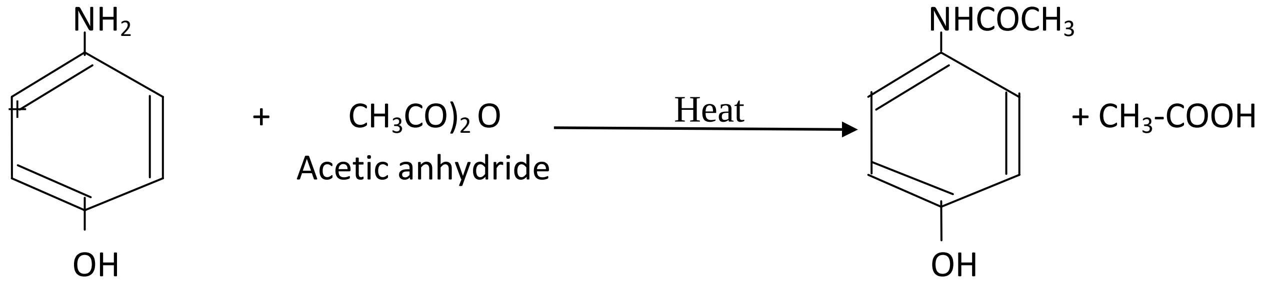
OBSERVATIONS

REACTION:



p-Amino phenol p-hydroxy acetanilide(p-Acetamol)

Calculation of Theoretical Yield

Mass of p-amino phenol = 2.0 g

Molecular formula: $\mathbf{C_6H_7NO} \longrightarrow \mathbf{C_8H_9NO_2}$

p-amino phenol p-acetamol

Molecular wt.= $(6\times12) + (7\times1) + (1\times14) + (1\times16) \rightarrow (8\times12) + (9\times1) + (1\times14) + (2\times16)$

= 109g **= 151g**

109 g of p-aminophenol on acetylation gives 151 g of p-acetamol

2.0 g of p-aminophenol on acetylation gives $\frac{151 \times 2}{109} = 2.77$ g of p-acetamol

EXPERIMENT NO.6.

AIM: PREPARATION OF para-HYDROXY ACETANILIDE (p-ACETAMOL) FROM para-AMINO PHENOL

Principle: When p-amino phenol is treated with acetic anhydride, it undergoes acetylation reaction to give its acetylated product para-acetamol or p-hydroxy-acetanilide. This reaction is an electrophilic substitution reaction.

Procedure: 2g of p-amino phenol is taken in a 100 cm^3 conical flask, 6 cm^3 of acetic anhydride is added into it. The contents of the flask is stirred vigorously and the flask is heated on a water bath so that the solid dissolves completely. The flask is cooled in an ice bath and the sides of the flask is scratched to induce crystallization. The solid obtained is filtered on a Buchner funnel and washed with cold water.

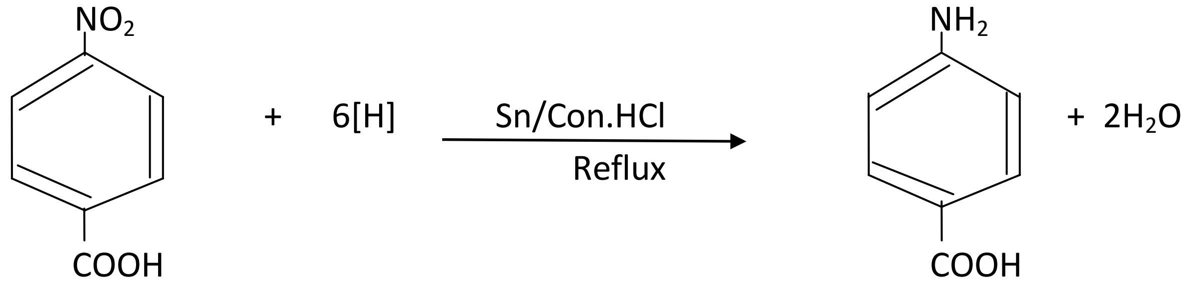
Recrystallisation: A pinch of the product p-acetamol is added to about 1 cm^3 of hot water and the solution is warmed slightly to dissolve the crystals and filtered. The filtrate is cooled to the room temperature slowly. Pure crystals of p-acetamol gets crystallised which are filtered, dried and kept for inspection. The melting point of the recrystallised sample is determined.

Report:

Theoretical Yield	Practical Yield
2.77 g	

<u>OBSERVATIONS</u>

<u>REACTION</u>:



p-nitro benzoic acid

p-amino benzoic acid

<u>Calculation of Theoretical Yield:</u> Mass of p-amino phenol = 2.0 g

Molecular formula: $\mathbf{C_7H_5NO_3} \longrightarrow \mathbf{C_7H_7NO_2}$

p-nitro benzoic acid p-amino benzoic acid

Molecular wt: (7x12) + (5x1) + (1x14)+ (3x16) → (7x12) + (7x1) + (1x14)+ (2x16)

= 151g **= 137g**

151 g of p-nitro benzoic acid on reduction gives 137 g of p-amino benzoic acid

2g of p-nitro benzoic acid on reduction gives = $\frac{137 \times 2}{151}$ = 1.815 g of p-amino-benzoic acid

EXPERIMENT NO.7.

AIM: PREPARATION OF para-AMINO BENZOIC ACID FROM para-NITRO BENZOIC ACID

Principle: When p-nitro benzoic acid is reduced using tin and con.HCl, it reduces to give its reduced product para-amino benzoic acid.

Procedure: 2g of p-nitro benzoic acid is placed in a 500 ml round bottomed flask, 6g of powdered tin and 12 ml of con.hydrochloric acid is added to it, a reflux condenser is attached to the flask and the contents are heated in low flame, when the reaction commences, the heat source is removed. The flask is gently shaken for 20 minutes so that all the tin dissolves and a clear solution is obtained. If the reaction is not completed, the contents of the flask is heated for further 5 minutes for completion.The contents of the reaction mixture are cooled and filtered. Ammonium hydroxide is added to the filtrate until it is just alkaline, then heated on a water bath for 25 mins. and then filtered. The precipitate is washed with hot water. The concentrated filtrate and the residue is acidified with glacial acetic acid and heated on water bath till crystals starts separating out. The crystals are separated and dried.

Recrystallization: A pinch of the product p-amino benzoic acid is added to about 1 cm^3 of hot water or alcohol and the solution is warmed slightly to dissolve the crystals and filtered. The filtrate is cooled to the room temperature slowly. Pure crystals of p-amino benzoic acid gets crystallized which are filtered, dried and kept for inspection. The melting point of the recrystallised sample is determined.

Report:

Theoretical Yield	Practical Yield
1.815 g	

OBSERVATIONS

REACTION:

$$2\ C_6H_5CHO + CH_3COCH_3 \xrightarrow{\quad\quad} C_6H_5CH = CH\text{-}CO\text{-}CH = CH\text{-}C_6H_5 + 2H_2O$$

Benzaldehyde Acetone Di-benzalacetone

Calculation of Theoretical Yield:

i) Density of Benzaldehyde = 1.04 $g.cm^{-3}$
ii) Volume of Benzaldehyde = 2.0 cm^3
iii) Mass of Benzaldehyde =1.04 x 2 = 2.08g.

Molecular formula: $\mathbf{C_7H_6O \xrightarrow{\quad\quad} C_{17}H_{14}O}$

Benzaldehyde Dibenzal-acetone

Molecular weight: (7x12) + (6x1) + (1x16) → (17x12) + (14x1) + (1x16)

= 106g = 234g

106 g of benzaldehyde on condensation gives 234 g of di-benzalacetone

2.1g of benzaldehyde on condensation gives = $\frac{234 \times 2.08}{106}$ = 4.591 g of di-benzal acetone

EXPERIMENT NO.8.

AIM: PREPARATION OF DI-BENZALACETONE FROM BENZALDEHYDE

Principle: When an alcoholic solution containing both acetone and two moles ofbenzaldehyde is made alkaline with sodium hydroxide, rapid condensation o cm3 urs with the formation of dibenzal-acetone or dibenzylidene-acetone.

Procedure: 2 cm^3(2.1g) of benzaldehyde and 0.8 cm^3 of pure acetone are dissolved in 20 cm^3 of alcohol in a 100 cm^3 conical flask. 4 cm^3 of 10% aqueous sodium hydroxide solution is diluted with 16 cm^3 of water. This dilute alkali is added to the conical flask which is stoppered and shaken vigorously for about 10 minutes (releasing the pressure from time to time if necessary). The mixture is then allowed to stand for 30 minutes, with o cm3 asional shaking. It is then cooled in ice water. Pale yellow crystals of dibenzalacetone separate out. The crystals are filtered on a Buchner funnel, washed with water to eliminate traces of alkali, and drained thoroughly. The crystals are dried using blotting paper.

Recrystallization: A pinch of di-benzalacetone is added to about 1 cm^3 of alcohol. The solution is warmed slightly to dissolve the crystals and filtered. The filtrate is cooled to the room temperature slowly. Pure crystals of di-benzalacetone gets crystallized which are filtered, dried and kept for inspection. The melting point of the recrystallized sample is determined.

Report:

Theoretical Yield	Practical Yield
4.591 g	

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OBSERVATIONS

REACTION:

$$\underset{\text{Benzaldehyde}}{C_6H_5\text{–}CHO} + [O] \xrightarrow[\text{Heat}]{KMnO_4} \underset{\text{Benzoic acid}}{C_6H_5\text{- }COOH} + H_2O$$

Calculation of Theoretical Yield:

i) Density of Benzaldehyde = 1.04$g.cm^{-3}$

ii) Volume of = 5.0 cm^3

iii) Mass of Benzaldehyde = 1.04 x 5 = 5.2g

Molecular formula: $$\underset{\textbf{Benzaldehyde}}{C_7H_6O} \longrightarrow \underset{\textbf{Benzoic acid}}{C_7H_6O_2}$$

Molecular weight: (7x12) + (6x1) + (1x16) → (7x12) + (6x1) + (2x16)

= 106g =122g

106 g of Benzaldehyde on oxidation gives 122 g of benzoic acid

5.2g of Benzaldehyde on oxidation gives $= \frac{122 \times 5.2}{106}$ = 5.985 g of benzoic acid

EXPERIMENT NO.9.

AIM: PREPARATION OF BENZOIC ACID FROM BENZALDEHYDE

Principle: Benzaldehyde undergoes oxidation in presence of alkaline potassium permanganate solution give benzoic acid

Procedure: 5 cm^3 of benzaldehyde and 20 cm^3 of sodium carbonate solution(2N) are taken in a 250 cm^3 round bottomed flask and 2 or 3 pieces of the porous porcelain are added. The flask is fitted with a reflux condenser. The condenser is cooled by passing cold water. 10 gms of $KMnO_4$ is dissolved 200 cm^3 of water. The solution is taken in a dropping funnel. The dropping funnel is inserted through the top of the condenser. The contents of the flask are kept steadily boiling and the permanganate solution is added in rapid drops until the solution in the flask acquires a permanent pink colour even after prolonged boiling (about 10 minutes), brown manganese dioxide is precipitated.

The flask is carefully removed and cooled. The precipitated manganese dioxide is filtered. The filtrate is acidified with dilute sulphuric acid and then treated with fresh sodium sulphite solution until the colour is discharged. On cooling, benzoic acid crystallizes out. It is filtrated on a Buchner funnel at the pump. The crystals are washed twice with a little water and dried by pressing them between two layers of filter paper. The yield is recorded.

Recrystallisation: A pinch of the product benzoic acid is added to about 1 cm^3 of hot water or alcohol and the solution is warmed slightly to dissolve the crystals and filtered. The filtrate is cooled to the room temperature slowly. Pure crystals of benzoic acid gets crystallized which are filtered, dried and kept for inspection. The melting point of the recrystallised sample is determined.

Report:

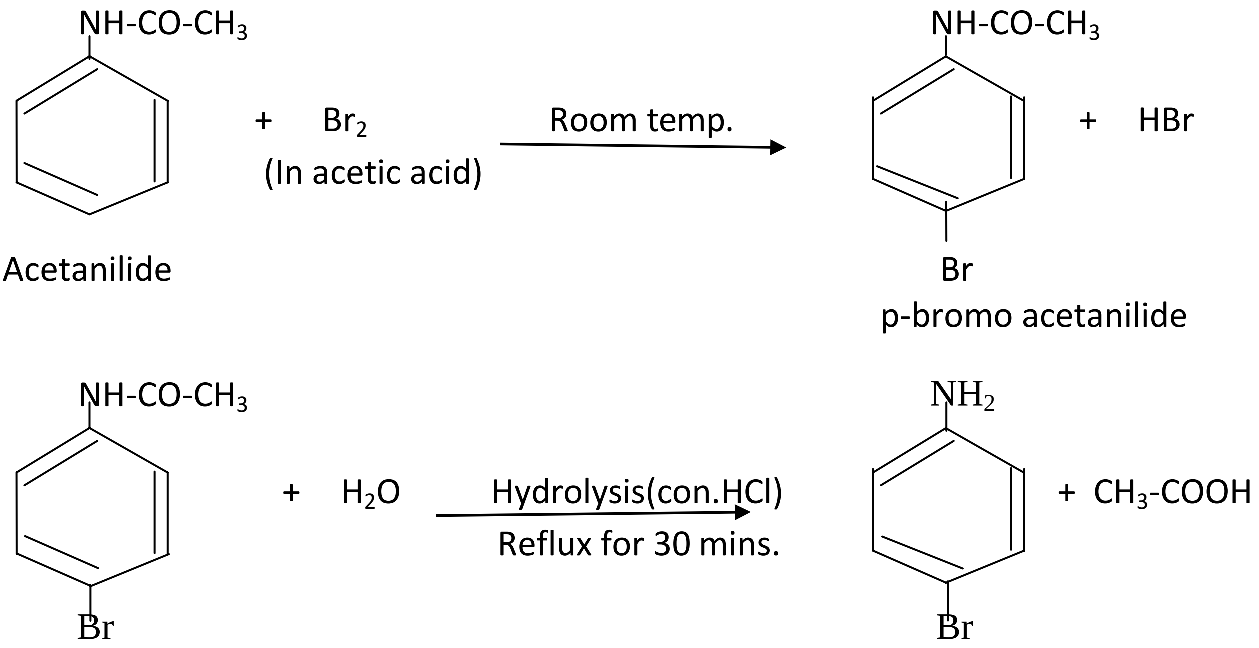
Theoretical Yield	Practical Yield
5.985 g	

EXPERIMENT NO.10.

AIM: PREPARATION OF p-BROMOANILINE FROM ACETANILIDE

Principle: Acetanilide on bromination gives p-bromo acetanilide which on acid hydrolysis yields p-bromo aniline.

REACTION:



Procedure:

Step-I: Preparation of p-bromo acetanilide from acetanilide

4.5g of acetanilide is dissolved in 20 cm^3 of glacial acetic acid with shaking. 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 30 minutes and then poured into cold water. The p-bromo acetanilide separates out. It is then filtered at pump using Buchner funnel, washed with sodium bi-sulphite solution, the with water and then dried.

Step-II: Preparation of p-bromo aniline from p-bromo acetanilide:

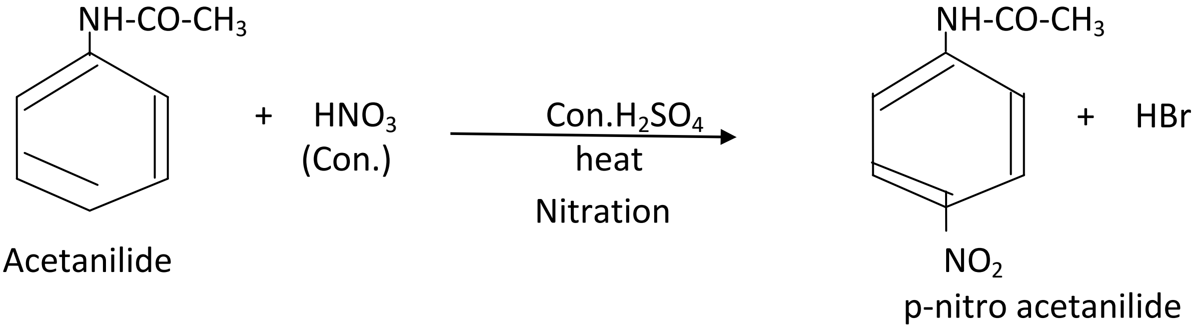
p-bromo acetanilide obtained is mixed with 10 cm^3 of con.HCl and the reaction mixture is refluxed for about 30 minutes. Then the reaction mixture is cooled under water, diluted with water and 10% solution of NaOH is added till the solution becomes basic. The crystals of p-bromo aniline separates out and are filtered at pump using Buchner funnel. It is then washed well with water and dried. A portion of it is recrystallised from equal volume of alcohol and water.

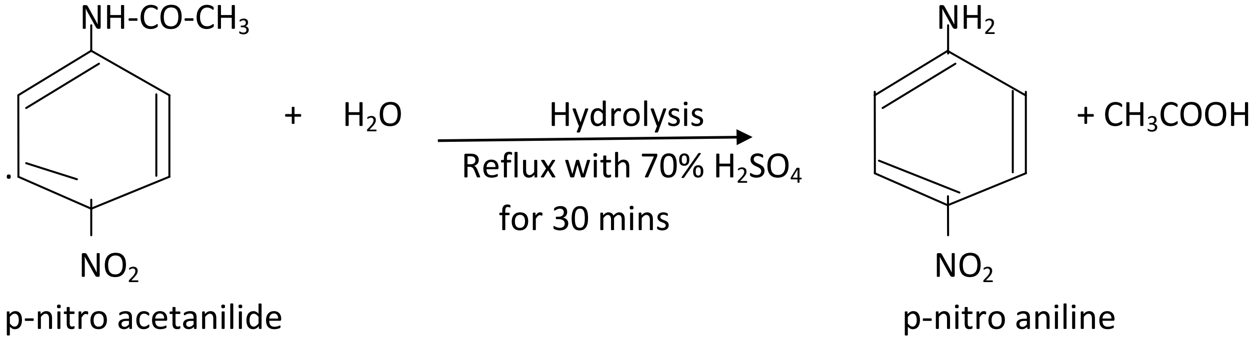
EXPERIMENT NO.11.

AIM: PREPARATION OF p-NITRO ANILINE FROM ACETANILIDE

Principle: Acetanilide on nitration gives p-nitro acetanilide which on acid hydrolysis yields p-nitro aniline.

REACTION:





Procedure:Step-I: Preparation of p-nitro acetanilide from acetanilide

4.5 g of acetanilide is dissolved in glacial acetic acid, little con. sulphuric acidis added and cooled in freezing mixture. To this 20 cm^3 of nitration mixute(1:1 mixture con.H_2SO_4 and con.HNO_3) is added in small portions carefully and the temperature is maintained below 10^{o}C during the addition. Then the reaction mixture is allowed to stand for one hour at room temperature. It is then poured into crushed ice. The precipitate of p-nitro acetani-lide is filtered at pump, washed with water, re-crystallized with alcohol and dried.

Step-II: Preparation of p-nitro aniline from p-nitro acetanilide:

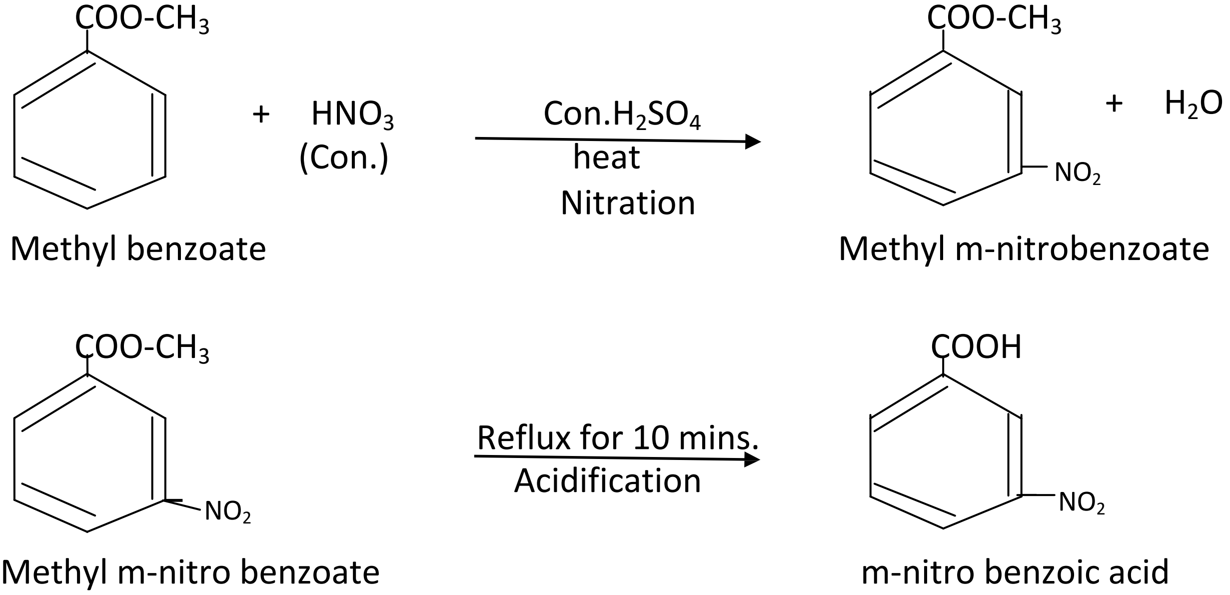
p-nitro acetanilide is refluxed with 70% sulphuric acid for about 30 minutes.p-nitro aniline is obtained. It is then poured into water and made alkaline with10% sodium hydroxide solution. The precipitate of p-nitro aniline is re-crystallized from hot water, dried and weighed.

EXPERIMENT NO.12.

AIM: PREPARATION OF m-NITRO BENZOIC ACID FROM METHYL-BENZOATE

Principle: Methyl benzoate on nitration gives methyl m-nitro benzoate which on hydrolysis yields m-nitro benzoic acid.

REACTION:



Procedure:Step-I: Preparation of methyl m-nitro benzoate from methyl- benzoate

4 cm^3 of methyl benzoate is dissolved in 6 cm^3 of con.sulphuric acid and cooledin a freezing mixture. 20 cm^3 of nitrating mixture(1:1mixture of con.H_2SO_4 and con.HNO_3) is added in small portions carefully. Keep the reaction mixture at room temperature for about 30 minutes. Then it is poured into crushed ice. The precipitate of methyl m-nitro benzoate is filtered at pump, washed with water and then with alcohol and dried.

Step-II: Preparation of m-nitro benzoic acid from methyl m-nitro benzoate

Methyl m-nitro benzoate is refluxed with sodium hydroxide solution for about 10 mins., m-nitro sodium benzoate acid is obtained. This m-nitro sodium benzoate on acidification gives m-nitro benzoic acid. It is then diluted with water, recrystallized from dil.HCl dried and weighed.

EXPERIMENT NO.13.

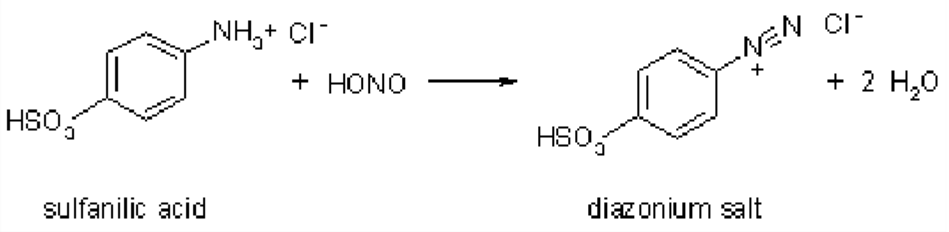
AIM: Preparation of Methyl orange by diazotization and coupling

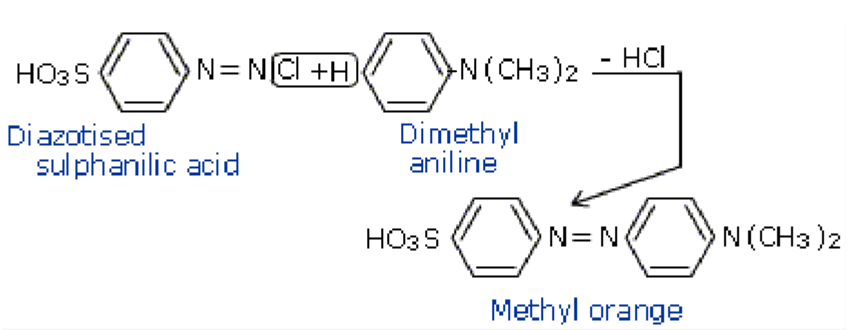
Principle: Methyl orange is prepared from sodium salt of sulphonilic acid which is diazotized and coupled with dimethyl aniline in basic medium.

Procedure: The method of preparation involves two steps

Step 1: Diazotisation: 5 g of sulphanilic acid placed in 100 cm^3 conical flask. It is dissolved in 50 cm^3 of 2.5% sodium carbonate solution by warming. The flask is cooled by placing it in ice water and a temperature of about 10°C is maintained. 1.8 g of sodium nitrite is dissolved in 5 cm^3water. This solution is introduced into the flask slowly with constant stirring. This solution is transferred into a beaker containing 5 cm^3 conc. hydrochloric acid and about 25 g of crushed ice. Diazotisation takes place forming diazobenzenesulphonate (diazotized benzene sulphanilic acid).

Step2: Coupling: In another 100 cm^3 conical flask 3.2 cm^3 and 2 cm^3 glacial acetic acid are placed. A solution of dimethyl aniline acetate is formed. This solution is added to the suspension of diazobenzenesulphonate solution slowly with vigorous stirring. The reaction mixture is allowed to stand at room temperature for about 10-15 minutes. To this 20 cm^3 of 20% sodium hydroxide solution is added slowly with constant stirring. A red solution of sodium salt of methyl orange is formed. 5 g of sodium chloride crystals is added the solution is boiled till all the salt is dissolved and then cooled in water. Methyl orange is separated out as an orange red crystalline solid. The solid is filtered, washed with little ethanol and dried. The solid is recrystallized from hot water and dried.





EXPERIMENT NO.14.THIN LAYER CHROMATOGRAPHY

AIM: SEPARATION OF GREEN LEAF PIGMENTS BY THIN LAYER CHROMATOGRAPHY

Principle: Thin layer chromatography is adsorption chromatography performed on open layers of adsorbent materials like silica gel on glass plate. Green leaves contains coloured pigments such as chlorophyll ‘a’, ‘b’, carotenes and xanthophylls. These can be separated by TLC technique using a mixture of benzene and acetone as developing solvents.

Procedure: The experiment consists of three steps:

1. Preparation of chromatographic plate: Silica gel is stirred with ethanol into a slurry. Two TLC glass plates are held back to back which are dipped in the silica gel slurry for proper spreading of slurry on the plates. The plates are then separated and dried at room temperature and dried at room temperature and then activated by heating in an oven between 100 and 250°C.

2. Preparation green leaf pigments: 2 cm^3 of hexane and 1 cm^3 of ethanol and green leaves are crushed in a mortar to get a paste, which is then diluted with small quantity of water and transferred into a separating funnel, shaken well and kept aside. By this method the organic and aqueous layers are separated. The organic layer is collected in 10 cm^3 of $Na_2S_2O_3$ in a small bottle, the aqueous layer is rejected.

3. Separation of pigments by TLC: 1.0 – 2.0 cm^3 of the pigment on the TLC plate at about 1.5cm from one end using a capillary tube. The spot is dried. 6.0cm^3 of the solvent mixture (7:3 mixture of benzene and acetone is used as a solvent for the separation of pigments) is transferred into a TLC jar. The spotted TLC plate is placed carefully into the jar. Care should be taken to maintain the level of the solvent well below the spot. The jar is covered with a glass plate. The solvent ascends on TLC plate due to capillary action and reaches the spotted point. The components present in the mixture are separated with respect to their R_f values. The plate is removed, dried and developed. The different colored spots separated are identified as chlorophyll a ,b, carbonates and xanthophylls(yellow) etc.

The various components present in a mixture can be identified using R_f values. R_f = Distance travelled by the compound
Distance travelled by the solvent

Since the solvent front travels faster than compound on TLC and hence the R_f values are expressed as decimal fractions.

Report: The various components present in green leaf extracted by TLC are

1) R_f value of green pigment = -------------
2) R_f value of yellow pigment = -------------
3) R_f value of orange pigment = -------------

Observation:

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

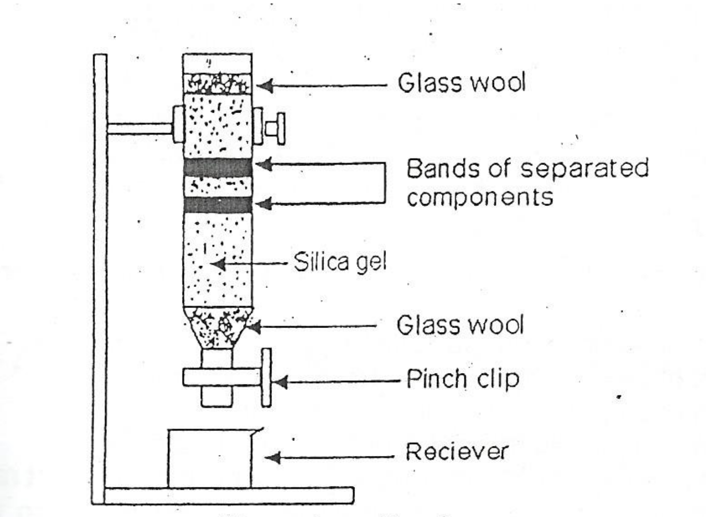
1) Distance travelled by the solvent = ------- R_f value of green pigment = ----------
2) Distance travelled by yellow pigment =-------- R_f value of yellow pigment = --------
3) Distance travelled by green pigment = ---------R_f value of orange pigment =--------
4) Distance travelled by orange pigment = --------

EXPERIMENT NO.15.COLUMN CHROMATOGRAPHY

AIM: SEPARATION OF A MIXTURE OF TWO ORGANIC COMPOUNDS BY COLUMN CHROMATOGRAPHY

Principle: Different adsorption capacity of organic compounds is the principle involved in the separation of mixture of organic compounds. The solute acts as the adsorbate and the stationary phase as the adsorbent. The constituents adsorbs at different parts of the column. The more adsorbate component adsorbs at the top and the least at the bottom of the column, different bands are formed. A proper solvent is used to elute the different compounds by elusion process.

Procedure:



Chromatographic column

The chromatographic column(glass tube) is vertically fixed to the stand. The lower end of which is attached with a pinch clip. The nozzle portion of the column is filled with glass wool or cotton. A slurry is prepared by fixing 10 g of silica gel and 30 cm^3 of alcohol in a beaker. The slurry is poured into the column. As the solvent flows out the column, the silica gel gets packed(Note: the silica gel should not be dried). A pinch clip is applied when the solvent is just above the level of silica gel. About 0.5 cm^3 of the mixture of methyl red and methylene blue(a pinch each in alcohol) is carefully loaded on the silica gel using a dropper. A little glass wool or cotton is placed on the top of packing. Alcohol is added to separate the components, when the solvent flows down the column, the two different bands of methylene blue and methylene red are formed and are collected in the different receivers during the elution.