AmbujaNeotia

THE NEOTIA UNIVERSITY

ज्ञानम् आत्म प्रदीपाय

MEDICINAL CHEMISTRY – I

IV SEMESTER (2nd YEAR B.PHARM)

PRACTICAL LAB MANUAL

1. ASSAY OF ASPIRIN

AIM:

To perform Assay of aspirin

REQUIREMENTS:

Aspirin, Sodium hydroxide solution (0.5N), Hydrochloric acid (0.5N),Phenol red indicator, Burette, Conical flask, Funnel, Beaker etc.

PROCEDURE:

a) STANDARDIZATION OF 0.5M HYDROCHLORIC ACID

Weighed accurately 0.75g of Anhydrous sodium carbonate previously heated at 270^0C. Dissolve in 100 ml of water and added 0.1ml of methyl red solution. Added the titrant slowly from the burette with constant stirring until the solution becomes faintly pink. Heated the solution.Cool and continue. If pink colour fades on heating continue this process until a faint pink colour is no longer affected by continous boiling.

Each ml of 0.5M HCl = 0.026495g of Na_2CO_3

b) ASSAY OF ASPIRIN

Weighed accurately 1.5g of aspirin and dissolved in 15ml ethanol added 50ml of 0.5M sodium hydroxide boil gently for 10 minutes, cool and titrated the excess alkali with 0.5M HCl using phenol red solution as indicator. Perform a blank determination the difference between the titration represent the volume of sodium hydroxide consumed.

Each ml of 0.05M NaOH = 0.04504g of C_9H_8

2. ASSAY OF PHENOBARBITONE

AIM :

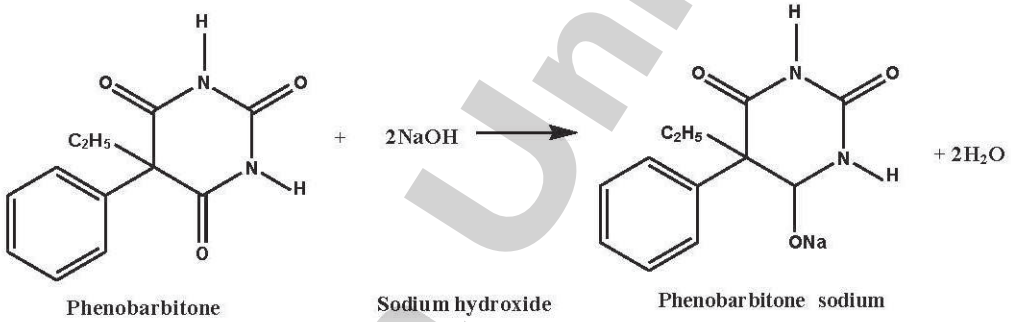
To perform the Assay of Phenobarbitone

REQUIREMENTS:

Sodium hydroxide, aldehyde free ethanol, benzoic acid, thymolphthalein solution, silver nitrate, pyridine and ether, conical flask, burette, beaker, pipette etc.

PRINCIPILE:

Phenobarbitone is assayed by non-aqueous titration. In this method, drug is dissolved in the pyridine and titrated with sodium hydroxide solution using thymolphthalein as an indicator.



Phenobarbitone Sodium hydroxide Phenobarbitone sodium

PROCEDURE:

a) STANDARDISATION OF SODIUM HYDROXIDE SOLUTION

Actually weighed 0.6g of benzoic acid and dissolved it in a mixture of 30ml of ethanol and 6ml of water and titrated with ethanolic sodium hydroxide solution using 0.2ml of thymolphthalein as indicator.

b) ASSAY OF PHENOBARBITONE

Weighed and powdered 20 tablets, Weighed a quantity of the powder containing about 0.1g (100 mg) of phenobarbitone in 5ml of pyridine add 0.25 ml of thymolphthalein solution and 10 ml of silver nitrate pyridine reagent and titrated with 0.1M ethanolic sodium hydroxide until a pure blue colour is obtained. Repeated the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

Equivalent factor: 1ml of 0.1M ethanolic sodium hydroxide=0.01161g of $C_{12}H_{12}N_2O_3$

REPORT

The percentage purity of Phenobarbitone was found to be =

CALCULATION

a) Standardization of 0.1M Sodium hydroxide solution

$$\text{Molarity of NaOH} = \frac{\text{Weight (W)}}{\text{Mol.wt of benzoic acid (122.12) X Volume (V)}}$$

Where,

W = Weight of benzoic acid (g)

V = Volume of NaOH solution consumed

b) Determination of Phenobarbitone

$$\% \text{ purity of phenobarbitone} = \frac{0.01161 \times V \times \text{Molarity(Calculated)} \times 100}{\text{Molarity (given)} \times W}$$

Where,

Molarity (calculated) = Molarity obtained from step (a)
V = Volume of Sodium hydroxide used
0.01161 is the equivalent factor
Molarity (given) = 0.1M
W = weight of sample

REPORT:

The percentage purity of Phenobarbitone was found to be =

3. ASSAY OF FUROSEMIDE

AIM:

To carry out the Assay of furosemide tablets

REQUIREMENTS:

Furosemide, dimethyl formamide, sodium hydroxide, bromothymol blue indicator,0.1N oxalic acid, Phenolphthalein indicator, conical flask, burette, beaker, funnel etc.

PRINCIPLE

It is assayed by aqueous acid base titration between weak acid furosemide and strong alkali sodium hydroxide. In this assay protophilic solvent dimethyl formamide is used which enhances the acidity of furosemide so that it can be titrated with sodium hydroxide. To make the effect of acid impurities present negligible a solvent blank determination is carried out.

PREPARATION AND STANDARDIZATION OF STANDARD SOLUTIONS

a) SODIUM HYDROXIDE. XM

Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium hydroxide in sufficient water to produce 1000ml.

b) STANDARDIZATION OF 0.1M SODIUM HYDROXIDE SOLUTION

Weighed accurately about 5g of potassium hydrogen phthalate previously dried at 120^0C for two hours dissolve in 75ml of carbon dioxide free water. Added 0.1ml of phenolphthalein solution and titrate with the sodium hydroxide until a permanent pink color is produced.

Each ml of 0.1M NaOH equivalent to 0.02042g of potassium hydrogen phthalate.

PROCEDURE:

a) **ASSAY METHOD BY (NEUTRALIZATION TITRATION)**

Weighed and powdered 20 tablets and Weighed accurately about a quantity of powder equivalent to 0.5g and dissolve in 40ml of dimethyl formamide and titrate with 0.1M sodium hydroxide using bromothymol blue as an indicator the end point shows the colour change from yellow to blue. Carry out a blank titration.

b) **ASSAY METHOD BY (UV SPECTROPHOTOMETRY)**

Weighed and powdered 20 tablets and Weigh accurately about a quantity of powder equivalent to 0.1g of furosemide and shake with 150ml of 0.1M sodium hydroxide for 10 minutes. Added sufficient 0.1M sodium hydroxide to produce 250ml and filter. Dilute 5ml to 200ml with 0.1M sodium hydroxide and measure the absorbance of the resulting solution at the maximum at about 271nm.Calculate the content of $C_{12}H_{11}ClN_2O_5S$ taking 580 as the value of A (1%, 1cm) at the maximum at about 271 nm.

REPORT:

The given sample contains ..... mg of furosemide.

4. ASSAY OF IBUPROFEN

AIM:

To carry out the assay of Ibuprofen tablet

REQUIREMENTS:

Ibuprofen,0.1N sodium hydroxide solution, phenolphthalein indicator, 0.1N oxalic acid solution, conical flask, burette, beaker etc.

PRINCIPLE:

Ibuprofen is determined by neutralization titration in which free carboxylic group is titrated with sodium hydroxide solution using phenolphthalein indicator. The amount of sodium hydroxide consumed in the reaction indicates the amount of ibuprofen present in the sample.

PROCEDURE:

PREPARATION AND STANDARDIZATION OF STANDARD SOLUTIONS SODIUM HYDROXIDE.XM

Solutions of any molarity xM may be prepared by dissolving 40x g of Sodium hydroxide in sufficient water to produce 1000ml.

STANDARDIZATION OF 0.1M SODIUM HYDROXIDE SOLUTION

Weighed accurately about 5g of potassium hydrogen phthalate previously dried at 120^0C for two hours dissolve in 75ml of carbon dioxide free water. Added 0.1ml of phenolphthalein solution and titrate with the sodium hydroxide until a permanent pink color is produced.

Each ml of 0.1M NaOH equivalent to 0.02042g of potassium hydrogen phthalate.
Phenolphthalein solution
A 1.0%w/v solution of phenolphthalein in ethanol(95%).

PROCEDURE:

Weighed and powdered 20 tablets. Weighed a quantity of powder containing about 0.4g of ibuprofen, dissolve in 100ml of ethanol (95%) and titrated with 0.1M sodium hydroxide using 0.2ml of phenolphthalein solution as indicator. Perform a blank determination and make necessary correction.

Each ml of 0.1 M sodium hydroxide is equivalent to 0.02063 g of C_{13} H_{18} O_2

REPORT

The given sample contains .....mg of Ibuprofen.

5. ASSAY OF CHLORPROMAZINE

AIM: To carry out the Assay of Chlorpromazine.

REQUIREMENTS:

Perchloric acid (0.1M),Chlorpromazine, mercuric acetate solution(5% w/v in acetic acid),crystal violet solution (0.2%w/v in acetic acid),acetone,methyl orange indicator, conical flask, burette, beaker, potassium hydrogen phthalate, glacial acetic acid, crystal violet indicator.

PRINCIPLE:

Chlorpromazine is estimated by non-aqueous titration which is suitable for titration of weak acid and weak base. In this non aqueous solvent like perchloric acid is utilized as a titrant and methyl orange is used as an indicator. Mercuric acetate is added in the non-aqueous titration inorder to remove the chloride ions. So as to prevent the interference of the chloride ion releasedby the titrant. The mercuric acetate replaces the halide ion in chlorpromazine with acetate ionwhich is a strong base. The end point is indicated by appearance of blue colour.

PROCEDURE:

a) STANDARDISATION OF PERCHLORIC ACID (0.1N)

Dissolved 0.5g of potassium hydrogen phthalate in 25ml of glacial acetic acid and added few drops of 5%w/v crystal violet indicator. Titrated the solution with 0.1N perchloric acid till blue green colour appears.

b) ASSAY OF CHLORPROMAZINE

Weighed accurately about 0.6g and dissolved in 200 ml of acetone. Added 15ml of mercuric acetate solution. Titrated with 0.1M perchloric acid, using a saturated solution of methyl orange in acetone as indicator. Perform a blank determination and make a necessary correction.

Each ml of 0.1M perchloric acid equivalent to 0.03553g of $C_{17}H_{19}ClN_2S,HCl$

REPORT:

The given sample contains .....mg of chlorpromazine.

6. ASSAY OF ATROPINE

AIM:

To carry out the assay of atropine

REQUIREMENTS:

Perchloric acid (0.1M),atropine,glacial acetic acid,crystal violet solution (0.2%w/v in acetic acid),acetone,methyl orange indicator, conical flask, burette, beaker.

PRINCIPLE:

Atropine is assayed by non-aqueous titration which is generally used for the titration of weak acid with weak base. In this titration non-aqueous solvent perchloric acid is used and crystal violet is used as an indicator. At the end point blue colour is obtained.

PROCEDURE:

a) STANDARDISATION OF PERCHLORIC ACID (0.1N)

Dissolved 0.5g of potassium hydrogen phthalate in 25ml of glacial acetic acid and few drops of 5%w/v crystal violet indicator. Titrated the solution with 0.1N perchloric acid till blue green colour appears.

b) ASSAY OF ATROPINE

Weighed accurately 400mg of atropine and dissolved it in 50ml of glacial acetic acid and added a drop of crystal violet indicator. Titrated this solution with 0.1N perchloric acid until green color is obtained end point.

7. PREPARATION OF BENZIMIDAZOLE

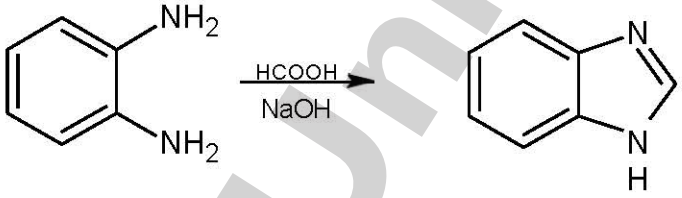
AIM: To prepare and submit benzimidazole from o-phenylenediamine.

REQUIREMENTS:

Round bottom flask, Beaker, Measuring cylinder,Waterbath, Buchner funnel, O-phenylene diamine, Formic acid(90%),Sodium hydroxide (10%)

PRINCIPLE:

The preparation of benzimidazole can be done by reaction between O-phenylene diamine with formic acid in presence of base i.e sodium hydroxide.It is a condensation type of reaction in which o-phenylene diamine condensed with formic acid to give benzimidazole with removal of two molecules of water.



O-phenylene diamine Benzimidazole

PROCEDURE:

Placed 27g of O-phenylenediamine in a round bottomed flask of 250ml and added 17.5g (16ml) of 90% formic acid. Heated the mixture on a water bath at 100^0C for 2 hour. Cooled and added 10% sodium hydroxide solution slowly, with constant rotation of the flask, until the mixture is just alkaline to litmus. Filter off the synthesized crude benzimidazole by using the pump wash with ice cold water.

Recyrstallisation: Dissolved the synthesized product in 400ml of boiling water, added 2g of decolorizing carbon and digest for 15minutes. Filter rapidly through Buchner funnel and a flask at the pump. Cool the filtrate to about 10^0C, filter off the benzimidazole, wash with 25ml of cold water and dry at 100^0C. The yield of pure benzimidazole is 25g (85%), m.p 171-172^0C.

CALCULATION

Here limiting reagent is O-phenylene diamine; hence yield should be calculated from its amount taken.

Molecular formula of O-phenylene diamine = $C_6H_8N_2$
Molecular formula of benzimidazole = $C_7H_6N_2$
Molecular weight of O-phenylene diamine = 108g/mole
Molecular weight of benzilidazole = 118g/mole
108g of O-phenylene diamine forms 118g benzimidazole

Therefore, 27g O-phenylene diamine will form……(X) g benzimidazole
X = (118 X 27)/108 = 29.5g
Theoretical yield = 29.5g
Practical yield = ...g
% yield = (practical yield) X 100
(theoretical yield)

REPORT:

Benzimidazole was synthesized from O-phenylene diamine and submitted.

8. SYNTHESIS OF BENZOTRIAZOLE

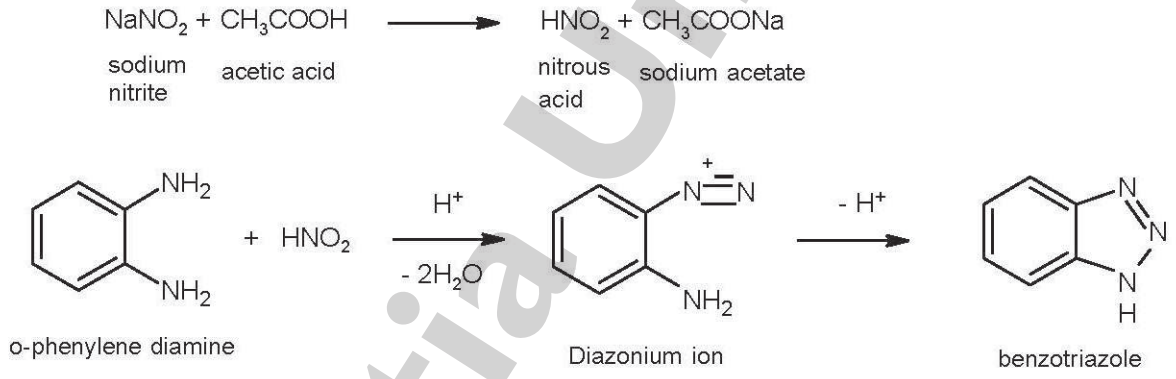
Aim- To synthesize and submit benzotriazole from o-phenylene diamine and report its percentage yield

CHEMICAL REQUIREMENTS:

o-phenylenediamine, glacial acetic acid, sodium nitrite

PRINCIPLE:

The sodium nitrite reacts with glacial acetic acid and liberates nitrous acid. The o-phenylene diamine reacts with nitrous acid and produce diazonium ion. When the structure and stereochemistry of diazonium ion are stable, intramolecular nitrogen coupling occurs and form benzotriazole directly.



CALCULATIONS

Molecular weight of o-phenylene diamine =
Molecular weight of benzotriazole =
---- g of o-phenylene diamine gives -----g of benzotriazole

1g of o-phenylene diamine =

---- g of o-phenylene diamine =

Theoretical yield =

Practical yield =

Percentage yield = $\frac{Practical\ yield}{theoretical\ yield}$ x 100

PROCEDURE:

Dissolve 1.3g of o-phenylenediamine in a mixture of 1.5ml of glacial acetic acid and 5ml water in a beaker. Stir until the solid dissolves, warm gently if necessary. Cool the solution to 15^0C. Stir well and add a solution of 2g of sodium nitrite in 2ml water. Reaction mixture become warm within 2-3 minutes and reaches a temperature of about 85^0C and then begins to cool. Colour changes from deep red to pale brown. Continue stirring for 15 minutes till the temperature fall about 35-40^0C. Thoroughly chill in ice bath for 30 minutes. Filter the product and wash with cold water.

USE:

Used in bulk drug industry as an important intermediate compound.

It is the basic nucleus present in anthelmintic drugs like mebendazole, thiabendazole etc.

REPORT:

Benzotriazole was prepared and submitted. The percentage yield was found to be ---------

9. SYNTHESIS OF 2,3-DIPHENYL QUINOXALINE

AIM:

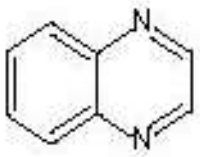
To synthesize and submit 2,3-diphenyl quinoxaline from o-phenylenediamine and report its percentage yield.

CHEMICAL REQUIREMENTS:

o-phenylenediamine, benzil, rectified spirit.

PRINCIPLE:

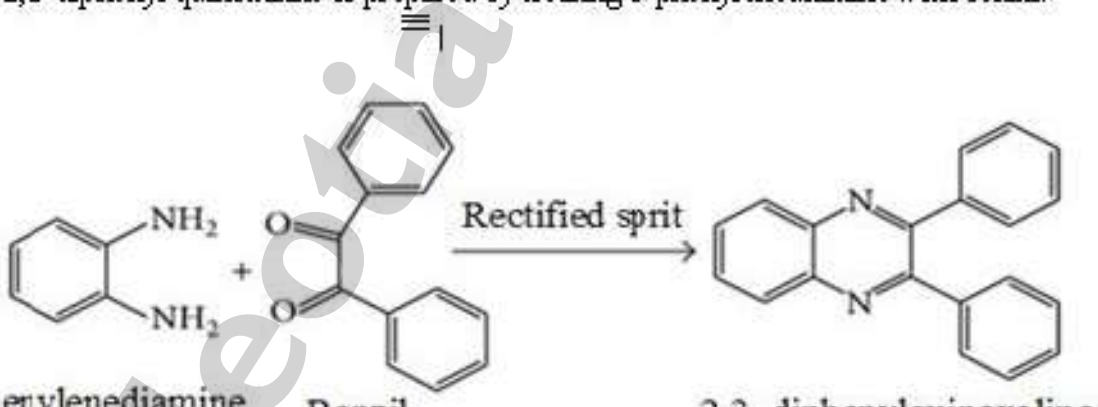
Quinoxalines are a type of heterocyclic compounds. They are also known as benzopyrazines.



benzopyrazine

pyrazine

Generally quinoxaline is formed by the condensation of o-phenylenediamine with diketones. Here 2,3-diphenyl quinoxaline is prepared by treating o-phenylenediamine with benzil.



o-phenylenediamine Benzil 2,3- diphenylquinoxaline

CALCULATIONS

Molecular weight of 2, 3-diphenyl quinoxaline =
Molecular weight of o-phenylene diamine =
----- g of o-phenylene diamine gives ----- g of 2, 3-diphenyl quinoxaline

1g of o-phenylene diamine =

=

----- g of o-phenylene diamine =

Theoretical yield =

Practical yield =

Percentage yield = $\frac{Practical\ yield}{theoretical\ yield}$ x 100

=

PROCEDURE:

Add a solution of 1.1g of o-phenylenediamine in 8ml rectified spirit to a warm solution of 2.1g of benzil in 8ml rectified spirit. Warm the mixture for 30 minutes in a water bath. Add water dropwise until slight cloudiness persists. Cool the solution and filter the product.

USE:

Quinoxaline derivatives are used as antimicrobial agents like levomycin.
They are also used in dyes.

REPORT:

2,3-diphenyl quinoxaline was prepared and submitted.
The percentage yield was found to be ---------

10. SYNTHESIS OF PHENYTOIN

AIM:

To prepare and submit recrystallized dried product of phenytoin and calculate

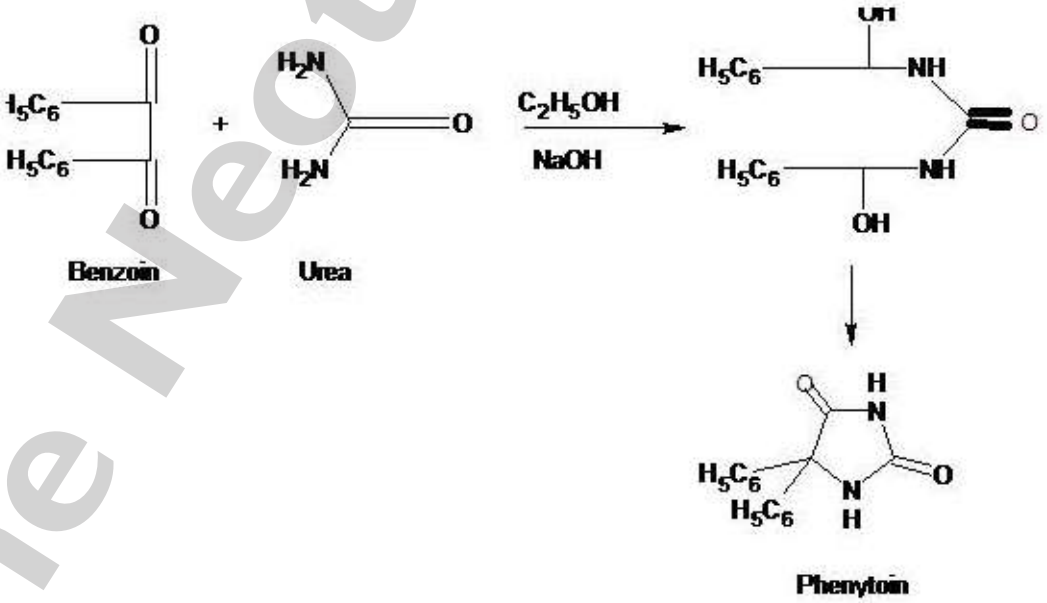
(i) Percentage yield
(ii) Melting point

REQUIREMENTS:

Chemicals used: Urea, Nitric acid, Benzoin, Sodium hydroxide, ethanol, conc:HCl
Apparatus used: Round bottom flask ,reflex condenser, funnel, beaker, filter paper, glass rod.

PRINCIPLE:

Phenytoin is 5,5-diphenyl imidazoline 2,4-dione.Benzil react with urea in the presence of alkali and alcohol to give phenytoin by pinacolone rearrangement.



PROCEDURE:

a) Preparation of Benzil from Benzoin:

Place 2g of benzoin and 5ml of concentrated HNO_3 in a round bottom flask and heat on a boiling water bath till crystalline benzoin is replaced by oily benzil.Pour the mixture in to beaker of cold water with stirring the oily benzil crystallize in to yellow salt.

b) Preparation of phenytoin from benzil:

Place 1g benzil, 1g urea ,5ml 30% aqueous sodium hydroxide and 20ml ethanol in a round bottom flask which is attached to reflux condenser and boil for 2hours.Cool the mixture to attain room temperature.Pour the mixture to 100ml water and,mix and allow to stand for 15minutes.Filter to remove insoluble biproducts.Render the filtrate strongly acidic with concentrated HCl .Cool the filtrate in ice cold H_2O.Filter the precipitate product dry and submit.

IDENTIFICATION

Experiment	Observation	Inference
To the sample solution add hydrochloric acid	White precipitate	Presence of phenytoin
To the sample add pyridine and copper sulphate Solution	Blue colour	Presence of phenytoin

REPORT:-

11. SYNTHESIS OF BENZOCAINE [ETHYL PARA AMINO BENZOATE]

AIM:

To synthesis recrystallized product of benzocaine from para amino benzoic acid and calculate

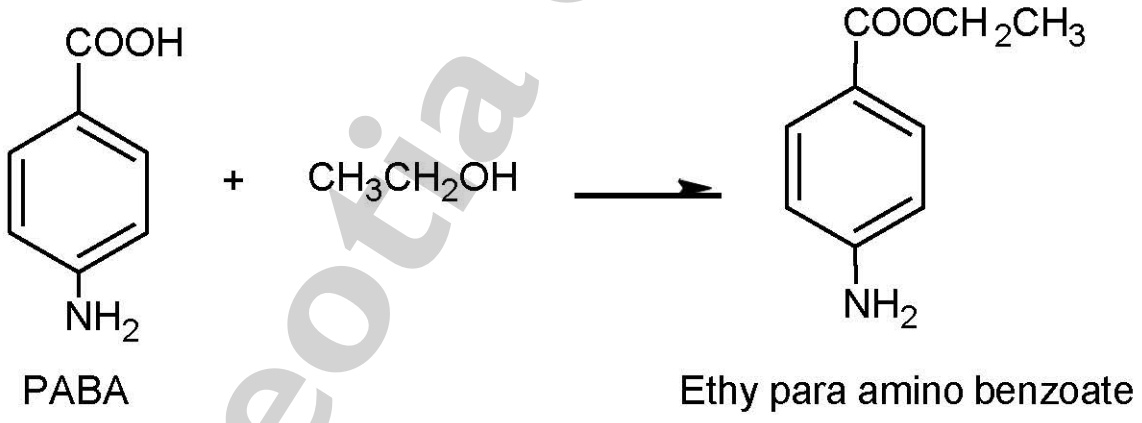
(i) Percentage yield
(ii) Melting point

REQUIREMENTS:

PABA, Conc:sulphuric acid, ethanol,reflux condenser, RB flask , beaker

PRINCIPLE:

Benzocaine is the ethyl ester of para amino benzoic acid(PABA).It can be prepaired from PABA and ethanol by fischer esterification.



PROCEDURE:

To a 100ml RB flask, add 8ml of ethanol, 4.12g of para amino benzoic acid(PABA) and 1.2ml of conc:H_2SO_4 keep the mixture under reflux for 1hour up on cooling reaction mixture sets to a solid mass of hydrochloride of ethyl para amino benzoate. Pour the hot solution in to excess of water(no hydrochloride) add Na_2CO_3 to the clear solution until it is neutral to litmus. Filter wash and dry the product.

IDENTIFICATION

EXPERIMENT	OBSERVATION	INFERENCE
To the sample solution add sodium nitrite and con.HCl and cool the mixture. To this add a solution of beta naphthol in sodium hydroxide. Maintain the temperature at 0 to 5°	Deep red colour	Benzocaine confirmed

REPORT:-