B.SC.-2ND SEMESTER

PRACTICAL FILE

(CHEMISTRY)

PRACTICAL FILE

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INDEX

S.r. No.	Name of the Experiment	Page No.	Date of Experiment	Date of Submission	Remarks
1.	To identify the given (reducing) sugar by Benedict's test.	1-2	24-5-22	25-5-22	
2.	To identify the given (non-reducing) sugar by iodine test.	3-4	27-5-22	28-5-22	
3.	To synthesize the osazone of glucose or fructose.	5-6	3-6-22	4-6-22	
4.	To isolate the proteins from leaves or seeds.	7-8	8-6-22	9-6-22	
5.	To determine the strength of the given glycine solution by formylation method.	9-12	10-6-22	11-6-22	
6.	To extract DNA from onion.	13-14	15-6-22	16-6-22	
7.	To synthesize acetyl salicylic acid (aspirin) by acetylation of salcylic acid and compare it with the ingredient of an aspirin tablet by TLC	15-17	19-6-22	20-6-22	

INDEX

S.r. No.	Name of the Experiment	Page No.	Date of Experiment	Date of Submission	Remarks
8.	To synthesize barbituric acid from urea and diethyl malonate.	18-20	22-6-22	23-6-22	

$$Na_2CO_3 + 2H_2O \longrightarrow 2\,NaOH + H_2CO_3$$

$$2\,NaOH + CuSO_4 \longrightarrow Cu(OH)_2 + Na_2SO_3$$

$$Cu(OH)_2 \longrightarrow CuO + H_2O$$

$$\text{D- Glucose} + 2CuO \longrightarrow \underset{\substack{\text{Cuprous oxide}\\(\text{Red ppt})}}{Cu_2O\downarrow} + \text{D- gluconic acid}$$

Expt. No..... Page No.....1..... Date...../...../.....

Experiment no. 1

Object- To identify the given (reducing) sugar by Benedict's test.

Principle-

Under alkaline conditions free aldehyde or ketone group present in the mono, di (except- sucrose) or oligo saccharides reacts with $Cu(OH)_2$ and reduced to red coloured Cu_2O compound.

Regents-

1. Solution A - Dissolve 1.73 g trisodium citrate (dehydrate) and 1g sodium carbonate (anhydrous) in 8ml of distilled water.

2. Solution B - Dissolve 1.73 g copper sulphate ($CuSO_4 \cdot 5H_2O$) in 20 ml water.

3. Benedict's reagent- Mix 0.8 ml of solution A and 0.2 ml of solution B before use. Always prepare fresh solution.

Procedure-

Take 1 ml given solution in a test-tube and add 1 ml of Benedict's reagent. After mixing heat the test tube in a boiling water bath for 5 min. A brick red precipitate. is observed in the test tube.

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Expt. No..... Page No....2..... Date...../...../.....

Observation:-

A brick red precipitate indicates a positive test for reducing sugar.

Result:-

The given sample of sugar is reducing sugar.

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Expt. No..... Page No.....3..... Date...../...../.....

Experiment no. 2

Object- To identify the given (non-reducing) sugar by Iodine test.

Principle-

Under mild acidic conditions non-reducing sugar such as starch forms blue colour complex when reacts with iodine.

Reagents-

1. Iodine solution- Prepare 0.005 N iodine solution in 3% potassium iodide (KI) solution. Store in brown bottle and preserve at room temperature.

2. 0.1 N HCl.

Procedure-

Take 1 ml of a test solution and add few drops of iodine solution.
Now add few drops of 0.1 N HCl.
Mix well and record the colour change.

Observation-

A blue colour indicates the presence of starch and brown colour for glycogen.

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Expt. No..... Page No.....9..... Date...../...../.....

Result -

The given sample of sugar is non-reducing sugar.

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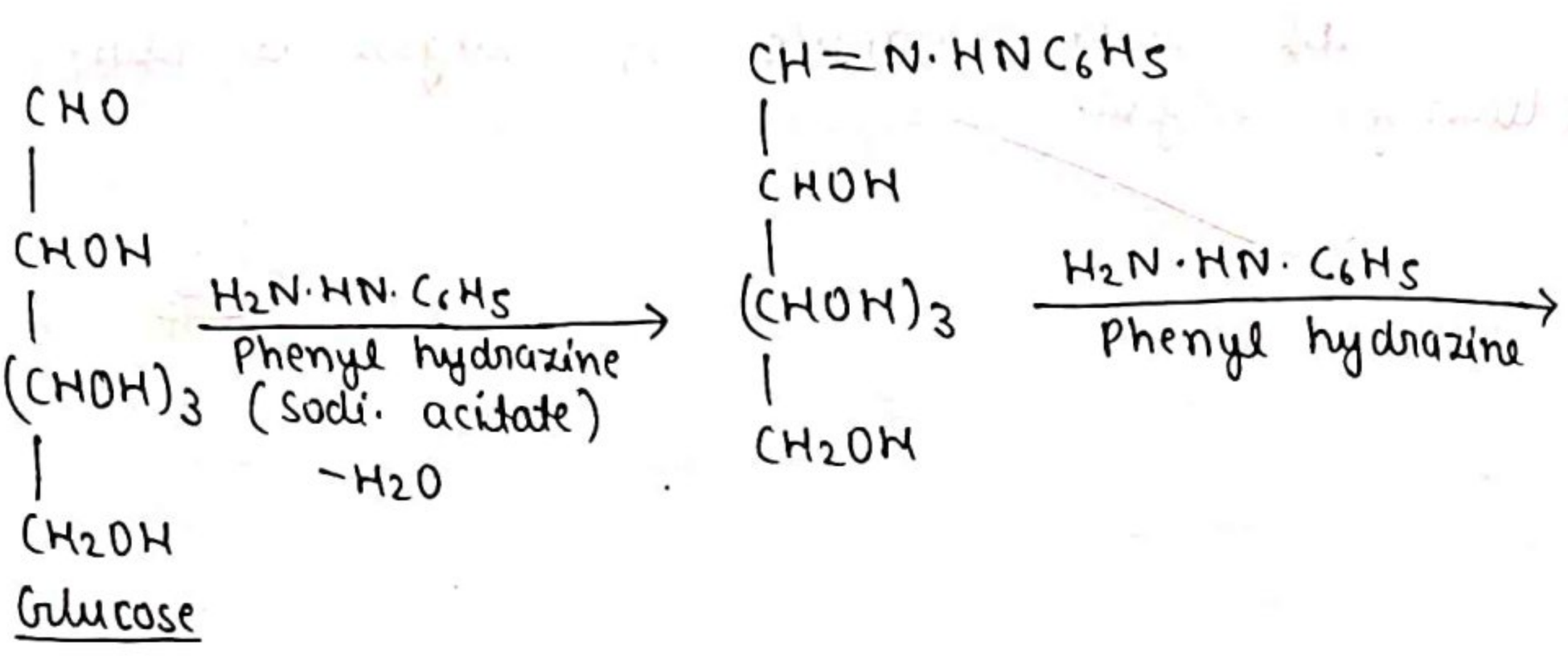
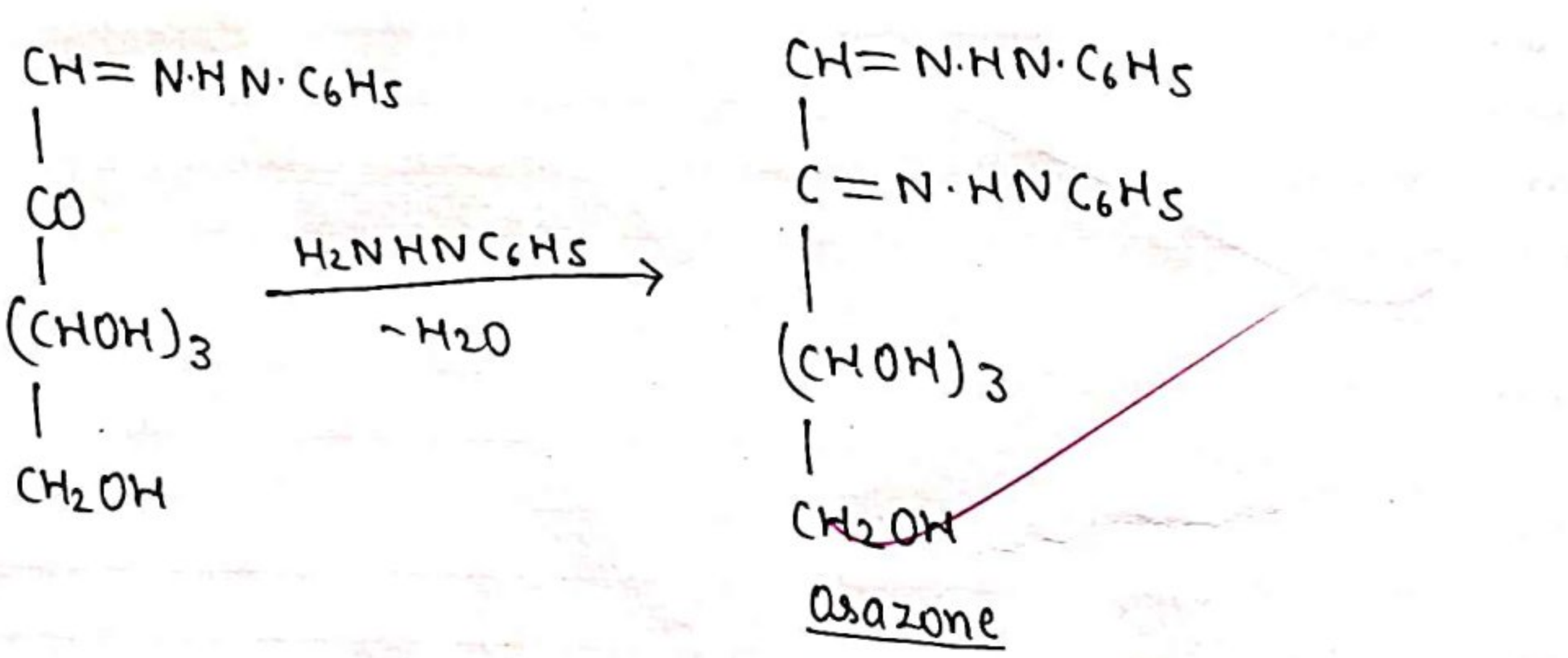


Glucose



Osazone

Expt. No..... Page No.....5..... Date...../...../.....

Experiment no. 03

Object:-

To synthesize the osazone of glucose or fructose.

Discussion:-

Carbohydrates such as d- glucose, fructose, l- arbinose, d- galactase, maltose, lactose etc. reacts with phenyl hydrazine in the presence of sodium acetate to form corresponding osazone. The reaction takes place in different steps.

Reagents:-

Glucose (or fructose) 2g, crystalline sodium acetate 5g, phenyl hydrazine HCl 5g and ethyl alcohal.

Procedure:-

In a round bottom flask take 2g of d- glucose, 5g crystalline sodium acetate, 5g phenyl hydrazine HCl and 40 ml of water. Shake the contents and loosely cork the flask and keep in a boiling water bath. After about eight minutes a solid starts to separate. Cool and filter off the yellow product. Recrystallise the product with ethanal and dry.

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Expt. No..... Page No.....6..... Date...../...../.....

Result-

Weight of recrystallized osazone is 6.9

Melting point of the product is 25°c

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Expt. No..... Page No.....7..... Date...../...../.....

Experiment no. 4

Object-

To isolate the proteins from leaves or seeds.

Principle-

To bring the pratein from the cell source into the solution is called isolation of pratein. In can be done by disrupting the cell membrane and cell wall. The animal cell is disrupted easily as compared to plant and microbial cell, because of absence of tough cell wall in animal cells. The isalation is done in an extraction buffer which consists of additives such as EDTA, DTT, glycerol, protease inhibitors.

Requirements-

Plant tissues such as fresh leaves, seeds etc. Extraction buffer consisting of 2M K_2HPO_4, 0.5 EDTA, 80% Glycerol, 1M DDT (Dithiatheital), Protease inhibitors, Mortar and Pestle.

Procedure-

Protein is isolated from leaves or seeds at 1-4°c. Weight 1g plant tissue and cut finally into small pieces. Grind them-

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in chilled pestle and mortar with 5 ml. chilled extraction buffer and pinch of clean sand. Grind the tissue to smooth paste. Transfer homogenate to centrifuge tube and spin in refrigerated centrifuge at 10,000 rpm for 20 minutes. Transfer the supernatant to clean and dry centrifuge tube. It can be used as a source of protein. The protein sample should be stored in refrigerator for further use or at -80°C for long term storage.

Result-

Isolated protein is colourless liquid.

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Expt. No..... Page No.....9..... Date...../...../.....

Experiment no. 5

Object- To determine the strength of the given glycine solution by formylation method.

Theory-

It is well known fact that glycine like amino acids from Zwitter ions in aqueous solution and can not be estimated directly by titration with standard alkali or acid solutions due to opposing effects of the amino (basic) and carboxylic group (acidic).

However, if the aqueous solution of amino acid is treated with an excess of a carefully neutralized solution of formaldehyde ($HCHO$), then it is possible to titrate it with a strong solution of alkali. Then reactions, invalved, may be shown as follows -

$$\underset{\text{Glycine}}{NH_2CH_2COOH} \rightleftharpoons NH_2CH_2COO^- + H^+ \rightleftharpoons \underset{\text{Zwitter ion}}{H_3\overset{+}{N}CH_2COO^-}$$

$$OH^- + H_3\overset{+}{N}CH_2COO^- \rightleftharpoons \underset{\text{Anion.}}{H_2NCH_2COO^-} \rightleftharpoons H_2O$$

The reaction is accompanied by the condensation of the anion with $HCHO$ to give a stable anion which is titrated with a solution of strong alkali.

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$CH_2O + H_2NCH_2COO^- \longrightarrow CH_2=N-CH_2COO^- + H_2O$

$CH_2=N-CH_2COO^- + Na^+ \longrightarrow CH_2=N-CH_2COONa$

Reagents-

(i) Glycine (A.R.) — 2g
(ii) Formalin solution (40%) — 50ml
(iii) Sodium hydroxide (A.R.) — 1g in 250 ml $\left(\frac{N}{10}\right)$
(iv) Phenolphthalein indicator.

Procedure-

(a) Preparation of standard glycine solution - Dissolve accurately weighed about 2.0 g of glycine in a little distilled water (taken in measuring flask of 250 mL) and make up to the mark with the same.

(b) Preparation of neutralized formalin solution - Take 50 ml of commercial formalin solution (40% aq. HCHO) in a conical flask and add about 8-10 drops of phenolphthalein. Now titrate in with N/10 NaOH solution until the solution has a faintly pink colour.

(c) Titration of the given glycine solution- Pipette out 25ml of the given glycine solution in the conical flask, add 2-3 drops of phenolphthalein indicator. Now add 10 ml of neutral formaldehyde solution. Shake it and titrate the acid solution

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($CH_2 \equiv N - CH_2COO^-$) with NaOH solution (N/10 approx.) till pink colour is just observed. Note down the volume of NaOH used in the actual formal titration. Concordant reading are obtained by repeating the process.

(d) Titration with standard glycine solution- Repeat the same procedure with the standard glycine solution.

Observations-

(i) Preparation of standard glycine solution.

(a) Weight of empty weighing tube = 2 g

(b) Weight of weighing tube + glucine = 3.5 g

(c) Weight of glycine (ii-i) = 1.5 g

(ii) Titration with the given glycine solution-

S. No	Glycine solution taken (ml)	Burette Reading		NaOH solution used ml	Correct Volume Used ml
		Initial	Final		
1.	25	0.0	16	16	16
2.	25	0.0	15	15	15
3.	25	0.0	16 / 15.6	16	16 / 14.3

that means 25 ml of given glycine ≡ Vg mL NaOH solution

(iii) Titration with standard glycine solution- Some table as in (ii) that means 25 mL of standard glycine solution ≡ Vs mL NaOH solution.

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Calculations-

Strength of the standard glycine solution =

$= 4 \times w$ g/litre.

$= 4 \times 2 =$ 8 g/Litre

Strength of given glycine solution.

$$= \frac{V_g}{V_s} \times 4 \times w \text{ g/litre}$$

$$= \frac{15.6 \times 4 \times 2}{14.3} = \frac{15.6 \times 8}{14.3}$$

$$= \frac{124.8}{14.3} = 8.72 \text{ g/Litre}$$

Result -

The strength of given glycine solution is $= \frac{4 \times w \times Vg}{Vs}$ g/litre

$= 8.72$ g/litre

Precautions - For neutrilization of formalin, NaOH solution should be added carefully and it must not be even in slight excess as this volume is not accounted in the calculations.

Teacher's Signature : .....

Expt. No..... Page No....13..... Date...../...../.....

Experiment no. 06

Object:- To extract DNA from onion.

Requirements:-

Onion, water bath, thermometer, centrifuge, ethanol (70%), mortar and pestle, centrifuge tubes, dry proteases etc. DNA release buffer, precipitation solution, DNA salt solution etc.

Procedure:-

Take about 10g pieces of onion in a mortar and crush it very well to get homogenous paste of onion. Add 0.20 ml DNA release buffer to the tube containing the onion paste. Invert the tube several times to slowly mix and transfer the sample from the joining tube to one of your labelled tubes. The DNA release buffer breakes open the onion cells releasing the DNA.

Now add 0.02 mL protease to the tube to digest and remove the cellular material and protein and release the DNA. Invert the solution and heat the solution at 50-55°c in water bath for about 1 hour. After this add 0.1 ml DNA salt solution to the tube. mix it very well. Centrifuge the tube for 5 minutes at 5000 rpm. Transfer the supernatant solution to other labelled tube. Add 0.8 ml precipitation solution, close the tube and slowly

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invert the tube several times to mix. White DNA may appear on standing the tube.

Result:-

DNA is separated as white precipitate from onion.

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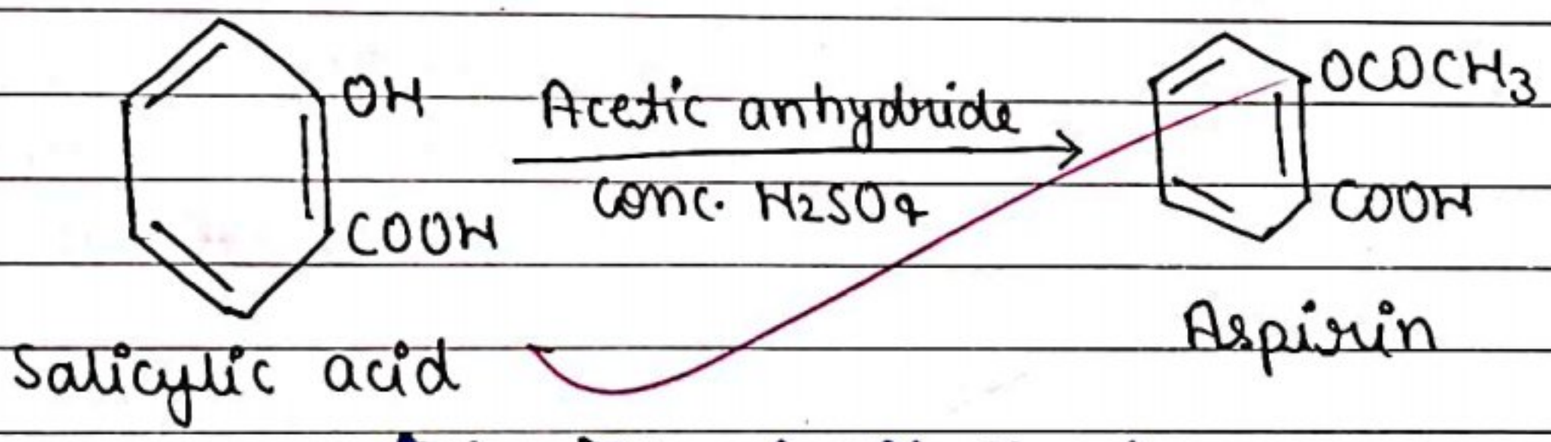
Expt. No..... Page No.....15..... Date...../...../.....

Experiment no. 07

Object- To synthesize acetyl salicylic acid (Aspirin) by acetylation of salicyclic acid and compare it with the ingredient of an aspirin tablet by TLC.

Principle- The phenolic group of salicylic acid can be easily acetylated either with acetyl chloride in the presence of pyridine or with acetic anhydride in presence of a little conc. H_2SO_4 as catalyst to yield acetyl salicylic acid (aspirin)



(I) First Method

Reagents- Salicylic acid 10 g, Pyridine 7.5 mL, Acetyl chlorine 7.3 mL (8.0 g)

Procedure-

Dissolve 10 g of salicylic acid in 7.5 mL of pyridine (dry) in a 100 ml conical flask. On account of the liberation of heat (exothermic reaction), the flask is cooled in ice bath. Immediately add 7.3 ml of acetyl chloride drop

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by drop from a separating funnel with continuous shaking the flask vigorously during addition. The heat of reaction causes the temperature of solution to rise rapidly. Maintain the temperature between 50-60°c by cooling the flask occasionally in cold water, if necessary. Then heat the reaction mixture on a boiling water bath for 5-6 minutes. On cooling a viscous semi-solid mass is obtained. Add to it 100 ml of ice cold water and stir vigoro-usly. The crude acetyl salicylic acid solidifies at once. Filter it at pump and wash throughly with cold water and drain well. Dry it on a porous plate and recrystalline either from a mixture of equal valumes of acetic acid and water or from benzene. The yield of colourless product (aspirin) is 19 g and m.p. 136°c

(II) Second Method

Reagents- Salicylic acid 7.5 g, Acetic acid anhydride 10.5 ml (11.2 g), conc. H_2SO_4 0.5 ml.

Procedure- In a 100ml flask, take 7.5 g of dry salicylic acid and 10.5 ml of redistilled acetic anhydride, add 0.5 ml of conc H_2SO_4. Shake the mixture thoroughly and heat on a water bath keeping the temperature at about 50-60°c for 15 minutes with continuous shaking. Allow the mixture to cool, add 100ml of water

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stir well and filter at the pump, wash thoroughly and drain well.

Recrystallise the crude, acetyl salicylic acid by dissolving it in 25 mL of hot alcohal and pouring the solution mixture until the solution becomes clear. Allow it to cool slowly. Beautiful needle like crystals of aspirin are obtained. The yield is 9.0 g and m.p. is 136°c

Result- Weight of crude aspirin = 4.5 g
Weight of recrystallised aspirin = 6.8 g
M.P. of pure aspirin = 135°c

Precautions-

(i) In the first method, the addition of acetyl chloride, to the solution of salicylic acid in pyridine, should be dropwise with continuous shaking so that the temperature may not rise above 50-60°C

(ii) Aspirin should not be crystalline from a solvent of high boiling point otherwise it may decompose.

(iii) For better crystallisation, aspirin should be dissolved in minimum quantity of hot ethanol and then water is added.

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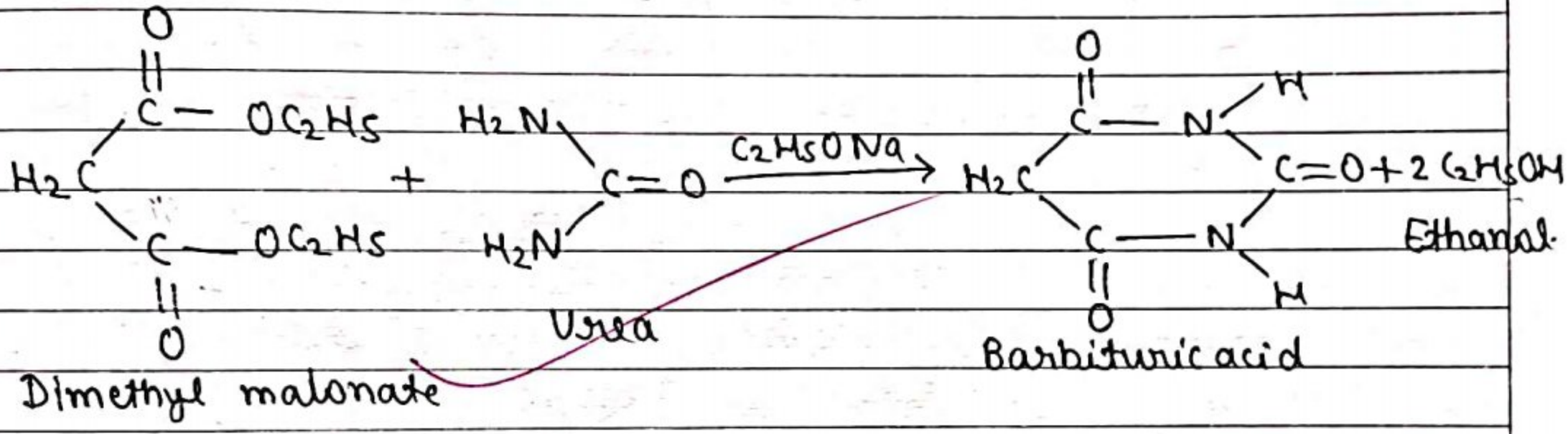
Expt. No..... Page No.....18..... Date...../...../.....

Experiment no. 08

Object:- To synthesize barbituric acid from urea and diethyl malonate.

Principle-

In the presence of sodium ethoxide (which may be prepared by the reaction of ethyl alcohal and sodium) urea is condensed with diethyl malonate to produce barbituric acid.



Reagents-

Urea (30g), diethyl malonate (80g), Absolute alcohal (250 mL), sodium (11.5g), calcium chloride (20g) and concentrated HCl (20mL)

Apparatus-

Round bottom flask, -2L, Reflux condenser, Buchner funnel, Measuring cylinder, Beaker etc.

Teacher's Signature : .....

Expt. No..... Page No.....19..... Date...../...../.....

Procedure-

Assemble a reflux condenser with a round bottom flask. Take 11.5 g clean sodium pieces and add 250 mL of absolute ethanol slowly and gradually. If the reaction is unduly vigorous, immerse the flask with ice. When all the sodium has completed reacted, add 80 g (76 mL) diethyl malonate, followed by a solution of dry urea (30 g) in 250 mL of hot (about 70°C) absolute ethanol. Shake the mixture, very well, attach a guard tube (containing solid calcium chloride) to the top of the condenser. Now reflux the mixture for 7 hours in an oil bath and heat to 110°C. A white solid will be separate out.

Treat the reaction mixture with hot water (about 50°C) 750 mL and then with concentrated HCl with constant stirring, until the solution will be acidic. Filter out, the filtrate is almost clear solution and leave it in the refrigerator overnight.

Filter the product by buchner funnel at the water pump. Wash the product with cold water, drain well and dry the product with cold water, drain well and dry the product at 100°C for 2 hours.

The yield is 50 g. The melting point of the product is 295°C.

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Expt. No..... Page No.....20..... Date...../...../.....

Result -

(i) The yield of product is 50 g.
(ii) The melting point of the product is 245°C

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