18BTC101J – BIOCHEMISTRY LABORATORY

Offered to

II YEAR B.TECH – BIOTECHNOLOGY

Specializations: Genetic Engineering / Regenerative Medicine

DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIOENGINEERING

SRM INSTITUTE OF SCIENCE AND TECHNOLOGY

KATTANKULATHUR

2020

SYLLABUS

BIOCHEMISTRY LABORATORY

Lab 1 – Introduction to commonly used instruments and laboratory safety – Video lecture

Exp. No.: 1 – Stoichiometric Calculations

Lab 2 - Preparation and measurement of pH of standard buffers

Exp. No.: 2 - Measurement of pH using pH meter

Exp. No.: 3 - Preparation of Biological Buffers

Lab 3 - Qualitative analysis of Monosaccharide in food samples

Exp. No.: 4 – Aldose - Reducing hexose sugar (Glucose)

Exp. No.: 5 – Ketose – Reducing hexose sugar (Fructose)

Exp. No.: 6 - Reducing pentose sugar (Aldose – Ribose/Ketose – Ribulose)

Lab 4 - Qualitative analysis of Disaccharides in food samples

Exp. No.: 7 – Reducing disaccharide (Maltose/Lactose)

Exp. No.: 8 – Non-Reducing disaccharide (Sucrose)

Lab 5 - Qualitative analysis of Polysaccharides in food samples

Exp. No.: 9 – Polysaccharide –Before/after hydrolysis with acid/enzyme (Starch)

Lab 6 - Estimate blood glucose, compares normal and diabetes mellitus samples

Exp. No.: 10 – Glucose estimation by Dinitro salicylic acid method

Lab 7 - Quantitative analysis of proteins

Exp. No.: 11- Estimation of proteins by Lowry's method

Lab 8 - Separation of amino acids on Thin Layer Chromatography

Exp. No.: 12 – Separation of amino acids by TLC

Lab 9 - Quantitative estimation of serum cholesterol

Exp. No.: 13 – Estimation of Cholesterol by Zak's method

REFERENCE: Biochemistry Laboratory Manual

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EXP NO: 1

DATE:

STOICHIOMETRIC CALCULATIONS

NORMALITY:

The normality of a solution is defined as the number of gram equivalents of a substance in 1 litre of the solution. Normality is denoted by the symbol 'N'. For example, if a solution is termed as 1N, it contains 1 gram equivalent of the substance in 1 litre of the solution.

$$Normality = \frac{number\ of\ gm\ equivalents}{litre}$$

where,

$$Number\ of\ gm\ equivalents = \frac{given\ weight\ (g)}{equivalent\ weight}$$

and

$$Equivalent\ Weight = \frac{molecular\ weight}{valency}$$

MOLARITY:

The molarity of a solution is defined as the number of moles of a substance in 1 litre of the solution. Molarity is denoted by the symbol 'M'. For example, if a solution is termed as 1M it contains 1 mole of the substance in 1 litre of the solution.

$$Molarity = \frac{number\ of\ gm\ moles}{litre}$$

where,

$$Number\ of\ gm\ moles = \frac{given\ weight\ (g)}{molecular\ weight}$$

MOLALITY:

The molality of the solution is defined as the number of moles of a substance in 1Kg of the solvent. Molality is denoted by the symbol ‘m’.

$$\boldsymbol{Molality = \frac{number\ of\ moles}{Kg\ solvent}}$$

where,

$$\boldsymbol{Number\ of\ moles = \frac{given\ weight\ (g)}{molecular\ weight}}$$

OSMOLARITY:

The osmolarity of the solution is defined as the number of osmoles of a solute per litre of the solution. Osmolarity is denoted by the symbol ‘Osm’ or ‘osmol’. Osmolarity is distinct from molarity because it measures osmoles of solute particles rather than moles of solute. The distinction arises because some compounds can dissociate in solution, whereas others cannot.

$$\boldsymbol{Osmolarity = \frac{number\ of\ Osmoles}{litre}}$$

OSMOLALITY:

The osmolality of the solution is defined as the number of osmoles of a solute per kg of the solvent. Osmolarity is denoted by the symbol ‘Osm’ or ‘osmol’.

$$\boldsymbol{Osmolality = \frac{number\ of\ Osmoles}{Kg\ solvent}}$$

CALCULATIONS:

EXP NO: 2

DATE:

MEASUREMENT OF pH USING pH METER

AIM:

To become familiar with operating the pH meter, and to learn how to use the Henderson-Hasselbalch equation to make buffer solutions at a desired pH value.

PRINCIPLE:

The pH meter measures the electrical potential developed across a pair of electrodes by dipping it into a solution. For the measurement of pH, an electrode system sensitive to changes in the hydrogen ion concentration of the solution is chosen. This electrode system consists of a sequence of electrodes whose potential varies with the pH of the solution.

DIGITAL PH METER

REQUIREMENTS:

1. 0.1N HCl
2. 0.1N NaOH
3. Colored Soft Drink
4. Colorless Soft Drink
5. Urine
6. Tap Water
7. Lime Juice
8. Pomegranate Juice
9. pH meter
10. Stirrer
11. Glass rod and etc…

PROCEDURE:

The pH meter is cleaned using distilled water and the probe is patted dry using soft tissue. After cleaning, the pH meter was calibrated using the standard buffers and cleaned again. The test sample was taken in a beaker and its pH was recorded. Following this, 1 drop of 0.1N HCl was added to the sample and the pH was recorded again. Next two drops of 0.1N HCl was added to the sample and the pH was recorded. Finally, 5 drops of HCl was added to the sample and the pH was recorded. The procedure was repeated using 0.1N NaOH in the place of HCl with a fresh sample and the pH was recorded.

RESULT:

OBSERVATIONS:

	Tap Water	**Milk**	**Fresh citrus juice**	**Colored Soft Drink**	**Colorless Soft Drink**	**Your own samples if any**
Test Sample (As it is pH)						
(+) 1 drop HCl						
(+) 2 drops HCl						
(+) 5 drops HCl						
(+) 1 drop NaOH						
(+) 2 drops NaOH						
(+) 5 drops NaOH						

EXP NO: 3

DATE:

PREPARATION OF BIOLOGICAL BUFFERS

AIM:

To prepare the biological buffers and to measure their pH.

PRINCIPLE:

Buffers may be mixtures of weak acids and their conjugate base or weak bases and their conjugate acids. They resist changes in pH upon addition of small amounts of strong acids or bases. In a physiological system, this property is vital for the maintenance of homeostasis. For eg. Most of the body fluids contain natural buffering systems such as blood, saliva, urine, mucous etc.

1. Citrate buffer (pH 5.5):

Stock solutions:

1. 0.1M Solution of Citric acid

   21.01g of citric acid is dissolved in 1000 ml of distilled water.
2. 0.1M Solution of Sodium citrate

   29.41 g of sodium citrate is dissolved in 1000 ml of distilled water.

PROCEDURE:

Pipette out exactly 16.0 ml of citric acid solution into a 100ml standard flask. To this add exactly 34.0 ml of sodium citrate. The solution is made up to 100ml with distilled water. The resultant solution is 0.1M citric acid-sodium citrate buffer whose pH is measured using a pH meter.

The pH meter is first standardized using standard buffer and the electrode is washed with distilled water. The pH of the citrate buffer is measured as 5.5.

2. Carbonate-Bicarbonate Buffer (pH 10.0):

Stock solutions:

1. Sodium Carbonate solution (0.2M)

   Dissolve 2.12 g of anhydrous sodium carbonate in 100 ml of water.
2. Sodium Bicarbonate Solution (0.2M)

Dissolve 1.68 g of sodium bicarbonate in 100 ml of water.

PROCEDURE:

Pipette out exactly 27.5 ml of sodium carbonate solution (0.2M). To this added 22.5ml of sodium bicarbonate solution (0.2M). The resultant solution is 0.1M carbonate buffer whose pH is measured using a pH meter.

The pH meter is first standardized using standard buffer and the electrode is washed with distilled water. The pH of the carbonate buffer was measured as 10.

3. Glycine - HCl Buffer (pH 2.5):

Stock solutions:

1. 0.2 M of Glycine

   Dissolve 1.501 g of Glycine in 100 ml of water.
2. 0.2 M of HCl

PROCEDURE:

Take 50mL of 0.2 M of Glycine solution and add 32.5mL of 0.2 M HCl solution and make up to 100mL. The resultant solution is Glycine -HCl buffer whose pH is measured using a pH meter.

The pH meter is first standardized using standard buffer and the electrode is washed with distilled water. The pH of the Glycine -HCl buffer is measured as 2.5.

4. Tris Buffer (pH 8.0):

Stock solutions:

1. 0.2 M of Tris

   Dissolve 1.121 g of Tris in 100 ml of water.
2. 0.2 M of HCl

PROCEDURE:

Take 50mL of 0.2 M of Tris solution and add 26.8mL of 0.2 M HCl solution and make up to 200mL. The resultant solution is Tris - HCl buffer whose pH is measured using a pH meter.

The pH meter is first standardized using standard buffer and the electrode is washed with distilled water. The pH of the Tris -HCl buffer is measured as 8.0.

5. Phosphate Buffer (pH 7.0):

Stock solutions:

1. Monobasic sodium phosphate solution (0.2M)

   Dissolve 2.78 g at monobasic sodium phosphate in 100ml of water

2. Dibasic sodium phosphate solution (0.2M)

   Dissolve 5.365g of dibasic sodium phosphate heptahydrate and 7.179 g of dibasic sodium phosphate deco carbonate in 100 ml of water.

PROCEDURE:

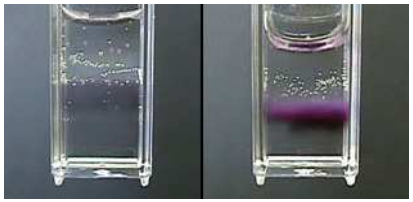
Mix 39 ml of monobasic sodium phosphate solution with 61ml of dibasic sodium phosphate solution. This is made up to 200 ml with distilled water. The resultant solution is 0.1 M phosphate buffer whose pH is measured using a pH meter.

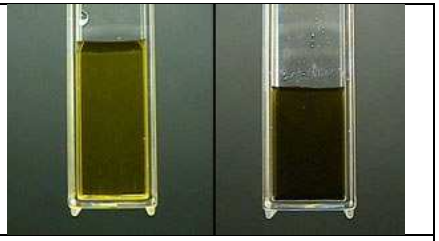
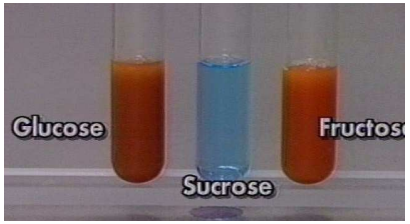
The pH meter is first standardized using standard buffer and the electrode is washed with distilled water. The pH of the phosphate buffer is measured as 7.

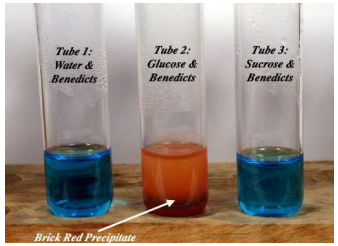
RESULT:

The buffers of the required pH were prepared.

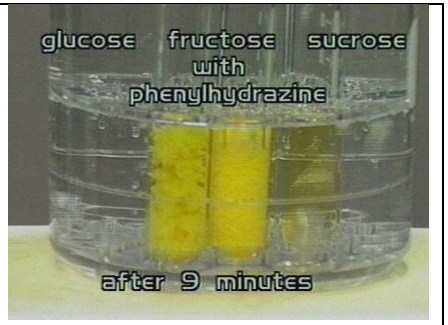
QUALITATIVE ANALYSIS OF SUGARS – GENERAL PROCEDURE

NAME	PRINCIPLE	PROCEDURE	OBSERVATIONS
Molisch's Test	Molisch's Test is a sensitive chemical test for all carbohydrates, and some compounds containing carbohydrates in a combined form, based on the dehydration of the carbohydrate by sulfuric acid to produce an aldehyde (either furfural or a derivative), which then condenses with the phenolic structure resulting in a red or purple-colored compound.	Place 2 mL of a known carbohydrate solution in a test tube, add 1 drop of Molisch's reagent (10% α-naphthol in ethanol). Pour 1-2 mL of conc. H_2SO_4 down the side of the test tube, so that it forms a layer at the bottom of the tube. Observe the color at the interface between two layers and compare your result with a control test. A brown color due to charring must be ignored and the test should be repeated with a more dilute sugar solution.	A red or purple-colored compound was observed at the interface. 
Iodine Test	Iodine dissolved in an aqueous solution of potassium iodide reacts with the starch producing a purple black color. The color can be detected visually with concentrations of iodine as low as 0.00002M at 20°C. However the intensity of the color decreases with increasing temperature and with the presence of water-miscible,	A few drops of 0.01 M iodine in 0.12 M KI are added to a 1% solution of the carbohydrate in question.	The immediate formation of a vivid blue color indicates amylose. With starch a blue-black coloration forms due to the poly iodide complex formed.

	organic solvents such as ethanol. Also the test cannot be done at very low pHs due to the hydrolysis of the starch under these conditions.		
Fehling's Test	Fehling's solution reacts with reducing sugars on heating and reduces the Cu (II) ion to Cu (I) producing a precipitate of red copper oxide.	**Fehling's I** consists of 7 g of hydrated copper (II) sulfate dissolved in 100 mL of distilled water. **Fehling's II** is made by dissolving 35 g of potassium sodium tartrate and 10 g of sodium hydroxide in 100 mL of dist. water. **Fehling's reagent:** Equal volumes of Fehling I and Fehling II are mixed to form a deep blue solution. To 1 mL of Fehling's solution A (aqueous solution of $CuSO_4$) add 1 mL of Fehling solution B (solution of potassium tartrate). Add 2 mL of the sugar solution mix well and boil.	A **red precipitate of cuprous oxide** forms at the end of the reaction. 
Barfoed's Test	Barfoed's reagent, cupric acetate in acetic acid is slightly acidic and is balanced so that is can only be reduced by monosaccharide but not less powerful reducing sugars. Disaccharides may also react with this reagent, but the reaction is much slower when	Barfoed's reagent consists of a 0.33 molar solution of neutral copper acetate in 1% acetic acid solution. The reagent does not keep well and it is therefore advisable to make it up when it is actually required. To 1-2 mL of Barfoed's reagent, add an equal volume of sugar	**A brick-red cuprous oxide precipitate forms** if reduction has taken place.

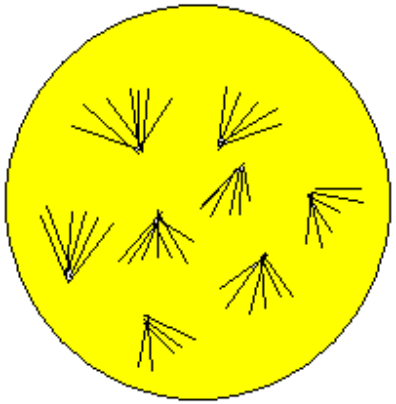
	compared to monosaccharides.	solution. Boil for 5 min. in a water bath and allow standing.	
Benedict's Test	Benedict's solution reacts with reducing sugars on heating and reduces the Cu(II) ion to Cu(I) producing a precipitate of red copper oxide. The resulting colour change depends on the type and concentration of sugar, so this test can be used semi-quantitatively to indicate approximate concentrations.	**Benedicts reagent** **Solution 1** Sodium citrate 86.5g Sodium carbonate (anhydrous) 50g Dissolve in 400mls H_2O **Solution 2** Copper sulphate $5H_2O$ 8.7g Dissolve in 50ml of H_2O. Add 2 to 1 with rapid stirring then dilute to 500 ml. To 2 ml sugar solution, add 2 ml of Benedict's reagent and stand the test tube in boiling water for a few minutes.	A colour change through green to yellow, brown and finally to red indicates the presence of reducing sugar. 
Bial's Test:	The components include orcinol, hydrochloric acid, and ferric chloride. A pentose, if present, will be dehydrated to form furfural which then reacts with the orcinol to generate a colored substance. The solution will turn bluish and a precipitate may form.	**Bial's reagent:** Dissolved 1.5gms of orcinol in 50 ml of conc. HCl and added 20 to 30 drops of 10% solution of ferric chloride in water. **Procedure:** Mixed 5ml of Bial's reagent with 5ml of sugar solution and kept in boiling water bath. 1 mL of sugar solution with 3 mL Seliwanoff's reagent (0.5 g resorcinol per liter 10% HCl) in boiling water.	

Seliwanoff's Test	Seliwanoff's Test distinguishes between aldose and ketose sugars. Ketoses are distinguished from aldoses via their ketone/aldehyde functionality. If the sugar contains a ketone group, it is a ketose and if it contains an aldehyde group, it is an aldose. This test is based on the fact that, when heated, ketoses are more rapidly dehydrated than aldoses.	Heat 1 mL of sugar solution with 3 mL of Seliwanoff's reagent (0.5g resorcinol per liter of 10% HCl) in boiling water.	In less than 30 seconds, a red color must appear for ketoses. Upon prolonged heating, glucose will also give an appreciable color.
Phenylhydrazine test	Osazone formation involves hydrazone formation at C-1 of an aldose (or C-2 of a ketose) and oxidation of C-2 (or C-1) of an alcohol group to a ketone (or an aldehyde). The new carbonyl group is also converted to a hydrazone. It has been suggested that the reaction stops here (rather than further oxidation at C-3, etc.) because of hydrogen-bonding stabilization of the osazone.	Place 0.2 g of the unknown sample in a test tube and add 0.4 g of phenylhydrazine hydrochloride, 0.6 g of crystallized sodium acetate, and 4 mL of distilled water. Place the test tube in a beaker of boiling water. Note the time that the test tube was immersed and the time of the precipitation. After 20 min, remove the test tube from the hot water bath and set it aside to cool. A small amount of the liquid and solid is poured on a watch glass. Tip the watch glass from side to side to spread out the crystals, and absorb some of the mother liquid with a piece of filter paper, taking care not to crush or break up the clumps of crystals. Examine the crystals under a low-power	The times required for the formation of the osazones can be a valuable aid in distinguishing among various sugars. The following figures are the times required for the osazone to precipitate from the hot solution: fructose, 2 min; glucose, 4-5 min; xylose, 7 min; arabinose, 10 min; galactose, 15-19 min; raffinose, 60 min; lactose, osazone soluble in hot water; maltose, osazone soluble in hot water; mannose, 0.5 min (hydrazone); sucrose, 30 min (owing to hydrolysis and formation of glucosazone).

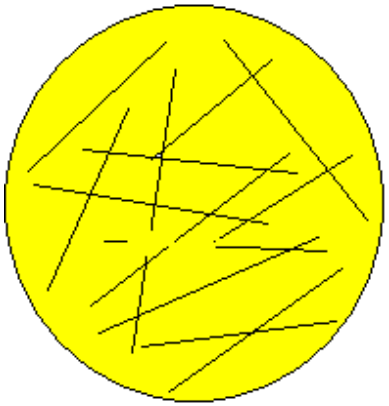
		microscope (about 80-100×), and compare with photomicrographs. The formation of tarry products due to oxidation of the phenylhydrazine may be prevented by the addition of 0.5 mL of saturated sodium bisulfite solution. This should be done before heating if it is desired to isolate the osazone and determine its melting point.	

OSAZONE CRYSTALS UNDER MICROSCOPE

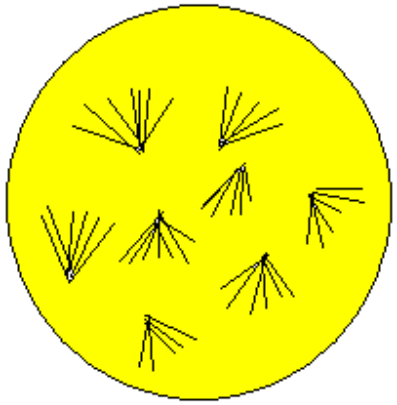
GLUCOSAZONE CRYSTALS



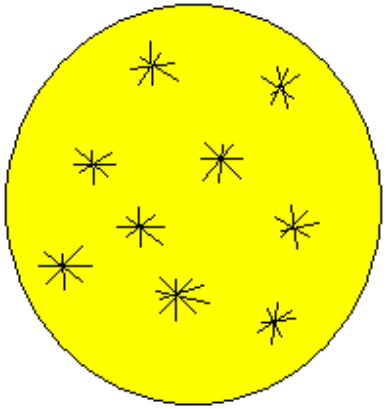
XYLOSAZONE CRYSTALS



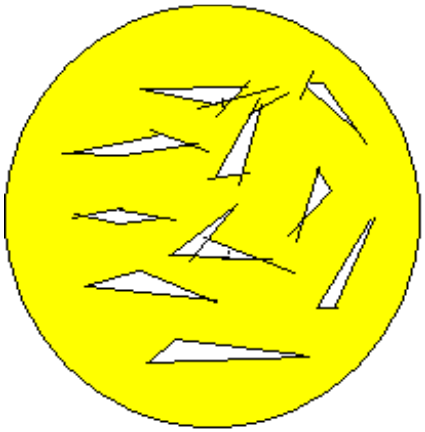
FRUCTOSAZONE CRYSTALS



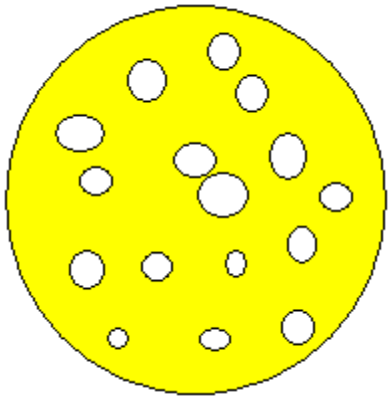
MALTOSAZONE CRYSTALS



GALACTOSAZONE CRYSTALS



LACTOSAZONE CRYSTALS



EXP NO: 4

DATE:

QUALITATIVE ANALYSIS OF SUGARS

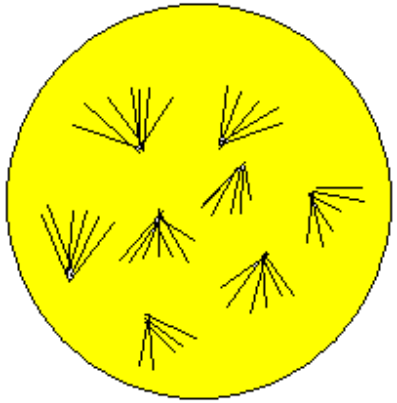
REDUCING MONOSACCHARIDE – ALDOSE (Glucose)

NAME	PROCEDURE	OBSERVATION	INFERENCE
Solubility Test	1. A small amount of the substance was dissolved in water and observed. 2. A small amount of the substance was dissolved in hot water and observed. 3. A small amount of the substance was dissolved in dilute HCl and observed. 4. A small amount of the substance was dissolved in alcohol and observed.	1. Soluble in water 2. Soluble in hot water 3. Soluble in dilute HCl 4. Soluble in alcohol	May be a monosaccharide
Molisch's Test	To 2 drops of Molisch's reagent add 2ml of sugar solution in a test tube and added 2ml of conc. H_2SO_4 by the sides of the tube	Reddish violet ring is formed at the junction of two liquids	Presence of carbohydrate.

Iodine Test	1 to 2 drops of iodine reagent is added 2 to 3ml of sugar solution.	No color change.	Absence of polysaccharide
Barfoed's Test	To 8 drops of sugar solution add 5ml of Barfoed's reagent placed in water bath for few minutes and allow to stand.	Red precipitate was formed	Presence of a monosaccharide .
Benedict's Test	To 8 drops of sugar solution added 5ml of Benedict's reagent in a test tube and kept in the boiling water bath for 2 minutes.	Blue colour turns orange.	Presence of reducing sugar.
Fehling's Test	To a few drops of sugar solution added 5ml of Fehling's solution and heated the mixture.	A brownish orange precipitate was obtained	Presence of reducing sugar.
Bial's Test:	Mixed 5ml of Bial's reagent with 5ml of sugar solution and kept in boiling water bath.	No green colour was observed	Absence of pentose sugar.
Seliwanoff's Test	To few drops of sugar solution added 5ml of Seliwanoff's reagent and kept it in boiling water bath for few minutes.	No cherry red colour was observed	Absence of ketose sugar.

Phenyl hydrazine test	**Reagent:** 20 gm of phenyl hydrazine chloride is dissolved in 120 ml of water, slightly warmed to get a solution and few ml of solution and 2 ml of glacial acetic acid is added. **Procedure:** To 2 to 3 ml of sugar solution added 2 to 3 drops of phenyl hydrazine, 8 drops of glacial acid and a pinch of sodium acetate and heated in boiling water bath.	Yellow precipitate is formed Fine needle or feather shaped crystals are formed within 10-12 minutes	Presence of reducing sugar. Presence of Glucosazone

OSAZONE CRYSTALS: GLUCOSAZONE CRYSTALS



RESULT:

The given sugar is identified to be **……**

EXP NO: 5

DATE:

QUALITATIVE ANALYSIS OF SUGARS

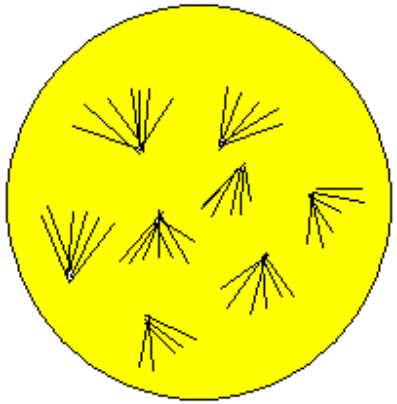
REDUCING MONOSACCHARIDE – KETOSE (Fructose)

NAME	PROCEDURE	OBSERVATION	INFERENCE
Solubility Test	1. A small amount of the substance was dissolved in water and observed. 2. A small amount of the substance was dissolved in hot water and observed. 3. A small amount of the substance was dissolved in dilute HCl and observed. 4. A small amount of the substance was dissolved in alcohol and observed.		
Molisch's Test	To 2 drops of Molisch's reagent add 2ml of sugar solution in a test tube and added 2ml of conc. H_2SO_4 by the sides of the tube		

Iodine Test	1 to 2 drops of iodine reagent is added 2 to 3ml of sugar solution.		
Barfoed's Test	To 8 drops of sugar solution add 5ml of Barfoed's reagent placed in water bath for few minutes and allow to stand.		
Benedict's Test	To 8 drops of sugar solution added 5ml of Benedict's reagent in a test tube and kept in the boiling water bath for 2 minutes.		
Fehling's Test	To a few drops of sugar solution added 5ml of Fehling's solution and heated the mixture.		
Bial's Test:	Mixed 5ml of Bial's reagent with 5ml of sugar solution and kept in boiling water bath. 1 mL of sugar solution with 3 mL Seliwanoff's reagent (0.5 g resorcinol per liter 10% HCl) in boiling water.		
Seliwanoff's Test	To few drops of sugar solution added 5ml of Seliwanoff's reagent and kept it in boiling water bath for few minutes.		

Phenyl hydrazine test	To 2 to 3 ml of sugar solution added 2 to 3 drops of phenyl hydrazine, 8 drops of glacial acid and a pinch of sodium acetate and heated in boiling water bath.		

OSAZONE CRYSTALS: FRUCTOSAZONE CRYSTALS



RESULT:

The given sugar is identified to be **……**

EXP NO: 6

DATE:

QUALITATIVE ANALYSIS OF SUGARS

REDUCING MONOSACCHARIDE – PENTOSE

NAME	PROCEDURE	OBSERVATION	INFERENCE
Solubility Test	1. A small amount of the substance was dissolved in water and observed. 2. A small amount of the substance was dissolved in hot water and observed. 3. A small amount of the substance was dissolved in dilute HCl and observed. 4. A small amount of the substance was dissolved in alcohol and observed.		
Molisch's Test	To 2 drops of Molisch's reagent add 2ml of sugar solution in a test tube and added 2ml of conc. H_2SO4 by the sides of the tube		

Iodine Test	1 to 2 drops of iodine reagent is added 2 to 3ml of sugar solution.		
Barfoed's Test	To 8 drops of sugar solution add 5ml of Barfoed's reagent placed in water bath for few minutes and allow to stand.		
Benedict's Test	To 8 drops of sugar solution added 5ml of Benedict's reagent in a test tube and kept in the boiling water bath for 2 minutes.		
Fehling's Test	To a few drops of sugar solution added 5ml of Fehling's solution and heated the mixture.		
Bial's Test:	Mixed 5ml of Bial's reagent with 5ml of sugar solution and kept in boiling water bath. 1 mL of sugar solution with 3 mL Seliwanoff's reagent (0.5 g resorcinol per liter 10% HCl) in boiling water.		
Seliwanoff's Test	To few drops of sugar solution added 5ml of Seliwanoff's reagent and kept it in boiling water bath for few minutes.		

Phenyl hydrazine test	**Reagent:** 20 gm of phenyl hydrazine chloride is dissolved in 120 ml of water, slightly warmed to get a solution and few ml of solution and 2 ml of glacial acetic acid is added. **Procedure:** To 2 to 3 ml of sugar solution added 2 to 3 drops of phenyl hydrazine, 8 drops of glacial acid and a pinch of sodium acetate and heated in boiling water bath.		

RESULT:

The given sugar is identified to be **……..**

EXP NO: 7

DATE:

QUALITATIVE ANALYSIS OF SUGARS

REDUCING DISACCHARIDE – LACTOSE/MALTOSE

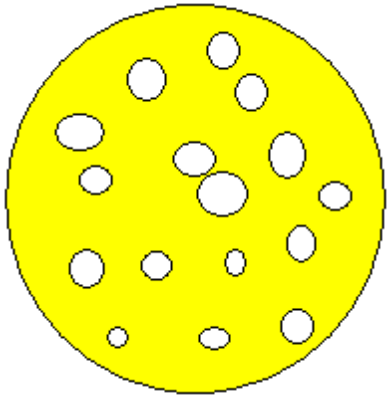
NAME	PROCEDURE	OBSERVATION	INFERENCE
Solubility Test	1. A small amount of the substance was dissolved in water and observed. 2. A small amount of the substance was dissolved in hot water and observed. 3. A small amount of the substance was dissolved in dilute HCl and observed. 4. A small amount of the substance was dissolved in alcohol and observed.		
Molisch's Test	To 2 drops of Molisch's reagent add 2ml of sugar solution in a test tube and added 2ml of conc. H_2SO4 by the sides of the tube		

Iodine Test	1 to 2 drops of iodine reagent is added 2 to 3ml of sugar solution.		
Barfoed's Test	To 8 drops of sugar solution add 5ml of Barfoed's reagent placed in water bath for few minutes and allow to stand.		
Benedict's Test	To 8 drops of sugar solution added 5ml of Benedict's reagent in a test tube and kept in the boiling water bath for 2 minutes.		
Fehling's Test	To a few drops of sugar solution added 5ml of Fehling's solution and heated the mixture.		
Bial's Test:	Mixed 5ml of Bial's reagent with 5ml of sugar solution and kept in boiling water bath.		
Seliwanoff's Test	To few drops of sugar solution added 5ml of Seliwanoff's reagent and kept it in boiling water bath for few minutes.		
Phenyl hydrazine test	**Reagent:** 20 gm of phenyl hydrazine chloride is dissolved in 120 ml of water, slightly warmed to get a solution and few ml of solution and 2 ml of glacial acetic acid is added.		

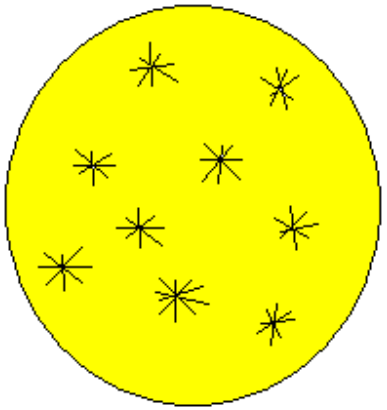
	Procedure: To 2 to 3 ml of sugar solution added 2 to 3 drops of phenyl hydrazine, 8 drops of glacial acid and a pinch of sodium acetate and heated in boiling water bath.		

OSAZONE CRYSTALS:

LACTOSAZONE CRYSTALS



MALTOSAZONE CRYSTALS



RESULT:

The given sugar is identified to be **……**

EXP NO: 8

DATE:

QUALITATIVE ANALYSIS OF SUGARS

NON- REDUCING DISACCHARIDE – SUCROSE

NAME	PROCEDURE	OBSERVATION		INFERENCE	
		BEFORE ACID HYDROLYSIS	AFTER ACID HYDROLYSIS	BEFORE ACID HYDROLYSIS	AFTER ACID HYDROLYSIS
Solubility Test	1. A small amount of the substance was dissolved in water and observed. 2. A small amount of the substance was dissolved in hot water and observed. 3. A small amount of the substance was dissolved in dilute HCl and observed. 4. A small amount of the substance was dissolved in alcohol and observed.	1. Soluble in water 2. Soluble in hot water 3. Soluble in dilute HCl 4. Soluble in alcohol	1. Soluble in water 2. Soluble in hot water 3. Soluble in dilute HCl 4. Soluble in alcohol	May be a sugar	May be a sugar

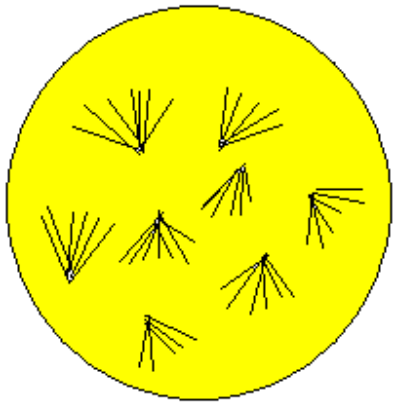
Molisch's Test	**Molisch's Reagent:** 15gms of napthol was dissolved in 150ml of 95% ethanol. **Procedure:** To 2 drops of Molisch's reagent add 2ml of sugar solution in a test tube and added 2ml of conc. H_2SO_4 by the sides of the tube	Reddish violet ring is formed at the junction of two liquids	Reddish violet ring is formed at the junction of two liquids		Presence of carbohydrate.
Iodine Test	**Iodine Solution:** 1gm of potassium iodine is dissolved in 100ml of water. To this 0.025gm of iodine is added. **Procedure:** 1 to 2 drops of iodine reagent is added 2 to 3ml of sugar solution.	No colour change.	No colour change.	Absence of polysaccharide	Absence of polysaccharide
Barfoed's Test	**Barfoed's reagent:** 13.3gms of crystalline cuprous acetate are dissolved in 200ml of water and 1.8ml of glacial acetic acid is added to the above solution.				

	Procedure: To 8 drops of sugar solution add 5ml of Barfoed's reagent placed in water bath for few minutes and allow to stand.	Red precipitate was not formed	Red precipitate was formed	Absence of monosaccharide	Presence of a monosaccharide .
Benedict's Test	**Benedicts reagent** 43.25gm of sodium cuprate and 45gm of anhydrous sodium carbonate are dissolved in 200ml of water. To the above solution 4.35gms of copper sulphate dissolved in 25ml of water is added and mixed well and then the solution is made upto 250 ml with water. **Procedure:** To 8 drops of sugar solution added 5ml of Benedict's reagent in a test tube and kept in the boiling water bath for 2 minutes.	No characteristic colour change	Blue colour turns orange.	Absence of reducing sugar	Presence of 2-4g reducing sugar.
Fehling's Test	**Fehling's Test** Prepare equal volume of solution (A) and (B) by mixing solution (A) 6.93 gm of $CuS0_4$ in 100ml of				

	water. Solution (B) 20gm of potassium hydroxide and 39.6 gm of sodium potassium tartrate. **Procedure:** To a few drops of sugar solution added 5ml of Fehling's solution and heated the mixture.	No characteristic observation	A brownish orange precipitate was obtained	Absence of reducing sugar	Presence of reducing sugar.
Bial's Test:	**Bial's reagent:** Dissolved 1.5gms of orcinol in 50 ml of conc. HCl and added 20 to 30 drops of 10% solution of ferric chloride in water. **Procedure:** Mixed 5ml of Bial's reagent with 5ml of sugar solution and kept in boiling water bath. 1 mL of sugar solution with 3 mL Seliwanoff's reagent (0.5 g resorcinol per liter 10% HCl) in boiling water.	No green colour was observed.	No green colour was observed	Absence of pentose sugar.	Absence of pentose sugar.
Seliwanoff's Test	**Seliwanoff's reagent:** Dissolve 50gms of dilute hydrochloric acid (1:2) in water.				

	Procedure: To few drops of sugar solution added 5ml of Seliwanoff's reagent and kept it in boiling water bath for few minutes.	No cherry red colour was observed.	Cherry red colour was observed	Absence of ketose sugar.	Presence of ketose sugar.
Phenyl hydrazine test	**Reagent:** 20 gm of phenyl hydrazine chloride is dissolved in 120 ml of water, slightly warmed to get a solution and few ml of solution and 2 ml of glacial acetic acid is added. **Procedure:** To 2 to 3 ml of sugar solution added 2 to 3 drops of phenyl hydrazine, 8 drops of glacial acid and a pinch of sodium acetate and heated in boiling water bath.	No characteristic observation.	Yellow precipitate is formed Fine needle or feather shaped crystals are formed within 10-12 minutes	Absence of reducing sugar	Presence of reducing sugar. Presence of Glucosazone

OSAZONE CRYSTALS: GLUCOSAZONE CRYSTALS



RESULT:

The given sugar is identified to be **……..**

EXP NO: 9

DATE:

QUALITATIVE ANALYSIS OF SUGARS

POLYSACHARIDE - STARCH

NAME	PROCEDURE	OBSERVATION		INFERENCE	
		BEFORE ACID HYDROLYSIS	**AFTER ACID HYDROLYSIS**	**BEFORE ACID HYDROLYSIS**	**AFTER ACID HYDROLYSIS**
Solubility Test	1. A small amount of the substance was dissolved in water and observed. 2. A small amount of the substance was dissolved in hot water and observed. 3. A small amount of the substance was dissolved in dilute HCl and observed. 4. A small amount of the substance was dissolved in alcohol and observed.	1. Insoluble in water 2. Partially Soluble in hot water 3. Insoluble in dilute HCl 4. Insoluble in alcohol	1. Soluble in water 2. Soluble in hot water 3. Soluble in dilute HCl 4. Soluble in alcohol	May be a Polysaccharide	May be a monosaccharide
Molisch's Test	**Molisch's Reagent:** 15gms of napthol was dissolved in 150ml of 95% ethanol.				

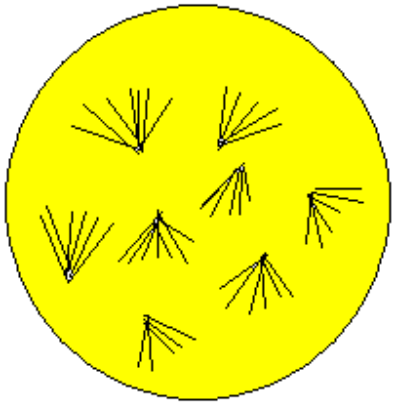
	Procedure: To 2 drops of Molisch's reagent add 2ml of sugar solution in a test tube and added 2ml of conc. H_2SO4 by the sides of the tube	Reddish violet ring is formed at the junction of two liquids	Reddish violet ring is formed at the junction of two liquids	Presence of carbohydrate.	Presence of carbohydrate.
Iodine Test	**Iodine Solution:** 1gm of potassium iodine is dissolved in 100ml of water. To this 0.025gm of iodine is added. **Procedure:** 1 to 2 drops of iodine reagent is added 2 to 3ml of sugar solution.	Blue colored solution was obtained.	No colour change.	Presence of polysaccharide	Absence of polysaccharide
Barfoed's Test	**Barfoed's reagent:** 13.3 gms of crystalline cuprous acetate are dissolved in 200ml of water and 1.8ml of glacial acetic acid is added to the above solution. **Procedure:** To 8 drops of sugar solution add 5ml of Barfoed's reagent placed in water bath for few minutes and allow to stand.	No characteristic colour change was observed	Red precipitate was formed	May be a polysaccharide .	Presence of a monosaccharide .
Benedict's Test	**Benedicts reagent** 43.25gm of sodium cuprate and 45gm of anhydrous				

	sodium carbonate are dissolved in 200ml of water. To the above solution 4.35gms of copper sulphate dissolved in 25ml of water is added and mixed well and then the solution is made upto 250 ml with water. **Procedure:** To 8 drops of sugar solution added 5ml of Benedict's reagent in a test tube and kept in the boiling water bath for 2 minutes.	No characteristic colour change was observed	Blue colour turns orange.	Absence of reducing sugar.	Presence of 2-4g reducing sugar.
Fehling's Test	**Fehling's Test** Prepare equal volume of solution (A) and (B) by mixing solution (A) 6.93 gm of CUS04 in 100ml of water. Solution (B) 20gm of potassium hydroxide and 39.6 gm of sodium potassium tartrate. **Procedure:** To a few drops of sugar solution added 5ml of Fehling's solution and heated the mixture.	No characteristic colour change was observed	A brownish orange precipitate was obtained	Absence of reducing sugar.	Presence of reducing sugar.
Bial's Test	**Bial's reagent:** Dissolved 1.5gms of orcinol in 50 ml of conc. HCl and				

	added 20 to 30 drops of 10% solution of ferric chloride in water. **Procedure:** Mixed 5ml of Bial's reagent with 5ml of sugar solution and kept in boiling water bath. 1 mL of sugar solution with 3 mL Seliwanoff's reagent (0.5 g resorcinol per liter 10% HCl) in boiling water.	No green colour was observed	No green colour was observed	Absence of pentose sugar.	Absence of pentose sugar.
Seliwanoff's Test	**Seliwanoff's reagent:** Dissolve 50gms of dilute hydrochloric acid (1:2) in water. **Procedure:** To few drops of sugar solution added 5ml of Seliwanoff's reagent and kept it in boiling water bath for few minutes.	No cherry red colour was observed	No cherry red colour was observed	Absence of ketose sugar.	Absence of ketose sugar.
Phenyl hydrazine test	**Reagent:** 20 gm of phenyl hydrazine chloride is dissolved in 120 ml of water, slightly warmed to get a solution and few ml of solution and 2 ml of				

	glacial acetic acid is added. **Procedure:** To 2 to 3 ml of sugar solution added 2 to 3 drops of phenyl hydrazine, 8 drops of glacial acid and a pinch of sodium acetate and heated in boiling water bath.	No yellow precipitate is formed.	Yellow precipitate is formed Fine needle or feather shaped crystals are formed within 10-12 minutes	May be a polysaccharide.	Presence of reducing sugar. Presence of Glucosazone

OSAZONE CRYSTALS: GLUCOSAZONE CRYSTALS



RESULT:

The given sugar is identified to be **……**

EXP NO: 10

DATE:

GLUCOSE ESTIMATION BY DINITRO SALICYLIC ACID METHOD

AIM:

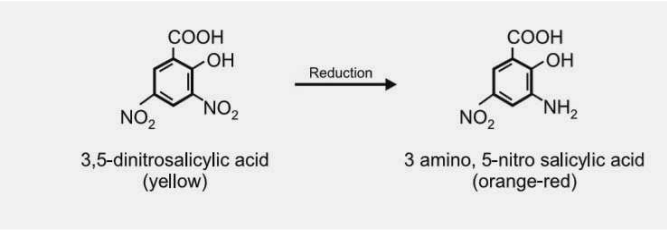
Estimation of reducing sugars from given sample(s) by Dintro salicylic Acid (DNS).

PRINCIPLE:

Reducing sugars have the property to reduce many of the reagents. One such reagent is 3,5-dinitrosalicylic acid (DNS).This method involves the oxidation of the aldehyde functional group present in, for example, glucose functional group is aldehyde. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions:

oxidation
aldehyde group ----------> carboxyl group

reduction
3,5-dinitrosalicylic acid ----------> 3-amino,5-nitrosalicylic acid



REACTION INVOLVING DNS (estimation of reducing sugars by DNS method)

REAGENTS

Dinitrosalicylic Acid Reagent Solution, 1%

Solution "A" is prepared by dissolving 300g of sodium potassium tartarate in about 500ml distilled water.

Solution "B" is prepared by dissolving 10 g of 3,5-dinitrosalicylic acid in 200 ml of 2N NaOH solution.

The dinitrosalycilate reagent is prepared by mixing solutions A and B and raising the final volume to 1 litre with distilled water.

Standard Glucose Solution: 0.1g anhydrous glucose is dissolved in distilled water and then raised the volume to 100 ml with distilled water.

PROCEDURE:

1. Take 7 clean, dry test tubes.
2. Pipette out standard sugar solution in different test tubes and make up the volume of all test tubes to 1 mL with distilled water.
3. Add 2 mL DNS reagent to all the test tubes and mix plug the test tube with cotton or marble and keep the test tube in a boiling water bath for 5 minute.
4. Take the tubes and cool to room temperature.
5. Add 7 ml of water after cooling the solution completely.
6. Read extinction at 540 nm against the blank.
7. All the tubes must be cooled to room temperature before reading, since the absorbance is sensitive to temperature.

RESULT:

GLUCOSE ESTIMATION BY DINITRO SALICYLIC ACID METHOD

OBSERVATIONS

Tube No.	**Glucose Std. Vol. (ml.)**	**Conc. of Glucose (µg/mL)**	**Test (ml)**	**Water (ml)**	**DNS reagent (ml.)**		**Water (ml.)**	**OD at 540nm.**
1	0.0	-	-	1.0	2.0	Heat for 5 min. in a boiling water bath	7.0	
2	0.2	200	-	0.8	2.0		7.0	
3	0.4	400	-	0.6	2.0		7.0	
4	0.6	600	-	0.4	2.0		7.0	
5	0.8	800	-	0.2	2.0		7.0	
6	1.0	1000	-	0.0	2.0		7.0	
7	-	-	0.4	0.6	2.0		7.0	

CALCULATION:

EXP NO: 11

DATE:

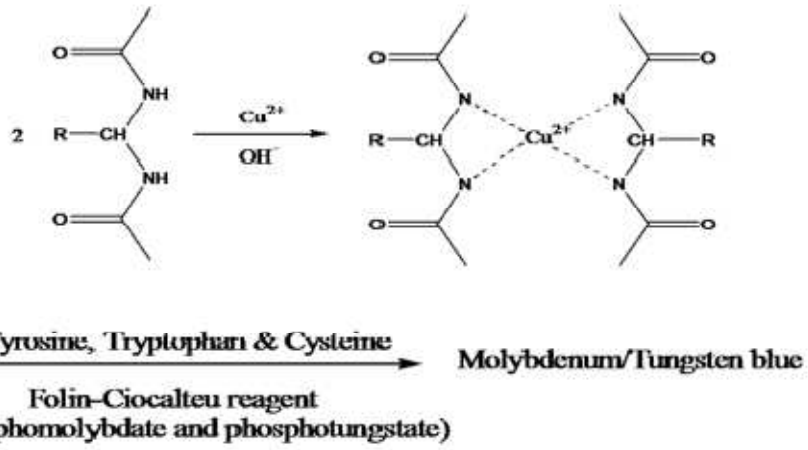
ESTIMATION OF PROTEIN BY LOWRY'S METHOD

AIM

To estimate the amount of protein present in the given sample by Lowry's method

PRINCIPLE

The Lowry protein assay is a biochemical assay for determining the total level of protein in a solution. The method combines the reactions of Copper ions with peptide bond under alkaline conditions, with the oxidation of aromatic protein residues. The Lowry method is best used with protein concentrations of 0.01-1mg/ml, and is based on the reaction of Cu^{2+} produced by the oxidation of peptide bonds, with Folin-Ciocalteu reagent. This reaction involves reduction of Folin's reagent and oxidation of aromatic (mainly Tryptophan and Tyrosine). In addition tyrosine and tryptophan residues of protein possess the reduction of phosphomolybdate and phosphotungstate component of the folin's phenol reagent to form bluish products. Experiments have shown that Cysteine is also reactive towards the reagent. The concentration of the reduced Folin's reagent is measured by absorbance maximally at 750nm. As a result, the total concentration of protein in the sample can be deduced from the concentration of Trp and Tyr residues that reduce the reagent.



REQUIREMENTS

1. Complex-forming Reagent

   Prepare fresh before use by mixing the following solutions in 100:1:1 proportion

   a. Solution A- 2% Na_2CO_3 in 2N NaOH
   b. Solution B- 1% $CuSO_4$
   c. Solution C- 2% sodium potassium tartarate

2. Folin's Reagent

   It is prepared in the ratio 1:2 with distilled water just before use.

3. Standard solution

   Prepare the standard 1mg/mL concentration of BSA.

PROCEDURE

1. Take a series of test tubes and add increasing volume of working standard solution to each tube.
2. Take 1ml of water as blank, and 0.5ml and 1ml of unknown protein solution in tubes T1 and T2, respectively.
3. Make up the volume in all the tubes to 1ml using distilled water.
4. Add 4.5ml of complex-forming reagent to all the tubes, and incubate the tubes at room temperature for 10 minutes.
5. Add 0.5ml of Folin's reagent to each tube, and incubate at room temperature for 30 minutes.
6. After incubation, read the absorbance at 640nm.
7. Plot a graph of OD versus protein concentration, and determine the concentration of the unknown protein sample from the graph.

RESULT

The graph of OD vs. protein concentration is plotted, and the protein concentration of the given sample is found to be----------

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

OBSERVATIONS

S.NO	REAGENTS	B	S_1	S_2	S_3	S_4	S_5	T_1	T_2
1	Volume of working standard (ml)	-	0.2	0.4	0.6	0.8	1.0	-	-
2	Concentration of working standard (µg)	-	200	400	600	800	1000	-	-
3	Volume of unknown solution (ml)	-	-	-	-	-	-	0.2	0.4
4	Volume of distilled water (ml)	1.0	0.8	0.6	0.4	0.2	-	0.8	0.6
5	Volume of alkaline copper reagent (ml)	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
6	Volume of Folin's phenol reagent	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
The contents are mixed well and kept at room temperature for 20 minutes. The blue color developed is read at 640nm									
7	Optical density 640nm								

CALCULATION:

EXP NO: 12

DATE:

SEPARATION OF AMINO ACIDS BY THIN LAYER CHROMATOGRAPHY

AIM:

To separate amino acids present in samples by Thin Layer Chromatography (TLC) method.

PRINCIPLE:

Chromatography is a technique used for the separation of closely related compounds in a mixture. This separation is aided by the difference in equilibrium distribution of components between the two phases- stationary phase and mobile phase. Thin layer chromatography is used to obtain quantitative as well as qualitative data. A TLC plate is made up of a thin layer of silica adhered to glass or aluminium support. The silica is the stationary phase and the solvent mixture is the mobile phase. Separation results from partition equilibrium of the components in the mixture. The separation depends on several factors: solubility of compounds, attraction between the compound and silica and size of compound. An important factor used in thin layer chromatography is the R_f value.

$$R_f = \frac{\text{Distance moved by substance from origin}}{\text{Distance moved by solvent from origin}}$$

Separation of amino acids by TLC: Amino acids interact to different extents with the silica based on their 'R' groups. The amino acids that interact strongly with silica move a small distance while those that do not interact strongly mover farther. Since amino acids are colorless compounds, ninhydrin is used to detect them. Ninhydrin reacts with α-amino acids those results in purple coloured spots (due to formation of complex - Ruhemann's purple). This method is used to separate amino acids chiefly for preparative purposes that are to purify them rather than simply analyze them.

MATERIALS REQUIRED:

1. Standard amino acids
2. Distilled water
3. Pre-coated TLC plate
4. TLC Chamber
5. Butanol
6. Acetic acid
7. Spraying reagent
8. Hot air oven
9. Blade and scissors
10. Pencil and ruler etc.

PREPARATION OF REAGENTS:

1. **Preparation of mobile phase:**
   Butanol, acetic acid and water were mixed in the ratio 4:1:1 respectively, for 5 ml.

2. **Preparation of ninhydrin solution:**
   0.3% solution of Ninhydrin in butanol containing 3 ml acetic acid

PROCEDURE:

1. The TLC plate was taken and after covering the silica side with paper, an appropriate size was chosen and cut with the help of scissors.
2. The smooth base of the TLC plate was determined and using a pencil, a straight, horizontal line about 0.5 cms from the base was drawn.
3. Spots were the standard and samples had to be loaded were marked at sufficient distance apart and numbered.
4. Using capillary tubes, a spot of the standard and each of the samples were made on the TLC plate. The plate was allowed to dry in air.
5. The TLC chamber was filled with 5 ml of solvent (mobile phase) and the TLC plate was lowered in an upright position into the chamber such that the solvent was only half-way to the horizontal line drawn.
6. The chamber was left undisturbed for one hour and the mobile phase was allowed to move up the silica by capillary action, carrying along with it, the sample molecules.
7. The plate was removed from the TLC chamber and briefly dried in the hot air oven at 60° Celsius after marking the distance to which the solvent had travelled, with a pencil.

8. Ninhydrin solution was sprayed on the TLC plate and it was again placed in the hot air oven for Ruhemann's purple spots to develop.
9. The TLC plate was removed from the hot air over upon development of colored spots/bands and observed.

OBSERVATION:

The TLC plate sprayed with ninhydrin solution was placed inside the hot air oven and allowed to develop. Ruhemann's purple colour bands were observed in the plate and Rf values were calculated.

$$\text{Rf} = \frac{\text{distance moved by solute}}{\text{distance moved by solvent}}$$

RESULT:

Rf Values for Amino acids on TLC

Amino acids	Cold condition		After final heating		Detection limit for ninhydrin (μg) (R_F)
	Observed colors	Detection limit (μg)	Observed colors	Detection limit (μg)	
Glycine	Deep orange	0.5	Deep pink	0.1	0.01 (0.03)
Alanine	Pinkish violet	0.1	Light pink/milky pink	0.1	0.009 (0.22)
Valine	Reddish pink	0.1	Reddish pink	0.05	0.01 (0.14)
Leucine	Bluish violet	0.5	Violetish pink	0.1	0.01 (0.09)
Isoleucine	Very light violet	1.0	Light violet	0.1	0.20 (0.32)
Serine	Deep pink	0.1	Deep bluish pink	0.1	0.008 (0.38)
Threonine	Yellowish orange/ivory	0.5	Yellowish pink/candy	0.1	0.05 (0.28)
Aspartic acid	Yellowish violet	0.2	Greyish violet	0.1	0.10 (0.12)
Aspargine	Light yellow/pale cream	1.0	Greyish yellow	0.1	0.10 (0.45)
Glutamic acid	Light violet	0.5	Light violet	0.1	0.04 (0.33)
Glutamine	Light violet	0.5	Light violet	0.2	0.10 (0.38)
Lysine	Reddish violet	0.2	Brick red	0.1	0.005 (0.42)
Histidine	Yellowish violet	0.1	Yellowish pink/petal	0.1	0.05 (0.18)
Arginine	Light pink/mauve	0.5	Pink	0.1	0.01 (0.05)
Phenyl alanine	Orangish violet	1.0	Greyish pink	0.2	0.05 (0.58)
Tyrosine	Light violet	1.0	Light pink	0.1	0.03 (0.51)
Tryptophan	Greyish violet	0.5	Pinkish violet	0.1	0.05 (0.55)
Cysteine	Yellowish violet	2.0	Pinkish violet	1.0	0.02 (0.41)
Cystine	Very light pink	2.0	Light pink	1.0	0.01 (0.35)
Methionine	Lilac/bluish violet	0.5	Bluish violet	0.2	0.01 (0.48)
Proline	Light yellow/off white	1.0	Grey/beige	0.2	0.10 (0.22)
Hydroxy proline	Pinkish violet	0.2	Yellowish brown	0.1	0.05 (0.34)

Amino acids	Mobile phases (M)											
	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}	M_{11}	M_{12}
Leucine	0.98	0.14	0.01	0.91	0.71	0.92	0.77	0.82	0.82	0.75	0.87	0.87
Isoleucine	0,97	0.21	0.01	0.92	0.72	0.92	0.76	0.81	0.81	0.77	0.85	0.81
Phenyl alanine	0.99	0.26	0.01	0.90	0.74	0.90	0.78	0.81	0.88	0.78	0.88	0.89
Tyrosine	0.97	0.22	0.02	0.92	0.75	0.91	0.80	0.83	0.86	0.77	0.90	0.88
Alanine	0.93	0.14	0.01	0.90	0.54	0.91	0.66	0.67	0.70	0.70	0.77	0.68
Lysine	0.97	0.06	0.01	0.27	0.07	0.31	0.60	0.10	0.08	0.11	0.08	0.07
Proline	0.92	0.10	0.01	0.75	0.47	0.76	0.36	0.45	0.46	0.53	0.58	0.58
Serine	0.94	0.14	0.01	0.88	0.61	0.91	0.59	0.65	0.70	0.71	0.75	0.68
Glutamic acid	0.95	0.17	0.01	0.70	0.68	0.77	0.54	0.47	0.41	0.58	0.64	0.49
Methionine	0.92	0.10	0.01	0.84	0.68	0.89	0.69	0.72	0.75	0.66	0.85	0.81
Arginine	0.92	0.07	0.01	0.24	0.05	0.28	0.09	0.10	0.06	0.05	0.07	0.05
Histidine	0.92	0.11	0.01	0.79	0.40	0.85	0.55	0.64	0.48	0.16	0.66	0.54
Tryptophan	0.99	0.14	0.01	0.83	0.77	0.89	0.63	0.83	0.88	0.78	0.88	0.83

CALCULATIONS:

EXP NO: 13

DATE:

ESTIMATION OF CHOLESTEROL BY ZAK'S METHOD

AIM:

To estimate the amount of cholesterol in the given food/serum sample.

PRINCIPLE:

Cholesterol is a steroid lipid, amphipathic in nature. It consists of basic cyclopentano perhydro phenothrene (CPPP) nucleus. It is synthesized in liver from Acetyl CoA. It acts as a precursor for steroid hormones and vitamin D.

In Zak's method, the proteins present in the sample are first precipitated by adding $FeCl_3$-CH_3COOH reagent. The protein free filtrate is treated with conc. H_2SO_4. In the presence of excess conc. H_2SO_4, cholesterol present in the sample gets dehydrated to form cholesta-3,5-diene, and by the catalytic action of Fe^{3+} ions a red colored complex is formed. The intensity of red color is measured at 560 nm.

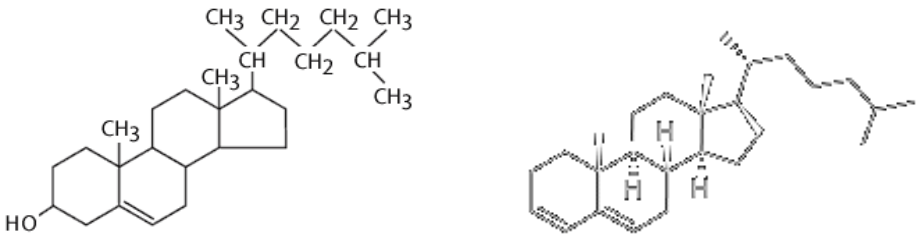


Figure: Structure of Cholesterol

REAGENTS REQUIRED:

1. Stock Standard Solution: About 100 mg of cholesterol is dissolved and made up to 100 ml with glacial acetic acid (concentration 1 mg / ml).

2. Working Standard: About 4 ml of stock solution was made up to 100 ml with ferric chloride acetic acid reagent (concentration in 0.04 mg / ml).

3. Ferric chloride of 0.05% in acetic acid.

4. Concentrated sulphuric acid.

5. Glacial acetic acid.

6. Preparation of unknown food sample: 20 ml of food sample and 40 ml of chloroform was added and centrifuged. The supernatant was used for estimation.

7. Glassware's and etc…

PROCEDURE

1) 0.5 ml to 2.5 ml of working standard was pipetted out into clean test tubes.

2) 0.5 ml and 1 ml of unknown sample supernatant was taken in test tubes.

3) The volume was made up to 5.0 ml with ferric chloride and 3.0 ml of concentrated sulphuric acid were added.

4) The test tubes were kept at room temperature for 15 minutes.

5) The absorbance was read at 540 nm.

6) Standard graph of OD vs. concentration of cholesterol was drawn for the values obtained. From the standard graph the amount of cholesterol present in the food sample was calculated.

RESULT:

The amount of cholesterol present in the given food sample is found to be……

ESTIMATION OF CHOLESTEROL BY ZAK'S METHOD

OBSERVATIONS

S. NO.	REAGENTS	B	S_1	S_2	S_3	S_4	S_5	T_1	T_2
1.	Volume of standard cholesterol (ml)	_	0.5	1.0	1.5	2.0	2.5	_	_
2.	Concentration of cholesterol (µg)	_	20	40	60	80	100	_	_
3.	Volume of food sample supernatant (ml)	_	_	_	_	_	_	1	1
4.	Volume of 0.05% ferric chloride acetic acid reagent (ml)	5	4.5	4	3.5	3	2.5	_	_
5.	Volume of conc. sulphuric acid (ml)	3	3	3	3	3	3	3	3
		Incubate the tubes for 15 minutes at room temperature							
6.	O.D at 540 nm								

CALCULATION: