KHYBER MEDICAL UNIVERSITY

LOGBOOK FOR BIOCHEMISTRY

1st YEAR BDS

CERTIFICATE

Name of Institution:

Full Name of Student:

Roll Number:

Class:

It is certified that _____
S/D of _____has fulfilled the requirement of practical work in Department of Biochemistry.

_____

Signature of Teacher

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Block A Module 1: FOUNDATION I

Block A

Module 1: Foundation I

Carbohydrates

Number of hours:8

Learning Outcomes:

1. Detection of Monosaccharide in a given Solution
2. Detection of unknown sugar in a solution
3. Detecting of Reducing and non-reducing Sugars

FOOD CONSTITUENTS.

The food we eat consists of substances called "NUTRIENTS" which are derived from animal and plant tissues. Nutrients are essential chemical substances obtained from food that sustain human health, growth, and energy, categorized into macronutrients (carbohydrates, proteins, fats) for energy and micronutrients (vitamins, minerals) for metabolic function. Water and fiber are also crucial components of a balanced diet.

Key Nutrient Classifications

Macronutrients: Required in large amounts to provide energy (calories).

Carbohydrates: Primary energy source.

Proteins: Build, repair, and maintain tissues.

Fats: Energy, insulation, and organ protection.

Micronutrients: Required in small amounts to support metabolism, immune function, and structural health.

Vitamins: Organic compounds (e.g., A, C, D, E, K).

Minerals: Inorganic elements (e.g., Calcium, Iron, Potassium).

Water: Essential for transporting nutrients and oxygen to cells.

Functions and Importance

Nutrients regulate gene expression, build cellular structures, provide fuel for daily activities, and help prevent diseases like diabetes and heart disease. A balanced diet, rich in varied foods, ensures the body receives the necessary nutrition. Carbohydrates, proteins and lipids are the major food constituents present in bulk in the food, whereas vitamins and minerals are in smaller amounts. Water is one of the most essential constituents, to think of life without it is impossible.

CARBOHYDRATES

Carbohydrates are defined as polyhydroxy aldehydes or polyhydroxy ketones. Consist of polyhydroxy aldehydes (glucose) or polyhydroxy ketones (fructose). Carbohydrates are widely distributed both in animal and plant tissues. The carbohydrates to be considered here are those which occur in mammalian tissues and are of importance in human nutrition. Vegetables contain a considerable amount of carbohydrates, but human beings lack some of the enzymes in their digestive systems to hydrolyze all of them. However, only a very few of the complex carbohydrates are broken down by the digestive enzymes into utilizable sugars which can be absorbed by the intestinal mucosa.

Carbohydrates are classified as:

a) Monosaccharides

i. Glucose.

ii. Fructose.

iii. Galactose.

b) Disaccharides

i. Lactose.

ii. Maltose.

iii. Sucrose.

c) Oligosaccharides

i. Maltotriose

d) Polysaccharides

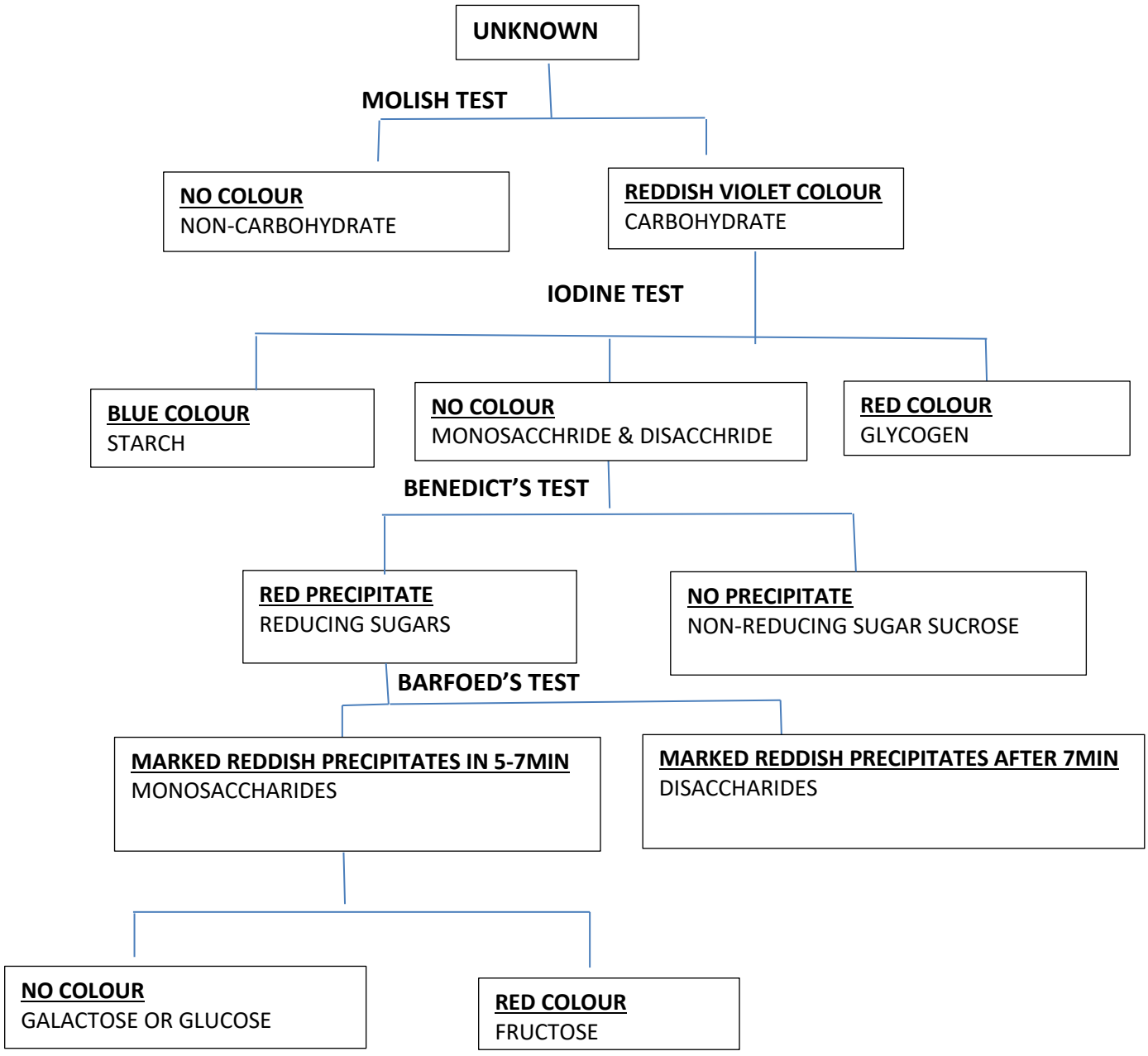
i. Starch.

ii. Glycogen.

iii. Dextrin.

Monosaccharides such as glucose, fructose and galactose and disaccharides like maltose and lactose are classed as "reducing sugars". These sugars can reduce in alkaline or slightly acidic solutions some of the metal ions such as Cu2+, Ag+, Bi^{3}+, and $Fe(CN)^{3}$.

Qualitative Tests of Carbohydrates:



Qualitative tests of Carbohydrates:

GENERAL REACTIONS OF CARBOHYDRATES

MOLISCH'S REACTION:

PRINCIPLE:

Sugars on reaction with dehydrating agents like concentrated strong acids (concentrated H_2SO_4) yield furfural and furfural derivatives, such as hydroxymethyl furfural, which condense with a-naphthol and give a reddish violet ring.

REAGENTS:

1. Molisch's Reagent:

i- alpha-naphthol,

ii-Ethyl alcohol

2. Concentrated H_2SO_4

3. Original solution (O.S.) - containing a carbohydrate.

PROCEDURE:

To 2ml of sugar solution (original solution) add 2 to 3 drops of Molisch's reagent

Mix thoroughly. Carefully pore 5 ml concentrated H_2SO_4 along the side of the test tube. Acid being heavier will form a layer beneath the sugar solution. The formation of a reddish violet ring at the junction of the two liquids indicates the presence of carbohydrates. This test is very sensitive and is given by all the carbohydrates.

REACTIONS GIVEN BY MONOSACCHARIDES AND DISACCHARIDES

1. COPPER REDUCTION TESTS:

Carbohydrates which give reduction tests have free aldehyde or ketonic groups, and are called "reducing sugars".

PRINCIPLE:

Alkaline copper reagents (Benedict's and Fehling's reagents) are reduced by the reducing sugars with the formation of yellow, orange or red precipitate.

The reaction of acid copper reagents (Barfoed's reagent) with reducing sugars slow and can be used to distinguish monosaccharides from disaccharides.

a. FEHLING'S TEST:

1. Fehling's reagent:

i. Solution A - Copper sulphate solution, and

ii. Solution B - Alkaline tartrate solution.

These solutions are preserved in separate bottles. Fehling's reagent is freshly prepared by mixing equal volumes of solution-A with solution-B.

2. Original solution (O.S.)-containing a carbohydrate.

PROCEDURE:

To 1ml of sugar solution (original solution) in a test tube, add 1ml of Fehling's reagent. Mix and boil carefully. The production of yellow or brownish-red precipitate of cuprous oxide indicates the presence of reducing sugars in the sample.

b. BENEDICT'S TEST:

1. Benedict's reagent:

i. Copper sulphate,

ii. Sodium Citrate, and

iii. Sodium Carbonate.

2. Original solution (O.S.)-containing a carbohydrate.

PROCEDURE:

To 5ml of Benedict's reagent in a test tube add 8 drops of sugar solution (original solution). Mix thoroughly and heat to boil for 2 minutes. Allow the tube to cool. The solution, in addition to formation of a precipitate, will change colour from blue to green, yellow, orange or red depending upon the amount of reducing sugar present.

This test can be used as a rough quantitative test for the clinical evaluation as shown in the following table:

OBSERVATIONS	CONCENTRATION OF SUGAR	CLINICAL EVALUATION
No colour change (Blue)	0.0%	Nil
Green coloured solution with no precipitate.	0.1%	Traces
Green coloured solution with yellow precipitate.	0.1-0.5%	+
Olive green coloured solution with yellow precipitate.	0.5-1%	++
Yellow orange coloured precipitate.	1-2%	+++
Brick red coloured precipitate	2% or more.	++++

c. BARFOED'S TEST:

1. Barfoed's Reagent:

i. Copper acetate, and

ii. Acetic acid.

2. Original solution (O.S.) - containing a carbohydrate.

PROCEDURE:

To 5ml of Barfoed's reagent in a test tube add 0.5ml of sugar solution (original solution). Mix thoroughly and place it in the boiling water bath. Note the time when signs of reduction i.e., formation of a red precipitate of cuprous oxide first appears in the test tube. The monosaccharides start forming precipitates in less than 7 minutes where as

The precipitates appearing after 7 minutes indicate the presence of disaccharides in the solution. SPECIAL REACTIONS GIVEN BY THE INDIVIDUAL SUGARS

TEST FOR FRUCTOSE (FREE OR COMBINED):

1. SELIWANOFF'S TEST:

PRINCIPLE:

Fructose on heating with the HCl, rapidly forms furfural, which on reaction with resorcinol gives red coloured compounds.

REAGENT:

1. Seliwanoff's Reagent:

i. Resorcinol, and

ii. Concentrated HCl.

2. Original solution (O.S.) - containing a carbohydrate.

PROCEDURE:

To 3ml of Seliwanoff's reagent in a test tube add 3 drops of a original solution (fructose solution) and heat the mixture to just boiling. A positive reaction is indicated by the production of a red colour. Ketoses free or combined (Fructose or Sucrose), give a red colour. Prolonged boiling with aldoses is bound to give a false positive result, as the aldoses also start giving a similar reaction.

HYDROLYSIS OF SUCROSE

Sucrose does not reduce Benedict's, Barfoed's or Fehling's reagents. Sucrose, upon hydrolysis, takes one molecule of water and breaks down into two molecules of monosaccharides ie, glucose and fructose.

PROCEDURE:

To 3ml of sugar solution (original solution) in a test tube add few drops of concentrated HCL Mix carefully and gently heat to boil. Cool under tap water. Add 1ml 5% NaOH drop by drop to the test tube. Heat to boil again. On completion of hydrolysis the solution will turn yellowish, indicating the presence of a reducing sugar. After acid hydrolysis it gives positive copper reduction tests.

REACTIONS GIVEN BY POLYSACCHARIDES

The polysaccharides are complex carbohydrates. They have high molecular weight. Few of them are appreciably digested in the alimentary canal by human beings. They are not reducing sugars and so cannot reduce Benedict's reagent. When starch and glycogen are boiled with dilute acids, they are hydrolyzed to glucose. The intermediate products formed during the course of hydrolysis, are dextrins and maltose.

1. IODINE TEST:

REAGENTS:

a. 0.01N Iodine solution.

b. Original solution (O.S.)-containing a carbohydrate.

PROCEDURE:

To 3ml of the starch solution (original solution) in a test tube, add 1-2 drops of the dilute iodine solution. Observe the production of a blue colour. The blue colour produced disappears on heating and it reappears on cooling the solution. Starch, glycogen and higher dextrins on reaction with dilute iodine solution form coloured compounds

Starch	Blue colour.
Amylodextrin.	Purple colour.
Erythrodextrin.	Red colour.
Glycogen.	Red colour.
Achrodextrin (lower dextrins), Disaccharides (Maltose) and Monosaccharides.	No change in colour.

HYDROLYSIS OF STARCH

Starch does not reduce Benedict's, Barfoed's or Fehling solution.

PROCEDURE:

To 3-5ml of sugar solution (original solution) in a test tube add 0.5ml of concentrated HCl. Mix carefully and gently heat to boil. Cool under tap water. Add 5% NaOH drop by drop to the test tube. Heat to boil again. On completion of hydrolysis the solution will turn yellowish, indicating the presence of a reducing sugar.

QUESTION/ANSWERS

1. Describe a test for the detection of sugar in urine?
A. Benedict's Test - To 5 ml Benedict's Reagent add 8 drops of urine, mix and heat to boil for 2 minutes. The change in the colour of solution indicates the presence of sugar in the urine.

2. What is the composition of Benedict's Reagent?
A. Benedict's Reagent contains: Sodium citrate, Sodium carbonate, Cupric sulphate and distilled water.

3. What is the use of Benedict's Test?
A. Benedict's Test is used to detect the presence of reducing sugars in any solution.

4. What is positive Benedict's Test?
A. A positive Benedict's Test gives various shades of colour from green, yellow, orange, red and brick-red colour depending upon the increasing concentration of reducing sugars in the solution.

5. What is the clinical significance of a positive Benedict's Test?
A. A positive Benedict's Test indicates the presence of reducing sugars particularly glucose in the urine. Being a qualitative test it indicates a rough estimate of glucose in the urine sample. This can be helpful in diagnosing and monitoring the level of glucose in patients with diabetes mellitus.

6. What is the pH required for Benedict's and Barfoed's Test?
A. pH for Benedict's Test is alkaline and for Barfoed's Test it is acidic.

7. What is reducing sugar?
A. All the sugars which have free aldehyde or ketone group are known as reducing sugars. They reduce the cupric (Cu**) to cuprous (Cu) ions.

8. Name the reducing sugars?
A. All the monosaccharides and disaccharides (i.e., glucose, fructose, galactose, lactose and maltose) except sucrose are the reducing sugars.

9. What is a non-reducing sugar?
A. The sugars which do not have any free aldehyde or ketone group are called non-reducing sugars e.g., sucrose and polysaccharides.

10. Why sucrose being disaccharide is a non-reducing sugar?
A. Sucrose being disaccharide consisting of glucose and fructose, both are reducing sugars but the aldehyde group of glucose and ketone group of fructose is linked together in glucosidic linkage, thus there is no free reducing group available.

11. What is the principle of Molisch's Test?
A. Carbohydrates are converted into furfural by concentrated H_2SO_4, which acts as a dehydrating agent. Furfurals form a coloured complex with a- naphthol (Molisch's Reagent) producing a violet ring at the junction of two liquids.

12. What is the use (or significance of Molisch's Test?
A. It is used for detection of carbohydrates in any solution.

13. How much is ++ sugar in the urine?
A. 0.5-1%

14. What is the significance of Barfoed's Test?
A. Copper in acid medium in Barfoed's reagent is reduced slowly and can be used to distinguish between monosaccharides and disaccharides e.g., A positive Barfoed's Test within or less than 7 minutes indicates the presence of monosaccharides, whereas, a positive Barfoed's Test after or more than 7 minutes indicates the presence of disaccharides in any solution i.e., it is faster in monosaccharides than in disaccharides.

15. What is the composition of Seliwanoff's Reagent?
A. It contains: i- Resorcinol, and ii- HCI.

16. What is the significance of Seliwanoff's Reagent?
A. Positive Seliwanoff's Test indicates the presence of keto-sugars; thus, it helps in differentiating between aldo- and keto- sugars.

17. What is the principle of Seliwanoff's Test?
A. The HCI in Seliwanoff's Reagent acts as a dehydrating agent, converting keto- sugars into furfurals, which form a cherry-red coloured complex with Resorcinol.

18. What is the use of lodine Test?
A. It indicates the presence of polysaccharides in any solution.

EXPERIMENT No.,
Date

DETECTION OF GLUCOSE IN A GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube, Burner, Glass slide, cover slip Microscope, Rack, Pipettes, Beaker and Water bath

Original solution (Glucose solution).

REAGENTS: Molisch's Reagent, lodine Reagent, Benedict's Reagent, Seliwanoff's Reagent, Conc. HCI, Conc. HzSO4, 5%1 NaOH & Barfoed's Reagent

No.	Tests	OBSERVATION	INFERENCE
1	MOLISCH'S TEST		
2	IODINE TEST		
3	BENEDICT'S TEST		
4	BARFOED'S TEST		
5	SELIWANOFF'S TEST		

RESULT: _____

EXPERIMENT No.,
Date

DETECTION OF FRUCTOSE IN A GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube, Burner, Glass slide, cover slip Microscope, Rack, Pipettes, Beaker and Water bath

Original solution (Glucose solution).

REAGENTS: Molisch's Reagent, lodine Reagent, Benedict's Reagent, Seliwanoff's Reagent, Conc. HCI, Conc. HzSO4, 5%1 NaOH & Barfoed's Reagent

No.	Tests	OBSERVATION	INFERENCE
1	MOLISCH'S TEST		
2	IODINE TEST		
3	BENEDICT'S TEST		
4	BARFOED'S TEST		
5	SELIWANOFF'S TEST		

RESULT: _____

EXPERIMENT No.,
Date

DETECTION OF GALACTOSE IN A GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube, Burner, Glass slide, cover slip Microscope, Rack, Pipettes, Beaker and Water bath

Original solution (Glucose solution).

REAGENTS: Molisch's Reagent, lodine Reagent, Benedict's Reagent, Seliwanoff's Reagent, Conc. HCI, Conc. HzSO4, 5%1 NaOH & Barfoed's Reagent

No.	Tests	OBSERVATION	INFERENCE
1	MOLISCH'S TEST		
2	IODINE TEST		
3	BENEDICT'S TEST		
4	BARFOED'S TEST		
5	SELIWANOFF'S TEST		

RESULT: _____

EXPERIMENT No.,
Date

DETECTION OF LACTOSE IN A GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube, Burner, Glass slide, cover slip Microscope, Rack, Pipettes, Beaker and Water bath

Original solution (Glucose solution).

REAGENTS: Molisch's Reagent, lodine Reagent, Benedict's Reagent, Seliwanoff's Reagent, Conc. HCI, Conc. HzSO4, 5%1 NaOH & Barfoed's Reagent

No.	Tests	OBSERVATION	INFERENCE
1	MOLISCH'S TEST		
2	IODINE TEST		
3	BENEDICT'S TEST		
4	BARFOED'S TEST		
5	SELIWANOFF'S TEST		

RESULT: _____

EXPERIMENT No.,
Date

DETECTION OF MALTOSE IN A GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube, Burner, Glass slide, cover slip Microscope, Rack, Pipettes, Beaker and Water bath

Original solution (Glucose solution).

REAGENTS: Molisch's Reagent, lodine Reagent, Benedict's Reagent, Seliwanoff's Reagent, Conc. HCI, Conc. HzSO4, 5%1 NaOH & Barfoed's Reagent

No.	Tests	OBSERVATION	INFERENCE
1	MOLISCH'S TEST		
2	IODINE TEST		
3	BENEDICT'S TEST		
4	BARFOED'S TEST		
5	SELIWANOFF'S TEST		

RESULT: _____

EXPERIMENT No.,
Date

DETECTION OF SUCROSE IN A GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube, Burner, Glass slide, cover slip Microscope, Rack, Pipettes, Beaker and Water bath

Original solution (Glucose solution).

REAGENTS: Molisch's Reagent, lodine Reagent, Benedict's Reagent, Seliwanoff's Reagent, Conc. HCI, Conc. HzSO4, 5%1 NaOH & Barfoed's Reagent

No.	Tests	OBSERVATION	INFERENCE
1	MOLISCH'S TEST		
2	IODINE TEST		
3	BENEDICT'S TEST		
4	BARFOED'S TEST		
5	SELIWANOFF'S TEST		

Perform tests after hydrolysis

a.	BENEDICT'S TEST		
b.	BARFOED'S TEST		

RESULT: _____

EXPERIMENT No.

Date

DETECTION OF UNKNOWN SOLUTION IN A GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube, Burner, Glass slide, cover slip Microscope, Rack, Pipettes, Beaker and Water bath

Original solution (Glucose solution).

REAGENTS: Molisch's Reagent, lodine Reagent, Benedict's Reagent, Seliwanoff's Reagent, Conc. HCI, Conc. HzSO4, 5%1 NaOH & Barfoed's Reagent

No.	Tests	OBSERVATION	INFERENCE
1	MOLISCH'S TEST		
2	IODINE TEST		
3	BENEDICT'S TEST		
4	BARFOED'S TEST		
5	SELIWANOFF'S TEST		

RESULT: _____

Solutions

Number of hours:2

Learning Outcomes:

1. Prepare of 0.9% NaCl.
2. Measure the PH of given solution. SOLUTIONS

Solution is a homogeneous mixture of a solid, liquid, or gaseous substance (the solute) in a liquid (the solvent) from which the dissolved substances can be recovered by crystallization or other physical processes.

TYPES OF SOLUTIONS:
There are different terms used for expressing the strength of biomedical solution.

I. PERCENT SOLUTION.
a. Weight by volume percent (w/v%)
Weight of the solute dissolved per 100 ml of the solvent.
b. Weight by weight percent (w/w%)
Weight of the solute dissolved per 100 grams of the solvent.
c. Volume by volume percent (v/v%)
Volume of solute dissolved per 100 ml (volume) of the solvent.

II. MOLAR SOLUTION (M).
A solution which contains molecular weight of a substance in grams per litre of the solution.
IM = Gram molecular weight/litre.
IM of HCl = 1+35.5 =36.5
1mM of HCl = 36.5 × 10-3
1µM of HCl 36.5 × 10-6

WATER OF CRYSTALIZATION:
Substances like CuSO4 exist in the form of crystals. Those molecules which hold molecules of water as integral part of their crystal lattice to form their molecular solution.
$CuSO_4.5H_2O$
IM 64+32+4(16)+10+5(16)
=64+32+64+10+80
= 160+10+80
=250
1M solution 250g $CuSO_4$/L

III. NORMAL SOLUTION:
Normal solution contains gram equivalent weight of the solute per litre of the solvent.
Eq. Wt. = Gm. Mol. Wt./Valency
Eq. Wt. of H_2SO_4 = 2(1) +32 + 16(4)
=2+32+64
= 98÷2
= 49

Valency = Number of replaceable hydrogen ions.

IV. MOLAL SOLUTION:

Number of moles of a substance dissolved in 1000 grams of water (w/w). Final volume is more than one litre.

V.SATURATED SOLUTION:

Saturated solution is that solution which normally contains the maximum amount of substance able to be dissolved at a given temperature. The solution can stay in equilibrium with an excess of the solute.

VI. SUPERSATURATED SOLUTION:

Supersaturated solution is one that contains a greater quantity of solid than can normally be dissolved at a given temperature. It is an unstable system. The excess of the solute above the saturation quantity crystallizes out when a crystal of the solute is added to it.

VII. HYPERTONIC SOLUTION:

Hypertonic solution is one that has an osmotic pressure greater than that of blood or plasma.

VIII. HYPOTONIC SOLUTION:

Hypotonic solution has osmotic pressure less than that of blood or plasma.

IX. ISO-OSMOTIC OR ISOTONIC SOLUTION:

Iso-osmotic or isotonic solution is that solution which has same osmotic pressure as that of blood serum or a reference solution.

X. COLLOIDAL SOLUTIONS:

Colloidal solutions are a macroscopically homogeneous system consisting of either single, large molecules or aggregations of smaller molecules suspended in a liquid. Colloidal particles are large and they cannot pass through the pores of ordinary parchment or collodion membranes. However, they are not large enough to settle out by gravity. There are two phases in a colloidal system - the finely divided particles called the dispersed phase, and the medium in which they are, usually a fluid, is the dispersion phase.

XI. BUFFER SOLUTION:

Buffer solution is a solution which tends to resist a change in pH on the addition of small amounts of acid or alkali.

QUESTION/ANSWERS

1. What is Normal solution?
A. A solution that contains gram equivalent weight of the solute per litre of the solvent.

2. What is an Acid?
A. Acid is proton donor.

3. What is a base?
A. Base is a proton acceptor.

4. What is a weak acid?
A. An acid which dissociates only partially e.g., H2CO3, CH3COOH.

5. What is a strong acid?
A. An acid which can completely dissociate e.g., HCl, H2SO4.

6. What is an indicator?
A. Indicators are weak organic acids which dissociate in a solution to give ions of different colour from the undissociated molecule e.g., phenolphthalein is colourless in acid and pink in alkaline medium.

7. What is pH?
A. pH is the negative log of hydrogen ion concentration (- log [H*]).

8. What is Hendersen-Hasselbalch Equation?
A. PH =pKa + log [Salt]/[Acid]

9. What is a Buffer solution?
A. Buffers are solutions which tend to resist changes in their pH on the addition of moderate amounts of acid or alkali.

10. What is Buffer capacity?
A. Is the ability of a buffer solution to resist pH changes.

11. What is the pH of blood?
A. pH of blood is 7.35 - 7.45

12. What is the pH of urine?
A. pH 4-6

13. What is Normal Saline?
A. Normal Saline is also called as Physiological or Isotonic Saline. Normal Saline solution consists of Sodium Chloride (NaCl) and water. It is 0.9 percent Sodium Chloride solution.

14. What are the uses of Normal Saline Solution?
A. Normal Saline has many uses. It is used to:
1.Clean wounds and abrasions.
2. Clean eyes.
3. Clear Sinuses.
4. Treat dehydration.
5. Given intravenous in hypovolemic conditions.

Experiment No.
Date:

PREPARATION OF 0.9% SODIUM CHLORIDE (NORMAL SALINE) SOLUTION.

METHOD:
Dilution method.

PRINCIPLE:
A measured known amount of solute that is sodium chloride is dissolved in distilled water. The solution of sodium chloride is then diluted to get the desired percentage solution.

APPARATUS:
Weighing machine, beaker, volumetric flask, funnel, stirrer.

REAGENTS:
1. Sodium Chloride (dry) [NaCl],
2. Distilled Water.

PROCEDURE:
Take 0.9 gram of Sodium Chloride in a beaker and add 50ml of distilled water in it. Stir the contents of the beaker until the solute is dissolved in the distilled water. Pour the contents of the beaker into the 100ml volumetric flask with help of a funnel. Now dilute the solution with distilled water up to the 100 ml mark. Mix well.

CALCULATIONS:
W1/V1=W2/V2
W_1 = Weight of Solute used.
V_1 =Volume of Solvent used.
W2 Required Weight of Solute
V2 Required volume of Solvent
By applying the equation $W_1 / V_1 = W2/V2$ find out the value for W2.
Wix V2=W2x V1
W2=W1x V2/V1
W_2 = X gm

TO PREPARE 1000 mL OF 0.9% SODIUM CHOLDRIDE SOLUTION

DETERMINATION OF PH OF A SOLUTION

pH, is the measurement of acidity or basicity of the solutions. In chemistry it is considered as the concentration of Hydrogen ions in a solution. The original concept of pH was proposed by a Danish Biochemist Søren Peder Lauritz Sørensen in 1909, as negative logarithm of hydrogen ion concentration in an aqueous solution.

pH=-log[H]

Arrhenius gave the definition of an acid as a substance that dissociates or ionizes to form hydrogen (H*) ions when dissolved in water, and a base is a substance that dissociates into hydroxyl (OH) ions when dissolved in water.

pH measurement ranges between 0 and 14. pH values below 7 are acidic whereas pH values above 7 are considered alkaline. pH value of 7 is taken as neutral.

METHODS FOR MASUREMENT OF PH

The methods for measuring pH in a given solution are:

1. Colorimetric/Indicator method,
2. Metal Electrode method,
3. Glass Electrode method, and
4. ISFET Electrode / Semiconductor method.

Colorimetric / Indicator method: Litmus paper is used to check the pH of the solution. It tells whether the solution is acidic if it turns red or basic if it turns blue. The litmus paper turns purple if the pH is close to neutral that is 7. The change in colour to red is when the pH is below 4.5 and it changes to blue the pH will be above 8.3. Exact pH of the substance cannot be determined by the use of a litmus paper.

Indicators are chemicals when added to acidic or alkaline solutions change colour. The indicators commonly used in laboratories are phenolphthalein, and methyl orange.

Phenolphthalein when added to basic or alkaline solution turns pink in colour whereas methyl orange when added to acidic solution changes to red colour.

Metal Electrode method: The method includes the hydrogen-electrode method, quinhydron-electrode method and antimony-electrode method. All the methods have their limitations and are not in frequent use nowadays.

Glass Electrode method: The method uses two electrodes, one being the glass electrode and the other is the reference electrode. It measures the voltage or potential difference generated between the two electrodes dipped in the solution. This is one of the most common methods used to determine pH of a solution.

ISFET Electrode / Semiconductor method: Ion-selective field effect transistor (ISFET) is a semiconductor chip which is resistant to damage in solution. This sensor replaces the glass electrode. The miniature size of the semiconductor chip is helpful in case of determination of pH in small amount of sample.

PH METER

A pH meter is a device used to measure the acidity or alkalinity of a solution. It determines the pH of a solution more accurately than the pH strips.

Principle of pH Meter:

The principle of pH meter is basically potentiometery. The variation in the electrical potential between the Measuring and Reference electrodes dipped in the given solution leads to flow of electrons resulting in generation of current which is measured with the help of a voltmeter.

PARTS OF PH METER

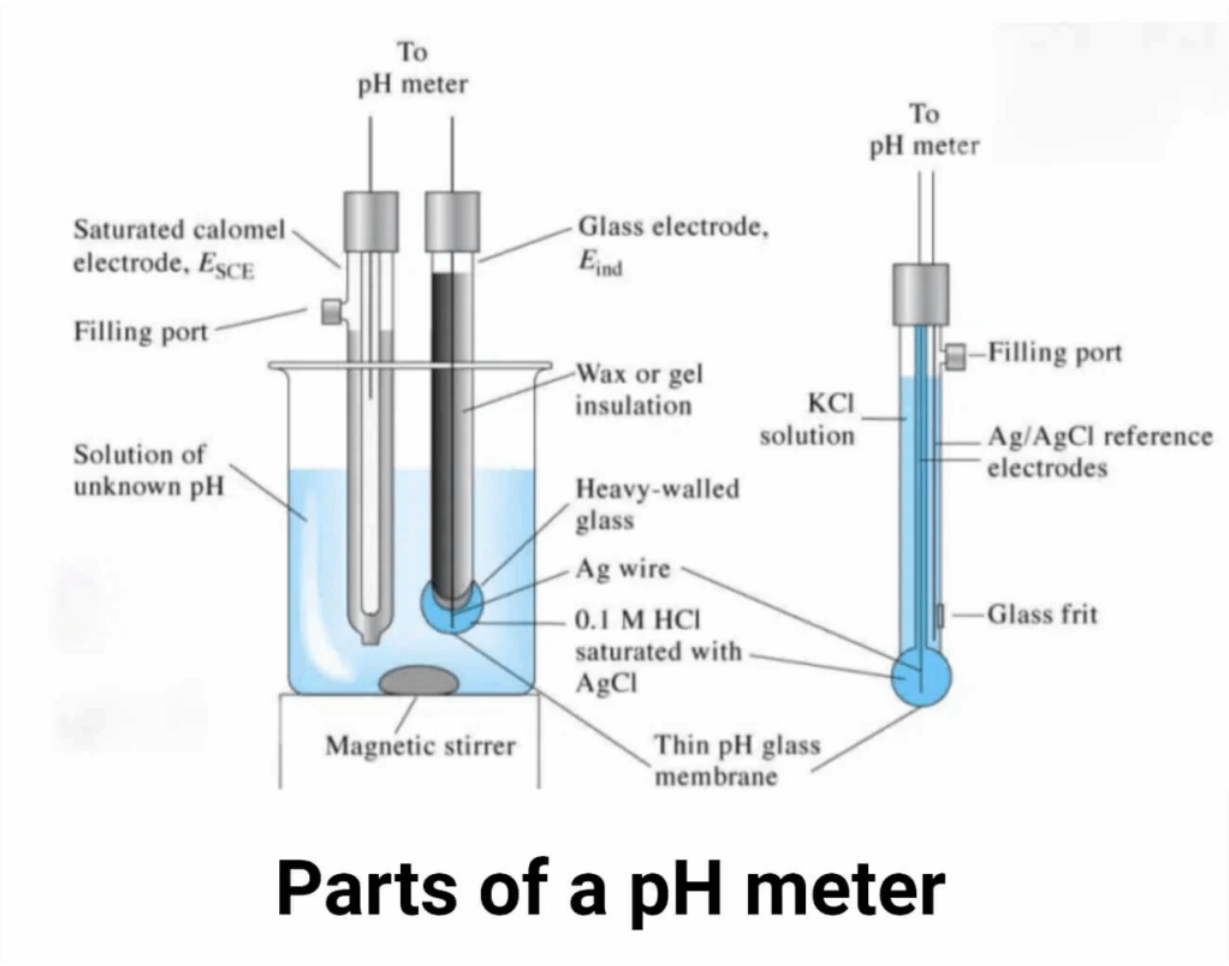
The pH meter consists of important components like Measuring Electrode, Reference Electrode and Voltmeter.

a. Measuring Electrode: The Measuring Electrode is a Glass Electrode also called Indicator Electrode or pH-Responsive Electrode. The Glass Electrode comprises of glass membrane that is very sensitive to concentration of hydrogen ions in a solution to be tested. The electric potential of a Glass Electrode is variable depending on the sample solution.

b. Reference Electrode: The Reference Electrode is considered as a standard. The electrical potential does not vary from sample to sample and remains constant.

The Reference Electrode is usually a silver chloride (AgCl) electrode and can also be of mercurous chloride (Hg_2Cl_2) electrode also called Calomel Electrode.

The pH meters usually have a Combination Electrode, which comprises both the Measuring and the Reference Electrodes in one unit.



Parts of a pH meter

Experiment No.:
Date:

TO DETERMINE THE PH OF A GIVEN SOLUTION

METHOD:

PROCEDURE:

RESULT:

Block B: CRANIOFACIAL

No Practical in block B

Block C: GIT & UGS

Block C

Number of hours:6

Learning Outcomes:

Perform the procedure of protein analysis.

PROTEINS

Proteins are large, nitrogenous compounds. They are polymers of amino acids, connected chains folded into a specific three-dimensional structure. Protein is found in every animal and with each other by peptide linkages. A protein molecule consists of one or more polypeptide Proteins are stored in seeds of many plants, and are required for the growth and development of vegetable cell. The enzymes and many of the hormones present in the body are protein in nature.

The major protein of milk is casein and that of egg white is ovalbumin.

Egg, milk and milk products, lean meat, fish, nuts, legumes, and some cereals are good sources of protein in our diet.

All proteins in all species, whatsoever their function or biological activity may be, are made up of 20 amino acids.

AMINO ACIDS FOUND IN PROTEINS

I. Having Aliphatic side chains:

a. Glycine,

b. Alanine,

c. Valine,

d. Leucine, and

e. Isoleucine.

II. Having side chains containing hydroxyl groups:

a. Serine,

b. Threonine, and

c. Tyrosine.

III. Having side chains containing sulphur atom:

a. Cysteine, and

b. Methionine.

IV. Having side chains containing acidic groups:

a. Aspartic acid,

b. Asparagine,

c. Glutamic acid, and

d. Glutamine.

V. Having side chains containing basic groups:

a. Arginine,

b. Lysine, and

c. Histidine.

VI. Having Aromatic rings:

a. Histidine,

b. Phenylalanine,

c. Tyrosine, and

d. Tryptophan.

VII. Imino acids:

a. Proline.

REACTIONS FOR PROTEINS

1. GENERAL REACTIONS:

i. BIURET REACTION:

This reaction is given by all substances containing two or more peptide amino acids do not give this reaction. The name "Biuret" was given to a compound which was linkages i.e. proteins and their hydrolytic products (proteoses and peptones). Dipeptides and produced after urea were heated at 180°C. This compound on reaction with dilute solution of copper sulphate gave a violet colour. Both biuret and peptides contain -CONH- (peptide linkages) and give positive biuret reaction, though biuret is not a protein in nature.

PRINCIPLE:

The peptide nitrogen atoms form a coordination complex with the cupric
ions and a violet colour is produced.

PROCEDURE:

To 2ml of original solution (protein solution) in a test tube add 2 drops of 2% copper sulphate solution and 1ml of 5% sodium hydroxide solution. Mix thoroughly. A violet colour is produced with proteins, a bluish violet colour with gelatin, whereas peptones will give a pink colour.

ii. HEAT COAGULATION OF PROTEINS

Albumins, globulins and other proteins on heating undergo coagulation whereas gelatin, peptones and peptides do not coagulate on heating.

PROCEDURE:

Fill 2/3rd of the test tube with original solution (protein solution). Heat to boil. A white coagulum is formed. Add few drops of 2% acetic acid to the boiled solution and note that the coagulum does not re-dissolve, but it persists.

2.SEPARATION OF THE ALBUMIN AND GLOBULINS

i. AMMONIUM SULPHATE SATURATION TEST:

Half-saturation (50% saturation) with ammonium sulphate will result in precipitation of globulins, whereas albumins are precipitated after full-saturation (100% saturation) of the test sample with ammonium sulphate.

a. 50% Ammonium Sulphate Saturation Test:

To 2ml of original solution (protein solution) add 2ml of saturated ammonium sulphate solution. Mix thoroughly. The solution is now half-saturated. A bulky precipitate of globulin is formed. Allow the precipitate to settle down and then filter through a filter into a clean and dry beaker. Filtrate if cloudy, should be re-filtered through the same filter paper, to get a clear filtrate. No precipitation will indicate the presence of peptones in the test sample paper

b. 100% Ammonium Sulphate Saturation Test:

To 2ml filtrate from 50% ammonium sulphate saturation test, add ammonium sulphate crystals. Shake vigorously in order to dissolve crystals. Continue adding ammonium sulphate crystals to the filtrate until some crystals remain un-dissolved at the base of Filter out the precipitated albumin. Absence of precipitation indicates the presence of peptones the test tube. The solution is now fully saturated and white precipitate of albumin is formed.

3. SEPARATION OF GELATIN

MAGNESIUM SULPHATE SATURATION TEST:

To the precipitate from the 50% ammonium sulphate saturation test, add 2ml of distilled water and heat, the precipitate dissolves. Cool under tap water. Now saturate the solution in the test tube with magnesium sulphate crystals. A white precipitate is formed indicating the presence of gelatin.

B. TESTS FOR AMINO ACIDS

1.GENERAL REACTIONS OF AMINO ACIDS (Free or Combined):

NINHYDRIN REACTION (Triketo Hydrindene Hydrate):

PRINCIPLE:

Ninhydrin reaction is given by both free and combined a-amino acids. Amino acids, on heating with ninhydrin, are oxidatively decarboxylated, producing carbon dioxide, ammonia (NH3), and an aldehyde. Reduced ninhydrin then reacts with the liberated ammonia and a blue or purple coloured complex is produced.

PROCEDURE:

To 1ml of original solution (protein solution) in a test tube add 2 - 3 drops of freshly prepared 0.5% ninhydrin solution and heat to boil. A blue or purple colour is produced if proteins, peptides or amino acids are present.

2.XANTHOPROTEIC REACTION (Test for Benzoid Radicals):

PRINCIPLE:

Nitration of benzoid radicals present in the amino acid side chain occurs due to reaction with nitric acid, giving the solution a yellow colouration.

PROCEDURE:

To about 1ml of original solution (protein solution) in a test tube add 5 drops of concentrated nitric acid. A white precipitate is formed due to denaturation of proteins by nitric acid. Heat to boil for half minute. The colour of the precipitate turns yellow and then partially gets dissolved to give a yellow-coloured solution. Cool under the tap water and add about 10 drops of strong aqueous ammonia, or sodium hydroxide. The yellow colour is intensified and changes to orange. Yellow colour solution indicates the presence of aromatic amino acids (Tyrosine, Tryptophan and Phenylalanine) in the protein. Phenylalanine like other amino acids contain benzene ring, but nitration of its benzene ring with nitric acid occurs with a great difficulty, and the process of nitration cannot be performed ordinarily by this method.

3. MILLON'S REACTION (Test for Hydroxybenzene Radicals):

Amino acid Tyrosine (Hydroxyphenylalanine) and other phenolic compounds give this reaction.

MILLON'S REAGENT:

Mixture of mercurous and mercuric nitrates.

PRINCIPLE:

The mercurous and mercuric nitrate reacts with the hydroxybenzene radicals (Phenols) forming a red coloured compound.

PROCEDURE:

To 2ml of original solution (protein solution) in a test tube add a few drops of the Millon's reagent. Boil gently for half a minute. The solution will turn red or a red precipitate will be formed. The proteins, on the addition of Millon's reagent, form a white precipitate first due to denaturation of proteins by mercury salts, which upon heating turn red. Tyrosine is the only amino acid which gives this reaction.

4. CYSTEINE TEST (Test for Sulphur):

PRINCIPLE:

Protein or amino acids when heated with sodium hydroxide, sulphur splits out as sodium sulphide, which on reaction with lead acetate, forms greyish brown to black precipitate of lead sulphide (PbS).

PROCEDURE:

To 1ml of original solution (protein solution) in a test tube add 1ml of 20% sodium hydroxide and 0.5ml of 2% lead acetate. A white precipitate is obtained due to denaturation of proteins. Now boil the mixture. The white precipitate turns grayish brown or black indicating the presence of sulphur. A positive reaction is due to the presence of a sulphur containing amino acid in the protein. This can be cysteine or methionine.

5.HOPKINS-COLE REACTION (Test for Tryptophan):

HOPKINS-COLE REAGENT:

Glyoxalic acid.

PROCEDURE:

To 1ml of original solution (protein solution) in a test tube add 1ml of Hopkins- Cole reagent. Mix thoroughly and add 1ml of concentrated sulphuric acid, pouring it down along the side of the test tube. A deep violet or purple ring forms at the junction of the two liquids. This indicates the presence of tryptophan. Gelatin and other proteins which do not contain tryptophan do not give this reaction.

6. ARGININE TEST (Test for Guanidine Group):

This test is specific for Arginine and indicates the presence of guanidine group in the arginine molecule.

PROCEDURE:

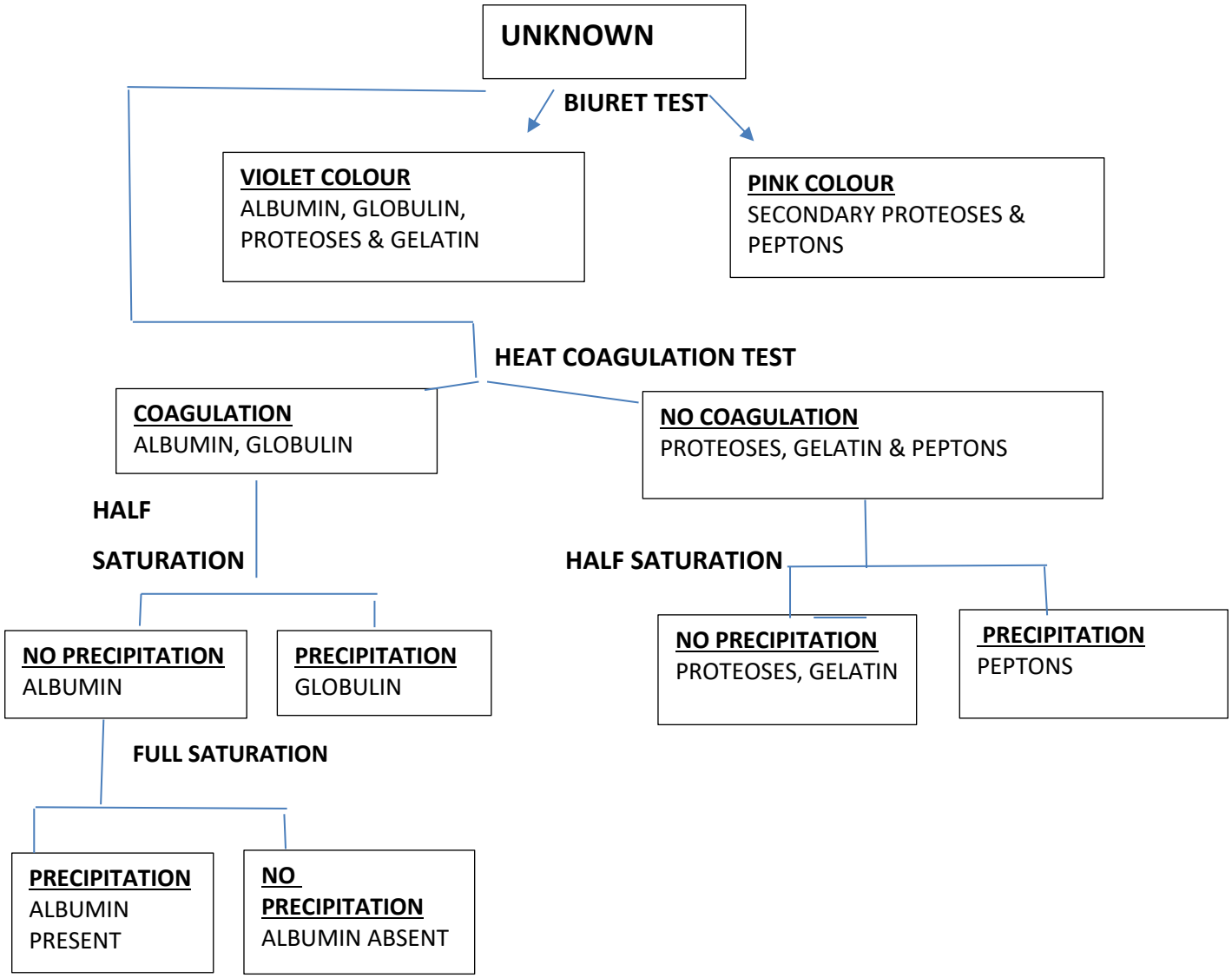
To 1ml of original solution (protein solution) in a test tube add 1ml of 5% sodium hydroxide and add 2 drops of 1% alcoholic a-naphthol mix and add 3 drops of sodium hypobromite (NaOBr) reagent. Mix it thoroughly. A bright red colour is formed. This confirms the presence of arginine.

7. TEST FOR PHOSPHATE

PROCEDURE:

To 1ml of original solution (protein solution) in a test tube add 1ml of ammonium molybdate reagent. Heat to boil. A yellow precipitate is formed. This confirms the presence of phosphate. Casein and other phosphoproteins give this test positive.

Qualitative Tests for Identification of Proteins



QUESTION/ANSWERS

1. What are Aromatic amino acids?

A Those amino acids which contain benzene ring are called aromatic amino acids.

2. Name the amino acids containing benzene ring?

A. These are phenylalanine, tyrosine and tryptophan.

3. Which of the amino acids can give positive Xanthoproteic Test?

A. All aromatic amino acids can give positive Xanthoproteic test.

4. Which of the amino acids can give positive Millon's Test?

A. Tyrosine, because it contains phenolic or hydroxyl-benzene group.

5. Which of the amino acids can give positive Hopkin-Cole Test?

A. Tryptophan, because it contains an indole ring.

6. Name the sulphur containing amino acids?

A. Methionine, cysteine, and cystine.

7. Name the simplest amino acid?

A. Glycine.

8. Which of the sulphur containing amino acid is an essential amino acid?

A. Methionine.

9. What is the significance of Ninhydrin Test?

A. A positive Ninhydrin Test indicates the presence of an amino acid (both in free or combined form) in any solution.

10. What is a Protein?

A. Proteins are nitrogenous compounds containing large number of amino acids linked through peptide linkages.

11. What is peptide linkage?

A. It is the linkage established between an alpha amino (a -NH2) group of one amino acid with alpha carboxyl (a-COOH) group of another amino acid, with the removal of 1 molecule of water.

12. What is a peptide?

A. A peptide consists of 2 or more amino acid residues joined by peptide linkage e.g., Glutathione.

13. What is a dipeptide?

A. A dipeptide consists of two amino acids joined by one peptide linkage.

14. What is tripeptide?

A. A tripeptide consists of three amino acids joined by two peptide linkages e.g., Glutathione.

15. What is a polypeptide?

A. Peptides containing more than 10 amino acids residues joined by peptide linkages.

16. What is the effect of cationic and anionic precipitants on proteins?

A. The proteins behave as cations in an acid medium and are precipitated with large acid radicals. In alkaline medium the proteins behave as anions and are precipitated with heavy metal ions.

17. What is Biuret?

A. Two molecules of urea on heating at 180° C, produce a compound called "Biuret".

18. What is the importance of Bluret Test?

A. Biuret test is performed to detect the presence of peptide linkages in a solution.

19. What is the minimum number of peptide linkages required for a biuret test to be positive (positive biuret test)?

A. A minimum of two peptide linkages must be present to give a positive biuret test.

20. What is an iso-electric point (iso-electric pH) of a protein?

A. It is the pH of electrical neutrality, i.e., the pH at which the protein will not migrate to either anode or cathode in an electrical field. At this pH the net charge on the molecule is zero.

21. What is denaturation of protein?

A. Denaturation can be defined as loss of biologic activity of a protein. Denaturation does not involve covalent bond cleavage, but results from a re-arrangement of secondary, tertiary and quaternary structure of protein molecule. Proteins become more digestible when get denatured.

22. What is the clinical importance of Heat Coagulation test?

A. This test can be used to detect proteins in the urine.

EXPERIMENT No.
Date.

DETECTION OF ALBUMIN IN THE GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, and Burner.

REAGENTS: Albumin solution, 2% CuSO, 5% NaOH, 2% acetic acid, 0.5% Ninhydrin solution, co HNO, strong aqueous ammonia, Millon's Reagent, 20% NaOH, 2% lead acetate, Hopkin Cole Reagent, conc. H.SO, 1% alcoholic a-naphthol, and NaOBr.

NO.	TEST	OBSERVATION	INFERENCE
1.	BIURET TEST		
2.	HEAT COAGULATION TEST		
3	AMMONIUM SULPHATE SATURATION TEST:		
a.	50% SATURATION TESTS.		
b.	100% SATURATION TESTS.		
4.	TESTS FOR INDIVIDUAL AMINO ACIDS:		
A	NINHYDRIN TEST.		
B	XANTHOPROTEIC TEST.		
C	MILLON'S TEST		
D	CYSTEINE TEST		
E	HOPKIN-COLE'S TEST.		
F	ARGININE TEST		
5.	TEST FOR PHOSPHATE.		

RESULT _____

EXPERIMENT No.
Date.

DETECTION OF GLOBULIN IN THE GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, and Burner.

REAGENTS: Albumin solution, 2% CuSO, 5% NaOH, 2% acetic acid, 0.5% Ninhydrin solution, co HNO, strong aqueous ammonia, Millon's Reagent, 20% NaOH, 2% lead acetate, Hopkin Cole Reagent, conc. H.SO, 1% alcoholic a-naphthol, and NaOBr.

NO.	TEST	OBSERVATION	INFERENCE
1.	BIURET TEST		
2.	HEAT COAGULATION TEST		
3	AMMONIUM SULPHATE SATURATION TEST:		
a.	50% SATURATION TESTS.		
b.	100% SATURATION TESTS.		
4.	TESTS FOR INDIVIDUAL AMINO ACIDS:		
A	NINHYDRIN TEST.		
B	XANTHOPROTEIC TEST.		
C	MILLON'S TEST		
D	CYSTEINE TEST		
E	HOPKIN-COLE'S TEST.		
F	ARGININE TEST		
5.	TEST FOR PHOSPHATE.		

RESULT _____

EXPERIMENT No.
Date.

DETECTION OF GELATIN IN THE GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, and Burner.

REAGENTS: Albumin solution, 2% CuSO, 5% NaOH, 2% acetic acid, 0.5% Ninhydrin solution, co HNO, strong aqueous ammonia, Millon's Reagent, 20% NaOH, 2% lead acetate, Hopkin Cole Reagent, conc. H.SO, 1% alcoholic a-naphthol, and NaOBr.

NO.	TEST	OBSERVATION	INFERENCE
1.	BIURET TEST		
2.	HEAT COAGULATION TEST		
3	AMMONIUM SULPHATE SATURATION TEST:		
a.	50% SATURATION TESTS.		
b.	100% SATURATION TESTS.		
4.	TESTS FOR INDIVIDUAL AMINO ACIDS:		
A	NINHYDRIN TEST.		
B	XANTHOPROTEIC TEST.		
C	MILLON'S TEST		
D	CYSTEINE TEST		
E	HOPKIN-COLE'S TEST.		
F	ARGININE TEST		
5.	TEST FOR PHOSPHATE.		

RESULT _____

EXPERIMENT No.
Date.

DETECTION OF PEPTONE IN THE GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, and Burner.

REAGENTS: Albumin solution, 2% CuSO, 5% NaOH, 2% acetic acid, 0.5% Ninhydrin solution, co HNO, strong aqueous ammonia, Millon's Reagent, 20% NaOH, 2% lead acetate, Hopkin Cole Reagent, conc. H.SO, 1% alcoholic a-naphthol, and NaOBr.

NO.	TEST	OBSERVATION	INFERENCE
1.	BIURET TEST		
2.	HEAT COAGULATION TEST		
3	AMMONIUM SULPHATE SATURATION TEST:		
a.	50% SATURATION TESTS.		
b.	100% SATURATION TESTS.		
4.	TESTS FOR INDIVIDUAL AMINO ACIDS:		
A	NINHYDRIN TEST.		
B	XANTHOPROTEIC TEST.		
C	MILLON'S TEST		
D	CYSTEINE TEST		
E	HOPKIN-COLE'S TEST.		
F	ARGININE TEST		
5.	TEST FOR PHOSPHATE.		

RESULT _____

EXPERIMENT No.
Date.

DETECTION OF UNKNOWN IN THE GIVEN SOLUTION

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, and Burner.

REAGENTS: Albumin solution, 2% CuSO, 5% NaOH, 2% acetic acid, 0.5% Ninhydrin solution, co HNO, strong aqueous ammonia, Millon's Reagent, 20% NaOH, 2% lead acetate, Hopkin Cole Reagent, conc. H.SO, 1% alcoholic a-naphthol, and NaOBr.

NO.	TEST	OBSERVATION	INFERENCE
1.	BIURET TEST		
2.	HEAT COAGULATION TEST		
3	AMMONIUM SULPHATE SATURATION TEST:		
a.	50% SATURATION TESTS.		
b.	100% SATURATION TESTS.		
4.	TESTS FOR INDIVIDUAL AMINO ACIDS:		
A	NINHYDRIN TEST.		
B	XANTHOPROTEIC TEST.		
C	MILLON'S TEST		
D	CYSTEINE TEST		
E	HOPKIN-COLE'S TEST.		
F	ARGININE TEST		
5.	TEST FOR PHOSPHATE.		

RESULT _____

END OF PRACTICAL LOGBOOK KHYBER MEDICAL UNIVERSITY

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