MLR INSTITUTE OF PHARMACY

(Approved by AICTE & PCI, New Delhi and Affiliated to JNTUH, Hyderabad)

Dundigal, Quthubullapur Mandal, Hyderabad 500043, R.R. Dist

CLASS

I/II B.PHARMACY

SUBJECT

PHARMACEUTICAL BIOCHEMISTRY

Practical Manual

About MLRIP

VISION

To be an educational Institute of par excellence and produce competent pharmacy professionals to serve the community through research and the ever-increasing needs of Industry.

MISSION

1. Imparting quality education and innovative research for various career opportunities.
2. Creating conducive academic environment to produce competent pharmacy professionals.
3. Indoctrination of students adorned with high human values and make them aware of their responsibility as health care professionals.

Program Educational Objectives

PEO 1: To produce graduates with sound theoretical knowledge and technical skills required for their career opportunities in various domains.

PEO 2: To incite the students towards research and to address the challenges with their innovative contributions for the benefit of the mankind.

PEO 3: To instill the essence of professionalism, ethical commitment to become a health care professional with sound integrity and adherence to the core human values in the service of the society.

PROGRAM OUTCOMES

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

LIST OF EXPERIMENTS

S.NO	NAME OF THE EXPERIMENT	PG.NO
	SAFETY PRACTICES IN THE PHARMACEUTICAL CHEMISTRY LABORATORY	
1	IDENTIFICATION OF CARBOHYDRATES(GLUCOSE,FRUCTOSE,LACTOSE,MALTOSE, SUCROSE & STARCH)	
2	IDENTIFICATION OF PROTEINS(ALBUNIM,CASEIN)	
3	QUANTITATIVE ANALYSIS OF REDUCING SUGARS(DNSA METHOD) & PROTEINS (BIURET METHOD)	
4	QUALITATIVE ANALYSIS OF URINE FOR ABNORMAL CONSTITUTENTS	
5	PREPARATION OF STANDARD BUFFER AND MEASUREMENT OF pH	
6	METHOD OF PREPARATION OF FOLIN-WU FILTERATE	
7	DETERMINATION OF BLOOD CREATININE	
8	DETERMINATION OF BLOOD SUGAR	
9	DETERMINATION OF SERUM TOTAL CHOLESTEROL	
10	STUDY OF ENZYMATIC HYDROLYSIS OF STARCH	
11	DETERMINATION OF SALIVARY AMYLASE ACTIVITY	
12	FACTORS EFFECTING ENZYMES(PH)	
13	STUDY THE EFFECT OF TEMPERATUE ON SALIVARY AMYLASE ACTIVITY.	
14	STUDY THE EFFECT OF SUBSTRATE ON SALIVARY AMYLASE ACTIVITY.	

SAFETY PRACTICES IN THE PHARMACEUTICAL CHEMISTRY LABORATORY

General:

Never work in the laboratory alone. Perform no unauthorized experiments. Do not use mouth suction to fill pipettes. Confine long hair and lose clothes while working in the laboratory. Wear shoes. Learn the location of and correct use of the nearest fire extinguisher. Learn the location of the safety shower and first aid kit and be prepared to give help to others.

Safety Glasses:

Safety glasses should be worn at all times while in the laboratory, whether you actively engage in experiment work or not contact lenses should never be worn in the laboratory because they cannot be removed rapidly enough if reagents accidently splash in the eye.

Fire:

Avoid unnecessary flames. Check the area near you for volatile solvents before lighting a burner. Check the area near you to flames. If you are about to begin working with a volatile solvent. Be particularly careful of the volatile solvents diethyl ether, petroleum ether (ligroin), benzene, methanol, ethanol and acetone.

Chemicals:

Handle every chemical with care. Avoid contact with skin and clothing. Wipe up spills immediately especially near the balances and reagent itself. Replace caps on bottles as soon as possible. Do not use an organic solvent to wash a chemical from the skin as this may actually increase the rate of absorption of the chemicals through the skin. Avoid the inhalation of organic vapors particularly aromatic solvents and chlorinated solvents. Use care in smelling chemicals and do not taste them unless instructed to do so. Drinking, eating or smoking in the laboratory is forbidden.

Disposal of Chemicals:

Dispose of chemicals as directed in each experiment. In general, small quantities of water soluble substances can be flushed down the drain with a large quantity of water. Water-insoluble substances and liquids should be placed in the waste containers provided. Chromium ion in the +6 oxidation state (orange) should be reduced to +3 state (green) with a mid reducing agent such as bisulphate before disposal.

Caution:

It has been determined that several chemicals that are widely used in the organic laboratory. (e.g. benzene and chloroform) cause cancer in test animals when administered in large doses, where the possible, the use of these chemicals is avoided in this book, in few cases, where suspected carcinogens are used, the precautions noted should be followed carefully. A case in point is chromium in the Cr^{+6} oxidation state. The dust of solid Cr^{+6} salts is carcinogenic.

Fire Burning Clothing:

Prevent the person from running and fanning the flames. Rolling the person on the floor will help extinguish the flames and prevent the flames if a safety shower is nearby, hold the person under the shower until flames are extinguished and chemically washed away. Do not use a fire blanked if a shower is near by. The blanket does not cool and smoldering continues. Remove contaminated clothing, wrap the person in a blanket to avoid shack, get prompt medical attention. Do not under any circumstances use a carbon tetrachloride (toxic) fire extinguisher and be very careful using a CO_2 extinguisher (the person may smoother)

CUTS:

Minor cuts:

This type of cut is common in the organic laboratory and usually arises from broken glass, wash the cut, remove any pieces of glass and apply pressure to stop the bleeding. Get medical attention.

Major cuts:

If blood is spurting, place a pad directly on the wound, apply firm pressure, wrap the injured to avoid shack and get immediate medical attention. Never use a tourniquet.

Adapted from safety in Academic chemistry laboratories, prepared by the American chemical society on chemical safety, March 1974.

Good Laboratory Practices:

1. Wear laboratory apron while working.
2. Use clean glassware.
3. Use strong acid and alkali carefully.
4. Do not displace the reagents from their respective places.
5. Do not interchange pipettes from one reagent to another without thorough cleaning.
6. Carry out the reactions carefully without harming the neighboring student.
7. Use the gas whenever necessary. Close the gas tap when not required.
8. Prepare your own reagents when necessary for correct results.
9. Do not consult your friends for doubts. Consults books and teachers for your problems.
10. Take signature of your teachers for all your assignments.
11. Leave the laboratory well prepared for the experiment concerned.
12. Write the laboratory records regularly

INTRODUCTION OF CARBOHYDRATES

Carbohydrates may be defined as poly hydroxy aldehydes or ketones or substances that yield aldehydes or ketones on hydrolysis. They have the empirical formula $(C.H_2O)_n$. These are also represented as hydrates of carbon. They are classified into

I. Based on the number of sugar units present- these are again classified into
   a) Monosaccharides
   b) Oligosaccharides.
   c) Polysaccharides.

II. Based on the functional group present monosaccharides are classified into
   a) Aldoses (which have aldehyde functional group)
   b) Ketoses(which have ketone functional group)

1. Monosaccharides: These are often called as simple sugars which cannot be hydrolysed into simple molecules. The general formula is $(C_nH_2O)_n$. These are further subdivided depending on the number of carbon atoms present in a sugar.

   Eg: triose(glyceraldehyde, dihydroxyacetone), tetrose(erythrose,threose,), pentose(arabinose, ribose, xylose), hexose(glucose, mannose, galactose,fructose), heptose.

2. Oligosaccharides: These carbohydrates yield 2-10 units of monosaccharides on hydrolysis.

   Eg: maltose, lactose, sucrose

   Maltose → glucose+glucose

   Lactose → glucose + galactose

   Sucrose → glucose + fructose

3. Polysaccharides: These carbohydrates yield more than 10 molecules of monosaccharides on hydrolysis. These are tasteless & non-sugars & they form colloids with water.

   Eg: starch, cellulose, agar, pectin, dextrin & glycogen.

IDENTIFICATION TEST FOR CARBOHYDRATES

EXPERIMENT	OBSERVATION	REMARKS
Molisch test: To 2ml of test solution add 2drops of molisch reagent &shake well. Now add H_2SO_4through the sides of the test tube.	Reddish violet coloured ring is seen at the junction of two liquids.	It is a sensitive chemical test for the presence ofcarbohydrates, based on the dehydration of the carbohydrate by sulfuric acid to produce an aldehyde derivatives which condenses with α-naphthol to form chromogen (red- or purple-colored compound).
Iodine test: To 2ml of test solution add 2drops of iodine.	Blue colour is seen	Specific test for non reducing sugar (starch, dextrin). Starch is made up of two polysaccharides- amylose & amylopectin. Iodine is trapped inside the helix and the complex is responsible for blue colour. On heating the helical coil unwinds causing denaturation. Iodine is released and the colour disappears. On cooling renaturation takes place.
Fehling's test: To 1ml of test solution add 1ml of fehling's A, fehling's B in equal volume & heat on water bath.	Reddish colour precipitate is seen.	Cupric hydroxide present in Fehlings solution when heated in the presence of reducing sugar gets reduced to yellow/ red cuprous oxide & gets precipitated. Hence, the formation of red coloured precipitate indicates the presence of reducing sugars in the test solution.
Benedict's test: To the test solution add Benedict's reagent.	Formation of yellow/ green/ red coloured precipitate.	Reducing sugar(free aldehyde or keto group) reduce cupric ion in benedicts solution to cuprous ion. Cuprous hydroxide formed is converted to red cuprous oxide while heating
Barfoed's test: To the test solution add Barfoed's	Appearance of red precipitate.	Copper acetate which is present in barfoed's reagent convert to copper oxide and give

reagent.		brick red precipitate when react with monosaccharide or disaccharides. Monosaccharde react fast while disaccharide react slowly.
Seliwanoff's test: To the test solution add seliwanoff's reagent & heat on a water bath.	Appearance of deep red colour.	In presence of conc. HCl ketoses underdo dehydration to yield furfural derivatives more rapidly than aldoses. These derivatives form a complex with resorcinol to yield a deep red colour. It is a timed colour reaction specific to ketoses. Upon continous heating aldoses get converted to ketoses & give a positive test.
Tollen's test: To the test solution add tollen's reagent & heat.	Siver mirror / black precipitate is observed.	Test used to distinguish between an aldehyde and a ketone. The diamine silver complex which is the main component of tollen's reagent oxidizes aldehydes & not ketones to a carboxylate ion & silver is reduced to elemental silver & aq NH_3. The elemental silver precipitates out of the solution on to the inner walls of the test tube giving a characteristic silver mirror.
Bial's test: To the test solution add Bial's reagent & heat	blue or green color muddy-brown or gray solution	This test is for the presence of pentoses. 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. Furfural from pentoses gives a blue or green color & hydroxymethylfurfural from hexoses may give a muddy-brown or gray solution.
Osazone test: to 5ml of test solution add 10 drops	Needle shaped crystals are seen.	

of glacial acetic acid, 1drop of phenylhydrazine, hydrochloride & double the amount of sodium acetate crystals. Mix the solution & heat for 5min, cool on a ice bath until crystals are formed. Observe it under microscope	Rhombic plate like crystals are seen. Sunflower shaped crystals are seen. Powder puff shaped crystals are seen.	Ketoses & aldoses react with phenylhydrazine to form phenylhydrazone which inturn reacts with 2 molecules of phenylhydrazine to form osazone.

Specific test for sucrose: This test is mainly based on the hydrolysis of sucrose by HCl & its conversion into glucose & fructose which can be detected by Tollen's, benedict's & seliwanoff's test.

Hydrolysis test: To 2ml of test solution add 2drops of HCl pink colour is developed which indicates the acidic nature of the solution. Boil the solution for 3min & cool it under tap water & neutralize with NaOH solution.	Formation of blue colour is seen	Neutralisation has taken place.

GENERAL PROCEDURE FOR THE IDENTIFICATION OF CARBOHYDRATES

EXPERIMENT	OBSERVATION	REMARKS
I. PRELIMINARY TEST		
Colour	Colourless	May be carbohydrate
Odour	Odourless	May be carbohydrate
Taste	Sweet	May be Reducing sugar
	Taste less	Non reducing sugar
State	Crystalline	May be monosaccharide or oligosaccharide.
	Amorphous	May be polysaccharide.
Solubility	Soluble in cold water	May be a sugar
	Insoluble in cold water, soluble in hot water & form colloids.	May be polysaccharide.
II. CHEMICAL TEST		
Molisch test: to 2ml of test solution add 2drops of molisch reagent & mix. Then add 2ml of conc. H_2SO_4 slowly from the sides of the test tube.	Reddish-violet coloured ring is seen at the junction of two liquids.	It is a sensitive chemical test for the presence of carbohydrates, based on the dehydration of the carbohydrate by sulfuric acid or hydrochloric acid to produce an aldehyde, which condenses with two molecules of phenol (α-naphthol), resulting in a red- or purple-colored compound.
Iodine test: To 2 ml of test solution add 2 drops of iodine solution.	Blue colour is seen	May be a non reducing sugar.
	No blue colour is seen	May be a reducing sugar
Fehlings test: To 1ml of test solution add 1ml each Fehlings A & B and heat.	Reddish brown colour precipitate is seen.	May be a reducing sugar.
	Reddish brown precipitate is not seen	May be a non reducing sugar.
Benedict's test: To the test solution add Benedict's	Reddish brown colour precipitate is seen.	May be a reducing sugar.

reagent.	Reddish brown colour precipitate is not seen	May be a non reducing sugar.
Barfoed's test: To the test solution add Barfoed's reagent & heat on a water bath.	Red precipitate is seen within short time.	May be a monosaccharides.
	Red precipitate is seen over a long time.	May be a oligosaccharides
	No red precipitate is seen	May be a polysaccharides
Seliwanoff's test: To the test solution add seliwanoff's reagent & heat on a waterbath.	Red colour is seen	Presence of ketone functional group.
	Red colour is not seen	Presence of aldehyde functional group.
Tollen's test: To the test solution add tollen's reagent & heat.	Silver mirror is deposited on the walls of the testtube.	Presence of aldehydes functional group.
	Black precipitate is observed.	Presence of ketone functional group.
Bial's test: To the test solution add Bial's reagent & heat	blue or green color	Presence of pentose sugar
	muddy-brown or gray solution	Presence of hexose sugar
Osazone test: to 5ml of test solution add 10 drops of glacial acetic acid, 1drop of phenylhydrazine.HCl & double the amount of sodium acetate crystals. Mix the solution & heat for 5min, cool on a ice bath until crystals are formed. Observe it under microscope	Needle shaped crystals are observed	Glucose,fructose &mannose is present.
	Sunflower shaped crystals	Maltose is present
	Powder puff shaped crystals.	Lactose is present
	Rhombic plate shaped crystals.	Galactose is present.

EXPERIMENT-I

IDENTIFICATION OF UNKNOWN CARBOHYDRATE SAMPLES-I

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TEST		
Colour		
Odour		
Taste		
State		
Solubility in water		
II. CHEMICAL TEST:		
Molisch's test: To 2ml of test solution add 2drops of molisch reagent &shake well. Now add H_2SO_4through the sides of the test tube.		
Iodine test: To 2ml of test solution add 2drops of iodine		
Fehling's test: To 1ml of test solution add 1ml of fehling's A, fehling's B in equal volume & heat on water bath.		
Benedict's test: To the test solution add Benedict's reagent.		
Barfoed's test: To the test solution add Barfoed's reagent.		
Seliwanoff's test: To the test solution add seliwanoff's reagent & heat on a water bath.		
Tollen's test: To the test solution add tollen's reagent & heat.		
Osazone test: To 5ml of test solution add 10 drops of glacial acetic acid, 1drop of phenyl hydrazine hydrochloride & double the amount of sodium acetate crystals. Mix the solution & heat for 5min, cool on a ice bath until crystals		

are formed. Observe it under microscope.		
Test for non-reducing sugars: (sucrose)		
Hydrolysis test: Add few drops of conc. HCl to 5ml of test solution & heat for 5min the add NaOH solution to make it slightly alkaline. Now perform Fehling's test, Benedict's test & Osazone test with the hydrolysed solution.		

Report: The given unknown sample was found to be …….. .

EXPERIMENT-I

IDENTIFICATION OF UNKNOWN CARBOHYDRATE SAMPLES-2

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TEST		
Colour		
Odour		
Taste		
State		
Solubility in water		
II. CHEMICAL TEST:		
Molisch's test: To 2ml of test solution add 2drops of molisch reagent &shake well. Now add H_2SO_4through the sides of the test tube.		
Iodine test: To 2ml of test solution add 2drops of iodine		
Fehling's test: To 1ml of test solution add 1ml of fehling's A, fehling's B in equal volume & heat on water bath.		
Benedict's test: To the test solution add Benedict's reagent.		
Barfoed's test: To the test solution add Barfoed's reagent.		
Seliwanoff's test: To the test solution add seliwanoff's reagent & heat on a water bath.		
Tollen's test: To the test solution add tollen's reagent & heat.		
Osazone test: To 5ml of test solution add 10 drops of glacial acetic acid, 1drop of phenyl hydrazine hydrochloride & double the amount of sodium acetate crystals. Mix the solution & heat for 5min, cool on a ice bath until crystals		

are formed. Observe it under microscope.		
Test for non-reducing sugars: (sucrose)		
Hydrolysis test: Add few drops of conc. HCl to 5ml of test solution & heat for 5min the add NaOH solution to make it slightly alkaline. Now perform Fehling's test, Benedict's test & Osazone test with the hydrolysed solution.		

Report: The given unknown sample was found to be …….. .

EXPERIMENT-I

IDENTIFICATION OF UNKNOWN CARBOHYDRATE SAMPLES-3

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TEST		
Colour		
Odour		
Taste		
State		
Solubility in water		
II. CHEMICAL TEST:		
Molisch's test: To 2ml of test solution add 2drops of molisch reagent &shake well. Now add H_2SO_4through the sides of the test tube.		
Iodine test: To 2ml of test solution add 2drops of iodine		
Fehling's test: To 1ml of test solution add 1ml of fehling's A, fehling's B in equal volume & heat on water bath.		
Benedict's test: To the test solution add Benedict's reagent.		
Barfoed's test: To the test solution add Barfoed's reagent.		
Seliwanoff's test: To the test solution add seliwanoff's reagent & heat on a water bath.		
Tollen's test: To the test solution add tollen's reagent & heat.		
Osazone test: To 5ml of test solution add 10 drops of glacial acetic acid, 1drop of phenyl hydrazine hydrochloride & double the amount of sodium acetate crystals. Mix the solution & heat for 5min, cool on a ice bath until crystals		

are formed. Observe it under microscope.		
Test for non-reducing sugars: (sucrose)		
Hydrolysis test: Add few drops of conc. HCl to 5ml of test solution & heat for 5min the add NaOH solution to make it slightly alkaline. Now perform Fehling's test, Benedict's test & Osazone test with the hydrolysed solution.		

Report: The given unknown sample was found to be …….. .

EXPERIMENT-I

IDENTIFICATION OF UNKNOWN CARBOHYDRATE SAMPLES-4

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TEST		
Colour		
Odour		
Taste		
State		
Solubility in water		
II. CHEMICAL TEST:		
Molisch's test: To 2ml of test solution add 2drops of molisch reagent &shake well. Now add H_2SO_4through the sides of the test tube.		
Iodine test: To 2ml of test solution add 2drops of iodine		
Fehling's test: To 1ml of test solution add 1ml of fehling's A, fehling's B in equal volume & heat on water bath.		
Benedict's test: To the test solution add Benedict's reagent.		
Barfoed's test: To the test solution add Barfoed's reagent.		
Seliwanoff's test: To the test solution add seliwanoff's reagent & heat on a water bath.		
Tollen's test: To the test solution add tollen's reagent & heat.		
Osazone test: To 5ml of test solution add 10 drops of glacial acetic acid, 1drop of phenyl hydrazine hydrochloride & double the amount of sodium acetate crystals. Mix the solution & heat for 5min, cool on a ice bath until crystals		

are formed. Observe it under microscope.		
Test for non-reducing sugars: (sucrose)		
Hydrolysis test: Add few drops of conc. HCl to 5ml of test solution & heat for 5min the add NaOH solution to make it slightly alkaline. Now perform Fehling's test, Benedict's test & Osazone test with the hydrolysed solution.		

Report: The given unknown sample was found to be …….. .

EXPERIMENT-I

IDENTIFICATION OF UNKNOWN CARBOHYDRATE SAMPLES-5

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TEST		
Colour		
Odour		
Taste		
State		
Solubility in water		
II. CHEMICAL TEST:		
Molisch's test: To 2ml of test solution add 2drops of molisch reagent &shake well. Now add H_2SO_4through the sides of the test tube.		
Iodine test: To 2ml of test solution add 2drops of iodine		
Fehling's test: To 1ml of test solution add 1ml of fehling's A, fehling's B in equal volume & heat on water bath.		
Benedict's test: To the test solution add Benedict's reagent.		
Barfoed's test: To the test solution add Barfoed's reagent.		
Seliwanoff's test: To the test solution add seliwanoff's reagent & heat on a water bath.		
Tollen's test: To the test solution add tollen's reagent & heat.		
Osazone test: To 5ml of test solution add 10 drops of glacial acetic acid, 1drop of phenyl hydrazine hydrochloride & double the amount of sodium acetate crystals. Mix the solution & heat for 5min, cool on a ice bath until crystals		

are formed. Observe it under microscope.		
Test for non-reducing sugars: (sucrose)		
Hydrolysis test: Add few drops of conc. HCl to 5ml of test solution & heat for 5min the add NaOH solution to make it slightly alkaline. Now perform Fehling's test, Benedict's test & Osazone test with the hydrolysed solution.		

Report: The given unknown sample was found to be …….. .

EXPERIMENT-I

IDENTIFICATION OF UNKNOWN CARBOHYDRATE SAMPLES-6

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TEST		
Colour		
Odour		
Taste		
State		
Solubility in water		
II. CHEMICAL TEST:		
Molisch's test: To 2ml of test solution add 2drops of molisch reagent &shake well. Now add H_2SO_4through the sides of the test tube.		
Iodine test: To 2ml of test solution add 2drops of iodine		
Fehling's test: To 1ml of test solution add 1ml of fehling's A, fehling's B in equal volume & heat on water bath.		
Benedict's test: To the test solution add Benedict's reagent.		
Barfoed's test: To the test solution add Barfoed's reagent.		
Seliwanoff's test: To the test solution add seliwanoff's reagent & heat on a water bath.		
Tollen's test: To the test solution add tollen's reagent & heat.		
Osazone test: To 5ml of test solution add 10 drops of glacial acetic acid, 1drop of phenyl hydrazine hydrochloride & double the amount of sodium acetate crystals. Mix the solution & heat for 5min, cool on a ice bath until crystals		

are formed. Observe it under microscope.		
Test for non-reducing sugars: (sucrose)		
Hydrolysis test: Add few drops of conc. HCl to 5ml of test solution & heat for 5min the add NaOH solution to make it slightly alkaline. Now perform Fehling's test, Benedict's test & Osazone test with the hydrolysed solution.		

Report: The given unknown sample was found to be …….. .

INTRODUCTION TO PROTEINS

Proteins are macromolecules made up of aminoacids. They may be defined as high weight mixed polymers of aminoacids joined together with peptide linkages(-CONH-).

They perform a variety of functions in the form of enzymes, hormones, anitibodies, coagulation factor, structural constituents of tissues, carriers of gases, metals, vitamins & in the maintenance of acid base balance & osmotic pressure. The physical, chemical & biological properties of proteins depends upon the aminoacids that they contain. The order in which they are present and special relationship of one aminoacids to another.

Proteins are chief constituents of all living matter. They contain carbon, hydrogen, nitrogen, sulphur & phosphorous. These proteins are made of amino acids which contain both the amino groups & carbonyl group hence they show both acidic & basic property. They are classified in to

a. Simple proteins
b. Conjugated proteins
c. Derived proteins

a. Simple proteins: The proteins which are made up of two amino acids are called as simple proteins. They can be subdivided on the basis of their stability & heat coagulability intoAlbumin, globulin, scleroproteins, prolamines, histones.

b. Conjugated proteins: These proteins are made up of amino acids & a non-protein part called prosthetic group. Depending upon the nature of the prosthetic group they are subdivide into glycoproteins, nucleoprotein, phosphoprotein, metalloprotein, flavoprotein, leukoprotein, lipoprotein, chromoprotein.

c. Derived proteins: These proteins are derived from native proteins. This can be further subdivided in to primary derived proteins & secondary derived proteins. These derived proteins are obtained from the denaturation of proteins.

1. **Primary derived proteins:** they are obtained due to intramolecular changes not involving hydrolysis & these include metaproteins, denaturaed proteins & coagulated proteins.
2. **Secondary derived proteins:** They are formed from the hydrolysis of native proteins & these include primary, secondary proteins & peptones.

IDENTIFICATION TESTS FOR PROTEINS

Test	observation	Remarks
Biuret test: To 2ml of sample solution add biuret reagent dropwise & mix.	A purple or pinkish-violet colour is observed.	Cupric ions in an alkaline medium forms a coordinate complex with the peptide bond. This give rise to a purple or pinkish-violet colour. This is a test for peptide linkage since all the proteins have peptide linkages. All proteins react +vely to biuret reagent.
Ninhydrin test: To 2ml of sample solution add 8-10 drops of ninhydrin reagent & boil it.	Blue colour is seen.	Ninhydrin reacts with α-amino acid group & is reduced to hydanton. These amino acid is inturn converted to an aldehyde containing 1-carbon atom less than amino acid, ammonia & CO_2 .The reduced form of ninhydrin & ammonia react with other molecule of amino acid to form blue coloured complex. Since all the proteins contain atleast 2 amino acid, all proteins give positive reaction to this test.
Heat coagulation: Fill 2/3 rd of the test tube with sample solution, add 4-5 drops of chlorophenol red & mix. A purple red colour develops then add 1% acetic acid dropwise until the colour changes to pale pink. Hold the test tube incline it slightly & heat the above part of the test tube.	A dense coagulum is formed in the upper part of the solution.	When a protein is heated, its physical, chemical & biological properties are changed due to breakdown of certain bonds. This is known as denaturation. However when proteins like albumin are heated at their isoelectric PH a series of changes occur involving dissociation of protein subunits (destruction of quaternary structure). Uncoiling of polypeptide chains (destruction of 2^0& 3^0 structures) & clumping of the uncoiled polypeptide (coagulation). Chlorophenol red is used to adjust the P^H of solution to isoelectric P^H of albumin which is 5.4.

		It gives a pink colour at this P^H, purple red above this P^H& yellow colour below this P^H.
Xanthoprotein test: To 2ml of protein solution add 1ml of conc. HNO_3, a white precipitate is formed. Then heat the solution, a little precipitate redissolves & the entire solution becomes yellow. Then cool the solution add 2ml of 40% NaOH & mix.	A deep yellow or orange is seen.	Proteins containing aromatic amino acids like tryptophan, tyrosine, phenylalanine answer this test. The phenyl groups of amino acids undergo nitration upon the addition of conc. HNO3 at at elevated temperature. The nitrophenyl groups import yellow colur to this solution upon the addition of alkali. These nitrophenyl groups ionize & give a deep yellow/ orange colour
Sakaguchi's test: To 2ml of sample solution, add 2 drops of α-naphthol, 2drops of NaOH, mix well & add sodium hypobromide.	Red colour is seen	This is a test for guanidine containing amino acids like arginine. In an alkaline medium α-naphthol combines with guanidine group of arginine to form a complex which is oxidized by sodium hypobromide to produce red colour.
Lead sulphide test: To 2ml of sample solution add 2ml of 40% NaOH, boil for a min. % add 5 drops of lead acetate solution.	A brown or black precipitate is formed.	When proteins containing amino acids like cysteine are heated or boiled with strong alkali, the organic sulphur is converted to sulphide. The addition of lead acetate causes precipitation of insoluble lead sulphide which is black in colour.
Neumann's test: Take a dry test tube with a pinch of sample & add 6 drops of conc.H_2SO_4& 2 drops of conc. HNO_3. Heat gently till the solution becomes dark. Cool it & add a drop of HNO_3& heat again until the solution becomes colourless and white fumes of conc.H_2SO_4 are	A canary yellow precipitate is seen.	This test is for phosphorous containing proteins like casein. Upon heating with conc. H2SO4 & conc. HNO3 the protein is digested & phosphorous is released. When ammonium molybdate is added it reacts with phosphorous in an acidic medium and forms canary yellow precipitate of ammonium phosphomolybdate.

evolved. Cool the solution & add 3ml of water followed by a drop of methyl red indicator & ammonium molybdate.		
Isoelectric point precipitation test: To 2ml of sample solution add 1drop of bromocresol green followed by 1% acetic acid dropwise until the colour changes to green.	A curdy white precipitate is seen.	The solubility of proteins is minimum at their isoelectric PH as they become electrically neutral at this P^H, most of the proteins can be precipitated by heating them at isoelectric P^H. Casein can be precipitated at its isoelectric P^H 4.6
Millon's test/ Cole's test: To 1ml of sample solution add 1ml of mercuric sulphate solution & boil gently for 30sec. & then add 2 drops of 1% sodium nitrate.	Red colour is seen.	This test is answered by the proteins containing hydroxyphenyl group like tyrosine. The red colour is due to the formation of mercuric phenolate when the nitrated phenol radical reacts with mercuric sulphate.
Aldehyde test: To 1ml of sample solution add a drop of formaldehyde solution mix thoroughly & hold the test tube in a slant position & add carefully 1ml of conc. H_2SO_4 through the sides of the test tube.	Violet colour is observed at the junction of two liquids.	The indole nucleus of tryptophan is oxidized in the presence of an oxidizing agent (H_2SO_4). This oxidized product reacts with aldehyde to give a violet coloured complex.
Pauly's test: To 0.5ml of sulfanilic acid add equal volume of 0.5% sodium nitrate. Allow it to stand for 1min. & add 1ml of sample solution, mix well & ad 2ml of	Red colour is seen.	Sulfanilic acid reacts with imidazole ring of histidine & phenolics hydroxyl group of tyrosine in alkaline medium to form red colour.

10% Na_2CO_3		
Saturation test:		
Half saturation test: Take 5ml of sample solution, add 5ml of saturated ammonium sulphate. Shake it vigorously & allow it to stand.	White/ slight precipitate is seen.	Proteins which are colloidal in nature are kept in solution by electric charges & the shell of hydration. When a neutral salt such as ammonium sulphate is added the electric charges are neutralized shell of hydration is removed as it has greater affinity to water than the colloid. Due to this protein molecules form a precipitate.
Full saturation test: Take 5ml of sample solution & saturate it with solid ammonium sulphate & allow it to stand.	White/ no precipitate is seen.	The amount of ammonium sulphate required to precipitate the colloid depends upon the surface area of protein molecules. Albumin which has a relatively larger surface area is precipitated by half & full saturation. Peptone which has very less surface area is not precipitated even by full saturation.

GENERAL PROCEDURE FOR IDENTIFICATION OF PROTEINS

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TESTS:		
State	Solid	May be a casein/ gelatin.
	Liquid	May be Albumin
	Semi solid	May be peptone
Texture	Granular	May be Casein/ gelatin.
	Amorphous	May be peptone
Colour	Colourless	May be a protein.
	Pale yellow	May be Peptone
Odour	Egg like	May be Albumin
	Milk like	May be Casein
	Meat like	May be a peptone
Solubility	Soluble in hot water	May be Gelatin
	Soluble in cold water	May be Albumin
	Soluble in acidic medium	May be Casein
	Soluble in alkaline medium	May be Casein
II. CHEMICAL TESTS:		
Biuret test: To 2ml of sample solution add biuret reagent and mix.	Purplish/ pinkish violet colour is seen.	Presence of proteins.
Precipitation of acidic agents: To 1ml of sample add equal volume of picric acid.	Thick precipitate of protein is observed.	Presence of protein.
Precipitation of heavy metals: To 1ml of sample add 5-10 drops of lead sulphate solution.	Thick precipitate of protein is observed	Presence of protein.
Ninhydrin test: To 2ml of sample add 8-10 drops of ninhydrin reagent & boil.	Blue colour is seen.	Presence of Amino acids.
Heat coagulation test: Fill 2/3 rd of the test tube with sample solution, add 4-5 drops of chlorophenol red & mix. A purple red colour	A dense coagulum is formed in the upper part of solution.	Heat coagulable protein like albumin & globulin are present.

develops then add 1% acetic acid dropwise until the colour changes to pale pink. Hold the test tube incline it slightly & heat the above part of the test tube.		
Xanthoprotein test: To 2ml of protein solution add 1ml of conc. HNO_3, a white precipitate is formed. Then heat the solution, a little precipitate redissolves & the entire solution becomes yellow. Then cool the solution add 2ml of 40% NaOH & mix.	A deep yellow or orange colour is seen.	Presence of aromatic amino acid in protein.
Sakaguchi's test: To 2ml of sample solution, add 2 drops of α-naphthol, 2drops of NaOH, mix well & add sodium hypobromide.	Red colour is seen.	Presence of arginine in protein.
Lead sulphide test: To 2ml of sample solution add 2ml of 40% NaOH, boil for a min. % add 5 drops of lead acetate solution.	A brown or black precipitate is formed.	Presence of sulphur containing amino acid in protein.
Neumann's test: Take a dry test tube with a pinch of sample & add 6 drops of conc.H_2SO_4& 2 drops of conc. HNO_3. Heat gently till the solution becomes dark. Cool it & add a drop of HNO_3& heat again until the solution becomes colourless and white fumes of conc.H_2SO_4 are evolved. Cool the solution & add 3ml of water followed by a drop of methyl red indicator & ammonium molybdate.	A canary yellow precipitate is seen.	Casein is present.
Isoelectric point precipitation test: To 2ml of sample solution add 1drop of bromocresol green followed by 1% acetic acid dropwise until the colour changes to green.	Curdy white precipitate is seen.	Casein is present.
Saturation test:		

Half saturation test: Take 5ml of sample solution, add 5ml of saturated ammonium sulphate. Shake it vigorously & allow it to stand.	White precipitate	Casein /gelatin is present.
	Slight precipitate	Albumin
Full saturation test: Take 5ml of sample solution & saturate it with solid ammonium sulphate & allow it to stand.	White precipitate	Albumin /casein / gelatin is present.
	No precipitate	Peptone is present.

EXPERIMENT -II

IDENTIFICATION TEST FOR UNKNOWN PROTEIN SAMPLE- 1

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TESTS:		
State		
Texture		
Colour		
Odour		
Solubility		
Appearance of solution		
II. CHEMICAL TESTS:		
Biuret test: To 2ml of sample solution add biuret reagent and mix.		
Precipitation of acidic agents: To 1ml of sample add equal volume of picric acid.		
Precipitation of heavy metals: To 1ml of sample add 5-10 drops of lead sulphate solution.		
Ninhydrin test: To 2ml of sample add 8-10 drops of ninhydrin reagent & boil.		
Heat coagulation test: Fill 2/3 rd of the test tube with sample solution, add 4-5 drops of chlorophenol red & mix. A purple red colour develops then add 1% acetic acid dropwise until the colour changes to pale pink. Hold the test tube incline it slightly & heat the above part of the test tube.		

Xanthoprotein test: To 2ml of protein solution add 1ml of conc. HNO_3, a white precipitate is formed. Then heat the solution, a little precipitate redissolves & the entire solution becomes yellow. Then cool the solution add 2ml of 40% NaOH & mix.		
Sakaguchi's test: To 2ml of sample solution, add 2 drops of α-naphthol, 2drops of NaOH, mix well & add sodium hypobromide.		
Lead sulphide test: To 2ml of sample solution add 2ml of 40% NaOH, boil for a min. % add 5 drops of lead acetate solution.		
Newmann's test: Take a dry test tube with a pinch of sample & add 6 drops of conc.H_2SO_4& 2 drops of conc. HNO_3. Heat gently till the solution becomes dark. Cool it & add a drop of HNO_3& heat again until the solution becomes colourless and white fumes of conc.H_2SO_4 are evolved. Cool the solution & add 3ml of water followed by a drop of methyl red indicator & ammonium molybdate.		
Isoelectric point precipitation test: To 2ml of sample solution add 1drop of bromocresol green followed by 1% acetic acid dropwise until the colour changes to green.		

Saturation test:		
Half saturation test: Take 5ml of sample solution, add 5ml of saturated ammonium sulphate. Shake it vigorously & allow it to stand.		
Full saturation test: Take 5ml of sample solution & saturate it with solid ammonium sulphate & allow it to stand.		

Report: The given unknown protein sample was found to be…….

EXPERIMENT II

IDENTIFICATION TEST FOR UNKNOWN PROTEIN SAMPLE-2

EXPERIMENT	OBSERVATION	INFERENCE
I. PRELIMINARY TESTS:		
State		
Texture		
Colour		
Odour		
Solubility		
Appearance of solution		
II. CHEMICAL TESTS:		
Biuret test: To 2ml of sample solution add biuret reagent and mix.		
Precipitation of acidic agents: To 1ml of sample add equal volume of picric acid.		
Precipitation of heavy metals: To 1ml of sample add 5-10 drops of lead sulphate solution.		
Ninhydrin test: To 2ml of sample add 8-10 drops of ninhydrin reagent & boil.		
Heat coagulation test: Fill 2/3 rd of the test tube with sample solution, add 4-5 drops of chlorophenol red & mix. A purple red colour develops then add 1% acetic acid dropwise until the colour changes to pale pink. Hold the test tube incline it slightly &		

heat the above part of the test tube.		
Xanthoprotein test: To 2ml of protein solution add 1ml of conc. HNO_3, a white precipitate is formed. Then heat the solution, a little precipitate redissolves & the entire solution becomes yellow. Then cool the solution add 2ml of 40% NaOH & mix.		
Sakaguchi's test: To 2ml of sample solution, add 2 drops of α-naphthol, 2drops of NaOH, mix well & add sodium hypobromide.		
Lead sulphide test: To 2ml of sample solution add 2ml of 40% NaOH, boil for a min. % add 5 drops of lead acetate solution.		
Newmann's test: Take a dry test tube with a pinch of sample & add 6 drops of conc.H_2SO_4& 2 drops of conc. HNO_3. Heat gently till the solution becomes dark. Cool it & add a drop of HNO_3& heat again until the solution becomes colourless and white fumes of conc.H_2SO_4 are evolved. Cool the solution & add 3ml of water followed by a drop of methyl red indicator & ammonium molybdate		
Isoelectric point precipitation test: To 2ml of sample solution add 1drop		

of bromocresol green followed by 1% acetic acid dropwise until the colour changes to green.		
Saturation test:		
Half saturation test: Take 5ml of sample solution, add 5ml of saturated ammonium sulphate. Shake it vigorously & allow it to stand.		
Full saturation test: Take 5ml of sample solution & saturate it with solid ammonium sulphate & allow it to stand.		

Report: The given unknown protein sample was found to be…….

EXPERIMENT NO: III

QUANTITATIVE ANALYSIS OF REDUCING SUGARS

Aim: To perform quantitative analysis of reducing sugars using DNS reagent.

Apparatus required: Volumetric flask, beaker, pipette, stirrer, measuring cylinder, test tubes, water bath & tripod stand.

Chemicals required: sodium potassium tartarate, NaOH, 3-amino 5-nitro salicylic acid.

Principle: Reducing sugars have the property to reduce many of the reagents. One such reagent is 3,5-dinitrosalicylic acid (DNS). This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in glucose and the ketone functional group in fructose. 3,5-DNS in alkaline solution is reduced to 3-amino 5-nitro salicylic acid.

Reagents Required

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

2. Dinitro salicylic acid reagent:

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

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

c. The dinitrosalycilate reagent is prepared by mixing solutions a & b and raising the final volume to 1 litre with distilled water.

Procedure: Pipette out 0.2-1.0ml of standard glucose solution into different test tubes. Make up the volumes to 2.0ml in each test tube with distilled water. Add 2.0ml of DNS reagent to each test tube and then place all the tubes in a boiling water bath for 15 min. Cool and add 16.0 ml of water. Mix the contents of the test tube thoroughly and read the absorbance of the solution in a calorimeter at 540 nm against the blank solution.

Prepare standard curves of the reducing sugars provided and use them to estimate the concentration of the unknown provided.

Report: Concentration of the given unknown sample solution was found to be ..... mg/ml.

EXPERIMENT NO: IV

QUANTITATIVE ANALYSIS OF PROTEINS

Aim: To perform quantitative analysis of Proteins by biuret reagent.

Apparatus required: Volumetric flask, beaker, pipette, stirrer, measuring cylinder, test tubes, water bath & tripod stand.

Chemicals required: Na-K tartrate,$CuSO_4$,0.2 N NaOH, egg albumin.

Principle: This is the most commonly used method based on the fact that the - CO - NH (peptide) group of proteins form a purple complex with copper ions in an alkaline medium. This test is given by compounds containing two or more peptide bond (CO-NH group). Since all proteins and peptides possessing at least two peptide linkage ie. tripeptide gives positive biuret test. Some substances like urea and biuret interfere because they possess the - CO – NH - group. The principle of biuret test is conveniently used to detect the presence of proteins in biological fluids. Alkaline CuSO4 reacts with compounds containing two or more peptide bonds to give a violet colored product which is due to formation of co-ordination complex of cupric ions with unshared electron pairs of peptide nitrogen and O2 of water.

Reagents Required:

1. 0.2 N NaOH.
2. Biuret reagent: Dissolve 1.5 gm of $CuSO_4$ and 6gms of Na-K tartrate in 250 mL 0.2 N NaOH solution and make up the volume to 500 mL with 0.2 N NaOH.
3. Protein standard solution: Dissolve 500 mg of egg albumin in 50 mL of H_2O. Make up the volume to 100 mL to get the final concentration of 5 mg/mL.

Procedure:

Pipette out standard protein solution into a series of tubes — 0.0, 0.2, ..., 1 mL and make up the total volume to 4 mL by adding water. The blank tube will have only 4 mL of water. Add 6 mL of biuret reagent to each tube and mix well. Keep the tubes at 37°C for 10 minutes during which a purple color will develop. The optical density of each tube is measured at 520 nm (green filter) using the blank reagent. Draw the graph to the known concentrate of a protein in an unknown solution

Result: Concentration of the given unknown sample solution was found to be ..... mg/ml.

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URINE ANALYSIS

Introduction: Urine is a chief excretory fluid eliminated through kidney. Most of the wastes are eliminated through inorganic substances. The composition of urine varies largely and mainly depends on the nature of dirt.

The individual takes a part from these variations. Any sample of normal urine contains organic and inorganic substances like chlorides, sulphates, phosphates, uric acid, creatinine, these are called low threshold substances as they get filtered very easily during "glomerular filtration"

Abnormal or pathological urine is that sample which contains essential substances of our body like glucose, bile, blood, proteins etc… These are highly threshold substances as they are not filtered during "glomerular filtration".

Collection of Urine:

Morning midstream of urine collected is clean glass or polythene container would serve the purpose. A random sample at any time of the day may be used for four routine purposes. However for special purposes like certain chemical investigations or for acid fast bacilli. A 24 hours collection is preferable for bacteriological purpose. Sterile screw capped or rubber capped container are used for some quantitative chemical examinations. Preservatives like toluene e, thymal, chloroform or 2N HCl are used.

Urine present in HCl is suitable for estimation of the urea, Ammonia and calcium while thymal preserves urine is suitable for sodium, potassium, chloride, urea, protein, amylase, reducing sugars.

PHYSICAL PROPERTIES OF NORMAL URINE

EXPERIMENT	OBSERVATION	INFERENCE
a) Volume	1) 1000- 1500ml/day	Normal
	2) More than 1500 ml/day	Polyuria due to excess water intake less perspiration or intake of high protein diet to diuretic substance like tea, alcohol , caffeine and diabetes insipidus
	3) less than 1000ml/day	Hard physical work, vomiting, diarrhoea, fever.
	4) No urine	Nephritis (abnormality of nephron)
b) Colour	1) If urine is pale yellow	Normal due to presence of urochrome and also normal due to less urine output
	2) Mark yellow	Seen after heavy meal.
	3) Dark yellow	Seen in abnormal condition, jaundice
	4) Brown colour	Abnormal condition like cholera, typhus
	5) Reddish colour	Abnormal condition due to haematuria
	6) Milky white	Abnormal due to chyluria
	7) Cloudy	Abnormal may be due to presence of epithelial mucous cells or due to presence of urea and phosphorous.
c) Odour	1) Peculiar odour	Normal
	2) Aromatic odour	Due to drug metabolism and microbial presence
	3) Unpleasant	Due to excess microbial presence
	4) Sweet odour	Due to ketosis (presence of sugars)
	1) 1.01 to 1.024	Normal

d) Specific gravity	2) less than 1.024	Due to decrease in water intake, excess kidney disorder or diabetic mellitus or excessive perspiration.
	3) Greater than 1.024	It is due to diabetes insipidus condition
e) pH	1) 6-7.5	Normal
	2) Acidic urine	Abnormally seen after high diet and abnormally seen in acidosis
	3) Alkaline urine	Abnormally seen in alkalosis

GENERAL PROCEDURE FOR QUALITATIVE ANALYSIS OF ABNORMAL CONSTITUENTS OF URINE

Experiment	Observation	Inference
1) Test for proteins:		
(i) Sulphosalicylic acid test : 3 ml of urine with sulpho salicylic acid drop by drop	White precipitate was formed	Protein is present
(ii) Haller's ring test (or) Nitric Acid Test: To the concentrated nitric acid add few ml of urine drop by drop	A white ring appears at the junctions of two liquids	Protein is present
(iii) Heat Coagulation test: 5 ml of urine is taken to which two drops of chlorophenol red is added, adjust the pH to pink colour by adding 1% acetic acid (or) 2% sodium carbonate, boil it for 2 min and add two drops of acetic acid again if needed	Turbidity (or) precipitation was seen	Protein is present
2) Test for Sugar :		
(i) Benedicts Test: 5 ml of urine with 5 ml of Benedicts and boiled for 2 min and then allowed to cool	At first green precipitate is formed which turns to yellow and finally red.	Sugars are present
ii) Fehling's test: 2 ml of Fehling's A and Fehling's B reagents is transferred into a test tube, boil for few minutes and add 2 to 3 ml of urine sample and again boil for few min.	Red (or) Yellow precipitate was formed	Sugars are present
3) Test for Ketone bodies:		

Ruther's Test: 5 ml of urine sample add solid ammonium sulphate and saturate it completely then add few drops of sodium nitro prusside solution and 2 ml of strong ammonia solution from the sides of test tube wait for ten minutes.	Permanganate colour develops	Ketone bodies are present
4) Test for Bile Salt:		
5 ml of urine sample is taken in a beaker and sulphur powder is sprinkled on its surface.	If the powder floats on the beaker	Bile salts are present
	If the powder sinks to the bottom	Bile salts are absent
5) Test for Bile Pigments:		
(i) Nitric acid test: 3 ml of conc. Nitric acid is taken in a test tube, to which urine is added slowly from the sides of test tube.	Fine play of colours are observed	Bile pigments are present
(ii) To 10 ml of urine add 2 to 3 drops of dilute hydrochloric acid and filter it through a filter paper and allow it to dry, then add a drop of conc. Nitric acid at the apex of paper.	Green, blue, violet, red and yellowish red colours are observed	Bile pigments are present
6) Test for blood:		
Benzidine test: a pinch of benzidine powder and 1 ml of glacial HNO_3 is added to test tube containing urine, shake for 1 minute and add 2 drops of H_20_2.	Green (or) blue colour is observed	Blood is present

PATHOLOGICAL CONDITIONS DUE TO ABNORMAL CONSTITUENTS PRESENT IN URINE

Abnormal constituents	Pathological conditions	Causes
Protein	Protonuria	Severe exercise, high protein diet, kidney diseases, pregnancy damage to lower urinary bladder and fasting conditions
Sugar	Glycosuria	Diabetics mellitus
Ketone bodies	Ketonuria	Excessive fatty metabolism usually seen in diabetes starvation and pregnancy
Bile pigments	Bilirubinuria	Due to obstructive haemolytic jaundice or due to obstructive hepatic jaundice
Blood	Haematuria	May be due to tuberculosis , renal stones ,cancer or acute inflammation of kidney

EXPERIMENT NO: 5

QUALITATIVE ANALYSIS OF URINE FOR ABNORMAL CONSTITUTENTS

Experiment	Observation	Inference
1) Test for proteins: **(i) Sulphosalicylic acid test :** 3 ml of urine with sulpho salicylic acid drop by drop **(ii) Haller's ring test (or) Nitric Acid Test:** To the concentrated nitric acid add few ml of urine drop by drop **(iii) Heat Coagulation test:** 5 ml of urine is taken to which two drops of chlorophenol red is added, adjust the pH to pink colour by adding 1% acetic acid (or) 2% sodium carbonate, boil it for 2 min and add two drops of acetic acid again if needed		
2) Test for Sugar : **(i) Benedicts Test:** 5 ml of urine with 5 ml of Benedicts' and boiled for 2 min and then allowed to cool **(ii) Fehling's test:** 2 ml of Fehling's A and Fehling's B reagents is transferred into a test tube, boil for few minutes and add 2 to 3 ml of urine sample and again boil for few min.		
3) Test for Ketone bodies: **(i) Ruther's Test:** 5 ml of urine sample add solid ammonium sulphate and saturate		

it completely then add few drops of sodium nitroprusside solution and 2 ml of strong ammonia solution from the sides of test tube wait for ten minutes.		
4) Test for Bile Salt: 5 ml of urine sample is taken in a beaker and sulphur powder is sprinkled on its surface.		
5) Test for Bile Pigments: (i) Nitric acid test: 3 ml of conc. Nitric acid is taken in a test tube, to which urine is added slowly from the sides of test tube. (ii) To 10 ml of urine add 2 to 3 drops of dilute hydrochloric acid and filter it through a filter paper and allow it to dry, then add a drop of conc. Nitric acid at the apex of paper		
6) Test for blood: Benzidine test: a pinch of benzidine powder and 1 ml of glacial nitric acid is added to test tube containing urine, shake for one minute and add two drops of hydroxide peroxide		

Report: The given sample does not contain----------,

it only contains----------.

Part- II

BLOOD ANALYSIS

METHOD OF PREPARATION OF FOLIN-WU FILTRATE

(OR)

PROTEIN FREE FILTRATE

Introduction: Folin-wu filtrate is essential to perform various experiments on blood for example

Estimation of sugar, urea, creatinine in blood

Reagents: Sodium tungstate 10% , 2/3N sulphuric acid

Procedure:

Collect 3ml of oxalated blood in 50 ml beaker add 21ml of distilled water add 3ml of sodium tungstate 10% and mix well add 3ml of 2/3N sulphuric acid by a graduated pipette drop by drop with constant shaking ,stopper with a rubber cork ,shake well and keep for 5 minutes. The colour of precipitat, gradually changes from red to brown.

Note: If the change in colour does not occur ,it means the precipitate is incomplete ,due to much use of oxalate as an anti coagulate in such an emerging the sample may saved by adding few drops of sulphuric acid while adding each drop shake vigorously till dark brown colour is obtained. Filter the above mixture in a funnel covered with filter obtain a clear filtrate as clear as water. Thus obtained filtrate is known as folin-wu filtrate.

Report: Folin-wu filtrate was prepared and submitted and the volume was found to be**…...**

EXPERIMENT -6

ESTIMATION OF GLUCOSE IN BLOOD

Aim: To estimate blood sugar level in a given blood sample

Apparatus: Test tubes, graduated pipette (5ml, 2ml), conical flask, glass rod, photo electric calorimetry

Chemicals required: Alkaline copper sulphate solution, phosphomolybdate, sodium tungstate 10%, 2/3 sulphuric acid, standard glucose solution no.1, standard glucose solution no.2, fluoride oxalate solution

Principle: In these method protein free filtrate is obtained(follin-wu filtrate) so that 10ml of filtrate corresponds to 1ml of blood sample protein free filtrate is obtained by precipitate of protein of blood by tungsten acid then the protein free filtrate containing glucose is heated with alkaline copper sulphate solution. Thus glucose reduces copper sulphates to form equivalent quantity of cuprous oxide. These cuprous oxide formed is reduced with phospho molybdic acid to produce corresponding equivalent quantity of molybdenum blue. The molybdenum blue gives intense blue colour the intensity of which is directly proportional to cuprous oxide which corresponds to the amount of glucose present in given sample follin-wu filtrate. The blue coloured obtained with test blood sample is compared with standard solution by similar procedure and by using photoelectric calorimeter. The optical density of test and standard is measured and concentration of glucose in blood can be calculated by using calorimetry principle

Preparation of reagent:

1) **Sodium tungstate:** Dissolve 10gms of sodium tungstate in 100ml of distilled water
2) **2/3 N sulphuric acid:** Dissolve 3.5gms of sulphuric acid in 100ml of distilled water
3) **Alkaline copper sulphate solution:**
    a) Anhydrous sodium carbonate 40gms, tartaric acid 7.5gms, distilled water 400ml

b) Crystalline copper sulphate 45gms, distilled water 200ml

Add solution A to solution B with constant stirring and make the final volume with distilled water to 1000ml.

4) **Phospho molybdic acid:** Phospho molybdic acid 70gm, sodium tungstate 10gm, 10% sodium hydroxide 10gm, distilled water 400 ml. Boil all the contents for 30min to remove ammonia and then add phosphoric acid 250 ml then add distilled water to make the volume up to 1000ml
5) **Benzoic acid solution:** 2.5gm of benzoic acid in 100ml of distilled water.
6) **Stock glucose solution:** (1gm/100ml) 1ml of stock of glucose in benzoic acid solution 100ml
7) **Standard glucose solution (no.1):** (20mg/100ml) 2ml of stock glucose solution in 100ml of benzoic acid solution.

Procedure:

Wash and clean the three test tube label as (U) unknown, std-1(standard-1),std-2(standard-2).To the test tube labeled as "U" take 2ml of follin-wu filtrate. In a test tube labeled as sdt-1 take 1ml of standard sugar solution or standard glucose solution(0.1mg of glucose).In a test tube labeled as std-2 take 1ml of standard glucose solution(0.2mg of glucose). To all the above tubes, add 1ml of alkaline copper sulphate solution. Keep the tubes in boiling water bath for 6-8 min. Remove from the water bath and add 1ml of phospho molybdic acid to all test tubes. Keep the test tube again in boiling water bath for 2 min and after 2 min cool it to room temperature.

Transfer the test tube contents in the conical flask labeled as "U", std-1, std-2 and add 25ml of distilled water to each conical flask mix well and compare the optical density by using photoelectric calorimeter at 420nm

Report:

EXPERIMENT -7

ESTIMATION OF SERUM CHOLESTROL

Aim: To estimate cholesterol in given sample of serum (blood)

Apparatus: Centrifuge, graduated pipette, photoelectric calorimetry, test tubes

Principle: Serum total cholesterol is estimated by **Zak's method.** Serum proteins are precipitate by ferric chloride : acetic acid reagent. Cholesterol undergoes dehydration in the presence of Conc. H_2SO_4 to give 3,5 –cholestadiene and this undergoes polymerization and sulphonation to give a red colour chromogen. A measure of the intensity of the colour indicates the concentration of cholesterol in the serum.

Preparation of reagents:

1. Ferric chloride : Acetic acid reagent: Dissolve 50mg of $FeCl_3$ in 100ml of glacial acetic acid. Use fresh reagent in the assay.

2. Standard cholesterol solution: Dissolve 100mg of cholesterol in 100ml of glacial acetic acid. dilute 1ml of this stock solution to 25ml with ferric chloride : acetic acid reagent.

Procedure: The glassware used in the estimation should be absolutely dry. Pipette 0.1ml of serum and 9.9ml of ferric chloride : acetic acid reagent into a centrifuge tube. Cover the tube with a stopper and mix well. Allow to stand for 15min. and then centrifuge. Transfer 5ml of the clear supernatant into a dry test tube marked T(Test). Into another test tube pipette 5ml of standard cholesterol solution marked S (Std). Into another test tube pipette 5ml of ferric chloride : acetic acid reagent marked B(Blank). Add 3 ml of Conc. H_2SO_4 into each test tube. Mix well and read the optical densities after 30min. at 560nm.

Report: The amount of cholesterol in 100ml of serum is ……mg.

EXPERIMENT-8

PREPARATION OF BUFFER SOLUTION AND MEASUREMENT OF pH

Aim: To prepare standard carbonate, phosphate & acetate buffers & measure their pH.

Apparatus required: Volumetric flask, beaker, pipette, stirrer, measuring cylinder &pH meter.

Chemicals required: KCl,HCl, NaOH, potassium hydrogen phthalate (KHP) and boric acid.

Principle: Buffers are defined as the solutions which resist to change in the pH by the addition of small amounts of acids or bases. Ex: The bicarbonate buffering system is used to regulate the pH of blood.

pH= Pka + log [salt]/[acid]

A buffer usually consist of a weak acid & its salt with a strong base or a weak base & its salt with a strong acid. These solutions are used in many biochemical experiments where the pH needs to be accurately controlled.

Buffer capacity: Buffer capacity, β, is a quantitative measure of the resistance of a buffer solution to pH change on addition of hydroxide ions.

Procedure:

Composition of standard buffer solution:

1) **HCl buffer (pH - 2.2):** Place 50ml of 0.2N potassium chloride add 7.8ml of 0.2N HCl in 250 ml flask and add water to make up to volume and check the pH.
2) **Acid phthalate buffer (pH - 3.8):** Place 50 ml of 0.2 N potassium hydrogen phthalate and 2.9ml of 0.2N HCl in 250ml flask and add water to make up the volume and check the pH.
3) **Neutralized phthalate buffer (pH-5):** Place 50 ml of 0.2N potassium hydrogen phthalate add 22.6 ml of 0.2N sodium hydroxide in 250 ml flask and add water to make up the volume and check the pH.
4) **Alkaline borate buffer (pH-8.2):** Place 50ml of 0.2N boric acid and KCl solution and add 39.1ml of 0.2N NaOH in 250ml flask and add water to make up the volume and check the pH.

Preparation of reagents:

1) **0.2N potassium chloride:** Dissolve 14.91 g of potassium chloride in water and dilute to 1000ml.
2) **0.2N HCl:** Place 17ml of concentrated HCl in water and dilute it to 1000 ml

3) **0.2N NaOH:** Dissolve 8.4gm of NaOH in sufficient water which is carbon dioxide free and dilute it to 1000ml.

4) **0.2 potassium hydrogen phthalate:** Dissolve 40.84 g of potassium hydrogen phthalate in water and make up the volume to 1000ml.

5) **0.2N boric acid and KCl:** Dissolve 12.36 g of boric acid and 14.91gm of KCl in water and make up the volume to 1000 ml.

Report: Standard buffer solutions were prepared & pH was measured.

EXPERIMENT-9

STUDY OF ENZYMATIC HYDROLYSIS OF STARCH

Aim: To study the enzymatic hydrolysis of starch.

Apparatus:

Chemicals: 1% starch solution, iodine solution.

Principal:

Procedure: Take 4 test tubes and to it add 1ml of 1% starch solution and 1ml of phosphate buffer solution. In a water bath place all the test tubes at temperatures 0^0C, 25^0C, 37^0C, and 95^0C. Then leave the test tube for few minutes and then add about 1ml of diluted saliva in each and every test tube. Now take 4 separate watch glasses and to it add 1 drop of iodine solution. In this iodine solution take 1 drop from each test tube and add to the iodine to check if any starch is left or all hydrolyzed. If any starch is left then it turns the iodine solution to blue colour. Measure the time required for hydrolysis of starch in all the test tubes.

flow chart:

mob

EXPERIMENT-9

DETERMINATION OF SALIVARY AMYLASE ACTIVITY

Aim: To determine the salivary amylase activity.

Apparatus: Beakers, pipettes, test tubes, glass-stoppered bottles, incubator set at 37°C.

Chemicals: starch paste, 0.02 N iodine solution.

Principle: ppt Enzyme amylase is found in saliva which is secreted by salivary glands in mouth palate. Amylase partially hydrolyses starch or glycogen into glucose and maltose. Salivary amylase acts at a temperature of 37 ^{0}C and pH of 6.6. When iodine solution is mixed with starch, blue colour is obtained. When starch is first hydrolyzed with amylase and then mixed with iodine solution, blue colour is not obtained because starch has been broken into glucose and maltose.

Procedure: Prepare 0.02 N iodine solutions in a glass stoppered bottle. Prepare 1% starch solution in a glass stoppered bottle. For collecting own saliva, rinse your month with warm water. Then take 20-25ml of warm water in mouth, rotate water with tongue for 2-3 minutes and collect the saliva solution in a beaker. This contains salivary enzyme amylase. In another beaker mix 5ml of 1% starch solution and 5ml of saliva solution. Incubate the beaker in an oven set at 37°C for one hour. Take two test tubes and mark them A and B, respectively. In each test tube add 2 drops of iodine solution. In test tube A add a drop of starch and saliva, mix the solution with a glass rod. With another pipette add a drop of starch solution only in test tube B. Note the colour change in both the test tubes.

Report: In test tube ___ , solution becomes blue while in test tube ___ it remains colourless as starch has been completely hydrolyzed.

EXPERIMENT-10

STUDY THE EFFECT OF TEMPERATURE ON SALIVARY AMYLASE ACTIVITY

Aim: To determine the effect of temperatures on salivary amylase activity.

Apparatus required: Beaker, test tubes, thermometer, stopwatch, Bunsen burner, tripod stand, glass rod, water bath, ice cubes.

Chemicals required: Starch suspension, saliva solution, Iodine solution.

Principle: All enzymes are proteinaceous in nature. At lower temperatures, the enzyme salivary amylase is deactivated and at higher temperatures, the enzyme is denaturated. Therefore, more time will be taken by enzyme to digest the starch at lower and higher temperatures. At 37° C, the enzyme is most active, hence, takes less time to digest the starch**.**

Procedure: Label the test tubes as A_1, A_2, B_1, B_2, C_1 and C_2 .Then transfer 1ml of 0.1% starch suspension into test tube A_1, B_1 and C_1. Transfer 2ml of saliva solution into the test tubes labeled A_2, B_2, and C_2. In water bath containing cold water keep test tubes A_1 and A_2 immersed. Then after 5 minutes transfer starch solution from test tube A_1 into the test tube A_2 and mix it thoroughly with the help of a glass rod. Now initiate the stopwatch and take one drop of mixture from A_2 and mix it with Iodine solution taken in a petri plate. Then repeat this test with iodine in a gap of every 10 minutes. Then record the time taken for complete hydrolysis of starch. Then throughout the experiment keep the test tubes with mixtures in the required temperature like warm water, hot water. For each temperature the time taken for complete hydrolysis of starch should be noted. This variation in time will show the effect of various temperature on salivary amylase activity.

Report:

EXPERIMENT-11

STUDY THE EFFECT OF SUBSTRATE CONCENTRATION ON SALIVARY AMYLASE ACTIVITY

Aim: To determine the effect of substrate concentration on salivary amylase activity.

Apparatus required: Beaker, test tubes, Bunsen burner, tripod stand, glass rod, water bath, ice cubes.

Chemicals required: Starch suspension, saliva solution, Iodine solution.

Principle:

Preparation of standard solution: Prepare starch solutions from the stock solution (1.0 mg/ml) into dilutions of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml from the starch stock solution.

Procedure:

Take 5 test tubes and label them as S_1 to S_5. In place 1ml of standard starch solution into their respective test tubes labeled as S_1 to S_5 .Take only 1ml of water in another test tube and label it as B. Add 1ml of salivary amylase solution in all the 6 test tubes. Mix it well and incubate all the test tubes at 370C for 10 minutes. Now add 1 ml of Iodine solution (5 g potassium iodide to 100 ml water. The dissolved potassium iodide is added with 1 g of iodine and is allowed to dissolve) in all the test tubes and place them in ice bath for 10 minutes. Now measure the intensity of colour developed (absorbance) in colorimeter at 590nm. Prepare a standard curve of Absorbance vs. Concentration.

Report: