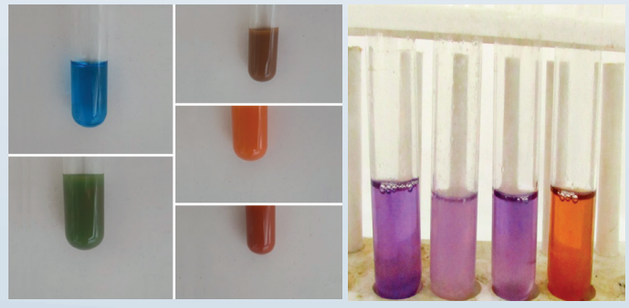
Section

1

Qualitative Analysis

1. Carbohydrate Analysis
2. Lipids Analysis
3. Protein Analysis
4. Urine Analysis



Chapter

1

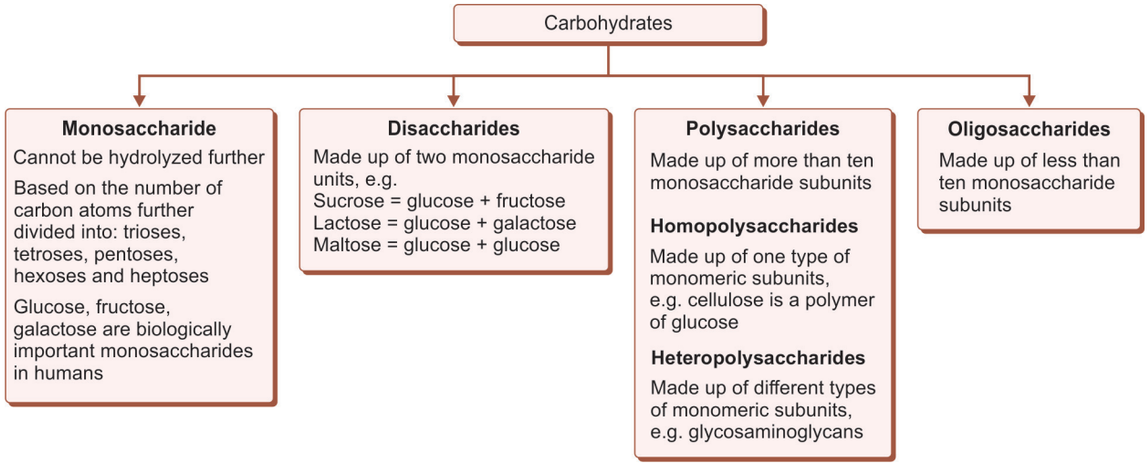
Carbohydrate Analysis

Definition

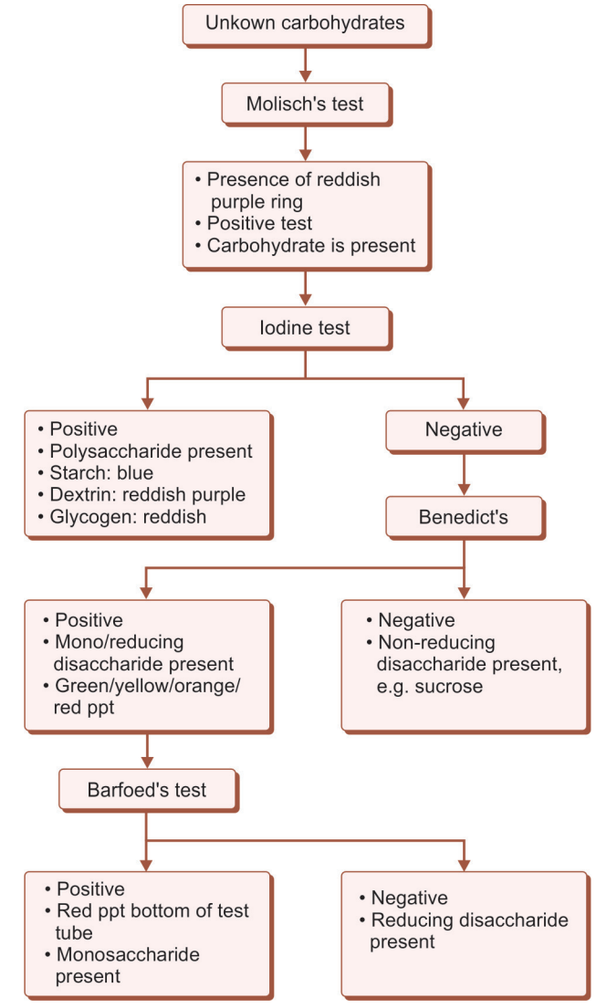
- Carbohydrates are aldehyde or ketone derivatives of polyhydroxy alcohols.
- Their main function is to provide energy.
- Glucose is the main form of carbohydrate absorb from the gut.
- Humans can synthesize glucose from non-carbohydrate sources like lactate, glucogenic amino acids, propionic acid, glycerol, pyruvate by a process known as gluconeogenesis.

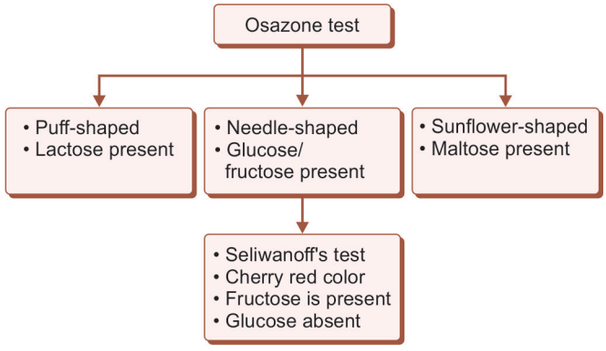
Oligosaccharides: Made up of less than ten monosaccharide subunits.

Classification of Carbohydrates



Identification of unknown carbohydrates





Experiment	*Principle*	*Observation*	*Inference*
Molisch's test (α-naphthol reaction)			
3 ml sugar solution + Molisch reagent (α-naphthol) + Conc sulfuric acid	Conc acid dehydrates sugar to form furfural/furfural derivatives which form complex with molisch reagent	Violet/purple ring at the junction of two liquids (Fig. 1.2)	Carbohydrates present in given solution
Iodine test			
3 ml polysaccharide solution + 0.05N iodine soln 2 drops	Formation of adsorption complex between polysaccharide and iodine	Starch — Blue Glycogen — Reddish Dextrin — Reddish brown (Fig. 1.3)	Polysaccharide is present
Benedict's test			
5 ml benedict reagent + 8 drops sugar soln + Boil for 2 min Benedict reagent: copper sulfate, sodium citrate, sodium carbonate	Reducing sugars have free aldehyde or ketone group, which reduces metallic ions. Benedict reagent has cupric ion which is reduced to cuprous ion	*Colour* — *Conn sugar* Green — 0.5 g Yellow — >0.5–1 g Orange — >1–2 g Red ppt — > 2g (Fig. 1.4)	Reducing sugars present
Barfoed test			
5 ml barfoed reagent + 0.5 ml sugar soln + Put in boiling water bath for 2 min	In acid medium free aldehyde or keto group reduces cupric ion into cuprous ion	Red ppt at the bottom of the test tube (Fig. 1.5)	Monosaccharides are present
Osazone test			
5 ml sugar solution + 300 mg phenyl hydrazine mixture + Heat in boiling water bath (15–45 min depends on type of sugar) Cool at room temperature Avoid rapid cooling	Carbonyl carbon and adjacent cabon react with phenyl hydrazine to form phenyl hydrazone, phenyl hydrazone reacts with two molecules of phenyl hydrazine to form osazones	Lactose — Puff-shaped Maltose — Sunflower-shaped Glucose — Needle-shaped Fructose — Needle-shaped (Fig. 1.7–1.9)	Confirms the presence of lactose, maltose, glucose and fructose
Seliwanoff's test			
3 ml Seliwanoff's reagent + 5 drops fructose + Heat to just boil	Seliwanoff reagent is resorcinol in dil HCl keto group are readily dehydrated by dil HCl to from hydroxymethylfurfural, which condenses with resorcinol to form red complex	Cherry red color (Fig. 1.6)	Fructose is present
Inversion test			
5 ml sucrose soln + 2 drops conc HCl + Boil for 2 mins and divide into two parts Part 1: Perform Benedict's test Part 2: Perform Seliwanoff's test	Sucrose hydrolyze into glucose and fructose when boiled with conc HCl	Part 1: Positive Benedict test Part 2: Positive Seliwanoff's test	Sucrose is present

Fig. 1.1: Reagents for carbohydrates identification

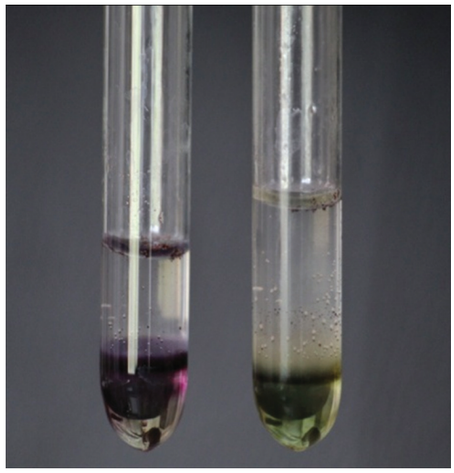


Fig. 1.2: Molisch's test

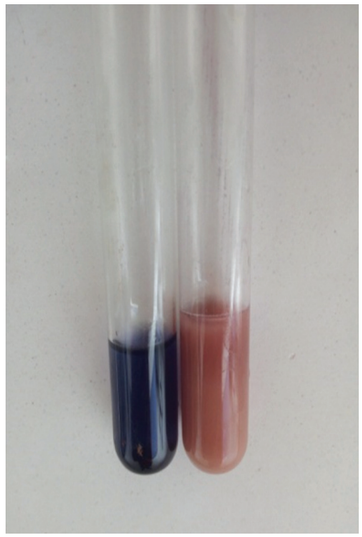


Fig. 1.3: Iodine test

Fig. 1.4: Benedict test: Green, yellow, orange, red

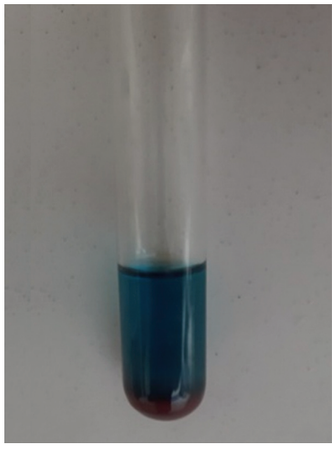


Fig. 1.5: Barfoed's test

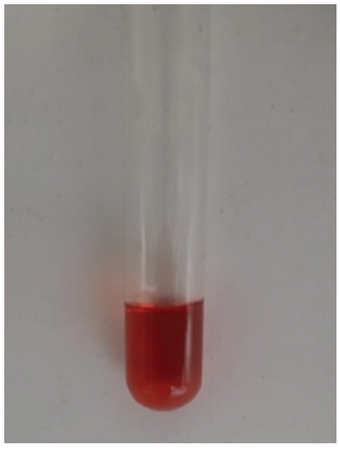


Fig. 1.6: Seliwanoff's test

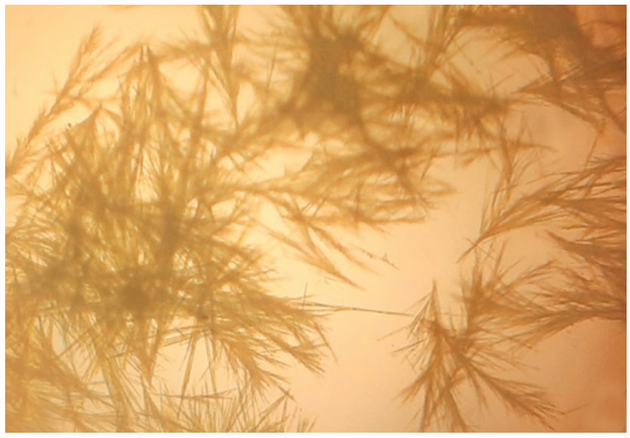


Fig. 1.7: Glucose, fructose-osazone

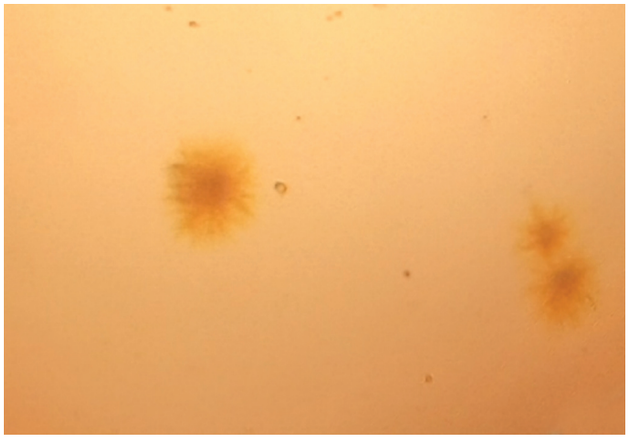


Fig. 1.8: Maltose-osazone

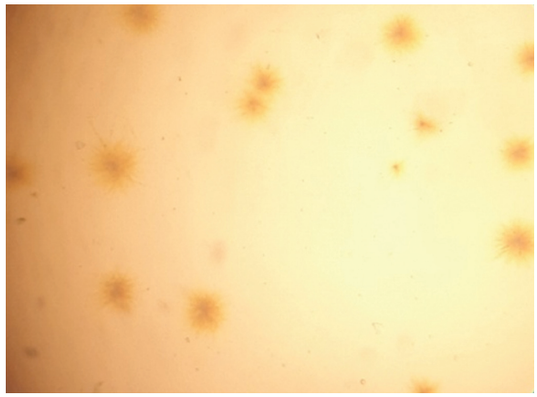


Fig. 1.9: Lactose-osazone

VIVA VOCE

Q1. Name of the group test of carbohydrates.

Ans. Molisch's test

Q2 Why Benedict test is semiquantative test?

Ans. Color of precipitate indicate quantity of sugar in solution.

Q3. Name of test for polysaccharides.

Ans. Iodine Test.

Q4. How to differentiate monosaccharide's glucose and fructose?

Ans. By Seliwanoff's test is positive for fructose.

Q5. Different shape of crystal by osazone test.

Ans. Needle shape—Glucose, fructose
Sunflower shape—Maltose
Cotton ball shape—Lactose

Q.6. Name of common sugar.

Ans. Sucrose

Q7. Carbohydrates preferred in diabetic patients.

Ans. Polysaccharides.

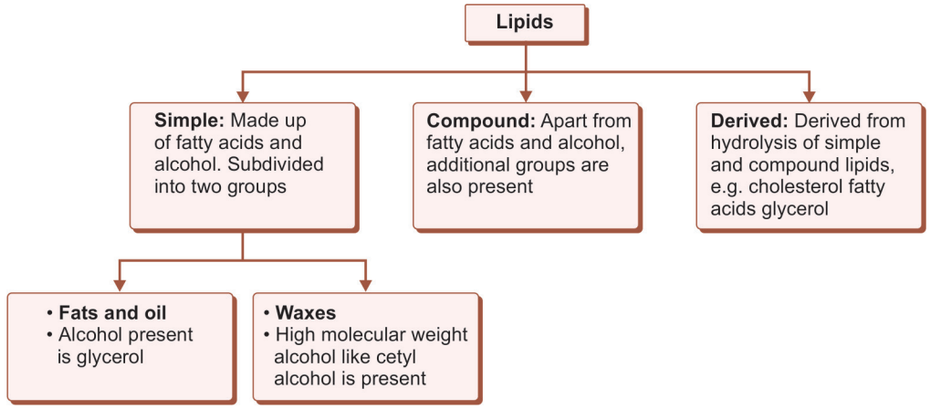
Chapter

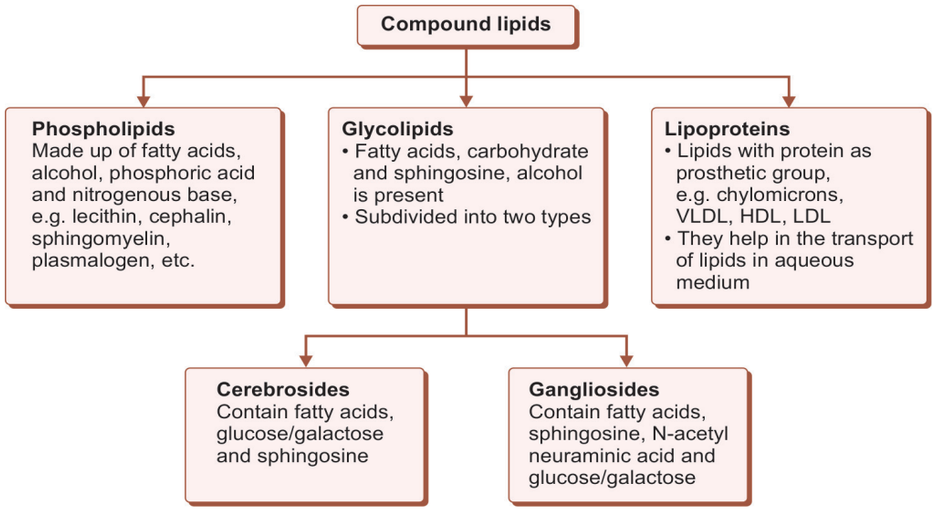
2

Lipid Analysis

Definition: Lipids are esters of fatty acids with alcohol, which are soluble in organic solvents and insoluble in water.

Classification of Lipids





Test	*Principle*	*Observation*	*Inference*
Solubility test: Reagents (ether, chloroform, benzene, CCl_4) **Procedure:** Take 4 test tube and fill 3 ml of water, ether chloroform, benzene CCl_4 in each test tube respectively + Add 5 drops of oil into each test tube + Shake well and allow to stand	This test is based on solubility of lipid in organic solvents and insoluble in water	Oil and water separate quickly in 1st test tube whereas clear solutions are formed in the case of organic solvents	Lipids are insoluble in water and soluble in organic solvents
Saponification test: **Procedure:** Take 5 drop oil and 5 ml of alkali in test tube. Keep test tube in boiling water bath and boil it till the solution become soapy + Cool it take out test tube and add $CaCl_2$	When oil and fat are boiled with alkali than both are hydrolyzed and liberated fatty acid form salts with alkali called soap and process is known as saponification	A white ppt of insoluble calcium soap is formed	Calcium salt of fatty acid is formed
Emulsification test: **Reagents** (0.5% Na_2CO_3 solution in water + 5% of Bile salt solution) **Procedure:** Take 5 ml of each water bile salt, Na_2CO_3, soap solution in separate + Add 5 drop of oil each + In first tube oil and water layer separates immediately indicating. Emulsion is unstable but in case of Na_2CO_3 layer separate longer time indicating emulsion is more stable	When oil and water are shaken together the oil is broken down into small droplets which are dispersed in water. This is known as oil in water emulsion. The water due to their high surface tension has a tendency to close together and separate as a layer. Bile salt, Na_2CO_3, soap solution lowers the surface tension so they are best emulsifying agents	• Oil and water separate quickly • Separation does not take place with soap, bile salt, Na_2CO_3	• Emulsification is unstable • Emulsification is stable with soap, bile salt, Na_2CO_3
Grease spot test reagent: (Ether) **procedure:** Take 3 ml of ether in test tube + Add 5 drop of oil to it shake well and put 1–2 drop of solution on filter paper wait 5	All lipids are grease in nature so this test is used as a group test for lipid	Ether evaporates and leaving behind a translucent spot on paper (Fig. 2.3)	Grease nature of lipid

Glycerol Analysis

Test	*Principle*	*Observation*	*Inference*
Dunstan's test Reagents: (borax solution + phenolphthalein indicator) **Procedure:** 3 ml of borax solution in test tube + add drop of phenolphthalein indicator pink color produced indicate medium is alkaline + add 20% glycerol drop by drop until pink color disappears indicate medium is acidic. Heat again until pink color reappears that indicate medium has become alkaline again	Borax hydrolyzed into NaOH (strong base) and boric acid (weak acid) after addition of glycerol it react with boric acid and forms strong acid (glyceroboric acid)	Medium is alkaline-pink color disappear. Medium is acidic than color will reappear (Fig. 2.1)	Presence of glycerol
Acrolein test: Reagents-solid $KHSO_4$ **Procedure:** Take dry test + add 1–2 drop of glycerol + add pinch of $KHSO_4^+$ heat it	On heating with $KHSO_4$ glycerol is dehydrated to form an unsaturated aldehyde (acrolein)	Pungent smell is produced	Presence of glycerol

Cholesterol Analysis

Procedure	*Principle*	*Observation*	*Inference*
Salkowski's test Reagent: con H_2SO_4 **Procedure:** Take 2 ml of cholesterol in dry test tube + add 2 ml of conc H_2SO_4 shake well and allow to stand	When sample is dissolved in chloroform and equal volume of H_2SO_4 is added. If cholesterol is present solution become blue red and change to violet red and H_2SO_4 become red with green fluorescence	Two layer separated chloroform layer-cherry red (Fig. 2.2) acid layer–green fluorescence	Presence of cholesterol
Libermann-Burchard's test reagent: Con H_2SO_4 Acetic anhydride **Procedure:** Take 3 ml of cholesterol in dry test tube + add 10 drop of acetic anhydride + add 1–2 drops of con H_2SO_4 mix well	Cholesterol is dehydrated by con H_2SO_4 and acetic anhydride leading to formation of 2,4 or 3,5 cholestadienes	Red color develops followed by blue and finally whole solution becomes green	Presence of cholesterol

Test for Unsaturation (Hubl's Iodine Test)

Procedure	*Principle*	*Observation*	*Inference*
Hubl's iodine test) Reagents-iodine 26 gm Mercuric chloride 30 gm C_2H_5OH 1000 ml. Procedure 3 ml of $CHCl_3$ + add Hubl's reagent drop by drop	Unsaturated fatty acid absorb iodine at double bonds until all the bonds are saturated with iodine	Color of iodine disappears	Show degree of unsaturation

Fig. 2.1: Dunstan's test

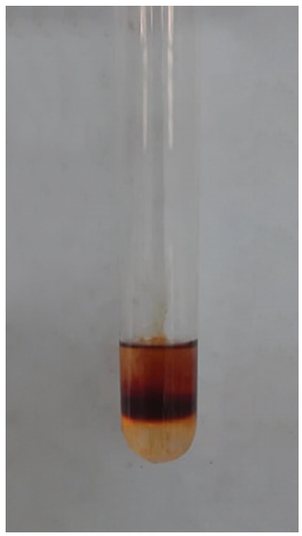


Fig. 2.2: Salkowski's test

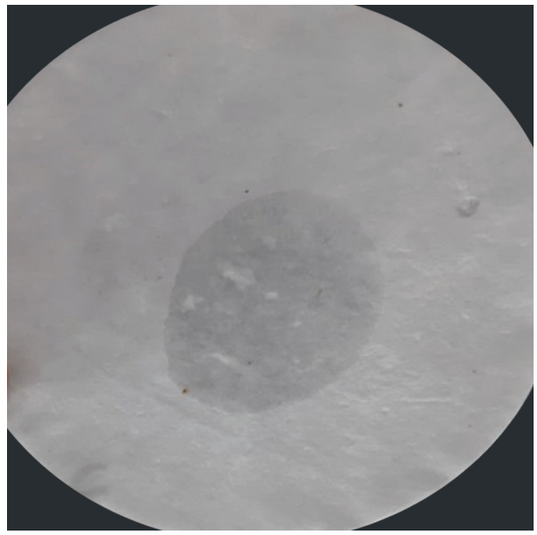


Fig. 2.3: Grease spot test

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Q1. Name of essential fatty acids.

Ans. Lionlic acid, linoleic acid.

Q2. Name of the test for cholesterol.

Ans. Salkowski, Liberman-Burchard reaction.

Q3. Name of ring present in cholesterol.

Ans. CPPP (cyclopentaperhydrophenenthrene).

Q4. Name of the test for unsaturation of fatty acids.

Ans. Hubl's iodine test.

Q5. What is the importance of emulsification?

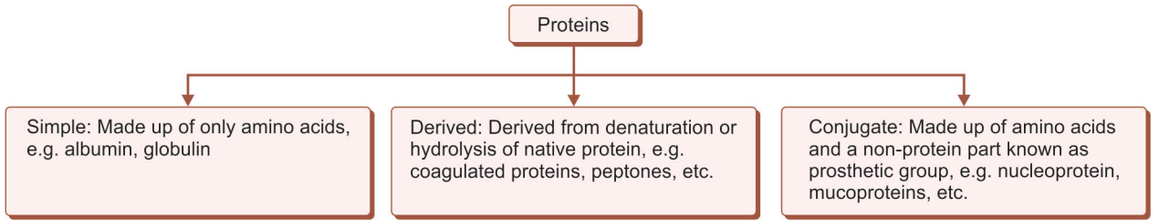
Ans. Digestion of lipids.

Chapter 3

Protein Analysis

Definition: Polymers of L-amino acids linked by peptide bond.

Classification of Proteins



Color Reactions

A. Biuret Test, Ninhydrin Test (Group Color Reaction for Amino Acids)

Test procedure	*Principle*	*Observation*	*Inference*
Biuret test **Reagents: 40%** NaOH **Procedure:** 2 ml protein solution + 1 ml NaOH mix + 1–2 drops of 0.5% $CuSO_4$	Violet color formation because of peptide linkage of protein with $CuSO_4$ in alkali medium provided by NaOH	Violet color presence (Fig. 3.1)	Peptide linkage, presence of protein
Ninhydrin **Reagents:** 0.1% Freshly prepared ninhydrin solution **Procedure:** 1 ml protein solution + 1 ml freshly prepared 0.1% ninhydrin solution mix and boil for 1 min, cool	Heating with ninhydrin (oxidizing agent) α amino acids give purple color	Violet—purple color (Fig. 3.2)	Presence of α amino acids **Exception:** Proline and hydroxyl proline

B. Xanthoproteic, Millon Nasse's, Hopkin's Cole's (Aromatic Amino Acids)

Test procedure	*Principle*	*Observation*	*Inference*
Xanthoproteic Reagents: Con. HNO_3, 40% NaOH **Procedure:** 3 ml protein solution + 1 ml HNO_3 heat for 1 min yellow, cool + 2 ml 40% NaOH mix-orange color	Heating of aromatic amino acids with HNO_3 lead to nitration of benzene group of amino acids, by ionization it give orange color	Yellow to dark yellow convert in to orange color (Fig. 3.3)	Presence of aromatic amino acids

Contd…

Test procedure	*Principle*	*Observation*	*Inference*
Millon Nasse's (tyrosine) reagents: 15% $HgSO_4$ in 6N H_2SO_4 (Millon Nasse's reagent), $NaNO_2$ freshly prepared **Procedure:** 4 ml solution + 1.5 ml Millon's reagent-Boil-cool 1 ml $NaNO_2$	Mercuration and nitration of phenolic group—development of color	Red-pink color developed in precipitate and solution (Fig. 3.4)	Tyrosine presence
Hopkin cole (aldehyde) reagents: Conc H_2SO_4, dilute formaldehyde **Procedure:** 2 ml protein solution + 1 ml formaldehyde mix + 2 ml H_2SO_4 along the side wall of test tube slowly	Indole ring combine with formaldehyde in presence of con H_2SO_4—violet ring	Violet ring at the junction (Fig. 3.5)	Tryptophan presence

C. Sakaguchi's (Test for Arginine)

Test procedure	*Principle*	*Observation*	*Inference*
Sakaguchi reagents: 10% NaOH, α napthol, 10% sodium hypobromite **Procedure:** 3 ml protein solution + 1 ml 10% NaOH mix + 2–3 drop of sodium hypobromite and mix well	Guanido group react with α napthol and sodium hypobromite and give red color complex	Presence of red color (Fig. 3.6)	Guanido group (arginine presence)

D. Lead Sulphide (Test for Sulfur Group)

Test procedure	*Principle*	*Observation*	*Inference*
Lead sulfide (cysteine and cystine) **Reagents:** 40% NaOH, 2% acetate **Procedure:** 3 ml protein + 2.5 ml 40% NaOH boil – 2 min – cool + 3–4 drop lead acetate	In alkali medium sulfur release as sodium sulfide and react with lead acetate form lead sulfide (black-brown color)	Brown black ppt form (Fig. 3.7)	Presence of sulfur con. amino acids (negative for casein and gelatin) lead

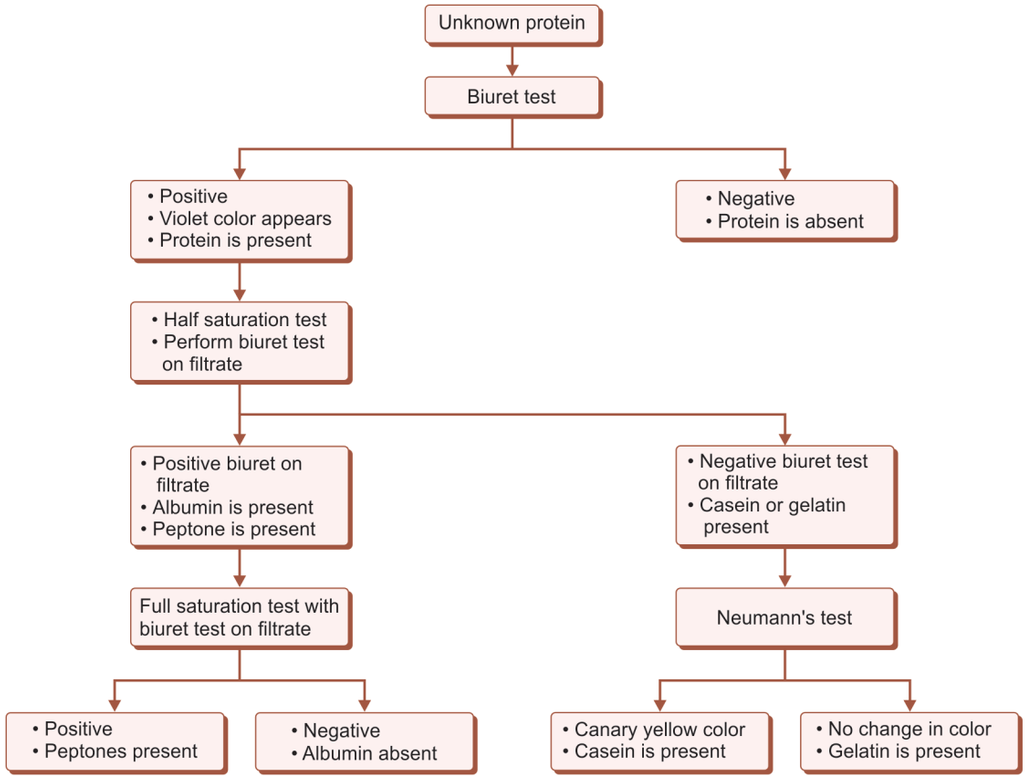
E. Neumann's (Test for phosphate group)

Test procedure	*Principle*	*Observation*	*Inference*
Neumann's (casein) **Reagents:** Ammonium molybdate, methyl red indicator, conc nitric acid, conc ammonia, conc H_2SO_4 **Procedure:** Dry pinch of casein + 6–8 drop of conc H_2SO_4 + 2–3 drop of conc HNO_3^- heat untill colorless. cool + methy indicator with 3 ml water red + ammonia-untill change in yellow + add conc HNO_3^- red, boil for 2 min, 2 ml ammonium molybdate, heat	Heating with conc H_2SO_4 + HNO_3 casein digested phosphorus remove, then react with ammonium molybdate its form ammonium phoshph-omolybdate-canary yellow color	Canary yellow color (Fig. 3.8)	Phosphorus group present

F. Coagulation and precipitation reaction

Test procedure	*Principle*	*Observation*	*Inference*
Heat coagulation 2/3 test tube with protein solution incline heat upper part + acetic acid	Denaturation of protein	Dense coagulum formed at upper part not dissolved adding of acetic acid (Fig. 3.9)	Presence of albumin and globulin (protein)
Precipitation reagents: Ammonium sulfate, $AgNO_3$, $HgNO_3$ sulfosalicylic acid	Shell of hydration, electrical charge	White ppt form	Albumin, casein, gelatin
Full saturation procedure: 3 ml protein + solid ammonium sulfate till saturation–allow to standing			
Half saturation procedure: 3 ml protein solution + saturated 3 ml, mix well-standing	Shell of hydration, electrical charge	White ppt form	Casein, gelatin
Heavy metals procedure: 3 ml protein + drop by drop heavy metals salt till ppt formation	Negatively charged protein react with metal and neutralize form ppt	Ppt form	Protein presence
Alkaloid reagents procedure: 3 ml protein + sulfosalicylic acid mixing	Acid base reaction form insoluble salt	White turbidity	Protein presence

G. Identification of Unknown Protein



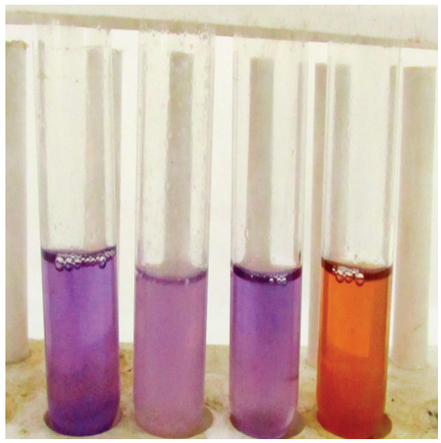


Fig. 3.1: Biuret test:
Albumin, casein, gelatin, peptone

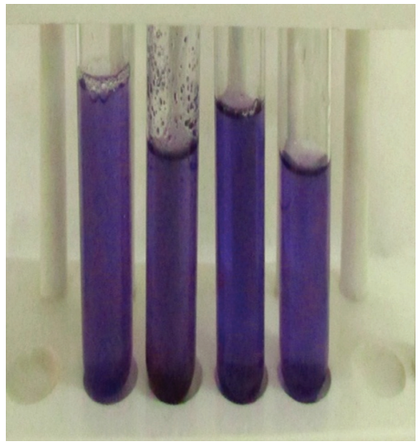


Fig. 3.2: Ninhydrin test:
Albumin, casein, gelatin, peptone

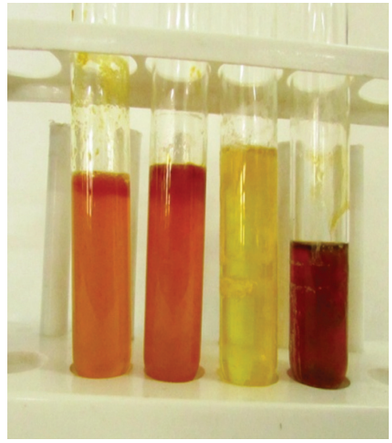


Fig. 3.3: Xanthoproteic test:
Albumin, casein, gelatin, peptone

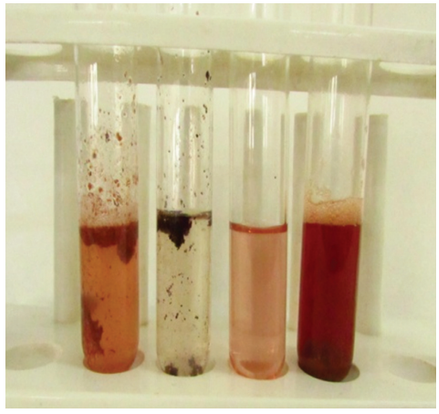


Fig. 3.4: Million Nasse's test:
Albumin, casein, gelatin, peptone

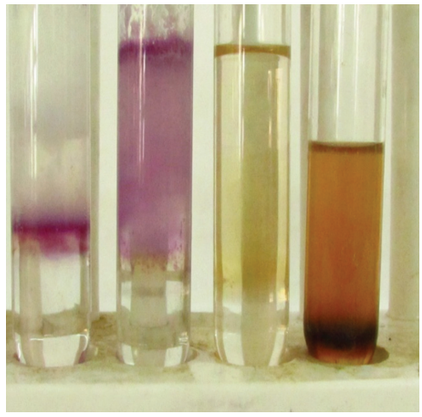


Fig. 3.5: Hopkin's Cole's test:
Albumin, casein, gelatin, peptone

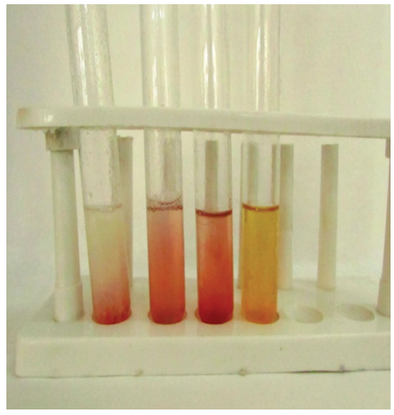


Fig. 3.6: Sakaguchi's test:
Albumin, casein, gelatin, peptone

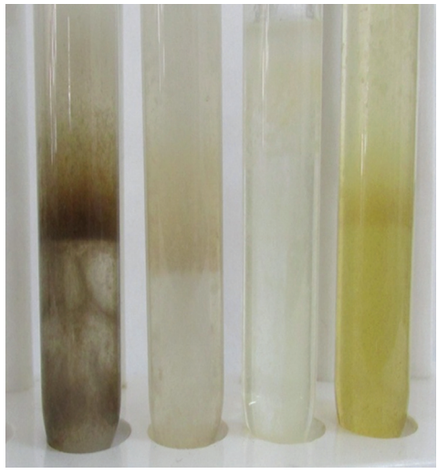


Fig. 3.7: Lead sulphide test:
Albumin, casein, gelatin, peptone

Note: From left to right albumin, casein, gelatin, peptone

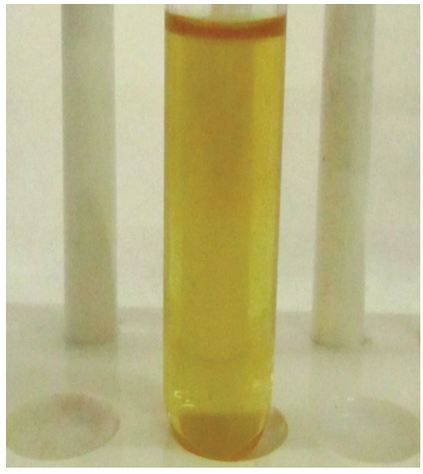


Fig. 3.8: Neumann's test: Casein

Fig. 3.9: Heat coagulation's test:
Albumin

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Q1. Name of the group test for proteins.

Ans. Biuret test.

Q2. Name of the test for aromatic ring containing amino acids.

Ans. Xanthoproteic acids.

Q3. Specific test for tyrosine.

Ans. Millons test.

Q4. Test name for tryptophan.

Ans. Hopkin's cole's.

Q5. Specific test for sulphur test.

Ans. Lead acetate.

Q6. Test for casein.

Ans. Neumann's test.

Q7. How to differentiate albumin and gelatin by saturation test?

Ans. Gelatin gives half saturation, while albumin gives full saturation.

Q8. Name of first class protein.

Ans. Albumin.

Chapter 4

Urine Analysis

NORMAL URINE

Urine is the excretory product of body, formed by kidneys. It is made up of water and water soluble waste products. Examination of urine gives us idea of renal function.

Normal Constituents of Urine

1. Water	90–95%
2. Urea	25–30 gm/day
3. Creatinine	1–1.2 gm/day
4. Uric acid	0.4–0.7 gm/day
5. Sodium	3–3.5 gm/day
6. Potassium	2–2.5 gm/day
7. Chloride	10–15 gm/day
8. Calcium	0.1–0.3 gm/day
9. Phosphate	0.7–1.2 gm/day
10. Sulfate	1–1.2 gm/day
11. Ammonia	0.6–0.8 gm/day

Physical Examination of Normal Urine

1. Appearance	Freshly void urine is clear, it becomes turbid on standing due to precipitation of phosphates
2. Odor	Aromatic odor, which becomes ammonical on standing
3. Color	Straw color
4. Volume	1–2 L/day, depends upon the intake of water
5. pH	Usually acidic, pH ranges from 4.5 to 8 post meal, pH of urine is alkaline; it is called alkaline tide
6. Specific gravity	1.010–1.025, measured with the help of urinometer, it tell us about the concentrating ability of kidneys

Test	*Principle*	*Observation*	*Inference*
Urea **1. Specific urease test reagents:** Soybean meal, phenol red **Procedure:** 4–5 ml of urine + pinch of soybean meal + 1–2 drop of phenol indicator mix and wait 10 mins	Urease in soybean meal acts on urea and liberate NH_3, which makes the medium alkaline. In alkaline medium phenolphthalein gives red color	Yellow color Red color (Due to NH_3) (Fig. 4.2a)	Presence of urea
2. Sodium hypobromite test reagents: Alkaline hypobromite **Procedure:** 4–5 ml sample + 2 ml alkaline hypobromite + mix	Breakdown of urea and release of nitrogen produce effervescence	Effervescence due to production of nitrogen (Fig. 4.2b)	Presence of urea
Calcium test reagents: Ammonium oxalate **Procedure:** Take 2–3 ml ammonium oxalate + 1–2 ml urine	Formation of calcium oxalate	White ppt of calcium oxalate (Fig. 4.4)	Presence of calcium
Phosphate test reagents: Ammonium molybdate **Procedure:** Take 1–2 ml urine + add con HNO_3 + heat + ammonium molybdate	Formation of phospho-molybdate	Canary yellow ppt (Fig. 4.5)	Presence of phosphate
Creatinine test Jaffe's test reagents: Alkaline picrate **Procedure:** 3–4 ml urine + 1 ml alkaline picrate	Formation of creatinine picrate	Orange red color (Fig. 4.3)	Presence of creatinine
Uric acid Benedict's test reagents: Sodium tungstste, arsenic pentoxide, phosphoric acid, HCl, anhydrous sodium carbonate **Procedure:** 4 ml urine + pinch of sodium carbonate + mix + 4–5 drop of above reagent + mix	In alkaline medium, arsenophosphotungstate of Benedict's uric acid regent is reduced to blue colored arsenophosphotungstate	Blue color develop	Presence of uric acid
Ammonia test reagents: Phenolphthalein indicatior **Procedure:** 9 ml urine + drop of phenolphthalein indicatior + drop of 0.1 N NaOH hold glass rod at the mouth of tube	Ammonia is alkaline in nature which turns phenolphthalein indicator pink	Tip of rod turns pink	Presence of ammonia

Fig. 4.1: Reagents

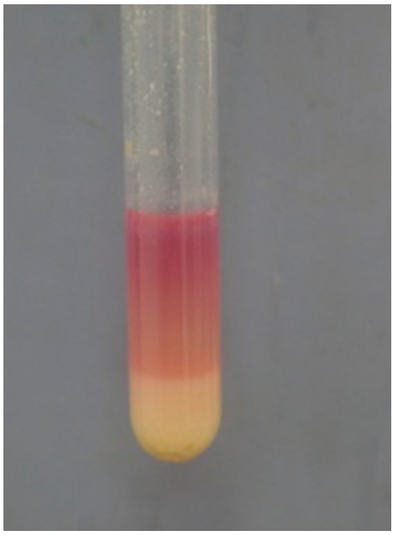

Fig. 4.2a: Test for urea, soybean meal

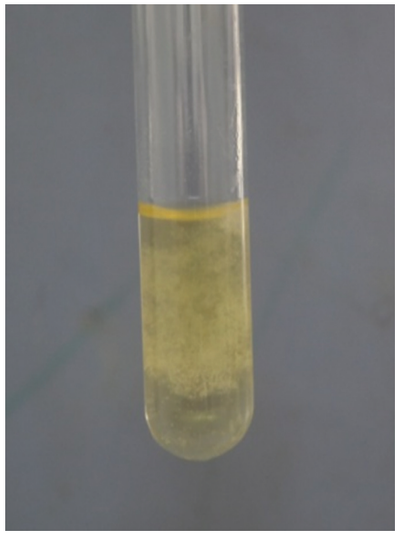

Fig. 4.2b: Sodium hypobromite test

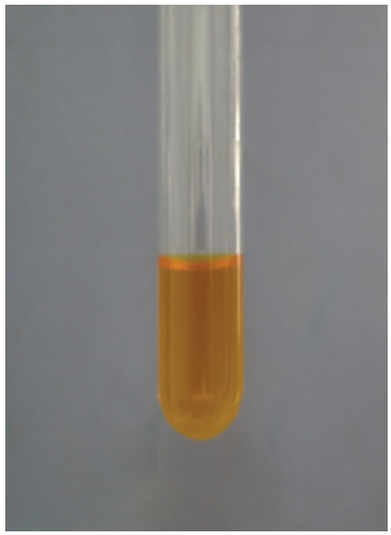

Fig. 4.3: Test for creatinine

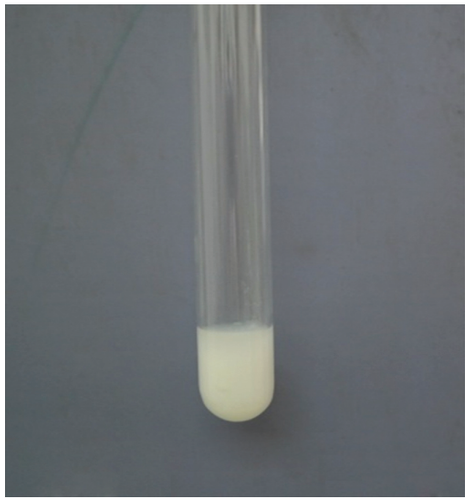

Fig. 4.4: Test for calcium

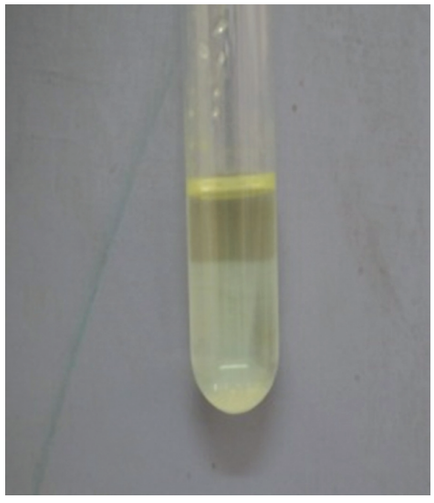

Fig. 4.5: Test for phosphate

PATHOLOGICAL URINE

Physical Examination of Pathological Urine

	Normal	*Pathological*
Appearance	Clear	a. Turbid in UTI
Odor	Aromatic	a. Fruity in diabetic ketoacidosis, starvation due to presence of ketone bodies b. Mousy odor in phenylketonuria c. Foul smell in UTI
Color	Straw color	a. Red in hematuria b. Brown to black in alkaptonuria c. Brown in hemoglobinuria
Volume	1–2 L/day	a. Oliguria <300 ml/day b. Polyuria >2500 ml/day seen in diabetes mellitus and diabetes insipidus c. Anuria <100 ml/day
pH	4.5–8	a. pH decreases in metabolic acidosis b. Increase in pH is seen in metabolic alkalosis and UTI
Specific gravity (SG)	1.010–1.025	a. Isothenuria SG is fixed at 1.010 seen in chronic renal failure b. SG >1.030 hyperosmolar urine, seen in diabetes mellitus, severe dehydration, adrenal insufficiency and nephrotic syndrome c. SG <1.008 hypos molar urine, seen in diabetes insipidus, compulsive polydipsia

Pathological Chemical Constituents Commonly seen in Urine

Abnormal constituents	*Pathological condition*
1. Glucose	a. Diabetes mellitus
2. Albumin	a. Nephrotic syndrome b. Eclampsia and pre-eclampsia c. Heart failure d. UTI e. Inflammatory kidney diseases
3. Blood	a. UTI b. Kidney stones c. Tumors related to renal system d. Hemoglobinopathies
4. Bile salt	a. Obstructive jaundice
5. Bile pigments	a. Obstructive jaundice
6. Urobilinogen	a. Obstructive jaundice
7. Pentose	a. Essential pentosuria
8. Bence-Jones protein	a. Multiple myeloma
9. Ketone bodies	a. Diabetic ketoacidosis
	b. Starvation
	c. Hyperemesis gravidarum

Abnormal Constituents of Urine (Pathological Urine)

Test	*Principle*	*Observation*	*Inference*
Reducing sugar: Benedict test **Reagents:** Sodium citrate + sodium carbonate + copper sulphate **Procedure:** 5 ml reagent + 7–8 drop of urine sample + boil 2–3 min + cool	Reducing sugars have free aldehyde or ketone group, which reduces metallic ions. Benedict reagent has cupric ion which is reduced to cuprous ion	**color** **Con** Green 0.5–1% yellow 1–1.5% orange 1.5–2% Brick red >2% blue No sugar (Fig. 4.6)	Presence of reducing sugar
Protein heat coagulation test-procedure: Fill ¾ test tube with sample + heat upper part + coagulum formed + add 2–3% CH_3COOH If coagulum is dissolves, indicate presence of phosphate	Denaturation of protein by heat	Coagulum is formed at upper part (Fig. 4.8a)	Presence of protein
Heller's test-reagents: HNO_3 **Procedure**: 3 ml of sample + add 3–4 ml of HNO_3 along the side wall of tube	Nitric acid cause precipitation of protein	White ring formed at junction of two liquid (Fig. 4.8b)	Protein present
Bence Jones proteins: **Procedure:** Heat 40–60°C– ppt + heat 100°C– dissolve ppt + cool again reapper		Heat–ppt disappear cool–reappear	Presence of Bence Jones proteins
Ketone bodies: Rothera's nitroprusside test reagents: Ammonium sulfate, sodium nitroprusside, liquor ammonia **Procedure**: 5 ml urine saturate with ammonium sulfate + sodium nitroprusside + shake + liquor ammonia side of tube	Ketone bodies form a purple colored complex with sodium nitroprusside in alkaline medium. β-hydroxybutyrate does not give this test	Purple ring formed at the junction of two liquid (Fig. 4.7)	Presence of ketone bodies
Bile salt Hay's sulfur test reagents—sulfur powder **Procedure:** 4–5 ml of sample in tube + add pinch of sulfur powder on surface	Bile salt reduces the surface tension of water	Sulfur powder sinks towards bottom (Figs 4.9a, and b)	Presence of bile salts
Bile pigments Fouchet's test reagent: ($FeCl_3$+TCA) 6 ml $BaCl_2$ add 10–11 ml urine sample + filter Add 1–2 drop of reagent	$BaCl_2$ reacts with sulfur to form barium sulfate	Green blue color appear	Presence of bilirubin
Urobilinogen Ehrlich's test-reagent: (p-dimethyl-aminobenzaldehyde) **Procedure:** 5–6 ml urine + 5 ml of reagent + wait 8–10 min + add 10 ml saturated sodium acetate	Urobilinogen in acidic medium react with Ehrlich's reagent to form colored compound	Pink, red color	Presence of urobilinogen
Blood pigment benzidine test-reagents: H_2O_2 **Procedure:** 2–3 drop saturate benzidine solution + add 3 ml sample + 4 drop H_2O_2	Peroxidase like activity of hemoglobin $H_2O_2 \rightarrow H_2O + O$ (O) oxidizes the benzidine to form blue/ green colored oxidized product	Blue green color develop	Presence of blood pigment

Fig. 4.6: Benedict's test

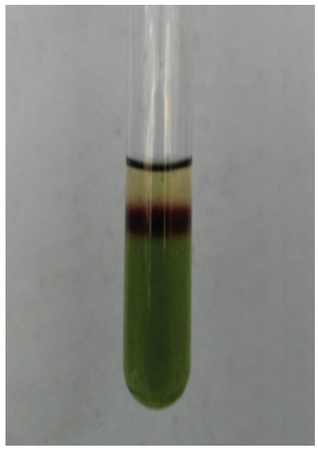

Fig. 4.7: Rothera's test

Fig. 4.8a: Heat coagulation test

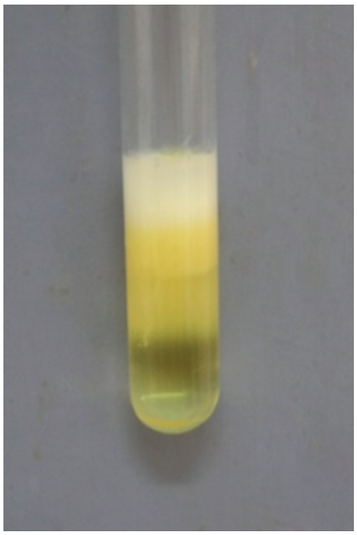

Fig. 4.8b: Heller's nitric acid test

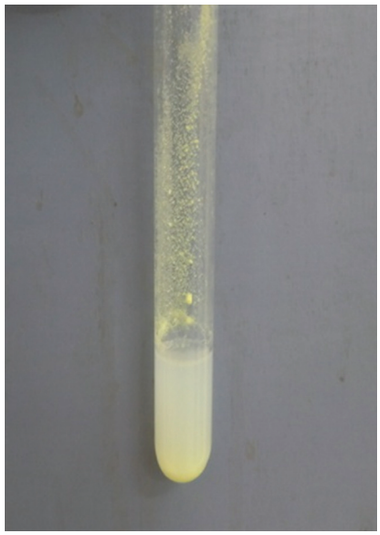

Fig. 4.9a: Hay sulfur test

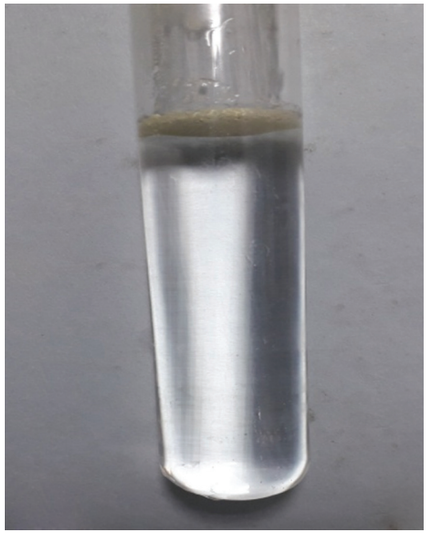

Fig. 4.9b: Control hay sulfur test

VIVA VOCE

Q1. What is the specific gravity for normal urine?

Ans. 1.010–1.025

Q2. How specific gravity changes in diabetes insipidus?

Ans. It is low, fixed below 1.010.

Q3. In which conditions color of urine sample can be changed?

Ans. Jaundice-deep yellow, red—hemoglobinuria, blood, dark amber—vitamin B complex therapy.

Q4. Name of ketone bodies.

Ans. Acetone, acetoacetate, beta hydroxy butyric acid.

Q5. When ketone bodies appear in urine?

Ans. Uncontrolled diabetes mellitus, severe starvation.

Q6. Write common causes for proteinuria.

Ans. Nephrotic syndrome, diabetic nephropathy, renal failure, CHD.

Q7. Commonly which protein come into urine?

Ans. Albumin.

Q8. Name of bile salts.

Ans. Sodium and potassium cholic and chenodeoxycholic acid.

Q9. Name of bile pigments.

Ans. Bilirubin, billiverdin.

Q10. Presence of Bence Jones protein in urine indicate…….

Ans. Multiple myeloma.

Q11. Name few conditions where specific gravity of urine increases.

Ans. Diabetes mellitus, proteinuria, presence of ketone bodies in urine.