S.A.RAJA PHARMACY COLLEGE

VADAKKANGULAM 627 116

SUBJECT: BIOCHEMISTRY

II SEMESTER B.PHARM

PRACTICAL LAB MANUAL

INTRODUCTION

GENERAL INSTRUCTIONS:

- Wear only cotton dress while working in the laboratory.
- Wear a white overcoat in the practical class.
- Maintain an observation book and bring it to every practical class.
- Do your practical work with concentration and avoid distractions and talking to your neighbours.
- Use reagents in prescribed amounts.
- Keep the reagents bottle in their places and do not interchange the stoppers, pipettes and droppers.
- Do not carry the common reagent to your table.
- Handle glassware carefully.
- While handling concentrated acids and alkalis, exercise caution and avoid mouth pipetting.
- No chemicals should be touched by fingers. Use the intended spatulas.
- Follow the first aid instructions when needed.
- While pipetting any solutions take care so that the tip of pipette is well inside the solution.
- Don't run the gas burner unnecessarily. Put them off when not required.
- Do every practical with theoretical knowledge and significance.
- Give your own observation for every test and inference according to your observation book.
- Every practical class is important. If you miss any class, do the relevant experiment during your leisure hours with permission from your teachers.
- Record the work done in the practical class neatly in the practical record book. Your record of work is assessed for internal marking. Brink your record note book after recording the previous experiment to every class.

FIRST AID EQUIPMENTS:

- First aid equipment is to be kept in the laboratory. The kit should contain the following in your box located in a central place. These should not be kept in a locked cupboard.
- 5% aqueous $NaCO_3$
- 2% aqueous $NaHCO_3$ in a dropper bottle
- 5% acetic acid
- Saturated solution of boric acid in a dropper bottle
- Glycerine tincture of iodine, Grease or Vaseline, Dettol, absorbent cotton, gauge, roller bandages, adhesive tape, scissors.

ACCIDENTS IN LABARATORY:

It may be caused by

1. Acid:

Splashes on the skin, splashes in the eye, swallowing while pipetting.

2. Alkalis

3. Toxic substances

4. Heat

- Open flames
- Hot liquids
- Inflammable liquids
- Explosions

5. Broken glass

6. Contamination by infected material

7. Electric shock

LABARATORY FIRST AID:

- Inhalation injury is best treated by removal to the uncontaminated and ventilated area. Irritation of the throat is removed by warm, soothing drinks.
- Chemical injuries to the eye by splashing require immediate attention by dilution of the affected area with plenty of water.
- Chemical injuries to the mouth by entry of strong chemicals (acids or alkalis) while pipetting needs immediate dilution with water and washing the mouth.
- Burn on the skin cools the area by ointment. Do not rub.
- In all the above cases after first aid treatments ask for prompt medical attention from casualty department.

INTRODUCTION TO CARBOHYDRATE

DEFINITION:

Carbohydrates are defined as poly hydroxy alcohols with aldehyde or ketone and their derivatives.

Carbon, hydrogen and oxygen are the elements which will compose the carbohydrate.

CLASSIFICATION:

Carbohydrates are divided into four major groups as follows,

Monosaccharide

Disaccharides

Oligosaccharides

Polysaccharides

MONOSACCHARIDE:

- They are simple sugars which cannot be hydrolysed by simple forms.
- They are represented by a general formula $C_n (H_2O)_n$
- Depending upon the carbon atoms they possess as trioses, tetroses, pentoses, hexoses, heptoses.
- If the aldehyde (CHO) is present in the structure that is aldoses
- If the ketone (-CO) group is present that is ketoses.

GENERAL FORMULA	**ALDOSES**	**KETOSES**
Trioses $C_3H_6O_3$	Glyceraldehyde Glyceroses	Dihydroxy acetone
Tetroses $C_4H_6O_4$	Erythrose	Erythrulose
Pentoses $C_5H_{10}O_5$	Ribose, xylose, Arabinose	Ribulose, Xylulose, Arabilose
Hexoses $C_6H_{12}O_6$	Glucose	Fructose, Ketoses

Simplest form of aldoses -Glyceraldehyde

Simplest form of ketoses -Dihydroxy acetone

Commonest aldoses - Glucose

Commonest ketone -Fructose

Natural glucose - α –D glucose

DISACCHARIDES:

These sugars yields two molecules of same or different molecule of monosaccharide on hydrolysis

They are represented by a general formula $C_n(H_2O)_{n-1}$

Maltose (Hydrolysis) → 2 molecules of glucose

Lactose (Hydrolysis) → glucose + galactose

Sucrose (Hydrolysis) → glucose + fructose

OLIGOSACCHARIDES:

- These carbohydrates yield 3 to 10 monosaccharide units on hydrolysis.
- The oligosaccharides are of plant orgin

Raffinose (Hydrolysis) → fructose+ Galactose + glucose

(Trisaccharides)

Stachyose (Hydrolysis) → 2 molecules of Galactose + glucose+ fructose

(Tetrasaccharides)

Verbascose (Hydrolysis) → 3 molecules of Galactose + glucose+ fructose

(Pentasaccharides)

POLYSACCHARIDES: (Glycons)

These carbohydrates yield more than 10 molecules of monosaccharide on hydrolysis. They have higher molecular weight. They are mostly insoluble in water and tasteless

General formula: $(C_6H_{10}O_5)_n$

Polysaccharides are two types

[A] HOMOPOLYSACCHARIDES: [HOMO GLYCONS]

These are the polymer of same monosaccharide units.

Eg. Starch, glycogen, inulin, cellulose, dextrin, dextran.

[A] HETROPOLYSACCHARIDES: [HETRO GLYCONS]

These are the polymer of different monosaccharide units or their derivatives.

Eg. Mucopolysaccharides (glucose amino glycons)

Hyaluronic acid

Chondroitin sulphate

Heparin

Keratan sulphate

BUFFER SOLUTION

INTRODUCTION:

A buffer solution is one that resists P^H change on the addition of acid or alkali. Such solution are used in many biochemical experiments where the P^H needg to be accurately controlled.

From the Henderson – Hasselbalmh equation, the P^H of a buffer solution depend on two factorg; one is the pK_a value and the other the ratio of salt to acid. This ratio is considered to be the same as the amount of salt and acid mixed together over the P^H range 4-10, where the concentration of hydrogen and hydroxyl ions is very low and can be ignored. Let us take the example acetate buffers consisting of a mixture of acetic acid and sodium acetate.

$$CH_3COOH \longleftrightarrow CH_3COO^- + H^+$$

$$CH_3COONa \longrightarrow CH_3COO^- + Na^+$$

Since acetic acid is only weakly dissociated, the concentration of acetic acid is almost the same as the amount put in the mixture; likewise the concentration of acetate ion man be considered to be the same as the concentration of sodium acetate placed in the mixture since the salt is completely dissociated.

DEFINITION OF P^H:

P^H is defined as the negative logarithm of the hydrogen ion concentration.

MEASUREMENT OF P^H:

The most convenient and reliable method for measuring P^H is by the use of a P^H meter which measures the e.m.f of a concentration cell formed from a reference electrode, the test solution, and a glass electrode sensitive to hydrogen ions.

GLASS ELECTRODE:

The glass electrode consists of a very thin bulb about 0.1 mm thick blown on to a hard glass tube of high resistance. Inside the bulb is a solution of hydrochloric acid (0.1 col/ litre)

connected to a platinum wire via a silver-silver chloride electrode, which is reversible to hydrogen ions. A potential is developed across the thin glass of the bulb which depends on the $\mathbf{P^H}$ of the solution in which it is immersed. This potential is not readily affected by salts, protein or oxidizing and reducing agents, so the electrode can be used in a wide variety of media.

The glass electrode in the test solution constitutes a half cell and the measuring circuit is completed by a reference electrode which is not sensitive to hydrogen ions.

CALOMEL ELECTRODE:

The reference electrode commonly used is the calomel electrode similar to that illustrated. The calomel electrode is stable, easily prepared and the potential with respect to the standard hydrogen electrode is accurately known.

Ex.No: 1

DATE:

PREPARATION OF CARBONATE BUFFER AND ITS MEASUREMENT OF P^H

AIM:

To prepare carbonate buffer and it measurement of P^H by using P^H mete.r

MATERIALS REQUIRED:

- Sodium bicarbonate
- Sodium carbonate
- Distilled water
- 100ml volumetric flask
- Beaker
- Stirrer.

PROCEDURE:

To 93 ml of 0.1M sodium bicarbonate in volumetric flask and 0.1M sodium bicarbonate was added to make up the volume up to 100ml. The P^H was measured by the P^H meter.

Preparation of 0.1M sodium bicarbonate

8.4gm of sodium bicarbonate in 1000ml of water

Preparation of 0.1M sodium carbonate

28.6 g of sodium carbonate in 1000ml of water**.**

REPORT:

Carbonate buffer was prepared and the P^H was found to be --------

Ex.No: 2

DATE:

ISOLATION OF CASEIN FROM MILK

AIM:

T o isolate and identify the casein from given sample of milk.

MATERIALS REQUIRED:

- Beaker
- Glacial acetic acid
- Funnel
- Filter paper
- Milk.

PRINCIPLE:

Milk is the O/W type of emulsion. It contains proteins, carbohydrates, vitamins and minerals. The chief protein present in the milk is casein and β- albumin.

Casein is the phosphate protein containing about 0.85% phosphorous and 0.7% sulphur. It contains about 15 amino acids and it is rich in essential amino acids. Its isoelectric point is 4.6 and its nitrogen content is 15.16%. It's available in two forms,

ACID CASEIN:

Warm skimmed milk is acidified with dil .H_2SO_4

RENNET CASEIN:

Skimmed milk is treated with an enzymes .when a rennet extract, glacial acetic acid is added with milk its isoelectric point brought to 4.6. Emulsion breaks and then the casein precipitate.

PROCEDURE:

100 ml of milk is warmed to 40^{o}C in the beaker. Cooled and diluted with glacial acetic acid drop wise. The mixture is stirred well by agitation. Titration is done till the complete separation of the casein. It's removed by muslin cloth. Dried in vaccum or open air till it forms amorphous mass. Remove it by knife and pressed with spatula to make powder form.

REPORT:

The casein was isolated from the milk.

EXP.NO. 3

DATE :

ESTIMATION OF URINARY CREATININE

AIM:

To estimate the amount of Creatinine present in the given urine sample

METHOD:

JAFFE'S METHOD

REAGENT:

Picric acid reagent -0.4M

0.75N Sodium hydroxide

PRINCIPLE:

Creatinine in urine reacts with picric acid in the presence ofsodium hydroxide to give an red colour compound of creatinine picrate. The intensity of the colour is proportional to the amount of creatinine present and is compared with that of a standard creatinine solution similarly treated. The readings are taken in a colorimeter at 520 nm.

PROCEDURE:

Dilute urine solution (test solution): Dilute 1ml of urine to 100ml with distilled water in a standard flask.Take 3 test tubes and lable them as blank, standard, and test and proceed as follows

REAGENT (ml)	**BLANK (ml)**	**STANDARD (ml)**	**TEST (ml)**
Distilled water	5		
Working standard soluti		5	
Sample solution			1
0.04M Picric acid	1	1	1
0.75N NaOH	1	1	1

Mix and allow to stands for 15 minutes. Measure the absorbance of standard, test and blank at 5 20nm.

NORMAL RANGE:

0.6-1.5mg/dl

REPORT:

The amount of Creatinine present in the given test solution was found to be ----------mg/dl

CALCULATION:

Absorbance of test (T) =

Absorbance of standard (s) =

Absorbance of blank (B) =

Urea concentration =

$$\frac{\text{Absorbance of test- Absorbance of blank}}{\text{Absorbance of std-Absorbance of blank}} \times \frac{\text{Con. Of std}}{\text{Vol.of urine}} \times 100$$

Ex.No:4

DATE:

GENERAL PROCEDURE FOR QUALITATIVE ANALYSIS OF CARBOHYDRATES

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	Action of heat: Heat a small amount of substance in a dry test tube.	A black residue is formed	Presence of carbohydrates
2	Action of conc.H_2SO_4 Take a small amount of sample solution in a dry test tube and add 2ml of conc. H_2SO_4	Solution becomes black in colour	Presence of carbohydrates
3	Molisch's test: To 2 ml of given sample solution add 5 drops of Molisch's reagent. Then slowly add 2ml of conc. H_2SO_4 along the sides of the test tube.	Violet ring is formed at the junction of two layers	Presence of carbohydrates
Principle: Carbohydrate when treated with conc. H_2SO_4 undergoes dehydration to give furfural derivatives. These furfural derivatives condense with phenols to form coloured products. The phenol used in Molisch's reagent is α-naphthol. In case of oligo and polysaccharides they are first hydrolysed into monosaccharide by an acid, which undergoes dehydration to form furfural or its derivatives. Any carbohydrates with more than four carbon atoms will give positive test.			
4	Benedict's test: To 5ml of Benedict's reagent add 8drops of given sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	An appearance of green, yellow, orange or brick red precipitate.	Presence of reducing sugars like glucose, fructose, lactose and maltose.
Principle: Benedict's reagent contains copper sulphate, sodium carbonate and sodium citrate. Carbohydrates with free aldehyde or ketone groups have reducing properties. The mild alkali sodium carbonate converts glucose into enediol. This enediol reduces copper sulphate to cuprous hydroxide that is unstable and decomposes on boiling to cuprous oxide. The precipitated cuprous oxide will have different shades of colour depending upon the concentration. Sodium citrate present in this reagent prevents the precipitation of cupric ion as cupric hydroxide by forming cupric sodium citrate complex. It also improves the self life of the reagent by preventing an interaction between sodium carbonate and copper sulphate.			
5	Fehling's test:		

	To the given sample solution add Fehling's A & B solution. Boil for 15 minutes on the water bath.	Brick red colour precipitate is formed	Presence of reducing sugars
6	Tollen's Mirror test: To 2ml of tollen's reagent add 2ml of sugar solution, mix well and boil for 2 minutes	Silver mirror is formed	Presence of reducing sugars
TEST TO DISTINGUISH MONOSACCHARIDE FROM DISACCHARIDES			
7	Barfoed's test: To 2ml of Barfoed's reagent add 1ml of sample solution and heat in boiling water bath for 2 minutes (or) boil directly for 30 seconds. Cool under running tap water.	Red precipitate is formed in 1 minute Red precipitate is formed in 5 minutes	Presence of monosaccharide Presence of disaccharides
Principle: Barfoed's reagent is cupric acetate in acetic acid solution. Here reduction of cupric ions is carried out in a mildly acidic medium. Since acidic medium is unfavourable for reduction usually the strongly reducing carbohydrate that is monosaccharide gives a positive test.			
TEST TO DISTINGUISE FRUCTOSE FROM GLUCOSE			
8	Seliwanoff's test: To 2.5ml of seliwanoff's reagent add 5 drops of sugar solution and heat the mixture to boil for 30 seconds and cool.	Cherry red colour is formed No cherry red colour is formed	Presence of fructose Presence of glucose
	Principle: The seliwanoff's reagent is resorcinol in conc. hydrochloric acid. The carbohydrates are converted into furfural derivatives by HCl present in the seliwanoff's reagent. Furfural derivatives of ketose sugar (fructose) condense with resorcinol to form a red colour compound.		
9	Foulger's test: Take 3ml of foulger's reagent and add 5 drops of sugar solution boil and leave the test tube in the rack	Blue colour is formed Green colour is formed	Presence of fructose Presence of glucose
Principle: The foulger's reagent is resorcinol in conc. sulphuric acid. The carbohydrates are converted into furfural derivatives by conc. sulphuric acid present in the foulger's reagent. Furfural derivatives of ketose sugar condense with resorcinol to form a blue colour compound.			
CONFIRMATORY TEST FOR DISACCHARIDES AND MONOSACCHARIDES			

		EXCEPT SUCROSE	
10	Osazone test: Take 10ml of sample solution in a dry test tube add one spatula full of phenyl hydrazine and two spatula full of sodium acetate and 1ml of glacial acetic acid. Mix well and filtered in a test tube. Filtrate is kept in boiling water bath for 30 minutes. And examine the shape of the crystal under the microscope.	Sunflower like appearance Dark yellow broomstick or needle like appearance Powder puff or badminton ball shape	Presence of maltose Presence of glucose and fructose. Presence of lactose
Principle: Reducing sugar when treated with phenyl hydrazine in the presence of sodium acetate (PH - 5) give characteristic yellow coloured crystals of osazone. Glucose and fructose give same type of osazone. Because they differ only at the first two carbon atoms which are masked by attachment of two molecules of phenyl hydrazine.			
		CONFIRMATORY TEST FOR SUCROSE	
11	Hydrolysis test: To 5ml of sample solution add 3 drops of conc. hydrochloric acid and boil for 1 minute. Cool and neutralize with 20% sodium carbonate solution till the effervescence ceases to the neutralized solution. Add 5ml of benedict's reagent and boil for 2 minutes and cool.	Red colour precipitate is formed	Presence of sucrose
Principle: Sucrose does not reduce Benedict's reagent but hydrolysed and neutralized product of sucrose will answer for Benedict's reagent. This indicating the presence of reducing sugar in the hydrolysis of sucrose.			
CONFIRMATORY TEST FOR POLYSACCHARIDES			
12	Iodine test: To the sample solution add few drops of N/50 iodine solution	Deep blue colour is formed	Presence of starch
Principle: The test depends upon the property of adsorption having the bigger polysaccharides molecules which adsorb the smaller iodine molecules and forms the blue coloured complex.			

Ex.No:5

DATE:

QUALITATIVE ANALYSIS OF UNKNOWN SAMPLE OF CARBOHYDRATES

SAMPLE [I]

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	Action of heat: Heat a small amount of substance in a dry test tube.	A black residue is formed	Presence of carbohydrates
2	Action of conc.H_2SO_4 Take a small amount of sample solution in a dry test tube and add 2ml of conc. H_2SO_4	Solution becomes black in colour	Presence of carbohydrates
3	Molisch's test: To 2 ml of given sample solution and add 5 drops of Molisch's reagent. Then slowly add 2ml of conc. H_2SO_4 along the sides of the test tube.	Violet ring is formed at the junction of two layers	Presence of carbohydrates
4	Benedict's test: To 5ml of Benedict's reagent, add 8drops of given sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	An appearance of green, yellow, orange or brick red precipitate.	Presence of reducing sugar like glucose, fructose, lactose and maltose.
5	Fehling's test: To the given sample solution, add fehling's A & B solution. Boil for 15 minutes on the water bath.	Brick red colour precipitate is formed	Presence of reducing sugars
6	Tollen's Mirror test: To 2ml of tollen's reagent add 2ml of sugar solution, mix well and boil for 2 minutes	Silver mirror is formed	Presence of reducing sugars

7	Barfoed's test: To 2ml of Barfoed's reagent, add 1ml of sample solution and heat in boiling water bath for 2 minutes (or) boil directly for 30 seconds. Cool under running tap water.	Red precipitate is formed in 1 minute	Presence of monosaccharide
8	Seliwanoff's test: To 2.5ml of seliwanoff's reagent add 5 drops of sugar solution and heat the mixture to boil for 30 seconds and cool.	No cherry red colour is formed.	Presence of glucose
9	Foulger's test: Take 3ml of foulger's reagent add 5 drops of sugar solution boil and leave the test tube in the rack	Green colour is formed	Presence of glucose
10	Osazone test: Take 10ml of sample solution in a dry test tube. Add one spatula full of phenyl hydrazine and two spatulas full of sodium acetate and 1ml of glacial acetic acid. Mix well and filtered in a test tube. Filtrate is kept in boiling water bath for 30 minutes. And examine the shape of the crystal under the microscope.	Dark yellow broomstick or needle like appearance	Presence of glucose and fructose.

REPORT

The given unknown sample was found to be

- **Carbohydrate**
- **Reducing sugar**
- **Monosaccharide**
- **Glucose**

Ex.No:6

Date:

QUALITATIVE ANALYSIS OF UNKNOWN SAMPLE OF CARBOHYDRATES SAMPLE [II]

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	Action of heat: Heat a small amount of substance in a dry test tube.	A black residue is formed	Presence of carbohydrates
2	Action of conc.H_2SO_4 Take a small amount of sample solution in a dry test tube and add 2ml of conc. H_2SO_4	Solution becomes black in colour	Presence of carbohydrates
3	Molisch's test: To 2 ml of given sample solution add 5 drops of Molisch's reagent. Then slowly add 2ml of conc. H_2SO_4 along the sides of the test tube.	Violet ring is formed at the junction of two layers	Presence of carbohydrates
4	Benedict's test: To 5ml of Benedict's reagent add 8drops of given sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	An appearance of green, yellow, orange or brick red precipitate.	Presence of reducing sugars like glucose, fructose, lactose and maltose.
5	Fehling's test: To the given sample solution add fehling's A & B solution. Boil for 15 minutes on the water bath.	Brick red colour precipitate is formed	Presence of reducing sugars
6	Tollen's Mirror test: To 2ml of tollen's reagent add 2ml of sugar solution, mix well and boil for 2 minutes	Silver mirror is formed	Presence of reducing sugars
7	Barfoed's test: To 2ml of Barfoed's reagent, add 1ml of sample solution and heat in boiling water bath for 2 minutes	Red precipitate is formed in 1 minute	Presence of monosaccharide

	(or) boil directly for 30 seconds. Cool under running tap water.		
8	Seliwanoff's test: To 2.5ml of seliwanoff's reagent, add 5 drops of sugar solution and heat the mixture to boil for 30 seconds and cool.	Cherry red colour is formed.	Presence of fructose
9	Foulger's test: Take 3ml of foulger's reagent add 5 drops of sugar solution boil and leave the test tube in the rack	Blue colour is formed	Presence of fructose
10	Osazone test: Take 10ml of sample solution in a dry test tube. Add one spatula full of phenyl hydrazine and two spatulas full of sodium acetate and 1ml of glacial acetic acid. Mix well and filtered in a test tube. Filtrate is kept in boiling water bath for 30 minutes. And examine the shape of the crystal under the microscope.	Dark yellow broomstick or needle like appearance	Presence of glucose and fructose.

REPORT:

The given unknown sample was found to be

- **Carbohydrate**
- **Reducing sugar**
- **Monosaccharide**
- **Fructose**

Ex.No:7

DATE:

QUALITATIVE ANALYSIS OF UNKNOWN SAMPLE OF CARBOHYDRATES SAMPLE [III]

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	Action of heat: Heat a small amount of substance in a dry test tube.	A black residue is formed	Presence of carbohydrates
2	Action of conc.H_2SO_4 Take a small amount of sample solution in a dry test tube and add 2ml of conc. H_2SO_4	Solution becomes black in colour	Presence of carbohydrates
3	Molisch's test: To 2 ml of given sample solution add 5 drops of Molisch's reagent. Then slowly add 2ml of conc. H_2SO_4 along the sides of the test tube.	Violet ring is formed at the junction of two layers	Presence of carbohydrates
4	Benedict's test: To 5ml of Benedict's reagent, add 8drops of given sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	An appearance of green, yellow, orange or brick red precipitate.	Presence of reducing sugars like glucose, fructose, lactose and maltose.
5	Fehling's test: To the given sample solution add Fehling's A & B solution. Boil for 15 minutes on the water bath.	Brick red colour precipitate is formed	Presence of reducing sugars
6	Tollen's Mirror test: To 2ml o tollen's reagent add 2ml of sugar solution, mix well and boil for 2 minutes	Silver mirror is formed	Presence of reducing sugars

7	Barfoed's test: To 2ml of Barfoed's reagen,t add 1ml of sample solution and heat in boiling water bath for 2 minutes (or) boil directly for 30 seconds & then Cool	Red precipitate is formed in 5 minutes	Presence of disaccharides
10	Osazone test: Take 10ml of sample solution in a dry test tube. Add one spatula full of phenyl hydrazine and two spatulas full of sodium acetate and 1ml of glacial acetic acid. Mix well and filtered in a test tube. Filtrate is kept in boiling water bath for 30 minutes. And examine the shape of the crystal under the microscope.	Powder puff or badminton ball shape	Presence of lactose.

REPORT:

The given unknown sample was found to be

- **Carbohydrate**
- **Reducing sugar**
- **Disaccharide**
- **Lactose**

Ex.No:8

DATE:

QUALITATIVE ANALYSIS OF UNKNOWN SAMPLE OF CARBOHYDRATES

SAMPLE [IV]

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	Action of heat: Heat a small amount of substance in a dry test tube.	A black residue is formed	Presence of carbohydrates
2	Action of conc.H_2SO_4 Take a small amount of sample solution in a dry test tube and add 2ml of conc. H_2SO_4	Solution becomes black in colour	Presence of carbohydrates
3	Molisch's test: To 2 ml of given sample solution add 5 drops of Molisch's reagent. Then slowly add 2ml of conc. H_2SO_4 along the sides of the test tube.	Violet ring is formed at the junction of two layers	Presence of carbohydrates
4	Benedict's test: To 5ml of Benedict's reagent, add 8drops of given sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	An appearance of green, yellow, orange or brick red precipitate.	Presence of reducing sugar like glucose, fructose, lactose and maltose.
5	Fehling's test: To the given sample solution add Fehling's A & B solution. Boil for 15 minutes on the water bath.	Brick red colour precipitate is formed	Presence of reducing sugar
6	Tollen's Mirror test: To 2ml of tollen's reagent add 2ml of sugar solution, mix well and boil for 2 minutes	Silver mirror is formed	Presence of reducing sugar
7	Barfoed's test: To 2ml of Barfoed's	Red precipitate is formed	Presence of

	reagent add 1ml of sample solution and heat in boiling water bath for 2 minutes (or) boil directly for 30 seconds. Cool under running tap water.	in 5 minutes	disaccharides
10	Osazone test: Take 10ml of sample solution in a dry test tube add one spatula full of phenyl hydrazine and two spatulas full of sodium acetate and 1ml of glacial acetic acid. Mix well and filtered in a test tube. Filtrate is kept in boiling water bath for 30 minutes. And examine the shape of the crystal under the microscope.	Sunflower like appearance	Presence of maltose.

REPORT:

The given unknown sample was found to be

- **Carbohydrate**
- **Reducing sugar**
- **Disaccharides**
- **Maltose**

Ex.No: 9

DATE:

QUALITATIVE ANALYSIS OF UNKNOWN SAMPLE OF CARBOHYDRATES SAMPLE [V]

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	Action of heat: Heat a small amount of substance in a dry test tube.	A black residue is formed	Presence of carbohydrates
2	Action of conc.H_2SO_4 Take a small amount of sample solution in a dry test tube and add 2ml of conc. H_2SO_4	Solution becomes black in colour	Presence of carbohydrates
3	Molisch's test: To 2 ml of given sample solution, add 5 drops of Molisch's reagent. Then slowly add 2ml of conc. H_2SO_4 along the sides of the test tube.	Violet ring is formed at the junction of two layers	Presence of carbohydrates
4	Benedict's test: To 5ml of Benedict's reagent add 8drops of given sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	No appearance of green, yellow, orange or brick red precipitate.	Absence of reducing sugars like glucose, fructose, lactose and maltose.
5	Fehling's test: To the given sample solution add fehling's A & B solution. Boil for 15 minutes on the water bath.	No Brick red colour precipitate is formed	Absence of reducing sugars
6	Tollen's Mirror test: To 2ml of tollen's reagent add 2ml of sugar solution, mix well and boil for 2 minutes	No Silver mirror is formed	Absence of reducing sugasr

7	Barfoed's test: To 2ml of Barfoed's reagent, add 1ml of sample solution and heat in boiling water bath for 2 minutes (or) boil directly for 30 seconds. Cool under running tap water.	Red precipitate is formed in 5 minutes	Presence of disaccharides
10	Hydrolysis test: To 5ml of sample solution add 3 drops of conc. hydrochloric acid and boil for 1 minute. Cool and neutralize with 20% sodium carbonate solution till the effervescence ceases to the neutralized solution add 5ml of benedict's reagent boil for 2 minutes and cool.	Red colour precipitate is formed	Presence of sucrose

REPORT:

The given unknown sample was found to be

- **Carbohydrate**
- **Non-Reducing sugar**
- **Disaccharides**
- **Sucrose**

Ex.No:10

DATE:

QUALITATIVE ANALYSIS OF UNKNOWN SAMPLE OF CARBOHYDRATES SAMPLE [VI]

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	Action of heat: Heat a small amount of substance in a dry test tube.	A black residue is formed	Presence of carbohydrates
2	Action of conc.H_2SO_4 Take a small amount of sample solution in a dry test tube and add 2ml of conc. H_2SO_4	Solution becomes black in colour	Presence of carbohydrates
3	Molisch's test: To 2 ml of given sample solution add 5 drops of Molisch's reagent. Then slowly add 2ml of conc. H_2SO_4 along the sides of the test tube.	Violet ring is formed at the junction of two layers	Presence of carbohydrates
4	Benedict's test: To 5ml of Benedict's reagent add 8drops of given sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	No appearance of green, yellow, orange or brick red precipitate.	Absence of reducing sugars like glucose, fructose, lactose and maltose.
5	Fehling's test: To the given sample solution add fehling's A & B solution. Boil for 15 minutes on the water bath.	No Brick red colour precipitate is formed	Absence of reducing sugars
6	Tollen's Mirror test: To 2ml o tollen's reagent add 2ml of sugar solution, mix well and boil for 2 minutes	No Silver mirror is formed	Absence of reducing sugars
7	Barfoed's test: To 2ml of Barfoed's reagent add 1ml of sample solution and heat in boiling water both for 2 minutes	No Red precipitate is formed in 1 minute No Red precipitate is formed in 5 minutes	Absence of monosaccharide Absence of disaccharides

	(or) boil directly for 30 seconds. Cool under running tap water.		
10	Iodine test: To the sample solution add few drops of N/50 iodine solution	Deep blue colour is formed	Presence of starch

REPORT:

The given unknown sample was found to be

- **Carbohydrate**
- **Non - Reducing sugar**
- **Polysaccharides**
- **Starch**

Ex.No:11

DATE:

REACTION OF PROTEIN

INTRODUCTION:

Proteins are high molecular weight mixed polymers of α-amino acids joined together with peptide linkage.

Proteins are macromolecules and they form colloidal systems. Most of them are hydrophilic, therefore, they are hydrated. Being colloids, they are charged. Hence proteins can be precipitated by dehydration and neutralization of the electrical charges, which they carry, to bring them to the isoelectric point P^H.

All Proteins do not contain the same amino acids. The various amino acid constituents of proteins may be identified by various colour reactions. Based upon physical and chemical properties and the presence of different amino acids, proteins in a given solution can be analyzed under the following headings,

*Precipitation of protein

* Colour reaction of protein

* Reaction of specific protein

Eg. Albumin, globulin, Metaprotein, proteoses and peptones, Casein, mucin and gelatin

PRECIPITATION OF PROTEIN:

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1.	PRECIPITATION BY SOLUTION OF HEAVY METALS : • Take 3ml of protein solution and add 5% mercuric nitrate or mercuric sulphate drop by drop and observe the precipitate. • Take 3ml of protein solution and add 10% lead acetate solution	White precipitate is obtained White precipitate is obtained	Presence of protein Presence of protein

	drop by drop and observe the precipitate.		
PRINCIPLE: Proteins exist as negatively charged ions(anions) in pH higher than their isoelectric pH. To such a solution if salt of heavy metals are added, positively charged metal ions can complex with protein anion and metal proteinates are formed which gets precipitated.			
2.	PRECIPITATION BY ALKALOIDS REAGENT: To 2ml of protein solution, add 2 drops of 20% sulphosalicylic acid.	White precipitate or turbidity appears.	Protein is precipitated by sulpho salicylic acid.
PRINCIPLE: Proteins exist as positively charged ions(cations) in pH lower than their isoelectric PH. Certain alkaloidal reagents have negative, which neutralize positive charges thus resulting in precipitation.			
3.	PRECIPITATION BY ALCOHOLS: Take a 2ml of protein solution add 2ml of alcohol.	White precipitate is formed.	Presence of protein
PRINCIPLE: Precipitation occurs by alcohol due to dehydration or denaturation and removal of charges of proteins.			
4.	PRECIPITATION BY NEUTRAL SALT: HALF SATURATION: Take 3ml of protein solution in a test tube; add an equal volume of saturated ammonium sulphate solution to it, mixed and allowed to stand. FULL SATURATION: Take 3ml of protein solution, add ammonium sulphate salt to it and keep on adding and at the same time mix till protein solution become saturated.	White precipitate is formed. White precipitate is formed.	Presence of protein Presence of protein

PRINCIPLE: Neutral salts like ammonium sulphate precipitate protein by neutralization of charges on the protein and dehydration.			
5.	PRECIPITATION BY ACIDS: Take 3ml of conc. nitric acid in a test tube; slowly add protein solution along the sides of the test tube.	White precipitate is formed at the junction of two layers.	Protein is precipitate by nitric acid.
PRINCIPLE: Conc. Acid causes denaturation of protein, which brings native proteins into insoluble acid metaprotein. Derived proteins like gelatin and peptone are not sufficiently denaturated by acids and thus do not get precipitated.			
6.	PRECIPITATION BY ALKALIES: To 3ml of protein solutions, add 2ml of 40% sodium hydroxide and observe.	Precipitation occurs No precipitation occurs	Presence of gelatin and casein. Presence of albumin and peptones.
	PRINCIPLE: Casein and gelatin get denatured by alkali but alkali metaprotein of albumin is soluble, hence not precipitated.		
7.	PRECIPITATION AT ISOELECTRIC pH To 3ml of casein solution add a drop of bromocresol green (BCG) solution. Note the colour; add 2% acetic acid drop by drop till colour changes to green.	Note the formation of precipitate of casein. Bromocresol green has a green colour at a pH of 4.6 which is the isoelectric point of casein	Presence of casein
PRINCIPLE: The solubility of protein is minimum at their isoelectric pH as the protein molecules become electrically neutral at this pH.			

Ex.No:12

DATE:

COLOUR REACTIONS OF PROTEINS AND AMINO ACIDS:

INTRODUCTION:

Proteins are polymers of amino acids and are macromolecules. Principal linkage in proteins is peptide bond which binds the various amino acids in it. Proteins give characteristic colour on treatment with certain reagents due to presence of different amino acids or a class of amino acids having a characteristic group or due to certain grouping in the protein molecules.

S.NO.	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**BIURET TEST:** To 3ml of protein solution add an equal volume of 5% NaOH and 3 to 4 drops of 1% copper sulphate.	Purple or pinkish purple is produced.	Presence of peptide bond of protein.
	PRINCIPLE: This test is positive for all compounds containing more than one peptide linkage. Since all proteins contain peptide linkages, they respond to this test. The purple or violet colour is due to formation of copper coordination complex between cupric hydroxide and the peptide bond. The colour intensity depends upon the presence of number of peptide linkages.		
2.	**XANTHOPROTEIN TEST:** To 3ml of protein solution add 1ml conc.nitric acid boil for about half a minute. Cool and observe yellow colour. Now, add 2ml of conc.ammonia or 40% sodium hydroxide and observe the change in colour and after addition of alkali.	There is depending of yellow colour	Presence of aromatic amino acid.
	PRINCIPLE: There is nitration of the phenyl groups of aromatic amino acids on heating with nitric acid. The nitrophenyl groups give yellow colour to the solution. On addition of alkali, the nitrophenyl groups get ionized giving deep yellow colour to the solution.		

3.	**MILLON'S TEST:** To 1ml of protein solution add 1ml of 10% mercuric nitrate (prepared in 10% sulphuric acid). Boil gently for half a minute. Cool and add 3 drops of 1% solution of sodium nitrate. Mix and observe.	The red colour solution or precipitate or both are obtained	Presence of tyrosine in a solution
	PRINCIPLE: Sodium nitrate reacts with sulphuric acid to form nitrous acid. The protein gets precipitated by the mercuric sulphate, and boiling exposes the reacting groups. The exposed reacting groups (Phenol group of tyrosine) react with nitrous acid and give red colour precipitate.		
4.	**ALDEHYDE TEST: (REACTION FOR TRYPTOPHAN)** To 3ml of protein solution add 2 drops of 1 in 500 formalin and 1 drop of 10% mercuric sulphate (prepared in 10% sulphuric acid. Mix well thenadd gently through the side of the test tube about 3ml conc. Sulphuric acid.	Violet ring is formed at the junction of the layer	Presence of tryptophan in protein.
	PRINCIPLE: Aldehyde (aldehyde) react with the oxidized product of the indole nucleus of tryptophan to give violet coloured complex (sulphuric acid with mercuric sulphate is used as oxidizing agent in this reaction).		
5.	**SAKAGUCHIS' TEST:** To 5ml of protein solution add 5 drops of 5 % sodium hydroxide and 4 drops molichs' reagent. Mix and add 10 drops of bromine water.	A caramine red colour developed.	Presence of arginine
	PRINCIPLE: In an alkaline medium α-napthol combine with the guanidine group of arginine to form a complex which is oxidized by bromine to produce a caramine red colour.		

6.	**SULPHUR TEST:** To 3ml of protein solution add an equal volume of 40% sodium hydroxide and boil for 3 minutes. Cool and then add 1ml of lead acetate solution.	A dark grey or black precipitate is obtained.	Presence of sulphur containing aminoacid cysteine and cystine. Methionine does not answer to this test due to presence of thioether linkage which does not allow the release of sulphur in this reaction.
	PRINCIPLE: When cysteine and cystine are boiled with strong alkali, organic sulphur is converted to sulphide (Na_2S). This sodium sulphide react with lead acetate to form a black grey precipitate of lead sulphide		
7.	**NINHYDRIN TEST:** To 3ml protein solution add 3 drops of ninhydrin reagent in the (0.1% in acetone) heated to boil and cool.	A bluish purple colour is obtained. Yellow colour is obtained	Presence of α-amino acid. Presence of proline and hydroxyl proline.
	PRINCIPLE: Ninhydrin react with free α-amino acid to give bluish purple colour. Ninhydrin is a powerful oxidizing agent and causes oxidative decarboxylation of alpha amino acid producing an aldehyde. The reduced ninhydrin then react with ammonia and another molecule of ninhydrin and produces a bluish purple colour compound.		
8.	**MOLISCH'S TEST:** To 2ml of protein solutions add 6 drops of 1% molisch's reagent, add 2ml conc. Sulphuric acid along the side of the test tube.	Violet ring is formed at the junction of the solutions.	Presence of glycoprotein
PRINCIPLE: Carbohydrates present in protein when treated with conc. H_2SO_4 undergo dehydration to give furfural derivatives. These furfural derivatives condense with phenols to form coloured products. The phenol used in Molisch's reagent is α-naphthol. In case of oligo and polysaccharides they are first hydrolysed into monosaccharide by acids,which undergoes dehydration to form furfural or its derivatives. Any carbohydrates with more than four carbon atoms will give positive test.			

Ex.No: 13

DATE:

IDENTIFICATION TEST FOR ALBUMIN

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	**XANTHOPROTEIN TEST:** To 3ml of protein solution add 1ml conc.nitric acid boil for about half a minute. Cool and observe yellow colour. Now, add 2ml of conc.ammonia or 40% sodium hydroxide and observe the change in colour and after addition of alkali.	yellow colour is appears	Presence of aromatic amino acid.
2	**MILLON'S TEST:** To 1ml of protein solution add 1ml of 10% mercuric nitrate (prepared in 10% sulphuric acid). Boil gently for half a minute. Cool and add 3 drops of 1% solution of sodium nitrate. Mix and observe.	The red colour precipitate is obtained	Presence of tyrosine in a solution
3	**SULPHUR TEST:** To 3ml of protein solution add an equal volume of 40% sodium hydroxide and boil for 3 minutes. Cool and then add 1ml of lead acetate solution.	No dark grey or black precipitate is obtained.	Absence of sulphur containing aminoacid cysteine and cystine. Methionine does not answer to this test due to presence of thioether linkage which does not allow the release of sulphur in this reaction.

4	**HELLER'S TEST:** Take 3ml of con. nitric acid and add 2ml of urine along the sides of test tube.	A white ring of meta proteins appears at the junction of the fluids.	Presence of protein like albumin & globulins.
5	**PRECIPITATION BY NEUTRAL SALT:** HALF SATURATION: Take 3ml of protein solution in a test tube; add an equal volume of saturated ammonium sulphate solution to it, mixed and allowed to stand. FULL SATURATION: Take 3ml of protein solution, add ammonium sulphate salt to it and keep on adding and at the same time mix till protein solution become saturated.	No white precipitate is formed. White precipitate is formed.	Absence of Globulin, Casein. Presence of Albumin

REPORT:

The given sample was found to be Albumin.

Ex.No: 14

DATE:

IDENTIFICATION TEST FOR CASEIN

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1	**XANTHOPROTEIN TEST:** To 3ml of protein solution add 1ml conc.nitric acid boil for about half a minute. Cool and observe yellow colour. Now, add 2ml of conc.ammonia or 40% sodium hydroxide and observe the change in colour and after addition of alkali.	yellow colour is appears	Presence of aromatic amino acid.
2	**MILLON'S TEST:** To 1ml of protein solution add 1ml of 10% mercuric nitrate (prepared in 10% sulphuric acid). Boil gently for half a minute. Cool and add 3 drops of 1% solution of sodium nitrate. Mix and observe.	The red colour precipitate is obtained	Presence of tyrosine in a solution
3	**SULPHUR TEST:** To 3ml of protein solution add an equal volume of 40% sodium hydroxide and boil for 3 minutes. Cool and then add 1ml of lead acetate solution.	No dark grey or black precipitate is obtained.	Absence of sulphur containing aminoacid cysteine and cystine. Methionine does not answer to this test due to presence of thioether linkage which does not allow the release of sulphur in this reaction.
4	**HELLER'S TEST:** Take 3ml of con. Nitric	No white ring of meta proteins appears at the	Absence of protein like albumin & globulins.

	acid and add 2ml of urine along the sides of test tube.	junction of the fluids.	
5	**PRECIPITATION BY NEUTRAL SALT:** **HALF SATURATION:** Take 3ml of protein solution in a test tube; add an equal volume of saturated ammonium sulphate solution to it, mixed and allowed to stand. **FULL SATURATION:** Take 3ml of protein solution, add ammonium sulphate salt to it and keep on adding and at the same time mix till protein solution become saturated.	White precipitate is formed. No white precipitate is formed.	Presence of Protein like Globulin ,Casein. Absence of Albumin
6	**NEUMANN' TEST FOR ORGANIC PHOSPHOROUS:** Take 5ml of solution add 0.5ml of 40% sodium hydroxide.Heat for one minute and cool spontaneously. Add 0.5ml of con.nitric acid and 1ml of saturated ammonium molybdate solution.	A canary yellow colour or precipitate is formed.	Presence of inorganic phosphorous.
7	PRECIPITATION AT ISOELECTRIC pH To 3ml of casein solution add a drop of bromocresol green (BCG) solution. Note the colour; add 2% acetic acid drop by drop till colour changes to light green.	Note the formation of precipitate of casein. Bromocresol green has a green colour at a pH of 4.6 which is the isoelectric point of casein	Presence of casein

REPORT:

The given sample was found to be Casein.

Ex.No: 15

DATE:

QUALITATIVE ANALYSIS OF LIPIDS

INTRODUCTION:

Lipids are insoluble in water but soluble in solvents like alcohol, ether, chloroform etc. The lipids in the human body consist of neutral fats, phospholipids, cerebrosides, cholesterol and cholesterol esters, free fatty acids.

Lipids are transported in blood as lipoprotein, after combination with the apoproteins in the liver and to some extent in the small intestines. The different lipids carried by lipoproteins include triacylglycerols, phospholipids, cholesterol and its esters and free fatty acids. As lipids are hydrophobic they are necessarily converted into hydrophilic lipoprotein for transport in the aqueous medium of blood.

GENERAL PROCEDURE FOR QUALITATIVE ANALYSIS OF LIPIDS

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	FILTER PAPER TEST: Place a drop of given sample in its filter paper and allow to dry and observe after 5 minutes	Transparent spot occurs	Presence of lipids
2.	GREASE –SPOT TEST: Put a drop of oil over a piece of ordinary writing paper PRINCIPLE: All the lipids are greasy in nature	A translucent spot occurs	Presence of fat
3.	SOLUBILITY TEST: Take 2 dry test tubes and add 2ml of water, ether, to test tube 1, 2, respectively, now add 1 drop of oil to each test tube and shake well. PRINCIPLE: Oil is insoluble in polar solvents (water) but soluble in non polar solvents (ether, ethyl alcohol, chloroform and benzene)	In water oil is broken into small droplets and floats on the surface. But in other solvents oil is disappears	Presence of lipids

4.	EMULSIFICATION TEST: To 2ml of water in a test tube add one drop of oil and shake vigorously. Allow the test tube to stand and observe.	In water oil is broken into small droplets and floats on the surface.	Presence of lipids
	PRINCIPLE: When oil and water which are immiciscible are shaken together the oil is broken up into very tiny droplets which are dispersed in water. This is known as oil in water emulsion.		
5.	SAPONIFICATION TEST: To the ten drops of given oil 20 drops of 40% potassium hydroxide or 20% sodium hydroxide are mixed in a test tube and kept in a boiling water bath for 10 minutes till the solution is clear. Cool this mixture and divide into 4 parts • Two test tubes are taken, in one test tube take 5ml of water and other test tube take chloroform and add 3 drops of given sample solution in each test tube. • To 2ml of sample solution add 3 drops of conc. HCl. • To 2ml of sample solution add 2% calcium chloride • Add 2ml of sample solution to 2ml of saturated sodium chloride	Dissolve in water and precipitate in chloroform Fatty acids separate out White precipitate is formed. Precipitate is formed	Fats when treated with alkali hydroxide liberate fatty acids. This reaction of alkaline to form salt is called soaps. Soaps are insoluble in chloroform, but soluble in water. Conc. Acid cause hydrolysis of soap Precipitate by calcium salts is called saponification. Precipitate by sodium salts is called saponification.
6.	TEST FOR UNSATURATION: • Take 1ml of given oil, dissolve in 1ml of alcohol and2 drops of bromine water.	Decolourisation of bromine water takes place. No decolourisation of bromine water takes place	Presence of unsaturated fat. Presence of saturated fat.

	• Take 1ml of given oil, dissolve in 1ml of alcohol and 2 drops of $KMno_4$ water.	Decolourisation is occurs No decolourisation is occurs.	Presence of unsaturated fat. Presence of saturated fat.
	PRINCIPLE: Lipids are two types. They are saturated and unsaturated. Saturated lipids are liquid at room temperature. Unsaturated lipids are liquid at room temperature. Higher is the degree of unsaturation, lower is the temperature required to liquify it. Unsaturated fatty acids can react with halogens like bromine or iodine due to the presence of double bonds. Bromine goes into the solution forming a dibromide. In other words, the colour of bromine solution gets discharged. But when all bonds are saturated, bromine solution gives its own colour.		
7.	TEST FOR STEROIDS: • SALKOWSKI'S TEST: Take 5ml of sample in chloroform in a dry test tube; add gently along the sides, an equal volume of conc. sulphuric acid. Observe the upper chloroform layer and the lower acid layer.	The acid layer develops a yellow colour with green fluorescence. The chloroform layer will give a relay of colour from bluish red to gradually violet colour.	Presence of cholesterol
	PRINCIPLE: Cholesterol is dehydrated by sulphuric acid to form 3, 5 cholestadine. Polymers of this react with sulphuric acid to form their sulphuric acid derivatives, which give various colours.		
	• LIEBERMANN- BURCHARD REACTION: To about 2ml of sample dissolve in chloroform in a dry test tube and add 2ml of acetic anhydride and 2-3 drops of conc. Sulphuric acid. Mix and stand for a few minutes in the dark.	An emerald green colour develops.	Presence of cholesterol
	PRINCIPLE: Addition of sulphuric acid to cholesterol in the presence of acetic anhydride gives a green chromphore. Acetic anhydride removes any trace of moisture.		

Ex.No: 16

DATE:

QUALITATIVE ANALYSIS OF LIPIDS

SAMPLE-I

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	FILTER PAPER TEST: Place a drop of given sample in its filter paper and allow to dry observe after 5 minutes	Transparent spot occurs	Presence of lipids
2.	GREASE –SPOT TEST: Put a drop of oil over a piece of ordinary writing paper.	A translucent spot occurs	Presence of fat
3.	SOLUBILITY TEST: Take 2 dry test tubes and add 2ml of water, ether, to test tube 1, 2, respectively, now add 1 drop of oil to each test tube and shake well.	In water oil is broken into small droplets and floats on the surface. But in other solvents oil is disappears	Presence of lipids
4.	EMULSIFICATION TEST: To 2ml of water in a test tube add one drop of oil and shake vigorously. Allow the test tube to stand and observe. Now add a few drops of soap solution to the same and shake. Allow to stand and observe.	In water oil is broken into small droplets and floats on the surface. Oil is now seen in minute droplets suspended in the liquid.	Presence of lipids Presence of lipids
5.	SAPONIFICATION TEST: To the ten drops of given oil 20 drops of 40% potassium hydroxide or 20% sodium hydroxide are mixed in a test tube and kept in a boiling		

	water bath for 10 minutes till the solution is clear. Cool this mixture and divide into 4 parts • Two test tubes are taken, in one test tube take 5ml of water and in other test tube take chloroform and add 3 drops of given sample solution in each test tube. • To 2ml of sample solution add 3 drops of conc. HCl. • To 2ml of solution add 2% calcium chloride • Add 2ml of sample solution to 2ml of saturated sodium chloride	Dissolves in water and precipitate in chloroform Fatty acids are separated out. White precipitate is formed. Precipitate is formed	Fats when treated with alkali hydroxide liberate fatty acids. This reaction of alkaline to form salt is called soaps. Soaps are insoluble in chloroform, but soluble in water. Conc. Acid cause hydrolysis of soap Precipitate by calcium salts called saponification. Precipitate by sodium salts called saponification.
6.	TEST FOR UNSATURATION: • Take 1ml of given oil, dissolve in 1ml of alcohol 2 drops of bromine water. • Take 1ml of given oil, dissolve in 1ml of alcohol 2 drops of $KMno_4$ water.	Decolourisation of bromine water takes place. Decolourisation is occurs	Presence of unsaturated fat. . Presence of unsaturated fat.
7.	TEST FOR STEROIDS: • SALKOWSKI'S TEST: Take 5ml of sample in chloroform in a dry test tube; add gently along the sides, an equal volume of conc. sulphuric acid. Observe the upper chloroform layer and the lower acid layer.	The acid layer develops a yellow colour with green fluorescence. The chloroform layer will give a relay of colour from bluish red to gradually	Presence of cholesterol

		violet colour.	
	• LIEBERMANN- BURCHARD REACTION: To about 2ml of sample dissolve in chloroform in a dry test tube and add 2ml of acetic anhydride and 2-3 drops of conc. Sulphuric acid. Mix and stand for a few minutes in the dark.	An emerald green colour develops.	Presence of cholesterol

REPORT:

The given sample contain

(i). **Lipids**

(ii). Unsaturated lipids

(iii). Cholesterol

Ex.No: 17

DATE:

GENERAL PROCEDURE FOR ANALYSIS OF NORMAL CONSTITUENT OF URINE

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Examination: a) Appearance	Clear	Normal urine is clear and transparent.
	b)Colour	Straw coloured	Due to the presence of urochrome.
	c)PH	6	PH of the normal urine is 4.8-7.0
	d)Specific gravity	1.018	Normal specific gravity of urine is 1.016-1.022
2.	Test for inorganic constituent: a) Test for chloride: To the 5ml of urine sample, add con. nitric acid and 1ml of 3% silver nitrate.	A white precipitate of silver chloride is formed.	Presence of chloride in urine
	b)Test for calcium and Phosphates: Take 10ml of urine add 2ml of ammonium hydroxide boil it & cool, then filter it. Filtrate is	White precipitate is formed.	

	collected and the precipitate is dissolved in quantity of acetic acid and divided into 2 parts. i) To one part, add 1ml of 5% potassium oxalate solution. Ii) To the 2nd part, add 1ml con. nitric acid if necessary boil and add 1ml of ammonium molybdate reagent and mix well. c) Test for ammonia: Take 5ml of urine in a test tube & add a drop of phenolphthalein and add 2% sodium carbonate drop by drop until the colour becomes faint pink. Allow to boil and hold a glass rod dipped in phenolphthalein at the mouth of the test tube. d) Test for inorganic sulphate: Take 5ml of urine in a test tube. Add 1ml of con. Hydrochloric acid. Mix well and add 5ml of 10% barium chloride solution. Filter it and use the filtrate for next experiment.	Yellow colour precipitate occurs. Phenolphthalein indicator in the glass rod changes to pink colour. A white precipitate of barium sulphate is formed.	Presence of calcium. Presence of phosphate. Presence of ammonia. Presence of inorganic sulphate.
3.	Test for organic constituent:	Precipitate is formed.	Due to the presence of

	Collect the above filtrate & allow it to boil.		organic constituent.
4.	Test for urea: Take 5ml of urine in a test tube & add 2 drops of freshly prepared alkaline sodium hypobromite solution.	Effervescence is produced.	Alkaline hypobromite react with urea which forms gases like nitrogen & co_2
5.	Test for creatinine: Jaffe's test: 3ml of urine and 3ml of water are taken in 2 separate test tubes. 1ml of saturated picric acid and 10 drops of 10% sodium hydroxide are added to both the test tubes and mixed. Wait for 5 minutes.	Orange red colour is developed in the test tube containing urine.	Due to the formation of creatinine picrate
6.	Test for uric acid: Take 3ml of urine and add 1ml of 1% sodium carbonate and 1ml of dil. Phosphotungstic acid.	Blue colour is appeared.	Presence of uric acid.

REPORT:

The constituents of normal urine are

- **Chloride**
- **Calcium**
- **Phosphate**
- **Ammonia**
- **Inorganic sulphate**
- **Organic constituent**
- **Urea**
- **Creatinine**
- **Uric acid**

Ex.No:18

DATE:

GENERAL PROCEDURE FOR ANALYSIS OF ABNORMAL CONSTITUENT OF URINE

(Pathological constituents of urine)

The commonly encountered pathological chemical constituents of urine are

1. **PROTEIN: May be albumin or globulin**

2. BLOOD: Haemoglobin, Erythrocytes

3. REDUCING SUGAR: Usually glucose and in special cases lactose, galactose, pentose and rarely fructose

4. KETONE BODIES: Acetone, aceto acetic acid

5. BILE SALTS & BILE PIGMENTS: Sodium and potassium salts of glycol/ taurcholic acids,Bilirubin

6. PORPHOBILINOGEN

7. UROBILINOGEN

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1.	**TEST FOR PROTEIN:** a)Heat coagulation test: Fill ¾ of a test tube with the urine acidified with 2% acetic acid mix & heat the upper portion.	Coagulation occurs	Presence of protein
	Principle: Urine contains mainly albumin which is a heat coagulable protein		
	b) Sulphosalicylic acid test: To 5ml of urine, add 1ml of 20% Sulpho salicylic acid.	White precipitate is formed	Presence of protein
	Principle: Protein is precipitated by Sulpho salicylic acid by removal of charges on the protein		
	c)Heller's test: Take 3ml of con. Nitric acid and	A white ring of meta proteins appears at the	Presence of protein

	add 2ml of urine along the sides of test tube.	junction of the fluids.	
2.	**TEST FOR BLOOD:** a)Ortho-Tolidine test: To 6 drops of freshly prepared O-Tolidine add 6 drops of hydrogen peroxide and 4 drops of previously boiled urine.	A transient dark green colour is formed	Presence of haemoglobin
	Principle: Heme part of haemoglobin has peroxidise like activity which release the nascent oxygen from H_2O_2. This nascent oxygen oxidizes O-Tolidine which gives green colour. Colour disappears after few minutes.		
	b)Benzidine test: To 6 drops of freshly prepared benzlidine add 6 drops of hydrogen peroxide and 4 drops of previously boiled urine.	A transient dark green colour is formed	Presence of haemoglobin
3.	**TEST FOR REDUCING SUGAR:** Benedict's test: To 5ml of Benedict's reagent, add 8 drops of urine sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	Colour changes from blue to green, yellow, orange or brick red precipitate.	Presence of reducing sugar.
	Principle: Benedict's reagents contain copper sulphate, sodium carbonate and sodium citrate. The mild alkali sodium carbonate converts glucose into enediol. This enediol reduces copper sulphate to cuprous hydroxide that is unstable and decomposes on boiling to cuprous oxide. The precipitated cuprous oxide will have different shades of colour depending upon the concentration.		

	Sodium citrate present in this reagent prevents the precipitation of cupric ion as cupric hydroxide by forming cupric sodium citrate complex. It also improves the self life of the reagent by preventing an interaction between sodium carbonate and copper sulphate.		
4.	**TEST FOR KETONE BODIES:** a)Rother's test: Take 5ml of urine and fully saturated with solid ammonium sulphate. This is to remove substances, which may interfere with the test. Then, add5 drops of a freshly prepared solution of sodium nitro prusside and gently shake. Then add a few ml of con.ammonia and mix it.	A permanganate colour appears.	Presence of acetone
	Principle: The nitro prusside in alkaline medium(due to con. Ammonia) react with ketone group to form a permanganate colour.		
	b)Gerhardt's test: To about 5ml urine in a test tube, add drop wise 10% ferric chloride	A wine red colour appear	Presence of aceto acetic acid
5.	**TEST FOR BILE SALTS & BILE PIGMENTS**: a)Hay's test: (for bile salt) Take two test tubes one with 5ml urine (A) and other with 5ml of water (B). Now, gently sprinkle flowers of sulphur into both.	Test tube (A) containing sulphur powder sink.	Presence of bilesalts
	Principle: Hay's test is based on the fact that bile salts lower the surface tension of urine		

	allowing the sulphur to sink.		
	b) Fouchet's test : (For bile pigments) Take 5ml of urine add a few crystals of magnesium sulphate and shake the tube till it dissolves. Now add 10% barium chloride in excess (about 10ml). A precipitate of barium sulphate is formed. The bile pigment get adsorbed to the precipitate of barium sulphate. Filter the contents of the tubes, the filter may be discarded. Dry the precipitate by using filter paper. To the dry precipitate add a drop of fouchets reagent that contain ferric chlorides as the oxidising agent	Green colour appear	Presence of bile pigments
	Principle: Ferric chloride reagent act as a oxidizing agent it oxidises bilirubin to biliverdin (green) or bilicyanin (blue)		
6.	**TEST FOR UROBILINOGEN AND PORPHOBILINOGEN:** Ehrlich's diazo test: To 5ml of urine add 5ml of ehrlichs diazo reagent mix well and allow it to stand for 10 mts.Add 5ml of saturated sodium	Chloroform layer changes to pink colour Aqueous layer changes to pink colour	Presence of Urobilinogen Presence of Porphobilinogen.

	acetate and mix. Now add 5ml of chloroform and shake vigorously for a few seconds and allow the layers to separate.		

EX.NO:19

DATE:

QUALITATIVE ANALYSIS OF ABNORMAL CONSTITUENT OF URINE

SAMPLE- I

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Protein:** a)Heat coagulation test: Fill ¾ of a test tube with the urine acidified with 2% acetic acid mix & heat the upper portion.	Coagulation occurs	Presence of protein
	b) Sulphosalicylic acid test: To 5ml of urine add 1ml of 20% Sulpho salicylic acid.	White precipitate is formed	Presence of protein
	c)Heller's test: Take 3ml of con. nitric acid add 2ml of urine along the sides of test tube.	A white ring of meta proteins appears at the junction of the fluids.	Presence of protein
2.	**Test for blood:** a)Ortho-Tolidine test: To 6 drops of freshly prepared O-Tolidine add 6 drops of hydrogen peroxide and 4 drops of previously boiled urine.	No transient dark green colour is formed	Absence of haemoglobin
	b)Benzidine test: To 6 drops of freshly prepared benzlidine add 6 drops of hydrogen peroxide and 4 drops of previously boiled urine.	No transient dark green colour is formed	Absence of haemoglobin

3.	**Test for reducing sugar:** Benedict's test: To 5ml of Benedict's reagent add 8 drops of urine sample. Boil over a flame for 2 minutes or place them in a boiling water bath for three minutes and allow to cool.	Colour changes from blue to orange precipitate.	Presence of reducing sugar.
4.	**Test for ketone bodies:** a)Rother's test: Take 5ml of urine and fully saturated with solid ammonium sulphate. This is to remove substances, which may interfere with the test. Then, add5 drops of a freshly prepared solution of sodium nitro prusside and gently shake. Then add a few ml of con.ammonia and mix it.	No permanganate colour appear	Absence of acetone
	b)Gerhardt's test: To about 5ml urine in a test tube, add drop wise 10% ferric chloride	No wine red colour appears.	Absence of aceto acetic acid
5.	**Test for bile salts & bile pigments:** a)Hay's test: (for bile salt) Take two test tubes one with 5ml urine (A) and other with 5ml water (B). Now gently sprinkle flowers of sulphur into both.	Test tube (A) containing sulphur powder does not sink.	Absence of bilesalts

	b) Fouchet's test : (For bile pigments) Take 5ml of urine add a few crystals of magnesium sulphate and shake the tube till it dissolves. Now add 10% barium chloride in excess (about 10ml). A precipitate of barium sulphate is formed. The bile pigments get adsorbed to the precipitate of barium sulphate. Filter the contents of the tubes, the filter may be discarded. Dry the precipitate by using filter paper. To the dry precipitate add a drop of fouchets reagent that contains ferric chlorides as oxidising the agent.	No Green colour appears.	Absence of bile pigments
6.	**Test for Urobilinogen and porphobilinogen**: Ehrlich's diazo test: To 5ml of urine add 5ml of ehrlichs diazo reagent mix well and allow it to stand for 10 mts. Add 5ml of saturated sodium acetate and mix. Now add 5ml of chloroform. Shake vigorously for a few seconds and allow the layers to separate.	No Chloroform layer changes to pink colour No Aqueous layer changes to pink colour	Absence of Urobilinogen Absence of Porphobilinogen

REPORT:

The given unknown sample contain

1. Protein

2. Reducing sugar

EX.NO: 20

DATE:

QUALITATIVE ANALYSIS OF ABNORMAL CONSTITUENT OF URINE

SAMPLE- II

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Protein:** a)Heat coagulation test: Fill ¾ of a test tube with the urine acidified with 2% acetic acid mix & heat the upper portion.	No Coagulation occurs	Absence of protein
	b) Sulphosalicylic acid test: To 5ml of urine add 1ml of 20% Sulpho salicylic acid.	No White precipitate is formed	Absence of protein
	c)Heller's test: Take 3ml of con. nitric acid add 2ml of urine along the sides of test tube.	No white ring of meta proteins appears at the junction of the fluids.	Absence of protein
2.	**Test for blood:** a)Ortho-Tolidine test: To 6 drops of freshly prepared O-Tolidine add 6 drops of hydrogen peroxide and 4 drops of previously boiled urine.	No transient dark green colour is formed	absence of haemoglobin
	b)Benzidine test: To 6 drops of freshly prepared benzlidine add 6 drops of hydrogen peroxide and 4 drops of previously boiled urine.	No transient dark green colour is formed	Absence of haemoglobin

3.	**Test for reducing sugar:** Benedict's test: To 5ml of Benedict's reagent add 8 drops of urine sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	Colour changes from blue to orange precipitate.	Presence of reducing sugar.
4.	**Test for ketone bodies:** a)Rother's test: Take 5ml of urine and fully saturated with solid ammonium sulphate. This is to remove substances, which may interfere with the test. Then, add5 drops of a freshly prepared solution of sodium nitro prusside and gently shake. Then add a few ml of con.ammonia and mix it.	No permanganate colour appear	Absence of acetone
	b)Gerhardt's test: To about 5ml of urine in a test tube, add drop wise 10% ferric chloride	No wine red colour appears.	Absence of aceto acetic acid
5.	**Test for bile salts & bile pigments:** a)Hay's test: (for bile salt) Take two test tubes one with 5ml urine (A) and other with 5ml water (B). Now gently sprinkle flowers of sulphur into both.	Test tube (A) containing sulphur powder sinks.	Presence of bilesalts

	b) Fouchet's test : (For bile pigments) Take 5ml of urine add a few crystals of magnesium sulphate and shake the tube till it dissolves. Now add 10% barium chloride in excess (about 10ml). A precipitate of barium sulphate is formed. The bile pigments get adsorbed to the precipitate of barium sulphate. Filter the contents of the tubes, the filter may be discarded. Dry the precipitate by using filter paper. To the dry precipitate add a drop of fouchets reagent that contains ferric chlorides as the oxidising agent.	No Green colour appear	Absence of bile pigments
6.	**Test for Urobilinogen and porphobilinogen**: Ehrlich's diazo test: To 5ml of urine add 5ml of ehrlichs diazo reagent mix well and allow it to stand for 10 mts.Add 5ml of saturated sodium acetate and mix. Now add 5ml of chloroform. Shake vigorously for a few seconds and allow the layers to separate.	No Chloroform layer changes to pink colour No Aqueous layer changes to pink colour	Absence of Urobilinogen Absence of Porphobilinogen

REPORT:

The given unknown sample contains

1**. Reducing sugar**

2. Bile salts

EX.NO:21

DATE:

QUALITATIVE ANALYSIS OF ABNORMAL CONSTITUENT OF URINE

SAMPLE- III

S.NO.	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Protein:** a)Heat coagulation test: Fill ¾ of a test tube with the urine acidified with 2% acetic acid mix & heat the upper portion.	Coagulation occurs	Presence of protein
	b) Sulphosalicylic acid test: To 5ml of urine add 1ml of 20% Sulpho salicylic acid.	White precipitate is formed	Presence of protein
	c)Heller's test: Take 3ml of con. Nitric acid add 2ml of urine along the sides of test tube.	A white ring of meta proteins appears at the junction of the fluids.	Presence of protein
2.	**Test for blood:** a)Ortho-Tolidine test: To 6 drops of freshly prepared O-Tolidine add 6 drops of hydrogen peroxide and 4 drops of previously boiled urine.	No transient dark green colour is formed	Absence of haemoglobin
	b)Benzidine test: To 6 drops of freshly prepared benzlidine add 6 drops of hydrogen peroxide and 4 drops of previously boiled urine.	No transient dark green colour is formed	Absence of haemoglobin

3.	**Test for reducing sugar:** Benedict's test: To 5ml of Benedict's reagent add 8 drops of urine sample. Boil over a flame for 2 minutes or place in a boiling water bath for three minutes and allow to cool.	No colour changes from blue to orange precipitate.	Absence of reducing sugar.
4.	**Test for ketone bodies**: a)Rother's test: Take 5ml of urine and fully saturated with solid ammonium sulphate. This is to remove substances, which may interfere with the test. Then, add5 drops of a freshly prepared solution of sodium nitro prusside and gently shake. Then add a few ml of con.ammonia and mix it.	No permanganate colour appears	Absence of acetone
	b)Gerhardt's test: To about 5ml urine in a test tube, add drop wise 10% ferric chloride	No wine red colour appear	Absence of aceto acetic acid
5.	**Test for bile salts & bile pigments:** a)Hay's test: (for bile salt) Take two test tubes one with 5ml urine (A) and other with 5ml water (B). Now gently sprinkle flowers of sulphur into both.	Test tube (A) containing sulphur powders sink.	Presence of bile salts

	b) Fouchet's test : (For bile pigments) Take 5ml of urine add a few crystals of magnesium sulphate and shake the tube till it dissolves. Now add 10% barium chloride in excess (about 10ml). A precipitate of barium sulphate is formed. The bile pigments get adsorbed to the precipitate of barium sulphate. Filter the contents of the tubes, the filter may be discarded. Dry the precipitate by using filter paper. To the dry precipitate add a drop of fouchets reagent that contains ferric chlorides as oxidising the agent.	No Green colour appear	Absence of bile pigments
6.	**Test for Urobilinogen and porphobilinogen**: Ehrlich's diazo test: To 5ml of urine add 5ml of ehrlichs diazo reagent mix well and allow it to stand for 10 mts.Add 5ml of saturated sodium acetate and mix. Now add 5ml of chloroform. Shake vigorously for a few seconds and allow the layers to separate.	No Chloroform layer changes to pink colour No Aqueous layer changes to pink colour	Absence of Urobilinogen Absence of Porphobilinogen

REPORT:

The given unknown sample contains

1**. Proteins**

2. Bile salts