School of Pharmacy

B.Pharmacy (2nd Semester)

LIST OF EXPERIMENTS FOR B PHARMACY (2nd semester) BIOCHEMISTRY

1	**To demonstrate the different methods of measuring pH and their significance**	
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3	**To perform qualitative tests for reducing sugars.**	
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7	To carry out quantitative estimation of reducing sugars(glucose) by di-nitrosalicylicacid(DNSA) method in given sample	
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12	**To carry out estimation of the enzyme activity using maltose standard curves**	
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Experiment 1

Different Methods Of Measuring pH And Their Significance

AIM:

To demonstrate the different methods of measuring pH and their significance

Equipment required: pH meter, pH Strips Material required: Buffers (sample)

Learning objectives: To understand the principle and working of various method of determining pH

Theory/Principle/Background of the topic:

There are millions of chemical substances in the world, some are acids, others are bases and some are neutral.

Acids and Bases

Acids are substances that produce free hydrogen ions (H" ions) when dissolved in water. Bases are substances that produce hydroxyl ions (OH ions) when dissolved in water. Acidic solutions are rich in hydrogen ions and basic solutions are poor in hydrogen ions.

Some acids dissociate only partly, releasing very small amounts ofH' ions, and are called weak acids. Others dissociate completely, releasing large amounts of H ions, and are called strong acids. In the same way, bases that dissociate partly are called weak bases and those that dissociate completely are called strong bases.

pH is defined as the negative logarithm of the hydrogen ion concentration. This definition of pH was introducedin 1909 by the Danish biochemist, Soren Peter Lauritz Sorensen.

It is expressed mathematically as:

pH = -log [H*]

where: [H+] is hydrogen ion concentration in mol/L

This value ranges from 0 to 14 pH. Values below 7 pH exhibit acidic properties.

Values above 7 pH exhibit basic (also known as caustic or alkaline) properties Since 7 pH is the center of the measurement scale, it is neither acidic nor basic, it is called "neutral." The pH of pure water is 7. i.e., [H*1 -[OH"] 10 - 7 at 25°C. So ph =7

Methods of Measuring pH:

pH in an aqueous solution can be measured in a variety of ways.

A. Colorimetric method:

pH paper

pH paper is a strip of special paper that is prepared by dipping the strip in different chemical compounds and then drying it. It can be used to find the approximate pH of any solution. These paper changes colour when immersed in acidic or basic solutions. To determine the pH, the pH paper is dipped in a given sample solution and the colour developed in the paper is compared

with the colour chart and the approximate pi of the solution can be identified It is commerically available as test papers.

Universal indicator

Universal indicator is a pH indicator composed of different chemical compounds. indicator is poured in the sample solution, the mixture obtained exhibits a smooth colour change over a pH value ranging from 1-14 that indicates the acidic or basic property of the solution. It is commercially available as test solutions A universal indicator is typically composed of water, propun-1-ol, phenolphthalein sodium salt, sodium hydroxide, methyl red.bromothymol blue monosodium salt, and thymol blue monosodium salt

Advantages:

• This method is simple

Disadvantages:

• A high degree of accuracy cannot be expected due to errors

*Various erors include,

- Error due to high salt concentration in the test liquid

• Eror due to the temperature of the test liquid

- Eror due to organic substances in the test liquid

B. Electrometric method:

This method uses a pH-sensitive glass eloctrode, a reference electrode and a pH meter

The working of the electrode is described according to the Nemst Equation given by I9th century Chemist. Hemmann Walther Nemst (1864–1941). For hydrogen ion activity,

where n=1, Nemst factor is 59 mv for every ten-fold change in activity at 250C. This means that for every pH unit change, the total potential will change 59.16 mV.

It is expressed as E =E0-2.3RT/nf log ai

where

E= total potantial in milivolts between two electrodes

E0=. Standard potential of the ion

R= univenalgasconstant in Joule mole/calvin

T =absolute temperahure in Kelvin)

N=.charge of the lon

F=Faraday oonstart (in Coulombs/mol)

Ai= activity of the ion

A pH meter measures essentially the electro-chemical potential between a known liquid inside the glass electrode (membrane) and an unknown liquid outside.

The glass electrode connists of a sturdy glass tube with a thin glass bulb welded to it, Inside is known solution of potassium chloride (KCI) buffered at a pH of 7.0. A silver electrode with a silver chloride tip makes contact with the inside solution. To minimise electronic interference, the probe shielded by a foil shield, often found inside the glass electrode.

2. Outline of procedure:

For very precise measurement, the pH meter should be calibrated before each measurement. The calibration should be performed with at least two buffer solutions with known pH. For general purposes, buffer solutions with pH 4. pH 7 and pH 9.2 can be used. For more precise measurements 3 buffer solution calibration preferred.

Application of pH determination:

All human beings and animals rely on intermal mechanisms to maintain the pH level of their blood The blood flowing through our veins must have a pH between 7.35 and 7.45, Exceeding this range by as little as one-tenth of a pH unit could prove fatal.

• To attain high crop yield, by conditioning ficld to correct pH value.

• Checking the changing composition and causc of rain to prevent crop from detrimental effects of Acid rain

• Proper pH econtrol keeps milk from tuming sour, makes strawberry jelly gel, and prevents shampoo from stinging your eyes.

• Neutralization of effluent in steel, pulp and paper, chemical, and pharmaccutical manufacturing

• Hexavalent chromium destruction

• Cyanide destruction

• Reverse osmosis

• Odor scrubbers

• Pharmaccutical manufacturing

• Chemical and petrochemical manufacturing

•Cooling tower control

Required results: Determination of pH of the given sample

Parameters: pH

Cautions: After each single mesurement, the bulb is rinsed with distilled water or deionised water to remove any traces of solution being measured Then the bulb is blotted with a blotting paper to remove remaining water that could dilute the sample and alter the reading. When not in use, the bulb must be kept wet at all times to avoid dehydration of the pH sensing membrane.

Experiment 2

Prepare Standard Buffers (Citrate, Phosphate And Carbonate) And Measurement Of pH

AIM:

To prepare standard buffers (citrate, phosphate and carbonate) and measurement of pH.

Equipment required pH meter Materials required Citric acid, Sodium citrate, Diethyl barbituric acid, Sodium diethyl barbititrate, monobasic and dibasic sodium phosphate

Learning objectives

To understand the components of respective buffer and technical operation of pH meter.

Theory/Principle/Background of the topic:

A buffer is a solution of weak acid and conjugate base or weak base and conjugate acid usod to resist pH change with added solute. Buffer solutions are resistant to pH change because of the presence of an oquilibrum between the acid (HA) and its conjugate base (A-). When some strong acid is added to a buffer, the equilibrium is shifted to the left, and the hydrogen ion concentration increases by less than expected for the amount of strong acid added. Buffer solutions are necessary in biology for keeping the correct pH for proteins to work. Buffers can be prepared in multiple ways by creating a solution of an acid and its conjugate base. electncal potential developed by pair of electrode pins in a solution

The pH meter measures For measurement of pH, an electrode system sensitive to change in H+ ion concentration of solution is taken. The electrode system consists of sequence of electrode raise with pH (H+ concentration of the solution).

Outline of procedure

a. Reagents preparation for citrate buffer:

Citric acid Dissolve 2.101 gm of citric acid in 100ml distilled water.

Sodium citrate solution 0.1 M: Dissolve 2.941gm of sodium citrate in 100ml distilled water

Procedure for preparation of citrate buffer (pH: 2.5):

a) 46 Sml of citric acid with 3.5ml sodium citrate solution and upto 100 ml with distilled water

b) It corresponds to 0.1 M citrate buffer and standardized with pH meter and measures the pH of the prepared solution,

c) This gives citrate buffer at pH 2.5,

b. Reagents preparation for phosphate buffer (pH: 6.8):

Monobasic: Dissolve 2.78gm of sodium dihydrogen phosphate in 100ml of distilled water Dibasic sodium phosphate (0.2M): Dissolve 5.3gm of disodium hydrogen phosphate or 7.17 gm sodium hydrogen phosphate in 100ml distilled water.

Procedure for preparation of citrate buffer:

A) 39 ml of dihydrogen sodium phosphate is mixed with 61 ml of disodium hydrogen phosphate.

b) This made up to 200ml with distilled water

c) This gives phosphate (Po4)2 buffer of 0.2M

d) Standardized pH meter with standard buffer.

e) Wash electrode with distilled water and introduced it into phosphate buffer prepared of f) the pH of solution is 68

c. Reagents preparation for carbonate buffer: Diethylbarbituric acid. Sodium diethyl barbitutrate

d. Procedure for preparation of eitrate buffer (pH: 6.8):

a) Dissolve 2.85gm of diethyl barbituric acid and 14.2gm of sodium diethyl distilled water and upto I liter. This gives the barbitone buffer.

b) The pH meter is first standararised with pH buffer.

c) Wash electrode with distilled water and introduced into barbitone buffer prepared, the pH of solution is 6.8.

Required results

Presence of desirable pH of the respective buffer

Parameters: pH

Cautions: Care should be taken while handling caustic acids like conc. suiphuric acid, nitric acid or hydrochloric acid.

Experiment – 3

Qualitative Tests For Reducing Sugars

AIM:

To perform qualitative tests for reducing sugars.

Equipment required: Water bath

Materials required Molisch's Reagent, lodine solution, Fehling's reagent A, Fehling's reagent B, Benedict's qualitative reagent, Barfoed's reagent, Seliwanoff 's reagent, Bial's reagent, Phenylhydrazine hydrochloride, Sodium acctate, Glacial acetic acid, Glucose, fructose, Microscope

Learning objectives: To characterize and identify unknown carbohydrates present in an unknown solution on the basis of various qualitative tests.

Theory/Principle/Background of the topic:

A carbohydrate is an organic compound with the general fomulaCm(H2O)n, that is, consists only of carbon, hydrogen and oxygen, with the last two in the 2:1 atom ratio.

Molisch's Test (general test): This is a common test for all carbohydrates larger than tetrosesThe is on the basis that pentoses and hexoses are dehydrated by conc. sulphuric acid to form furfural hydroxymethylfurfural, respectively. These products condense with a-naphthol to form purple condensation product

Test for reducing and non-reducing sugars

Reducing sugar: The ability of a sugar to reduce alkaline test reagents depends on the availabilie of an aldehyde or keto group for reduction reactions. These sugars, therefore, become potentil agents capable of reducing Cu+2 to Cut, Ag+ to Ag etc.

Fehling's Test: This forms the reduction test of carbohydrates. Fehling's solution contains biuk alkaline cupric hydroxide solution, heated with reducing sugars gets reduced to yellow of cuprous oxide and is precipitated. Hence, formation of the vellow or brownish-red colole precipitate helps in the detection of reducing sugars in the test solution.

Benedict's test: As in Fehling's test, free aldchyde or keto group in the reducing sugarn the cupric hydroxide in alkaline medium to red colored cuprous oxide. Depending on concentration of sugars, yellow to green color is developed. All monosaccharides are reducing sugars as they all have a free reactive carbonyl group. Some disaccharides, like maltose, have exposed carbonyl groups and are also reducing sugars, but less reactive than monosaccharides.

Barfoed's Test: Barfoed's test is used to detect the presence of monosaccharide (reducing) sugars in solution Barfoed's reagent, a mixture of cthanoic (acetic) acid and copper(II) acetate, is combined with the solution and boiled. A red copper(Il) oxide precipitate is formed will indicates the presence of reducing sugar. The reaction will be negative in the presence of disaccharide sugars because they are weaker reducing agents This test is specific for monosaccharides. Due to the weakly acidic nature of Barfoed's reagent, it is reduced only by monosaccharides. Disaccharides may also react with this reagent, but the reaction is much slower when compared to monosaccharides.

Seliwanoff's test: Seliwanoff s Test distinguishes between aldose and ketose sugars. Ketoses are distinguished from aldoses via their ketone/aldehyde functionality. This test is based on thc fact that, when heated, ketoses are more rapidly dehydrated than aldoses.

Bial's test is used to distinguish between pentoses and hexoses. They react with Bial's reagent and are converted to furfural,Orcinol and furfural condense in the presence of ferric ion to form a colored product. Appearance of green colour or precipitate indicates the presence of pentoses and formation of muddy brown precipitate shows the presence of hexoses.

Sr no.	Test	Observation	Inference
1	Molisch's Test In a test tube, add 2 ml of the test carbohydrate solution and 2 drops of a-naphthol solution. Carefully incline the tube and pour dropwise conc. H2SO using a dropper, along the sides of the tube	A deep violet coloration is produced at the junction of two layers.	Presence of carbohydrates
2	Fehling's test About 2 ml of sugar solution is added to about 1 ml of Fehling's solution A (aqueous solution of CuSO4) and 1 ml of Fehling solution B (solution of potassium tartrate). Mix well and then boiled for 10 min.	A red precipitate of cuprous oxide is formed	Presence reducing sugar of (glucose, fructose, maltose, lactose)
3	Benedict's test To 2 ml of Benedict's solution, add 5-6 drops of the test solution and shake cach tube. Place the tube in a boiling water bath and heat for 3	Color change from blue to green, yellow, orange according amount present	Presence reducing sugars of (glucose, fructose, maltose, lactose)

	minutes. Remove the tubes from the heat and allow them to cool.		
4	Barfoed's reagent test To 1-2 mL of Barfoed's reagent, add an equal volume of sugar solution. Boil for 1 min, in a water bath and allow to stand for few minutes.	a brick-red cuprous oxide precipitate in the bottom and along sides of tube test immediately	Presence of Monosaccharides (glucose, fructose)
5	Seliwanoffr's test Heat 1 mL of sugar solution with Seliwanoff's reagent (0.5 g resorcinol liter 10% HCI) in boiling water.	less than 30 seconds, apearance of cherry red colorr Upon prolonged heating, appearance of red colour	Presence of ketoses (Fructose) Presence of aldoses (Glucose)
6	Bial's test To 5 ml. of Bial's reagent add 2-3 ml of kest solution and warm gently in a hot water bath for 2minutes is indicative of pentoses	The formation of a bluish green product pentosesnuddy brown products	Presence of pentoses Presence of hexoses

Required results: The qualitative test should indicate presence or absence of carbohydrates.

Parameters: visible and microscopic estimation

Cautions Care should be taken while handling caustic acids like conc. sulphuric acid or hydrochloric acid..

Experiment 4

Qualitative Tests For Non-Reducing Sugars

AIM:

To perform qualitative tests for non-reducing sugars

Equipment required: Water bath

Materials required: Molisch's Reagent, lodine solution, Fehling's reagent A, Fehling's reagent B, Benedict's qualitative reagent, Barfoed's reagent, Seliwanoff 's reagent, Bial's reagent, Phenylhydrazine hydrochloride, Sodium acetate, Glacial acetic acid, Glucose, fructose, Microscope

Learning objectives: To characterize and identify unknown carbohydrates present an unknown solution on the basis of various qualitative tests.

Theory/Principle/Background of the topic:

A carbohydrate is an organic compound with the general formula Cn(H20)n, that is, consists only of carbon, hydrogen and oxygen, with the last two in the 2:1 atom ratio.

Molisch's Test (general test): This is a common test for all carbohydrates larger than tetroses. The test is on the basis that pentoses and hexoses are dehydrated by conc. sulphuric acid to form furfural or hydroxymethylfurfural, respectively. These products condense with a-naphthol to form purple condensation product.

Test for non-reducing sugars

Non-reducing sugar: A number of sugars especially disaccharides polysaccharides have glycosidic linkages which involve bonding a carbohydrate (sugar) molecule to another one, and hence there is no reducing group on the sugar, like in the case of sucrose, glycogen, starch and dextrin

lodine Test: This test is used for the detection of starch in the solution. The blue-black colour is due to the formation of starch-iodine complex. Starch contain polymer of a-amylose and amylopectin which forms a complex with iodine to give the blue black colour.

Osazone Test: The ketoses and aldoses react with phenylhydrazine to produce a phenylhydrazone which further reacts with another two molecules of phenylhydrazine to yield osazone. Needle-shaped yellow crystals are produced by glucose and fructose, whereas lactosazone produces mushroom shaped crystals. Crystals of different shapes will be shown by different osazones. Flower-shaped crystals are produced by maltose.

Outline of procedure

Sr no.	Test	Observation	Inference
1	Iodine test 2 drops of iodine solution are added to 2 ml of the test solution and contents are mixed gently.	Blue-black colour is observed.	Presence of polysaccharides (Starch)

2	Osazone test To 0.5 g of phenylhydrazine hydrochloride add 0.1 gram of sodium acctate and ten drops of glacial acetic acid. Add 5 mL of test solution to this mixture and heat under boiling water bath for about half an hour. Cool the solution slowly and examine the crystals under a microscope.	Needle-shaped yellow crystals osazone mushroom shapcd or powder puff shaped flower-shaped crystals No osazone formed	Glucose, fructose Lactose Maltose Sucrose (disaccharides)

Required results: The qualitative test should indicate presence or absence of carbohydrates

Parameters: visible and microscopic estimation

Cautions: Care should be taken while handling caustic acids like conc. sulphuric acid, nitric acid or hydrochloric acid.

Experiment –5

Qualitative Tests For Proteins

Aim : To perform qualitative tests for proteins.

EquipmentsRequired :pH meter, pipette, measuring cylinder, volumetric flask and beakers.

Materials required : Egg albumin, cow's milk,NaOH solution, 1% $CuSO_4$ solution, Conc. HNO_3, Ninhydrin solution, Millon's reagent.

Learning objectives : To detect the presence of proteins from a given unknown sample.

Theory/Principle/Background of the Topic :

Protein is an important macronutrient essential for survival. They are constituent of cells and hence are present in all living bodies. 10-35% of calories should come from protein. Protein is found in meats, poultry, fish, meat substitutes, cheeses, milk etc.

Proteins are large biological molecules composed of α-amino acids(Amino acid in which amino group is attached to α-carbon, which exist as zwitter ions and are crystalline in nature). They contain carbon, hydrogen, oxygen, nitrogen and sometimes phosphorus and Sulphur.

Casein contains a fairly high number of proline residues, which do not interact. There are also no disulfide bridges. As a result, it has relatively little tertiary structure. It is the name for a family of related phosphoproteins(αS1, αS2,κ). These proteins are commonly found in mammalian milk, making up 80% of the proteins in cow's milk and between 20% and 45% of the proteins in human milk.

The **albumins**(formed"(egg) white; dried egg white") are a family of globular proteins, the most common of which are the serum albumins.

Albumins are commonly found in blood plasma and differ from other blood proteins in that they are not glycosylated. Substances containing albumins, such as egg white, are called *albuminoids*.

Amino acids are molecules contain both amino(NH_2) and carboxylic(COOH) group. Amino aicd molecules undergo condensation reaction to form a specific type of linkage known as peptide linkage.

Depending on the number of amino acid molecules involved in the condensation reaction, the products formed are classified as;

Dipeptide

They are the products formed by the condensation of two α-amino acid molecules.

Tripeptide

They are formed by the condensation of three α-amino acid molecules.

If large number of amino acid molecules combine, the product formed is called polypeptide.

Biuret Test

This test is used to detect the presence of peptide bond. When treated with copper sulphate solution in presence of alkali (NaOH or KOH), protein reacts with copper(II) ions to form a violetcoloured complex called biuret.

Xanthoproteic test

It is an identification test of protein and it gives a positive result with those proteins with amino aicd carrying aromatic group. When protein is treated with hot concentrated nitric acid, a yellow coloured substance is formed. The yellow colour is due to xanthoproteic acid which is formed by the nitration of certain amino acids present in protein such as tyrosine and tryptophan.

Ninhydrin test

This is a test for amino acids and proteins with free $-NH_2$ group. When such as $-NH_2$ group reacts with ninhydrin, an intense blue complex is formed.

Millon's test

When egg albumin is treated with Millon'sreagent, it first gives a white colored precipitate which then changes to brick red on boiling. Millon'sreagent(Hg/HNO_3) gives positive results with proteins containing the phenolic amino acid "tyrosine".

Denaturation by heat (heat coagulation test)

Heat disrupts hydrogen bonds of secondary and tertiary protein structure while the primary structure remains unaffected. The protein increases in size due to denaturation and coagulation occurs.

Reduced Sulphur test

Proteins containing Sulphur(in cysteine and cystine) give a black deposit of lead sulfide (PbS) when heated with lead acetate in alkaline medium .

1. **Outline of procedure;**

S.No.	Test	Observation	Inference
1.	**Biuret test** Take a small quantity of the dispersion and add 2ml of NaOH solution into it. Now add 4-5 drops of 1% $CuSO_4$	Bluish violet colour is formed,.	Presence of protein (general)

	solution and warm the mixture for about 5 minutes.		
2.	**Xanthoproteic test** Take about 2ml of the sample in a test tube and few drops of conc.HNO_3 into it and heat the test tube	Yellow precipitate.	Presence of protein(containing tyrosine (yellow color) or tryptophan(orange color))
3.	**Ninhydrin test** Take 2ml of the sample in a test tube and add 3-4 drops of Ninhydrin solution and boil the contents.	Intense blue color is formed.	Presence of protein
4.	**Millon's test** Take 1-2 ml of the sample in a test tube and add 2 drops of Millon's reagent.	White precipitate which changes to brick red on boiling.	Presence of protein containing tyrosine (both albumin and casein).
5.	**Heat coagulation test** Put 2ml of protein solution in a test tube, incline it and heat to boiling	A permanent clotting and coagulation.	Presence of albumin
6.	**Reduced Sulphur test** To 1 ml of protein solution in a test tube, add 2 drops of 10% sodium hydroxide solution and 2 drops of lead acetate. – Mix well and put in a boiling water bath for few minutes	If a black deposit is formed If slight black turbidity is obtained	Albumin Casein due to its lower content of sulfur

Clinical significance:

Normal level: human serum albumin in adults (>3 y.o) is 3.5 to 5 g/dL. For children less than three years of age, the normal range is broader, 2.9-5.5 g/dL.

Low albumin (hypoalbuminemia) may be caused by liver disease, nephrotic syndrome, burns, protein-losing enteropathy, malabsorption, malnutrition, late pregnancy, artefact, genetic variations and malignancy.

High albumin (hyperalbuminemia) is almost always caused by dehydration, retinol (Vitamin A) deficiency.

Required results :

Parameters : Presence of amino acids and proteins

Cautions :glass assembly should be rinsed with distilled water after measuring pH of solution. Millon's reagent contain's mercury (II) nitrate in concentrated nitric acid – highly toxic, corrosive and strong oxidant.

Experiment no.6

Urine Analysis For Abnormal Constituents.

Aim : To carry out urine analysis for abnormal constituents.

Equipmentsrequired : Water bath

Materials required : Benedicts's reagent, sulphosalicylic acid, nitric acid, ammonium sulphate, ammonium hydroxide, Sulphur powder.

Learning objectives : To identify the abnormal constituents in urine sample using qualitative analysis.

Theory/Principle/Background of the topic:

Substances which are not present in easily detectable amounts in urine of normal healthy individuals but are present in the urine under certain diseased conditions are said to be Abnormal constituents of urine.

Normally, urine contains water and wastes, such as urea, uric acid, creatinine, and some ions, However, some of these substances may be abnormally elevated, which usually indicated that something is wrong with the body.

The following are some of the abnormal constituents of urine and some possible causes.

Albumin

Albumin is a type of protein, which is a normal component of plasma – the fluid component of blood. When albumin is found to be excessive in urine, it may indicate that the tiny filtering units in the kidney, called nephrons, are damaged or destroyed. Elevated albumin in the urine is termed albuminuria.

Albumin, the protein, is denatured by sulphosalicylic acid a coagulation, heat coagulation test and Heller's nitric acid test (Nitric acid causes precipitation of protein).

Bilrubin

Bilrubin, when modified by the kidneys, contributes to the classical yellow color of urine. It is a by product that results from the breakdown of hemoglobin – the red pigment in red blood cells. When levels of bilirubin in urine is above normal, the condition is called bilirubinuria. This may indicate liver disease or obstructive biliary disease. This is identified in urine using Hay's test and Gemlin test.

Glucose

The presence of glucose or blood sugar in urine is called glucosuria. It may indicate that the person has diabetes. It can be detected using Benedict's test of carbohydrates. Normal urine also contains a trace of glucose and glucuronates, but their amount is too small to cause reduction in Benedict's test. In diabetes mellitus and in renal glycosuria, glucose is found is urine. This gives a Benedict's test positive.

Ketonebodies

The presence of ketonebodies in the urine may indicate diabetes or anorexia. It may be elevated during fasting and starvation. It can be detected using Rothera's test. In this test acetoacetic acid forms a complex with nitroprusside in alkaline solution developing a permanganate colour.

Others abnormal constituents can be microbes, blood, white blood cells etc.

Outline of Procedure :

S.No.	Test	Observation	Inference
1.	**Test for glucose : Benedict's Test** To about 5 ml of Benedict's reagent, add 0.5 ml of urine and boil for 2 min.	Blue color appears Light green, green, yellow, brick red precipitates appears	Sugar absent Reducing sugar present
2.	**Test for albumin :Sulphosalicylic acid test :** Add a few drops of sulphosalicylic acid to 2 ml of urine.	Turbidity appears	Indicates the presence of albumin
3.	**Heat coagulation test:** Fill 3/4th of the test tube by urine. Heat the upper 1/3rd of the test tube by a small flame.	Tubidity appears on the heated portion of the tube	Indicates the presence of albumin
4.	**Heller's Nitric acid test:** To 3ml of nitric acid in a tube add 3 ml of urine by the wall of the tube in such a way that the two liquids do not mix	White ring appears at the junction of the two fluids	Indicates the presence of albumin
5.	**Test for ketone bodies:** **Rothera's test:** Saturate 5ml of urine with ammonium sulphate by shaking vigorously. Then add 2 drops of freshly prepared 5% solution of sodium nitroprusside and 1 ml of ammonium hydroxide. Allow it to stand in a rack for a while.	A permanganate color develops just above the layer of the undissolved ammonium crystals	Indicates the presence of ketone bodies
6.	**Test for Bilrubin: Gemlin Test:** To 5 ml of concentrated nitric acid in a test tube add an equal volume of urine carefully so that two layers are formed.	Various colored rings(green, blue, violet, reddish yellow and red) are formed at the junctions of the two liquids	Presence of bilirubin
7.	**Hay's test** Fill the half of the test tube with urine and another test tube with water. Sprinkle gently some Sulphur	The Sulphur powder spontaneously sinks in the test tube containing urine.	Presence of Bile salts

	powder on the surface of two liquids.		

Required results: Identification of abnormal constituents in given urine sample

Parameter: Visible identification.

Caution: Be careful in handling of corrosive chemical in the lab.

Experiment No.7

Quantitative Estimation Of Reducing Sugars (Glucose) By Di-nitrosalicylicacid(DNSA) Method

Aim: To carry out quantitative estimation of reducing sugars(glucose) by di-nitrosalicylicacid(DNSA) method in given sample.

Equipments required: Spectrophotometer/Colorimeter to determine the absorbance in given range, micropipette and tips, test tube stand.

Materials required: Distilled water, Sulphuricacid(Concentrated), Phenol Solution(5%)

Learning objectives: Students will learn about the quantitative estimation of the sugars in a given sample.

Theory/Principle/Background of the topic:

Carbohydrates are a major source of metabolic energy, both for plants and for animals and they also serve as a structural material (cellulose), a component of the energy the transport compound ATP, recognition sites on cell surfaces, and one of three essential components of DNA and RNA.

Carbohydrates are classified according to their molecular size and solubility. Carbohydrates are classified into groups according to the number of individual simple sugar units:

1. **Monosaccharides:** They are the simplest form of sugar and are usually colorless, water soluble and crystalline solids, e.g glucose, fructose, galactose etc.
2. **Disaccharides:** It is formed when two monosaccharides undergo condensation reaction and water soluble, e.g, sucrose, lactose etc.
3. **Polysaccharides:** It is formed when more than two monosaccharides units bound together by glycosidic bonds, e.g, starch, glycogen, cellulose etc.

Principle:

3, 5-Dinitrosalicylic acid(DNSA) is used extensively in biochemistry for the estimation of reducing sugars. It detects the presence of free carbonyl group(C=O) of reducing sugars. This involves the oxidation of the aldehyde functional group (in glucose) and the ketone functional group (in fructose). During this reaction DNSA in reduced to 3-amino-5-nitrosalicylic acid (ANSA) which under alkaline conditions is converted to a reddish brown coloured complex which has an absorbance maximum of 540 nm.

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of known chemical substance.

A spectrophotometer is an instrument that measures the amount of photos (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected. Depending on the range of wavelength of light source, it can be classified into two different types:

- **UV-visible spectrophotometer :**uses light over the ultraviolet range(185-400 nm) and visible range(400-700 nm) of electromagnetic radiation spectrum.
- **IR spectrophotometer:** uses light over the infrared range(700-15000 nm) of electromagnetic radiation spectrum.

In visible spectrophotometry, the absorption on the transmission of a certain substance can be determined by the observed color.

The basic structure of spectrophotometers. It consists of a light source, a collimator, monochromator, a wavelength selector, a cuvette, a photoelectric detector, and a digital display or meter. Detailed mechanism is described below.

A spectrophotometer, in general, consists of two devices; a spectrometer and a photometer. A spectrometer is a device that produces, typically disperses and measures light. A photometer indicated the photoelectric detector that measures the intensity of light.

- **Spectrometer:** It produces a desired range of wavelength of light. First a collimator (lens) transmits a straight beam of light (photons) that passes through a monochromator (prism) to split it into several component wavelengths (spectrum). Then a wavelength selector (slit) transmits only the desired wavelengths, as shown in figure 1.
- **Photometer:** After the desired range of wavelength of light passes through the solution of a sample in cuvette, the photometer detects the amount of photos that is absorbed and then sends a signal to a galvanometer or a digital display.

The relationship between absorbance and concentration is given by the lambert-beet law and is written mathematically as:

$A=\varepsilon\kappa$

Where **A** is the absorbance, a unit less quantity.

εis the molar absorptivity constant (a constant of the compound having units L*mol-1*cm-1) and

l is the path length over which the light interacts with the sample in cm.

c is the concentration.

In a more practical sense, the absorbance is defined as the negative logarithm of the transmittance. This is given mathematically as:

$$A = -\ logT = -\ logI/I_0$$

Where I is the intensity of the light beam when the sample is present; and I_0 is the intensity of the light beam when everything but the sample is present.

(in some cases this is called a background or a blank)

The molar extinction coefficient is given as a constant and varies for each molecule. Since absorbance does not carry any units, the units, for **ε**must cancel out the units of length and concentration. As a result, **ε**has the unitsL*mol-1*cm-1. The path length is measured in centimeters. Because a standard spectrometer uses a cuvette that is 1 cm in width, *l* is always assumed to equal 1 cm. Since absorption, **ε**and path length are known, we can calculate the concentration c of the sample.

Outline of procedure:

1. Take eight tubes and label them as Blank 1 to 7.
2. Make dilutions of glucose standards with concentrations of 40, 80, 120, 160 and 200 µg per 200 µl by transferring respective amount of glucose from the standard glucose solutioin (1 mg/ml) and adjusting it to a total volume of 200 µl by adding distilled water as mentioned in Table 2
3. Add 0.5 ml of DNSA reagent to all the eight test tubes. Mix well.
4. Keep in boiling water bath for 15 minutes.
5. Add 0.5 ml of 40% of Potassium sodium tartrate (Rochell's salt) solution and mix it well.
6. Switch on the spectrophotometer and select the wavelength of 540 nm. First take the absorbance(OD) of Blank and make it zero.
7. Take the OD of all the tubes(No. 1-7). Wash the cuvettes each time after taking OD.

Tube no.	**Blank**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Conc. Of Glucose(µg)	0.0	40	80	120	160	200	Test Sample 1	Test Sample 2
Vol. of Glucose std. taken(µl)	0.0	40	80	120	160	200	200 µl	200 µl
Vol. of distilled water added(µl)	200	160	120	80	40	0.0	200 µl	200 µl
Vol. of DNSA added(ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vol. of 40% Rochell's salt added	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Absrobance at 540 nm								

8. Plot a standard curve of absorbance at 540 nm on "Y" axis versus concentration of glucose in µg/200 µl on "X" axis.
9. Record the value "x" of unknown from graph corresponding to the OD reading of the test samples.

Required Results: The concentration of glucose in the unknown sample

Parameters: Graph OD vsconcentration(µg/µl) (Colorimeter) or Absorbance vs concentration (µg/µl) (Spectrophotometer)

Caution: Careful handling of harmful chemicals i.e., sulphuric acid and phenol during the practical in laboratory.

Experiment No. 8

QUANTITATIVE ESTIMATION OF PROTEINS BY BIURET METHOD

Aim: To carry out quantitative estimation of proteins by Biuret method

Equipments Required: Spectrophotometer/Colorimeter to determine the absorbance in given range, Micropipette and tips. Test Tube stand.

Materials Required: NaOH, Biuret reagent, Sodium Azide, Protein standard

Learning Objectives:

Theory/Principle/Background of the topic:

Proteins are essential to human life. They are macromolecules which are assembled on as on needed basis. They regulate may bodily functions and can functions as enzymes.

Proteins form between 50 and 70% of a cells dry weight and are found in all cells, secretions, fluids and excretions of the body. The concentration of proteins in the body ranges from 6.0 g/dL to 8.3 g/dL. The most abundant protein is albumin which can make up to 60% of the total protein concentration.

The **Biuret Method** which is the most widely used method for total protein determination, relies on the complexationof Cu^{2+} by the function groups involved with the peptide bond. A minimum of two peptide bonds is needed for the complexation to occur.

Upon complexation, a violet color is observed. The absorbance of the Cu^{2+} -protein complex is measured at 540 nm and compared to a standard curve.

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance.

A spectrophotometer is an instrument measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected.

The relationship between absorbance and concentration is given by the Lambert-Beer Law and is written mathematically as:

$A=\varepsilon\kappa$

Where **A** is the absorbance, a unit less quantity.

εis the molar absorptivity constant (a constant of the compound having units L*mol-1*cm-1) and

l is the path length over which the light interacts with the sample in cm.

c is the concentration.

In a more practical sense, the absorbance is defined as the negative logarithm of the transmittance. This is given mathematically as:

$$A = -\ logT = -\ logI/I_0$$

Where I is the intensity of the light beam when the sample is present; and I_0 is the intensity of the light beam when everything but the sample is present.

(in some cases this is called a background or a blank)

The molar extinction coefficient is given as a constant and varies for each molecule. Since absorbance does not carry any units, the units, for **ε**must cancel out the units of length and concentration. As a result, **ε**has the unitsL*mol-1*cm-1. The path length is measured in centimeters. Because a standard spectrometer uses a cuvette that is 1 cm in width, *l* is always assumed to equal 1 cm. Since absorption, **ε**and path length are known, we can calculate the concentration c of the sample.

Outline of procedure: Reagents

1) Biuret Reagent: Dissolve 9g of sodium potassium tartarate in 500ml of 0.1M sodium hydroxide solution. add 3g copper sulphate($CuSO_4.5H_2O$) and dissolve. Add 5g potassium iodide and make the volume to 1 litre with 0.1M sodium hydroxide.
2) Alkaline tartarate: Dissolve 9g of sodium potassium tartarate in 500 mg of 0.1 M sodium hydroxide , add 5g of potassium iodide and make the volume to 1 litre with 0.1M sodium hydroxide.
3) Protein standard: (5 g/L), dilute 2ml of bovine albumin protein standard solution to 25ml with water. Measure accurately 3ml portions into tubes and store at -20˚C until required.

Procedure:

1) Take tube 1 and add 0.2ml of plasma or serum in 2,8 ml water, label it as test/sample(T) tube.
2) Take another test tube and add 3 ml standard protein solution, label as standard (S).
3) Then take third test tube and add 3 ml of water for blank (B) in separate tube.
4) Add 5 ml of biuret reagent to each test tube and mix.
5) Either stand for 30 min or incubate at 37˚C for 10 min.
6) After cooling, measure the absorbance against reagent blank at 540 nm or with ilford 625 green light filter.
7) If the specimen is turbid, milky or highly pigmented, carry a attest and stand blank using alkaline tartarate reagent instead of biuret reagent.

Required results: The estimation of protein concentration in unknown sample

Serum Total Proteins: T or (T-B) X 15

S or (S-B)

Interpreation: The normal range is 6 to 8.3 g/dL or 60-83 g/L

The measurement of total protein concentration is of limited value. It may be altered by changes in plasma-volume, an increase is caused by dehydration and a decrease from overloading with water. Otherwise significant increase occurs in conditions associated with increase in total globulin and a decrease is usually a result of fall in albumin, for example in nephritic syndrome, protein losing enteropathy, burns, severe hemorrhages, reduced synthesis in liver diseases and malnutrition.

Caution: Careful handling of chemicals and equipments.

Experiment 9

EstimationOf The Concentration Of Cholesterol In Given Sample

AIM:

To estimate the concentration of cholesterol in given sample.

Equipments required: colorimeter

Materials required: Ferric chloride-acetic acid reagent, serum sample, sulphuric acid

Learning objectives: Quantitative analysis of creatinine in serum.

Theory/Principle/Background of the topic:

Cholesterol is a lipid sterol that is produced in and transported throughout the bloodstream in eukaryotes. Cholesterol is a critical compound used in the structure of cell membranes, hormones, and cell signaling. It is an essential component of animal cell structure in order to maintain permeability and fluidity. Cholesterol is a precursor for steroid hormones including the adrenal gland hormones cortisol and aldosterone, sex hormones progesterone, estrogens, and testosterone, and bile acids and vitamin D.

Determining circulatory levels of lipoproteins is critical to the diagnosis of lipid transport disorders. High levels of cholesterol and cholesteryl esters (hypercholesterolemia) have been associated with cardiovascular disease such as atherosclerosis and heart disease, although lower levels (hypocholesterolemia) may be associated with cancer, depression, or respiratory diseases.

Method of Zak

Serum is treat with ferric chloride-acetic acid reagent to precipitate the proteins. The protein free supernatant (containing ferric chloride) is treated with conc. Sulphuric acid. A reddish purple color is developed which is measured colorimetrically at 560 nm.

Outline of procedure: Reagents preparation:

1. Stock ferric chloride-acetic acid reagent: Dissolve I g FeCl.6H20 in glacial acetic acid (aldehyde free)

2. Working ferric chloride-acetic acid reagent: Dilute 5 ml stock to 100 ml with acetic acid.

3. Stock standard cholesterol solution (Img/ml). Dissolve 100 mg cholesterol in acetic acid and make final volume to 100 ml with acetic acid. Store in the cold

4. Working cholesterol standard solution (0.04 mg/ml). Dilute 4.0 ml stock to 100 ml with working ferric-chloride-acetic acid reagent. Store in the cold.

5. Sulphuric acid, concentrated (A.R.)

Procedure:

1. To 0.2 ml of serum in a 15 ml glass stopper centrifuge tube, add 9.8 ml of working ferric chloride-acetic acid reagent.

2. Mix and minutes and then centrifuge.

3. Mark three test tubes S, T and B for standard, test and blank respectively.

4. Measure 5.0 ml of protein free supernatant of serum in T and add 3.0 ml of concentrated H2SO4 to all the three tubes.

5. Mix, stand for 30 min and read standard and test at 560 nm against blank.

Calculations:

Serum cholesterol (mg/100 ml) = u/s X 100/0.2 X 0.4 u/s X 200

Cautions: 1. All personnel working in the laboratory must wear gloves and laboratory coats.

2. The improper handling of blood samples from patients with infectious diseases hepatitis or HIV, can lead to infection.

3. The contaminated area is cleaned with a solution of sodium hypochlorite.

Experiment 10

Enzymatic Hydrolysis Of Starch Using Salivary Amylase

AIM:

To carry out enzymatic hydrolysis of starch using salivary amylase

Equipments required: Water bath, pipette

Materials required: Saliva solution, starch solution, 1% lodine solution

Learning objectives: Students will learn the basics of enzymatic hydrolysis

Theory/Principle/Background of the topic:

All living beings need energy to survive It is from the food we consume that we get our energy We know that the energy we are getting is by the process of digestion that breaks down the complex substance of starch into simpler molecules of glucose, which are further metabolized into CO2 and water through the process of glycolysis The human digestive tract starts at the mouth and ends at the anus.

In the Beginning

The digestion of the food starts as soon as we put food in our mouth Our teeth cut the food into small pieces and the salivary glands secrete saliva that mixes with these food materials The saliva contains an enzyme called salivary amylase which hydrolyses starch into maltose The complete digestion of starch occurs only in the small intestine by the action of pancreatic amylase The activity of enzymes is strongly affected by several factors, such as temperature and pH.

Effect of Temperature

All enzymes are proteinaceous in nature. At a lower temperature, the enzyme salivary amylase is deactivated and at the higher temperature, the enzyme is denatured. Therefore, more time will be taken by an enzyme to digest the starch at lower and higher temperatures. Optimum temperature for the enzymatic activity of salivary amylase ranges from 32 °C to 37 °C. The optimum temperature means that the temperature at which the enzyme shows the maximum activity At this optimum temperature, the enzyme is most active and hence, takes less time to digest the starch

Effect of pH

The optimum pH for the enzymatic activity of salivary amylase ranges from 6 to 7. Above and below this range, the reaction rate reduces as enzymes get denatured. The enzyme salivary amylase is most active at pH 6.8. Our stomach has high level of acidity which causes the salivary amylase to denature and change its shape. So the salivary amylase does not function once it enters the stomach.

Principle: The activity of salivary amylase on starch can be studied by using the lodine test. If we add saliva on starch, the salivary amylase present in saliva gradually acts on starch and convert into maltose. Starch keeps on giving blue colour with iodine till it is completely digested in maltose. At this point, no blue colour is formed. This is the end point or achromic point.

Outline of procedure:

Collection of Saliva: Rinse mouth thoroughly with cold water and ensure that it does not contain any food particles. Now take about 20ml of luke warm water in the mouth and gargle for about three minutes so that saliva mixes up well with it. Spit this into a beaker,.filter if there is any suspended impurity. Clear filtrate is saliva solution and contains enzyme ptyalin.

Preparation of starch solution: Take about 0.5 g of starch in a 100 ml beaker and add enough water to make a paste. Dilute the paste by adding 50 ml water and boil for about 5 min,

Enzymatic hydrolysis of starch:

1.Pipette out 5ml of the starch (substrate) solution and add 2 ml of the saliva (containing enzyme) solution into the test tube.

2. Mix the solution well by shaking the tube carefully and start a stop watch. 3) After one minute take out two drops of the mixture solution from the test tube with the help of a dropper and transfer it into another test tube containing about one ml of 1% iodine solution, note the color produced, if any

4. Repeat this test after every one minute taking two drops of the mixture solution and fresh % iodine solution continue until the test shows no blue color. Record the time and blue color intensity

5. Absence of blue color on addition to iodine solution means absence of starch in the solution. That is whole of the starch has got digested or hydrolysed

1. **Required results**: Observation color intensity.

Caution: Students should be careful in handling corrosive chemical and use equipment under supervision only.

Experiment 11

Study The Effect Of Temperature On The Activity Of Alpha Amylase

AIM:

To study the effect of temperature on the activity of alpha amylase

Equipmentsrequired:Colorimeter, Water bath

Materials required: a) Dinitrosalicylic acid reagent (DNS Reagent)

b) 1% starch solution: Ig of starch in 100ml 0.05M phosphate buffer (pH6.9)

c) 2N NAOH solution

Learning objectives: To give idea of effect of temperature on enzyme activity and to analyze how temperature affects the enzyme activity

Theory/Principle/Background of the topic:

Rate of reactions including those catalyzed by enzymes rise with increase in temperature based on the Arrhenius equation k=Ae-Ea/RT, where K is the kinetic rate constant the reaction, A is the Arrhenius constant also known as the frequency factor. AG is standard free energy of activation (KJ) which depends on entropic and enthalpic factors is the universal gas constant and T is the absolute temperature. Increase in temperature of system will increase the number of collisions of the enzyme and substrate per unit time Th within limits, the rate of the reaction will increase.

Outline of procedure

a) Pipette out 0.5ml of the substrate solution and 0.5ml of the enzyme solution into two separate test tubes. Then both the substrate and the enzyme preincubated in separate test tubes at 10°C for 5 minutes.

b) After preincubation at that temperature, the substrate solution from the test tube mixed and incubated for 5 minutes at that temperature for the reaction to take place.

c) At the end of the incubation time, add 0.5ml of the 2N NAOH to arrest the reaction followed by 1.0ml of DNS reagent.

d) Repeat the above three 25°C, 37°C, 55°C and 80°C.

e) After the incubation, all the tubes placed in a boiling water bath for 5min and cooled to room temperature.

f) The absorbance of all the tubes read at 540nm. Enzyme activity is measured by intense color and increased OD.

g) Plot graph of OD vs temperature.

Required results: Change in absorbance w.r.t change in temperature, Report the temperature the OD is maximum, as optimum temperature

Graphs: O.D vs temperature.

Cautions: a) All tubes must be cooled to room temperature before taking readings since extinction is sensitive temperature change.

b) Handle the glass wares carefully.

Experiment 12

Estimation Of The Enzyme Activity Using Maltose Standard Curves

AIM:

To carry out estimation of the enzyme activity using maltose standard curves.

Equipments required: Colorimeter spectrophotometer

Materials required: Starch, Maltose, dinitrosalicyclic acid, sodium hydroxide, salivary amylase.

Learning objectives: Students will leam the basics of enzymatic hydrolysis and quantitative estimation of enzyme activity.

Theory/Principle/Background of the topic:

An amylase is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into sugar The ney and salivary gland make amylase (alpha amylase) to hydrolyse dietary starch into disaccharides trisaccharides which are converted by other enzymes to glucose to supply the body with en All amylases are glycoside hydrolases and act on a-1,4-glycosidic bonds. Amylases are usc fermentation of starch in beer industry, as flour additive, in molecular biology etc.

Rate of reactions including those catalyzed by enzymes rise with increase in temperature based on the Arrhenius equation K= Ae-AEa/RT, where K is the kinetic rate constant for the reaction, A is the Arrhenius constant also known as the frequency factor. AG is the standard free energy of activation (KJ) which depends on entropic and enthalpic factors, R is the universal gas constant and T is the absolute temperature. Increase in temperature of a system will increase the number of collisions of the enzyme and substrate per unit time. Thus within limits, the rate of the reaction will increase.

Maltose is a disaccharide made up of two subunits of glucose monomers. It is also called malt sugar is present in germinating grain, in a smaller amount in com syrup, and also is a product of the portal hydrolysis of starch. Maltose is considered as an important constituent in making fermented barley which is used to brew beer.

Maltose is a reducing sugar (those carbohydrates which have free aldehyde or keto group can reduce Fehling's and Benedict's reagents are called reducing sugars). Maltose can be used as a standard for estimating reducing sugar in unknown samples Constructing a standard curve / graph for maltose helps us to estimate concentration of reducing sugars present in an unknown sample and for determining the activity of amylase enzyme in forthcoming experiments. The standard curve for maltose is usually constructed using 3, 5- di nitro salicylic acid (DNS) as the reagent. Maltose reduces the pale yellow coloured alkaline 3,5-Dinitro salicylic acid (DNS) to the orange- red coloured, 3 amino,5 nitro salicylic acid.

The intensity of the colour is proportional to the concentration of maltose present in the solution [as per Beer Lambert's law]. This intensity change in colour is measured using a colorimeter as the absorbance at 540nm wavelength. Wave length is set to 540 nm because it is the region where orange-red colour absorbs. A series of solutions containing varying concentrations of maltose are prepared in test tubes and a known quantity of DNS is added to each. These test tubes are then heated on a water bath for few minutes and their optical densities are measured using a colorimeter. A graph is then plotted with amount of maltose on X axis and the observed optical density at Y axis. The plot thus obtained is called a standard maltose curve.

Outline of procedure:

Procedure for the standard curve:

1. First prepare the I mg/ ml solution of Maltose (10 mg in 10 ml of distilled water)

2. Then transfer I ml of solution from stock and made up to 10 ml (100 ug/ml)

3. Pipette out standard maltose solution in the range of 0.2, 0.4, 0.6, 0.8 and 1 ml, into 5 separate test tubes.

4. A test tube containing a blank solution is also prepared.

5. Using distilled water, bring the volume up to 2ml in each test tube, including the test tube containing the blank solution.

6 Add I ml of DNS reagent to each tube and cover the test tubes with aluminum foil.

7. Heat the contents in the test tubes in a boiling water bath for 5 minutes. 8. Cool the test tubes to room temperature, after taking them out of the water bath.

9. Then make the volume to 10 ml and mix well.

10. Record the intensity of dark orange red color at 540 nm as the 'absorbance' or OD. I1. Plot a graph with the concentration of maltose on X axis Vs OD at 540nm (A 540 nm) on Y axis.

Preparation of test:

a) Pipette out 0.5 ml of the substrate solution and 0 .5 ml of the enzyme solution into two separate test tubes. Then both the substrate and the enzyme pre-incubated in separate test tubes at 10°C for 5 minutes.

b) After pre-incubation at that temperature, the substrate solution from the test tube mixed and incubated for 5 minutes at that temperature for the reaction to take place.

c) At the end of the incubation time, add 0.5ml of the 2N NAOH to arrest the reaction followed by 1,0ml of DNS reagent.

d) After the incubation, all the tubes placed in a boiling water bath for 5 min and cooled room temperature.

e)The absorbance of tube is read at 540nm. Enzyme activity is measured by intense color and increased OD from the standard curve.

Required results: Calculation of concentration of test from standard curve

Parameters: Estimation of OD from graph OD vs concentration

Caution: Students should be careful in handling corrosive chemical and use equipments under supervision only .

Experiment 13

Quantitative Determination Of Glucose By Glucose Oxidase-Peroxidase

AIM:

To study the quantitative determination of glucose by glucose oxidase-peroxidase.

Equipments required: Centrifuge, Colorimeter.

Materials required. Thiourea , glacial acetic acid, ortho-toluidine, benzoic acid, glucose

Learning objectives: Estimation of glucose in serum

Theory/Principle/Background of the topic:

Glucose oxidase is an enzyme extracted from the growth medium of Aspergillusniger. Glucose oxidase catalyse the oxidation of Beta D- glucose present in the plasma to D glucono -1 5 - lactone with the formation of hydrogen peroxide; the lactone is then slowly hydrolysed to D-gluconic acid. The hydrogen peroxide produced is then broken down to oxygen and water by a peroxidase enzyme, Oxygen then react with an oxygen acceptor such as ortho toluidine which itself converted to a coloured compound, the of which can be measured colorimetrically.

Glucose is a simple sugar which is a permanent and immediate primary source of energy to all of the cells in our body. The glucose in blood is obtained from the food that you cat. This glucose gets absorbed by intestines and distributed to all of the cells in body through bloodstream and breaks it down for energy Body tries to maintain a constant supply of glucose for your cells by maintaining a constant blood glucose concentration. The concentration of glucose in blood, expressed in mg/dl, is defined by the term glycemiaThe value of blood sugar in humans generally ranges from 70 - 100 mg/dl. Blood sugar levels are regulated by the hormone insulin and glucagon which act antagonistically. These two hormones are secreted by the islet cells of the pancreas, and thus are referred to as pancreatic endocrine hormones.

When the blood glucose levels are high, insulin hormone secreted which causing liver to convert more glucose molecules into glycogen and when the blood glucose levels are low glucagon secreted and act on liver cells to promote the breakdown of glycogen to glucose and increases the blood glucose concentrations. Essentially blood glucose levels determine the time of secretion of these hormones. The blood glucose level is easily changed under the influence of some external and internal factors such as body composition, age, physical activity and sex. Diabetes is a disease related by the abnormal metabolism of blood sugar and defective insulin production. So blood sugar levels are an important parameter for the study of diabetes.

The level of glucose circulating in blood at a given time is called as blood glucose level. The blood glucose level varies at different time on various part of the day. Hypoglycemia is a possible side effect of diabetes medications in which blood glucose level drops below 70mg/dl. In people with diabetes, the body doesn't produce enough insulin or respond to insulin properly The result is that sugar builds up in the Page 10 blood stream, damaging the body's organs, blood vessels and nerves. This condition in which too much sugar in the blood stream is called hyperglycemia.

Outline of procedure:

a) Label sufficient test tubes for blank, standard solutions and test.

b) Place 5 ml of ortho-toluidine reagent in each tube. c) Test: Add 0.2 ml of serum and mix.

d) Blank: Add 0.2 ml of distilled water and mix.

e) Standards: Add 0.2 ml of each of the working glucose standard solutions to test tubes labelled as SI, S2, etc.

f) Place all the tubes in a boiling water bath for 12 minutes. Then cool all the tubes for 5 minutes in cold water.

g) Read the absorbance for each tube against the blank at 630 nm.

h) Calculate the concentration of glucose in the sample from the standard curve.

Experiment 14

Estimate Creatinine Level In Given Sample

AIM:

To estimate Creatinine level in given sample

Equipments required: Centrifuge, colorimeter

Materials required: picric acid -0.04M (9.16g/L), sodium hydroxide -0.75N, sodium tungstate - 10%, 2/3 N sulphuric acid, creatinine standard stock -100mg%, working standard-3mg%.

Learning objectives: Quantitative analysis of creatinine in serum.

Theory/Principle/Background of the topic:

Creatinine is a waste product formed in muscle by creatine metabolism and is excreted in the urine. Estimation of serum creatinine by Jaffe's alkaline picrate method is based on the following principle: creatinine in alkaline medium reacts with picric acid to form a red tautomers of creatinine picrate the intensity of which is measured at 520nm. Outline of procedure:

Preparation of reagents:

- Sodium tungstate, 10 %
- Sulphuric acid, 2/3 N
- Picric-acid, saturated: An excess of picric acid is allowed to stand with water with occasional shaking
- Sodium hydroxide, 10 %: 10 gm of sodium hydroxide is dissolved in water and diluted to 100 ml.
- Alkaline picrate: Five volumes of saturated picric acid and one volume of 10% sodium hydroxide are mixed just before use.
- Stock standard creatinine solution: 100 mg of creatinine is dissolved in and diluted to 100 ml with 0.1 N hydrochloric acid.
- Working standard creatinine solution: 2 ml of stock standard creatinine solution is transferred into a 1000 ml volumetric flask. I ml of conc. Hydrochloric acid is added. The volume is made up with water. This solution contain 0.01 mg of creatinine/5ml.

Procedure:

1) Measure 7 ml of water in a test tube.
2) Add I ml of serum and I ml of 10% sodium tungstate.
3) Mix and add 1 ml of 2/3N sulphuric acid with constant shaking.
4) Let it stand for a few minutes and filter.
5) Transfer 5 ml of the filtrate into tube labeled ' unknown'.
6) Measure 5 ml of the working standard creatinine solution in a tube labeled 'Standard" and 5 ml of water in a tube labeled Blank".
7) Add 2.5 ml of alkaline picrate solution to each tube and mix.
8) Let the tubes stand for 10 minutes.
9) Read "Unknown' and 'Standard' against Blank' at 520 nm or using a green filter.

Calculations:

Serum creatinine (mg/100 ml) =u/s X 0.01/0.5 X 100 = u/s X 2
Where u, Unknown s, Standard

Required results:

Parameters: Analysis of creatinine in serum

Interpretation: The normal range of serum creatinine is 0.6-1.5 mg/100 ml. the range of creatinine in whole blood is higher, Increasedcreatinine levels are seen in renal failure and other renal diseases

Cautions: a) All tubes must be cooled to room temperature before taking readings since extinction is sensitive to temperature change.
b) Handle the glass wares carefully.