Glass Apparatus

Test tubes, funnels, beakers, pipettes, measuring cylinders, glass-stoppered bottles, spirit lamps, titration flasks, conical flasks, reagent bottles. Besides these, filter papers, cotton, test tube holders, test tube stands are also required.

Note : All the glass apparatus must be chemically and physiologically cleaned. For example, a test tube should be washed first with vim and then it should be dried. After removing moisture, rinse the tube with chromic acid solution. Then wash the test tube 9 times with tap water and 3 times with distilled water. Now, the test tube is cleaned properly and is ready for experiments.

BIOCHEMICAL TESTS

Some important test on Carbohydrates, Proteins, Fats and Enzymes have been described here.

1. Test on Carbohydrates

Carbohydrates are abundantly found in the plants and as glycogen in the animals. In animals they are found in free stored state as glycogen or in combination with proteins as glycoprotein. The name carbohydrate is given because they are composed of **carbon, hydrogen and oxygen atoms**. H_2 and O_2 are found in the same proportions as in water (H_2O). Chemically carbohydrates are aldehyde and ketone derivatives of alcohols (aldoses and ketoses). In general, carbohydrates are white solids, sparingly soluble in organic liquids, except for certain polysaccharides, soluble in water. Many carbohydrates are of low molecular weight and having sweet taste. Carbohydrates are classified into 3 groups :

(1) **Monosaccharides** or simple sugars ($C_6H_{12}O_6$),
(2) **Di-** and **tri-saccharides** or compound sugars,
(3) **Polysaccharides**.

A. Monosaccharides

Monosaccharides or simple sugars occur abundantly in nature in the form of glucose and fructose. They occur in white crystalline form easily soluble in water and hot alcohol and practically insoluble in organic solvents like absolute alcohol, ether and acetone, etc. They are optically active and being aldehydes and ketones show common reactions.

In alkaline solution all the monosaccharides and many disaccharides behave as reducing agents and are easily oxidised by various reagents as silver and copper, etc. Most of the quantitative analysis for sugars depend upon the measurement of the reduction of Cu^{++} to Cu^{+} by alkaline sugar solutions.

B. Experiments with Glucose and Fructose

Make 0.2% and 2% solutions of the Dextrose-D or Fructose and perform the following experiments in order to identify the reducing action of glucose and fructose :

Experiment (1) **Reduction of methylene blue.**

Procedure : In a test tube take 3 cc of distilled water, then add a drop of methylene blue (1%). The water becomes blue coloured. Add 0.5 cc of 40% NaOH. Boil the solution. Colour is not discharged, blue colour remains. Add 1 cc of 0.2% glucose or fructose solution and boil.

Result : The solution is **decolourised** due to formation of leuco-methylene blue, the reduction product of methylene blue.

Experiment (2) **Reduction of alkaline ferricyanide.**

Procedure : In a test tube take 3 cc of 1% potassium ferricyanide solution and add 1 cc of 40% NaOH solution. Boil the solution. Add 0.2% glucose solution to the hot solution drop by drop and keep on boiling.

Result : The yellow colour of the ferricyanide begins to fade and finally **decolourise**.

Experiment (3) **Tommer's test. Reduction of alkaline copper sulphate.**

Procedure : In a test tube take 2 cc of 0.5% copper sulphate solution, then add 2 cc of 0.2% glucose solution and mix. Add 2 cc of 40% NaOH solution. A clear blue solution is obtained. Glucose acts as a solvent for cupric hydroxide $Cu(OH)_2$ and prevents its precipitation. Boil.

Result : Yellow or red precipitate of Cu_2O is formed due to the reduction of $CuSO_4$.

$$(OH)_2 - O - Cu_2O + 2H_2O$$

Experiment (4) **Fehling's test.**

Procedure : Take 5 cc of Fehling's solution and boil. There is no change of colour on the formation of precipitate, (in case of colour change and precipitate formation reject the solution). Add 1 cc of glucose solution and boil again.

Result : Colour changes with the formation of **yellow** or **brick-red precipitate of Cu_2O.**

Experiment (5) **Benedict's test.**

Procedure : In a test tube 5 cc of Benedict's reagent, then add 0.5 cc of glucose solution and heat to boiling. Boil for 2 minutes. Cool the solution under tap water.

Result : Green, yellow or red precipitate of Cu_2O is formed.

Experiment (6) **Picric acid test.**

Procedure : In a test tube take 3 cc of 2% glucose solution, then add 1 cc of picric acid saturated solution and then add 1 cc of 40% NaOH.

Result : Picric acid is reduced to picramic acid with the formation of **red colour**.

$$C_6H_2OH(NO_2)_3 + 3H_2 \rightarrow C_6H_2OH.\ NH_2(NO_2)_2 + 2H_2O$$

Experiment (7) **Nylander's test.**

Procedure : In a test tube take 5 cc of 2% glucose solution, add 0.5 cc of Nylander's reagent and heat to boiling and keep on boiling for 2 minutes.

Result : The solution becomes **dark black,** as bismuth sub-nitrate is reduced to bismuth.

$$Bi(OH)_2NO_3 + KOH \rightarrow Bi(OH)_3 + KNO_3$$

$$2Bi(OH)_3 + 3O \rightarrow 2Bi + 3H_2O$$

Experiment (8) **Rapid furfural test.**

Procedure : Take 1 cc of 2% fructose solution, add 6 drops at α-naphthol, then add 5 cc of conc. HCl in a test tube and boil.

Result : As the mixture begins to boil, **deep purple colour** appears.

C. Disaccharides

Disaccharides include lactose, maltose and sucrose.

(1) Lactose : It is found in milk and also in the urine of women during pregnancy and lactation.

(2) Maltose : It is the final product of starch hydrolysis.

(3) Sucrose : Abundant in plants as a reserve food material.

Lactose and maltose are reducing sugars and give positive Tommer's Fehling's, Benedict's and Barfoed's tests. Sucrose gives positive Rapid Furfural and Seliwanoff s tests. Lactose and maltose form osazones with characteristic crystalline forms and they can be classified by microscopic examination. Lactosazone forms mushroom-shaped crystals, glucosazone forms needle-shaped crystals, while maltosazone forms flower-shaped crystals. Sucrose does not form osazone. For the osazone formation, prepare 0.5% glucose and fructose solutions, 2% lactose, 2% maltose and 2% sucrose. In a test tube take 5 cc of sugar solution and add

10 drops of glacial acetic acid, then add a knife point of phenylhydrazine hydrochloride, then add twice the amount of sodium acetate crystals, give a little heat to the solution to dissolve the solids. Filter the solution and keep the filtrate in boiling water bath for 20 minutes, then remove and examine.

2. Test on Proteins

Proteins, found in animals and plants, are important building blocks formed by amino acids, condensed together by peptide linkage. All proteins contain carbon, hydrogen, oxygen, nitrogen and with a few exceptions sulphur also. The alimentary composition of proteins consists of approximately C = 45-55%, H = 6-8%, O = 19-25% and N = 14-20%. Proteins have high molecular weight. They contain free amino and carboxyl groups and so they can combine with bases and acids depending upon the pH of the medium. On hydrolysis, proteins break into peptones, proteoses, peptides and amino acids. Chemical behaviour of the proteins is due to the amino acids in the protein molecules.

For experiments fresh 5% solution of egg white is prepared. Egg white is filtered through cotton. Dissolve 5 cc of egg white into 95 cc of distilled water for 5% egg white solution. Proteins show both precipitation and colour reactions.

A. Precipitation of Proteins with Heavy Metals

Experiment (1) **Mercuric chloride test.**

Procedure : In a test tube take 3 cc of 5% egg white solution, then add mercuric chloride drop by drop.

Result : White turbidity is produced first which becomes thick and granular. The heavy metal salts precipitate protein solutions. This precipitate is generally soluble in excess of the salt solutions.

Experiment (2) **Ferric chloride test.**

Procedure : Take 3 cc of 5% egg white solution and add 0.5% ferric chloride solution drop by drop.

Result : On addition of first drop, turbidity appears and it increases on addition of subsequent drops. If $FeCl_3$ is added in excess, the turbidity disappears.

B. Precipitation of Proteins by Alkaloid Reagents

Experiment (3) **Sulphosalicyclic acid test.**

Procedure : It a test tube take 3 cc of 5% egg white solution and add 20% sulphosalicylic acid.

Result : **White precipitate** is obtained.

Experiment (4) **Esbach's test.**

Procedure : Take 3 cc of 5% egg white solution in a test tube and .a little Esbach's reagent.

Result : **Yellowish precipitate** is formed. By this method the quantity of albumen in urine is also estimated.

Experiment (5) **Tannic acid test.**

Procedure : In a test tube, take 3 cc of 5% egg white solution and then add 5 drops of freshly-prepared tannic acid solution.

Result : Brownish and non-granular precipitate is formed.

Experiment (6) **Hellers' test.**

Procedure : Take 3 cc of concentrated HNO_3 in a test tube and then add very carefully 3 cc of 5% egg white solution by means of a pipette in such a manner that it forms upper layer. Mix gradually by rotating between palms.

Result : A white ring is formed at the junction of the two solutions.

Experiment (7) **Acetic acid-potassium ferrocyanide test.**

Procedure : In a test tube take 3 cc of 5% egg white solution, then add 3 drops of glacial acetic acid, and 3 drops of potassium ferrocyanide solution.

Result : White precipitate is obtained. The precipitation is due to the proteoses, which dissolves on boiling and reappears on cooling.

C. Colour Reactions of Proteins

Proteins show colour reactions which are due to the presence of constituent radicals in the complex protein molecule. Since different proteins contain different groups, all proteins do not give positive reaction with all colour experiments. Sometimes, non-proteins or prosthetic groups also respond to certain colour reactions and hence several tests must be done before drawing any conclusion. For colour reaction experiments prepare 5% egg white solution.

Experiment (8) **Biuret reaction.**

Procedure : Take 3 cc of 5% protein solution in a test tube, add 1 cc of 40% NaOH solution to make it strongly alkaline, and then add 2 drops of 1% copper sulphate solution.

Result : Violet or pink colour appears. This reaction is due to the peptide linkage and so it is positive with all proteins.

Experiment (9) **Ring biuret test.**

Procedure : Take 3 cc of 5% or even more dilute egg white solution is a test tube, add 1 cc of 40% NaOH, than add by means of a pipette 1 cc of 1% copper sulphate over the surface of the liquid very gently so that the 2 fluids do not mix. Rotate gently.

Result : A pink or violet ring is formed at the junction of the two fluids. Proteoses and peptones give rose colour. Gelatin gives bluish pink or violet colour.

Experiment (10) **Xanthoproteic reaction for tyrosine, phenylalanine and tryptophane.**

Procedure : Take 3 cc of 5% egg white solution in a test tube, add 1 cc of conc. HNO_3 and boil. First white precipitate is formed which changes to yellow. The liquid also becomes yellow. Cool the test tube and add excess of 40% NaOH or ammonia to make alkaline.

Result : The yellow colour changes to **orange**. The proteoses and peptones do not form precipitate with HNO_3 but their solution turns yellow to orange in the presence of alkali. The precipitate is due to the formation of metaproteins insoluble in HNO_3 (nitric acid). The yellow colour is due to nitro-compounds from the protein molecule containing benzene ring. When made alkaline, the nitro-compounds ionize freely and produce deep yellow or orange colour.

Experiment (11) **Millon's test for tyrosine.**

Procedure : In a test tube take 3 cc of 3% egg solution, and 2 cc of mercuric sulphate reagent by pipette and boil cautiously for a minute. A yellowish precipitate generally formed. Cool the tube and add 2 drops of 1% $NaNO_2$ (sodium nitrite). Heat again.

Result : The solution and the precipitate become red showing the presence of tyrosine.

Experiment (12) **Aldehyde test for tryptophane.**

Procedure : In a lest tube take 3 cc of protein solution (5% egg white), then add one drop of 0.2% of 40% formalin, then add 0.5 cc of mercuric sulphate reagent. Shake well and then add 2.0 cc of conc. H_2SO_4. Shake.

Result : Violet or purple colour develops. Sometimes, a little heat is required for the colour to appear.

Experiment (13) **Glyoxalic acid test for tryptophane.**

Procedure : In a test tube take 3 cc of 5% egg white solution for protein and add 3 cc of glyoxalic reagent. Now add this solution very carefully to another test tube containing 5 cc of conc. H_2SO_4 in such a manner that the two fluids do not mix. Rotate the tube gently.

Result : Purplish violet colour develops at the junction of the fluids. The purple or violet colour is due to the presence of tryptophane, which forms condensation product with the aldehyde.

Experiment (14) **Arginine test for arginine.**

Procedure : In a test tube take 3 cc of 5% egg white protein solution, then add 2 drops of 1% α-naphthol solution, then add 1 cc of 40% NaOH solution, and then add 2 drops of sodium hypobromide (NaOBr).

Result : Bright colour is obtained. This reaction is specifically meant for arginine which is present in all proteins.

Experiment (15) **Sulphur test for cystine and cysteine.**

Procedure : In a test tube take 3 cc of protein solution (5% egg white), then add 5 drops of lead acetate which causes precipitation. Now add 40% NaOH drop by drop till the precipitate dissolves. Boil.

Result : Black or brown precipitate is formed, which shows the presence of cystine or cysteine group.

Experiment (16) **Molisch's test for carbohydrate group attached to protein molecule.**

Procedure : In a test tube take 3 cc of 5% egg white protein solution, then add 2 drops of 5% alcoholic thymol; now incline the tube and gently add 3 cc of conc. H_2SO_4 (the acid should go by the side of the tube wall) in such a way that the fluids do not mix. Rotate the tube gently.

Result : Purple-violet ring, at the junction of the fluids, is formed which shows the presence of carbohydrate group attached to the protein molecule.

Experiment (17) **Ninhydrin test.**

Procedure : Take 1 cc of 5% egg white protein solution and add 4 drops of 0.1% ninhydrin solution and boil for one minute. Cool the test tube.

Result : Blue colour develops. The test gives positive results by all amino acids and their derivatives except proline and hydroxyproline.

3. Test on Fats and Oils

Fats and oils are found abundantly in plants and animals forming distinct foodstuff. Fats have double caloric value than the carbohydrates. Fats have greasy feel with low melting point. They are soluble in organic solvents like ether, chloroform and alcohol and insoluble in water. Fats are hydrolysed by boiling acids and alkalines. Simple fat is glycerol which forms esters with 3 molecules of the same different acids and the most common acids are :

(1) $CH_3(CH_2)_4.COOH$ — palmitic acid

(2) $CH_3(CH_2)_{16}.COOH$ — stearic acid

(3) $CH_3(CH_2)_7.CH = CH(CH_2)_7.COOH$ — oleic acid.

For experiments with fats, olive oil is quite suitable.

Experiment (1) **Solubility test.**

Procedure : In separate test tubes marked A, B, C and D, take 0.5 cc of water in test tube A, 5 cc of ether in test tube B, 5 cc of chloroform in test tube C, and 5 cc of alcohol in test tube D. Add 3 drops of oil, preferably olive oil, in each test tube drop by drop.

Result : Test tube A = **oil is not miscible and it floats.**
Test tube B = **oil is miscible.**
Test tube C = **oil is miscible.**
Test tube D = **oil sinks to bottom; on heating oil dissolves.**

Experiment (2) Acrolin test.

Procedure : In a dry test tube take 0.5 cc of olive oil, then add knife point of sodium or potassium hydrogen sulphate and mix thoroughly by a glass rod and heat.

Result : Observe **irritating odour** of Acrolin. The glycerol present gives Acrolin on dehydration.

Experiment (3) **Emulsification of fats.**

Procedure : In a test tube take 3 cc of neutral olive oil, then add 2 drops of oleic acid, mix by shaking to form rancid oil. Now add 2 drops of this rancid oil to another test tube already containing 3 cc of dilute caustic soda.

Result : The acid dissolves in alkali forming a soap, which entangles oil by diffusion to form emulsion.

Experiment (4) **Salting out.**

Procedure : Take 10 drops of olive oil in a test tube, add 2 cc of alcoholic caustic soda, boil carefully. Soap solution is obtained. Divide this solution equally in 3 separate test tubes marked A, B and C. To test tube A add 3 cc of conc. HCl or H_2SO_4, to B add sodium chloride powder and to C add 2% calcium solution. Observe.

Result : Test tube A = small oil globules separate and float on the surface.
Test tube B = white precipitate separates and floats on the surface.
Test tube C = white precipitate of calcium soap is formed.

4. Test on Enzymes

Experiment (1) **To demonstrate action of salivary enzyme amylase (ptyalin).**

Principle : Enzyme amylase is found in saliva, which is secreted by salivary glands in mouth palate. Amylase partially hydrolysed (breaks) starch or glycogen into glucose and maltose. Salivary amylase acts at a temperature of 37°C and at a pH of 6.6 (acidic). When iodine solution is mixed with starch, blue colour is obtained. When starch is first hydrolysed with amylase and then mixed with iodine solution, blue colour is not obtained because starch has been broken into glucose and maltose.

Requirements : Beakers (100 cc), pipettes, test tubes, glass-stoppered bottles, plain or cavity slides, staining tubes, cavity blocks (small), toluene, starch paste, 0.02N iodine solution, incubator set at 37°C.

Procedure : (1) Clean all glass apparatuses first with vim. Dry them and then rinse with potassium dichromate sulphuric acid solution. Wash glass apparatuses 9 times with tap water and 3 times with distilled water. In case chromic acid solution is not available, wash them with vim, tap water and then with distilled water. Let all the apparatuses become dry. Keep them inverted over blotting paper for 24 hours.

(2) Prepare 0.02 N iodine solution in a glass stoppered bottle.

(3) Prepare 1% starch solution (1 gm of starch powder + 100 cc of distilled water) in glass stoppered bottle.

(4) For collecting own saliva, rinse your mouth with warm water quickly. Then take 20-25 cc of warm water in mouth, rotate water with tongue for 2-3 minutes and collect the saliva solution in a beaker. This contains salivary enzyme **amylase**.

(5) In another beaker mix 5 cc of 1% starch solution and 5 cc of saliva solution. Incubate the beaker in an oven set at 37°C for one hour.

(6) Take two cavity blocks or staining tubes and mark them A and B respectively with glass marking pencil. In each cavity block or a staining tube, add 2 drops of iodine solution. In cavity block A add a drop of starch-saliva mixed solution with a pipette. With another pipette add a drop of starch solution only in cavity block B.

Result : In cavity block B, solution becomes blue while in cavity block A it remains colourless as starch has been hydrolysed into glucose and maltose demonstrating activity of enzyme amylase.

Experiment (2) **To demonstrate action of pepsin on protein.**

Principle : Pepsin is a proteolytic enzyme secreted by gastric glands in the stomach. It is secreted in inactive form, called **pepsinogen,** which becomes active in the presence of HCl and then is called **pepsin. Pepsin hydrolyses proteins** into **peptones proteoses**. The enzyme acts at a temperature of 37°C and with a pH highly acidic (1.5 to 2.2). Both pepsin and pepsinogen have been prepared in a crystalline form. To demonstrate pepsin, **casein** protein is used. Pepsin acts on casein forming precipitate.

Requirements : Glass apparatuses as mentioned in case of amylase. Clean them as mentioned earlier. In addition **casein**, thymol, acetic acid, glycerol, HCl and sodium acetate. A hand centrifuge or high speed electrically operated centrifuge, centrifuge glass tubes and an incubator set at 37°C.