VIVEKANANDA COLLEGE

(Residential & Autonomous – A Gurukula Institute of Life – Training)

College with Potential for Excellence

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DBT STAR COLLEGE SCHEME

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Biochemistry and Biophysics lab Manual

Qualitative Test for Carbohydrate

S.No	Experiment	Observation	Inference
1.	Fehling's Test: Take A and B fehling's solution equal amount in test tube (2ml). Add 2ml of sample and boil.	A bright red colour precipitate is formed	It indicates the presence of sugar.
2.	Benedict's Test: Take 1 ml of sample in a test tube, add 5 drops of Benedict's solution, boil it and cool.	Formation of yellowish orange red precipitate.	It indicates the presence of glucose.
3.	Iodine Test: Take 1 ml of sample in a test tube and add few drops of iodine followed by 1-2 drops of NaoH, mix the content	Blue colour is disappeared	It indicates the presence of starch.

Qualitative Test for Carbohydrate

Aim:

To test the presence of carbohydrate in the given sample.

Background information:

Carbohydrates are polyhydroxy aldehydes or ketones and derivatives of them. They are the chief source of energy in the food of human and many other animals. The carbohydrates are classified into monosaccharides. Disaccharides, Trisaccharides, Oligosaccharides and Polysaccharides.

Monosaccharides are single contain only one molecule of sugar and they cannot be broken into simpler substances by hydrolysis. They are further subdivide according to the number of carbon atom contain in structure.

Materials required:

Test tube, Test tube holder, Spirit lamp, Reagents etc.

Procedure:

1. Fehling's test for reducing sugar:

Take equal volume of fehling's solutions A and B (2ml) in a test tube. Mix well and bil the contents. No colour change is noted and then add 2ml of given sample solution. Heat the content in the test tube. A bright red precipitate shows the presence of reduced sugar.

2. Benedict's Test:

Take 1 ml of sample in a test tube. Add 5 drops of Benedict's solution and boil the content. After boiled cool the content in a water bath. A yellowish orange red precipitate shows the presence of glucose.

3. Iodine Test:

Take 2 ml of 1% starch solution in a test tube, add few drops of iodine. Appearance of blue colour indicates the presence of starch. Then heat the solution the blue colour rapidly disappear. Then few drops of NaoH gets the solution disappearance of blue colour. This is because the NaoH removes free iodine. Which is necessary for the appearance of blue colour.

Result:

The qualitative test for carbohydrate are performed as given in the procedure and results are presented in the table.

Qualitative Test for Protein

S.No	Experiment	Observation	Inference
1.	Ninhydrin Test: In a test tube take 2ml of sample and add 1ml of ninhydrin solution, boil it and cool.	Appearance of blue colour	Presence of Protein.
2.	Xanthoprotein Test: In a test tube take 2ml of sample, add few drops of Conc.HNo3 and heat and cool. Add NaoH till yellow into orange.	Appearance of yellow to orange colour.	Presence of Protein.
3.	Biuret Test: In a test tube, take 2ml of sample with 2ml of 10% NaoH, it becomes pink then add 0.5% of CuSo4 it converts pink into violet.	Appearance of pink colour changes into violet while adding CuSo4	Presence of Protein.

Qualitative Test for Protein

Aim:

To test the presence of Protein in the given sample.

Background information:

Proteins are the most important compound of animals and plant tissue. They are associated with the structure and function of living system. Proteins are hydrolysed to produce peptone which is further hydrolysed and split into aminoacids. Mostly the proteins are soluble in weak or strong solution. Some proteins were coagulate while heating.

Materials required:

Test tube, Test tube holder, Stand, Spirit lamp, Ninhydrin solution, Conc. HNO3, Ammonium hydroxide, 10% sodium hydroxide solution, 0.5% Cupric sulphate solution, Protein sample (Egg albumin).

Procedure:

1. Ninhydrin Test:

Take 5 ml of the sample solution in a test tube .Add 0.5 ml of 0.1% of Ninhydrin solution. Heat it and cool, the solution turns into blue colour. It indicates the presence of Protein.

2. Xanthoprotein Test:

Take 5 ml of given sample solution in a test tube, add conc. HNO3 to about 1/3 of the volume. Gently heat the content. A yellow colour develops and cool it. Add Sodium Hydroxide till the yellow changes into orange.

3. Biuret Test:

Take 3ml of the given solution in a test tube and add an equal volume of 10% NaoH solution. Mix the contents thoroughly, pink colour appears first, then add 0.5% Cupric Sulphate solution drop by drop. Mix the contents the colour changes from bluish pink into violet colour.

Result:

The qualitative test for protein are performed as given in the procedure and results are presented in the table.

Qualitative Test for Lipids

S.No	Experiment	Observation	Inference
1.	Translucent Test: Place drops of water on a paper similarly place drops of given sample on another paper and warm them gently.	No translucent spot develops in water paper. Translucent spot developed in oil paper.	Absence of Lipid Presence of Lipid.
2.	Solubility Test: Take water and sample in equal volume in a test tube mix thoroughly.	The sample insoluble in water, but soluble in alcohol.	Presence of lipid.
3.	Sudan Test: Take Sudan III and water in test tube, mix well. A few grains of Sudan III are added in the oil sample.	No Change Oil were sedimented in bottom, deep orange red colour appear.	Absence of Lipid Presence of Lipid.

Qualitative Test for Lipids

Aim:

To test the presence of lipid in the given sample.

Background information:

Fats are esters of fatty acid and glycerol which are insoluble in water but soluble in alcohol other than chloroform. They are food resource in animals and plants.

Materials required:

Test tube, Test tube holder, Stand, Spirit lamp, Coconut oil, Paper, Alcohol, Chloroform, Sudan III.

Procedure:

1. Translucent Test:

Place a drop of given sample on a piece of paper and allow it to dry, warm gently, the translucent spot appears on the paper. It indicates the presence of fat substances.

2. Solubility Test:

1ml of lipid is taken in a test tube, add 1ml of alcohol or chloroform and mix well. Observe the nature of the solubility.

3. Sudan Test:

Sudan III is a dye that is insoluble in water. But soluble in fat. Place several grains of the dye in a test tube containing water and shacked well. Note that the water is coloured.

Now add several drops of oil to the test tube and let into stand for some time. The oil is collected at the bottom and become deep orange which indicates that the Sudan III is dissolved completely in the oil liquid.

Result:

The qualitative test for lipid are performed as given in the procedure and results are presented in the table.

Qualitative Test for Uric Acid

S.No	Experiment	Observation	Inference
1.	A pinch of sample is taken in a test tube add 5ml of distilled water and few drops of 10% NaoH, mix thoroughly. Boil it add few drops of Con.HCl. Finally a drop of solution is placed in a clean glass slide. Observe the uric acid crystals under low power of microscope.	Uric acid crystals such as rhomboid, wedge, rectangular shapes were seen	Presence of uric acid crystals.

Qualitative Test for Uric acid

Aim:

To demonstrate the presence of uric acid crystal in the excreta of birds.

Background information:

Terrestrial animals excrete their nitrogenous wastes in the form of uric acid, they are called uricotelic animals.

Materials required:

Test tube, Test tube holder, Stand, Sprit lamp, Bird excreta, Mortar and pestle, Conc. HCl, 10% NaoH.

Procedure:

A pinch of sample is taken in a test tube, add 5 ml of distilled water and few drops of 10% NaoH. Mix thoroughly and boil the contents. Then few drops of Conc. HCl is added. Finally a drop of this solution over a clean glass slide, allow it to dry. Observe the crystals under the low power of microscope.

Observation:

Presence of different type of uric acid crystal such as rhomboid, prismatic, wedge, rectangular, hexagonal shaped structures were seen under the microscope.

Test for albumin, sugar, urea in urine sample

S.No	Experiment	Observation	Inference
1.	Test for albumin: Take 2ml of sample in a test tube, add 3ml of Conc.HNO3	A white precipitate doesn't appear at the junction of two liquids.	Absence of albumin
2.	Test for sugar: Take 3ml of sample, add 3ml of Benedict reagent, boil and cool it.	Bluish green with yellow precipitate occurs or brick red precipitation occurs	Presence of sugar
3.	Test for urea: Take 3ml of urine sample, add 3 drops of phenol red and then add small amount of urease powder.	Appearance of red colour.	Presence of urea

Aim:

To test the presence of albumin, sugar and urea in the given sample of urine.

Background information:

Urine is an excretory product of the body forms in kidneys. The examination of urine for changes in characteristics and detection of abnormal constituents. An important test for suspected renal damage as well as systemic disease. The normal urine contains organic and inorganic

constituents. The nitrogenous constituents are urea, uric acid and creatine. A small amount of sugar is present in the normal urine i.e. 1.15gm out of this glucose is present in the concentration of 50-300mg / 24 hours. But due to some inborn errors of metabolism the glucose level may go higher in the urine presence of albumin, sugar and urea are tested by simple experiments.

Materials required:

Test tube, Test tube holder, Stand, Spirit lamp, conc.HNO3, Benedict's reagent, Urease powder.

Procedure:

1. Test for albumin: (Heat coagulation test)

Take 2ml of sample, add 3ml of conc. HNO3. A white precipitate appear at the junction of two liquids, which shows presence where no white precipitate is appear,Which shows absence of albumin in urine sample.

2. Test for sugar: (Benedict's test)

3ml of urine sample is taken in a test tube, add 3ml of Benedict's reagent with it. Gently boil for two minutes and cool. Whether it express two colour shows presence of sugar.

Bluish green with yellow precipitation – 0.2% sugar

Deep brick red precipitation – 1.2%

Deep yellow to orange precipitation -0.5%

3. Test for urea: (Urease test)

Take 3ml of urine sample, add 3 drops of phenol red, there will be no colour. Then a small amount of urease powder is added into the test tube,Which converts sample into red colour, which shows the presence of urea in the given sample.

Result:

The given sample contains urea, sugar but albumin is absent.

Effect of temperature on salivary amylase activity

S.No	Temperature	Time Taken (Sec)	Rate of activity
1.			
2.			
3.			

Aim:

To demonstrate the effect of temperature on salivary amylase activity in human.

Background Information:

Enzymes are biocatalysts which take part in biological reactions. Theenzyme activity is greater at optimum temperature, Like any chemical reaction, the velocity of an enzymatic reactions also increase with temperature upto a certain level, this can be demonstrated by the amylase activity.

Saliva is viscous, colourless fluid made up of 80 – 90% of water with an average pH of 6.8. It contains α-amylase, bicarbonate calcium, sodium, potassium etc. Amylase is an enzyme which converts starch into maltose.

Materials requires:

Boiling test tube, test tube stand, holder, cotton, paraffin wax, funnel, glass rod, stop watch, 1% starch, lugol's iodine etc.

Procedure:

A small piece of wax is chewed to induce saliva secretion which is collected in a test tube. From this 10% enzyme is prepared by mixing 1ml of concentrated saliva with a 9ml distilled water.

Take 2ml of 1% starch and 2ml of phosphate buffer (6.8 pH) in a boiling test tube. To this add 1ml of 10% saliva (coenzyme starch), then incubate the reaction mixture at room temperature (24ºC). Amylase acts on the substrate by converting starch into Maltose.

1% Lugol's iodine drops are placed on a white tile. Thereaction mixture may be mixed with iodine reacts with starch to give deep blue colour in the initial stage. When starch is digested no colour changed. It is observed in the iodine drops. This is the achromic end point.

By this method, the test tube time taken for achromic complete digestion of starch is noted using a stop watch. The same experiment is represented at two different (lower / higher) temperature at 5ºC intervals. The results are presented in a table.

Result:

As the temperature is increased, the rate of enzyme activity will also increase significantly.

Estimation of Ascorbic acid

Samples	**Burette readings**		**Volume of the dye (ml)**	**Concordant value (ml)**	**End point**
	Initial (ml)	Final (ml)			
Unknown sample (juice)					
Blank (distilled water)					
Standard Ascorbic acid					

Aim:

To estimate the amount of ascorbic acid present in the given sample.

Principle:

Ascorbic acid (Vitamin C) reduced 2, 6 dichlorophenol indophenol a coloured dye to colourless form in acidic medium. The vitamin gets oxidises to dehydro ascorbic acid. Though the dye is blue in colour, the appearance of a pale pink colour indicates the end point of titration.

Standard ascorbic acid stock solution: (1mg / 1ml)

i) Weigh 100 mg of ascorbic acid transfer it carefully into a 100 ml volumetric flask. Dissolve the vitamin and make up the volume into 100ml with oxalic acid solution (4%).

ii) Working standard (0.1mg / 1ml) dilute 10ml of the stock solution to 100ml with 4% oxalic acid in a 100ml volumetric flask.

Reagents:

i) oxalic acid solution 4%

ii) Dye solution

Procedure:

Pipette out 5ml of the working standard solution of ascorbic acid into a 100ml of conical flask. Add 40ml of oxalic acid solution and titrate the contents against the dye solution taken in glass burette. The appearance of a pale pink colour which persists for few minutes, indicates the end point of the titration. There is no need for addition of an indicator as this dye is a self-indicator. Repeat the titration thrice. The volume of dye consumed is equivalent to the amount of vitamin C.

Weight 10g of citrus fruit extract the juice into 100ml beaker. Filter the extract through a glass funnel plugged with filter paper. Take the extract into a 100ml volumetric flask make up the volume to 100ml with 4% oxalic acid. Make 1:10 dilution of this solution with 4% oxalic acid. Before titration use 5ml sample volume for titration. Past the procedure is similar to standard titration.

Calculation:

Ascorbic acid content (mg / 100g) of sample) is calculated by the following equation.

$$\text{Vitamin (mg.100g)} = \frac{0.5\text{mg}}{V2\text{ml}} \times \frac{V1\text{ml}}{5\text{ml}} \times \frac{100\text{ml} \times 10}{\text{weight of the sample (g)}} \times 100$$

Where

V1 – volume of the dye consumed for standard ascorbic acid (ml)
V2 – volume of dye consumed for the sample (ml)
x10 – Dilution factor.

Result:

The amount of ascorbic acid present in the given sample is 21.2 mg / 100ml.

Preparation of Haemin Crystals

Aim:

To prepare and mount haemin crystals of human blood.

Principle:

The blood pigment haemoglobin is commonly found in the blood of all vertebrates. It gives colour to the blood. It is a conjugated protein consisting of non-protein part called Haem or haematin consisting of phorpyring group combined with iron. Haemoglobin is readily split into its globin and Haemin means of heat and acetic acid.

Material requires:

Sterilized needle, sprit lamp, cotton, glass slide, glacial acetic acid and compound microscope.

Procedure:

Take a drop of human blood on a clean glass slide. Make a thin smear and dry it. Scarp the dry blood in the centre of the slide. Add one or two drops of conc. glacial acetic acid. Place a cover slip on the slide. Gently heat the slide over a sprit lamp until the bubbling clears. Examine the slide under the low power of the microscope.

Results:

The haemin crystal found in the human blood.

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