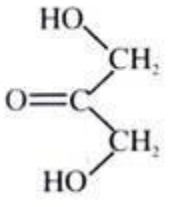
## Qualitative and Quantitative Tests for Carbohydrates

One of the most important constituents in our food is glucose which we usually obtain in the form of starch from plant sources. In our body glucose is readily utilized or is stored as glycogen.

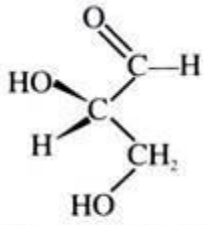
The metabolic processes in our body are mainly centred on glucose, which is a member of a large class of organic compounds called carbohydrates. These are generally referred to as sugars. Carbohydrates contain C, H and O atoms. Usually, H and O are present in the ratio of 2:1, just as in water; hence the name carbohydrates are in use.

Carbohydrates in general have either an aldehyde group (as in glucose) or a keto group (as in fructose). Those containing an aldehyde group (as in glucose) are called as aldoses and those containing keto groups are called ketoses. They may also be referred to on the basis of the number of carbon atoms contained in them; for example, both glucose and fructose are hexoses as they have six carbon atoms in them.

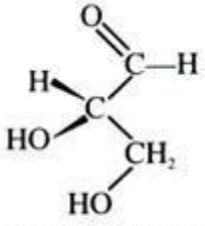
Ribose and deoxyribose are pentoses because they have five carbon atoms. Both of them are, however, aldoses. Similarly, arabinose is an aldopentose. They are also trioses, tetroses, heptoses, etc. Some carbohydrates are formed by the combination of two sugars for instance; the common sugar sucrose contains both glucose and fructose. Therefore, sucrose is referred to as a 'disaccharide', whereas glucose and fructose are monosaccharide's. There are many disaccharides like sucrose, e.g., maltose (glucose + glucose), lactose (galactose + glucose) and so on.



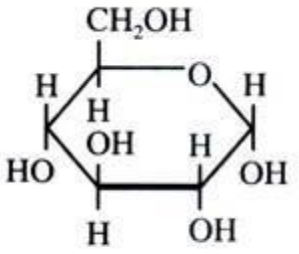
Dihydroxyacetone
(a ketose)



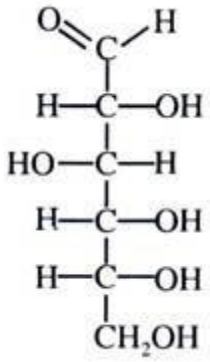
D-Glyceraldehyde
(an aldose)



L-Glyceraldehyde
(an aldose)




α-D-Glucopyranose




## Qualitative Test for Carbohydrates:

Most of the tests of the carbohydrates are based on their reducing properties (due to the presence of reducing aldehyde or ketone groups). Fehling's test, benedict's test are the example of this. The unspecific Molisch's test for carbohydrates is one of the examples of some tests which are based on the formation of furfural or furfural derivatives in presence of concentrated acids. Specific complex formation is sometimes used as specific test for carbohydrates. Formation of phenylhydrazone is one such example. For testing polysaccharides, iodine is found to be very useful.

**These tests will be discussed below:**

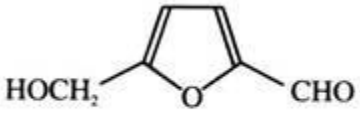
***1. Molisch's Test:***

**Principle:**

Alcoholic alpha naphthol forms furfural and furfural derivatives, such as hydroxymethylfurfural, by the concentrated sulphuric acid acting on the sugar. This compound forms a reddish-violet coloured ring at the junction of the two liquids. Molisch's reagent is 5% solution of alpha naphthol in alcohol.

**Procedure:**

Add 2 drops of Molisch's reagent to 2 ml of sugar solution in a test tube. Mx thoroughly. Add 2 ml of conc. $H_2SO_4$ by the side of the test tube slanting the tube. Then erect the test tube slowly. The formation of reddish violet ring at the junction of two liquids indicates the presence of carbohydrates.




Hydroxymethylfurfural

Concentrated solution of organic compounds may give a red instead of a violet colour due to the charring action of the sulphuric acid. In case of doubt the experiment should be repeated on a more diluted solution of the substance to be tested.

***2. Iodine Test:***

**Principle:**

The composition of the blue or red or wine red coloured substance is not well defined. This may be an adsorption complex of starch or dextrins or glycogen with iodine rather than a definite compound. Iodine reagent is 0.5 ml of iodine diluted to 5 ml with distilled water. Potassium iodide is added to the reagent solution in order to make the iodine more soluble in water.

**Procedure:**

Add 1 or 2 drops of dilute iodine solution to 2-3 ml of dilute starch or dextrin or glycogen solution. A blue, red and brown colour develops in case of starch, dextrin and glycogen respectively. In case of starch, the blue colour disappears on heating and reappears on cooling. But the red colour and the brown colour in cases of dextrin and glycogen respectively, do not reappear on cooling as in case of starch.

***3. Bial's Test for Pentoses:***

To 1 ml of sugar solution in a test tube add 3 ml of concentrated HC1 and 0.5 ml of Bial's reagent. Heat the tube in a boiling water bath for one minute. Record your observations with different sugars. The Bial's reagent is prepared by dissolving 3 gm of orcinol and 0.1 gm of ferric chloride in 100 ml of ethanol. This is a sensitive test for the detection of pentoses.

Heating with strong acid converts the pentose to furfural which then reacts with the coloured compound produced when orcinol and ferric chloride react with each other. A blue green compound is finally formed. This reagent reacts with many sugars but under the condition described above only pentoses yield blue- green colour.

***4. Seliwanoff 's Test***

To the sugar (2 ml) add 2 ml of seliwanoff s reagent. A blank without sugar should also be prepared to judge the colour change. Place the tubes in boiling water for exactly 1 min. Note the colour change, if any, and then continue the heating for 5 minutes and periodically observe the colour change. Seliwanoff s reagent is 0.5% resorcinol in conc.

HCl diluted 1:1 with water. Ketoses (naturally fructose) give fiery red colour. Aldoses (glucose, etc.) give the test weakly and slowly. If the boiling is prolonged, positive test is obtained with glucose (or maltose) due to its partial conversion to fructose. This test is also given by sucrose which is hydrolysed during the course of the test yielding fructose as one of the products.

***5. Reduction Tests:***

Carbohydrates with free aldehyde or ketone groups have the ability to reduce solutions of various metallic ions.

**These properties are mentioned below:**

**A. Fehling's Test:**

**Principle:**

Carbohydrates with free aldehyde or ketone groups reduce copper sulphate to cuprous oxide forming a yellow or brownish red coloured precipitate. Fehling's reagent is prepared freshly by mixing equal volumes of two stock solutions A and B. Solution A is 6.93 grams of $CuSO_4.5H_2O$ per 100 ml of water and Solution B is 20 grams of KOH and 34.6 grams of sodium potassium tartarate (Rochelle salt) per 100 ml solution.

**Procedure:**

Add a few drops of sugar solution at a time to 5 ml of Fehling's solution and heat the mixture after each addition. The production of yellow or brownish red cuprous oxide precipitate indicates the presence of reducing sugars.

**B. Benedict's Test:**

Add 5mL of Bennedict's qualitative reagent to the sugar solution, and place the test tube boiling water bath for 2 minutes. In case of reducing sugars there will be an appearance of red precipitate. Bennedict's qualitative solution is prepared by dissolving 173 gm. of sodium citrate and 100 gm. sodium carbonate and 100 ml of water, by heating.

If there is any turbidity, it should be removed by filtration. Copper sulphate solution (17.3 gm. copper sulphate in 100 ml water) is slowly added with constant stirring to the citrate-carbonate solution and the volume is made up to 11. This test is based on the modification of Fehling's test by Benedict. The difficulties faced by Fehling's test are, therefore, not faced in case of Bennedict's test.

In the presence of even small quantities of reducing sugars the entire body of the solution will be filled with a precipitate which is red. In the case of non-reducing sugar (say sucrose) the solution

will remain perfectly clean. This reagent is routinely used and found to be reliable in the examination of urine for pathological amounts of sugars.

**C. Barfoed's Test:**

Mix 5 ml of Barfoed's reagent with 1 ml of carbohydrate solution in a test tube and heat in a boiling water bath for 10 min. Appearance of a red precipitate of cupric oxide ($Cu_20$) indicates the presence of reducing sugar. Barfoed's reagent is prepared by dissolving 13.3 gm. of neutral copper acetate in 200 ml of water and then adding 1.8 ml of glacial acetic acid.

Barfoed's test is also copper reduction test but this test differs basically from Fehling's test or Bennedict's test as it is carried out in acidic medium instead of alkaline medium. Under acidic conditions the reduction takes place efficiently. Monosaccharide's respond quickly to the test whereas disaccharides respond slowly.

When the sugar solution is boiled in contact with the reagent the disaccharide is hydrolysed by acetic acid present in the reagent and the positive test is obtained. Chloride interferes with this assay as it causes the formation of a green precipitate the urine cannot be tested by this method as it contains chloride.

**Confirmatory Tests:**

**I. Phenyl hydrazine test:**

Take about 300 mg of phenyl hydrazine mixture (discussed below), to it add a few drops of glacial acetic acid and then 5 ml of sugar solution. Shake well and heat in a boiling water bath for 30 to 45 minutes. Take the tube out of the water bath and allow it to cool slowly. Yellow crystals of osazones will appear. Examine the crystals under the microscope and describe the nature of crystals.

Phenyl hydrazine mixture is prepared by mixing equal weights of phenyl hydrazine hydrochloride and anhydrous sodium acetate. The mixing is to be done thoroughly in a mortar. Glucose and Fructose give identical osazones called glucosazones and fructosazones because excepting the first two carbons (which are used in formation of osazone) the remaining four carbon atoms have same configuration in both of them.

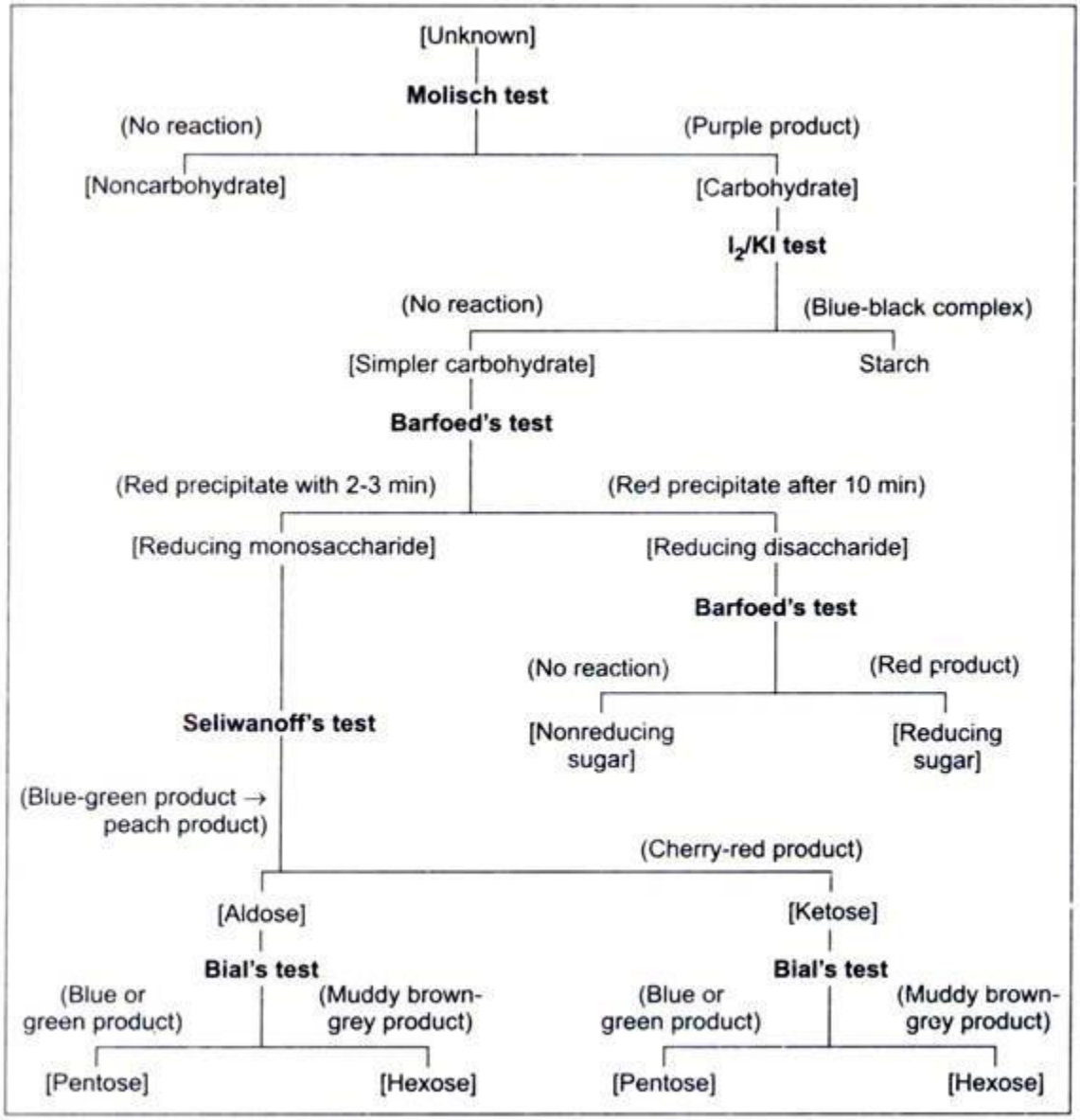



**Fig. 18.1:** Flow chart for classifying unknown carbohydrate

Sucrose as such does not form any osazone because it has no reducing group available for reaction with phenyl hydrazine. On hydrolysis, however, it gives rise to osazone. Osazones of disaccharides are soluble in hot water. Therefore, in their cases the osazones do not precipitate during heating. They appear only after cooling.

**Quantitative Tests of Carbohydrates:**

***1. Estimation of Glucose by Benedict's Method:***

During qualitative analysis of sugars we have already learnt that glucose reduces copper sulphate in Benedicts reagent under alkaline conditions and a red precipitate is formed. This qualitative method has been exploited for its use in quantitative analysis.

The Benedicts quantitative reagent contains the following ingredients, copper sulphate, sodium carbonate, sodium or potassium citrate, potassium thiocyanate and potassium ferrocyanide. Of these, copper sulphate has to be very accurately measured as the amount of copper sulphate reduced will correspond to the amount of glucose present in solution.

Due to presence of potassium thiocyanate in Benedict's reagent a white precipitate of cuprous thiocyanate instead of red precipitate of cuprous oxide will be formed when copper sulphate is reduced. As the precipitate formed is white it is very easy to determine the end point. Blue tint of Benedict's reagent disappears completely at this point.

The small amount of potassium ferrocyanide added helps to prevent the oxidation of cuprous oxide. Sodium or potassium citrate added does not allow the formation of copper carbonate. The alkaline condition is produced by sodium carbonate which is a mild alkali in comparison with NaOH and is, therefore, less destructive for the sugar. The Bennedict's reagent prepared as follows is stable for long periods of time.

To prepare quantitative Benedict's reagent 18.0 gm. of crystalline copper sulphate is dissolved in 100 ml of water (solution A). Further, 100 gm. of sodium carbonate, 200 gm. of anhydrous sodium citrate and 125 gm. of potassium thiocyanate are dissolved in 800 ml of water with heating (solution B). If solution B is not clear it should be filtered. Solution A is added slowly to solution B with stirring. Then 5 ml of potassium ferrocyanide solution is added and the volume is finally made up to 1 litre after cooling.

The reaction of $CuSO_4$ with glucose is quite complicated and a number of molecules of $CuS0_4$ are reduced by one molecule of glucose. Therefore, it is not possible to write the stoichiometric equation for reaction between $CuSO_4$ and glucose. But it has been found that 25 ml of the above mentioned quantitative reagent corresponds to 50 mg glucose. Determination of the unknown amount of glucose will be based on this.

**Procedure:**

Pipette out in a conical flask 25 ml of the Benedict's quantitative reagent. Add about 5 to 10 gm. of $Na_2CO_3$ and a few porcelain chips to the flask to prevent bumping. Heat the contents of

conical flask to boiling and then run in the glucose solution from a burette at first rapidly and then slowly until the blue colour becomes fade.
Allow it to boil for 2-3 minutes more and add glucose solution drop by drop till the solution becomes colourless. Note down the volume of the glucose solution used and calculate the percentage of glucose in solution as described below. Sometimes the solution in the flask becomes too much concentrated due to evaporation of water. To avoid it more water may be added.

Suppose 20 ml of the glucose solution is required to titrate 25 ml of Benedict's quantitative reagent. As 25 ml of the Benedict's quantitative regent is equivalent to 50 mg of glucose, hence 20 ml of the solution contains 50 mg of glucose. Therefore, 100 ml of the glucose contains 50×100/ 20 =250 mg of glucose and the strength of the solution 250 mg per cent.

**Estimation of Lactose by Benedict's Quantitative Regent:**
Principle is same as for glucose, only difference being 25 ml of Benedict's quantitative regent is equivalent to 67 mg of lactose.

Even sucrose after acid hydrolysis can be estimated by this method.

***2. Glucose Oxidase Method for Estimation of Glucose:***
In this method, the aldehyde group of β-D-Glucose is oxidized by glucose oxidase to give gluconic acid and hydrogen peroxide.

β-D-Glucose + $H_2O$ + $O_2$ → gluconic acid + $H_2O_2$
The hydrogen peroxide may be broken down to water and oxygen by a peroxidase and if an oxygen acceptor is present, it will convert to a coloured compound which can be measured. The reagent usually used is oxidation product of phenol condensed with 4-aminophenazone to give a coloured product as in determination of alkaline phosphatase.