Carbohydrates

Carbohydrates may be defined as polyhydroxy aldehydes or polyhydroxy ketones or compounds which produce them on hydrolysis.

Classification of Carbohydrates

Carbohydrates can be classified into three major groups: **monosaccharides, oligosaccharides and polysaccharides.**

a- Monosaccharides are the simplest carbohydrates and they cannot be further hydrolyzed into smaller units under normal conditions. Examples of **monosaccharides** are glucose, galactose, fructose and mannose

Classification of monosaccharides:

The monosaccharides are classified into different categories, based on the number of carbon atoms, and the functional group.

1- According to the number of carbon atoms present.

a- Trioses – sugar units containing three carbon atom e.g. glyceraldehydes
b- Tetroses – sugar units containing four carbon atom e.g. threose, erythrose
c- Pentoses – sugar units containing five carbon atom e.g. xylose, arabinose, rhamnose, ribose and ribulose.
d- Hexoses – sugar units containing six carbon atom e.g. glucose, fructose, mannose and galactose.
e- Heptoses – sugar units containing seven carbon atom e.g. cymarose

2- According on functional groups.

1. Aldoses- (containing aldehydic group) e.g. glucose, arabinose and galactose
2. Ketoses- (containing ketone group) e.g. fructose.

3- According on the number of carbon atoms and functional groups.

a) Aldosugar

1. Aldotrioes- (containing three carbon atoms and aldehyde group) and e.g. glyceraldehyde
2. Aldotetroes- (containing four carbon atoms and aldehyde group) and e.g. erythrose
3. Aldopentooes- (containing five carbon atoms and aldehyde group) and e.g. ribose
4. Aldohexoes- (containing six carbon atoms and aldehyde group) and e.g. glucose

b) Ketosugars

1. Ketotrioes- (containing three carbon atoms and ketone group) e.g. Dihydroxy acetone.
2. Ketotetroes- (containing four carbon atoms and ketone group) and e.g. erythrulose
3. Ketopentoes- (containing five carbon atoms and ketone group) and e.g. ribulose
4. Ketohexoes- (containing six carbon atoms and ketone group) and e.g. fructose

Common monosaccharides

No. of carbon atoms	Generic name	Aldoses (with aldehyde group)	Ketoses (with keto group)
3.	Triose	Ex: Glyceralde-hyde	Ex: Dihydroxy-acetone
4.	Tetrose	Erythrose	Erythrulose
5.	Pentose	Arabinose Xylose Ribose	Xylulose Ribulose
6.	Hexose	Glucose Galactose Mannose	Fructose
7.	Heptose		Sedoheptulose

b- Oligosaccharides are compound sugars containing 2 to 10 molecules of the same or different monosaccharides. Based on the number of monosaccharide units present, the oligosaccharides are further classified into disaccharide, trisaccharide,

tetrasaccharide, ------. A disaccharide consists of two monosaccharides that are linked together by a type of a bond called glycosidic bond such as sucrose, maltose, and lactose. Trisaccharide consists of three monosaccharides that are linked together by glycosidic bond

c- Polysaccharides are the macromolecular polymers of monosaccharide units linked by glycosidic bond. Based on the type of monosaccharide units present, the polysaccharides are classified into: **homopolysaccharides** (they are made up of one type of monosaccharide units) and **heteropolysaccharides** (they are made up of two or more different types of monosaccharide units or their derivatives). Starch, pectin, glycogen, cellulose, and heparin are examples of polysaccharides.

Tests for carbohydrates

Several qualitative tests have been devised to detect carbohydrates. These tests will utilize a test reagent that will yield a color change after reacting with specific functional groups of the compounds being tested.

Monosaccharides are the building blocks of carbohydrates. **Di, oligo and polysaccharides** on hydrolysis in presence of mineral acid yield monosaccharide units. Monosaccharides are soluble in water and practically insoluble in organic solvents like chloroform, ether and in absolute alcohols. These are optically active compounds and respond to various color reactions and identification tests.

The chemical properties of carbohydrates vary depending upon the number of hydroxyl groups and the presence or absence of –CHO/= CO groups. These variations are the basis in the development of colour reactions to identify the carbohydrates.

Some important points:

1. Most of the tests and reactions described are not quantitative and volumes are approximate, despite these facts some tests do not work if quantities greatly in excess of those stated are used.

2. **Do not** place your pipettes in reagent bottles as this leads to contamination.

3. In most tests, it is important to apply a control test using water instead of the solution under examination. If you are in doubt about the result of a test, perform the reaction with a suitable known compound.

4. In this experiment, sugar samples are given in their solid state. To perform each procedure, you should prepare your own sugar solution by taking very small amounts of solid sugars.

5. When you need to boil your sample in a test tube, prepare a hot water in a large beaker and put your test tube inside the beaker. **Do not** forgets to put boiling chips in the beaker.

Safety Precautions:

-Wear your safety goggles

Waste Disposal:

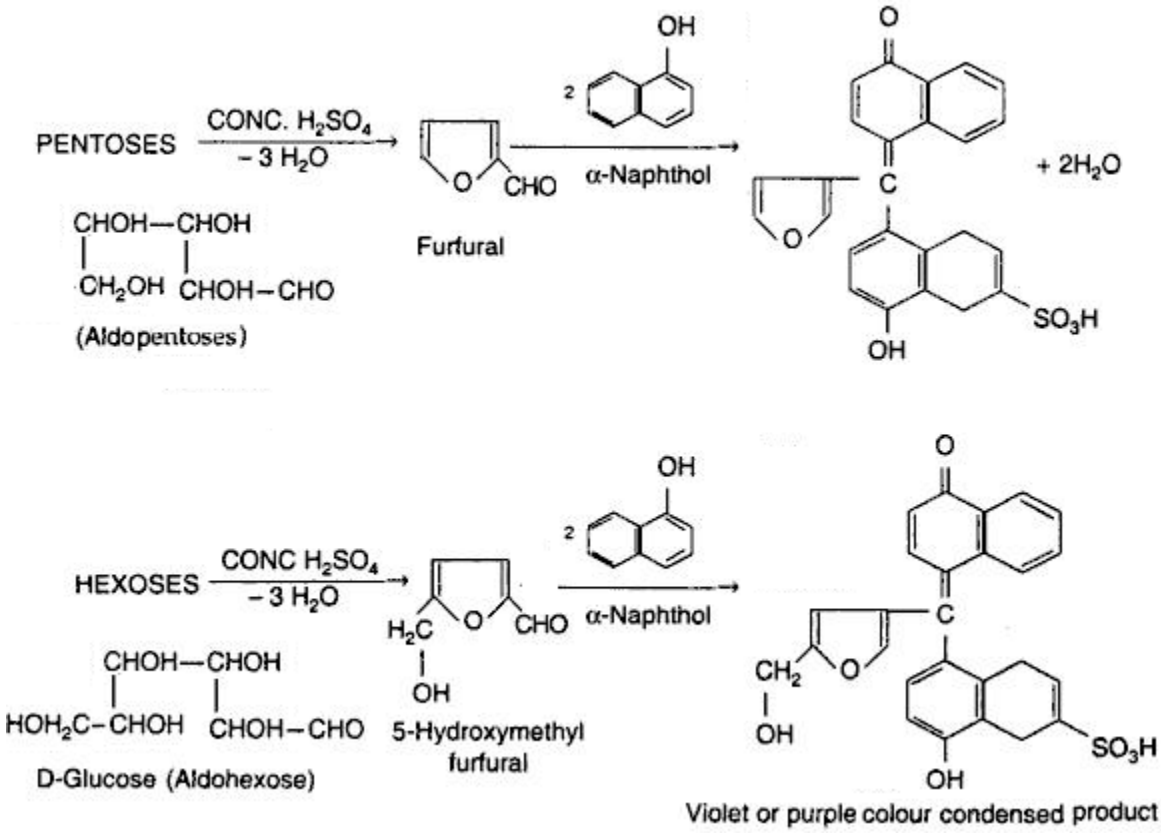
-All waste must be placed in the waste containers in one of the fume hoods

Test 1. Molisch's Test for Carbohydrates

The Molisch test is a general test for the presence of carbohydrates and are used for distinguish between carbohydrates and other compounds.

Carbohydrates when treatment with strong concentrated sulphuric acid H_2SO_4, undergoes dehydration to give furfural or furfural derivative which on condensation with alpha-naphthol yields a violet or purple colored complex whose exact structure is unknown.

If oligosaccharides or polysaccharides are present, they are first hydrolyzed glycosidic bonds to give the monosaccharides by concentrated sulphuric acid which are then dehydrated to form furfural or furfural derivatives. Pentose yield furfural and hexoses yield hydroxymethyl furfural.



Reagents:

1- Sugar solution (5%).

2- Molisch's reagent (5% α-naphthol in ethanol, prepare fresh).

3- Concentrated sulphuric acid H_2SO_4**.**

Procedure:

1- In a clean and dry test tube, Place 1 mL of a sugar solution in a test tube, add 3 drops of Molisch's reagent and mix.

2- Pour carefully 1 mL of conc. H_2SO_4 down the side of the test tube, so that it forms two layers at the bottom of the tube. Appearance of violet or purple ring at the junction of two layers indicates the presence of carbohydrates.

If the purple layer is difficult to see, mix slowly and carefully (large amounts of heat is generated) and observe the color. If the concentration of sugar is high enough all the solution becomes purple color.

Note:

1- Appearance of green ring is not a positive reaction. This is due to interaction of the alpha- naphthol with the acid.

2- Trioses, and tetroses, do not give this reaction because they do not possess the necessary minimum 5 carbon atoms for furfural formation.

Test 2. Benedict's Test for Reducing Sugars

This is a specific test for identification of reducing sugars and used for differentiation between reducing sugars and non-reducing sugars. It is a reduction test carried out in an alkaline medium.

Reducing sugars are those sugars; in a solution have free aldehydes or ketone groups in their structure. While non-reducing sugars are sugars do not have free aldehydes or ketone groups in their structure.

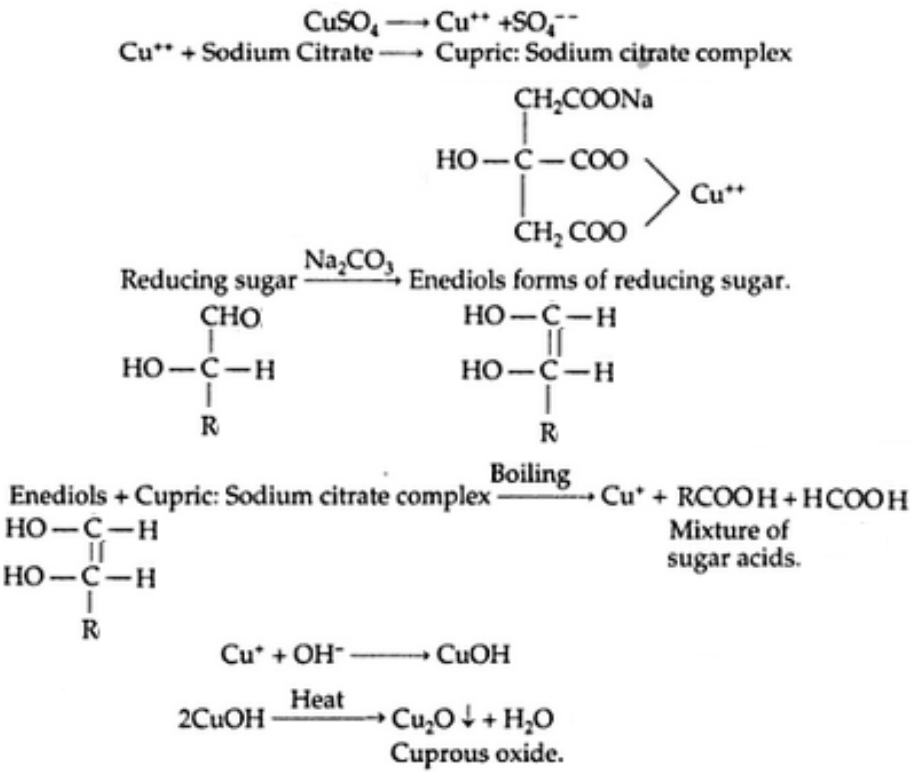
All monosaccharides are reducing sugars; they all have a free aldehydes or ketone groups. Some disaccharides have free aldehydes or ketone groups and are also reducing sugars. Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution. Polysaccharides are also non-reducing sugars.

Carbohydrates with free aldehyde groups or free ketone groups in alkaline medium reduce copper sulphate of benedict's solution to cuprous oxide on boiling, forming a red colored precipitate depending on the concentration of the sugar.

$$RCHO + 2\,Cu^{2+} + 4\,OH^{-} \longrightarrow RCOOH + \underset{\text{Red precipitate}}{Cu_2O} + 2\,H_2O$$

The Mechanism:

Reducing sugars under alkaline conditions form enediols. These enediols are unstable and decomposed to yield a mixture of sugar acids which are powerful reducing agents reduce cupric ion (Cu^{++}) to cuprous ion (Cu^{+}) as a cuprous hydroxide (CuOH). If the cuprous hydroxide in alkaline solution is heated, the black cuprous oxides (Cu_2O) precipitate, which indicates the presence of reducing sugar.



Reagents:

1- Sugar solution (5%).

2- Benedict reagent (To prepare one liter of Benedicts reagent: mix 173 g of tri-sodium citrate and 100 g of sodium carbonate with 800 ml of distilled water. Warm to dissolve, then cool and filter, add more distilled water to make the volume to 850

ml. Dissolve 17.3 g of copper sulphate in 100 ml distilled water and stir slowly into the first solution. Bring the volume to 1 litter with distilled water).

Procedure:

1- Add 5 drops of the sugar solution to 2.5 mL of Benedict's reagent, and shake the tube.

2- Place the tube in a boiling water bath and boil for 3 minutes.

3- Remove the tubes from the heat and allow them to cool. Formation of red precipitate indicates the presence of reducing sugars.

Note:

1- The role of copper sulphate is to provides cupric ions (Cu^{++}) in solution, sodium carbonate to makes medium alkaline, and sodium citrate- prevents the precipitation of cupric ions as cupric hydroxide by forming a loosely bound cupric- sodium citrate complex which on dissociation gives a continuous supply of cupric ions.

2- This test is useful for diagnosis of diabetes mellitus. The test is semi-quantitative because the color of the precipitate gives approximate percentage of sugar excreted in the urine.

Caution:

Benedict's reagent is caustic, rinse thoroughly with water if you get this solution on your skin or clothing.

Test 3. Barfoed's Test for Monosaccharides

Barfoed'stest is used for differentiates between monosaccharides and disaccharides as monosaccharides give positive test while disaccharides give negative.

Barfoed's test is similar to Benedict's test but the reduction carried out in an acidic medium. Reducing monosaccharides reduce the cupric ions formed in acidic medium to red colored cuprous ions within three minutes. The precipitate of cuprous

oxide is less dense than Benedict's test, therefore, it's recommended to leave the tube to stand to allow the precipitate to settle.

$$RCHO + 2\,Cu^{2+} + 2\,H_2O \longrightarrow RCOOH + \underset{\text{Red precipitate}}{Cu_2O} + 4H^+$$

The mechanism:

$$(CH_3COO)_2Cu + 2H_2O \longrightarrow 2CH_3COOH + \underset{\text{Cupric hydroxide}}{Cu(OH)_2}$$

$$Cu(OH)_2 \xrightarrow{\Delta} CuO + H_2O$$

$$\underset{\text{D-glucose}}{RCHO} + 2CuO \longrightarrow \underset{\text{D-gluconic acid}}{RCOOH} + \underset{\text{Cuprous oxide (Red ppt.)}}{Cu_2O\downarrow}$$

Reagents:

1- Sugar solution (5%).

2- Barfoeds reagent (dissolves 13.3 gm of copper acetate in 200 ml of distilled water and adds 1.3 ml of glacial acetic acid, mix and cool).

Procedure:

1- Add 1 mL of the sugar solution to 2 mL of freshly prepared Barfoed's reagent.

2- Place test tubes into a boiling water bath and boil for 3 minutes.

3- Remove the tubes from the bath and allow cooling. Formation of a brick red precipitate indicates the presence of monosaccharides.

Note:

1- The role of copper acetate is to provides cupric ions (Cu^{++}) in solution and glacial acetic acid is to provides acid medium.

2- Do not boil the solution for longer period because the overheating disaccharides may hydrolyze to monosaccharides and give positive test.

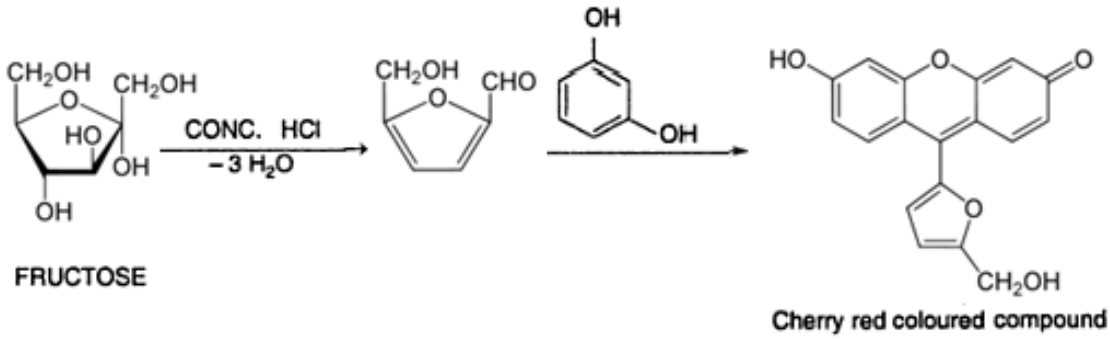
3- This test should not be performed in urine, other fluids containing chloride as it interferes with the test.

Test 4. Seliwanoffs Test for Ketoses

This test is specific for ketose sugar and hence is used to distinguish between aldose and ketose sugars.

Ketose sugars on treatment with conc. hydrochloric acid (HCl) forms furfural or its derivatives which on condensation with resorcinol gives a cherry red colored complex.

The Polysaccharides and oligosaccharides when hydrolyzed by acid yields simpler sugars; Then the dehydrated ketose reacts with the resorcinol to produce a deep cherry red color. Fructose is the common sugar that gives a positive test.



Sucrose (A disaccharide consisting of fructose and glucose) will also give Seliwanoff's test positive because the acidity of reagent is sufficient enough to hydrolyze sucrose to glucose and fructose but Benedicts test will be negative.

Reagents:

1- Sugar solution (5%).

2- Seliwanoff's reagent (Dissolve 0.5 g of resorcinol in 1000 ml of 3M HCl).

Procedure:

1- Add 0.5 ml of the sugar solution to 2 mL of Seliwanoff's reagent to a test tube and mix well.

2- The test tube is heated in a boiling water bath for 5 minutes. The formation of a red color within 2 min indicates a positive result for ketoses (Fructose and Sucrose).

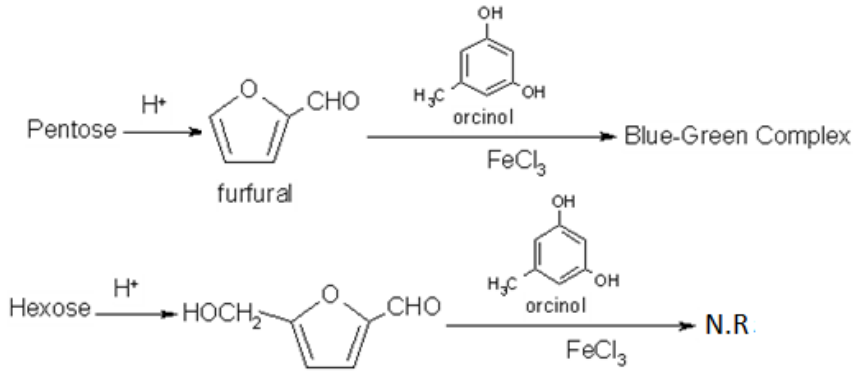
Note:

Aldoses do not react positively with this test but overheating of the solution (aldoses) will give this test because of their conversion to ketoses which has a faint pink color by hydrochloric acid.

Test 5. Bial's Test for Pentoses

This test is specific for pentoses and used for distinguishes between pentose and hexose sugars.

Pentose reacts with Bial's reagent (Orcinol in Conc. HCl and traces of $FeCl_3$ as catalyst) to form furfural, which condenses with orcinol to produce blue-green product. It is necessary to use dilute sugar solutions with this test (0.02 M).



Reagents:

1- Sugar solution (5%).

2- Bials oricinol reagent (Dissolve 1.5 gm of oricinol in 500 ml of conc. HCl and add 20 drops of solution of $FeCl_3$10%).

Procedure:

1- Add 0.5 mL of the sugar solution to 1.5 mL of Bial's reagent.

2- Gently heat the tube to boiling.

3- Allow the tube to cool. Formation of a blue- green color indicates the presence of pentose sugars.

Test 6. Iodine Test for polysaccharides:

This is a general test for polysaccharides. Polysaccharides can be differentiated from other carbohydrates (mono and disaccharides) by this test.

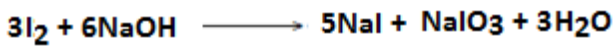
Starch is the most common polysaccharide. It is a mixture of amylose and amylopectin. Starch is insoluble in water but forms a colloidal solution on heating. Starch has no reducing activity.

When iodine (I_2) is added to starch, form a deep blue-black coordinate starch-iodine complex. This color is due to the absorption of iodine into the open spaces of the amylase molecules present in starch. Amylopectins, which are the other types of molecules present in starch, react with iodine to give purple color, while glycogen gives red color with iodine.

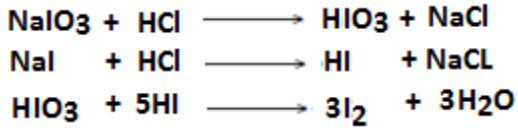
This test is sensitive against the temperature and the medium of the solution, so the reaction must be occur in the room temperature.

1- The blue color of solution disappears on heating because the helical structure of amylase is disrupted and it loses its iodine binding capacity. The blue color of solution reappears on cooling due to the regaining of helical structure of amylase and the iodine binding capacity is also recovered.

2- In alkaline medium: the blue color of solution disappears by the addition of NaOH because the helical structure of amylase is disrupted and it loses its iodine binding capacity.



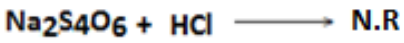
The blue color of solution reappears by the excess addition of HCl due to the regaining of helical structure of amylase and the iodine binding capacity is also recovered.



3- By the addition of sodium thiosulphate, the blue color of solution disappear because sodium thiosulphate react with I_2

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

And the color not reappears by the excess addition of HCl because $Na_2S_4O_6$ is stable.



$$Na_2S_4O_6 + HCl \longrightarrow N.R$$

Reagents:

1- Sugar (polysaccharide) solution (1%).

2- Iodine solution: (Dissolve 1 g iodine and 10 g KI in water and make up to 500 mL).

Procedure:

1- Add 6 drops of iodine solution to 3 mL of sugar solution (and observe the color.

2- A dark blue-black color is a positive test for amylose in starch. A red or brown color indicates the presence of other polysaccharides. Record your results. A positive test for glycogen is a brown-red color.

Divide the solution into three equal parts:

1- **Part one:** Take 1 ml of the solution and heat it, the color of solution disappears and reappears after cooling.

2- **Part two:** Take 1 ml of the solution and add few drops of NaOH solution (2%), the blue color of solution disappears, and reappear after the excess addition of HCl.

3- **Part three:** Take 1 ml of the solution and add few drops of sodium thiosulphate, the blue color of solution disappear, and the color not reappears by excess addition of HCl.

Note:

1- Monosaccharides and disaccharides are too small to trap iodine molecules and do not form dark colors with iodine.

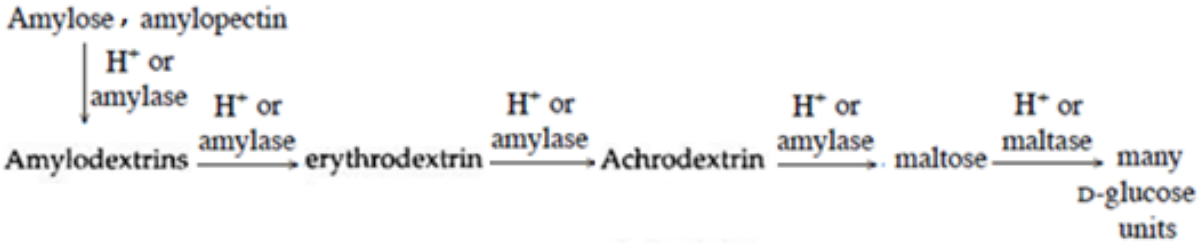
2- Formation of color depends upon the type of polysaccharides.

Polysaccharides	**Color with iodine**
Starch: Amylose	Blue color
Amylopectin	Purple color
Glycogen	Brown red color
Dextrin	Brown to colorless
Cellulose or Inulin	No color
Disaccharides and monosaccharides	No color

Test 7: Hydrolysis of polysaccharides

Polysaccharides contain only one reducing group for several hundred or more residues so that they are effectively non-reducing. Acid hydrolysis gives the constituent monosaccharides, which are then tested for specified tests.

In the laboratory, we use water and acid to hydrolyze starches, which produce smaller saccharides such as maltose. Eventually, the hydrolysis reaction converts maltose to glucose molecules. In the body, enzymes in our saliva and from the pancreas carry out the hydrolysis. Complete hydrolysis produces glucose, which provides about 50% of our nutritional calories.



Reagents:

1- Sugar solution (starch) (1%).

2- 2N of concentrate HCl (prepared by diluting one ml of concentrate HCl to 4 ml of water, and mix well.

3- Sodium carbonate solution.

Procedure:

1- Take 30 ml of starch solution (1%). Add 6 ml of (2N) HCl

2- Divide the solution in six equal parts (i.e. 4 ml each) in six different tubes and keep the tubes in a boiling water bath. Remove the tubes sequentially from the boiling water bath at intervals of 5, 10, 15, 20, 25, and 30 minutes.

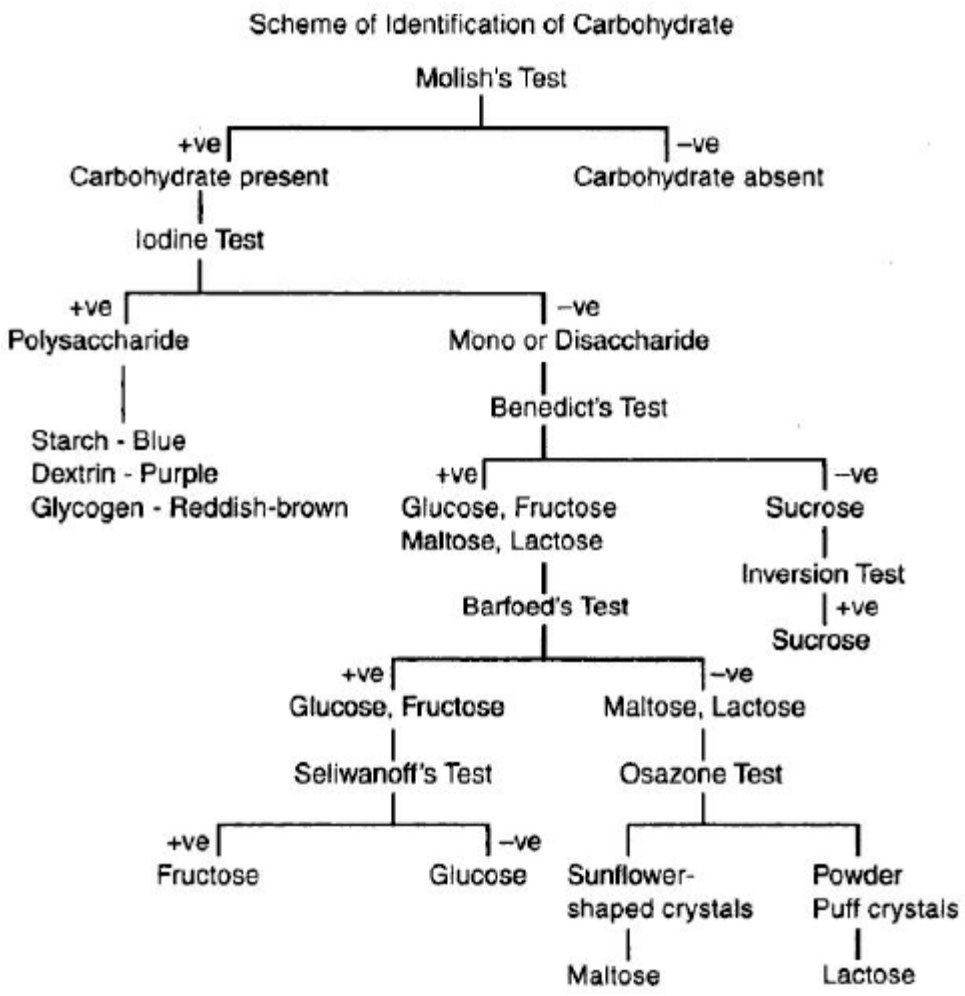
3- Divide the solution from each tube into two equal parts. This will give two sets:

Set- A: Perform iodine test.

Set –B: Perform Benedicts qualitative test, after making the solution alkaline (I.e. by neutralizing the acidity of the solution with sodium carbonate).

Time	Color with iodine	Benedicts test	Reduction of hydrolysis	Product
1 minute	Blue	Blue	No reduction	Starch
5 minute	Violet	Green	Reduction starts **(+)**	Amylodextrins
8 minute	Reddish violet	Red	Initiation of reduction **(++)**	Amylo and
12 minute	Red	Red	Partial reduction **(+++)**	erythrodextrin
20 minute	No color	Red	Completely reduced **(++++)**	Achrodextrin
25 minute	No color	Red	Completely reduced **(++++)**	Glucose
30 minute	No color	Red	Completely reduced **(++++)**	Glucose

The acidified starch takes about 20 minutes for complete hydrolysis.



Molisch test

How can you distinguish between carbohydrates with other compounds?

What is the role of sulphuric acid in molisch test?

Appearance of green ring is not a positive reaction in molisch test, why?

Trioses, and tetroses, do not give a positive reaction in molisch test, why?

Write the reaction of glucose with molisch reagent?

Write the reaction of ribose with molisch reagent?

Benedicts test

Define

Reducing sugar, non-reducing sugar

How can you distinguish between reducing and non-reducing sugars?

How can you distinguish between sucrose and maltose sugar?

How can you distinguish between sucrose and lactose sugar?

How can you distinguish between sucrose and glucose sugar?

Write the reaction of Benedicts test with glucose

Write the reaction of Benedicts test with maltose

Write the reaction of Benedicts test with sucrose

What is the role of copper sulphate in Benedicts test?

Sucrose and polysaccharides will not react with Benedict's solution, why?

Benedicts test carried out in an alkaline medium, why?

Why Polysaccharides are non-reducing sugar

Barfoed's test

How can you distinguish between mono and disaccharides?

How can you distinguish between fructose and maltose sugar?

How can you distinguish between fructose and lactose sugar?

Barfoed's test carried out in an acidic medium, why>

Write the reaction of Barfoed's reagent with glucose

Write the reaction of Barfoed's reagent with fructose

Write the reaction of Barfoed's reagent with lactose

Write the reaction of Barfoed's reagent with sucrose

What is the role of copper sulphate in Barfoed's test?

In Barfoed's test, do not boil the solution in for longer period, why

Seliwanoffs test

How can you distinguish between aldose and ketose sugars?

How can you distinguish between fructose and galactose sugars?

By which test, you can distinguish between aldose and ketose sugars.

What is the role of hydrochloric acid in Seliwanoffs test?

Is Fructose give a positive result with Seliwanoffs test?

Is Sucrose give positive with Seliwanoff's test?

How can you distinguish between fructose and galactose?

How can you distinguish between fructose and glucose?

Aldoses do not react positively with this test but overheating of the solution (aldoses) will give this test, why?

Bial's test

How can you distinguish between pentose and hexose sugars?

How can you distinguish between ribose and fructose sugars?

How can you distinguish between ribulose and glucose sugars?

How can you distinguish between Glucose and ribulose sugars?

What is the role of Orcinol in Conc. HCl in Bial's test?

Iodine test

How can you distinguish between starch and glucose?

How can you distinguish between starch and lactose?

How can you distinguish between starch and sucrose?

How can you distinguish between starch and ribose?

Iodine test is sensitive against the temperature and the medium of the solution, so the reaction must be occur in the room temperature, why?

In iodine test the blue color of solution disappears on heating, why?

In iodine test, the blue color of solution disappears by the addition of NaOH, why?

In iodine test, the blue color of solution reappears by the excess addition of HCl, why?

In iodine test, the blue color of solution disappear by the addition of sodium thiosulphate, why?

Why monosaccharides and disaccharides are not give positive results with iodine test?

Hydrolysis of polysaccharides

Why Polysaccharides give positive result with iodine test after 5 minutes of hydrolysis with HCl?

Why Polysaccharides give negative result with iodine test after 20 minutes of hydrolysis with HCl?

Why Polysaccharides give negative result with benedict test after 5 minutes of hydrolysis with HCl?

Why Polysaccharides give positive result with benedict test after 20 minutes of hydrolysis with HCl?

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