2. QUALITATIVE TESTS OF CARBOHYDRATE

Carbohydrates are the most abundant biomolecules on Earth. Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis. Many, but not all, carbohydrates have the empirical formula (CH2O)n; CnH2nOn. All common monosaccharides and disaccharides have names ending with the suffix "-ose." (exp. Glucose)

There are three major size classes of carbohydrates: monosaccharides, oligosaccharides, and polysaccharides.

2.1 Monosaccharides

Monosaccharides, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose. Monosaccharides are colorless, crystalline solids that are freely soluble in water but insoluble in nonpolar solvents. Most have a sweet taste.

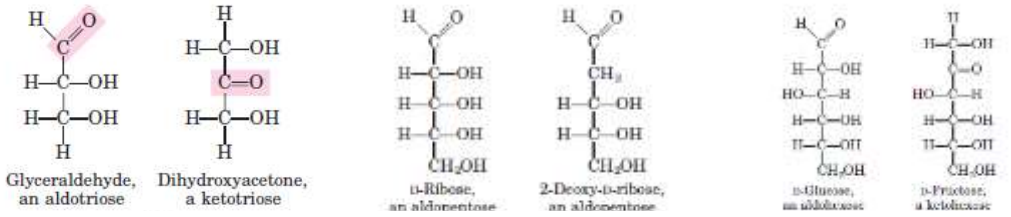
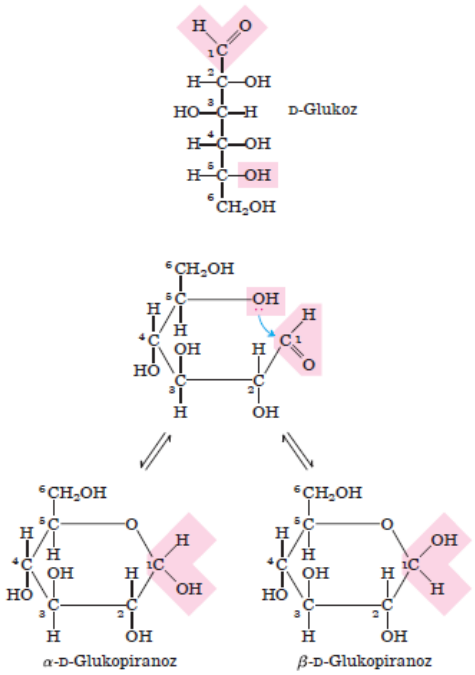


Figure 2.1 Formula of some impotant monosaccharides

In fact, **in aqueous solution**, all monosaccharides with five or more carbon atoms in the backbone occur predominantly as cyclic (ring) structures in which the carbonyl group has formed a covalent bond with the oxygen of a hydroxyl group along the chain. The formation of these ring structures is the result of a general reaction between alcohols and aldehydes or ketones to form derivatives called **hemiacetals or hemiketals**, which contain an additional asymmetric carbon atom and thus can exist in two stereoisomeric forms. The hemiacetal (or carbonyl) carbon atom is called the anomeric carbon. The anomers of α and β.

Figure 2.2 Alpha (***α***) and beta (**β**) anomers of glucose



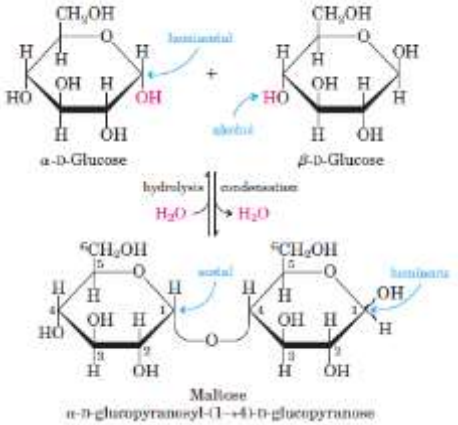
2.2 Disaccharides

A **disaccharide** is the sugar formed when two monosaccharides (simple sugars) are joined by glycosidic linkage. There are three common disaccharides: **maltose, lactose, and sucrose**.

Disaccarides consist of two monosaccharides joined covalently by an **O-glycosidic bond**, which is formed when a hydroxyl group of one sugar reacts with the anomeric carbon of the other. This reaction represents the formation of an acetal from a hemiacetal (such as glucopyranose) and an alcohol (a hydroxyl group of the second sugar molecule). Glycosidic bonds are readily hydrolyzed by acid but resist cleavage by base. Thus disaccharides can be hydrolyzed to yield their free monosaccharide components by boiling with dilute acid.

The disaccharide **maltose contains two D-glucose residues** joined by a glycosidic linkage between C-1 (the anomeric carbon) of one glucose residue and C-4 of the other. Because the disaccharide retains a free anomeric carbon (C-1 of the glucose residue on the right), **maltose is a reducing sugar**. Its abbreviated name is Glc (α1→4)Glc

Figure. 2.3 Formation of disaccharide maltose



The disaccharide **lactose**, which yields **D-galactose and D-glucose** on hydrolysis, occurs naturally **only in milk**. The anomeric carbon of the glucose residue is available for oxidation, and thus lactose is a **reducing disaccharide**. Its abbreviated name is

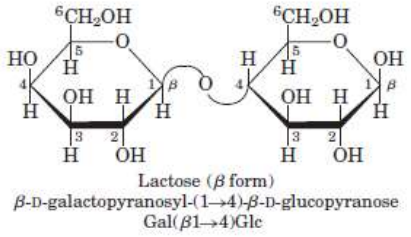


Figure 2.4 Beta lactose

Sucrose (table sugar) is a disaccharide of **glucose and fructose**. It is formed by plants but not by animals. In contrast to maltose and lactose, sucrose contains no free anomeric carbon atom; the anomeric carbons of both monosaccharide units are involved in the glycosidic bond. Sucrose is therefore **a nonreducing sugar**. Its abbreviated name is Glc(α→2β)Fru.

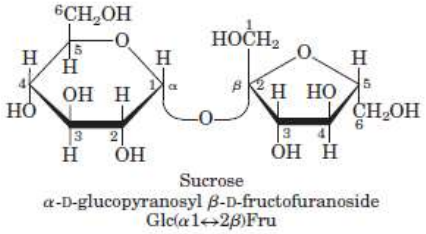


Figure 2.5 Sucrose (saccarose)

2.3 Polysaccharides (Glycans)

The **polysaccharides are** the **most** abundant **carbohydrates** in **nature**. Polysaccharides are very large polymers composed of tens to thousands of monosaccharides joined together by glycosidic linkages. Polysaccharides, also called **glycans**, differ from each other in the identity of their recurring monosaccharide units, in the length of their chains, in the types of bonds linking the units, and in the degree of branching.

Homopolysaccharides contain only a single type of monomer; **heteropolysaccharides** contain two or more different kinds. Some homopolysaccharides serve as storage forms of monosaccharides **that are used as fuels**; **starch and glycogen** are homopolysaccharides of this type. Other homopolysaccharides (such as cellulose and chitin) act as structural elements in plant cell walls and in the outer shells of invertebrate animals.

Heteropolysaccharides provide extracellular support for organisms of all kingdoms. For example, the rigid layer of the bacterial cell envelope (the peptidoglycan) is composed in part of a heteropolysaccharide built from two alternating monosaccharide units.

The most important storage polysaccharides are starch in plant cells and glycogen in animal cells. **Starch** contains two types of glucose polymer, **amylose and amylopectin**. **Amylose** consists of long, unbranched chains of D-glucose residues connected by (1→4) linkages. Such chains vary in molecular weight from a few thousand to more than a million. **Amylopectin** also has a high molecular weight (up to 100 million) but unlike amylose is highly branched. The glycosidic linkages joining successive glucose residues in amylopectin chains are (1→4); the branch points (occurring every 24 to 30 residues) are (1→6) linkages.

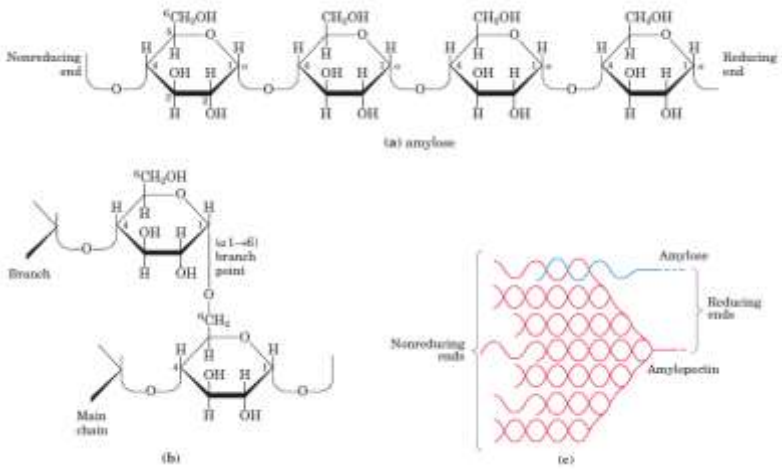


Figure 2.6 Amylose and amylopectin, the polysaccharides of starch.

2.4 EXPERIMENTS

General Tests for Carbohydrates

Carbohydrates are resistant to weak acids, but strong acids are able to degrade the glycosidic bonds and produce monosaccharides. Monosaccharides are dehydrated in acidic medium and yields furfurals and derivatives.

The presence of carbohydrates is indicated by the development of colors when the unknown is treated with strong acids and appropriate phenols (resorcinol, orcinol etc). Strong acids convert carbohydrates to hydroxymethylfurfural (HMF), also 5-(hydroxymethyl) furfural, is an organic compound formed by the dehydration reactions of carbohydrates. The colored substances probably are condensation product between the phenols and hydroxymethylfurfural.

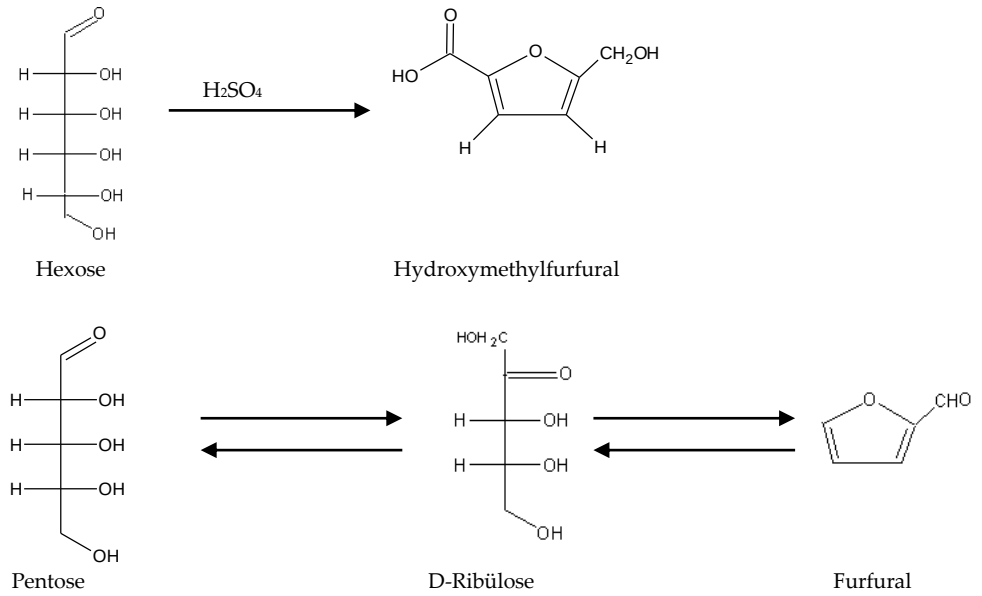


Figure 2.7 Dehydration reactions of carbohydrates when treated with strong acids and formation furfural

2.4.1.Molisch test

It is a general test used to detect the presence of carbohydrates. Except of amino sugars, sugar alcohols and carboxyclic acids, Molisch test can be used for all carbohydrates

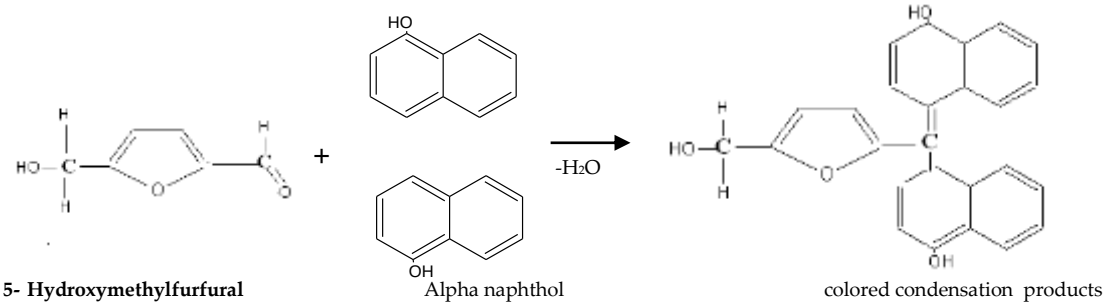
Principle of the Experiment: It is a useful test for identifying any compound which can be dehydrated to furfural or hydroxymethylfurfural in the presence of H_2SO_4. Alpha (α) naphthol reacts with the furfural to form purple colored condensation products.

Procedure:

1. Add 2 drops of Molisch reagent (prepared dissolving 0.1g of α-naphthol in 2 ml of ethanol) to 2 ml of the sugar solution to be tested and mix.

2. Incline the tube, and GENTLY add 2 ml of concentrated sulfuric acid down the side of the test tube.

3. A purple color at the interface between the sugar and acid indicates a positive result



5- Hydroxymethylfurfural Alpha naphthol colored condensation products

Figure 2.8 Molisch test reactions

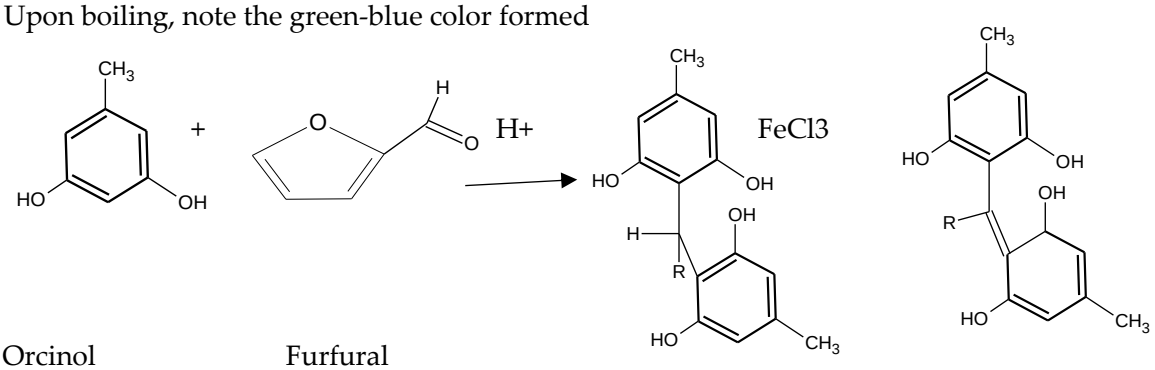
2.4.2 Bial's Test:

Bial's Test is performed to determine the presence of pentoses (5C sugars).

Principle of the Experiment: The components of this reagent are resorcinol, HCl, and ferric chloride. In this test, the pentose is dehydrated to form furfural and the solution turns bluish and a precipitate may form. Perform this test with ribose.

Procedure:

1. To 5 mL of Bial's reagent, add 2-3 drops of sugar solution and boil.
2. Upon boiling, note the green-blue color formed



Orcinol Furfural

Figure 2.9 Bial's Test reactions

Solutions:

Ribose: 1 g muscle tissue (meat) is boiled in 20 ml 1 N PCA (per chloric acid) and filtrated.

BİAL's reagent: 100 ml 30% HCl, 0.2 g resorcinol and 4 drop of 10% $FeCl_3$ is mixed.

2.4.3 Seliwanoff's (Resorcinol) Test for Ketohexoses

This test is specific for ketoses. Seliwanoff's test used to distinguish ketoses from aldoses.

Principle of the Experiment: Fructose (ketose) will give a cherry red color due to ketoses react with HCl faster than aldoses and yields hydroxy methyl furfural. Hydroxy methyl furfural reacts with resorcinol to yield red colored complex.

Procedure:

1. Add 1-2 drop of fruit juice to 2 mL of the Seliwanoff Reagent.
2. Heat the solution in a boiling water bath for 5 minutes.
3. A deep cherry or red color indicates the presence of ketoses.

Solutions:

Seliwanoff Reactive: Dissolve 50 mg resorcinol in alcohol and add 100 ml (1:1) HCl solution on it.

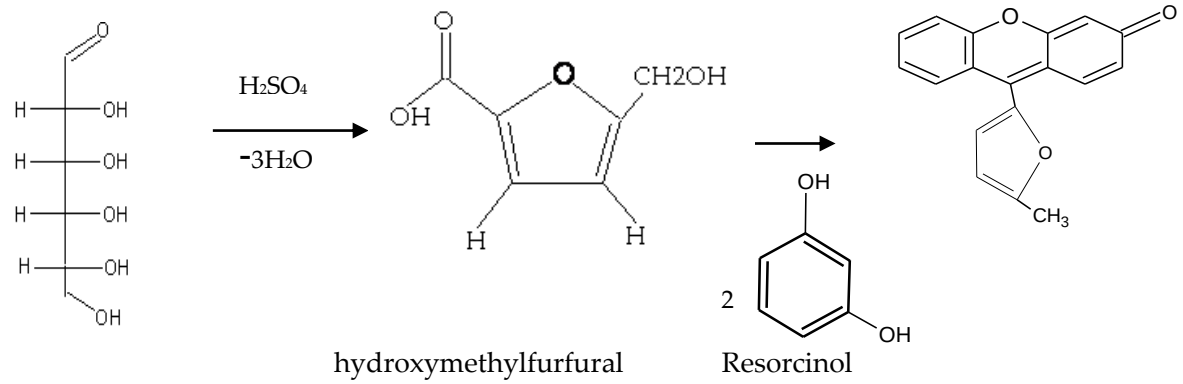


Figure 2.10 Seliwanoff's (Resorcinol) Test reactions

2.4.2 Carbohydrates as Reducing Sugars

A reducing sugar is any sugar that, in a solution, has an aldehyde or a ketone group. **Reducing sugar can act as reducing agents. This property can be used as a basis for the analysis of reducing sugars.** These sugars, therefore, become potential agents capable of reducing Cu^{+2} to Cu^{+}, Ag^{+} to Ag and so fort.

Most commonly used tests for detection of reducing sugars ;

Experiment 2.4.4 Methylene Blue Test

Reducing sugars are sugars which have free aldehyde or ketone group. As a result of oxidation of free aldehyde and ketone groups (in reducing sugars) to **carboxyclic acids** change the color of methylene blue solution from blue to colorless under alkaline conditions when they are heated. For example, glucose is oxidized to gluconate by oxygen.

Procedure:

1. Take 5 ml distilled water and 1 ml of methylene blue (0.1%) to test tube. Then add 2 drop of 2 N NaOH on it.
2. Heat the solution in water bath and add 4-5 drop of 2% glucose solution.
3. Observe that the blue color disappears at the end of the heating process. However, if the tube is shaken, solution turns into blue again.
4. Repeat the same procedure with tea sugar.

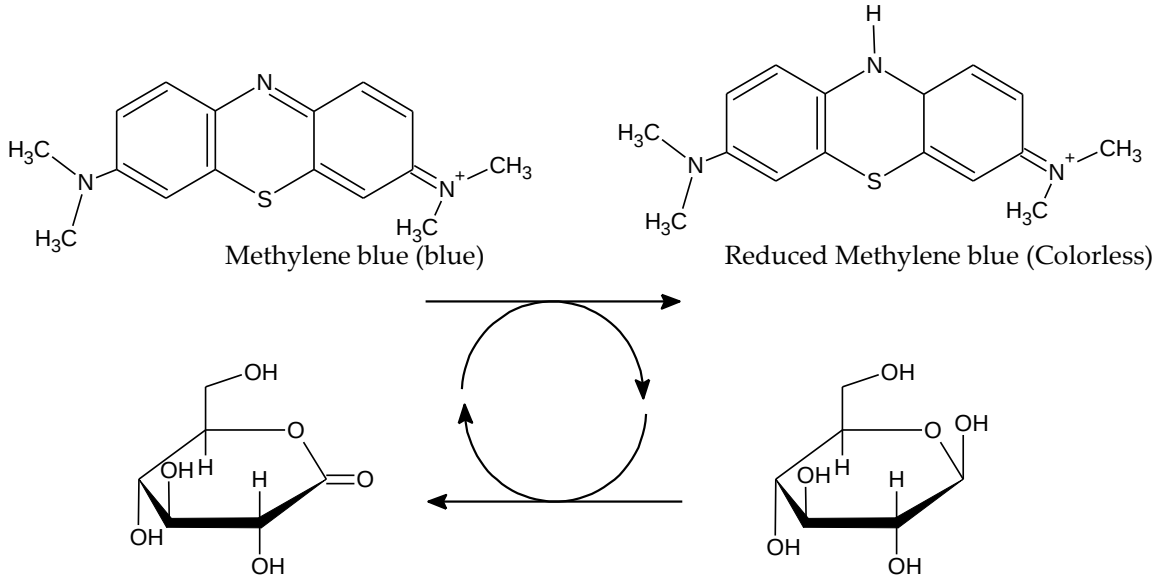


Figure 2.11 Methelene Blue test reaction

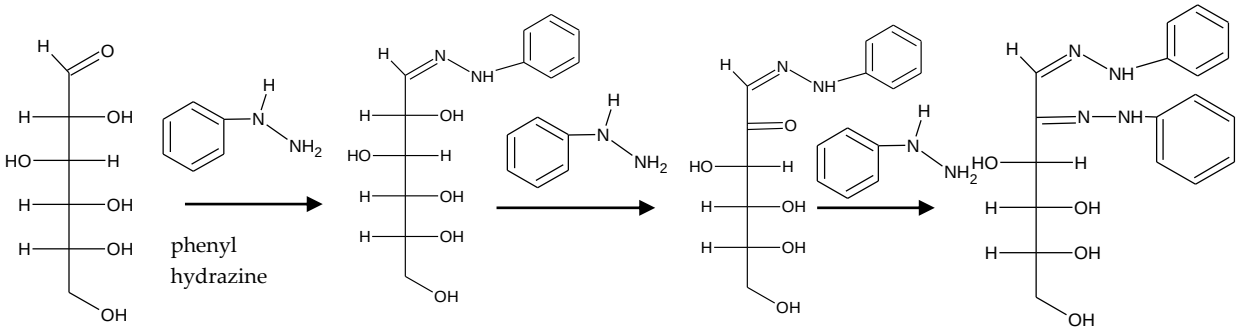
2.4.5 Osazone Test

Principle of the Experiment: All sugars containing free aldehyde or ketone group react with phenyl hydrazine when they are heated (together with phenyl hydrazine) to yield yellow crystalline solid called **osazone** which have definite crystal structures and melting points.

Characteristic osazones crystallines are produced when phenyl hydrazine bonds to 1st and 2nd carbon atoms of the monosaccharide. Thus, glucose, fructose and mannose produce the same osazone which resembles to yellow crop bundle. On the other hand, the osazone crystalline which is formed by galactose, looks like dark yellow buckeye.

Procedure:

1. Pour 5 ml 2% glucose solution to the test tube.
2. Add 10 drops of glacial acetic acid, 3 drop of phenyl hydrazine and 0.1 gram of sodium acetate to the test tube prepared in previous step.
3. Stir the solution and boil it in water bath 30 minutes.
4. Cool the solution slowly and observe the formed crystalline under a microscope.



Glucose phenyl hydrazone Ketophenyl hydrazone

Glucosazone Maltosazone Galactosazone Lactosazone

Figure 2.12 The formation of Osazones.

2.4.6 Benedict's test

Principle of the Experiment: The principle of Benedict's test is that when reducing sugars are heated in the presence of an alkali they get converted to powerful reducing species known as enediols.

Procedure:

1. Grate the onion and potato separately.
2. Squeeze their juice to different test tubes**.**
3. Follow the procedure indicated in the table given below

Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
0.5 mL Water	0.5 mL Onion juice	0.5 mL Potato juice	0.5 mL Glucose	0.5 mL Sucrose	0.5 mL Starch
1.5 mL Benedict	1.5 mL Benedict	1.5 mL Benedict	1.5 mL Benedict	1.5 mL Benedict	1.5 mL Benedict
All tubes are placed in boiling water for 5 minutes. The colour of the mixture serves as a guide to the amount of sugar.					

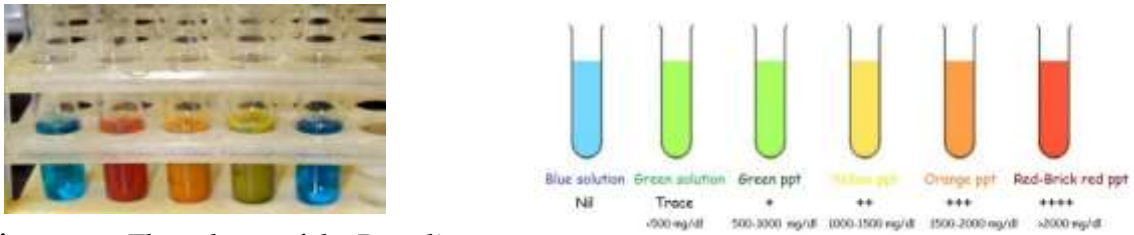


Figure 2.14 The colours of the Benedict test

Preparation of Qualitative Benedict's Reagent Solution:

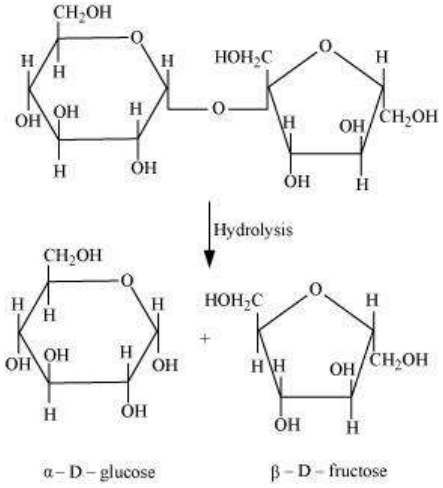
Dissolve 17.3 g sodium citrate, 10 g anhydrous sodium carbonate (Na_2CO_3) and 1.73 g copper sulphate ($CuSO_4.5H_2O$) in 100 ml distilled water.

2.5 Hydrolysis of Disaccharides and Polysaccharides

Principle of the Experiment: Disaccharides and polysaccharides are broken down to the monomer monosaccharide units by acids (for example, HCl and H_2SO_4) or enzymes.

The hydrolysis of sucrose in dilute acid or through the action of the enzyme **sucrase** (also known as invertase) gives an equimolar mixture of glucose and fructose.

Figure 2.15 The hydrolysis of sucrose



2.5.1 Acid Hydrolysis of Sucrose

Procedure:

1. Add 0.5 ml 10% HCl solution to 5 ml of 5% aqueous sucrose**.**
2. Heat the prepared solution in boiling water bath 30 minutes.
3. Allow the tube to cool to room temperature. After the solution cooled down, take 0.5 ml of this solution and apply the Benedict's Test (see Experiment 2.6). Please write down the hydrolysis products of sucrose and the colors which appear by Benedict's Test. Compare the results of this test with the result of previous test which was obtained from the unhydrolyzed sucrose.

1. Sucrose	Without hydrolysis →	**Benedict test**	→	Result and conclution
2. 10 mL of Sucrose	Boiling water hydrolysis, 10 % HCl →	cool to room temperature and take 1 mL of solution	**Benedict test** (Test 2.6) →	Result and conclution

Record the results on the report sheet.

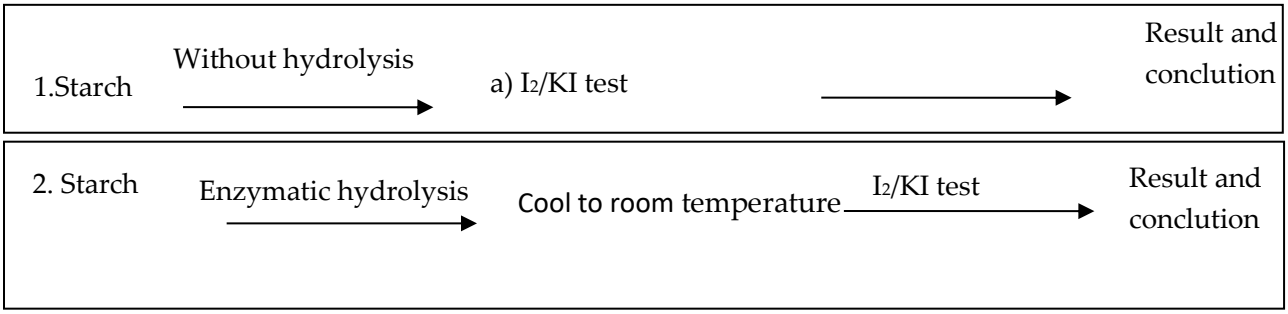
2.5.2 Enzymatic Hydrolysis of Starch by Amilase

Amylase is an enzyme produced primarily by the pancreas and the salivary glands to help digest carbohydrates. Amylase is an enzyme that hydrolysis alpha-bonds of large alpha-linked polysaccharides such as starch and glycogen, yielding maltose and dextrin. Amylase randomly affects α-1→4 bonds, belonging to the amylose structure of starch, and maltose units form. Amylase does not affect the α-1→6 bonds that belong to the amylopectin structure of starch. As a result of hydrolysis carried out with α- amylase, besides the maltose and glucose units, a large branched dextrin structure is formed in the media.

Figure 2.16 Enzymatic hydrolysis of starch by amilase

Procedure:

1. Add 0.1 (or 0.2, 0.3, 0.4 ml) amilase solution to 5 ml of 0.1% starch solution.
2. The prepared solution incubate in 37 oC for 30 minutes
3. Allow the tube to cool to room temperature and add 1 drop of Iodine-KI reagent (I_2/KI).



You can assess the relative enzyme activity as follows:

Starch hydrolysis	The resulting color with I2/KI
Starch	Blue-black
Amylodextrin	Violet
Erytrodextrin	Red
Achrodextrin	Light yellow
Maltose/Glucose	No color