University of Baghdad

College of Science for Women

Department of Chemistry

BIOCHEMISTRY LAB

(For Biology students/First class)

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Chapter 1: Carbohydrate Qualitative tests

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Carbohydrates, often referred to as carbs, are a type of macronutrient found in foods and drinks. They are one of three main nutrients, along with proteins and fats, that provide energy for the body's cells, tissues, and organs. Carbohydrates are classified into four main types based on their degree of polymerization and chemical structure:

1. **Monosaccharides:** These are simple carbohydrates with a single sugar unit. They have the chemical formula $C_6H_{12}O_6$. Monosaccharides can be further classified into aldoses and ketoses, and further into pentoses and hexoses based on the number of carbon atoms and the presence of an aldehyde or ketone group.

2. **Disaccharides:** These are composed of two monosaccharide units joined by a covalent bond known as a glycosidic linkage. Examples include sucrose and lactose. The formula for unmodified disaccharides is $C_{12}H_{22}O_{11}$.

3. **Oligosaccharides:** These are formed by the condensation of 2 to 9 monosaccharide units. They are a subset of polysaccharides and include malto-oligosaccharides like maltodextrins, and other oligosaccharides such as raffinose and stachyose.

4. **Polysaccharides:** These are complex carbohydrates composed of many monosaccharide units. They are further divided into:

- Starch: Composed of amylose and amylopectin, which are polysaccharides that store energy in plants and are broken down in the human body for energy.
- Non-starch polysaccharides: Include glycogen, cellulose, hemicellulose, pectins, and hydrocolloids. These are found in plant cell walls and are not easily digested by humans.

Each type of carbohydrate has a different role in the body and diet, with monosaccharides and disaccharides being the simplest forms and providing the body with immediate energy, while polysaccharides, especially starch, provide long-term energy storage. Oligosaccharides are found in a variety of foods and have different effects on digestion and health.

1. **Molisch's Test:** (General test of all Carbohydrates)

It is the general test for all carbohydrates (except trioses and tetroses Why?). Monosaccharides give a rapid positive test. Disaccharides and polysaccharides react slower. Why?

Principle:

The reaction is due to the formation of Furfural and it's derivatives by the dehydrating action of acid on sugar in the event of Sugar being (poly saccharide or disaccharide) the acid first hydrolyzes it into monosaccharides and then acts as a dehydrating agent.

The Molisch reagent dehydrates pentose's to form furfural. It dehydrates hexoses to form 5-hydroxymethylfurfural. The furfurals further react with α-naphthol present in the test reagent to produce a purple product.

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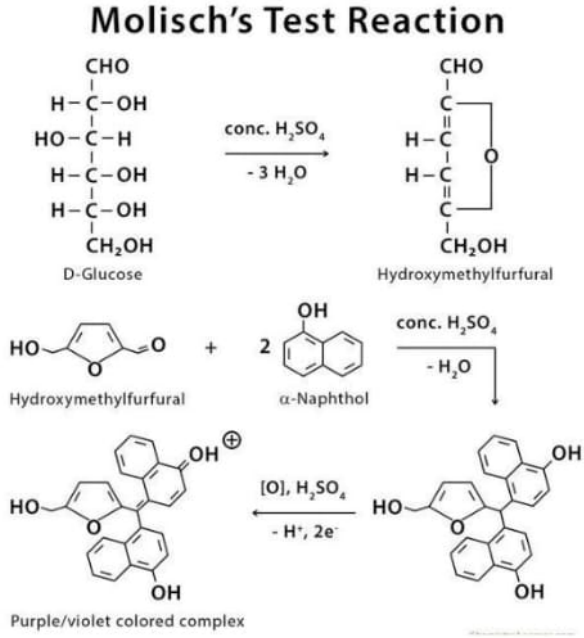

■ **Method:**

- 1ml sample solution + 2 drops of α-naphthol alcoholic and mix well.
- Slowly add a small amount of concentrated H_2SO_4 (sulfuric acid) down the sides of the sloping test tube without mixing to form ring at the interface of the two layers.

Lab results: Glucose, maltose, and starch will all display the purple ring compound at the interface of the acid and solution.
Limitations:
-Not specific to carbohydrates and can give false-positive results with furfurals or certain organic acids that can form similar products.
-Generalized test that cannot distinguish carbohydrates and further testing must be undertaken to identify thecarbohydrate.

2. **Benedict's Test:**

All reducing sugars* give positive Benedict's test (It is a redox reaction).

Principle:

Reducing sugars have free aldehyde or keton group, it under go converting in to enediol formsunder hot alkaline condition. The enediol are strong reducing agents converting cupric ions (Cu^{+2}) of the Benedict's solution into Cuprose ion which altimately oxide as a red precipitate red copper (I) oxide by aldehydes.

Benedict's reagent contains blue copper (II) sulfate ($CuSO_4$) · 5H2O which is reduced to red copper (I)oxide by aldehydes, thus oxidizing the aldehydes to carboxylic acids.

The copper oxide is insoluble in water and so precipitates. The color of the final solution ranges fromgreen to brick red depending on the concentration of reducing sugars present

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Method:

- 1ml sample solution + 1ml Benedict's reagent
- heat the mixture in Boiling of water Bath for (5min)
- Reddish brown precipitate.

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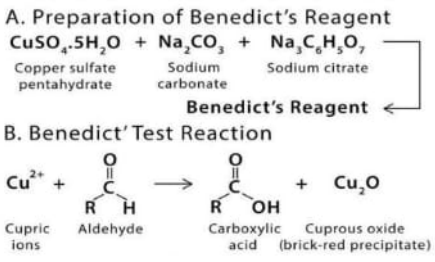
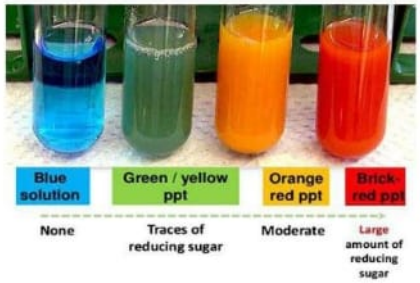
Results: Observe the color change in the solution. A greenish precipitate indicates a concentration of about 0.5 g%, yellow precipitate indicates 1 g%, orange indicates 1.5 g%, and red indicates 2 g% or higher concentration





***Note: Reducing sugars** are a type of sugar that can be reduced, meaning they can donate an electron to another compound. They are classified as reducing sugars if they have an open-chain form with an aldehyde group or a free hemiacetal group. This is because the carbonyl carbon of the sugar is oxidized to a carboxyl group during the reduction process.

Examples of reducing sugars include:

- Monosaccharides: Glucose, fructose
- Disaccharides: Sucrose, lactose

Non-reducing sugars, on the other hand, do not have an open-chain form with an aldehyde group or a free hemiacetal group. They are typically found in complex carbohydrates such as starch, where the sugar units are bonded together, making them unable to donate an electron.

Reducing sugars play a role in various biological processes, including energy transfer, as they can be oxidized to produce ATP, the energy currency of the cell. They are also used as substrates in fermentation processes, where they are broken down by yeast or bacteria to produce alcohol and carbon dioxide.