General Chemistry

University Of Fallujah
College Of Medicine

Lecture : ***Molish and Benedict Tests***

Stage : First stage 2st

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Date: / 3 / 2026

- **Carbohydrates** are aldehyde or ketone derivatives of polyhydric alcohols. They are widely distributed in plants and animals. Plants synthesize glucose by photosynthesis and it is converted mainly to storage form, the starch and structural frame work form, the cellulose .
- Animals largely depend on plant source to obtain carbohydrates though they can synthesize carbohydrates from non carbohydrates sources like glycerol and amino acids in their body (gluconeogenesis)

- Different carbohydrates are present in intracellular and extracellular fluids and are excreted in urine when the concentration of them rises in the blood as in certain diseases (glucose in urine in diabetes mellitus, fructose in urine in fructosuria , galactose in urine in galactosemia).

- The **classification** of carbohydrates will be useful for the detection of various types of carbohydrates by different chemical tests.
- 1. Monosaccharides: Cannot be hydrolyzed into simpler carbohydrates. trioses, tetroses, pentoses, hexoses, heptoses
- 2. Disaccharides: Give rise to two monosaccharide units upon hydrolysis :
- **Sucrose** (glucose + fructose)
- **Lactose** (glucose + galactose)
- **Maltose** (glucose + glucose)

3. Oligosaccharides: Yields less than ten monosaccharides
4. Polysaccharides: Contain more than ten monosaccharide units

Molish and Benedict Tests.

- Several **qualitative biochemical tests** are used to detect carbohydrates.
  Among the most important of these tests are:

1- Molisch Test – General test for carbohydrates

2- Benedict Test – Test for reducing sugars

- **Molisch Test** (α- Naphthol Reaction)
- **Observation**: A reddish violet ring appears at the junction of two liquids.
- **Principle:** Concentrated acid dehydrates the sugar to form furfural (in the case of pentoses) or furfural derivatives (hexoses and heptoses) which then condense with α-naphthol to give a reddish violet colored complex
- **Application of the test**: Used as a general test to detect carbohydrates.

  Monosaccharides , Disaccharides ,Polysaccharides

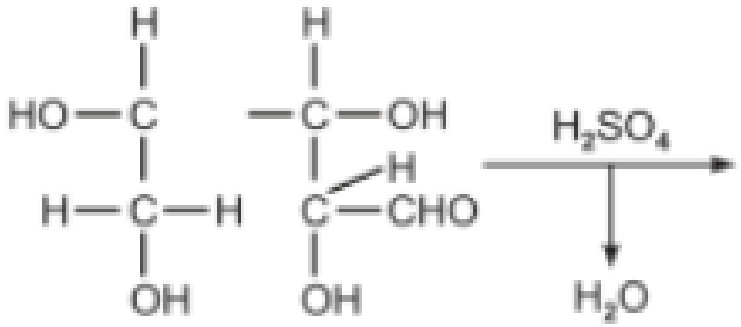
  Glycoproteins ,Glycolipids

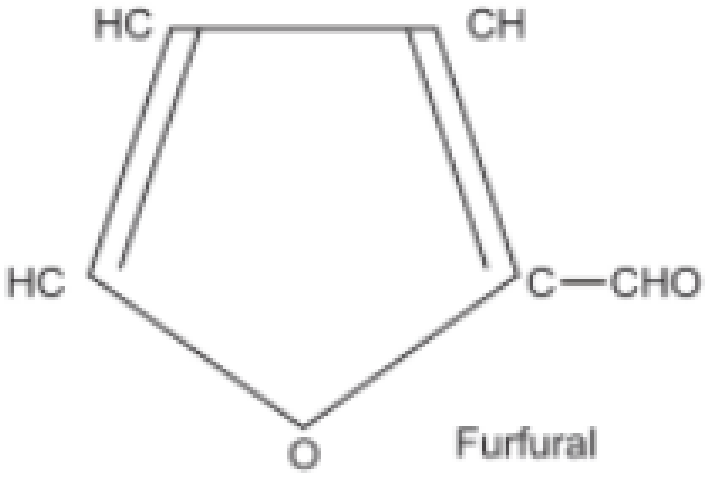
1. Instead of a violet ring appearance of **dark brown** indicates charring of sugar due to the heat generated during the addition of acid . It will become obvious when the concentration of the sugar solution is high. To avoid charring, dilute the sugar sample solution with water
2. Appearance of a **green color** while doing the test, which persist even after completion of the test suggest excess use of Molisch reagent than required or due to the presence impurities in the reagent.

- **Chemical Reaction**
- Dehydration of carbohydrate by sulfuric acid
- Formation of furfural derivatives
- Reaction with α-naphthol to produce violet complex

- **Procedure**
- Take **2 ml of test solution** in a test tube.
- Add **2 drops of Molisch reagent**.
- Carefully add **1–2 ml of concentrated sulfuric acid** along the wall of the test tube.
- Do not mix the layers.
- Observe the interface between the two layers.
- Formation of **purple or violet ring** at the junction of the two layers.

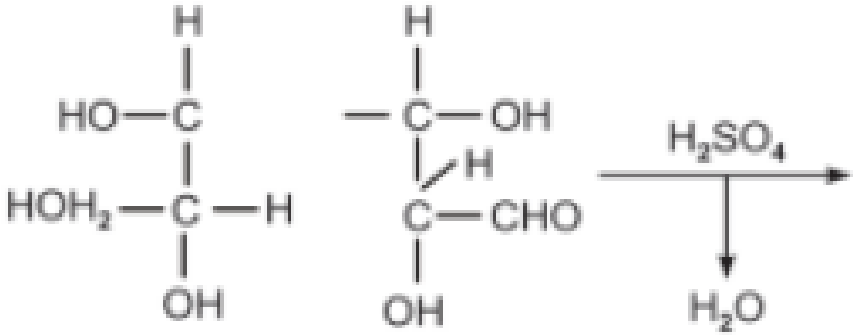
Pentose

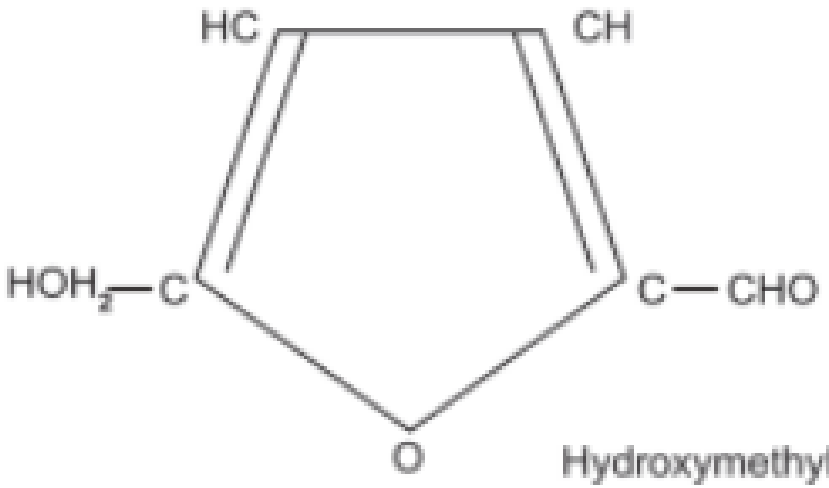




Furfural

Hexose





Hydroxymethylfu

- **Benedict Test**
- Benedict test is used to **detect reducing sugars**.
- Examples of reducing sugars:
- Glucose
- Fructose
- Lactose
- Maltose
- Non-reducing sugar example: Sucrose (unless hydrolyzed)

- **Observation:** The entire body of the solution will be filled with a precipitate, the color of which varies with the concentration of the sugar solution—green, yellow, orange or red. In the absence of reducing substance, blue color of the Benedict's reagent remains as such. The test is sensitive up to 0.1-0.15 gm% of sugar solution

- that is Benedict's test will not be positive with solutions containing less than 0.1-0.15 gm% of sugar). Inference: Reducing monosaccharides

- The color of the precipitate give an idea about the concentration of the sugar solution as shown below. Blue – absence of reducing sugar
- Green – up to 0.5 gm%
- Yellow – > 0.5 to 1.0 gm%
- Orange – > 1.0 to 2.0 gm%
- Brick red – ≥ 2 gm%

- **Principle of the Test**
- Reducing sugars have free aldehyde or ketone groups that can reduce Cu^{2+} ions in Benedict reagent to Cu_2O (cuprous oxide) during heating.
- This reaction produces a colored precipitate.
- Composition of Benedict Reagent
- Benedict reagent contains:
- Copper sulfate ($CuSO_4$)
- Sodium carbonate (Na_2CO_3)
- Sodium citrate

- **Procedure**
- Take **2 ml of Benedict reagent** in a test tube.
- Add **1 ml of the test sample**.
- Heat the mixture in a **boiling water bath for 3–5 minutes**.
- Observe the color change.

- **Applications in Medical Laboratories**
- These tests are used in:
- Biochemistry laboratories
- Urine glucose detection
- Carbohydrate analysis
- Educational laboratory experiments