Ministry of higher education and scientific research
AL-Mustaqbal University college
Department of medical physics

Stage two

practical biochemistry

Lecture 3

Qualitative analysis of Carbohydrate

((Bial`s test))

By

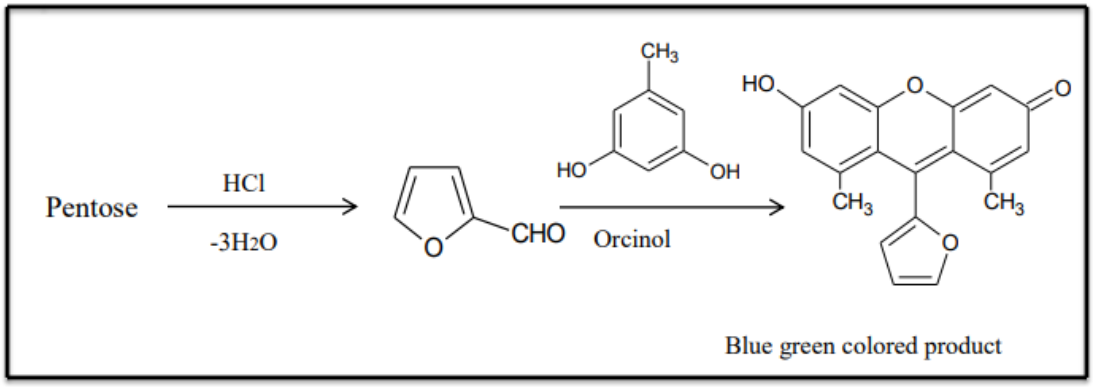
MSc. Elham Faisal

MSc. Doaa Nassr MSc. Issa farahan

- ✓ Monosaccharides are the simplest carbohydrates since they cannot be hydrolyzed to smaller carbohydrates.
- ✓ Monosaccharides is hexoses, containing 6 carbon, 12 hydrogen, and 6 oxygen molecules in slightly varied configurations.
- ✓ The three most common monosaccharides are **glucose, fructose, and galactose**.

Bial`s test:

- This test is used to distinguish between pentose monosaccharide and hexose monosaccharide.
- Pentose interact with concentrated hydrochloric acid to get furfural, then furfural reacts with orcinol to get a blue green colored solution.
- The hexose interacts with concentrated hydrochloric acid to get a hydroxyl methyl furfural which react with orcinol to get a brown colored solution.



Principle :

1- Bial`s reagent is prepared by adding 1.5 gm of orcinol in 500 ml concentrated hydrochloric acid, then 1 ml of 10 % ferric chloride is added to the solution

2- The test reagent dehydrates pentoses to form fufural and dehydrates hexoses to form 5-hydroxymethyl fufural, fufural reacts with orcinol and ferric chloride to produce blue-green complex, while 5-hydroxymethyl fufural produce muddy-brown color complex.

$$\text{Pentose} + H^+ \longrightarrow \text{Fufural}$$
$$\text{Fufural} + \text{Orcinol} + FeCl_3 \longrightarrow \text{Blue-green complex}$$

$$\text{hexose} + H^+ \longrightarrow \text{5-hydroxymethyl fufural}$$
$$\text{5-hydroxymethyl fufural} + \text{Orcinol} + FeCl_3 \longrightarrow \text{muddy-brown complex}$$

Procedure:

1. In clean dry test tube add 1 ml of 5% ribose solution (pentose).

2. In the second test tube add 1 ml of 5% glucose solution (hxose).

3. For each tube add 2.5 ml of Bial's reagent and mix well.

4. Keep both tubes in boiling water bath for one minutes and allow the tubes to cool down to room temperature.

5. Observe the appearance of blue-green color for ribose, and brown color for glucose.

Result Interpretation:

- **Positive Test**: The presence of a bluish-green color indicates the presence of pentoses.
- **Negative Test**: Absence of bluish-green color indicates absence of pentoses. Given that in our experiment we have used glucose solution, a brown color is observed indicating presence of hexoses.

1. On prolonged heating, glucoronates might also give a blue-green colored precipitate which might result in false-positive results.
2. The color produced might be different with different sugars, and the concentration might not be proportional to the intensity at higher levels.