

Kafrelsheikh University
1
Faculty of Pharmacy



Practical Biochemistry-
Second level students

CARBOHYDRATES

SECTION (1)

MONOSACCHARIDES

Carbohydrates

1. Definition:

Carbohydrates are organic substances containing **C, H, and O**. They are **polyhydroxy-aldehydes** or **polyhydroxy-ketones**, or **any compound giving these substances on hydrolysis**.

2. Functions:

- a.** Providing **energy** for the body is its **primary function**.
- b.** Acting as a **storage form of energy** in the body.
- c.** Serving as **cell membrane components** that mediate some forms of **intercellular communication**.
- d.** Serving as a **structural component** of many organisms, including the **cell walls** of bacteria, the **exoskeleton** of many insects and the **fibrous cellulose** of plants.

3. Classification:

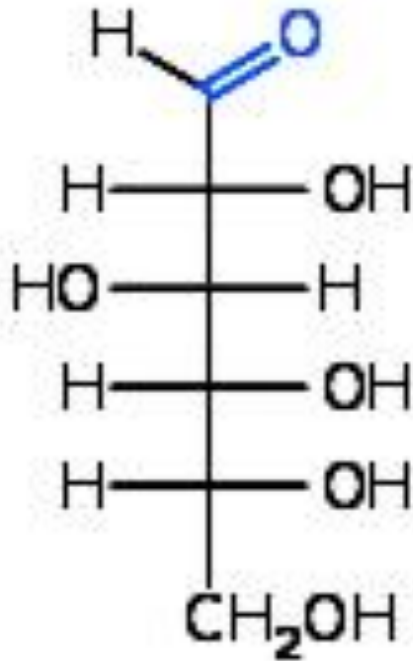
•According to the number of sugar units:

1. **Monosaccharides** (contain **one** sugar unit).
2. **Oligosaccharides** (contain **2-10** sugar units).
3. **Polysaccharides** (contain **more than 10** sugar units).

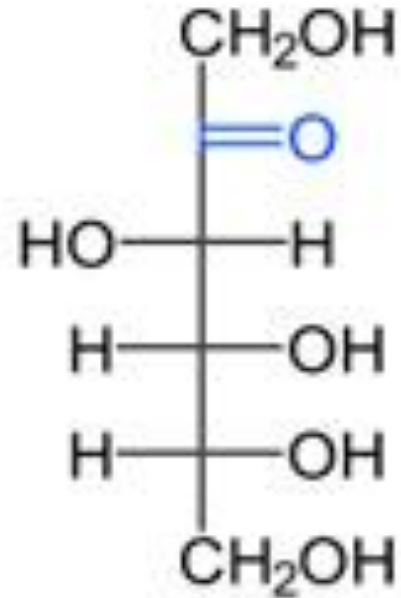
•According to their most oxidized functional group:

1. **Aldoses:** Carbohydrates with an **aldehyde** as their most oxidized functional group.
2. **Ketoses:** Carbohydrates with a **ketone** as their most oxidized functional group.

(1) Monosaccharides



Glucose



Fructose

General scheme for carbohydrates:

I. Physical properties:

Examine the **color, odor, aspect** and its **reaction to litmus paper**.

II. Chemical properties:

A. Molisch's reaction

B. Reactions for reducing sugars:

1. Fehling's test
3. Barfoed's test
5. Osazone test

2. Benedict's test
4. Moor's test

C. Ketose test

A. Molisch's reaction:

General reaction for **all** carbohydrates.

Procedure:

- Place about 1ml of sugar solution in a test tube.
- Add 2-3 drops of alcoholic α -naphthol, then shake.
- Add conc. sulfuric acid drop by drop carefully on the wall of the tube & don't shake.

Observation:

Violet ring at the junction of the 2 layers spreads by shaking.



Comment:

- 1- Dehydration of sugar to furfural in fructose or hydroxymethyl furfural (HMF) in glucose by conc. sulfuric acid.
- 2- Condensation of furfural or HMF with 2 molecules of alpha naphthol forming violet ring.

B. Reactions for reducing sugars

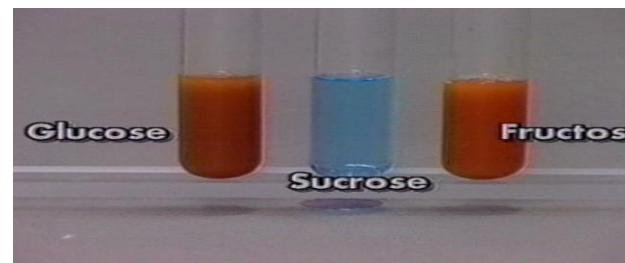
1. Fehling's test:

Procedure:

- Mix 1ml of Fehling A and 1ml of Fehling B in a clean test tube.
- Add 1 ml of the fehling mixture to 1ml sugar and heat for 1-2 min on direct flame.

Observation:

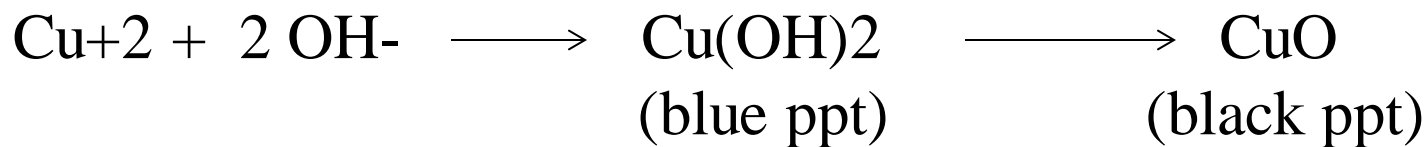
Red precipitate of cuprous oxide.



-Fehling A is a **copper sulfate solution**.

-Fehling B contains **sodium hydroxide** and **Rochelle salt (sodium potassium tartrate)**.

-Role of Rochelle salt: It act as chelating agent for Cu^{+2} to prevent its reaction with NaOH and formation of black cupric oxide.



Comment:

-Reduction of Cu^{2+} to Cu_2O (Due to the presence of free carbonyl group). It is a reducing sugar.

2. Benedict's test:

Procedure :

-Heat 1ml of Benedict's reagent with 1ml of the sugar solution for 1-2 min. on direct flame.

Observation:

Red precipitate of cuprous oxide.

-Benedict's reagent is composed of **copper sulfate, sodium carbonate and sodium citrate.**

-Role of citrate: as Rochelle salt in fehling's test.

- Benedict's reagent is a single solution.

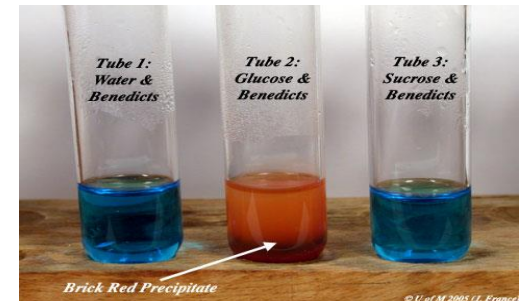
-Advantages:

1- The alkali is weaker and easier to handle.

2- More sensitive than Fehling's as it is not readily reduced by urates (as in urine) as Fehling's reagent.

Comment:

-Reduction of Cu^{2+} to Cu_2O (Due to the presence of free carbonyl group). It is a reducing sugar.



3. Barfoed's test:

Procedure:

- Mix 1ml of the sugar solution with 1ml of Barfoed's reagent.
- Heat in a boiling water bath for 3 minutes.

Observation:

Red precipitate of cuprous oxide.

-Barfoed's reagent contains **cupric acetate** in **dilute acetic acid**.

Comment:

-Reduction of Cu^{2+} to Cu_2O (Due to the presence of free carbonyl group). It is a reducing sugar.

4. Moore's test (Action of Alkali):

Procedure:

-Add 1ml of sugar solution to 1ml of **30% sodium hydroxide** solution then heat on direct flame for 1-2 minutes.

Observation:

Yellowish brown color and caramel odor.

- The odor is intensified by carefully acidifying it with diluted sulfuric acid.

Comment:

-It is a reducing sugar containing free carbonyl groups which undergo aldol condensation (caramelization) reaction in alkaline medium.

5. Osazone test (Phenyhydrazine test):

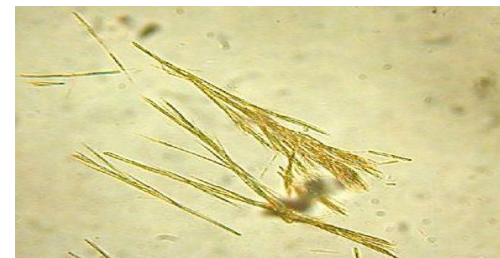
Procedure:

- To 5ml sugar in a test tube, add 10 drops of **glacial acetic acid**.
- Add about 1g of a freshly-prepared **mixture of phenylhydrazine hydrochloride and sodium acetate**.
- Mix well and heat in a boiling water bath for about 5-10 minutes.
- Cool.
- With a clean pipette, remove a few osazone crystals and examine them under the microscope.



Observation:

Yellow crystals of glucosazone & fructosazone (Brush-shaped or Raphides).



Comment:

-Due to the reaction of free aldehydic or ketonic group of one sugar molecule with 3 molecules of phenyl hydrazine HCl in three successive steps: **condensation, oxidation then condensation**.

C. Reaction for differentiation between glucose and fructose

Ketose test:

Procedure:

- Add 1ml of sugar solution to 3ml of conc. HCl solution.
- Heat for 1-2 min. on direct flame.

Observation:

- Fructose: Reddish brown color.**
- Glucose: Colorless.**



Comment:

- **In case of Fructose:** Dehydration of fructose and formation of furfural which is reddish brown in acidic medium.
- **In case of Glucose:** Dehydration of glucose and formation of hydroxymethyl furfural (HMF) which is colorless in acidic medium.

