BCM 101
BIOCHEMISTRY
Week 2 Practical
"Chemistry of carbohydrates"

Carbohydrates are a universal currency of energy stores and metabolic intermediates for living organisms. They have the general formula **[C(H_2O)]n**, which accounts for their name, "**carbohydrates**" (or hydrates of carbon). They are not truly hydrates of carbon, but are polyhydroxy compounds that contain a carbonyl group (C=O).

The aim of this practical session is to:

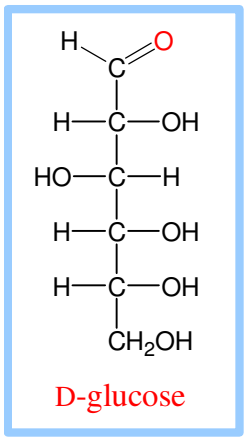
1. Obtain a simplified knowledge about different types of carbohydrates.
2. Identify and differentiate between the different types of carbohydrates.

Classification of carbohydrates

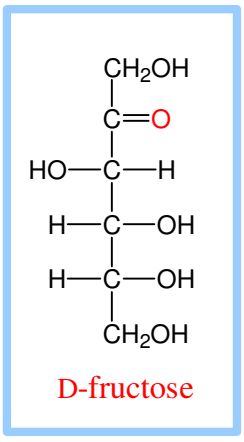
Carbohydrates are classified according to **4** different characteristics:

A. According to the number of carbon atoms in the sugar chain:
   1. **Trioses**: contain 3 carbon atoms (e.g. glycerose).
   2. **Pentoses**: contain 5 carbon atoms (e.g. ribose).
   3. **Hexoses**: contain 6 carbon atoms (e.g. glucose).

B. According to the terminal function group in the sugar chain:
   1. **Aldoses**: contain terminal aldehyde group (-CHO) (e.g. glucose).
   2. **Ketoses**: contain terminal ketone group (C=O) (e.g. fructose).



D-glucose



D-fructose

C. According to the number of sugar subunits:

1. **Monosaccharides**: they contain a single sugar unit (e.g. glucose & fructose).
2. **Disaccharides**: they are made up of two monosaccharide units linked together.
   e.g. Sucrose (glucose + fructose)
   e.g. Lactose (glucose + galactose)
   e.g. Maltose (glucose + glucose)
3. **Polysaccharides**: they are made up of many monosaccharide units linked together. Important polysaccharides include starch, cellulose, and glycogen, which are all made up of glucose molecules linked in different arrangements.

D. According to the reducing activity of the sugar unit:
Carbohydrates that can undergo oxidation are called reducing sugars. This depends on the presence of an exposed carbonyl group.

1. **Reducing sugars**: all monosaccharides & many disaccharides (e.g. lactose and maltose) are reducing sugars.
2. **Non-reducing sugars**: e.g. sucrose

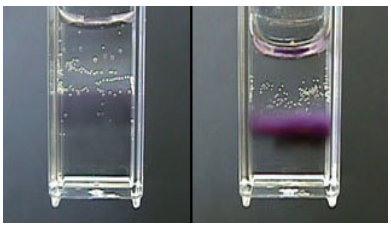
Qualitative tests for Carbohydrates

A- General Test for Carbohydrates

Molisch's test:

***Principle*:**

Concentrated sulphuric acid causes dehydration of all carbohydrates to give "**furfural**" compounds, that react with **α-naphthol** (Molisch's reagent) giving a violet or purple colored complex.



***Procedure*:**

1- In a test tube, add 2 ml of the test carbohydrate solution + 2 drops of **α-naphthol** solution.

2- Carefully pour dropwise 1ml conc. H_2SO_4, using a dropper, on the inner wall of test tube.

Observation:

A "**violet colored ring**" appears at the junction between the two layers.

B- Reactions of reducing sugars

General principle:

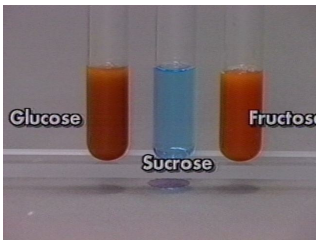
Sugars with free aldehyde groups can reduce either cupric ions in alkaline medium (**Fehling's** or **Benedict's** reagents) or in acid medium (**Barfoed's** reagents) to produce red or orange colored precipitate of cuprous ions. They can also reduce silver ions in alkaline medium (**Tollen's** reagent) to produce metallic silver giving a silver mirror. Heating in a boiling water bath is necessary for these reactions.

$$Cu^{2+} + e^{-} \xrightarrow{\text{Reducing sugar}} Cu_2O \downarrow$$

1- Reduction of Fehling's solution:

- **Fehling A** ($CuSO_4$ dissolved in dil. H_2SO_4)
- **Fehling B** (sodium potassium tartarate dissolved in dil. NaOH)

***Procedure*:**

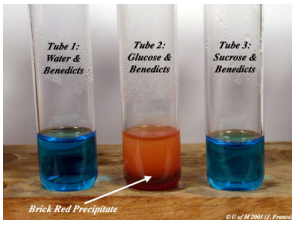


1- In a test tube add 2 ml of the test carbohydrate solution and put it in a boiling water bath **(tube 1).**
2- In another test tube add equal volumes of Fehling A & Fehling B and put it in a boiling water bath for 3 minutes **(tube 2).**
3- Mix the content of test tubes 1 & 2 and observe any change in color or precipitate formation.

***Observation*:**

Any change in color from deep blue (cupric ion) to green, yellow, orange or red (cuprous oxide) indicates a positive reaction.

2- Reduction of Benedict's reagent:



Benedict's reagent is $CuSO_4$ dissolved in H_2O alkalinized with sodium carbonate and sodium citrate.

***Procedure*:**

1- To 2 ml of **Benedict's** reagent, add 5 drops of the test carbohydrate solution and mix well.
2- Place the test tube in a boiling water bath for 5 minutes and observe any change in color or precipitate formation.

Observation:
Any change in color from deep blue (cupric ion) to green, yellow, orange or red (cuprous oxide) indicates a positive reaction.

3- Reduction of Tollen's reagent :

Tollen's reagent is ammoniacal silver nitrate solution.

***Procedure*:**

1- To 2 ml of **Tollen's** reagent, add 1 ml of the test carbohydrate solution and mix well.

2- Place the test tube in a boiling water bath for 5 minutes.

Observation:
Observe the appearance of a silver mirror.

C- Tests for Individual Carbohydrates

1- Osazone test:

***Principle*:**

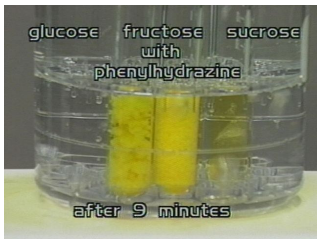
- Compounds containing the (-CO-CHOH) group form crystalline **osazone** compounds when heated with phenyl hydrazine.
- Osazone crystals have a characteristic shape under the light microscope and help in the identification of the sugar type.
- The reaction is stepwise; first, phenyl hydrazine reacts with carbonyl group of the sugar to form **phenyl hydrazone**, which then reacts with two further molecules of phenyl hydrazine to form the **osazone**.

$$\diagdown\!\!\diagup C{=}O + \text{phenyl hydrazine} \longrightarrow \text{Phenyl hydrazone}$$

$$\text{Phenyl hydrazone} + 2\ \text{phenyl hydrazine} \longrightarrow \text{osazone}$$

- **N.B.** the time of formation of the crystals, and whether the osazone is precipitated from hot solution or after cooling, can help in the identification of the type of carbohydrate.

***Procedure*:**



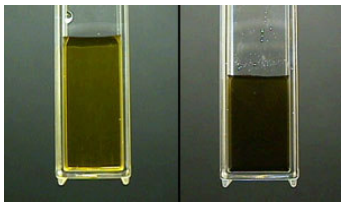
1- Acidify 3 ml of sugar solution with 5 drops of glacial acetic acid.
2- Add 1 ml of phenyl hydrazine solution and 1 ml of sodium acetate solution, mix well.
3- Place the test tube in a boiling water bath.

Observation:

- **Osazones** of monosaccharides (glucose & fructose) are formed on hot after about 15 minutes, and have the same crystal shape needles under the microscope.
- **Osazones** of reducing disaccharides (maltose & lactose) are formed after a longer time (up to 30 minutes) and crystal appears slowly after cooling and can be distinguished under the microscope as follows:
  Lactose gives crystals in the form of a tuft of needles.
  Maltose gives crystals in the form of broad needles.

2- Iodine Test:

Iodine solution gives a blue color with starch that disappears on heating and reappears on cooling. Iodine solution also gives a brown color with glycogen.



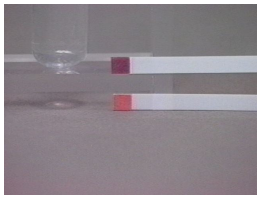
***Procedure*:**

1- Add 2 drops of diluted iodine solution to the test carbohydrate solution and observe the color obtained.
2- Compare the color obtained with that of water and iodine (Blank).

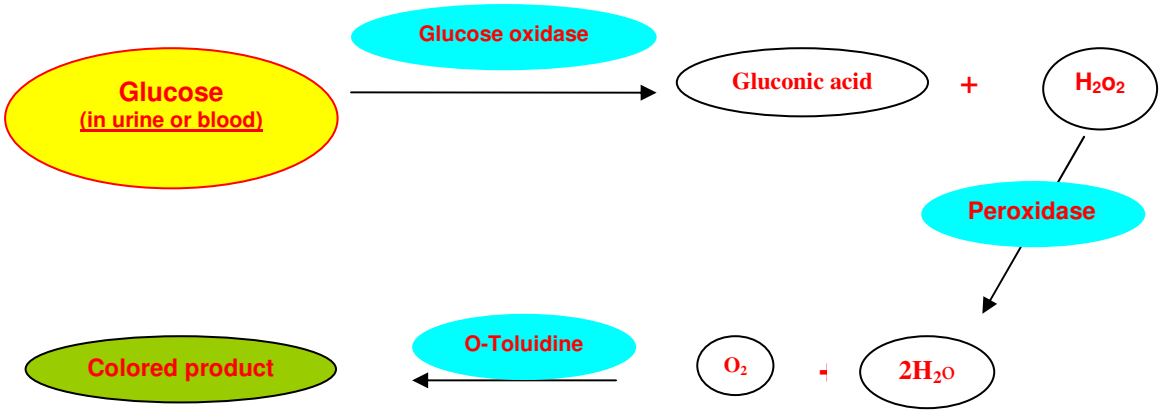
3- Biochemical test for glucose:

This method is commercially used in dipsticks used for testing the presence or absence of glucose in urine or blood.

The dipstick contains the enzyme glucose oxidase, peroxidase and o-Toluidine.



***Principle*:**



Clinical significance:

This test is used for qualitative determination of glucose in biological fluids (e.g. urine or blood) and subsequent diagnosis of "**diabetes mellitus**".

Laboratory Exercise:

Label 5 test tubes, one for each provided carbohydrate solution (glucose, fructose, lactose & starch) and perform the following tests:

1. Molisch's test.
2. Fehling's test.
3. Benedict's test.
4. Osazone test.
5. Iodine Test.

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Student Name: ..... **Student number:** .....

Laboratory exercise:

Record your observations for each carbohydrate solution in the provided table.

Sample	Molisch's test	Fehling's test	Benedict's test	Osazone test	Iodine Test
Glucose					
Fructose					
Sucrose					
Lactose					
Maltose					
Starch					