Carbohydrates

• ***Carbohydrates:*** *Are the most abundant and diverse class of organic compounds occurring in nature.*

• *They play* ***a key role in the evolution of life*** *due to creating a direct link between the sun and chemical energy.*

The aim of the experiments:

• *Understanding simple tests for the* ***identification of carbohydrates*** *in a given sample.*

Theory:

- ***Carbo****=Carbon,* ***Hydrate****=Water (Hydrogen+Oxygen)*
- ***General formula:*** *$(CH_2O)_n$*
- *They have important* ***structural and metabolic*** *roles in both* ***animals and plants.***
- *Commonly used for* ***food and energy storage.***

Classification of carbohydrates

- ***Monosaccharides:*** *Cannot be hydrolyzed into a more simple form.*
- ***Disaccharides:*** *Products of* ***two*** *monosaccharide units.*
- ***Oligosaccharides:*** *Products of* ***three to ten*** *monosaccharides.*
- ***Polysaccharides:*** *Products of* ***more than ten*** *monosaccharide units.*

Examples of carbohydrates

• Monosaccharides:
• Can be further classified into:

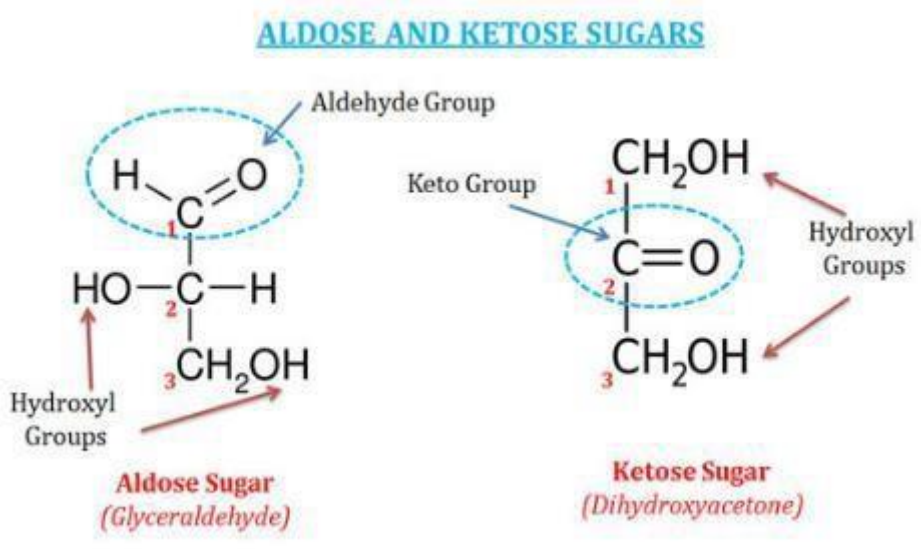
No. of carbon atoms Trioses

Tetroses – Pentoses – Hexoses – Heptoses

Aldehyde or Ketone group

Aldoses – Ketoses

Monosaccharides structure:



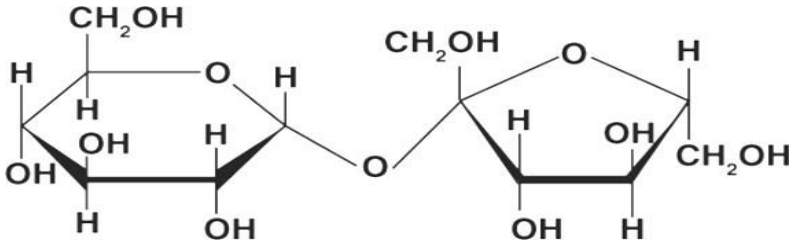
Monosaccharides examples:

	Aldoses	***Ketoses***
Trioses ($C_3H_6O_3$)	*Glyceraldehyde*	*Dihydroxyacetone*
Tetroses ($C_4H_8O_4$)	*Erythrose*	*Erythrulose*
Pentoses ($C_5H_{10}O_5$)	*Ribose*	*Ribulose*
Hexoses ($C_6H_{12}O_6$)	*Glucose*	*Fructose*

Other examples of carbohydrates include:

• Disaccharides:

• *Lactose, Maltose, and Sucrose.*



• Polysaccharides:

- Starch and Dextrin.

Can be linear or branched polymers.

Also, *sometimes they are classified as* ***Hexosans*** *or* ***Pentosans*** *depending on their yield when hydrolyzed.*

Qualitative and Quantitative tests for carbohydrates

To identify the presence of carbohydrates in a sample:

1 - Molisch's test
2 - Fehling's test
3 - Benedict's test
4 - Tollen's test
5 - Iodine test

Carbohydrate identification tests

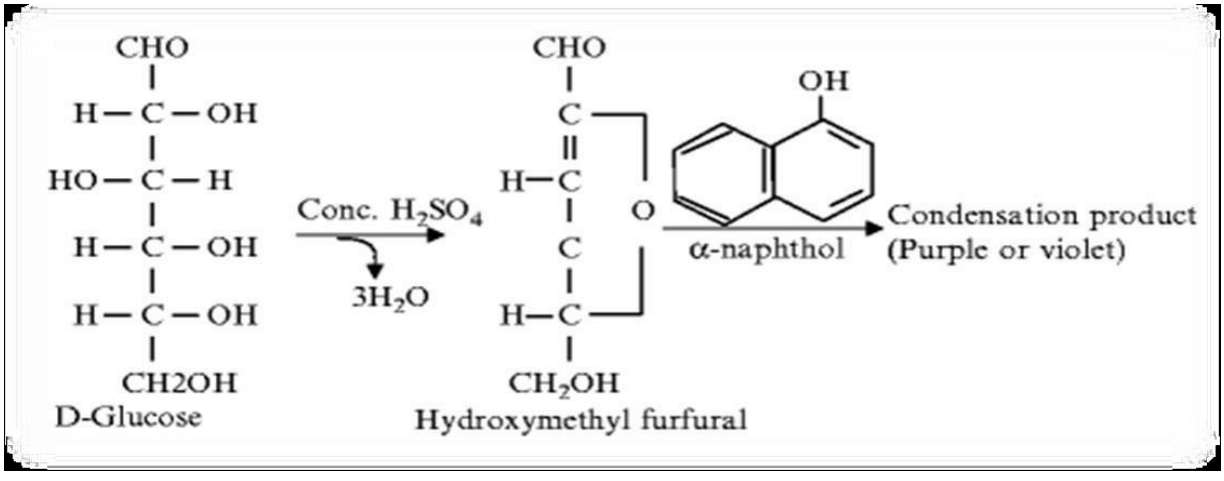
1 - Molisch's test:

General *test for carbohydrates.*

Concentrated ***sulfuric acid*** *converts the carbohydrate into* ***furfural*** *or any of its' derivatives, which in turn* ***reacts with α–naphtol*** *to form a* ***purple colored product****.*

Purple or violet ring is a positive result *for the presence of carbohydrates in the sample.*

Molisch's chemical reaction:



Molisch's Procedure:

*1. Take **2ml of the given sample solution** in a clean test tube.*

*2. Add **2-3 drops of Molisch reagent** slowly.*

*3. Add **concentrated sulfuric acid** along the sides of the test tube.*

*4. Notice the **separation** of the sample (Acid: bottom, sample: top).*

*5. If a **violet ring** is observed in the middle, then the **presence of carbohydrates** is confimed.*

2. Fehling's test:

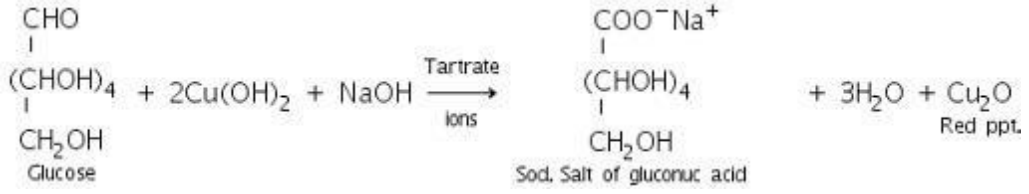
*This test identifies **reducing sugars.***

*Needs **boiling water bath.***

*The copper ions in Fehling's solution (+3) state is reduced to (+2) oxidation state, and **red cuprus oxide** is precipitated in the **alkaline medium.***

***Red precipitate is a positive result** for the presence of reducing sugars and carbohydrates.*

Fehling's chemical reaction:



Fehling's Procedure:

*1. Take **2ml of the given sample solution** in a clean test tube.*

*2. Add **1ml of Fehling A solution** slowly.*

*3. Add **1ml of Fehling B solution** slowly.*

4. Keep the solution in a boiling water bath for ≈10 minutes.

*5. If a **red precipitate** is observed, then the **presence of carbohydrates and reducing sugars** is confimed.*

3. Benedict's test:

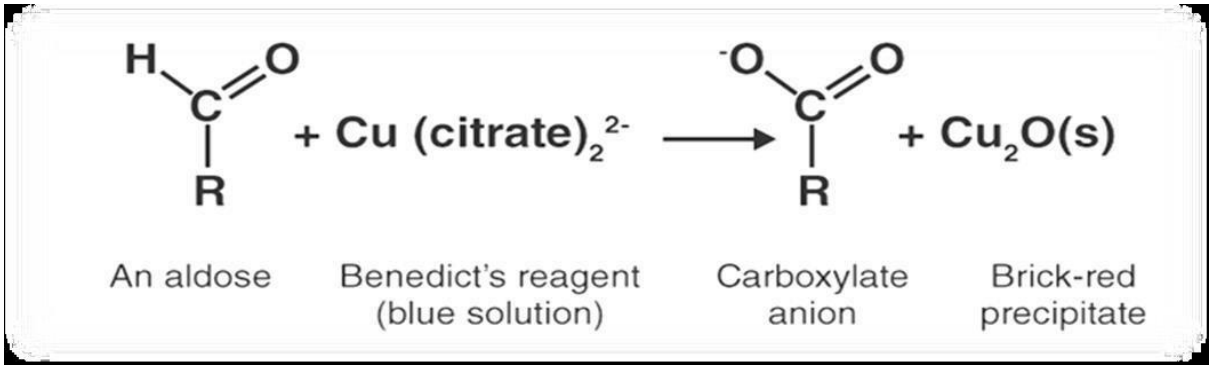
*This test identifies **reducing sugars.***

*In **Alkaline medium, sodium carbonate** converts glucose to **enediol**, which in turn reduces **cupric to cuprous** forming **cuprous hydroxide**.*

*Upon boiling, **red precipitate of cuprous oxide** is formed.*

***Red precipitate is a positive result** for the presence of reducing sugars and carbohydrates.*

Benedict's chemical reaction:



Benedict's Procedure:

*1. Take 3**ml of the given sample solution** in a clean test tube.*

2.** Add 3**ml of Benedict's reagent.

3. Boil the solution for ≈2 minutes.

4. Cool the solution and observe the test tube.

*5. If a **red/green/yellow precipitate** is observed, then the **presence of carbohydrates and reducing sugars** is confimed.*

4. Tollen's test:

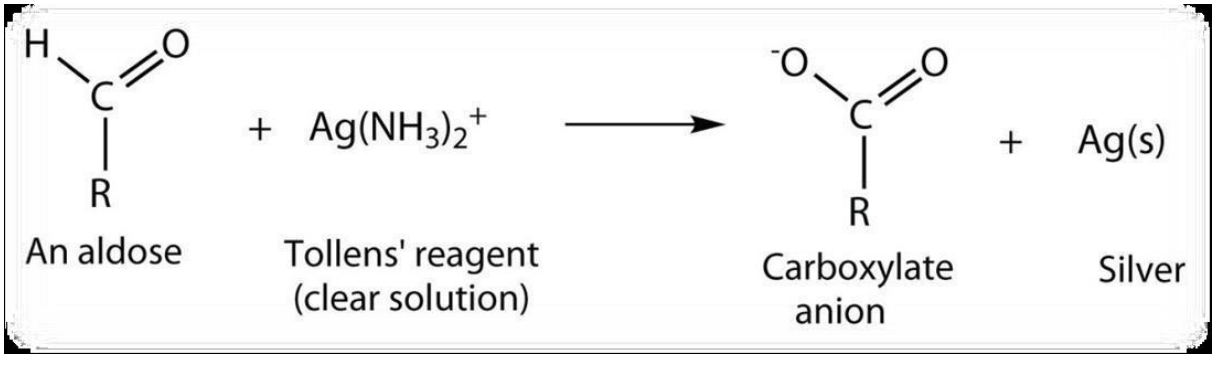
*This test identifies **reducing sugars.***

*Reacts with carbohydrates to form a **silver mirror** along the inner walls of the tube.*

Silver ions** are reduced to **metallic silver.

***Silver mirror is a positive result** for the presence of reducing sugars and carbohydrates.*

Tollen's chemical reaction:



Tollen's Procedure:

*1. Take **3ml of the given sample solution** in a clean test tube.*

2.** Add **3ml of Tollen's reagent.

3. Keep the test tube in boiling water for ≈10 minutes.

*4. If a **shiny silver mirror** is observed, then the **presence of carbohydrates andreducing sugars** is confimed.*

5 - Iodine test:

*This test identifies **starch.***
***Starch** reacts with **Iodine solution ,** and upon **cooling** a blue color appears.*
***Blue color is a positive result** for the presence of starch.*

Iodine test chemical reaction:

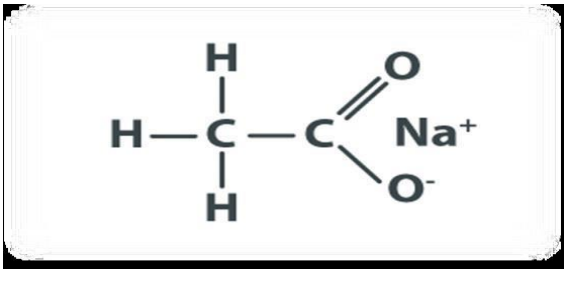
Iodine Test Procedure:

*1. Take **3ml of the given sample solution** in a clean test tube.*

2.** Add **3 drops of Iodine solution.

3. Observe the change in color.

*4. If a **blue color** is observed, then the presence of **starch** is confimed.*

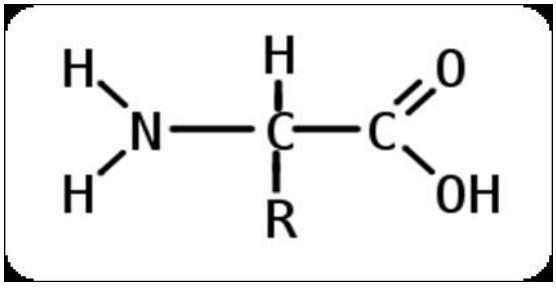


Sodium acetate

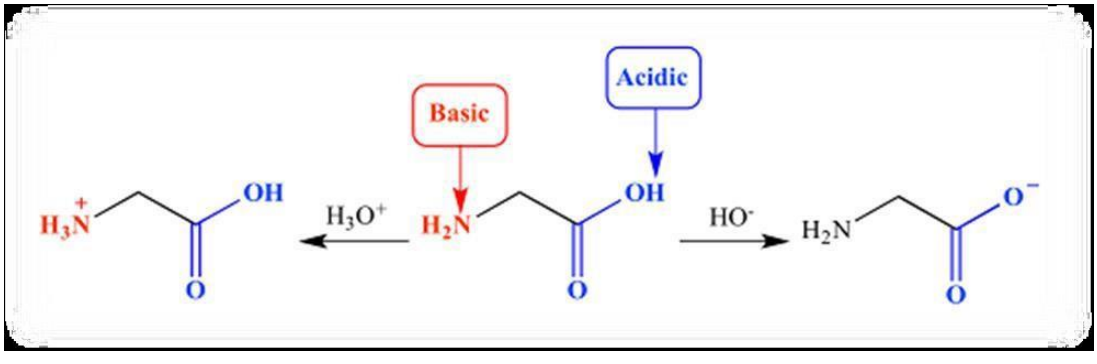
Amino Acids

Amino acids:

are molecules containing an amine group, a carboxyl acid group, and side chain.



Amino acids of the general formula **$RCH(NH_2)COOH$** are amphoteric, meaning that they can behave as amines in some reactions and as carboxylic acids in others.



At certain pH known as the isoelectric point an amino acid will have no net charge.

If the number of positive charges and negative charges is equal, then the molecule is called a Zwitterion, which has zero charge.

The net charge on the molecule is affected by the pH of its surrounding environment and can become more positive or negative due to the gain or loss of protons, respectively.

The importance of amino acids

Amino acids are critical to life; due to being the building blocks for proteins, and also being intermediates in metabolic pathways.

Classification of amino acids

Amino acids are generally classified according to the properties of their side chain.

The side chain can make an amino acid a 1weak acid or a 2weak base, and a hydrophile if the side chain is polar, or 4hydrophobe if it is nonpolar.

Specific reactions for individual amino acids

1. Millon's test:

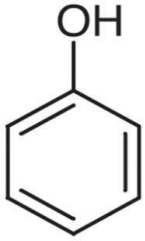
- Specific test to phenol containing structures. (Tyrosine, β-naphthol).
- Millon's reagent contains mercury dissolved in concentrated HNO_3 .
- Red precipitate is a positive test result for phenols in the sample.

Phenol:

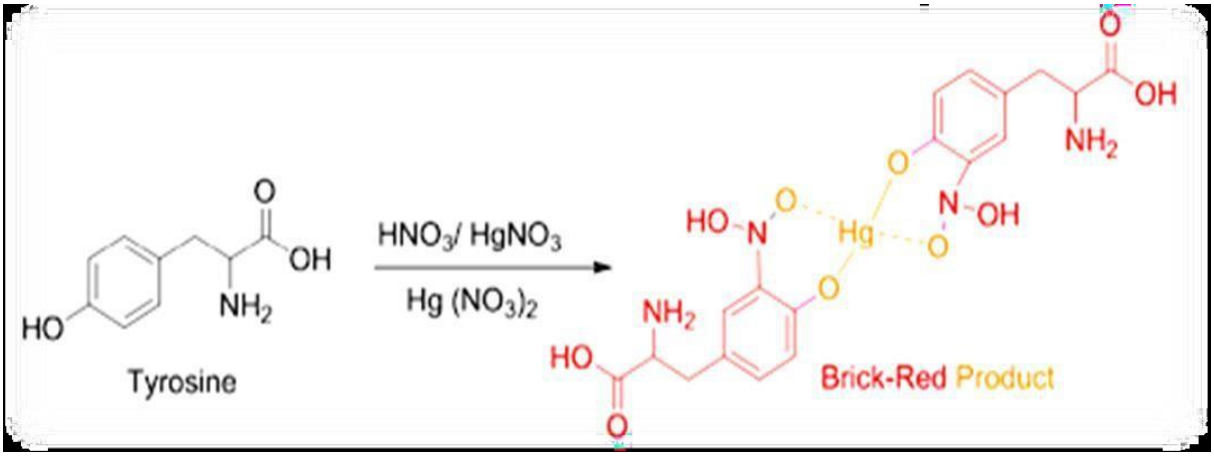
Also called carbolic acid, is an aromatic organic compound with the molecular formula (C_6H_5OH).

It is a white crystalline solid that is volatile.

The molecule consists of a phenyl group ($-C_6H_5$) bound to a hydroxy group ($-OH$). Mildly acidic, and requires careful handling because it can cause chemical burns.



Millon's chemical reaction:



Millon's Procedure:

1. Take **2ml of the amino acid** solution in a clean test tube.
2. Add **1-2 drops of Millon's** reagent.
3. Warm the solution in boiling water bath for ≈ 10 min.
4. The appearance of a **red colour** is a positive test result for the presence of phenols in the sample.
5. **Note:** This test is specific for phenols, if a sample also gives positive ninhydrin result THEN it is considered a phenolic amino acid.

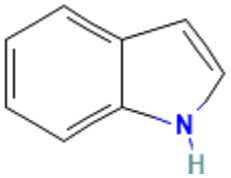
2. Hopkin's Cole test:

Specific test to indole group containing structures. (**Tryptophan**).

The glyoxylic aicd in glacial acetic acid reacts with the indole group in the presence of concentrated H_2SO_4.

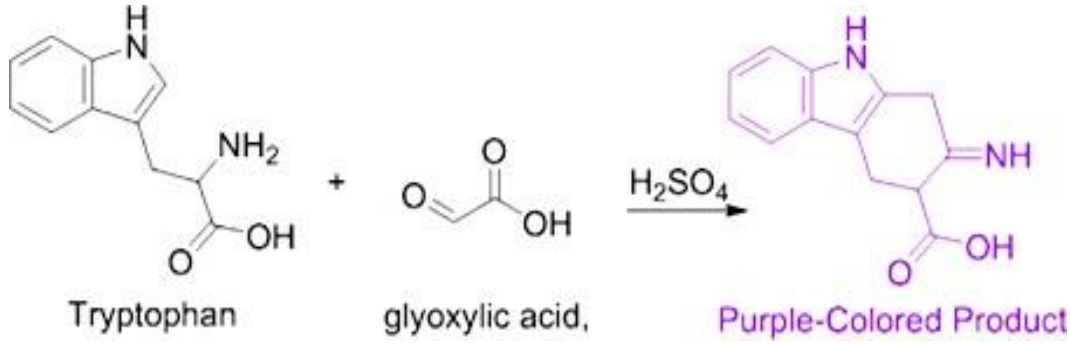
Purple colour is a positive test result for indole containing substances in the sample.

Indole:



- An aromatic heterocyclic organic compound
- with the formula (C_8H_7N).
- It consists of a six-membered benzene ring,
- fused to a five-membered pyrrole ring.

Hopkin's Cole chemical reaction:



Hopkin's Cole Procedure:

1. Take **2ml of amino acid** solution in a clean test tube.
2. Add **5 drops of Hopkin's** Cole solution.
3. Slowly add **2ml of H_2SO_4** along the side of the tube.
4. Notice the separation (Acid:bottom, Sample: Top).

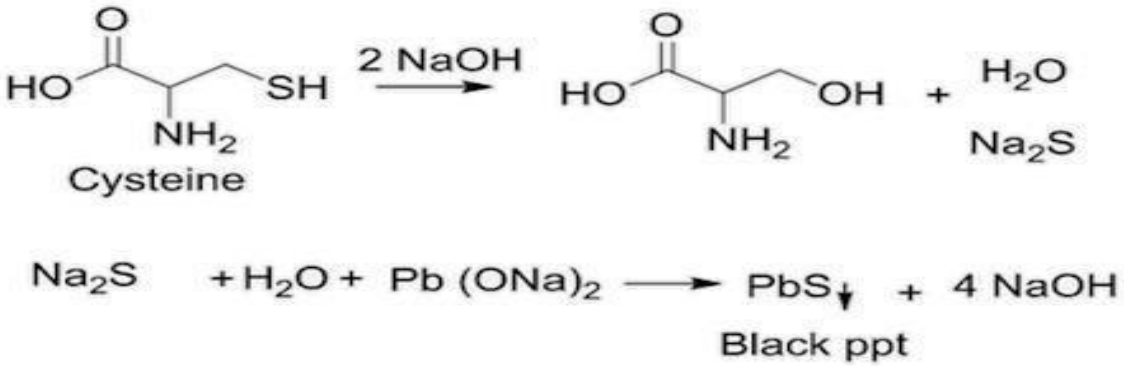
5. The appearance of a purple ring is a positive test result for the presence of indole in the sample.

6. **Note:** This test is specific for indole, if a sample also gives positive ninhydrin result THEN it is considered an indole derivative amino acid.

3. Lead-sulfide test:

Specific test for sulfide containing amino acids. (Cysteine, cystine).
When cystine is boiled with 40% NaOH, some of the sulfure in its structure will be converted to sodium sulfide Na_2S.
Sodium plumbate causes the precipitaion of PbS in the alkaline medium. (Brown colour is positive)

Lead-sulfide chemical reaction:



Lead-sulfide Procedure:

1. Take **2ml of amino acid** solution in a clean test tube.
2. Add **1 ml of 40% NaOH** solution.
3. Add **3 drops of 10%** Lead acetate solution.
4. Boil the mixture for ≈ 2 minutes.

5. The appearance of a brown precipitate is a positive test result for thepresence of sulfides in the sample.

6. Note: This test is specific for sulfide, if a sample also gives positive ninhydrin result THEN it is considered an sulfide containing amino acid.