Cihan University of Sulaimaniya

Practical Biochemistry

Lecture 1&2 :

Introduction & Identification of Carbohydrates

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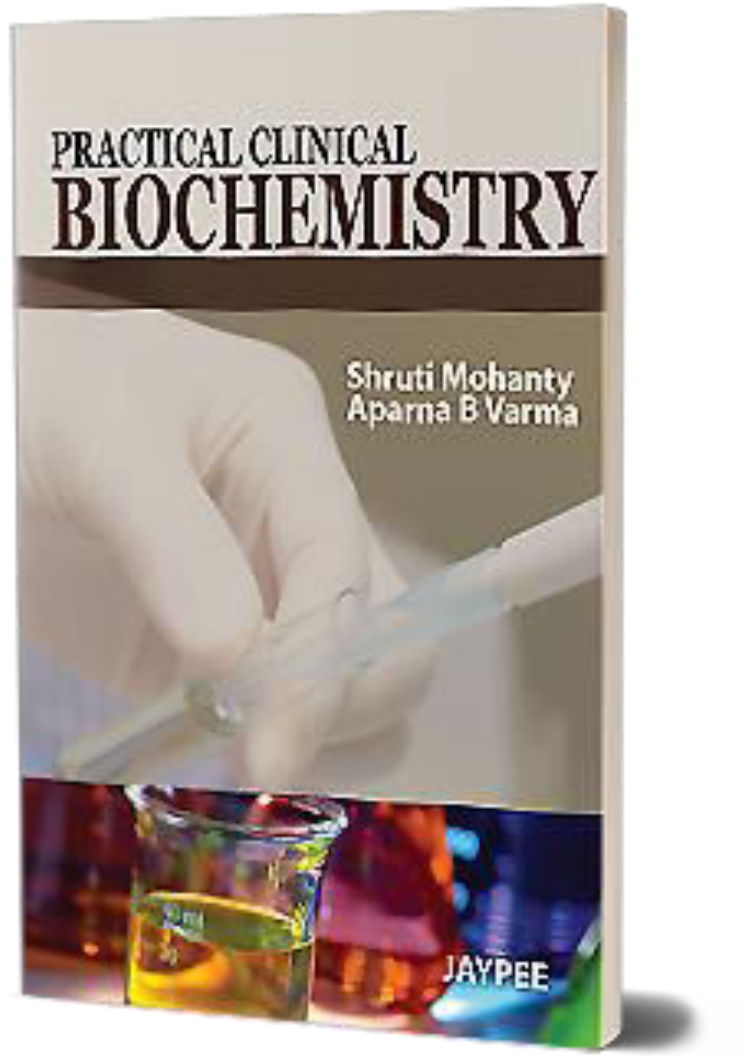
Introduction: Lesson Plan

Week Number	Subject- Practical
Week #1	Lab saefety-Introduction
Week #2	Identification of Carbohydrates-Molisch test, Iodine test
Week #3	Identification of Carbohydrates- benedicts and Barford's tests
Week #4	Carbohydrate qualitative tests(seliwanoff, Osazone, Fehling, Bails) Tests
Week #5	Identification of Amino acids- Ninhydrin Test
Week #6	Identification of Protein- Biurret Test
Week #7	Precipitation of proteins/solubility
Week #8	Reaction of Lipid - Saponification
Week #9	Reaction of Lipid Part II –Acrolein Test
Week #10	Introduction to lipids solubility/ Acrolein test
Week #11	Introduction to protein/ color reaction of proteins (Salkowaki, Liberman- Burchard) Test.
Week #12	Thin layer chromatography
Week #13	Precipitation of proteins/solubility
Week #14	Measurement of glucose by Digital Glucometer
Week #15	Final Exam



Introduction

- Carbohydrates are the most abundant class of organic compounds found in living organisms.
- Carbohydrates are a major source of energy from our diet.

- Carbohydrates have a wide range of functions, including:

1. providing a dietary calories for most organisms
2. acting as a storage form of energy in the body
3. serving as cell membrane components that mediate some forms of intercellular communication.
4. serve as a structural component of many organisms, including the cell walls of bacteria, the exoskeleton of many insects, and the fibrous cellulose of plants.

Introduction

They originate as products of photosynthesis, an endothermic reductive condensation of carbon dioxide requiring light energy and the pigment chlorophyll.

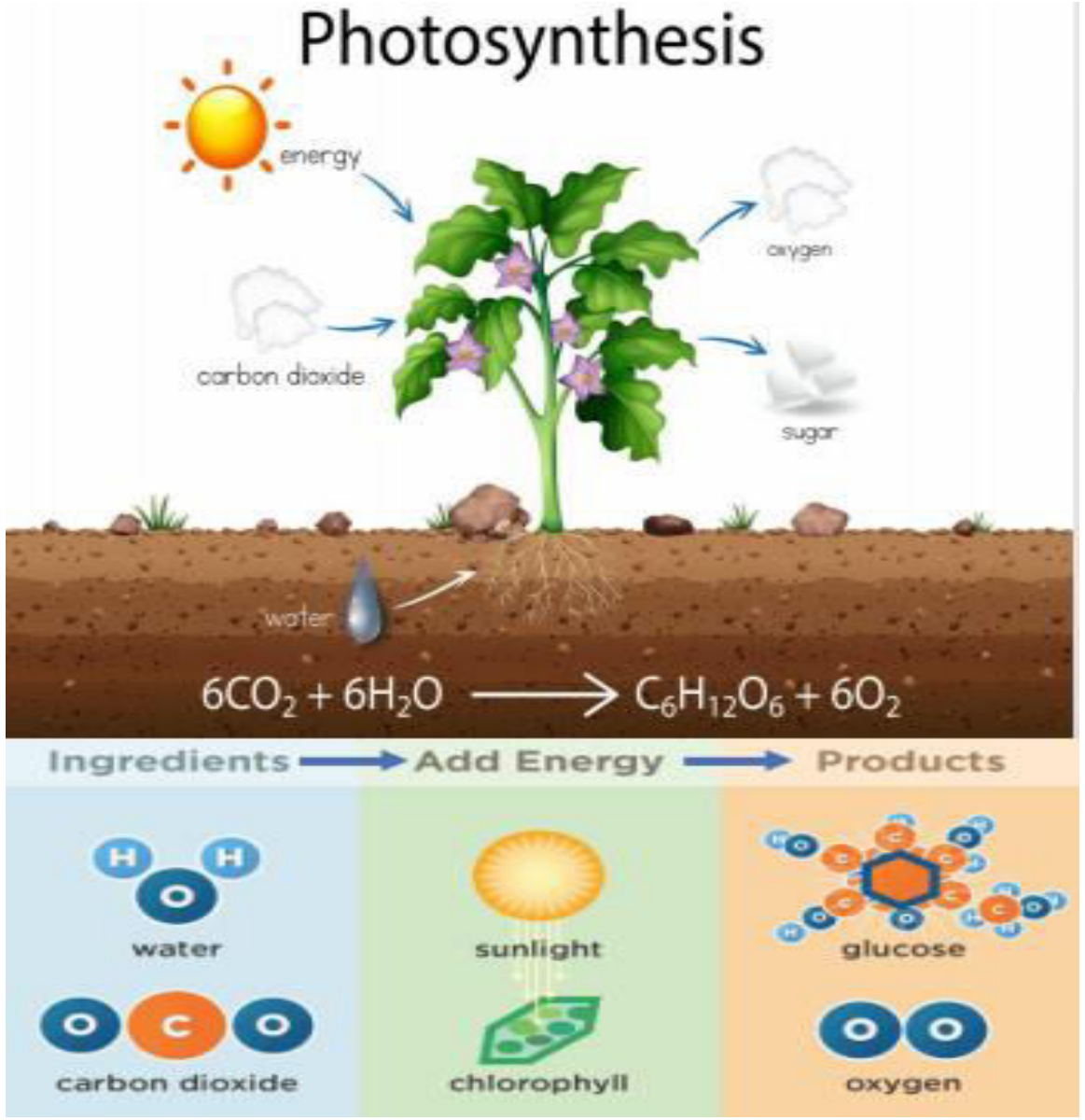


Figure 1: Photosynthesis reaction and formation of Carbohydrate

CLASSIFICATION OF CARBOHYDRATES

CARBOHYDRATES

Saccharides
(Sugars)

Physical Properties
* Low molecular weight
* Soluble in water
* Sweet to taste

Composition

Monosaccharides
Simple sugars

Disaccharides
Double sugars

Diagrammatic representation

General formula

$(CH_2O)_n$ When n=3 to7

$C_{12}H_{22}O_{11}$

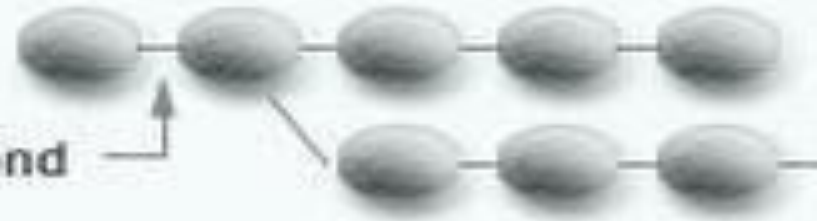
Common examples

Glyceraldehyde, Glucose
Fructose, Galactose,
Ribose sugar

Maltose,Sucrose
Lactose

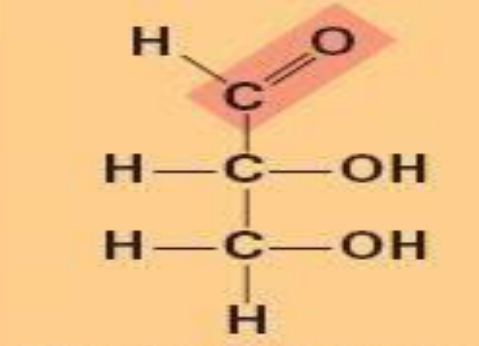
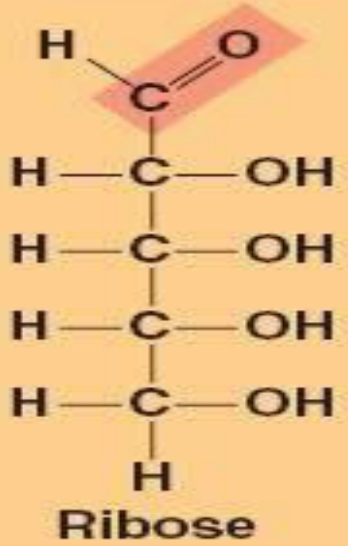
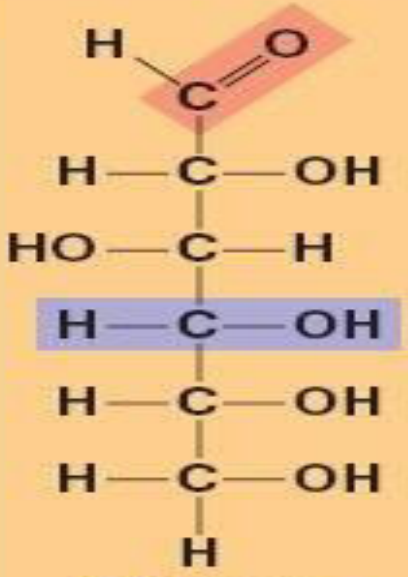
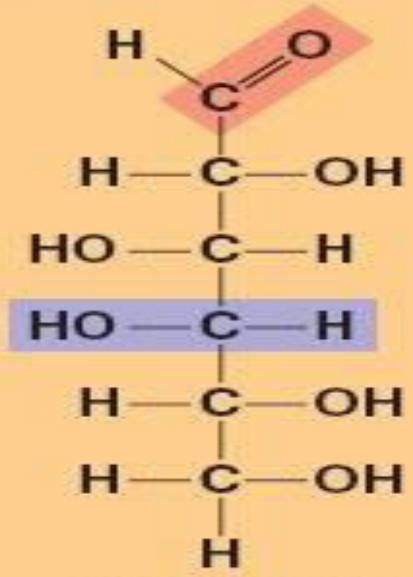
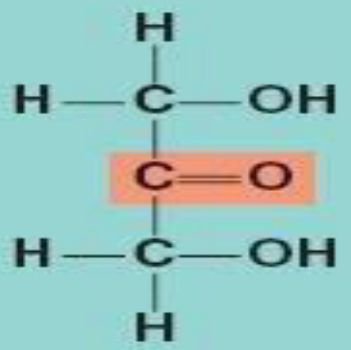
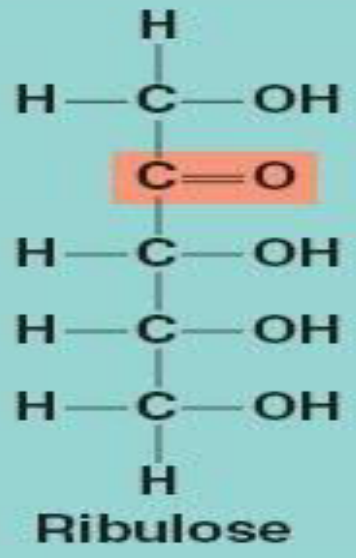
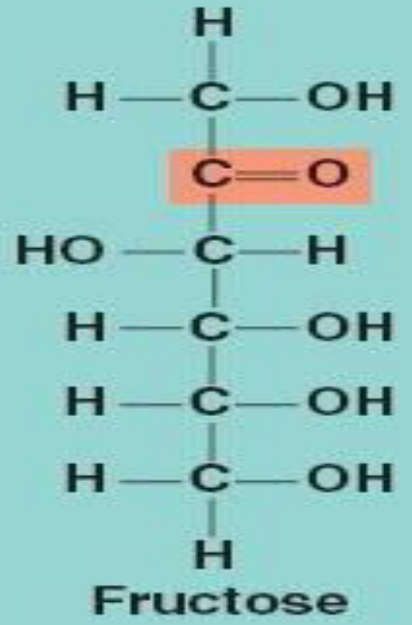
Polysaccharides
(Complex sugars)
* High molecular weight
* Insoluble in water
* Tasteless

Multiple sugars



$C_x(H_2O)_n$

Starch, Glycogen
Cellulose, Lignin, Chitin

	Triose sugars ($C_3H_6O_3$)	Pentose sugars ($C_5H_{10}O_5$)	Hexose sugars ($C_6H_{12}O_6$)	
Aldoses	 Glyceraldehyde	 Ribose	 Glucose	 Galactose
Ketoses	 Dihydroxyacetone	 Ribulose	 Fructose	

Introduction

Tests in the laboratory

- **Qualitative**
- Only give information of the presence or absence of a compound.
- **Semiquantitative**
- Approximation on the range of substance present in the sample.
- **Quantitative**
- Gives an exact amount of the substance present in the sample.

Classification of Carbohydrates

- **Monosaccharides:**

  -Examples: Glucose, Fructose, Galactose

- **Disaccharides:**

  - Examples: Lactose, maltose and sucrose

- **Oligosaccharides:**

  - three to ten monosaccharides

- **Polysaccharides:** more than ten monosaccharide units.

  - Examples: Glycogen, Starch, Fibers (cellulose)

Qualitative Tests for Carbohydrates

• Molisch Test

❑ **Objective:**

To identify **carbohydrates from other biomolecules**

❑ **Principle of Molisch's test:**

- Molisch's test is **a general test for all carbohydrates**. In this test, carbohydrates when reacted with conc. H_2SO_4 get **dehydrated** to form **furfural and its derivatives**.
- When monosaccharide are treated with conc H_2SO_4 or conc HCl, -OH group of sugar are removed in the form of water and furfural is formed from pentose sugar and hydroxymethyl furfural is formed from hexose sugar. These products reacts with sulphonated α- naphthol to give **a purple (violet red) colored complex**.

Figure 3: Molish test

Introduction

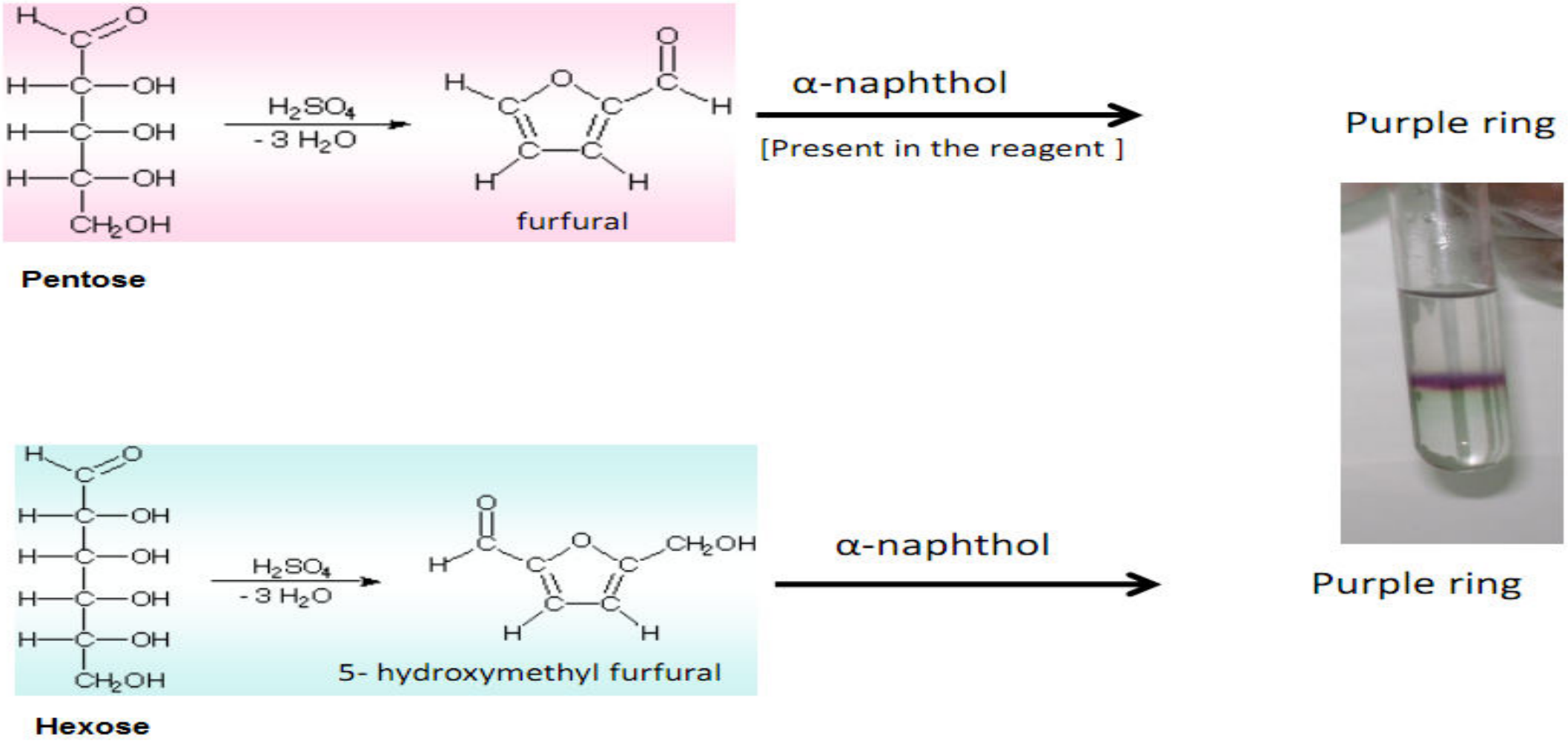


Figure 4: Molisch test reaction ,which is happen in 2 steps , first one formation of Aldehyde and the second one is formation of complex

Molisch's Test Procedure

1- Add 2ml of 1% solutions of sucrose and starch into three separate test tubes.

2- Add 2 drops of Molisch's reagent into each test tube and mix well by shaking.

3- Pour slowly and carefully about 1 ml of concentrated Sulphuric acid down the side of the tubes to form a layer below the sugar solution. A **reddish – violet ring** at the junction appears and the color distributed all over the solution.

4- Report your observations and explain your results.

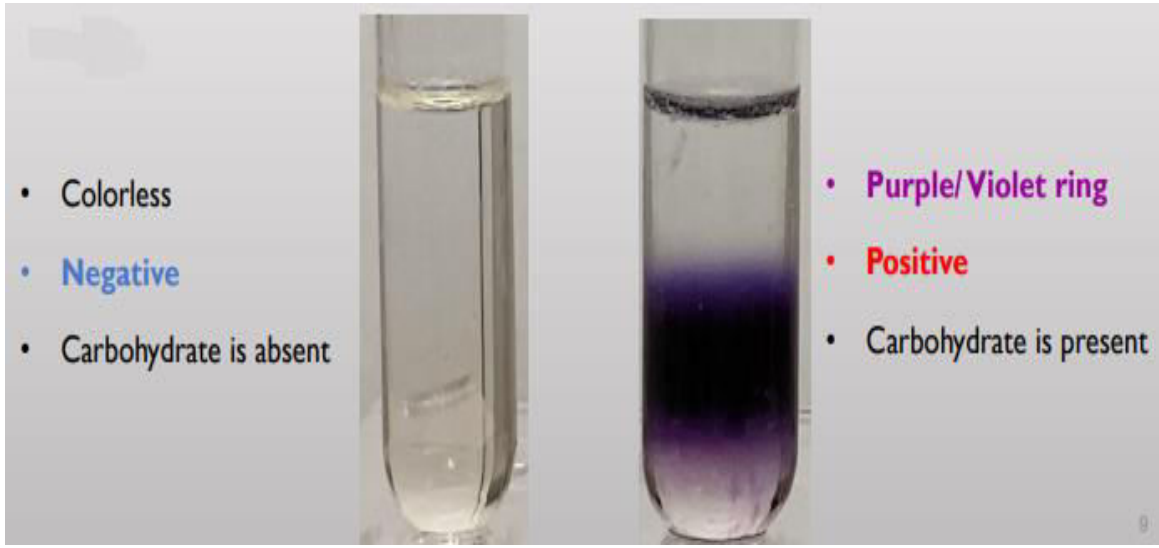


Figure 5: Positive and negative results of Molisch test

Iodine test

- **Objective**
  - Distinguishes Starch from other carbohydrates.
  - Iodine test is a chemical test for Polysaccharides ,
  - which is used to distinguish polysaccharides from mono- or disaccharides like starch , dextrin, and glycogen.
  - A polysaccharide is a large molecule made of many smaller monosaccharides. Monosaccharides units bound together by glycosidic bond to create polysaccharides.

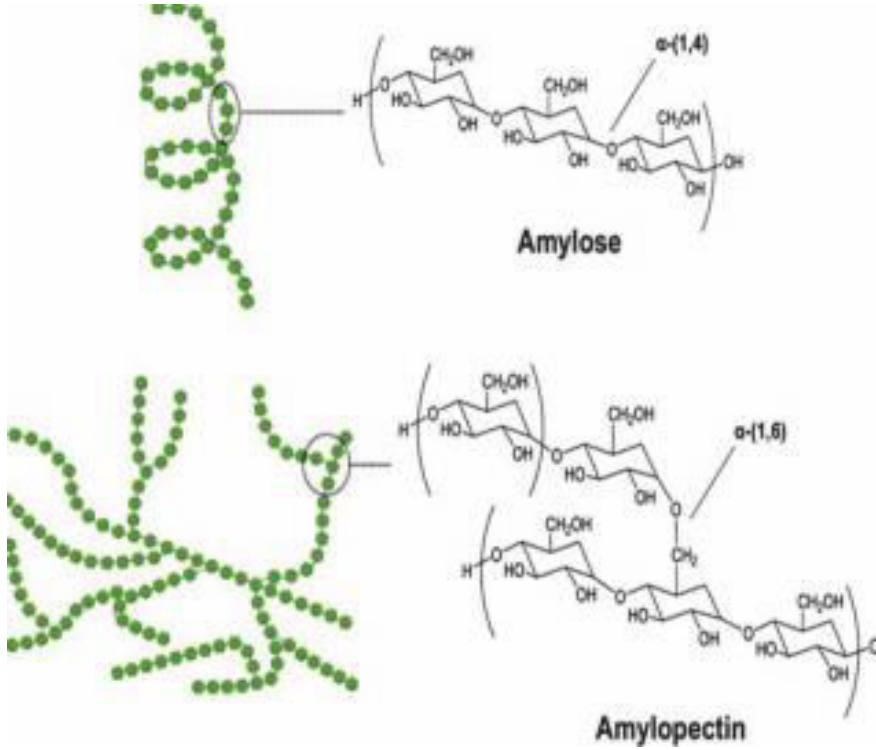


Figure 6: A starch molecules contains hundreds of glucose molecules in either occasionally branched chains (amylopectin) or un-branched chains (amylose)

Basic Principle

Starch when reacted with I2 forms compound that gives **blue color**. On heating or on addition of alkali like NaOH or KOH, color disappears. This reaction is only physically association where I2 traps in the coiled structure of polysaccharide. On heating or on addition of alkali; the coiled structure becomes linear and the I2 molecules are released and the color disappears.

- **Starch** produce intense blue color
- **Glycogen** produces a red – brown color.

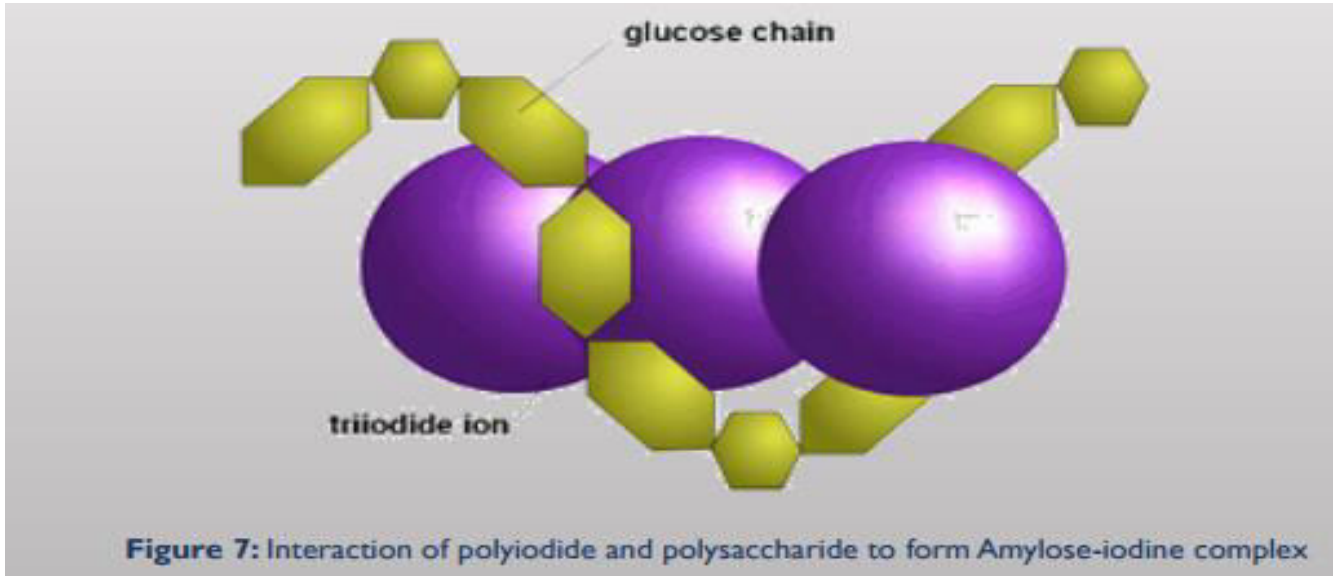


Figure 7: Interaction of polyiodide and polysaccharide to form Amylose-iodine complex

Procedure

1- Into **3 test t**ubes **add** about **2 ml** of 1% solution of **sucrose, starch, and distilled water** [serve as a blank] , each solution in a separate tubes.

2- Add **few drops of iodine** solution into each tube.

3- **Mix** the contents of each tube and **compare the color** of the blank to that of the other tubes.

4- Report the color which you observed in each tube.

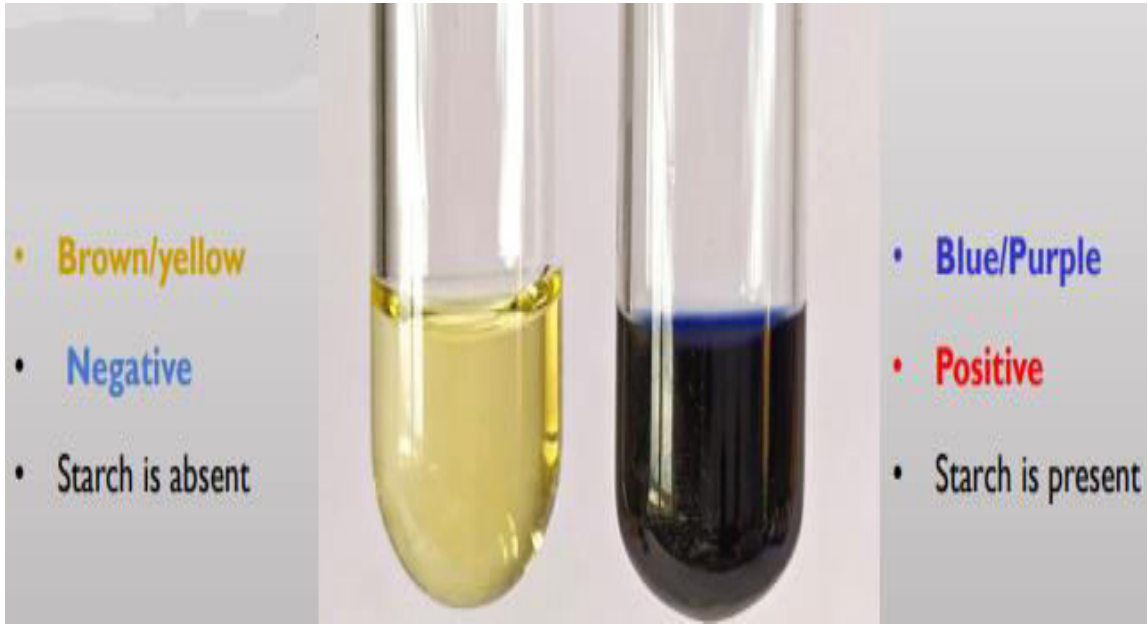


Figure 8: Iodine Test result's for starch sample.

Lab Questions

1. **Which types of carbohydrates does the Molisch test detect?**

2. **Name the complex formed by the addition of concentrated sulfuric acid to sugar solution.**

3. **Can the Molish test differentiate between different type of carbohydrate, like glucose, fructose, and sucrose? Why or why not?**

4. **Why glucose does not give positive result with iodine test but not starch?**

Thank You
Any Questions?