CLS 281

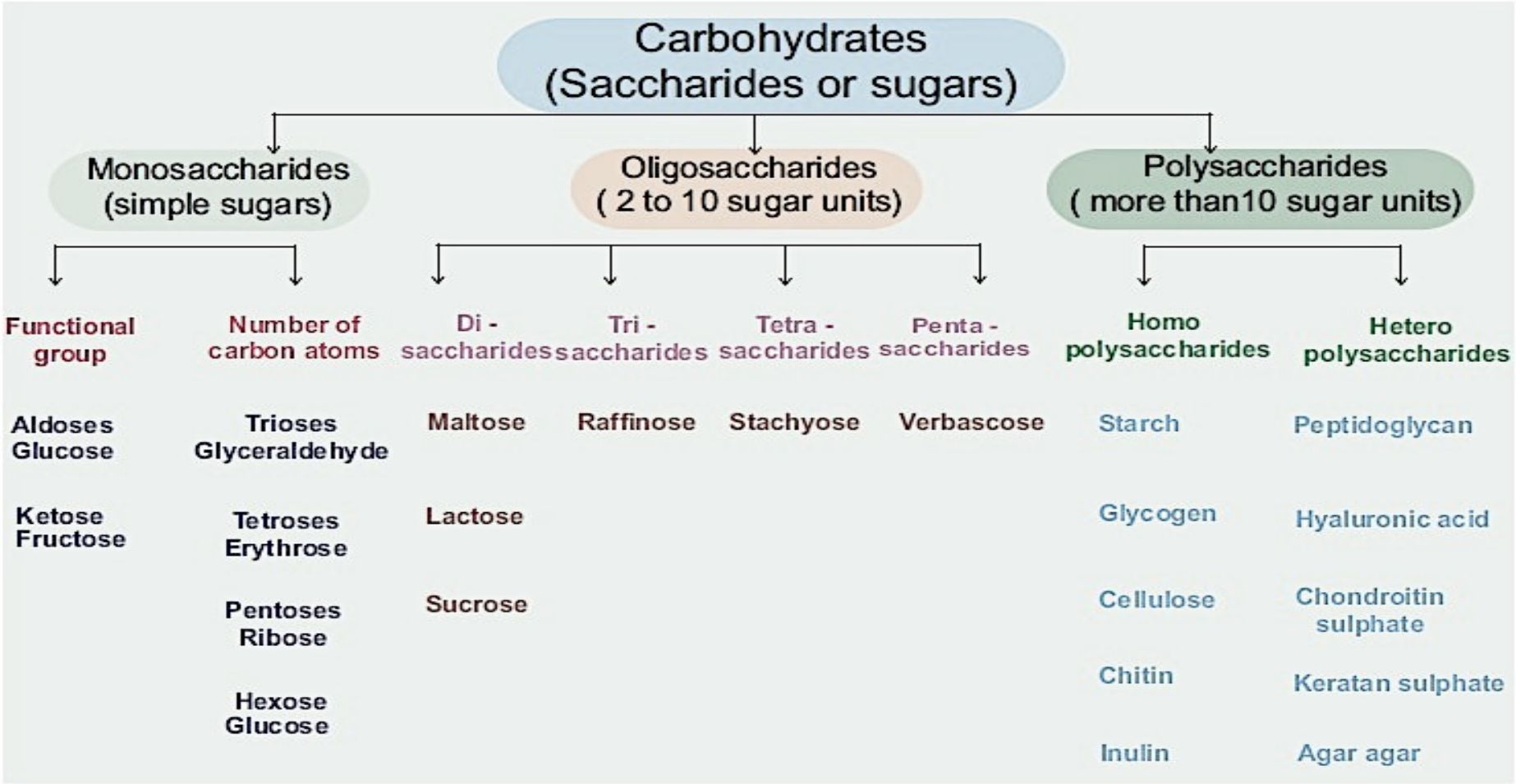
Basic Biochemistry and Biomolecules

جامعة الملك سعود
King Saud University
1957

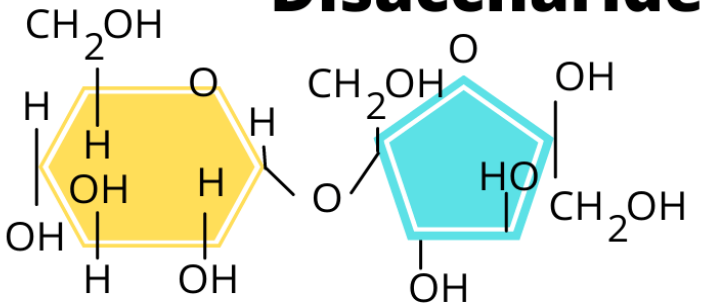
Experiment 6

Color Tests for Specific Carbohydrates: Ketoses, Pentoses, and Polysaccharides.

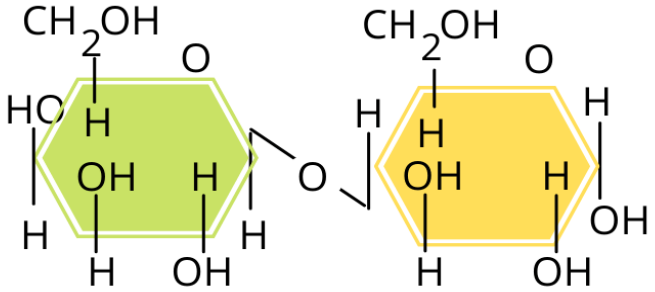
Carbohydrate Classification



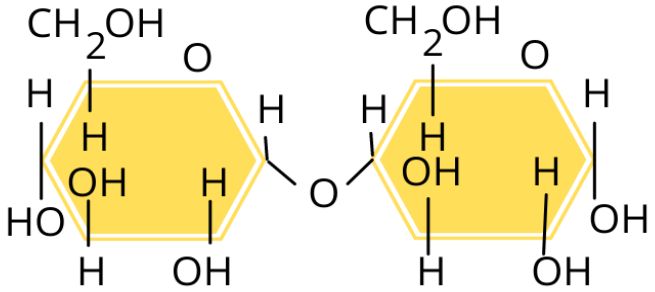
Disaccharide Examples



Sucrose
glucose + fructose

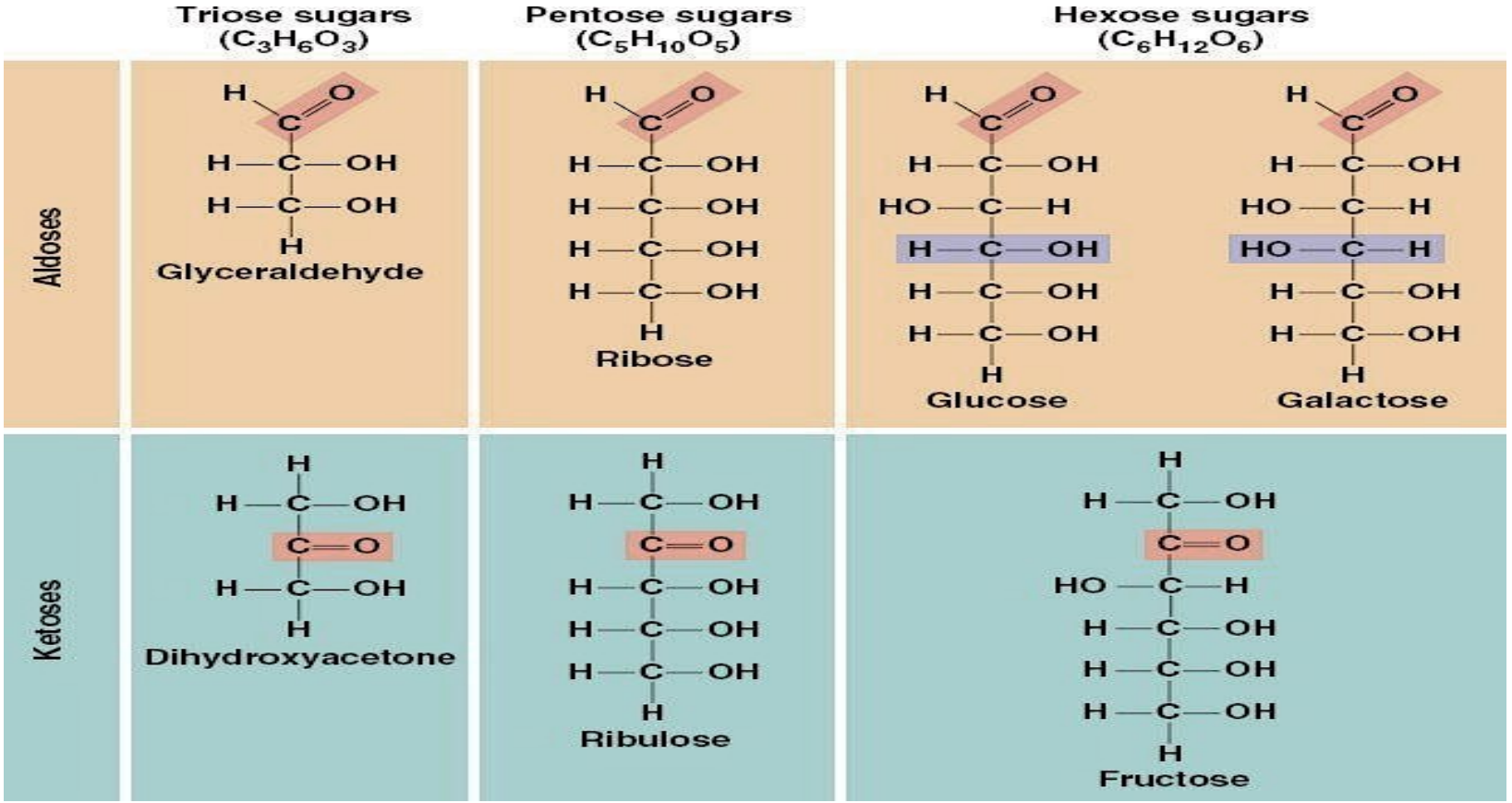


Lactose
galactose + glucose



Maltose
glucose + glucose

Aldose vs Ketose



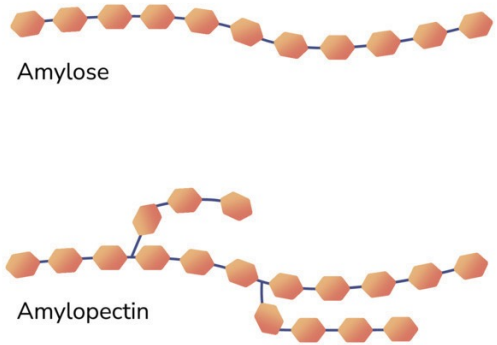
Aldose has a carbonyl group at the end of the carbon chain.
Ketose has a carbonyl group in the middle of the carbon chain.

Classification of Important Sugars

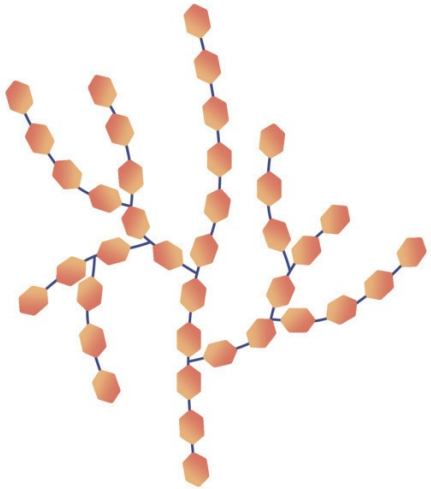
	Aldoses	Ketoses
Trioses ($C_3H_6O_3$)	Glycerose (glyceraldehyde)	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose, galactose, mannose	Fructose
Heptoses ($C_7H_{14}O_7$)	—	Sedoheptulose

Most Common Polysaccharides

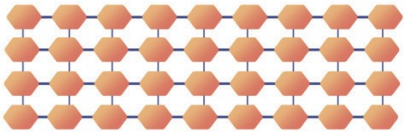
Starch



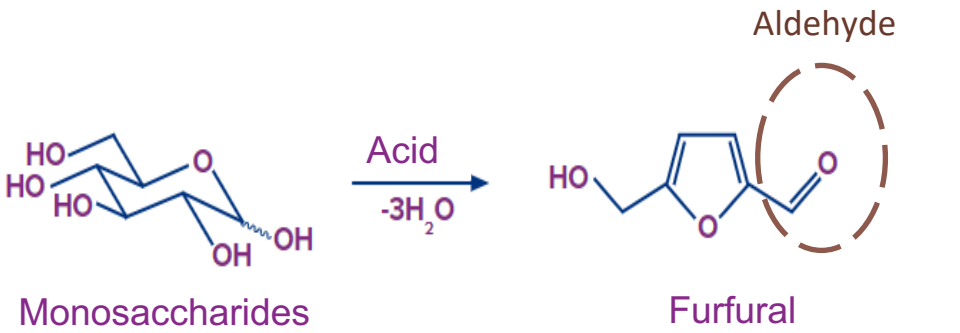
Glycogen

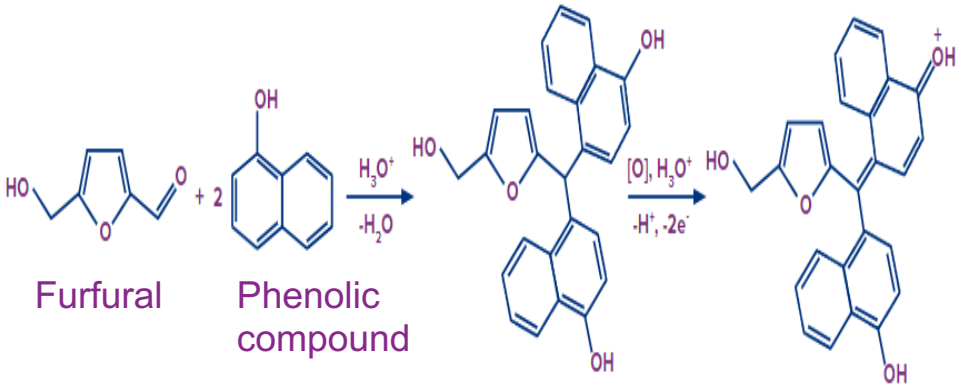


Cellulose



Dehydration of Monosaccharides into Furfural





Today’s experiments

1. Seliwanoff's Resorcinol Test.
2. Bial's Orcinol Test.
3. Iodine Test.

01 Seliwanoff's Resorcinol Test

- **Aim**
  - This test is used to distinguish between ketoses and aldoses monosaccharides.

- This test gives **fast**, clear **positive** results with ketohexoses. Thus, it's a specific test for ketohexoses.

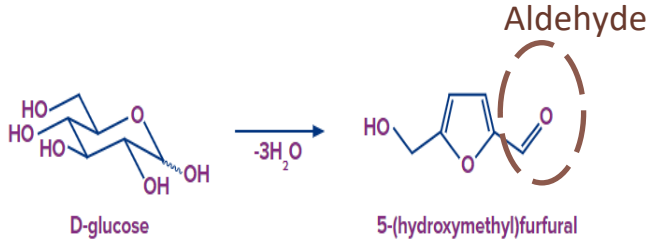
- **Why ketohexoses?**
  - **The Ketohexoses** are about 20 to 25 times faster than aldohexoses in producing furfural derivatives. And form considerably more furfural derivatives.

- If you end the reaction within 1 minute, you get a positive result with the fastest sugar (ketohexoses).

- **Note:**
  - Free or bounded ketohexoses can respond to the test. e.g. sucrose that contains fructose.

01 Seliwanoff's Resorcinol Test Principle

- **Seliwanoff's Resorcinol Reagent**
  - HCL
  - Distilled Water (D.W)
  - Phenolic compound (Resorcinol)
- **Principle**



Carbohydrate undergoes dehydration upon the introduction of concentrated Hydrochloric acid, resulting in the formation of **furfural** (aldehyde).

Ketohexoses Hexoses + HCL → Hydroxymethyl furfural + 3 H2O

Hydroxymethyl furfural + Resorcinol → Red product + 3 H2O

This aldehyde undergoes condensation along with Resorcinol.

= Heat

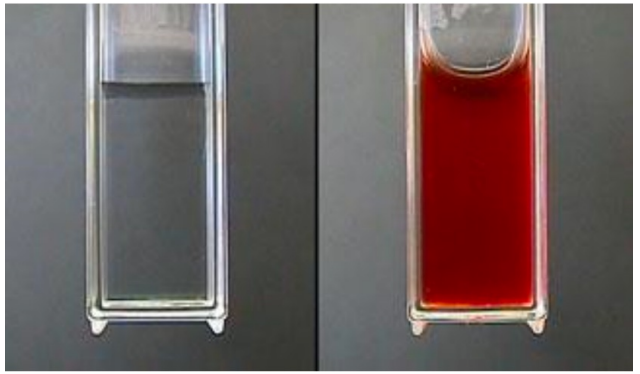
01 Seliwanoff's Resorcinol Test Procedure

Note: swirl the samples and reagent bottles before use to aspirate homogonous solution.

Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
1	Sample	1% Fructose	1% Glucose	1% Sucrose	1% Sorbose	1% Xylose	D.W
	Volume	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops
2	Resorcinol Reagent	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
3	Mix and Incubate in a boiling water bath for 1 min. Observe the color >>> record your result as (result after 1 min)						
4	Continue incubation for 4 min. Observe the change in color. >>> record your result as (result after 4 min)						
	Result 1 min						
	Result 4 min						

01 Seliwanoff's Resorcinol Test Result

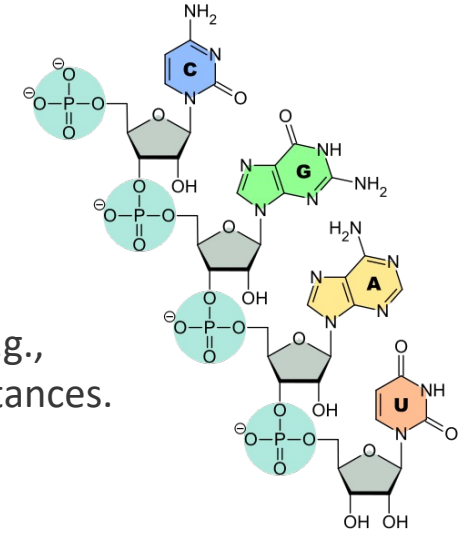
- **Positive Result**
  - Ketohexoses → red complex
- **Interference**
  1. Aldohexoses (glucose) → light yellow to faintly pink color
  2. Pentose → blue to green color
- **How to solve this interference?**
  - Make the concentration of **HCL** less than 12%.
  - Make the **incubation** period shorter than 1 minute.
  - If **aldohexose**, e.g., glucose, is present, it must not be in a concentration greater than 2%.

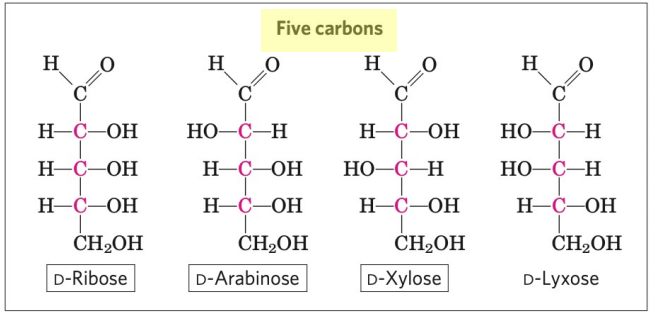


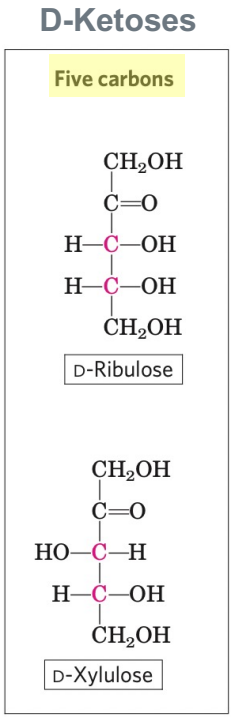
02 Bial's Orcinol Test

- **Aim**
  - It is a simple, rapid qualitative test for pentoses.
- **Usage**:
  - It can be used For quantitative assay of **pentoses** (e.g., **Ribonucleic acid**) in the absence of interfering substances.
- **Bial's Reagent contains:**
  - HCL
  - Phenolic compound (Orcinol)
  - Ferric chloride

  (used to increase the sensitivity of the test).







02 Bial's Orcinol Test Principle

- **Principle**

Pentose + HCL → furfural + 3 H2O

Carbohydrate undergoes dehydration upon the introduction of concentrated Hydrochloric acid, resulting in the formation of **furfural.**

furfural + orcinol → Blue-green color product + 3 H2O

Furfural undergoes condensation along with orcinol.

= Heat

02 Bial's Orcinol Test Procedure

Note: swirl the samples and reagent bottles before use to aspirate homogonous solution.

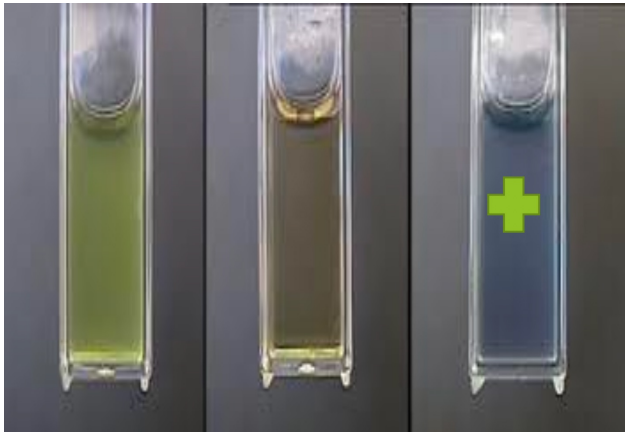
Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
1	Sample	1% Xylose	1% Glucose	1% Fructose	1% Lactose	1% Starch	D.W
	Volume	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop
2	Bial’s Reagent	3 ml	3 ml	3 ml	3 ml	3 ml	3 ml
3	Incubate in a boiling water bath for 3-5 mins. Observe the change in color formed.						

02 Bial's Orcinol Test Result

- **Result**
  - **Blue-green color product**

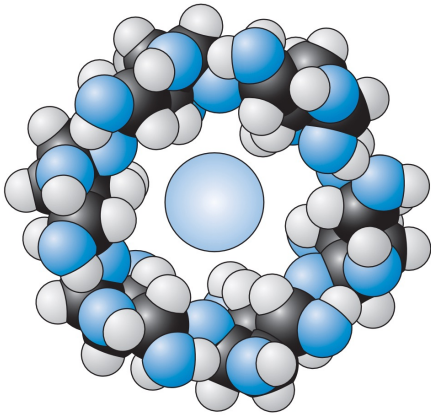
- **Interference in this test:**

  - Hexoses generally react to form green, red, or brown products.
  - However, all of these hexoses colors are considered negative results.

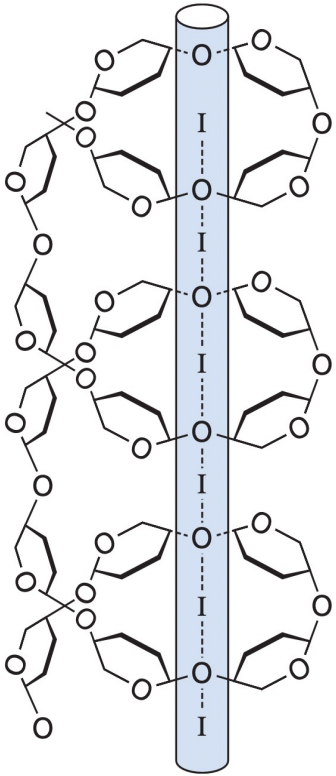


03 Iodine Test for Polysaccharide

- **Aim**
  - The iodine test is used for the detection of polysaccharides.
- **Reagent**
  - Iodine
- **Principle**
  - Iodine forms colored adsorption complexes with polysaccharides.
  - Color is due to the coordination complex between the helically coiled polysaccharide chains and the iodine centrally located within the helix.



(*b*)

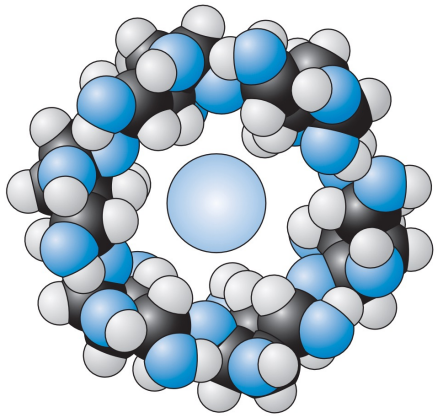


(*a*)

03 The Starch-Iodine Complex

- In the presence of **starch**, the iodine will fit into the center of the coild polysaccharides chain and form a chain of 6 iodine molecules (I_6) inside the helix, and the color turns into an intense blue complex.

- A similar complex is formed with other polysaccharides but will show a different color.



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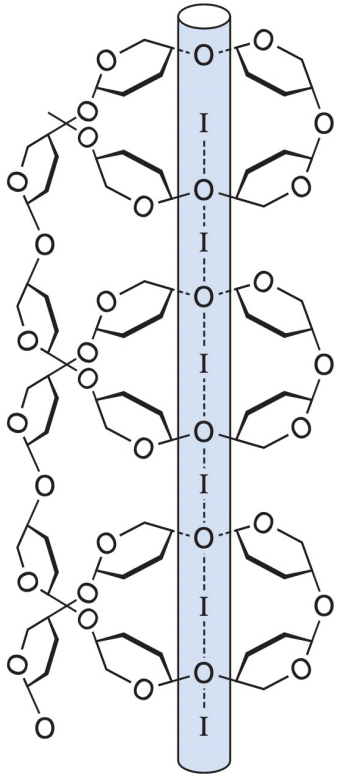


(a)

(a) Schematic structure of the starch-iodine complex. The amylose chain forms a helix around I_6 units.
(b) View down the starch helix, showing iodine inside the helix.

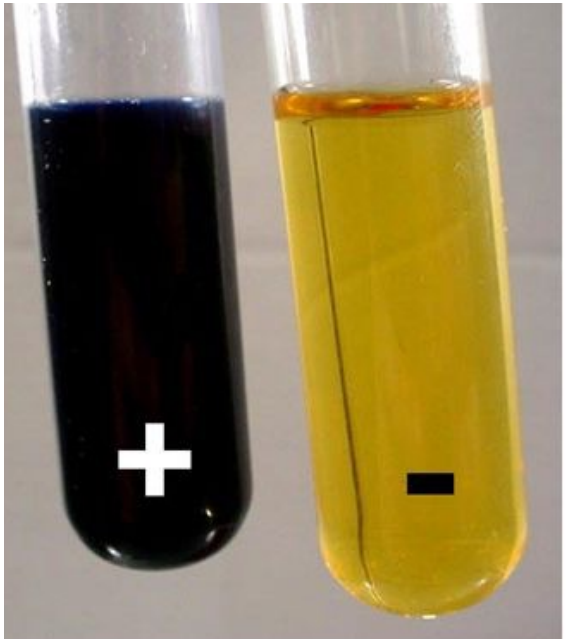
03 Iodine Test Procedure

Note: swirl the samples and reagent bottles before use to aspirate homogonous solution.

Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5
1	Sample	1% Starch	1% Glycogen	1% Dextrin	1% Cellulose	D.W
2	Volume	2 ml	2 ml	2 ml	2 ml	2 ml
3	Iodine Reagent	2 drops	2 drops	2 drops	2 drops	2 drops
4	Observe the color and record the result.					

03 Iodine Test Result

- Starch + Iodine → **Blue to black color**
- Dextrin + Iodine → **Red to violet color**
- Glycogen + Iodine → **Red to brown color**



Summary of Color Tests for Specific Carbohydrates

Test	Detect	Reagent	Principle	Positive Result	Negative Result	Interference	Note
Seliwanoff's Resorcinol Test	Specifically for the detection of Ketohexoses	**Seliwanoff's Resorcinol Reagent:** HCL, Distilled Water (D.W), Phenolic compound (Resorcinol).	**Hexoses + HCL → Hydroxymethyl furfural + 3 H2O** **Hydroxymethyl furfural + Resorcinol → Red product + 3 H2O**	**Red complex**	Others	**1-** Aldohexoses (glucose) → light yellow to faintly pink color 2-Pentose → blue to green color.	This test is used to distinguish between ketoses and aldoses monosaccharides. This test gives **fast**, clear **positive** results with ketohexoses.
Bial's Orcinol Test	**Pentoses**	**Bial's Reagent contains:** HCL, Phenolic compound (Orcinol), Ferric chloride (used to increase the sensitivity of the test).	**Pentose + HCL → furfural + 3 H2O** **furfural + orcinol → Blue-green color product + 3 H2O**	**Blue-green color product**	Others	Hexoses generally react to form green, red, or brown products. However, all are considered negative.	It can be used For quantitative assay of pentoses (e.g., **Ribonucleic acid**) in the absence of interfering substances

Summary of Color Tests for Specific Carbohydrates

Test	Detect	Reagent	Principle	Positive Result	Negative Result
Iodine Test	**Polysaccharide**	Iodine	●Iodine forms colored adsorption complexes with polysaccharides. ●Color is due to the coordination complex between the helically coiled polysaccharide chains and the iodine centrally located within the helix.	●Starch → **Blue to black color** ●Dextrin → **Red to violet color** ●Glycogen → **Red to brown color**	Others

Guideline for writing the lab report

Total: 5 marks

All the following information should be included in your report:

a) Course # (CLS 281)
b) Experiment title
c) Date of the experiment
d) Student's names and university ID#
e) Section #

The lab report is broken down into 6 sections:

1. Experiment **title**
2. The **aim** of the experiment (objective, or what the test detects specifically) (1 mark)
3. **Principle** (chemical reaction) (1 mark)
4. **Methodology** (written in **steps**, not in tables)
5. **Result** (1 mark)
6. **Interpretation or Comme**nt (2 mark)

Deadline: Next lab Submission: via email