Carbohydrates tests

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Introduction

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

Common examples

Glyceraldehyde, Glucose
Fructose, Galactose,
Ribose sugar

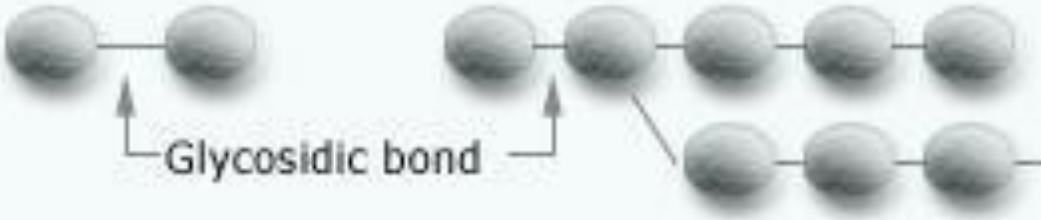
Polysaccharides
(Complex sugars)

Physical Properties
* High molecular weight
* Insoluble in water
* Tasteless

Composition

Multiple sugars

Diagrammatic representation



General formula

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

Common examples

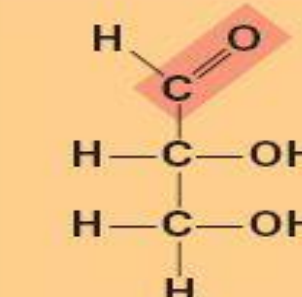
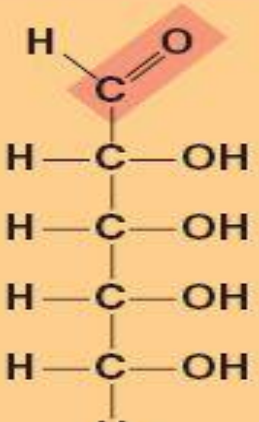
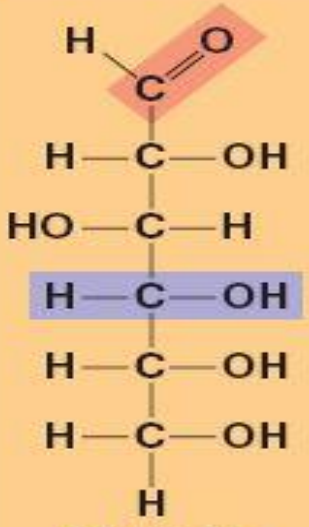
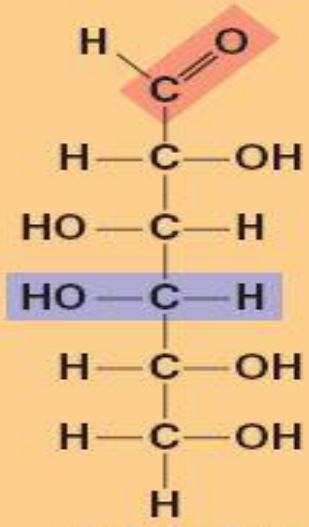
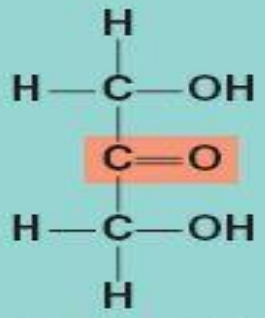
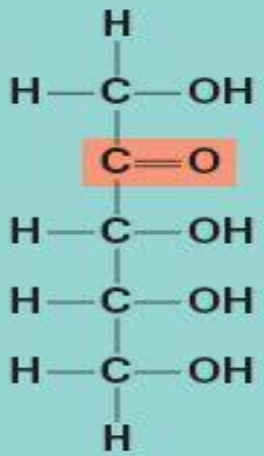
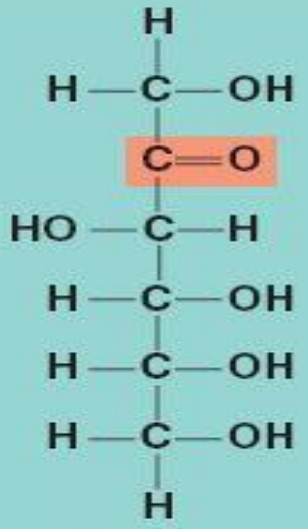
Maltose,Sucrose
Lactose

General formula

$C_x(H_2O)_n$

Common examples

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	

Today experiments:

- **General Color Tests for Carbohydrates.**
- **Reducing Properties tests.**

General Color Tests for Carbohydrates

1/Molisch Test:

2/ Anthrone Test:

aim

General test to detect charbohydrates.

1/Molisch Test

Principle:

The monosaccharid + H_2SO_4 **heat** → **furfrual (or furfural derivatives)** $-3H_2O$

Principle:

furfural(or it's derivatives)+ α-NaphtholA (2phenolgroups) ⟶ purple ring at the interface.

N.B:

If the carbohydrate is an oligosaccharide (e.g. disaccharide, trisaccharide …etc) or a polysaccharide, the **hydrolysis** of the carbohydrate acetal linkage occurs simultaneously with the dehydration reaction (in polysaccharide the color develops slower).

N.B:

A negative result by this reaction is a very good evidence of the absence of carbohydrates, but a positive test is indication to the probable presence of carbohydrate

Just for your information

- Thymol may be used as a reagent instead of α-Naphthol.
- Thymol is more stable than α-Naphthol, and can be applied to insoluble carbohydrates like cellulose or wood.
- resorcinol also is used instead of α-Naphthol.

Procedure

	Tube 1	Tube 2	Tube 3
0.5% starch	10 drops		
0.5% sucrose		10 drops	
0.5% glucose			10 drops
Water	2 ml	2 ml	2 ml
α-Naphthol	2 drops	2 drops	2 drops
	Mix		
Con. H2SO4	3 ml	3 ml	3 ml

A purple ring at the interface is indicative of a carbohydrate.

New Procedure

	Tube 1	Tube 2	Tube 3
0.5% starch	2 ml		
0.5% sucrose		2 ml	
0.5% glucose			2 ml
α-Naphthol	2 drops	2 drops	2 drops
	Mix		
Con. H2SO4	2ml	2ml	2ml

A purple ring at the interface is indicative of a carbohydrate.

How to perform the test:

Two ml of a sample solution is placed in a test tube. Two drops of the Molisch reagent (a solution of -napthol in 95% ethanol) is added. The solution is then poured slowly into a tube containing two ml of concentrated sulfuric acid so that two layers form.

http://www.harpercollege.edu/tm-ps/chm/100/dgodambe/thedisk/carbo/molisch/molisch.htm

Anthrone Test:

Principle

The monosaccharid + H_2SO_4 **heat** → **furfrual (or furfural derivatives)**

$-3\,H_2O$

Furfural or furfural derivatives + Anthrone ⟶

green to blue green color

characteristics

1.It is very sensitive, it will give a positive reaction with filter paper (cellulose).

characteristics

2. [carbohydrate] $\propto$ color intensity

so

It can be used for quantitative determination of glycogen, inulin and sugar of blood.

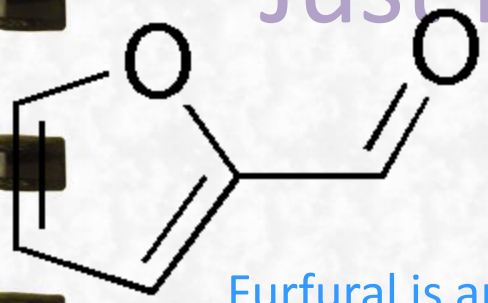
characteristics

3.It can be used as qualitative test, since different sugars dehydrate at different rates and produce a variety of colors.

characteristics

4.Furfural give green color but can be differentiated by the fact that the test is rapidly obscured by brown precipitate when the sample is diluted with 50% H_2SO_4 or glacial acetic acid.

Just for your information



Furfural is an organic compound derived from a variety of agricultural byproducts, including corncobs, oat, wheat bran, and sawdust. The name furfural comes from the Latin word furfur, meaning bran, referring to its usual source.

It is not a carbohydrate.

Procedure:

	Tube 1	Tube2	Tube 3	Tube4
Filter paper	Small piece			
0.5%starch	1 drop			
0.5%sucrose		1 drop		
0.5%glucose			1 drop	
Blank				1 drop
Water	1 ml	1 ml	1 ml	1 ml
Anthrone reagent	3 ml	3 ml	3 ml	3 ml

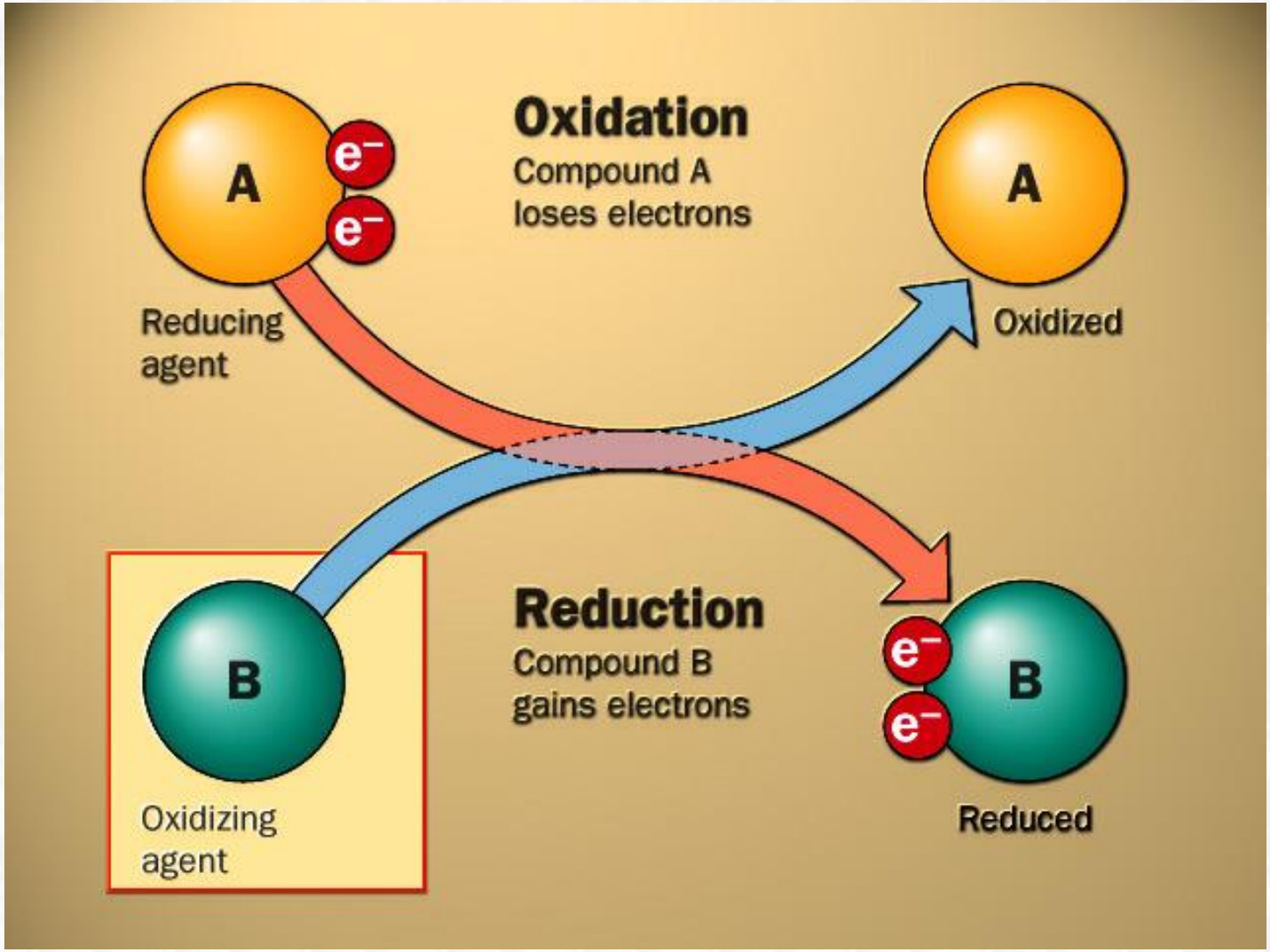
Heat for 3 mins in boiling water bath.

Green to blue green = positive.

Reducing Properties tests:

- ✓Benedict's Test
- ✓Barfoed's Test

Introduction:



reducing sugar:

is sugar that has free or potentially free aldehydic or ketonic group.

The reducing sugars include:

(1) All of the simple sugars (monosaccharides).

(2) A few of the disaccharides (including lactose and maltose, but not sucrose because it has no free aldehyde or ketone group).

(3) None of the polysaccharides.

N.B. hydrolysis of polysaccharides and sucrose makes them reducing agents

Monosaccharides'S properties:

- Monosaccharides are **stronger reducing agents** towards Cu++ than the disaccharide.
- Monosaccharides act as a reducing agent in weakly acid solution.

Background

- If a suspension of copper hydroxide in alkaline solution is heated, then

black cupric oxide is formed:

$$Cu(OH)2 \longrightarrow CuO + H2O$$

- However, if a reducing substance is present, then rust-brown cuprous oxide is precipitated:

$$2Cu(OH)2 \longrightarrow Cu2O + H2O + O2$$

Benedict's Test:

Principle:

Cu^{++} + Reducing sugar $\rightarrow$ Cu2O(insoluble yellow to red sugar)

N.B. this test is done under alkaline ph and heat conditions.

Note on the result:

The concentration of the suger afects the color of the test.

green orange red brown →

blue-sugar absent; green-0.5% sugar; yellow-1% sugar; orange-1.5% sugar; brick red-2 % or more sugar.

characteristics

- Benedict modified the original Fehling's test to produce a single solution which is more convenient for tests, as well as being more stable than Fehling's reagent.

- Benedict's test is a rapid and general test for reducing sugars.

Benedict's reagent components:

COPONENT	FUNCTION
copper sulphate	Acts as a source of Cu^{++}
sodium citrate	❑Citrate in Benedict's reagent act as a **complexing agent** to form deep, blue, stable, soluble complex ions with Cu++, ❑this is done to **prevent the precipitation** of copper sulphate .
sodium carbonate	Causes the alkalinity of the reagent

Procedure:

A)

1%Starch	1%Lactose	1%Sucrose	1%Fructose	1%Xylose	1%Glucose	Blank
1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Benedict's reagent						
5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml

- Incubate in boiling water bath for 5 mins.
- Observe changes in the color of solutions and formation of any precipitate.

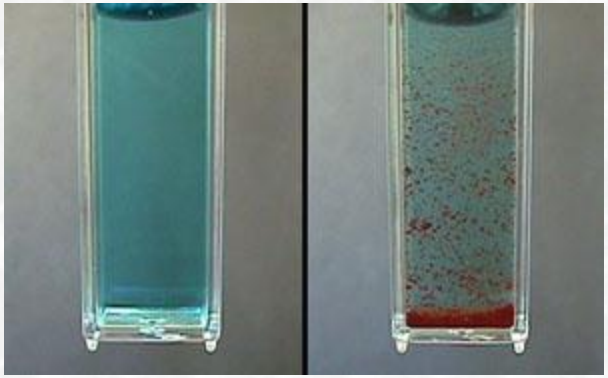
B) Hydrolysis step:

	1% Starch	1% Sucrose
	5 ml	5 ml
HCL (3M)	10 drops	10 drops
	Incubate for 5 mins. In boiling water bath	
	Take 1 ml	
Benedict's Reagent	5 ml	5 ml

- Incubate in boiling water bath for 5 mins.
- Compare the results with those obtained without acid treatment.

Barfoed's Test:

Principle:



Cu^{++} + Reducing sugar $\longrightarrow$ Cu_2O (a reddish precipitate)

N.B. this test is done under acidic ph and heat conditions

Barfoed's reagent components:

COMPONENT	FUNCTION
copper acetate.	Acts as a source of Cu^{++}
acetic acid	Provides the low ph (acidic).

characteristics

- By use of the Barfoed's reagent we can distinguish monosaccharides from disaccharides
- This happens by controlling such conditions as :
  - PH
  - time of heating
  - sugar concentration.

characteristics

- This test is positive with solutions of all monosaccharides of con. 0.1% and above.
- Disaccharide do not produce any reduction unless they are present in very high con.

characteristics

Aldose and ketose sugar reduce this reagent, but hexoses act more rapidly and more vigorously than reducing disaccharide.

Procedure:

A)

1%Starch	1%Maltose	1%Sucrose	1%Fructose	1%Xylose	1%Glucose	Water
5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Barfoed's Reagent						
5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml

Mix, incubate all the tubes in boiling water bath for 3.5 mins (or more).

Note any change in color or clarity of the solution.

Red Precipitate = positive

b)Hydrolysis step

	1% Starch	1% Sucrose
	2 ml	2 ml
Barfoed's Reagent	5 ml	5 ml

- Incubate in boiling water bath for 3.5 mins (or more)
- Note any change in color formed or clarity of solution

Red Precipitate = positive


Thank You