CLS 281

Basic Biochemistry and Biomolecules

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King Saud University
1957

Experiment 5

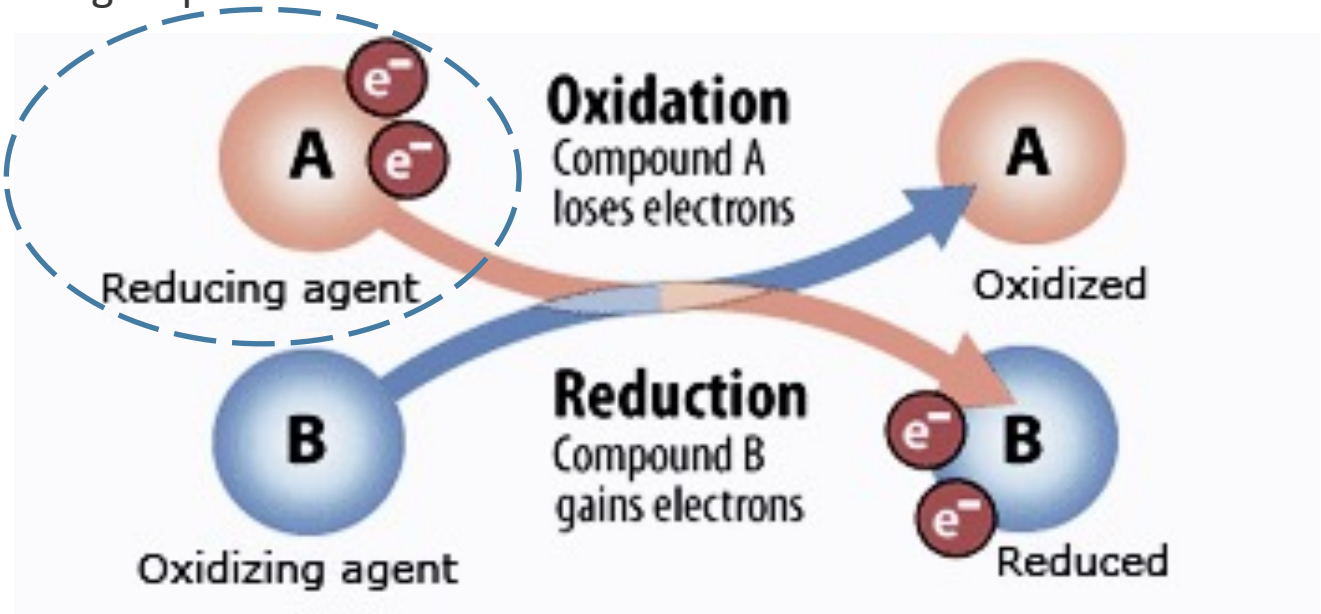
Reducing Properties Tests of Carbohydrates

Reducing Properties tests

1. Benedict's Test
2. Barfoed's Test

Reduction of Carbohydrate

- A reducing sugar is a sugar that has a free or potentially free aldehydic or ketonic group.

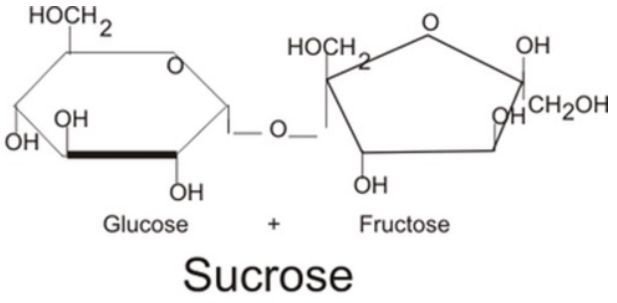


- Oxidation is the loss of electrons
- Reduction is the gain of electrons

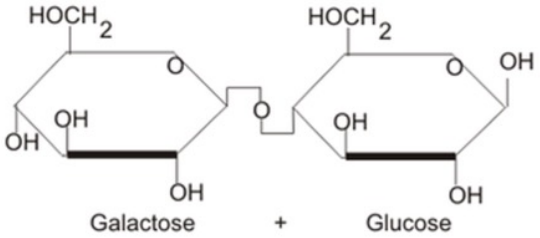
Reduction of Sugars

- The reducing sugars include:
  1. All of the simple sugars (monosaccharides)
  2. 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

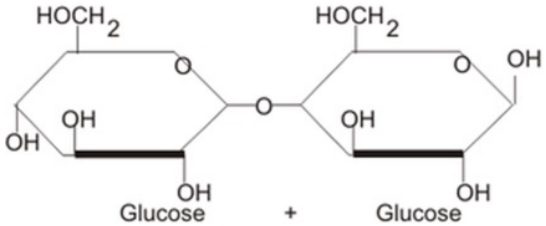
- The hydrolysis of polysaccharides and sucrose makes them reducing agents.



Sucrose



Lactose



Maltose

Background

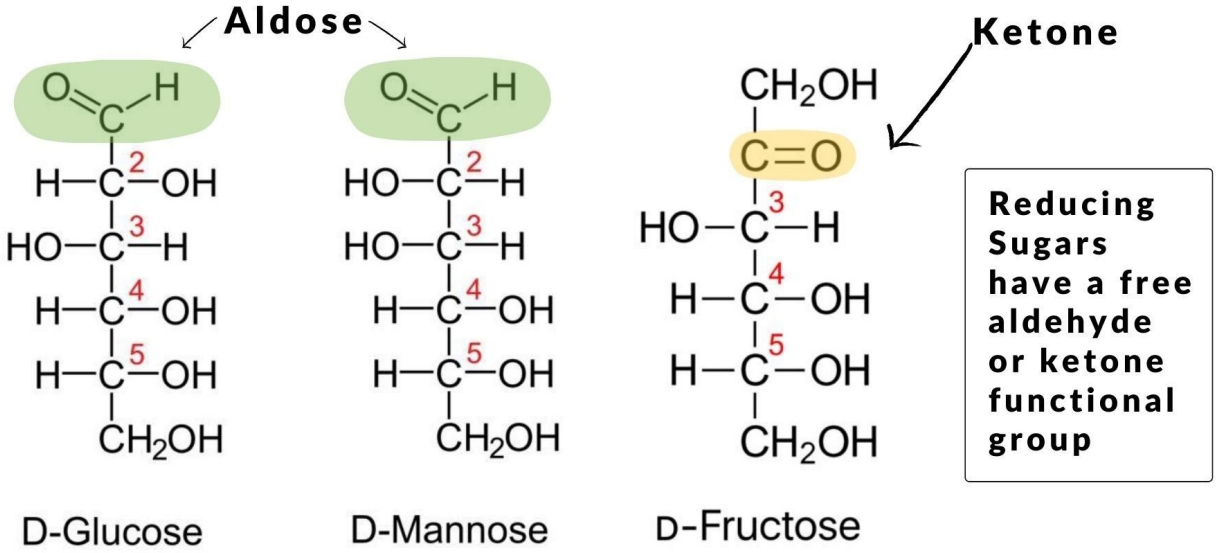
- When blue cupric hydroxide suspension in an **alkaline solution** is heated, it's oxidized into insoluble **black cupric oxide.**
  - $Cu(OH)_2 \rightarrow CuO + H_2O$

- However, if a reducing agent like certain sugars is present, the cupric hydroxide is reduced to insoluble yellow or red (brick) or rusty-brown cuprous oxide precipitate.
  - $2Cu(OH)_2 \rightarrow Cu_2O + H_2O + O_2$

01 Benedict's Test

- Carbohydrates with a free or potentially free aldehyde or ketone group have reducing properties in alkaline solutions.
- In addition, monosaccharides act as a reducing agent in weakly acid solutions.
- **Aim**
  - Detect the presence of reducing sugars (monosaccharides and some disaccharides), which have free ketone or aldehyde functional groups.
- 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.

Monosaccharides' properties



- Monosaccharides are stronger reducing agents towards Cu++ than the disaccharide.

- Monosaccharides act as a reducing agent in weakly acid solutions.

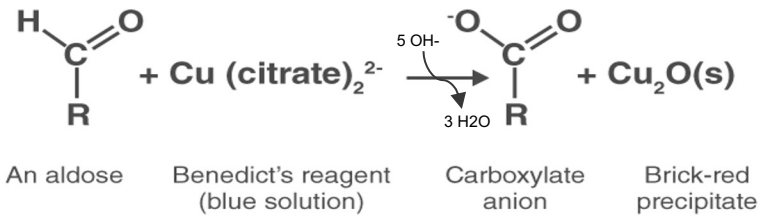
01 Benedict's Test Principle

Benedict's Reagent

- Composed of copper sulphate and sodium citrate, and sodium carbonate.

Principle

- Cu++ + Reducing sugar → Cu2O (insoluble yellow to red sugar)
  - N.B. this test is done under alkaline pH and heat conditions.



- Sucrose does not reduce Benedict's solution because it has no free aldehyde or ketone group.

Reagent Component	Function
Copper Sulphate	**Acts as a source of Cu++**
Sodium Citrate	• **Citrate in Bendict's reagent acts as a complexing agent to form deep blue <u>stable soluble complex ions with Cu++.</u>** • **This is done to prevent the precipitation of copper sulphate during storage.**
Sodium Carbonate	**Provides the alkalinity environment which is required for the redox reaction.**

01 Benedict's Test Procedure

Part 1:

Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
1	Sample	1 ml of 1%Starch	1 ml of 1%Lactose	1 ml of 1%Sucrose	1 ml of 1%Fructose	1 ml of 1%Xylose	1 ml of 1%Glucose	1 ml of Blank
2	Benedict's reagent	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml

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

- For sucrose and starch, do acid hydrolysis, then run again with Benedict's reagent.

01 Benedict's Test Procedure

Part 2: Hydrolysis step

Steps	Tube No.	Tube 1	Tube 2
1	Sample	5 ml of 1%Starch	5 ml of 1%Sucrose
2	HCL (3M)	10 drops	10 drops
3		Incubate for 5 mins. In boiling water bath	
4		Take 1 ml	
5	Benedict's Reagent	5 ml	5 ml

6. Incubate in a boiling water bath for 5 mins.

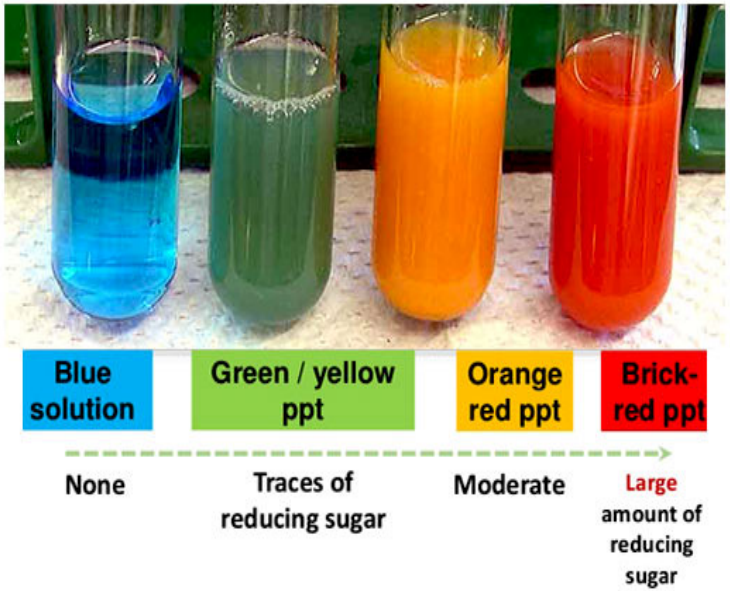
7. Compare the results with those obtained without acid hydrolysis treatment.

01 Benedict's Test Result

- **Positive result >>>** Formation of a green, orange, red-brown precipitate within three minutes. Reducing sugars present. Example: Glucose.
- **Interpreting the Results of Benedict's Test:**
  - **The color of the result changes with the concentration of the sugar in the sample.**

Color of the Precipitate	% of Reducing Sugar
Green	0.5%
Yellow	1%
Orange	1.5%
Red	2% or more

- **Negative result >>>** No color change (Blue). Reducing sugars absent. Example: Sucrose.



02 *Barfoed's Test*

- Aim
  - To detect reducing carbohydrates.
  - To distinguish reducing monosaccharides from disaccharides.

- Monosaccharides are stronger reducing agents towards Cu++ than the disaccharide.

- This test is positive with solutions of all monosaccharides of con. 0.1% and above.

- Sucrose is very easily hydrolyzed by the dilute acid reagent, and the liberated fructose is exceedingly reactive in this test.

- Disaccharides do not produce any reduction unless they are present in very high con.

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

02 Barfoed's Test Principle

- **Barfoed's Reagent**
  - Composed of copper acetate in acetic acid.

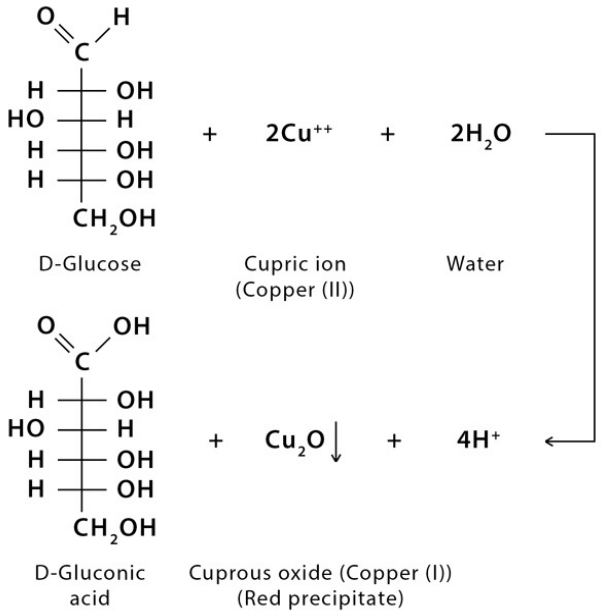
- By use of Barfoed's reagent, we can distinguish monosaccharides from disaccharides by controlling such conditions as pH and time of heating.

- **Principle**

  Reducing sugar + Cu++ → Cu2O

  (a reddish precipitate)
  - This test is done under acidic pH and heat conditions
- Under the acidic conditions of the Barfoed's test, the cuprous ion precipitates to the red cuprous oxide, which settles to the bottom of the tube. (The solution still remains dark blue).

Reagent Component	Function
Copper acetate	**Acts as a source of Cu++**
Acetic acid	**Provides the low (acidic) pH.**



02 *Barfoed's Test Procedure*

Part 1:

Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
1	Sample	5 ml of 1%Starch	5 ml of 1%Maltose	5 ml of 1%Sucrose	5 ml of 1%Fructose	5 ml of 1%Xylose	5 ml of 1%Glucose	5 ml of Water
2	Barfoed's Reagent	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml

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

- Note any change in color or clarity of the solution.

02 Barfoed's Test Procedure

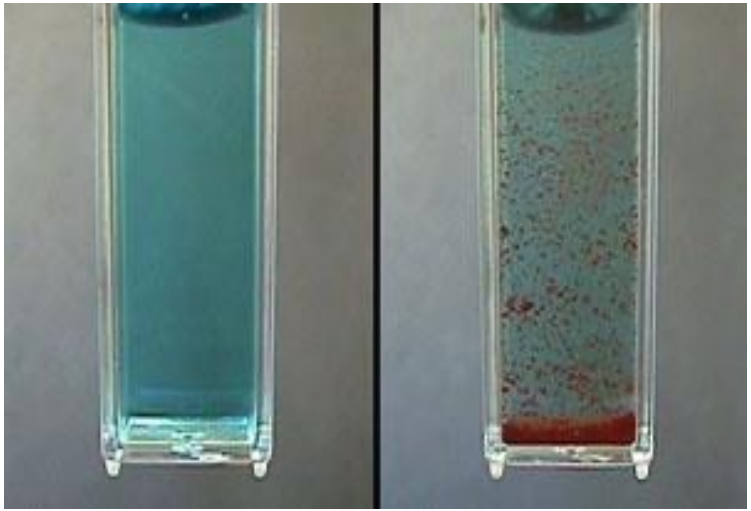
Part 2: Hydrolysis step

Steps	Tube No.	Tube 1	Tube 2
1	Sample	2 ml of 1%Starch	2 ml of 1%Sucrose
2	Barfoed's Reagent	5 ml	5 ml

3. Incubate in a boiling water bath for 3.5 mins or more.

4. Note any change in color formed or clarity of a solution.

02 Barfoed's Test Result



Blue Solution	Red Precipitation
Carbohydrates absent	Within few minutes - monosaccharides After 3 minutes- disaccharides

Summary of General Color Tests for Carbohydrates

Test	Detect	Reagent	Principle	Positive Result	Negative Result	Note
Molisch Test	Detect the presence of **carbohydrates** in a given sample.	- concentrated sulphuric acid H_2SO_4. - Phenol-type molecules **(α-Naphthol).**	1- Hydrolysis of sugar to monosaccharides. 2-Monosaccharides + H_2SO_4 → furfrual (of furfural derivatives) + 3 H_2O (dehydration reaction) 3- furfrual (of furfural derivatives) + α-Naphthol (2 phenol groups) → **purple ring** at the interface. (condensation reaction)	**purple ring color**	Colorless	Monosaccharides give a **rapid** positive test. Disaccharides and polysaccharides react **slower**.
Anthrone Test	Detect the presence of **carbohydrates** in a given sample.	- concentrated sulphuric acid H_2SO_4. - Phenol-type molecules **(anthrone)**	1- Hydrolysis of sugar to monosaccharides. 2-Monosaccharides + H_2SO_4 → furfrual (of furfural derivatives) + 3 H_2O (dehydration reaction) 3-The reaction of furfural with Anthrone reagent resulted in **a Blue-green complex.**	**Blue-green complex.**	Reagent color	It is very sensitive. It will give a positive reaction with filter paper (cellulose).

Reduced sugar

- All monosaccharides.
- Two of the disaccharides (lactose and maltose)

Non-reduced sugar

- Sucrose (Disaccharide)
- Polysaccharides

- The hydrolysis of **polysaccharides** and **sucrose** makes them reducing agents.

Summary of tests that detect reduced sugars

Test	Detect	Reagent	Principle	Positive Result	Negative Result	Note
Benedict's Test	Detect the presence of reducing sugars (monosaccharides and some disaccharides), which have free ketone or aldehyde functional groups.	Copper sulphate + sodium citrate + sodium carbonate. (Alkaline Medium)	Cu++ + Reducing sugar → Cu2O (insoluble yellow to red sugar) N.B. this test is done under alkaline pH and heat conditions.	Green- yellow – orange- red- brown (depending on the concentration of carbohydrate in the sample)	Blue Cu++ reagent color	Benedict's test is a rapid test for reducing sugars.
Barfoed's Test	- To detect reducing carbohydrates . - To distinguish reducing monosaccharides from disaccharides.	Copper acetate + acetic acid. (week acidic Medium)	Cu++ + Reducing sugar → Cu2O (a reddish precipitate) This test is done under acidic pH and heat conditions.	Reddish precipitate	Clear blue Cu++ reagent color	By use of Barfoed's reagent, we can distinguish monosaccharides from disaccharides by controlling the time of heating.

Report Criteria

Total: 5 marks

1- Course # (CLS 281)
2- Experiment title
2- Date of the experiment
4- Student's names and university ID#
5- Section #
6- Experiment title
5- The aim of the experiment (objective, or what the test detects specifically) (1 mark)
6- Principle (chemical reaction) (1 mark)
7- Methodology (written)
8- Result (1 mark)
9- Interpretation or Comment (2 mark) – the result has to be interpreted based on each sample.

Deadline: Next lab
Submission: Handout next lab