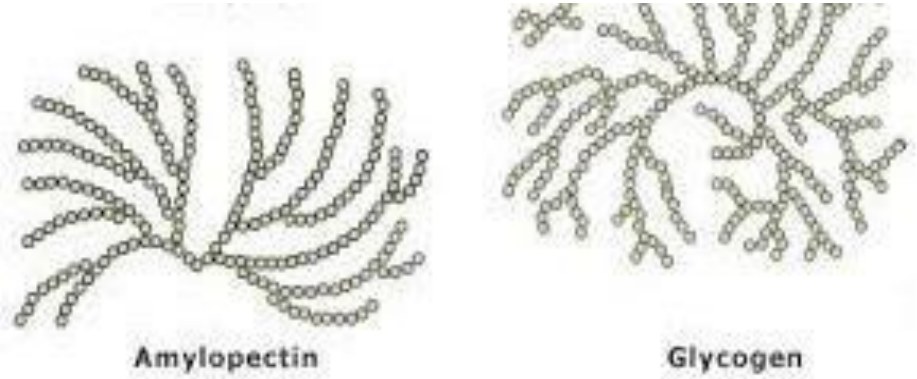
Qualitative analysis of carbohydrates II



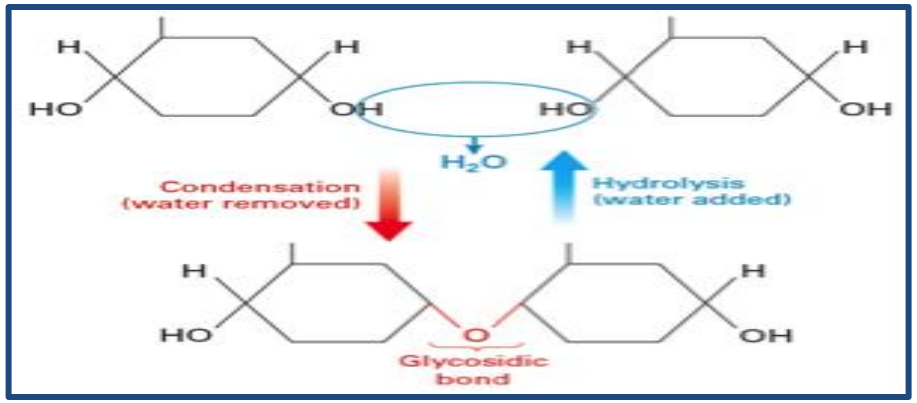
Complex sugars consist of more than one unit of monosaccharide, it could be:

-**Disaccharides** contain two monosaccharide units.

- **Oligosaccharides** contain 3-9 monosaccharide units.

- **Polysaccharides** can contain more than 9 monosaccharide units.

Complex carbohydrates can be broken down into smaller sugar units through a process known as **hydrolysis**.



-Polysaccharides can either be :

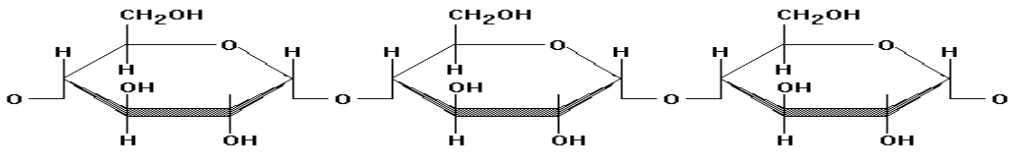
1. **homopolymeric** (same repeating monosaccharide unit).

2.**heteropolymeric** (mixture of monosaccharaides).

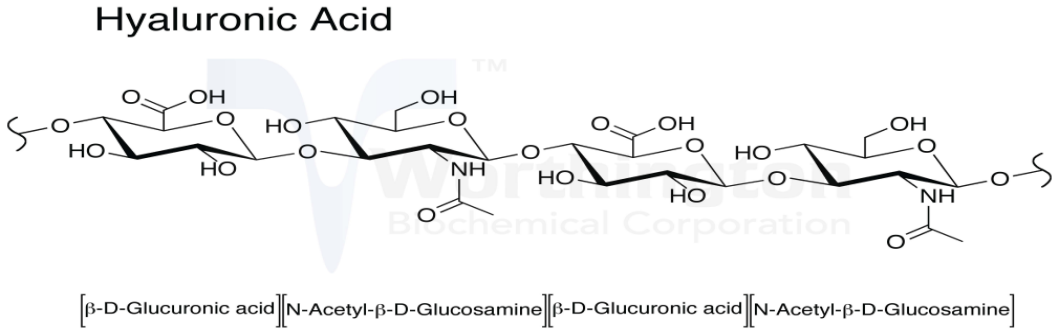
-Plants and animals store glucose in the form of very large polysaccharide glucose **homopolymers** .

-The glucose homopolymer present in plants to store glucose is called **starch**, while the glucose homopolymer present in animal cells is called **glycogen**.

starch



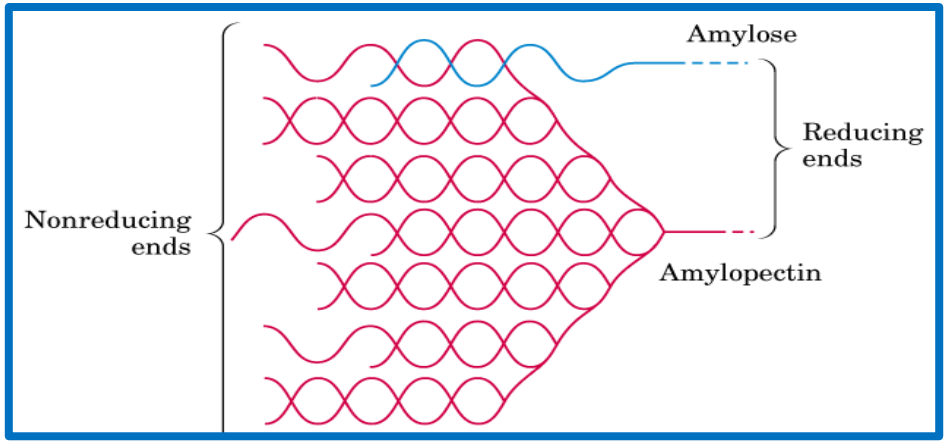
Hyaluronic Acid

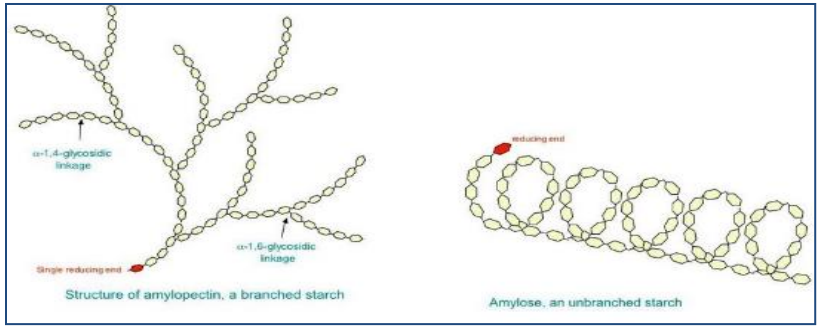


Starch consists of : amylose and amylopectin ..

-Amylose is a Linear helical polymer of glucose linked by α-1,4 glycosidic bonds (100 units).

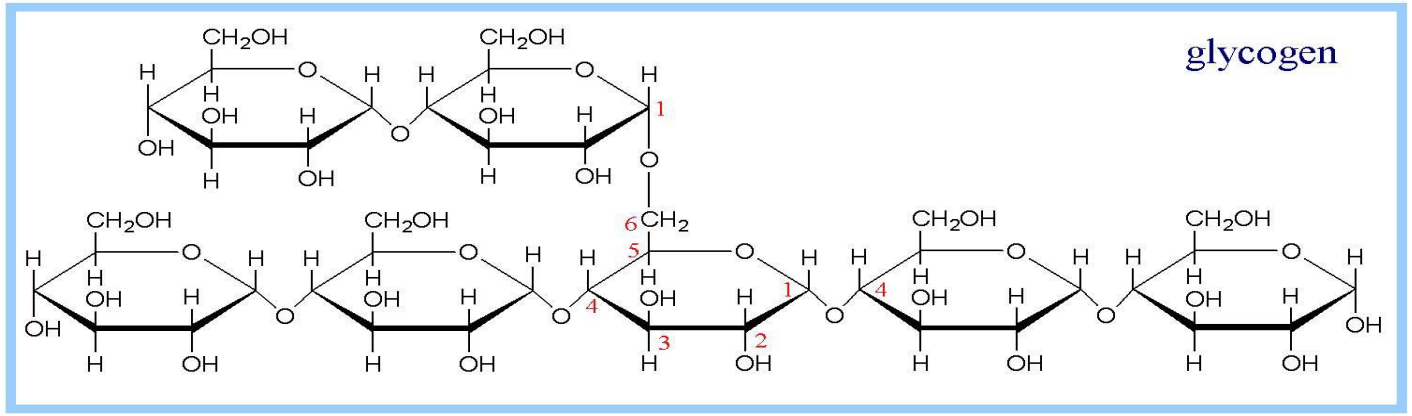
-Amylopectin Branched polymer containing glucose linked by α-1,4 glycosidic bonds. Branch points has α-1,6 glycosidic bonds, (100,000 units).





Glycogen

- Is a branched polysaccharide of D-glucose which contains both α(1 → 4) and a(1 → 6) is similar in structure to amylopectin.
But glycogen has **more** (1 → 6) branches.



Practical Part

1-Sucrose hydrolysis Test:

Objective:

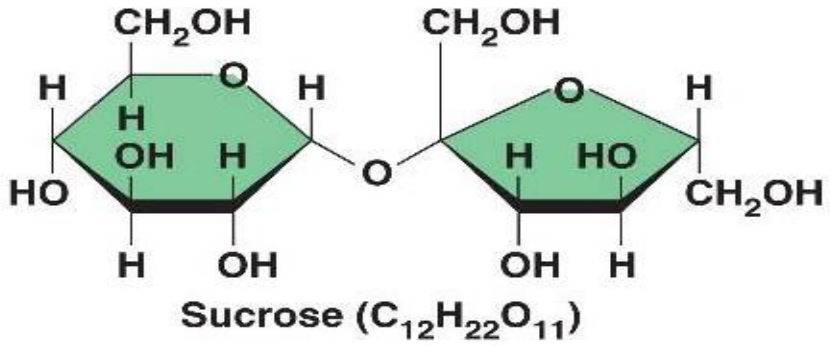
To identify the products of hydrolysis of di- and polysaccharides.

-This test is used to convert sucrose (non-reducing disaccharide) to glucose and fructose (reducing mono saccharides).

Principle:

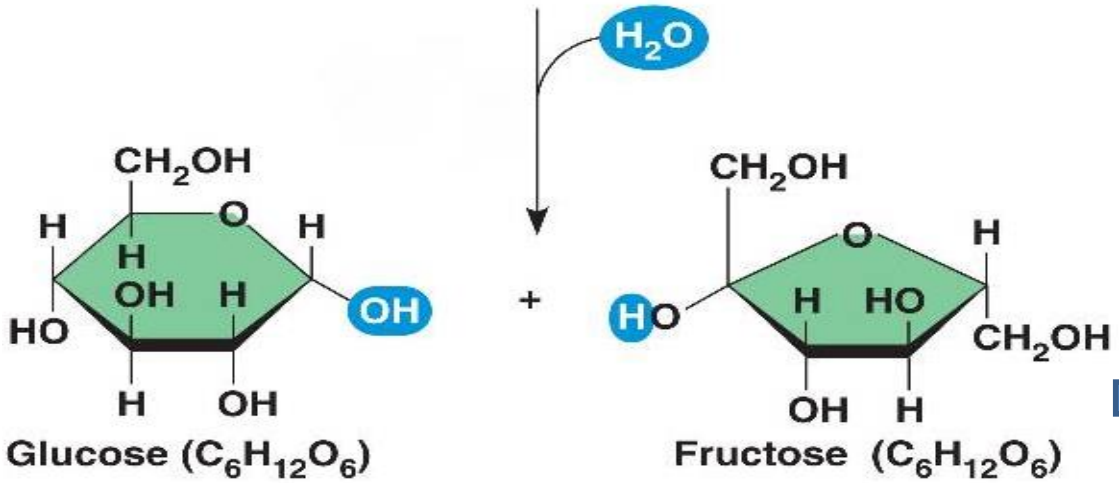
- **Sucrose is a non-reducing** disaccharide so it does not reduce the Cu++ solution (Bendict's test) because the glycosidic bond is formed between the two hemiacetal bonds**.** So there is no free aldehydic or ketonic group to give positive reducing properties.

- When glycosidic bond **hydrolysed** (in the presence of acid) sucrose will broken to glucose + fructose (which are **reducing sugars**) are then able to give positive reducing test.



Sucrose ($C_{12}H_{22}O_{11}$)

No result with bendict



Glucose ($C_6H_{12}O_6$)

Fructose ($C_6H_{12}O_6$)

Positive result with bendect

Method:

1- Set up tow tubes add to each one 4ml of a sucrose solution ,Lable the tube : (Sucrose with HCL, Sucrose without HCl)

2- To only one tube add four drops of concentrated hydrochloric acid (HCl)

3-Heat both in boiling water bath for 15 minutes.

4- After 15 minutes of heating add 4 drops of concentrated NaOH to each tube (?)

5-From the tube containing HCl take 2ml in tow tube to do Benedict's test and Seliwanoff's test , lable the tube (Benedict +HCl) and (Seliwanoff‘+HCl)

Add 2 ml of Benedict's reagent and 2.5 ml of Seliwanoff's reagent WHAT do expect?

6-From the tube which contain only sucrose take 2 ml to do Benedict's test only (add 2 ml of Benedict's reagent) WHAT do expect?

Result

Sucrose with HCL		Sucrose without HCL
Benedict's test	Seliwanoff's test	Benedict's test

Sucrose+ HCl
(+) Seliwanoff's test

Sucrose+ HCl
(+) Benedict's test

Sucrose only
(-) Benedict's test

2-The Iodine/Potassium Test:

Objective:

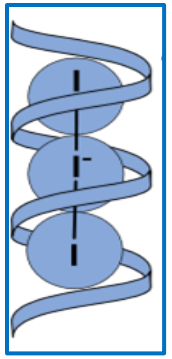
- To detect the presence of starch in a sample.

- This test used to distinguish between polysaccharides and mono or oligo saccharides.

Principle:

Starch forms deeply **blue color complex** with iodine. Starch contains α- amylose, a helical saccharide polymer and amylopectin. Iodine forms a large complex with α-amylose helix. This complex absorbs light and reflects the blue light only. Simple oligosaccharides and mono saccharides do not form this complex.

Note that other polysaccharides like glycogen may give other colors (red).



Method

-Two ml of a sample solution is placed in a test tube.

- Add 2drops of iodine solution and one ml of water. Shake it well

- A positive test is indicated by the formation of a **blue complex**.

- **Take a half of the tube of starch and heat it in boiling water bath for 10 min**

compare between the tow tubes and write your observation.

Result

Tube	Observation	Comments
(Starch + Iodine) without heating		
(Starch + Iodine) after heating		
(Glucose+ Iodine)		

Glucose + iodine

Starch + iodine
[before heating]

Starch + iodine
[after heating]

3-Hydrolysis of Starch:

Objective:

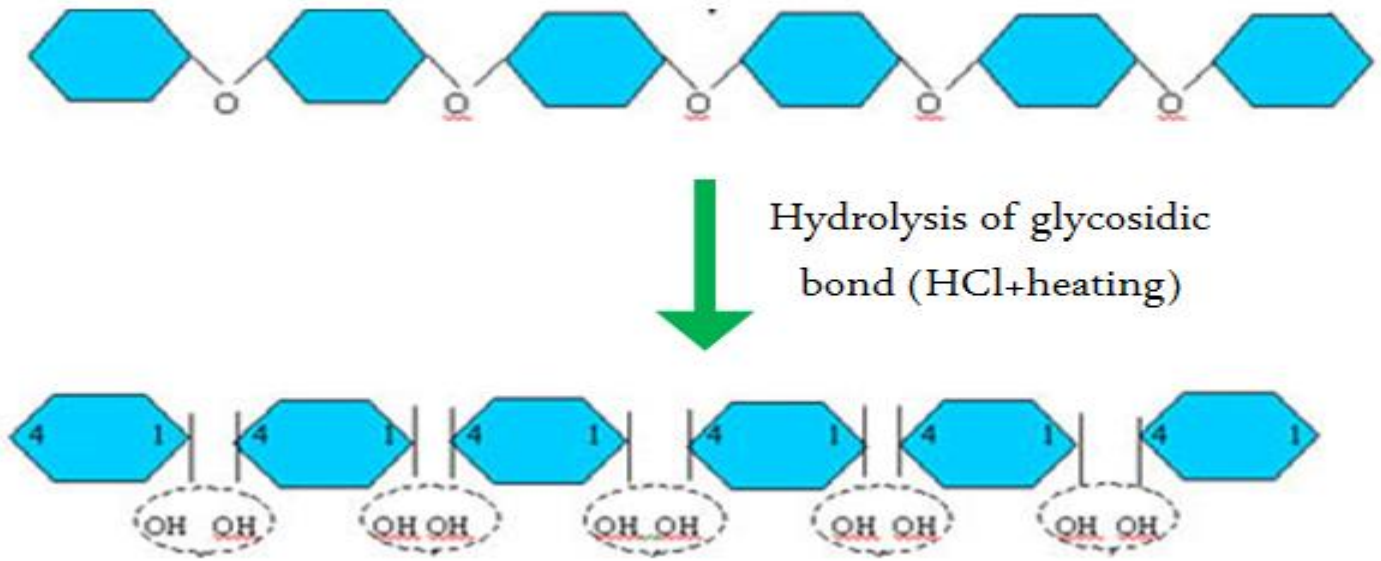
To establish the **effect of concentrated HCL** on a glycosidic bond in starch.

Principle:

Although starch has free hemiacetal in the terminal glucose residue, it **has no reducing properties**, because the percentage between the free residues is **very low** in comparison to the whole molecule.

Heating starch solution in acid medium hydrolyses the glycosidic bonds giving many free **glucose** residues. These glucose molecules give **reducing properties** to the hydrolysis product.

- This experiment illustrates the conversion of starch (non-reducing sugar) to a reducing sugar **by the action of hydrochloric acid** at **boiling point**. The longer the starch is exposed to the acid the further hydrolysis proceeds.



Method

1-Two ml of starch in large tube

2- Add 1ml of Hydrochloric acid, heated in boiling water bath for 10 mints. then cold solution

3-Add 1ml of sodium hydroxide to become base

4-Divided in two tube (a,b)

5- In tube (a) add 1 ml of iodine solution and note the result. WHAT do expect?

6- In tube (b) add 1 ml of Benedict reagent, mix and heated for 3 mint and record result. WHAT do expect?

Result:

Starch with HCL	
Benedict's test	Iodine test

Starch with HCL
[After heating]

[-] Iodine test

[+] Benedict's test

Questions:

How you can convert non-reducing sugar to reducing?

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

Explain why the blue color disappears upon heating?

Although starch has free hemiacetal bonds it is non reducing sugar, explain?