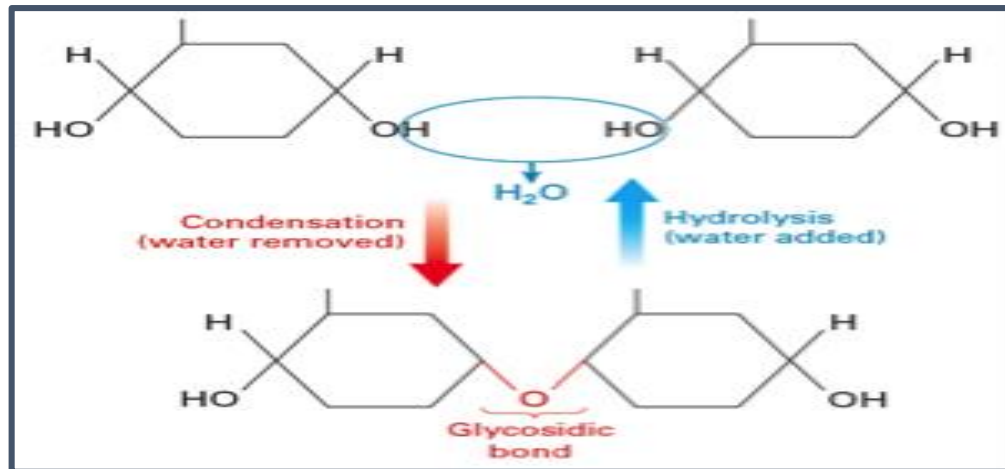


Qualitative tests of Carbohydrates-II-

BCH302 [Practical]

Complex carbohydrate:

- Complex sugars consist of more than one unit of monosaccharide, it could be:
 1. **Disaccharides:** contain **two** monosaccharide units.
 2. **Oligosaccharides:** contain **3-9** monosaccharide units.
 3. **Polysaccharides:** can contain **more than 9** monosaccharide units.
- Complex carbohydrates can be broken down into smaller sugar units through a process known as hydrolysis.

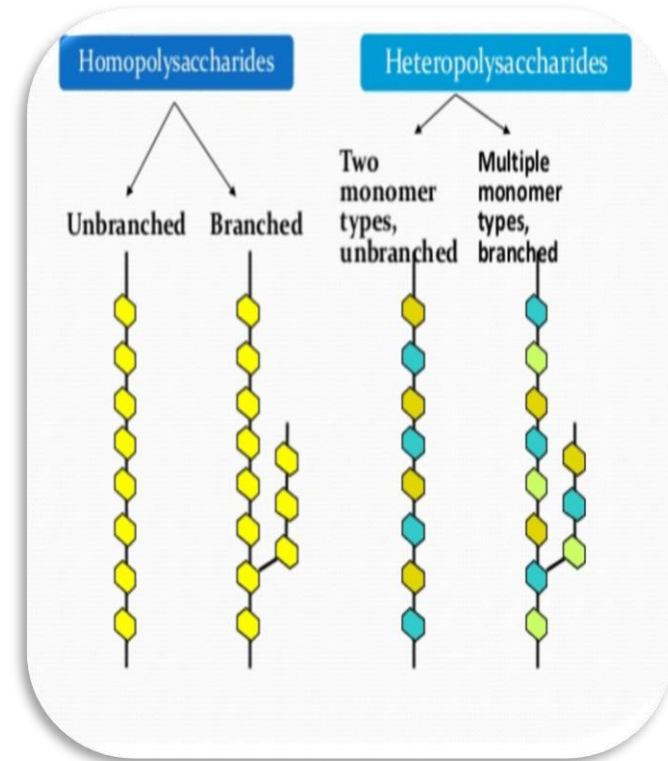


Polysaccharides :

- Polysaccharides can either be :

- homopolymeric (same repeating monosaccharide unit).
- heteropolymeric (mixture of monosaccharaides).

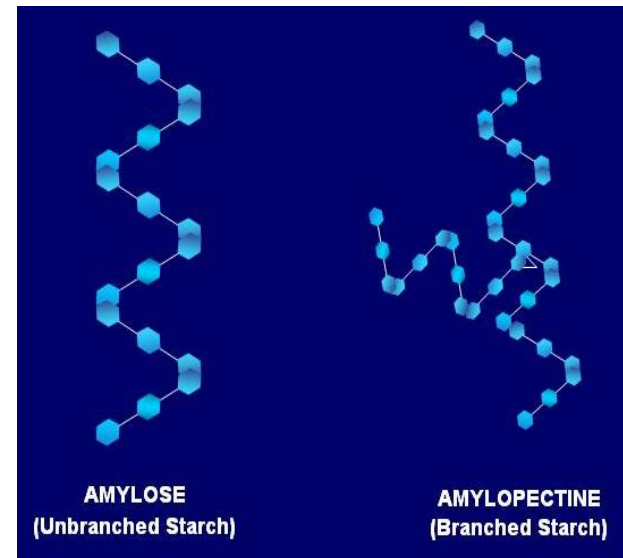
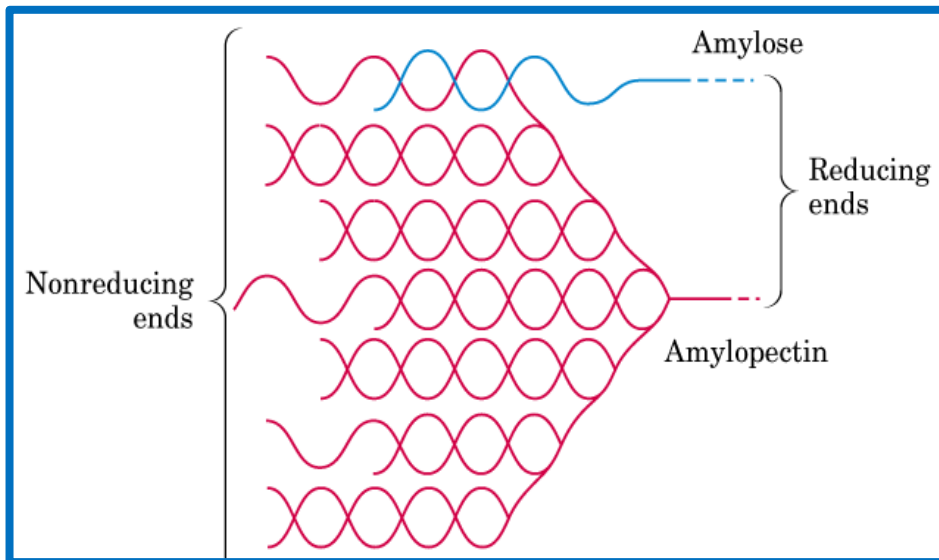
- Plants and animals store glucose in the form of very large polysaccharide glucose homopolymers.

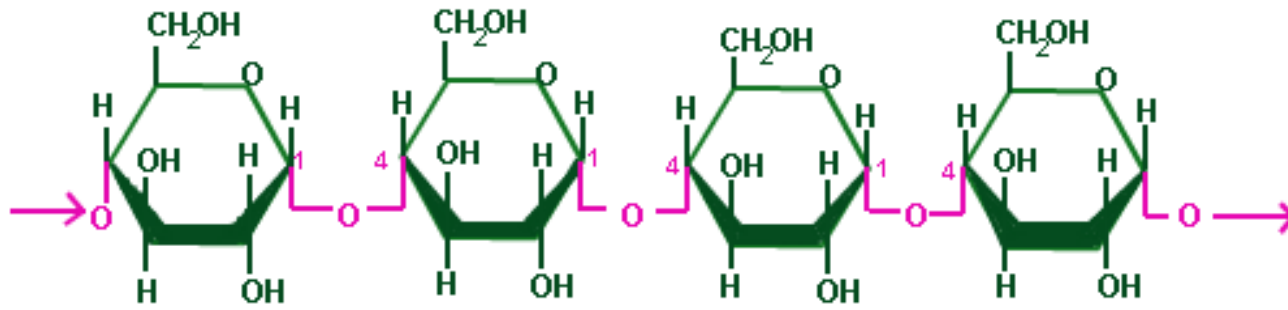


- The glucose homopolymer present in **plants** to store glucose is called **starch**.
- While the glucose homopolymer present in **animal** cells is called **glycogen**.

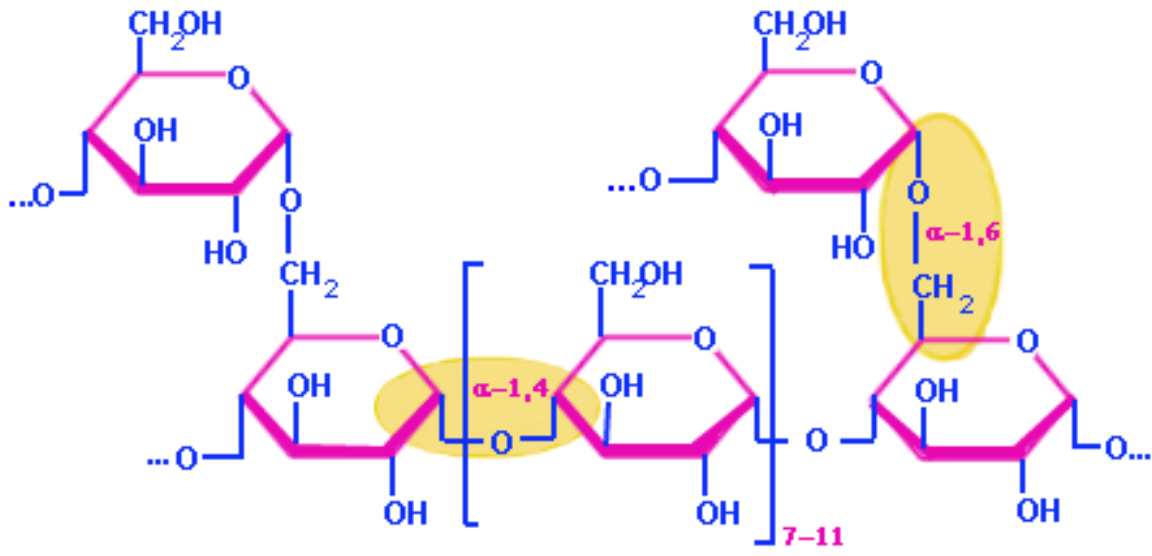
Starch:

- **Starch consists of two forms: amylose and amylopectin.**
 - **Amylose** is a Linear helical polymer of glucose linked by α -1,4 glycosidic bonds (100units).
 - **Amylopectin** is a Branched polymer containing glucose linked by α -1,4 glycosidic bonds, branch points has α -1,6 glycosidic bonds, (100,000 units).





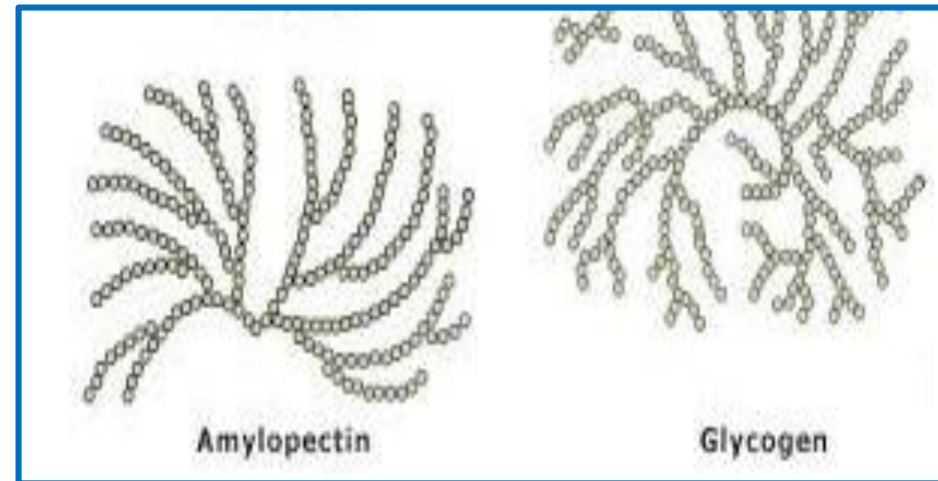
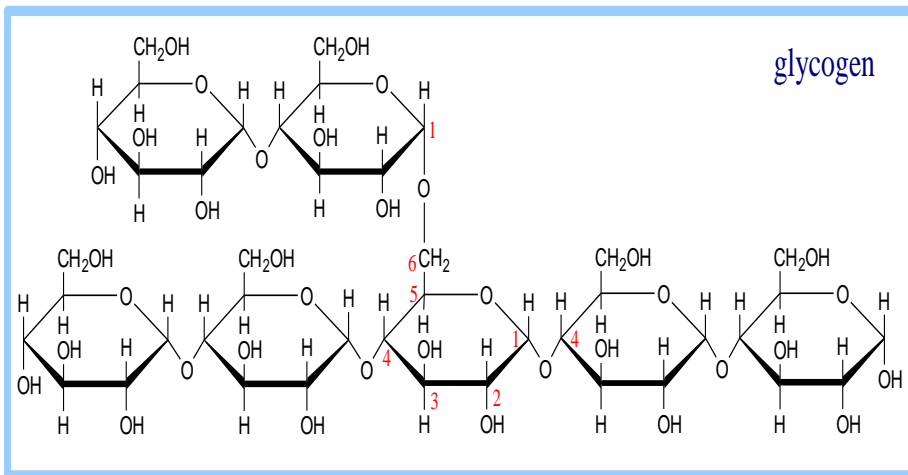
Amylose



Amylopectin

Glycogen:

- **Glycogen**, is a branched polysaccharide of D-glucose which contains both $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ is similar in structure to amylopectin.
→ But glycogen has **more $\alpha(1\rightarrow6)$ branches**.



Practical part

Qualitative tests of complex carbohydrates

1

Sucrose hydrolysis Test.

2

The Iodine/Potassium Iodide Test: for polysaccharides.

3

Hydrolysis of Starch.

Experiment 1 : Sucrose hydrolysis Test

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

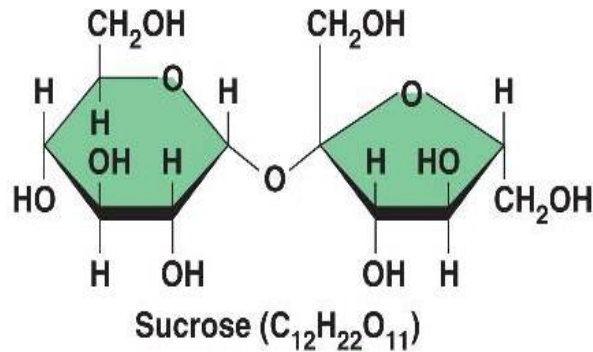
Objective:

- To identify the products of hydrolysis of sucrose (disaccharide).

Principle:

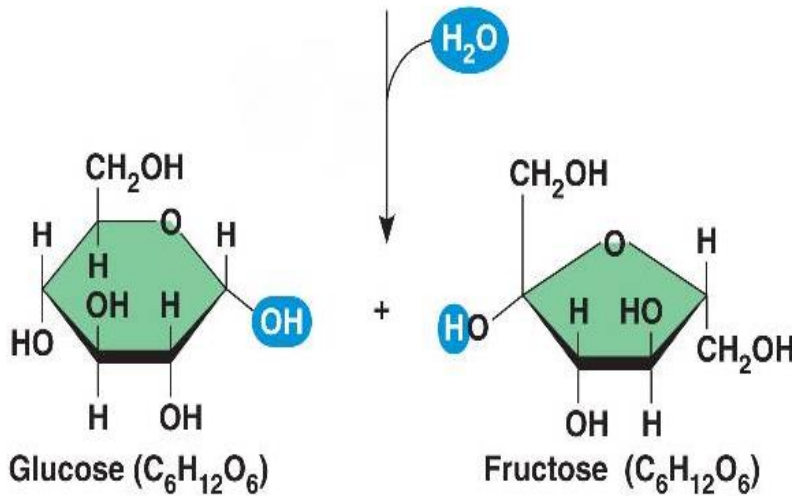
- Sucrose is a non-reducing disaccharide so it does not reduce the Cu^{++} solution (Benedict's and Fehling's test) because the glycosidic bond is formed between the two hemiacetal bonds.
- So there is **no free aldehydic or ketonic** group to give positive reducing properties.
- When glycosidic bond **hydrolysed (in the presence of acid)** to glucose + fructose (**which are reducing sugars**) are then able to give positive reducing test.

Non-reducing
disaccharide



-ve result
with
benedict

Reducing
monosaccharides



+ve result
with
benedict

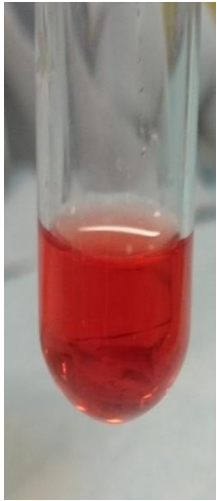
Experiment 1 : Sucrose hydrolysis Test

Method:

1. Set up two tubes, add to each one 4ml of a sucrose solution, label the tube: (Sucrose with HCL, Sucrose without HCL)
2. To only one tube add 7 drops of concentrated hydrochloric acid (HCl). **CARFULLY**
3. Heat both tubes in boiling water bath for 15 minutes.
4. Add 15 drops of concentrated NaOH to each tube (**why?**).
5. From the tube containing HCl take 2ml in two tube to do Benedict's test and Seliwanoff's test, label the tube (Benedict +HCl) and (Seliwanoff +HCl)
6. Add 2 ml of Benedict's reagent and 2.5 ml of Seliwanoff's reagent .**WHAT do expect?**
7. 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?**

Results:

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

Experiment 2 : The Iodine/Potassium Iodide Test

Objective:

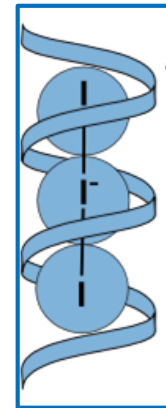
- This test used to distinguish between polysaccharides and mono or oligosaccharides.
- To detect the presence of starch in a sample.

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:

Other polysaccharides like glycogen may give other colors (**red**).



Experiment 2 : The Iodine/Potassium Iodide Test

Method:

1. Label two tubes A and B.
2. **In tube A:** add 2ml of starch and 2drops of iodine solution and one ml of water. Shake it well
3. A positive test is indicated by the formation of a **blue-black complex**.
4. Take a half of the tube A and heat it in boiling water bath for 3 min compare between the two tubes and write your observation. **WHAT do expect?**
5. **In tube B:** : add 2ml of glucose and 2drops of iodine solution. Record your results.

Results:

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



Glucose + iodine
(-ve)



Starch + iodine
(+ve)

Heating →



Starch + iodine

Experiment 3 : Hydrolysis of Starch

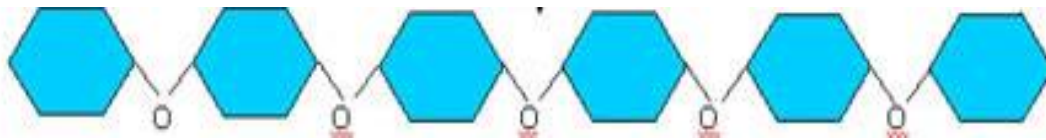
- This experiment illustrates the conversion of starch (**non-reducing sugar**) to glucose (**reducing sugar**) by the action of **hydrochloric acid at boiling point**. The longer the starch is exposed to the acid the further hydrolysis proceeds.

Objective:

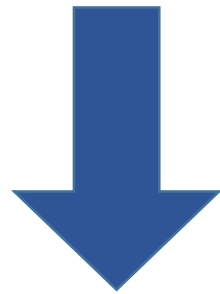
- To establish the effect of concentrated HCl on a glycosidic bond in starch.

Principle:

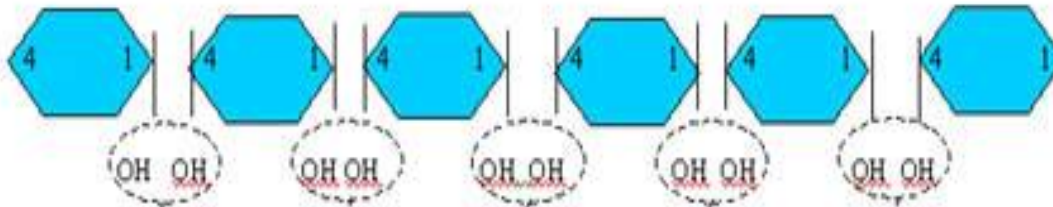
- Although starch has free hemiacetal in the **terminal glucose residue**, it has **no reducing properties**, because the percentage between the free residues is very low in comparison to the whole molecule.
- **Heating starch solution in acid medium** hydrolysis the glycosidic bonds giving many free glucose residues → These glucose molecules give reducing properties to the hydrolysis product.



Non-reducing



Hydrolysis of glycosidic
bond (HCl+heating)



Reducing

Experiment 3 : Hydrolysis of Starch

Method:

1. Two ml of starch in large tube.
2. Add 15 drops of Hydrochloric acid, heated in boiling water bath for 10 mints. then cold solution .
3. Add 25 drops of sodium hydroxide to become basic.
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?**

Results:

Starch with HCL	
Benedict's test	Iodine test

Starch with HCL
[After heating]



[-ve] Iodine test



[+] Benedict's test