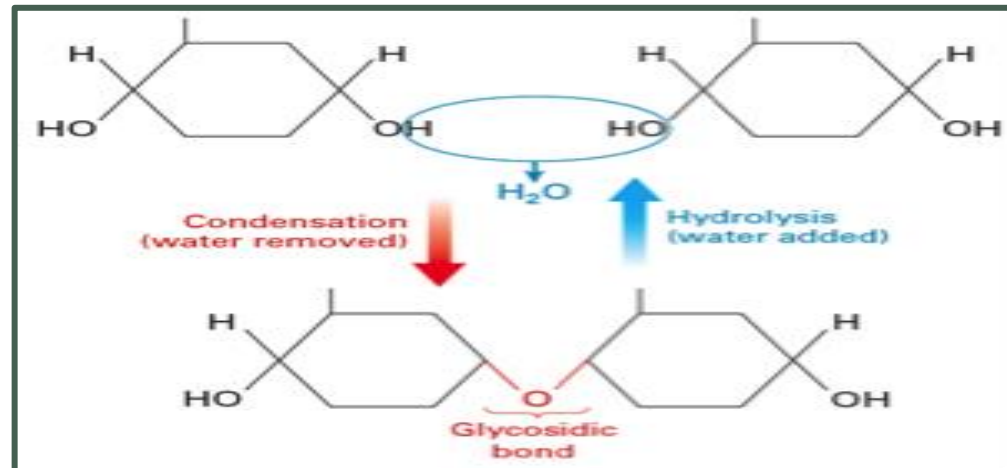


# Qualitative Tests of Carbohydrate-II

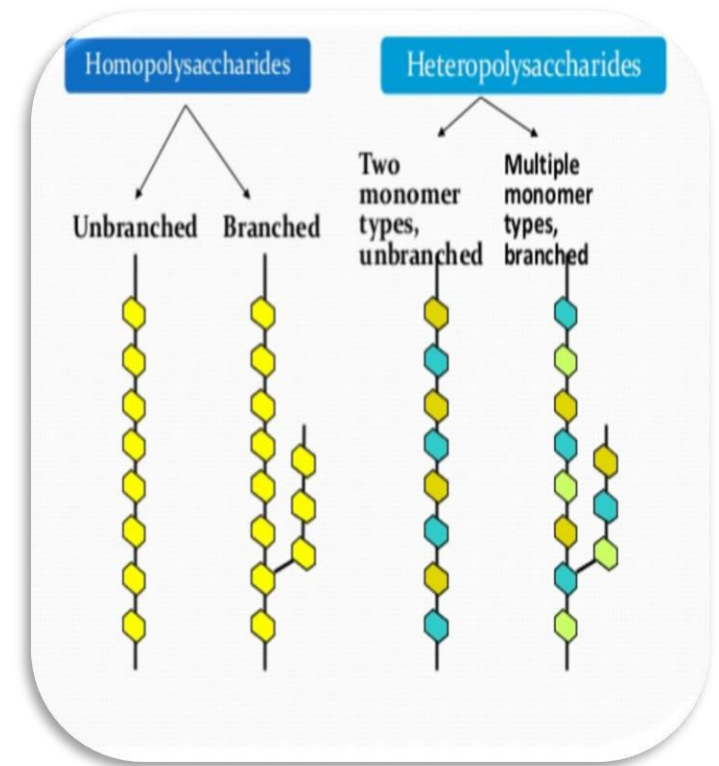
# Complex carbohydrates :

- 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 :**
  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.



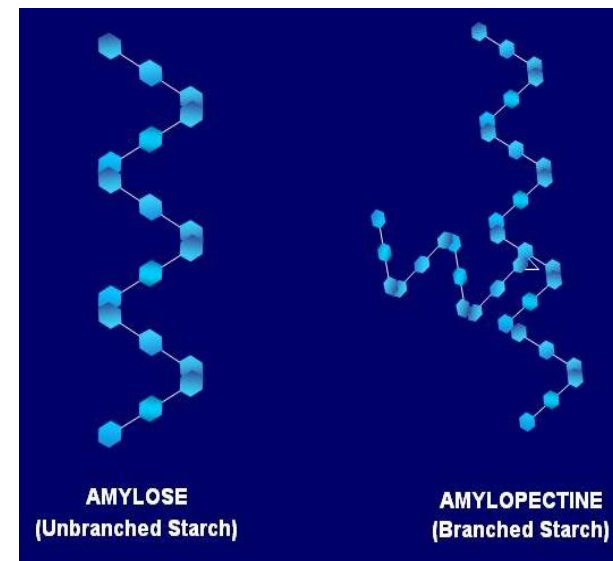
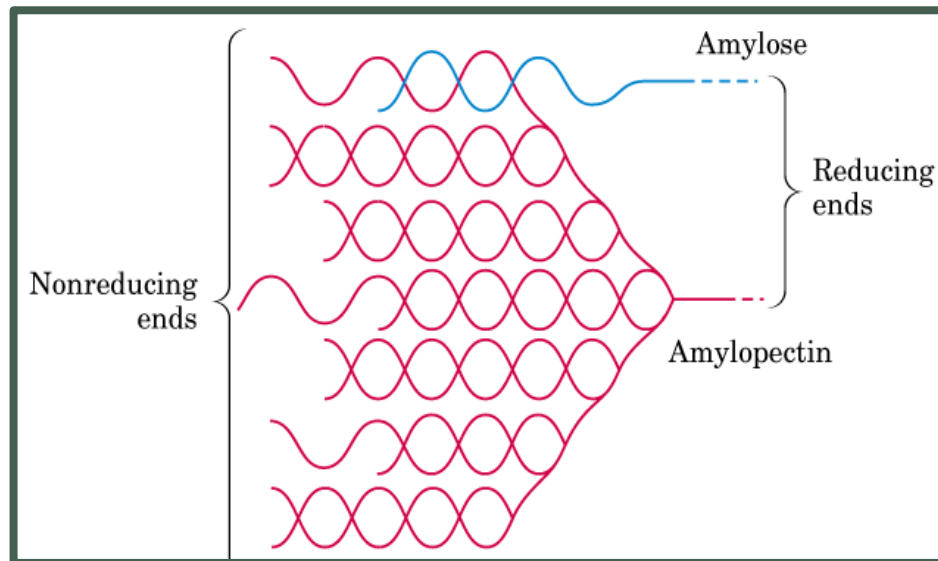
- The glucose homopolymer present in **plants** to store glucose is called **starch**.
- While the glucose homopolymer present in **animal** cells is called **glycogen**.

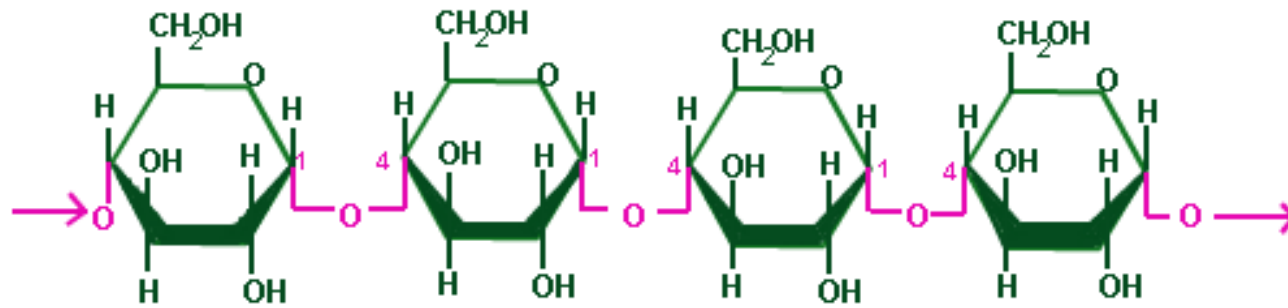
# Starch:

➤ **Starch consists of two forms: amylose and amylopectin.**

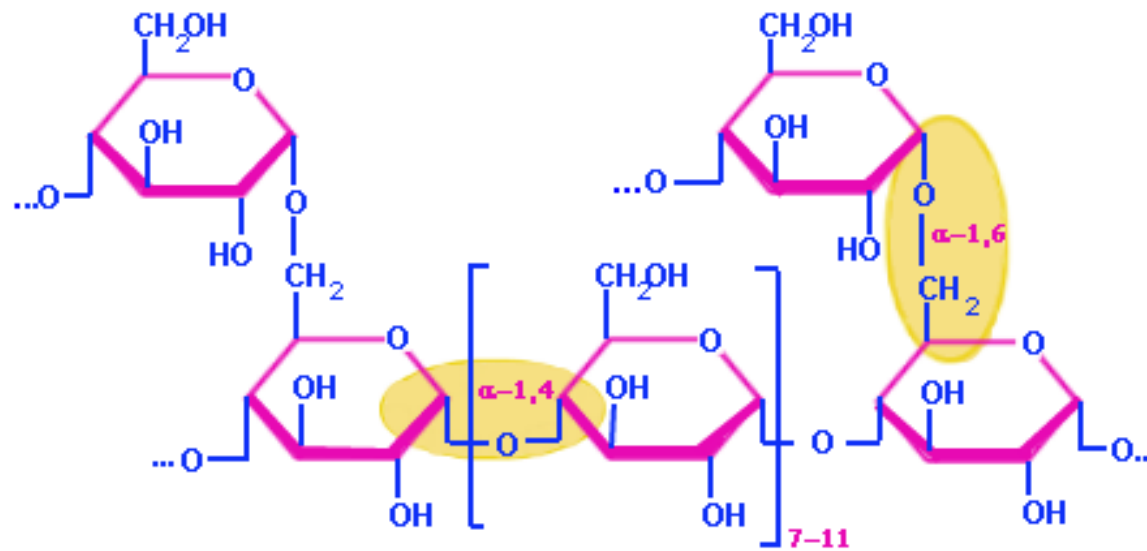
➔ **Amylose** is a Linear helical polymer of glucose linked by  $\alpha$ -1,4 glycosidic bonds (100 units).

➔ **Amylopectin** is a Branched polymer containing glucose linked by  $\alpha$ -1,4 glycosidic bonds, branch points has  $\alpha$ -1,6 glycosidic bonds, (100,000 units).





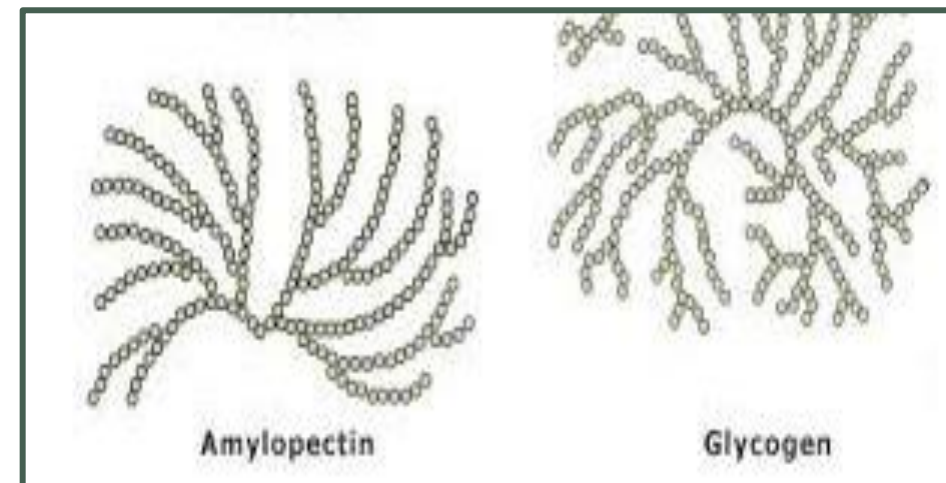
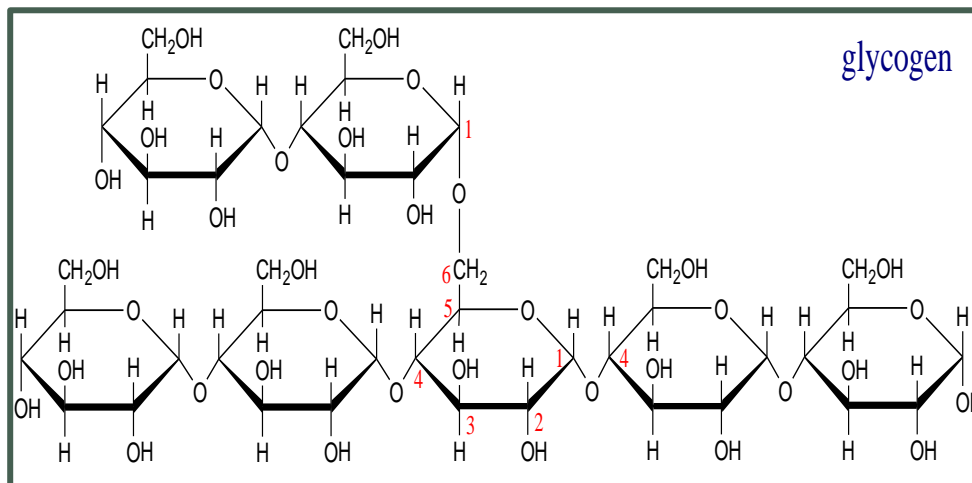
Amylose



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

# Chemical properties of carbohydrates:

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- 1 Sucrose hydrolysis Test.
- 2 The Iodine/Potassium Iodide Test.
- 3 Hydrolysis of Starch.



# Experiment I : Sucrose hydrolysis Test

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

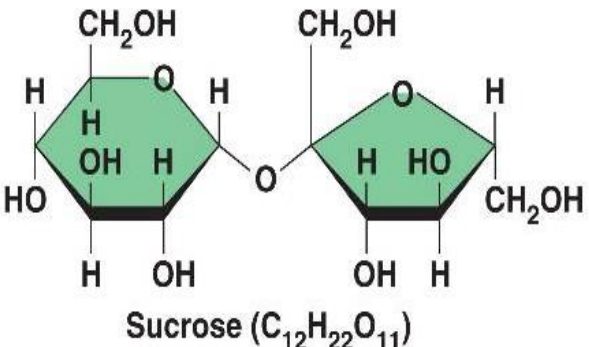
➤ **Objective:**

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

➤ **Principle:**

- **Sucrose is a non-reducing disaccharide** so it does not reduce the  $\text{Cu}^{++}$  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.

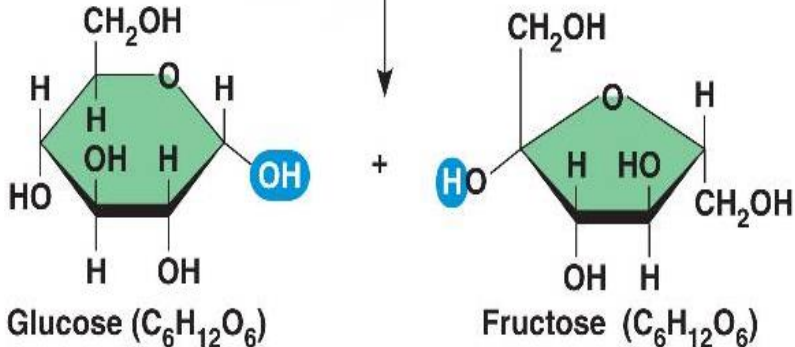
Non-reducing  
disaccharide



-ve result  
with  
benedict

H<sub>2</sub>O Heating + acid

Reducing  
monosaccharides



+ve result  
with  
benedict

# Experiment I : 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 2 ml in two tubes 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 you 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

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## ➤ Objective:

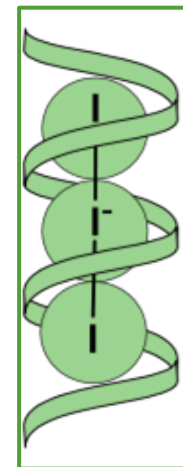
- This test used to distinguish between polysaccharides and mono or oligosaccharides.
- To detect the presence of starch in a sample.

## ➤ Principle:

- Starch forms deeply **blue color** complex with iodine.
- Starch contains  $\alpha$ - amylose, a helical saccharide polymer and amylopectin. Iodine forms a large complex with  $\alpha$ -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

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## ➤ Method:

1. Label two tubes A and B.
2. **In tube A:** add 2 ml of starch and 2 drops 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 2 drops 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

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➤ 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.

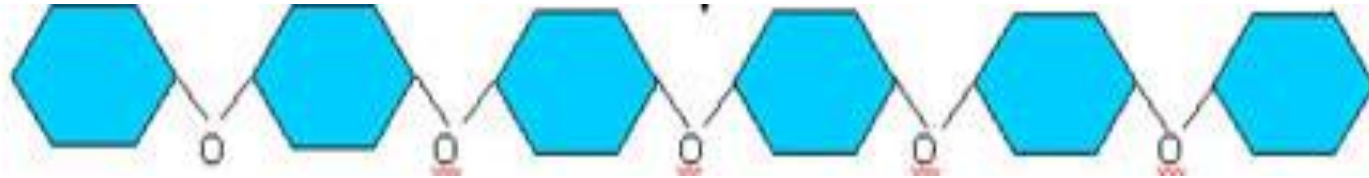
➤ **Objective:**

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

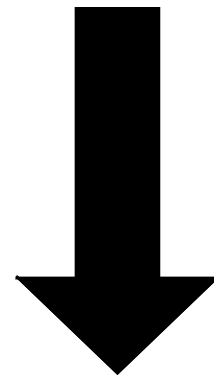
➤ **Principle:**

- 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.

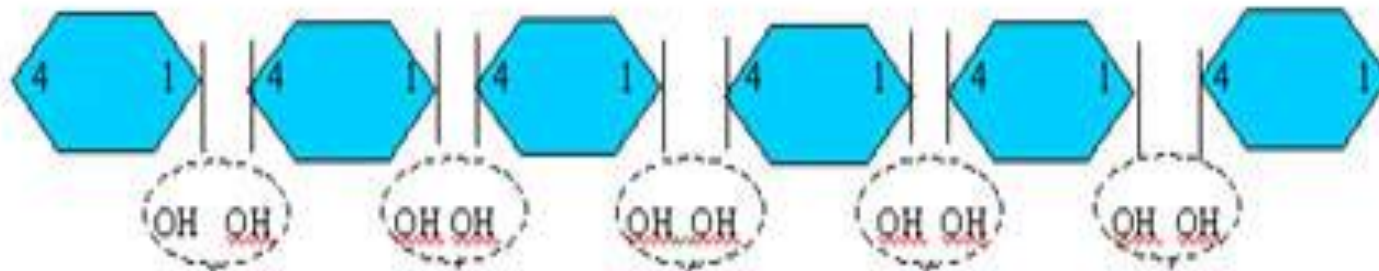




Non-reducing



**Hydrolysis of glycosidic  
bond (HCl+heating)**



Reducing

# Experiment 3: Hydrolysis of Starch

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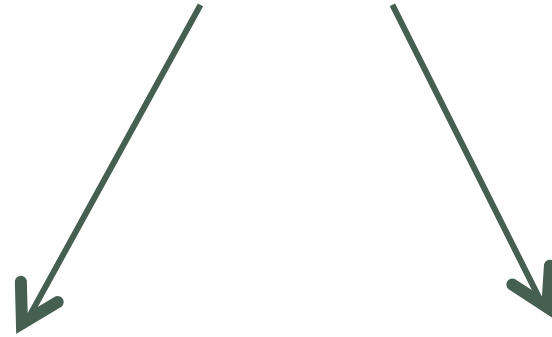
## ➤ Method:

1. Two ml of starch in large tube.
2. Add 15 drops of hydrochloric acid, heated in boiling water bath for 10 mins. 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

# Homework:

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- Explain why the blue color disappears upon heating?**