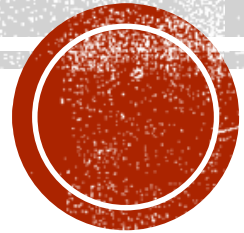
ACIDIC HYDROLYSIS OF GLYCOGEN

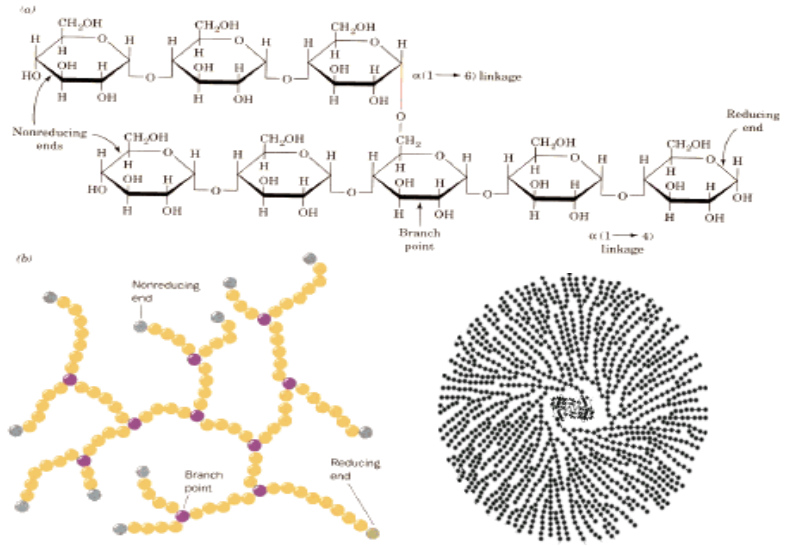


- OBJECTIVE:

- To examine the polysaccharide nature of glycogen and show that hydrolysis increases the number of reducing groups.

STRUCTURE OF GLYCOGEN:

- The structure of the glycogen molecule is **fan-like; with long chains of glucose residues** linked by α-1, 4 glycosidic bonds, with α-1, 6 links at the branch points.
- So, the whole glycogen molecule has only one free reducing end, where the C1 of a glucose residue is free (exposed).
- Thus the glycogen molecule is **essentially non-reducing.**



HYDROLYSIS OF GLYCOGEN:

- Hydrolysis converts glycogen from a non-reducing substance into reducing substances.
- Hydrolysis of the glycogen molecule with acid results in splitting of all its glyosidic bonds giving only glucose molecules as the product.
- Enzymes are more specific in the bond type they split.
- Thus salivary amylase (α-amylase) will randomly split only α-1 ,4 glycosidic bonds and produce a mixture of products consisting of **glucose, maltose and malttriose** molecules

METHOD:

2 **Addition of NaOH**

Tubes	Diluted glycogen	PS buffer	HCl		Time of hydrolysis (min)	NaOH	PS buffer	DNS reagent		H2O
1	0.4 ml	---	0.6 ml	--	0	1 ml	0.5 ml	2 ml	*Boiling water bath for 10 min* ↓ *Cool down*	5.5 ml
2		---		*Boiling water bath in intervals of 4 min*	4 min					
3		---			8 min					
4		---			12 min					
5		---			16 min					
6		---			20 min					
7		---			24 min					
8		---			28 min					
9		---			32 min					
Blank	---	0.4		--	0					

1 **Acidic hydrolysis of glycogen**

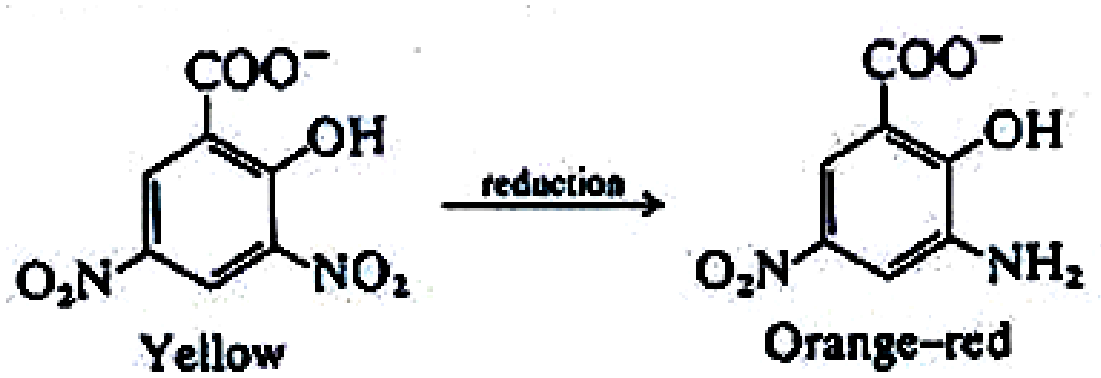
Mix well (total volume 10 ml in each tube) ↓

3 **Determination of reducing gp. no. by DNS**

Read the absorbance at 540 nm against blank

PRINCIPLE

- The increase in the number of reducing groups resulting from the hydrolysis is determined using 3, 5-dinitrosalicylic acid (DNS).
- In **alkaline** solution it is reduced to 3-amino-5- nitro salicylic acid, which is orange-red.
- **Absorbance is determined at 540 nm.**



Yellow

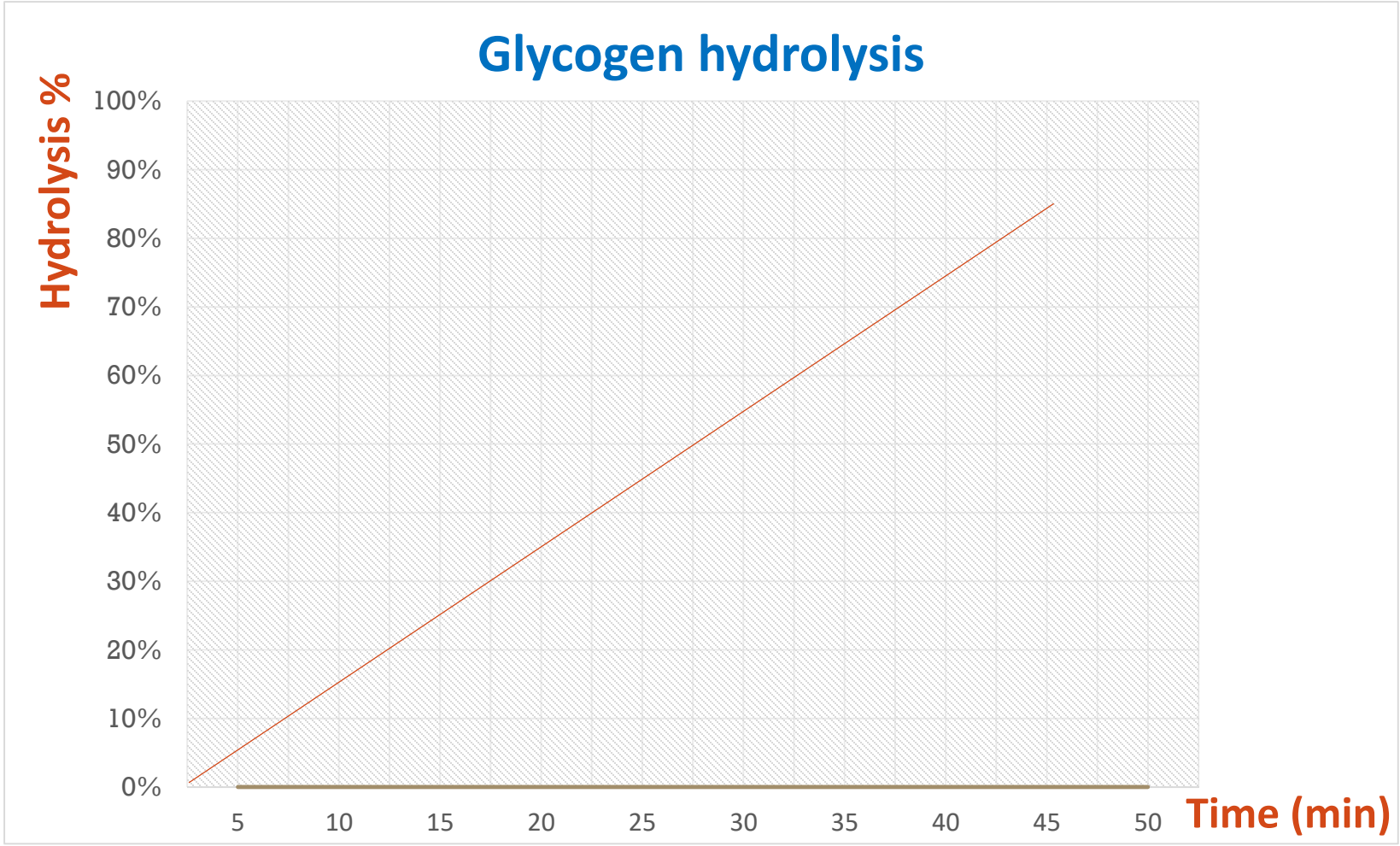
Orange-red

- RESULTS:

Tubes	Time (min)	Abs at 540nm	Hydrolysis %
1	0		
2	4		
3	8		
4	12		
5	16		
6	20		
7	24		
9	28		
10	32		

- **Hydrolysis %= Abs x 100**
- **Example:**
- **Abs = 0.123**
- **Hydrolysis % =** 0.123 x 100 = 12.3

- RESULTS:



Note: Acidic Hydrolysis increases the number of reducing groups with increasing time

- QUESTION:

Why is NaOH used in the protocol of acidic hydrolysis of glycogen?

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2/