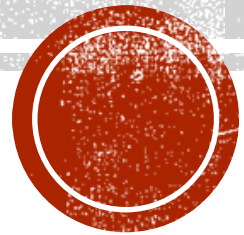
ENZYMATIC HYDROLYSIS OF GLYCOGEN AND DETERMINATION OF GLUCOSE

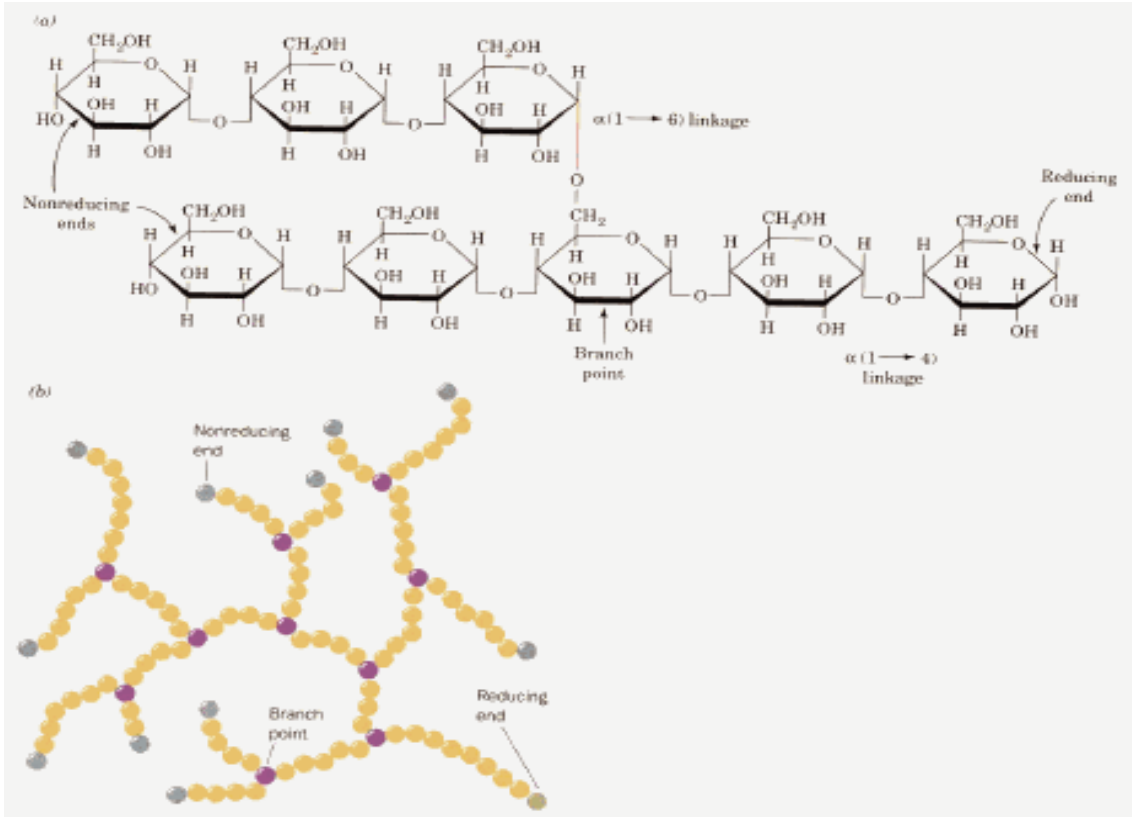


- OBJECTIVE:

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

INTRODUCTION:

- The structure of the glycogen molecule is **fan-like; with long chains of glucose residues** linked by 1, 4-glycosidic bonds, with 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).

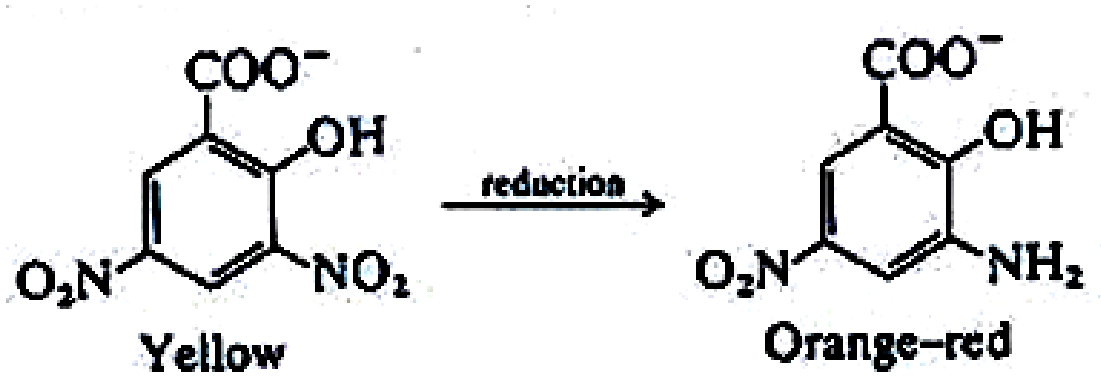


- Thus the glycogen molecule is **essentially non-reducing.**
- Hydrolysis converts glycogen from a non-reducing substance into reducing substances.
- Hydrolysis of the glycogen molecule with acid results in splitting of all its glyosidic bonds giving only glucose molecules as the product.
- Enzymes are more specific in the bond type they split.

- Thus salivary amylase (α-amylase) will randomly split only 1, 4- glycosidic bonds and produce a mixture of products consisting of **glucose, maltose and malttriose** molecules.
- The increase in the number of reducing groups is determined using 3, 5-dinitrosalicylic acid (DNS) in lkaline solution.

THEORY

- Several reagents can be used to assay reducing sugars such as 3, 5 dinitrosalicylic acid in one of the compounds.
- In **alkaline** solution it is reduced to 3-amino-5- nitro salicylic acid, which is orange-red.
- **Absorbance is determined at 540 nm.**



ACIDIC HYDROLYSIS OF GLYCOGEN

Tubes	Diluted glycogen	0.05 mol/l PS buffer	2 mol/l HCl		Time of hydrolysis (min)	1.2 mol/l NaOH	0.05 mol/l phosphate buffer	DNS reagent		H2O
1	0.4	---	0.6	--	**0**	1 ml	0.5	2	**Boiling water bath for 10 min ↓ Cool down**	5.5
2	0.4	---	0.6	**Boiling water bath in intervals of 4 min**	**4 min**	1 ml	0.5	2		5.5
3	0.4	---	0.6		**8 min**	1ml	0.5	2		5.5
4	0.4	---	0.6		**12 min**	1 ml	0.5	2		5.5
5	0.4	---	0.6		**16 min**	1 ml	0.5	2		5.5
6	0.4	---	0.6		**20 min**	1 ml	0.5	2		5.5
7	0.4	---	0.6		**24 min**	1 ml	0.5	2		5.5
8	0.4	---	0.6		**28 min**	1 ml	0.5	2		5.5
9	0.4	---	0.6		**40 min**	1 ml	0.5	2		5.5
Blank	---	0.4	0.6	--	**0**	1 ml	0.5	2		5.5

Mix well (total volume 10 ml in each tube)

↓

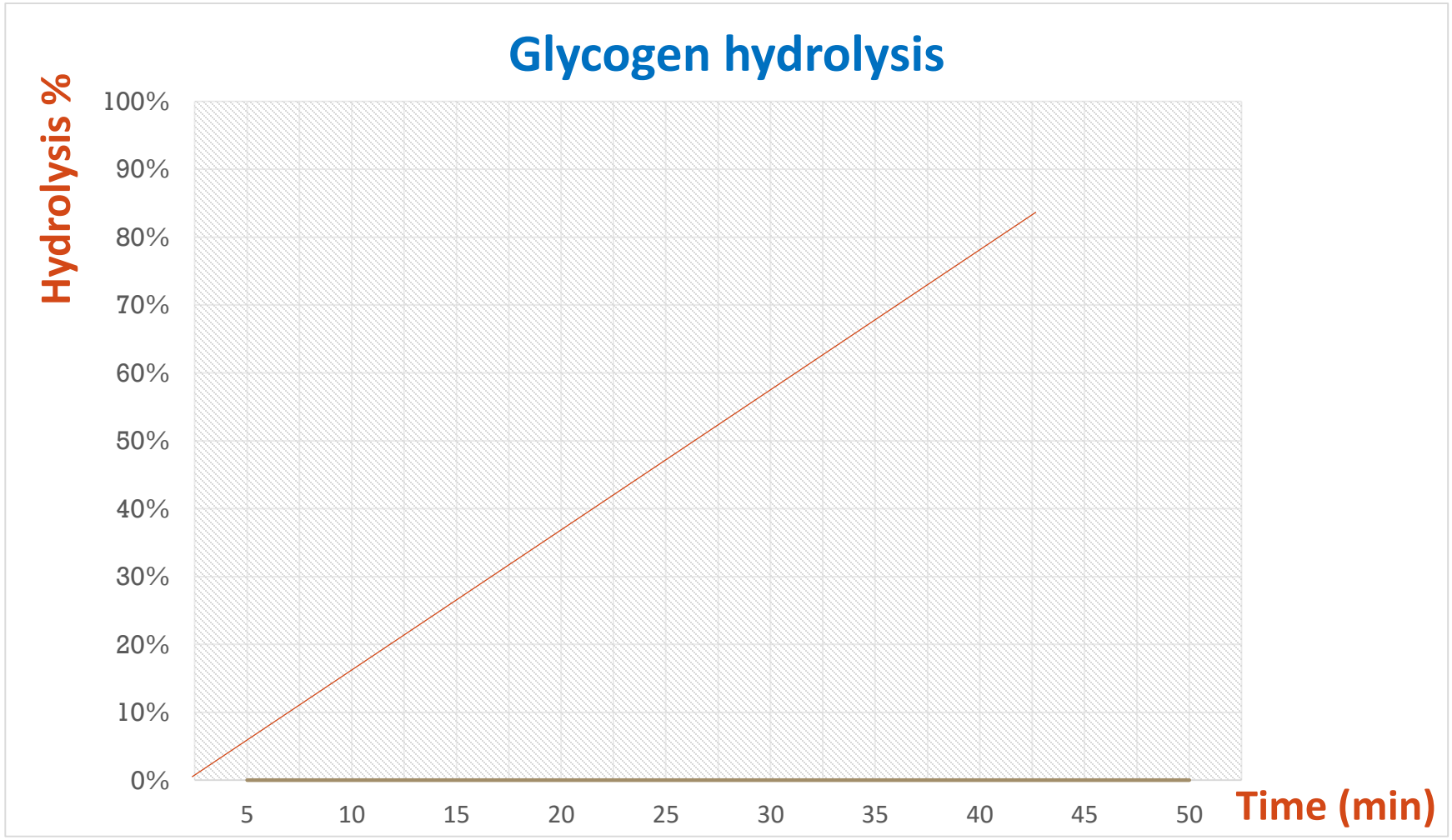
Read the absorbance at 540 nm against the blank sample (tube 10)

- RESULT:

Tubes	Time (min)	Abs (nm)	Hydrolysis %
2	4		
3	8		
4	12		
5	16		
6	20		
7	24		
9	28		
10	40		

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

- RESULT:


Glycogen hydrolysis
Hydrolysis %
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
5
10
15
20
25
30
35
40
45
50
Time (min)

Thank you