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# Diploma in Pharmacy 2<sup>nd</sup> Year

## Biochemistry & Clinical Pathology

### Experiment

To study the hydrolysis of starch from salivary amylase enzyme.

#### Aim:

To study the hydrolysis of starch from salivary amylase enzyme.

#### Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 65 – 70

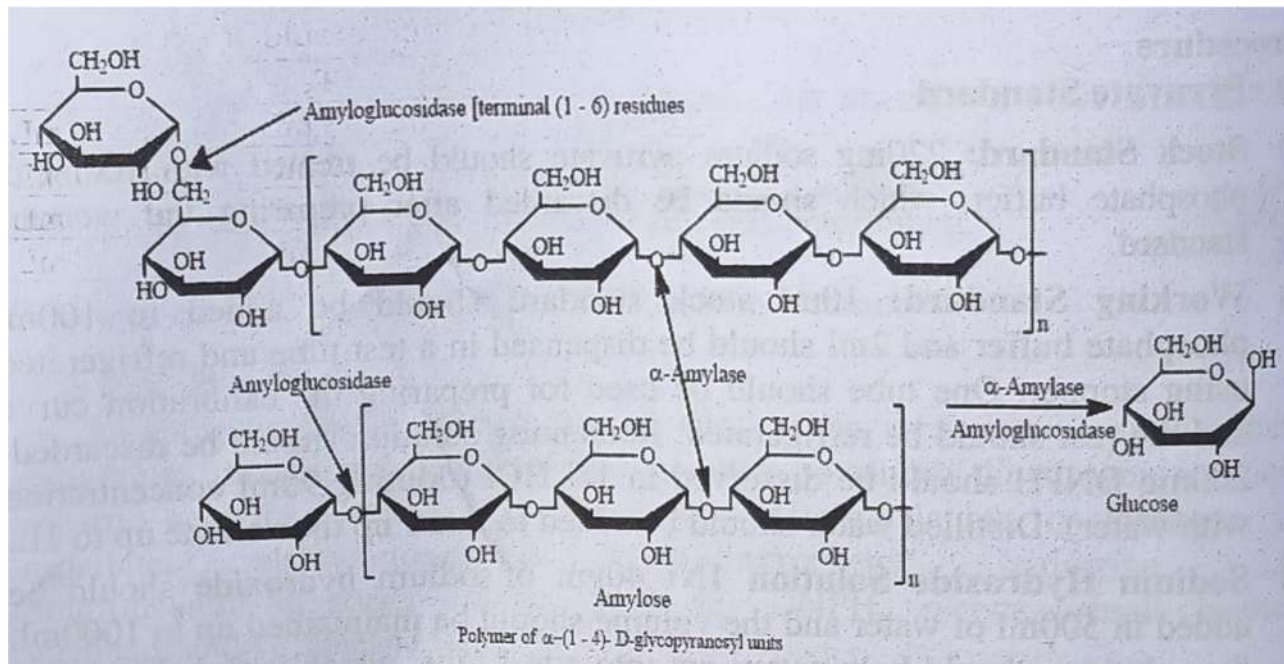
#### Materials Required

Starch, phosphate buffer solution, iodine solution, test tube, flask, pipette and stir rod.

#### Theory :

Starch is made up of mainly two parts:

- 1) **Amylose:** A linear polymer composed of D-glucose units that are  $\alpha$ -1-4 bound. About 20-30% of the structure is made up by this polysaccharide.
- 2) **Amylopectin:** It is a polysaccharide with highly branched polymer containing bonded glucose units from  $\alpha$ -1-6.



The enzyme amylase breaks down large alpha-linked polysaccharides like starch and glycogen to produce maltose and dextrin. It is the most common type of amylase present in people and other mammals. Amylase is found in saliva. This form of amylase is also known as "ptyalin".

Maltose units are formed when the enzyme randomly affects  $\alpha$ -1-4 bonds in the amylose structure of starch.  $\alpha$ -1-6 bonds that are part of the amylopectin structure of starch are unaffected by amylase. A large branching dextrin structure is formed in the media as a result of hydrolysis process carried out with-amylase, in addition to the maltose and glucose units.

## Optimum Conditions for $\alpha$ -Amylase

- 1) Optimum pH: 5.6-6.9
- 2) Human Body Temperature:  $37^{\circ}\text{C}$
- 3) Presence of Certain Anions and Activators:
  - i) Chloride and bromide is most effective
  - ii) Iodide is less effective
  - iii) Sulphate and phosphate is least effective

# Procedure

## Part-A

- 1) 1ml. of 1% starch solution and 1ml. phosphate buffer solution of pH = 6.8 should be added into 4 test tubes.
- 2) Each test tube should be placed into water baths at 0°C, 25°C, 37°C and 95°C, respectively.
- 3) After a few minutes 1ml. diluted (1/10) saliva should be added into each test tube.
- 4) 1 drop of iodine solution should be placed on 4 watch glasses and 1 drop of this solution should be taken from each test tube to check for any remaining starch (starch gives blue colour with iodine solution).
- 5) Hydrolysis time of 1ml starch for each test tube should be recorded.

## Part-B

- 1) 7 test tubes should be taken and the following solution should be prepared:

Reagent	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>
1% Starch Solution	0.2mL	0.5 mL	1 mL	2 mL	3 mL	4 mL	5 mL
Distilled Water	4.8mL	4.5 mL	4mL	3 mL	2 mL	1 mL	0 mL
Phosphate Buffer	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Saliva (1/20 diluted)	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL

- 2) Each test tube should be shaken thoroughly.
- 3) Each mixture should be examined for colour with iodine every minute.
- 4) Changes in colour should be recorded.

## Result :

Hydrolysis of starch from salivary amylase enzyme was studied.