

EXERCISE 11

ASSAY OF SALIVARY AMYLASE ACTIVITY

Structure

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11.1 INTRODUCTION

We all know that all enzymes are proteins and known as biocatalysts. In units 14 and 15 of course BZYCT-135 you have studied about factors affecting enzyme activity. You might have studied about different types of enzymes and importance of amylases like salivary and pancreatic amylases in these Units and even in your lower classes. However, in this Exercise, you will be assaying the activity of on an important enzyme i.e., salivary amylase. Amylase enzyme is essential for the digestion of starch, a plant storage polysaccharide. You are advised to watch the model video available at You Tube link (<https://www.youtube.com/watch?v=iaqxTaXit9M>) before going to start the Exercise. There are few changes in the operational steps explained in the video with that of the procedure explained in this Exercise. However, the core aim of the Exercise remains same.

Objectives

After performing this exercise, you should be able to:

- ❖ describe the principle behind amylase action;
- ❖ prepare salivary amylase;
- ❖ explain the role of pH and temperature on amylase enzyme activity; and
- ❖ discuss the significance of amylase in the digestion.

11.2 MATERIALS REQUIRED

Beakers, Test tubes, Test tube stand, Funnel, Iodine solution, Starch solution, NaCl solution, Phosphate Buffer solution, Distilled water, Cotton, Thermometer.

Preparation of Reagents

Phosphate Buffer: Weigh 13.872 grams of potassium dihydrogen phosphate and 35.084 grams of disodium hydrogen phosphate separately and dissolve in small volume of distilled water and make up to 1000 mL and adjust the pH to 6.8.

1% Starch Solution: Weigh 1 gram of starch powder and dissolve in 100 mL of distilled water.

1% NaCl Solution: Weigh 1 gram of NaCl crystals and dissolve in 100 mL of distilled water.

11.3 PRINCIPLE

The presence of starch in a solution can be detected by using Iodine solution as an indicator. Iodine is deep blue/black in colour in the presence of starch. As starch is broken down to dextrin, the iodine turns to a brown/red colour followed by a pale brown/yellow colour when the enzyme has completed the hydrolysis (Fig.11.3).

The colour change of iodine determines how far the reaction has progressed at different times. The point at which there is no change in the colour of iodine is referred to as the achromic point. Salivary Amylase is very active at 37°C and at pH 6.8.

The following flow chart displays the distinct color developed upon the interaction of salivary amylase with starch. It also describes different colors developed during the conversion of polysaccharide to disaccharides i.e., starch to maltose.

Distinct colours developed during reaction

Salivary Amylase at 37°C and 6.8 pH
↓
Starch (Blue black)
↓
Amylodextrin (Dark Brown)
↓
Erythrodextrin (Red Brown)
↓
Achrodextrin (Orange)
↓
Maltose (Yellow)

11.4 PROCEDURE

- i) **Enzyme Preparation:** Prepare a very thin layer of absorbent cotton, wet it with distilled water and squeeze out the water completely. Put the cotton layer down the rim of the funnel. Take a small piece of paraffin wax or dental grade cotton chew for few minutes. Later the chewed paraffin or dental grade cotton should be expectorated on to the cotton along with saliva. Store the filtered saliva in the test tube. The collected saliva can be further diluted to study the activity of the amylase enzyme (Fig. 11.1).

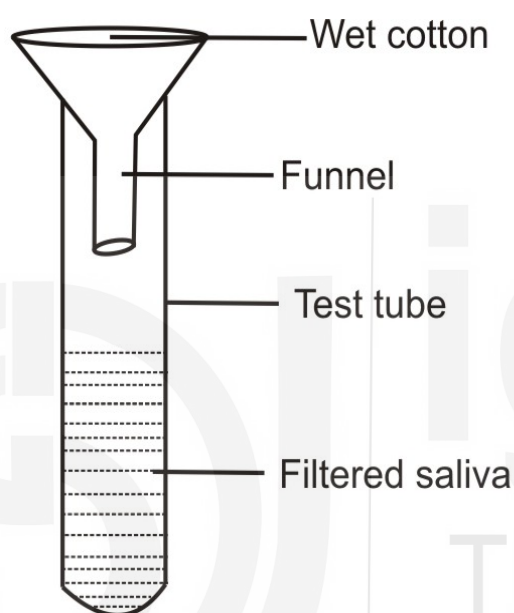


Fig. 11.1: Experimental setup for enzyme collection.

- ii) **Experimental Setup:** Take two clean and dry test tubes and label them as Control and Test. To both the tubes add starch solution, phosphate buffer and NaCl solution as shown in Table 11.1 (except distilled water and saliva).

Table 11.1: Protocol for Amylase Assay.

S.NO.	STARCH	BUFFER	NaCl	ENZYME
Control	5 mL	2 mL	2 mL	1 mL distilled water
Test	5 mL	2 mL	2 mL	1 mL dilute saliva

- iii) **Preparation of Digestive Mixture:** After adding the reagents as shown in Table 11.1, place the test tubes in a water bath and adjust the temperature to 37° C. When the contents of the tubes reached to 37°C, add 1 mL of dilute saliva (1:10) to the 'Test' tube and 1 mL of distilled water to the 'Control' tube (sample setup shown in Fig. 11.2).

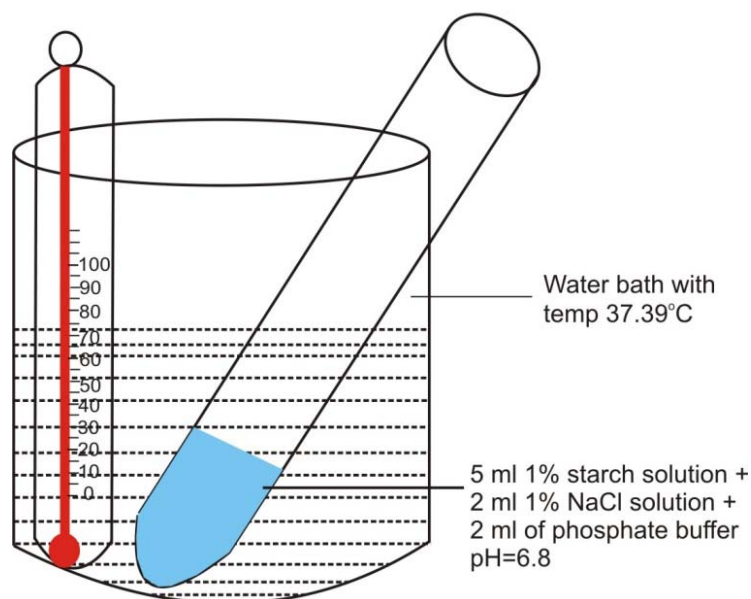


Fig.11.2: Experimental setup for starch digestion.

- iv) **Finding Achromic Point:** Take two clean tile grooves and label them as Control and Test. Mark the tile columns in minutes ranging from 0-12 (Fig. 11.3). Add few drops of dilute iodine solution in each groove in almost identical quantity. Now add two drops from each Control and Test tubes to the first groove of tile containing iodine solution. At the end of every minute, repeat this step for 12 times i.e., for a span of 12 minutes. Record the time when no change in colour (achromic point) of iodine solution is observed (Fig. 11.3).

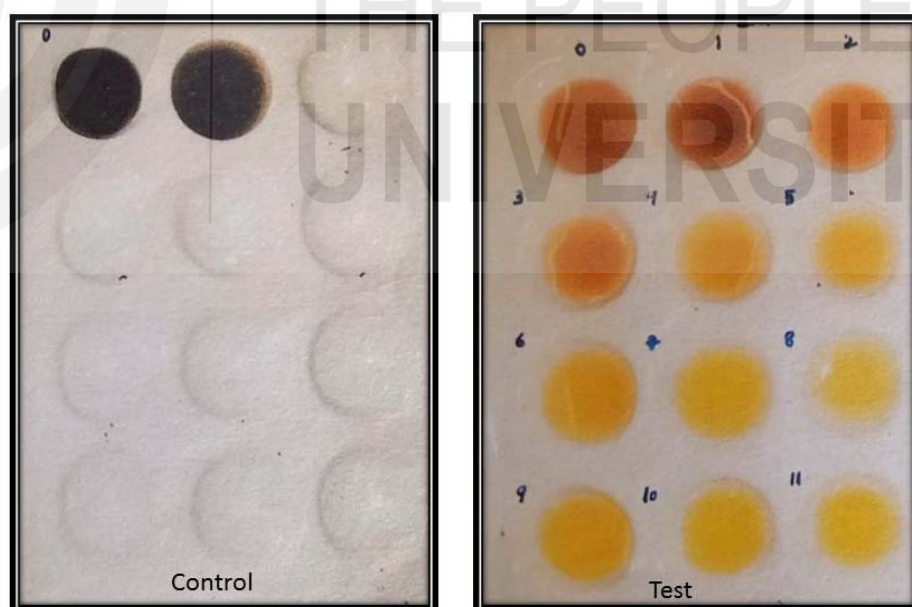


Fig. 11.3: Tile grooves showing salivary amylase test.

11.5 OBSERVATIONS

The change in the colour of the iodine solution indicates the progress of starch digestion. Iodine also acts as an inhibitor by preventing further digestion of starch by the enzyme amylase.

11.6 RESULTS

Amylase concentration of saliva is expressed as amylase unit (U). Amylase unit is the amount of enzyme required to digest 5 mL of 1% starch solution to the achromic point.

The amylase **enzyme activity** is expressed in U/ml. One unit of the enzyme is defined as the amount of enzyme required to liberate 1.0 mg of maltose from starch in 1 min at pH 7.0 at 37 °C.

Source: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4175699/>

CALCULATIONS

Achromic point = ____ minutes

$$\text{Amylase Unit (U)} = \frac{\text{Dilution Factor}}{\text{Time taken to reach achromic point}}$$

(Dilution Factor = 1/10)

Result = ____ U of enzyme is required to digest 5 mL of 1% starch at 37° C.

11.7 DISCUSSION

The salivary amylase enzyme is secreted by salivary glands, which are three pairs in humans. These are parotid, sublingual and submandibular. However, pancreas also secretes amylase that is known as pancreatic amylase. Amylase assay has certain clinical importance in the diagnosis of diseases like acute pancreatitis, perforated duodenal ulcer and cholecystitis.

Starch is a helical molecule. When mixed with saliva these starch molecules are hydrolysed by the enzyme amylase into dextrins, then maltose and finally into glucose. Amylase, like other enzymes, works as a catalyst. All catalysts are enzymes but all enzymes are not catalysts. A catalyst is a substance that fastens a chemical reaction but does not become part of the end product. Amylase digests starch by catalyzing hydrolysis, which is spitted by the addition of a water molecule. The presence or absence of starch can be confirmed by several tests such as the Iodine test, Benedict's and Fehling's test (Table 11.2). In general, a blue-black colour indicates the presence of starch. In this Exercise, iodine solution was used. The standardization of dilution of the enzyme is required to finish the reaction within 5-7 mins at its optimum conditions.

Table 11.2: Different products and colors developed during starch hydrolysis.

Starch hydrolysis products	Colour of Iodine indicator
Starch	Deep Blue/Black
Dextrin's	Dark Brown/red
Maltose or Glucose	Pale brown/Yellow (no change)

11.8 PRECAUTIONS

1. Rinse the mouth with water before expectorating the saliva.
2. Test-tubes should be thoroughly cleaned.
3. Dilutions should be accurate.
4. The temperature of water bath should be maintained at 37°C- 39°C
5. Equal quantity of enzyme-substrate should be added in the groove.
6. Remember values should be noted only after addition of digestive mixture in the groove

11.9 TERMINAL QUESTIONS

1. Fill in the blanks:
 - a. gland secretes amylase enzyme.
 - b. The products of starch digestion are
 - c. is the pH at which amylase enzyme shows its optimum activity
 - d. Development of color indicates the presence of starch.
 - e. is the unit used to express enzyme activity.

FURTHER READING

1. Experimental Biochemistry: A student Companion. Beedu Sashidhar Rao and Vijay Deshpande. ISBN 81-88237-41-8, I.K. International Pvt. Ltd.
2. Practical Biochemistry: for medical, dental and allied courses. 2nd edition, Dr. G. Rajagopal and Dr.B.D. Toora. ISBN 81-901769-5-1, Ahuja publishing house.
3. Preparative Organic Chemistry CHE-08 (L), Chemistry Lab-III. ISBN 81-7263-333-5, Published by Indira Gandhi National Open University, 1993 (Reprint December-2006).
4. Segel, I.H. Biochemical Calculations. 2nd ed. John Wiley & Sons. Inc. New York (1976).
5. Laboratory manual of Microbiology and Biotechnology (second edition), K.R. Aneja. ISBN 978-93-87025-49-3. MEDTECH a division of Scientific international (Pvt. Ltd).