

# Exercise 28

**Aim:** To study the effect of pH on the action of salivary amylase.

**Principle:** Optimal activity for most of the enzymes is generally observed between pH 5.0 and 9.0. However, a few enzymes, e.g., pepsin are active at pH values well outside this range. Above and below this range, the reaction rate reduces as enzymes get denaturated.

**Requirement:** Glass wares: test tubes, beakers, dropper, funnel; Chemicals: NaCl,  $\text{Na}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ , iodine crystals, potassium iodide, Buffer solutions of pH 4 and 9, Equipments: water bath or oven, thermometer; Miscellaneous: cotton, rubber, distilled water.

## Preparation of reagents

- Buffer solutions of pH 4 and 9 can be prepared by dissolving buffer tablets in appropriate amount of distilled water as indicated on the paper.

## Procedure

The first four initial steps are the same as in previous Experiment 26.

- Prepare three sets of indicator test tubes (8 to 10 in each set) in three separate test tube stands. Label test tube stands as A (for 6.8 pH), B (for 4 pH) and C (for 9 pH). In each test tube take 0.5 ml of iodine solution.
- In a test tube, take 5 ml of 1% starch solution, 1 ml of 1% NaCl solution and 1 ml of pH 6.8 buffer solution, mark it as control tube or A. In a second test tube, take 5 ml of 1% starch solution, 1 ml of 1% NaCl solution and 1 ml of pH 4 buffer solutions, mark it as experimental tube 'B'. In a third test tube, take 5 ml of 1% starch solution. 1 ml of 1% iodine solution and 1 ml of pH 9 buffer solution. Mark it as experimental tube 'C'.
- Transfer 1 ml of dilute saliva into each test tube and mix the two thoroughly. Place all three test tubes in water bath set at 37°C.
- Take a drop from each of the experimental tubes with the help of dropper and add to the corresponding indicator tubes containing iodine solution. Note this time as zero minute reading.
- At intervals of every 2 minutes repeat the above steps and note the change in colour of iodine solution. Continue this till the colour of iodine does not change.

### Exercise 28

- Note the time taken for different experimental tubes till they do not give any colour with iodine.

Time minute	Tube 'A'	Tube 'B'	Tube 'B'
0	Blue colour	Blue colour	Blue colour
2	-----	-----	-----
4	-----	-----	-----
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Calculate the time taken to reach the achromatic point in tubes A, B and C. Find out whether in any of the three tubes achromatic point was not observed.

### Discussion

On the basis of following questions draw your conclusion:

- At which pH is the reaction optimum?
- Did all three sets of tubes reach achromatic point? If not, why so?
- What inference do you draw about enzyme activity from your experiment?

### Questions

1. How many pairs of salivary glands are found in human beings?
2. What is an enzyme?
3. Why are enzymes mentioned as biocatalysts?
4. Why is NaCl solution added in the starch solution while testing salivary amylase activity?
5. What are the end products of salivary amylase activity?
6. What is achromatic point?
7. What is the optimum temperature and pH for salivary amylase action?
8. What is the need for secretion of pancreatic amylase into the intestine?
9. What do you mean by optimum temperature, pH and denaturation of enzyme?
10. How will you confirm that there is complete digestion of starch?