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# Effect of Substrate Concentration on Salivary Amylase Activity

Experiment 10 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

## Aim

To demonstrate starch hydrolysis by amylase and investigate the named reaction condition.

## Principle

Amylase cleaves internal alpha-1,4 glycosidic bonds in starch, producing smaller carbohydrates. Iodine-reactive chains diminish during hydrolysis. pH, temperature, enzyme concentration and substrate concentration must be controlled to make comparisons meaningful.

## Requirements

- Assigned amylase, buffered starch, dilute iodine, water blanks and controlled baths.
- Matched tubes, pipettes, well plate, timer and calibrated colour measurement when required.

## Procedure

### 1. Prepare controls

Use instructor-provided human salivary amylase or a characterised teaching preparation. Prepare a no-enzyme starch control and a no-starch blank. Keep total reaction volume, buffer pH and enzyme amount fixed unless that is the stated variable.

### 2. Prepare the comparison

Prepare starch concentrations such as 0.10, 0.25, 0.50 and 1.00% at equal final reaction volume; keep enzyme, pH and temperature fixed.

### 3. Start together

Add enzyme last and start the timer. Mix uniformly without adding iodine to the reaction tube; iodine belongs in the separate test wells.

### 4. Follow hydrolysis

Withdraw equal small aliquots into iodine-containing wells at fixed intervals and record the colour change from blue-black towards the iodine blank. For quantitative substrate comparisons, use a calibrated remaining-starch or reducing-sugar measurement during the initial linear interval.

### 5. Record and compare

Measure the endpoint time only at identical starting substrate concentration. For varied substrate concentrations, calculate initial rates from matched calibrated readings; 1/time alone is not a valid Michaelis-Menten comparison.

### 6. Graph

Plot initial rate against final substrate concentration. Discuss saturation without claiming an exact Km or Vmax from sparse data.

## Observations and Record

| Condition / replicate | Time or initial-rate reading | Control / observation |
|---|---|---|
| _____ | _____ | _____ |
| _____ | _____ | _____ |
| _____ | _____ | _____ |

## Worked Example

The numbers below are illustrative; enter your own observations in the record.

- If calibrated product rises from 0.010 to 0.050 µmol in the first 20 seconds, initial rate = (0.050 - 0.010) / (20/60) = 0.120 µmol/min.
- A plateau at higher substrate may support saturation only when enzyme amount, reaction interval and conditions remain identical.

## Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

## Precautions

- Do not share saliva or mouth-pipette. Use assigned preparations and dispose of biological material through the laboratory system.
- Take equal aliquots at scheduled times. Keep iodine out of the main reaction and use controls for colour comparisons.
- Heating can change starch behaviour as well as enzyme activity; interpret controls and repeat with a fresh enzyme aliquot.

## Viva Questions and Answers

### 1. What bonds does salivary amylase mainly cleave?

Internal alpha-1,4 bonds; it does not directly hydrolyse every starch linkage.

### 2. What does an iodine-negative result establish?

The tested aliquot no longer contains enough iodine-reactive starch chains to give the observed colour.

### 3. Why distinguish initial rate from endpoint time?

Initial rate evaluates reaction progress before major substrate depletion; endpoint time depends on starting substrate amount.

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