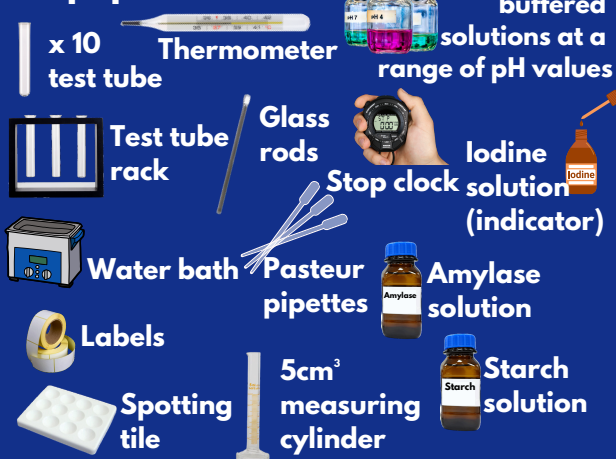


Equipment



Aim: Investigate the effect of pH on the rate of reaction of amylase

Enzymes

Biological catalysts which speed up chemical reactions in living organisms

Amylase

Produced in the salivary gland and pancreas



Iodine

Iodine is an eye irritant
Always wear goggles

Method:

Buffer solutions keep the reaction at a fixed pH
35°C is close to body temperature

Preparation

- 1) Heat water bath to 35°C.
- 2) Put 2cm³ of each buffered solution into separate test tubes. **Label each tube with the pH value**
- 3) Add 4cm³ of starch solution to 5 different tubes. **Label each as 'Starch'**
- 4) Leave a thermometer in one of the starch test tubes to monitor the temperature throughout. **Label as 'Amylase'**
- 5) Add 10cm³ of Amylase solution to one, different test tube.

Results (combined with another group)

pH of solution	Time for amylase to completely break down the starch (seconds)
4	101
5	42
6	21
7	32
8	59
9	81

- 6) Place all tubes into a water bath. Allow all solutions to reach 35°C.

REQUIRED PRACTICAL 7 ENZYMES

- 7) Meanwhile, put one drop of iodine solution into each spotting tile depression.



Continuous Sampling

- 8) Put a drop of starch solution in the first depression of the tile.
- 9) When at 35°C, add the 2cm³ of your first pH buffered solution to one starch tubes and mix with a glass rod.
- 11) Use the pipette to add 2cm³ of amylase solution to the mixture. Start the stop clock immediately.
- 12) Stir continuously for 10 secs then remove one drop of the mixture with the glass rod. **After mixing, the tube must be kept in the water bath**
- 13) Put this drop on the second depression of the tile. **Rinse glass rod with water between droplets!**
- 14) Every 10 seconds, use the glass rod to remove one drop and put each on the next depression of the tile. **This indicates all starch has been converted to sugars**

Repeat sampling until the iodine does not change colour.

Repeat steps 9-14 with the other buffer solutions with new pipettes, glass rods and spotting tiles.

Analysis of Results

"The results show that the time taken for all the starch to be digested by the amylase decreases from pH4 to pH6, and then increases again. Therefore, the optimum pH for this enzyme is pH 6."

Potential improvements?

- Using more accurate measuring apparatus e.g. a calorimeter
- Taking mean of several repeats at each pH
- Using a narrower range of pH

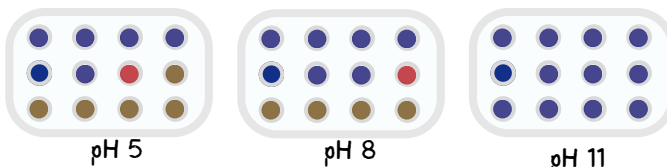
Brown → Blue-black

Starch is present

Brown → Brown

Starch is no longer present

What you might see...



Exam Style Questions - Enzymes

1) Amylase is the enzyme that controls the breakdown of starch to glucose. Describe how the student could investigate the effect of pH on the breakdown of starch by amylase. (4 marks)

2) Students investigated the effect of bile on the digestion of fat by lipase. They followed the following protocol:

- Mixed milk and bile in a beaker
- Put the pH sensor of a pH meter into the beaker
- Add lipase solution
- Recorded the pH at 2-minute intervals
- Repeated steps 1 to 4, but used water instead of bile.

a) Suggest two variables that the student should have controlled in this investigation. (2 marks)

b) They students found that by the end of each test the beaker with water and bile both had the same pH of 7.6. Suggest a reason for this. (1 mark)

3) A student wanted to investigate the effect of temperature on the activity of amylase. To do this, they timed how long it took for an iodine solution to turn yellow-brown in the presence of starch and amylase at different temperatures.

Table 1 shows the results.

a) Explain why the iodine solution remained blue-black in the investigation at 75°C. (2 marks)

Temperature in °C	Time taken for solution to turn yellow-brown
15	5
30	2
45	7
60	12
75	Remained blue-black

Table 1

b) Explain how amylase breaks down starch. Answer in terms of the 'lock and key theory'. (3 marks)

c) Describe how the student could extend the investigation to determine the effect of a different factor on amylase activity. (2 marks)

Exam Style Questions - Enzymes

1) Amylase is the enzyme that controls the breakdown of starch to glucose. Describe how the student could investigate the effect of pH on the breakdown of starch by amylase. (4 marks)

One mark for each of the following:

- Range of at least 3 pH values / use of buffer solutions
- Named controlled variables (e.g. amount / concentration of starch or amylase, use water bath/electric heater to control temperature)
- Use iodine to make qualitative observations
- Observe colour changes at different pH
- Do repeats at each pH

2) Students investigated the effect of bile on the digestion of fat by lipase. They followed the following protocol:

- Mixed milk and bile in a beaker
- Put the pH sensor of a pH meter into the beaker
- Add lipase solution
- Recorded the pH at 2-minute intervals
- Repeated steps 1 to 4, but used water instead of bile.

a) Suggest two variables that the student should have controlled in this investigation. (2 marks)

One mark for each of the following up to a maximum of two marks:

- Type of milk
- Volume of milk
- Volume of bile and water (should be the same)
- Volume of lipase
- Concentration of glucose
- Temperature

b) They students found that by the end of each test the beaker with water and bile both had the same pH of 7.6. Suggest a reason for this. (1 mark)

One mark for any one of the following:

- All milk/fat has been digested
- The same amount of fatty acids present
- (Lower pH) denatures the enzyme/lipase (allow enzyme won't work at a low pH)
- All reactants used up

3) A student wanted to investigate the effect of temperature on the activity of amylase. To do this, they timed how long it took for an iodine solution to turn yellow-brown in the presence of starch and amylase at different temperatures.

Table 1 shows the results.

a) Explain why the iodine solution remained blue-black in the investigation at 75°C. (2 marks)

Mark one - Enzyme / amylase is denatured OR enzyme/amylase has stopped working

Mark two - Starch is broken down OR starch is still present

Temperature in °C	Time taken for solution to turn yellow-brown
15	5
30	2
45	7
60	12
75	Remained blue-black

Table 1

b) Explain how amylase breaks down starch. Answer in terms of the 'lock and key theory'. (3 marks)

Mark one - Starch / substrate binds to active site (of enzyme)

Mark two - Because shape of active site and substrate are complementary (allow shape of starch/substrate and active site allow them to fit together)

Mark three - A chemical reaction occurs to produce smaller molecules OR bonds between starch molecules are broken to produce smaller molecules (allow sugars/maltose)

c) Describe how the student could extend the investigation to determine the effect of a different factor on amylase activity. (2 marks)

Mark one - Keep temperature constant

Mark two - Change a named factor (e.g. pH, enzyme) and test a range of values of this factor (e.g. concentration, substrate, inhibitor concentration)