

pH and enzyme activity

investigate how enzyme activity can be affected by changes in pH

For Pearson Edexcel International GCSE Biology 4BI1 and Science (Double Award) 4SD0. **Biology only** This practical is not in the Double Award and is only examined on Paper 2. Teacher answers start on a new page.

Name

Date

Aim

To investigate how pH affects the time taken for amylase to break down starch.

Background

Amylase is an enzyme that breaks down starch. Like all enzymes it has an **active site** whose shape is held by bonds between the amino acids of the protein. The substrate fits into the active site, so the shape must be right for the enzyme to work.

Each enzyme works best at its **optimum pH**. As the pH moves away from the optimum, the extra hydrogen ions or hydroxide ions interfere with the bonds that hold the enzyme in shape, so the active site changes shape and the substrate fits less well. At extreme pH values the enzyme is **denatured** and the substrate no longer fits.

A **buffer solution** keeps its pH almost constant when the enzyme and starch are added. The time for all the starch to be broken down is found by adding samples of the mixture to iodine solution, which is blue-black with starch and stays orange-brown when no starch is left.

Hypothesis

Amylase will break down starch in the shortest time at about pH 7, and will take longer at lower and higher pH values because the active site changes shape and the enzyme is denatured.

Variables

Independent	pH of the reaction mixture (using buffer solutions of pH 3, 5, 7, 9 and 11)
Dependent	Time taken for the starch to be completely broken down (min), shown when the iodine stays orange-brown
Control	• Temperature (all tubes in one water bath at 35 °C) • Volume and concentration of the amylase solution • Volume and concentration of the starch solution • Volume of buffer solution added • Volume of the mixture sampled and the volume of iodine in each well

Equipment

- Starch solution (1%), 2 cm³ per tube
- Amylase solution (1%), 2 cm³ per tube
- Buffer solutions at pH 3, 5, 7, 9 and 11, 1 cm³ per tube
- Iodine solution in a dropping bottle
- Spotting tile with 6 or more wells
- Water bath at 35 °C (a 250 cm³ beaker of water on a thermostatic heater, or hot and cold water) and a thermometer
- Test tubes, test tube rack and marker pen

- Syringes or graduated pipettes (2 cm³ and 5 cm³)
- Dropping pipettes or glass rods for sampling
- Stopwatch or timer
- Eye protection

Risk assessment

Hazard	Risk	Precaution
Hot water at 35 °C and a kettle	Low risk of scalds if hot water is spilled while preparing the bath	Fill beakers no more than half full, stand them on a heatproof mat, and let kettle water cool before use.
Iodine solution	Irritates eyes and skin and stains clothes and skin	Wear eye protection; wash splashes off skin with water; clear spills straight away.
Buffer solutions at pH 3 and pH 11	Mild irritants to eyes and skin	Wear eye protection and wash any splashes off with plenty of water.
Amylase solution	Enzymes can cause allergic reactions, and the dust from powders can affect breathing	Use the solution (not powder), avoid contact with skin and eyes, and wash hands after the practical.

Method

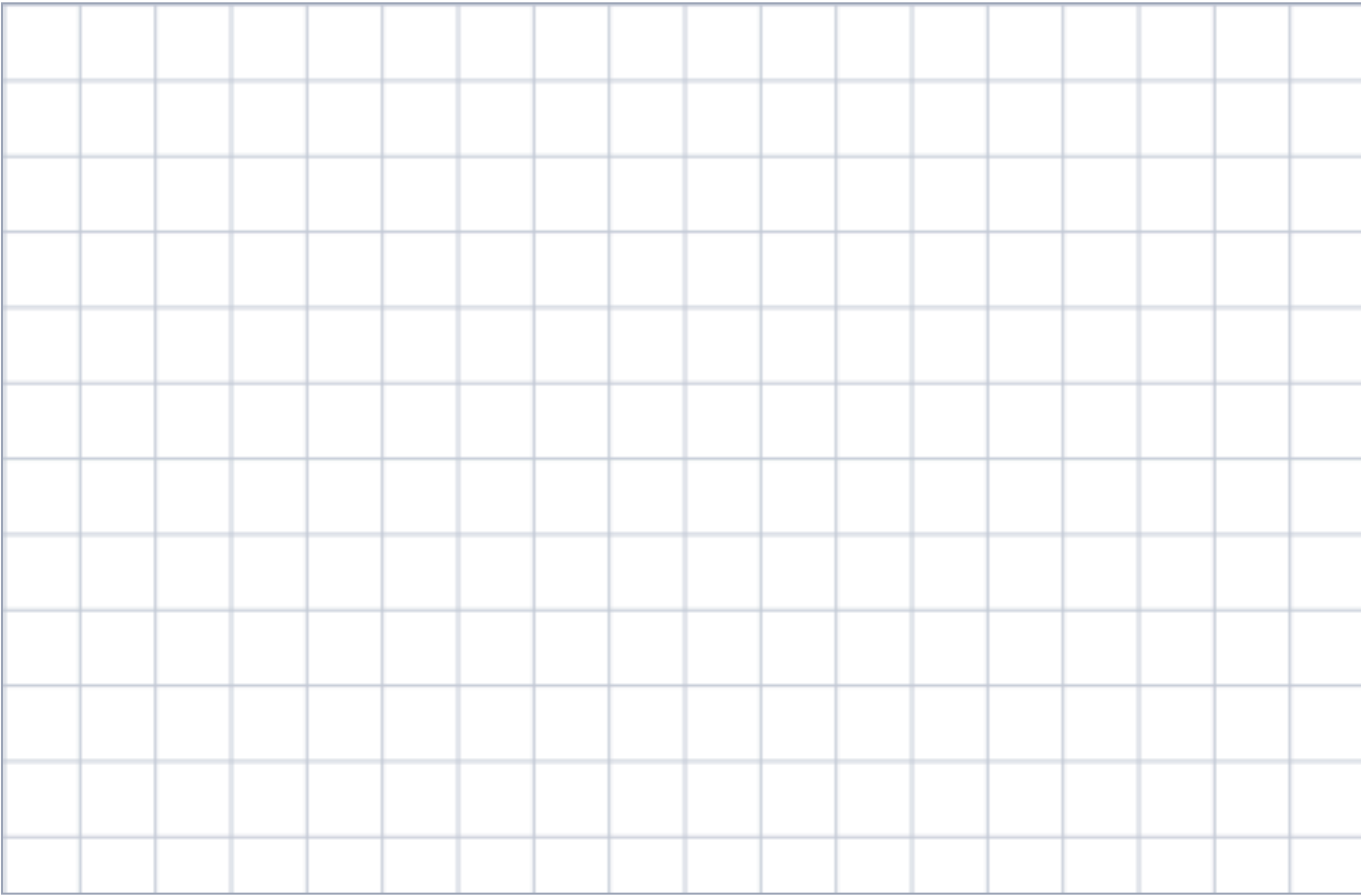
1. Put on eye protection. Set up a water bath at 35 °C and check the temperature with a thermometer.
2. Put one drop of iodine solution into each well of a spotting tile (at least 6 wells for each pH).
3. Label a test tube for each pH. Put 2 cm³ of starch solution into each tube, then add 1 cm³ of the matching buffer solution.
4. Label a second set of tubes and put 2 cm³ of amylase solution in each.
5. Stand the starch and buffer tube for the first pH and its tube of amylase in the water bath for 5 minutes.
6. Pour the amylase into the starch and buffer tube, mix, return it to the water bath and start the stopwatch at once.
7. After 30 seconds, use a clean dropping pipette to transfer one drop of the mixture into the first well of iodine solution. Record the colour.
8. Take a further drop every 30 seconds and add it to the next well. Continue until the iodine stays orange-brown.
9. Record the time when the iodine first stays orange-brown. If it is still blue-black after 20 minutes, record '>20 min'.
10. Repeat steps 5–9 for each of the other pH values, using fresh tubes and a clean pipette each time.
11. Repeat the whole investigation at least twice more and calculate the mean time at each pH.
12. Calculate the rate at each pH as $1 \div \text{mean time (min}^{-1}\text{)}$ and plot a graph of rate against pH.

Results

Time for starch to be broken down by amylase at different pH values					
pH	Time 1 (min)	Time 2 (min)	Time 3 (min)	Mean time (min)	Rate, 1 ÷ mean time (min ⁻¹)
3					
5					
7					
9					
11					

Graph

Plot rate (1 ÷ mean time, min⁻¹) on the y-axis against pH on the x-axis. Mark each point with a cross and draw a smooth curve of best fit through them. Read off the pH with the highest rate: this is the optimum pH for the amylase to the accuracy of the pH values tested.



Exam questions 10 questions · 29 marks

- 1

State the independent variable and the dependent variable in this investigation.

[2 marks]
-

2 Name two variables that the student must keep the same in every tube. **[2 marks]**

.....

.....

.....

.....

3 Explain why the student uses buffer solutions rather than adding an acid or alkali to change the pH. **[2 marks]**

.....

.....

.....

.....

4 The mean time for the starch to be broken down at pH 9 was 4.5 minutes. Calculate the rate of reaction, using $\text{rate} = 1 \div \text{time}$. Give your answer to 2 significant figures. **[2 marks]**

Answer =

.....

.....

.....

.....

5 Use the table to describe the effect of pH on the time taken for starch to be broken down. **[3 marks]**

Time for starch to be broken down	
pH	Mean time (min)
3	12.7
5	3.3
7	1.2
9	4.5
11	16.8

.....

.....

.....

.....

.....

10 Explain how a change in pH affects the structure and function of an enzyme such as amylase. **[6 marks]**

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

Teacher answers

Practical 3: pH and enzyme activity. The example results are practice data, not real measurements.

Example results

Example results (practice data). Rate is calculated from the mean time as shown.

pH	Time 1 (min)	Time 2 (min)	Time 3 (min)	Mean time (min)	Rate, $1 \div \text{mean time (min}^{-1}\text{)}$
3	13.5	12.0	12.5	12.7	0.08
5	3.0	3.5	3.5	3.3	0.30
7	1.0	1.5	1.0	1.2	0.83
9	4.0	4.5	5.0	4.5	0.22
11	17.0	15.5	18.0	16.8	0.06

Conclusion

The starch was broken down fastest at pH 7 (mean time 1.2 min, rate 0.83 min^{-1}), so this amylase has an optimum pH close to 7, although it may lie between pH 5 and pH 9 because no pH values in between were tested. As the pH moved further from 7 in either direction the time increased, so the rate fell, with the slowest reactions at pH 3 (12.7 min) and pH 11 (16.8 min). Acid or alkaline conditions disrupt the bonds that hold the enzyme's shape, so the **active site** changes shape and the enzyme is **denatured**. Fewer starch molecules fit into the active sites, so the reaction is slower. The results support the hypothesis. Testing pH 6 and pH 8 would locate the optimum more precisely.

Errors and improvements

Error	Effect on the results	Improvement
The buffer may not hold the pH exactly when the enzyme and starch are added	The actual pH of the mixture differs from the buffer value, so the optimum is misplaced	Use fresh buffers, add enough buffer, and check the pH of the final mixture with a pH meter.
Sampling every 30 seconds, so end-point times are approximate	Fast reactions (around pH 7) cannot be distinguished accurately	Sample every 10 or 15 seconds near the end-point, or use a colorimeter to follow the loss of starch.
Temperature of the water bath changes during the investigation	Different tubes react at different temperatures, so the pH is not the only variable that changes	Use a thermostatically controlled water bath and check with a thermometer; put tubes in the bath for 5 minutes before mixing.
Judging the end-point colour by eye	Random error in deciding when the iodine stays orange-brown	Compare with an orange-brown drop of iodine on the tile; have the same person judge each time; repeat and calculate a mean.
Only five pH values are tested	The optimum pH cannot be placed precisely, and the shape of the curve between points is uncertain	Test more pH values close to the peak, such as pH 6 and pH 8.

Mark scheme

One mark for each point unless a maximum is given. Words in brackets are not needed for the mark.

1. [2]

- independent: pH (of the buffer solution) (1)
- dependent: time taken for the starch to be broken down / for the iodine to stay orange-brown (1)

2. [2]

- temperature (1)
- volume / concentration of enzyme (1)
- volume / concentration of starch (1)
- volume of buffer (1)
- Max 2

3. [2]

- a buffer keeps the pH constant / at a known value (1)
- adding acid or alkali gives a pH that is hard to control / the pH could change when the starch and enzyme are added or during the reaction (1)

4. [2]

- $1 \div 4.5$ (1)
- $0.22 \text{ (min}^{-1}\text{)}$ (1) award 2 marks for the correct answer with no working; allow 0.222 for 1 mark only

5. [3]

- shortest time at pH 7 (1)
- time increases as the pH gets lower or higher than 7 (1)
- longest time at pH 11 / correct use of data, for example 16.8 min at pH 11 and 12.7 min at pH 3 (1)

6. [3]

- the enzyme / amylase is denatured (1) reject enzyme killed
- the shape of the active site changes (1)
- starch (substrate) no longer fits / fewer enzyme-substrate complexes form (1)

7. [2]

- only pH values 5, 7 and 9 were tested close to the peak / values between were not tested (1)
- the optimum could be anywhere between pH 5 and pH 9 / test pH 6 and 8 to confirm (1)

8. [1]

- the end-point is judged objectively / not by eye (1)
- it gives a continuous / more accurate measure of starch remaining over time (1)
- Max 1

9. [6]

Level 3 5-6	A logical method covering buffer solutions of at least four pH values, mixing of enzyme and starch at a controlled temperature, use of iodine and timing to the end-point, control of at least two variables, and repeats with a mean
Level 2 3-4	A method with most of the key steps, with limited control of variables or reliability, or one key step missing
Level 1 1-2	A basic method with little detail, for example 'add acid and see how fast it works'

Indicative content

- label tubes for each pH; add starch and buffer; equal volumes of amylase; place in a water bath at a fixed temperature, for example 35 °C, for 5 minutes; mix and start a stopwatch; sample every 30 s into iodine on a spotting tile; end-point when the iodine stays orange-brown; record time; repeat and calculate a mean; control temperature, volumes and concentrations; rate = $1 \div \text{time}$

10. [6]

Level 3 5-6	A full explanation linking pH change to bonds in the protein, change in active site shape, denaturation and reduced substrate binding, and mentioning the optimum pH
Level 2 3-4	Explains that the shape changes and the substrate no longer fits, but without clear links to the bonds or the optimum
Level 1 1-2	States that the enzyme stops working at the wrong pH with little explanation

Indicative content

- each enzyme has an optimum pH; enzyme is a protein with a specific 3D shape; shape held by bonds between amino acids; extreme pH (too acidic or alkaline) breaks these bonds; shape of the active site changes; enzyme is denatured; substrate no longer fits / fewer enzyme-substrate complexes; reaction slows or stops

Exam tips

- The pH curve has a peak, not a plateau. Describe it as 'rate increases to an optimum then decreases' and quote data from the table or graph.
- Say that the enzyme is 'denatured' because the 'active site changes shape'. Do not say the enzyme is 'killed' or 'dead'.
- Do not mix up the two practicals. Below the optimum temperature, explain with kinetic energy and collisions; for pH, explain that the change in pH affects the bonds that hold the active site in shape.
- If asked why a buffer is used, say it keeps the pH constant, so that pH is the only variable that changes.
- Not all enzymes have the same optimum pH. Pepsin in the stomach works best at about pH 2, for example, while amylase works best at about pH 7.