

The effect of pH on enzyme activity

Investigate the effect of pH on enzyme activity

For Edexcel GCSE Biology 1BI0 and Combined Science 1SC0. Teacher answers start on a new page.

Name

Date

Aim

To investigate how pH affects the time taken for amylase to break down starch, and to find the optimum pH for the enzyme.

Background

Enzymes are biological catalysts. Each enzyme has an **active site** with a specific shape that fits its **substrate**. **Amylase** is an enzyme that breaks down the substrate starch into the sugar maltose.

The **pH** of the solution affects the bonds that hold the enzyme in shape. At an extreme pH the shape of the active site changes, the substrate no longer fits and the enzyme is **denatured**. Each enzyme works fastest at its **optimum pH**.

Starch turns iodine solution from orange-brown to blue-black. When all of the starch has been broken down, a drop of the mixture no longer turns iodine blue-black. The time taken for this to happen is a measure of the enzyme activity: the shorter the time, the faster the reaction.

A **buffer solution** keeps the pH of the mixture constant, so pH is the only thing that changes. The rate of reaction can be calculated from the time: $\text{rate} = 1 \div \text{time}$.

Hypothesis

The starch will be broken down fastest at about pH 7, because amylase from saliva has an optimum pH close to neutral. At pH values further from this, the shape of the active site will change and the enzyme will work more slowly.

Variables

Independent	pH of the mixture (using buffer solutions of different pH values)
Dependent	Time taken for all the starch to be broken down (s), shown when iodine solution stays orange-brown
Control	• Temperature (35 °C, using a water bath) • Volume and concentration of amylase solution • Volume and concentration of starch solution • Volume of buffer solution • Volume of iodine solution in each well of the spotting tile • Time between samples (10 s)

Equipment

- Amylase solution, 1% (2 cm³ per test)
- Starch solution, 1% (2 cm³ per test)
- Buffer solutions at pH 4, 5, 6, 7, 8 and 9 (2 cm³ per test)
- Iodine solution in a dropper bottle

- Spotting tile
- Water bath set at 35 °C
- Thermometer
- Test tubes and test tube rack
- Three 5 cm³ syringes, or graduated pipettes, for measuring volumes
- Dropping pipettes or glass rods
- Stopwatch
- Marker pen for labelling tubes
- Eye protection

Risk assessment

Hazard	Risk	Precaution
Iodine solution	Irritates eyes and skin and stains skin and clothing.	Wear eye protection. Wipe up spills at once. Wash hands if iodine gets on skin.
Amylase	Enzymes can irritate the eyes and skin, and dust or splashes can trigger an allergic reaction in some people.	Wear eye protection. Use solutions only, not powder. Wash hands after the practical.
Buffer solutions at pH 4 and pH 9	May irritate eyes.	Wear eye protection. Rinse splashes from skin with water.
Water bath and glassware	Warm water can spill. Broken glass can cause cuts.	Keep the water bath away from the table edge and mop up spills to prevent slips. Tell the teacher if glass breaks.

Method

1. Put on eye protection. Set the water bath to 35 °C and check with a thermometer.
2. Label six test tubes with the pH values 4 to 9. Put 2 cm³ of starch solution in each tube and add 2 cm³ of the matching buffer solution.
3. Label six more test tubes with the pH values and put 2 cm³ of amylase solution in each.
4. Put drops of iodine solution into the wells of a spotting tile. Use one row of wells for each pH, with a drop in every well.
5. Stand the tubes in the water bath for 5 minutes, so that the contents reach 35 °C.
6. Start with pH 7. Add the 2 cm³ of amylase to the starch and buffer mixture, mix with a clean dropping pipette and start the stopwatch at once.
7. After 10 s, use the dropping pipette to put one drop of the mixture into the first iodine well. Record the colour.
8. Repeat every 10 s with a new well each time, until the drop of the mixture no longer turns blue-black (it stays orange-brown).
9. Record the time when the iodine first stays orange-brown. This is the time taken for all the starch to be broken down.
10. Rinse the pipette. Repeat steps 6 to 9 for each of the other pH values, using the correct tubes and a fresh row of iodine wells each time.
11. Repeat the whole experiment at least once more and calculate the mean time for each pH.
12. Calculate the rate of reaction for each pH: rate = $1 \div \text{mean time}$, in s⁻¹. To avoid very small numbers, multiply by 1000 and record the rate in $\times 10^{-3} \text{ s}^{-1}$ (for example, $1 \div 40 \text{ s} = 0.025 \text{ s}^{-1} = 25 \times 10^{-3} \text{ s}^{-1}$).
13. Plot a graph of rate against pH and draw a smooth curve through the points.

Results

Time taken for all the starch to be broken down at different pH values				
pH	Time taken, trial 1 (s)	Time taken, trial 2 (s)	Mean time (s)	Rate ($\times 10^{-3} \text{ s}^{-1}$)
4				
5				
6				
7				
8				
9				

Graph

Plot a line graph with pH on the x-axis (4 to 9) and rate of reaction ($\times 10^{-3} \text{ s}^{-1}$) on the y-axis, starting from zero. Plot each mean point with a small cross and join them with a smooth curve (not ruled lines between points). The peak of the curve shows the optimum pH.



Exam questions 10 questions · 27 marks

1 A student investigates the effect of pH on the time taken for amylase to break down starch. State the independent variable and the dependent variable in the investigation. [2 marks]

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2 The tubes were kept in a water bath at 35 °C during the investigation. Explain why. **[2 marks]**

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3 The student used buffer solutions. Explain why buffer solutions were used. **[2 marks]**

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4 The amylase solution and the starch solution were each warmed in the water bath for 5 minutes before they were mixed. Explain why. **[2 marks]**

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5 Describe how the student would know that all the starch had been broken down. **[2 marks]**

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6 At pH 6, the times taken in two trials were 70 s and 80 s. Use the mean time to calculate the rate of reaction in s⁻¹. Give your answer to 2 significant figures. **[3 marks]**

Answer =

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- 7** The table shows some of a student's results. State the optimum pH shown by the results. **[3 marks]**
Give a reason for your answer.

Mean time taken for starch to be broken down	
pH	Mean time (s)
5	125
6	75
7	40
8	85

- 8** The mean time taken at pH 4 was 260 s. Explain why this is longer than the time taken at pH 7. **[3 marks]**

- 9** Suggest two improvements to the investigation that would make the results more accurate. **[2 marks]**

10 Describe a method to investigate the effect of pH on the activity of amylase. Include the apparatus you would use and the variables you would control. **[6 marks]**

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Teacher answers

Core practical 2: The effect of pH on enzyme activity. The example results are practice data, not real measurements.

Example results

Example results (practice data)				
pH	Time taken, trial 1 (s)	Time taken, trial 2 (s)	Mean time (s)	Rate ($\times 10^{-3} \text{ s}^{-1}$)
4	250	270	260	3.8
5	120	130	125	8.0
6	70	80	75	13.3
7	40	40	40	25.0
8	80	90	85	11.8
9	190	210	200	5.0

Conclusion

The starch was broken down fastest at pH 7 (mean time 40 s, rate $25.0 \times 10^{-3} \text{ s}^{-1}$), so the optimum pH for this amylase is about 7. The time got longer, and the rate got slower, as the pH became more acidic or more alkaline. For example, at pH 4 the mean time was 260 s, which is 6.5 times longer than at pH 7. At pH values away from the optimum, the bonds that hold the enzyme in shape are affected. The shape of the active site changes, so the starch substrate no longer fits as well and fewer enzyme-substrate complexes form. At extreme pH the enzyme is denatured. The results agree with the hypothesis, but because only whole-number pH values were tested, the true optimum could be anywhere between about pH 6.5 and 7.5.

Errors and improvements

Error	Effect on the results	Improvement
The 10 s sampling interval is long compared with the shortest time (40 s).	The end time is only known to within 10 s, so the fastest rates are the least precise (a 10 s uncertainty in 40 s is 25%).	Sample more often (for example every 5 s), or use a colorimeter to follow the colour change continuously.
Deciding when the iodine stays orange-brown is a judgement by eye (human error).	Different people may record different end times, so results vary.	Compare each well with a white tile and a known orange-brown standard, and have the same person judge each time.
The enzyme starts working as soon as it touches the starch, and mixing takes time.	The start time is not exact.	Mix thoroughly and quickly in the same way each time, and start the stopwatch at the instant the enzyme is added.
Temperature of the mixture changing during the experiment, for example when taking samples out of the water bath.	Enzyme activity changes with temperature, so the results at one pH could be affected.	Leave the tubes in the water bath between samples and check the thermometer reading.
Only six pH values were tested and only two repeats.	The optimum is not located precisely and one anomalous result could change the mean.	Test more pH values near the optimum (for example 6.5, 7.0, 7.5) and repeat at least three times, ignoring anomalies when calculating the mean.

Mark scheme

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

1. [2]

- independent variable: pH (1)
- dependent variable: time taken for the starch to be broken down (1)

2. [2]

- temperature affects the rate of enzyme activity / enzymes are affected by temperature (1)
- so temperature is kept constant, to make sure it does not affect the results / so only pH changes (1)

3. [2]

- a buffer keeps the pH constant (1)
- so the pH does not change when the reaction happens / so the pH of each mixture stays at the value being tested (1)

4. [2]

- so both solutions are at the same, correct temperature before the reaction starts (1)
- because the reaction starts as soon as they are mixed / so the temperature of the mixture is 35 °C from the start (1)

5. [2]

- a drop of the mixture added to iodine solution (1)
- the iodine stays orange-brown / does not turn blue-black (1)

6. [3]

- mean time = $(70 + 80) \div 2 = 75 \text{ s}$ (1)
- rate = $1 \div \text{mean time}$, or $1 \div 75$ (1)
- 0.013 s^{-1} (1) allow 1.3×10^{-2} ; do not accept 0.0133 (not 2 significant figures) for the third mark
- Correct answer with no working gains 3 marks

7. [3]

- pH 7 (1)
- shortest time to break down the starch (1) allow quickest
- so the enzyme works fastest / highest rate of reaction at this pH (1)
- ignore 'the enzyme works best'

8. [3]

- pH 4 is far from the optimum pH of the enzyme (1)
- the shape of the active site changes / the enzyme is denatured (1) do not accept 'the enzyme is killed'
- so the starch (substrate) no longer fits the active site / fewer enzyme-substrate complexes form (1)

9. [2]

- take samples more often / at shorter time intervals (1)
- use a colorimeter to measure the colour change (1)
- test more pH values close to the optimum / smaller steps between pH values (1)
- compare colours against a standard / same person judges the end point (1)
- Max 2
- ignore 'repeat more times' (this improves repeatability, not accuracy)

10. [6]

Level 3 5-6	A clear, logical method that could be followed to give valid results. It states how pH is changed, how the reaction is timed or judged, and at least two control variables with how they are controlled. Repeats and a mean are included.
Level 2 3-4	A method with most of the main steps, but missing some detail such as how the end point is detected, how variables are controlled or repeats.
Level 1 1-2	A basic method with a few relevant points and little order.

Indicative content

- mix starch and buffer solutions of different pH values in separate tubes; add amylase and start a stopwatch; at regular intervals put a drop into iodine solution on a spotting tile; record the time when iodine stays orange-brown / no longer turns blue-black; control temperature with a water bath; control volume and concentration of amylase and starch; repeat and calculate a mean; calculate rate = $1 \div \text{time}$ and plot rate against pH.
- mix starch solution and a buffer solution of known pH in a test tube; use a range of buffers, for example pH 4 to 9; warm the tubes of starch-buffer and amylase in a water bath, for example at 35°C , before mixing; add amylase and start a stopwatch; every 10 s put a drop of the mixture into iodine solution on a spotting tile; record the time when iodine stays orange-brown / no longer turns blue-black; control temperature with a water bath; control volume and concentration of amylase and starch (same syringe or pipette); repeat at each pH and calculate a mean; calculate rate = $1 \div \text{time}$ and plot rate against pH.

Exam tips

- Learn the colour change for starch with iodine: orange-brown to blue-black. In this practical, the end point is when the colour stays orange-brown.
- Say the enzyme is denatured and the active site changes shape. Do not say the enzyme is killed, because enzymes are not alive.
- Describe a buffer as keeping the pH constant. Do not say it makes the pH 7.
- For rate questions, the time and the rate are inverse: a shorter time is a faster rate. $\text{Rate} = 1 \div \text{time}$.
- In graph questions, a smooth curve through the points shows an optimum. Do not join the dots with straight lines.