

The effect of pH on amylase

Investigate the effect of pH on the rate of reaction of amylase enzyme.

For AQA GCSE Biology 8461 and Combined Science: Trilogy 8464. Teacher answers start on a new page.

Name

Date

Aim

To find how pH affects the time taken for amylase to digest starch, using a continuous sampling technique with iodine solution.

Background

Enzymes are biological catalysts. **Amylase** breaks down starch into sugars (maltose). Each enzyme has an **active site** with a shape that fits its substrate. The substrate must collide with the enzyme and fit into the active site for the reaction to happen.

Each enzyme works best at a particular pH, called its **optimum pH**. If the pH is too high or too low, the bonds that hold the enzyme in shape break and the active site changes shape. The substrate no longer fits and the enzyme is **denatured**.

To follow the reaction, you test the mixture for starch using iodine solution. Starch turns iodine from orange-brown to blue-black. When all of the starch has been digested, the iodine stays orange-brown. This is called a **continuous sampling** technique, because you take samples from the same reaction mixture repeatedly. The shorter the time, the faster the rate of reaction.

Hypothesis

Amylase will digest the starch fastest at about pH 7 and more slowly at higher and lower pH, because the active site of the enzyme is changed when the pH is not at the optimum.

Variables

Independent	The pH of the buffer solution
Dependent	The time taken for all of the starch to be digested (s). The rate of reaction is $1 \div \text{time}$
Control	• Temperature (by using a water bath at 35 °C) • Volume and concentration of starch solution • Volume and concentration of amylase solution • Volume of buffer solution • Size of the drop of mixture added to the iodine

Equipment

- 1% amylase solution
- 1% starch solution
- Buffer solutions of pH 4, 5, 6, 7 and 8
- Iodine solution in a dropping bottle
- Spotting tile (or a white tile with dimples)
- Test tubes, a test tube rack and a marker pen
- Water bath set to 35 °C and a thermometer
- Syringes (or graduated pipettes) for measuring 2 cm³, one for each solution
- Dropping pipettes or glass rods for transferring samples

- Stopwatch

- Eye protection

Risk assessment

Hazard	Risk	Precaution
Amylase solution (enzyme)	Enzymes can irritate the skin and eyes, and may cause an allergic reaction.	Wear eye protection and wash off any splashes with water. Do not breathe in any dust or spray.
Iodine solution	It stains skin and clothing and irritates the eyes.	Wear eye protection. Wipe up spills and wash splashes off the skin with water.
Warm water bath	Spills of warm water and electrical hazard near water.	Keep the electrical equipment dry, and mop up spills straight away. Do not let the water boil.
Spills on the floor	Slipping.	Wipe up spills at once.

Method

1. Set a water bath to 35 °C. Using a pipette, put one drop of iodine solution into each dimple of a spotting tile (as many dimples as the number of samples you will take).
2. Use a syringe to put 2 cm³ of starch solution into a test tube. Use a different syringe to add 2 cm³ of pH 4 buffer solution. Label the tube with the pH.
3. In a second test tube, put 2 cm³ of amylase solution.
4. Stand both test tubes in the water bath for 5 minutes so that the solutions reach 35 °C.
5. Pour the amylase into the starch and buffer tube, mix by gently swirling and start the stopwatch straight away. Keep the tube in the water bath.
6. After 30 seconds, use a clean dropping pipette to remove one drop of the mixture. Add it to a drop of iodine solution on the spotting tile and record the colour.
7. Take another sample every 30 seconds and add it to a fresh drop of iodine. Continue until the iodine stays orange-brown. This is the end point.
8. Record the time at which the iodine first stayed orange-brown. If it is still blue-black after 10 minutes, record '> 600'.
9. Repeat steps 2 to 8 using buffer solutions of pH 5, 6, 7 and 8. Use clean syringes and put fresh drops of iodine on a clean spotting tile each time.
10. Repeat the whole experiment two more times so that you have three results for each pH.
11. Calculate the mean time for each pH. Calculate the rate of reaction for each pH: rate = 1 ÷ mean time, in s⁻¹.
12. Plot a graph of rate of reaction against pH. Draw a smooth curve through the points and read off the optimum pH.

Results

Time taken for the starch to be completely digested					
pH of buffer	Time, trial 1 (s)	Time, trial 2 (s)	Time, trial 3 (s)	Mean time (s)	Rate = 1 ÷ mean time (s ⁻¹)
4					
5					
6					
7					
8					

Graph

Plot a line graph. Put the pH of the buffer on the x-axis (4 to 8) and the rate of reaction (1 ÷ mean time, in s⁻¹) on the y-axis. Plot each point with a small cross and draw a smooth curve (not straight lines joining the points) that rises to a peak and falls again. Mark the optimum pH at the peak of the curve.



Exam questions 10 questions · 25 marks

1 State the independent variable and the dependent variable in this investigation. [2 marks]

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2 A buffer solution is added to each tube. Explain why. **[1 mark]**

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3 The test tubes are kept in a water bath at 35 °C. Explain why the temperature must be controlled. **[2 marks]**

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4 Every 30 seconds a drop of the mixture is added to iodine solution. Describe what the student sees when all of the starch has been digested, and explain why. **[2 marks]**

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5 In one trial the starch was completely digested in 50 seconds. Calculate the rate of reaction using the equation: $\text{rate} = 1 \div \text{time}$. Give the unit. **[2 marks]**

Answer =

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6 The table shows the time taken to digest starch at four different pH values. State the optimum pH for the enzyme and give a reason for your answer. **[2 marks]**

Time taken for starch to be digested	
pH	Time taken (s)
5	300
6	120
7	60
8	180

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7 Explain why the starch took much longer to digest at pH 4 than at pH 7. **[4 marks]**

8 The samples were taken every 30 seconds. Suggest how this limits the results and how the method could be improved. **[2 marks]**

9 The starch and the amylase are put in the water bath separately for 5 minutes before they are mixed. Explain why. **[2 marks]**

10 Describe a method to investigate how pH affects the time taken for amylase to digest starch. Include how you would make the investigation a fair test. **[6 marks]**

Teacher answers

Required practical 5: The effect of pH on amylase. The example results are practice data, not real measurements.

Example results

Example results (practice data): the starch had not been digested after 600 s at pH 4

pH of buffer	Time, trial 1 (s)	Time, trial 2 (s)	Time, trial 3 (s)	Mean time (s)	Rate = $1 \div \text{mean time (s}^{-1}\text{)}$
4	> 600	> 600	> 600	> 600	< 0.0017
5	240	270	240	250	0.0040
6	90	90	120	100	0.010
7	60	60	60	60	0.017
8	150	120	150	140	0.0071

Conclusion

The time taken to digest the starch was shortest at pH 7 (60 s), so the rate of reaction was highest at pH 7. The rate was lower at pH 6 and pH 8 and much lower at pH 5. At pH 4 no digestion was seen in 600 s. This shows that the optimum pH for this amylase is about 7. At pH values above or below the optimum, the shape of the active site changes. The enzyme is denatured, so the starch no longer fits the active site and is broken down more slowly or not at all. This supports the hypothesis. The values are only accurate to the nearest 30 s, because the samples were taken at 30 s intervals.

Errors and improvements

Error	Effect on the results	Improvement
Samples are only taken every 30 seconds.	The end point time is only accurate to the nearest 30 s, so differences between close pH values may not be seen.	Take samples more often (for example every 10 s), or use a colorimeter to measure the loss of the blue-black colour.
It is hard to judge when the iodine is orange-brown and not very pale blue-black (human judgement).	The end point may be recorded too early or too late, and different people may disagree.	Compare each drop with a reference drop of iodine, or use the same person each time. Use a white tile to see the colour clearly.
The enzyme and starch are not at the same temperature when they are mixed.	The temperature may alter the rate, so pH is not the only variable that changes.	Keep all solutions in the 35 °C water bath for 5 minutes before mixing and keep the mixture in the water bath.
Each sample taken removes some of the mixture.	The volume gets smaller, which could change the rate slightly.	Take drops of the same size and a small volume each time, or start with a larger volume.
Only one trial is done at each pH.	Anomalous results cannot be identified.	Repeat at least three times and calculate a 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: pH (of the buffer) (1)
- dependent: time taken for the starch to be digested / disappear (1)

2. [1]

- to keep the pH constant / at a set value (1)

3. [2]

- temperature affects the rate of reaction / the activity of the enzyme (1)
- so that only the pH affects the rate / it is a control variable (1)

4. [2]

- iodine stays orange-brown (1)
- because there is no starch left to turn it blue-black (1)

5. [2]

- $1 \div 50$ (1)
- 0.02 per second / 0.02 s^{-1} / $0.02 / \text{s}$ (1)

6. [2]

- pH 7 (1)
- it has the shortest time / the fastest rate of reaction (1)

7. [4]

- pH 4 is far from the optimum pH of the enzyme (1)
- the bonds that hold the enzyme in shape break / the shape of the active site changes (1)
- the enzyme is denatured (1)
- the substrate no longer fits the active site / fewer enzyme-substrate complexes form (1)

8. [2]

- time is only accurate to the nearest 30 s / differences between close pH values cannot be seen (1)
- take samples more often / every 10 s / use a colorimeter (1)

9. [2]

- so both reach the required temperature before the reaction starts (1)
- so that the reaction takes place at the same temperature from the beginning / temperature is controlled (1)

10. [6]

Level 3 5-6	A clear, logical method using a range of pH values, continuous sampling with iodine and a timer, with an end point stated. At least three variables are controlled and the results are processed or repeated.
Level 2 3-4	A method with most of the main steps (range of pH, samples tested with iodine, time recorded) and at least one variable controlled. Some detail may be missing.
Level 1 1-2	A few relevant points, for example mixing starch and amylase and testing with iodine, with little detail or control.

Indicative content

- put starch solution and a buffer of known pH in a tube and amylase in another tube; place both in a water bath at constant temperature
- mix the amylase with the starch and buffer and start a stopwatch
- every 30 seconds take a sample and add it to a fresh drop of iodine on a spotting tile
- end point: when the iodine stays orange-brown (no starch is left); record the time
- repeat for a range of pH values (for example pH 4 to 8) using a different buffer each time
- keep volumes and concentrations of starch, amylase and buffer the same, and the temperature constant
- repeat to calculate a mean time; rate = $1 \div \text{time}$; plot rate against pH to find the optimum

Exam tips

- The end point is when the iodine stays orange-brown, because all the starch has gone. Do not say 'the iodine turns clear'.
- A buffer keeps the pH constant. Say this clearly when asked why a buffer solution is used.
- At the wrong pH the enzyme is denatured: the shape of the active site changes and the substrate no longer fits. Do not say the enzyme is 'killed' (enzymes are not alive).
- A shorter time means a faster rate. Rate = $1 \div \text{time}$, and a graph of rate against pH should be a smooth curve with a peak at the optimum.
- Temperature must be controlled using a water bath or electric heater. Say that the starch and amylase are brought to the temperature first.