pH and the rate of an enzyme reaction

Answers, marking points and revision notes | AQA Combined Science 8464 and AQA GCSE Biology 8461

GCSE science required practicals

This practical online

Answers

1 Name the independent variable and the dependent variable in this investigation. (2 marks)

The independent variable is the pH. The dependent variable is the time taken for the starch to be broken down (which is used to find the rate).

Marking points

- independent: pH
- dependent: time for starch to disappear / rate of reaction

2 The tubes were kept in a water bath at 35 °C. Explain why the temperature had to be the same at every pH. (2 marks)

Temperature also changes the rate of an enzyme reaction. If the temperature changed between tests, the time would change for reasons other than pH, so it would not be a fair test.

Marking points

- temperature also affects the rate of enzyme activity
- so only pH should be changing / to make it a fair test

3 Describe how the students knew that all the starch had been broken down. Give the colours. (2 marks)

While starch is still there, a drop of the mixture turns the iodine blue-black. When a drop leaves the iodine orange-brown, all the starch has gone.

Marking points

- iodine turns blue-black while starch is present
- iodine stays orange-brown when the starch has all been broken down

4 Give **one** hazard in this practical and describe how to reduce the risk. (2 marks)

Iodine solution can irritate the eyes. Wear eye protection when adding drops to the spotting tile.

Marking points

- a hazard, e.g. iodine is an irritant / enzymes can cause allergies / broken glass
- a matching control, e.g. eye protection, avoid skin contact, keep tubes in a rack

5 Look at the results for pH 8. Identify the anomalous result and give one possible reason for it. (2 marks)

160 s on trial 3 is anomalous. A possible reason is that the students did not mix the amylase and starch properly, so the reaction was slower, or they used less amylase by mistake.

Marking points

- 160 s (trial 3) identified
- a sensible reason, e.g. poor mixing, wrong volume of amylase or starch, missed a sample, tube not warmed up

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6 Calculate the value of X, the mean time at pH 8. Leave out the anomalous result. (2 marks)

(90 + 100) ÷ 2 = 95 s

mean = (90 + 100) ÷ 2

mean = 190 ÷ 2

X = 95 s

Marking points

- uses only 90 and 100
- 95 s

7 Calculate the rate of reaction at pH 6 using rate = 1000 ÷ mean time. Use the mean time in the table. Give your answer to 3 significant figures. (2 marks)

1000 ÷ 73 = 13.69... = 13.7 (arbitrary units)

rate = 1000 ÷ mean time

rate = 1000 ÷ 73

rate = 13.698...

rate = 13.7 arbitrary units (3 significant figures)

Marking points

- 1000 ÷ 73
- 13.7 (allow 13.6 from the unrounded mean of 73.3 s)

8 A different way to work out a rate is rate = 1 ÷ time. Calculate the rate at pH 5 this way. Give your answer to 2 significant figures. (2 marks)

1 ÷ 147 = 0.0068 s^{-1}

rate = 1 ÷ mean time

rate = 1 ÷ 147

rate = 0.006802...

rate = 0.0068 s^{-1} (2 significant figures)

Marking points

- 1 ÷ 147
- 0.0068 s^{-1}

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9 **Higher tier**

Each test used 5 cm^3 of 1% starch solution, which contains 0.05 g of starch.

Calculate the mean rate at which starch was broken down at pH 7, in g/s. Give your answer in standard form to 2 significant figures. Show your working. (3 marks)

Rate = 0.05 ÷ 47 = 0.001063... g/s = 1.1×10^{-3} g/s

rate = mass of starch broken down ÷ time taken

rate = 0.05 g ÷ 47 s

rate = 0.001063... g/s

rate = 1.1×10^{-3} g/s (2 significant figures)

Marking points

- rate = mass of starch ÷ time
- 0.05 ÷ 47 = 0.001063...
- 1.1×10^{-3} g/s

10 Describe the effect of pH on the rate of the reaction. Use data from the table in your answer. (3 marks)

As the pH rises from 4 to 7 the time gets shorter, from 290 s to 47 s, so the rate increases. The fastest rate is at pH 7, so the optimum pH is about 7. Above pH 7 the time gets longer again, up to 230 s at pH 9, so the rate decreases.

Marking points

- rate increases from pH 4 up to pH 7
- fastest / optimum at about pH 7
- rate decreases above pH 7, with data quoted (e.g. 290 s at pH 4, 47 s at pH 7, 230 s at pH 9)

11 **Higher tier**

Explain why the reaction was very slow at pH 4. (3 marks)

At pH 4 the conditions are far from the optimum pH of amylase. The bonds holding the enzyme in shape are affected, so the shape of the active site changes. Starch molecules no longer fit the active site as well, so fewer enzyme-substrate complexes form and the starch is broken down more slowly.

Marking points

- pH 4 is far from the optimum / the enzyme starts to denature
- the shape of the active site changes
- the substrate (starch) no longer fits, so fewer enzyme-substrate complexes form

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12 The students took a sample every 10 seconds.

a. Explain why this limits how accurate the times are.

b. Suggest an improvement and explain why it is better.

(3 marks)

a. The starch could have run out at any moment during the 10 seconds before the sample that stayed orange-brown, so each time could be up to 10 s too long.

b. Use a colorimeter to measure the colour of a mixture of starch, iodine and amylase continuously. The end point is detected the moment the colour changes, rather than only at 10-second intervals, and it does not depend on judging colours by eye.

Marking points

- (a) the starch may have gone at any time in the 10 s gap, so the time is only known to the nearest 10 s
- (b) sample more often (e.g. every 5 s) or use a colorimeter
- (b) explanation: the end point is found more exactly / colour not judged by eye

13 The students only tested whole-number pH values. They concluded that the optimum pH of amylase is exactly 7. Explain why this conclusion may not be correct and how they could find the optimum more precisely.

(2 marks)

The true optimum could be anywhere between pH 6 and pH 8, because no values in between were tested. They should repeat the experiment using smaller steps of pH between 6 and 8, for example 6.5, 7.0 and 7.5.

Marking points

- the optimum could lie between pH 6 and pH 8 / only whole numbers were tested
- test more pH values in smaller intervals around pH 7

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14 Plan an investigation to find out how temperature affects the rate at which amylase breaks down starch.

(6 marks)

Set up water baths at a range of temperatures, for example 20, 30, 40, 50 and 60 °C, checking each with a thermometer. Put one drop of iodine into each well of a spotting tile. Measure 2 cm^3 of amylase into one tube and 5 cm^3 of starch with 1 cm^3 of pH 7 buffer into another, using syringes. Stand both tubes in the first water bath for 5 minutes so they reach that temperature. Mix them together, keep the tube in the water bath and start the stopclock. Every 10 seconds put a drop of the mixture onto the iodine, and stop when the iodine stays orange-brown, recording the time. Repeat at each temperature, and repeat each temperature three times so that a mean time can be found and anomalies spotted. Keep the pH, the volumes and concentrations of amylase and starch, and the sampling interval the same each time. Calculate the rate as 1000 ÷ mean time and plot rate against temperature to find the optimum temperature. Wear eye protection because iodine is an irritant.

Marking points (any 6, with the method in a logical order)

- a range of at least four temperatures set with water baths, checked with a thermometer
- measured volumes of amylase and starch (with syringes or pipettes)
- warm the solutions separately to the temperature before mixing
- sample the mixture onto iodine on a spotting tile at regular intervals
- record the time when the iodine stays orange-brown
- repeat each temperature and calculate a mean
- control variables: pH (buffer), volumes and concentrations of enzyme and starch
- calculate rate = 1000 ÷ time and plot rate against temperature
- a safety point, e.g. eye protection with iodine

How it is marked

- 5-6 marks: a clear, workable plan with a range of temperatures, pre-warming before mixing, sampling onto iodine at set intervals, the end point described, repeats with means, controls named and the rate worked out.
- 3-4 marks: the main steps are there (different temperatures, mixing amylase and starch, testing with iodine) but some detail is missing, such as pre-warming, the end point colour, repeats or controls.
- 1-2 marks: some relevant points, such as heating the mixture or testing with iodine, but the plan is unclear or would not give a rate at each temperature.

Revision notes: the method

Aim: To find out how pH affects the rate at which the enzyme amylase breaks down starch, by timing how long it takes for the starch to disappear.

Equipment

- amylase solution
- starch solution (1%)
- buffer solutions covering pH 4 to pH 9
- iodine solution in a dropping bottle
- spotting tile
- water bath set to 35 °C
- test tubes and a test tube rack
- 3 syringes or graduated pipettes (one for each solution)
- dropping pipette
- stopclock

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- thermometer
- glass rod

Method

1. Put one drop of iodine solution into each well of a spotting tile.
2. Use a syringe to put 2 cm^3 of amylase solution into one test tube and 5 cm^3 of starch solution into another.
3. Add 1 cm^3 of the pH 4 buffer to the starch solution.
4. Stand both tubes in the water bath at 35 °C for 5 minutes so they reach the same temperature.
5. Pour the amylase into the starch and buffer, mix well and start the stopclock straight away.
6. Every 10 seconds, use a dropping pipette to put one drop of the mixture into the next well of iodine on the spotting tile.
7. Keep sampling until the iodine stays orange-brown, which shows there is no starch left, and record that time.
8. Rinse the equipment and repeat with each of the other buffers, pH 5, 6, 7, 8 and 9.
9. Repeat each pH three times and calculate a mean time, then work out a rate using rate = 1000 ÷ time.

Variables

- **Independent:** The pH of the reaction mixture (set using buffer solutions)
- **Dependent:** The time taken for the starch to be broken down, found when a sample no longer turns iodine blue-black (used to calculate the rate)
- **Control:** the temperature, kept at 35 °C with a water bath; the volume and concentration of amylase solution; the volume and concentration of starch solution; the volume of buffer solution; the time between samples

Hazards

Hazard	What could happen	How to reduce the risk
Iodine solution	irritates the eyes and skin and stains	wear eye protection and wash off any splashes at once
Amylase solution	enzymes can cause an allergic reaction if they get on the skin or in the eyes	wear eye protection, avoid skin contact and wash hands after the practical
Glass test tubes	cuts if a tube is knocked over or breaks	keep tubes in a rack and tell the teacher about any breakage rather than picking up glass by hand

Key points

- The starch has gone when a drop of the mixture leaves the iodine orange-brown, not blue-black
- A shorter time means a faster rate: rate = 1000 ÷ time (or 1 ÷ time)
- Buffers keep the pH fixed; the water bath keeps the temperature the same for every pH
- Warm the enzyme and starch separately before mixing, so the reaction starts at the right temperature
- Far from the optimum pH the active site changes shape, so starch no longer fits and the enzyme is denatured
- Sampling every 10 seconds means each time is only known to within 10 seconds

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