Aim: To investigate the effect of pH on the rate of amylase activity, using iodine solution to detect the presence of starch.

Background Theory

- Amylase is an enzyme that breaks down starch (substrate) into maltose (product).
- Enzymes have an active site with a specific complementary shape to their substrate.
- Each enzyme has an optimum pH — the pH at which it works fastest.
- Extreme pH changes the shape of the active site (denaturation) — substrate can no longer bind.
- Iodine solution turns blue-black in the presence of starch; colourless/orange when starch has been digested.

★ The induced fit model: the active site changes shape slightly to better accommodate the substrate.

★ Above the optimum: bonds holding the enzyme shape break permanently — denaturation is irreversible.

Equipment

- Amylase solution (1%)
- Starch solution (1%)
- Iodine solution
- Buffer solutions (pH 4, 6, 7, 8, 10)
- Spotting tile
- Pipettes
- Test tubes
- Water bath (35°C)
- Stopwatch
- Thermometer

Method

1. Set up a water bath at 35°C.

2. Add 2 cm^3 of starch solution and 1 cm^3 of pH 4 buffer to a test tube. Place in water bath for 3 minutes.

3. Add 2 cm^3 of amylase solution to the starch-buffer mixture. Start the stopwatch immediately.

4. Every 30 seconds, transfer one drop of the mixture to a well of the spotting tile containing iodine solution.

5. Record whether iodine turns blue-black (starch present) or remains orange-brown (starch digested).

6. Record the time when the iodine no longer turns blue-black (endpoint).

7. Repeat steps 2–6 for pH 6, 7, 8 and 10. Use a fresh spotting tile for each.

8. Repeat the whole experiment twice more and calculate the mean time for each pH.

Variables

Independent variable	pH of buffer solution (4, 6, 7, 8, 10)
Dependent variable	Time taken for starch to be fully digested (endpoint — iodine no longer turns blue-black)

Controlled variables	Temperature (constant water bath), concentration of amylase, concentration of starch, volume of solutions used

Results Table

pH	Time (s) — Trial 1	Time (s) — Trial 2	Time (s) — Trial 3	Mean time (s)

Analysis

- Plot a bar chart or line graph of mean time (y-axis) vs pH (x-axis).
- The pH with the shortest time = optimum pH for amylase.
- Describe the trend: as pH increases from 4, time decreases to a minimum at optimum, then increases again.

★ Explain: at extreme pH values the ionic bonds holding the tertiary structure of amylase break — active site shape changes permanently (denaturation).

★ Calculate the rate of reaction: rate = 1 ÷ time. A shorter time = a faster rate.

Exam Tip: Denaturation is PERMANENT. At low temperatures, amylase is just slow — not denatured. Examiners test this distinction frequently.

Common Mistake: Do not say iodine "disappears". Say it "remains orange-brown" or "does not turn blue-black". The iodine is still there — just no starch to react with.