

3.2 Factors that affect enzyme action

Name: _____ Class: _____ Date: _____

Total: 17 marks

KEY WORDS michaelis-menten constant 米氏常数 • competitive 竞争性 • inhibitor 抑制剂
• competitive inhibitor 竞争性抑制剂 • non-competitive 非竞争性

Objective

Build the skills to answer exam questions on the **factors that affect enzyme action** — temperature, pH, enzyme and substrate concentration, **inhibitors** 抑制剂, the **Michaelis-Menten constant** 米氏常数, and **immobilised enzymes** 固定化酶.

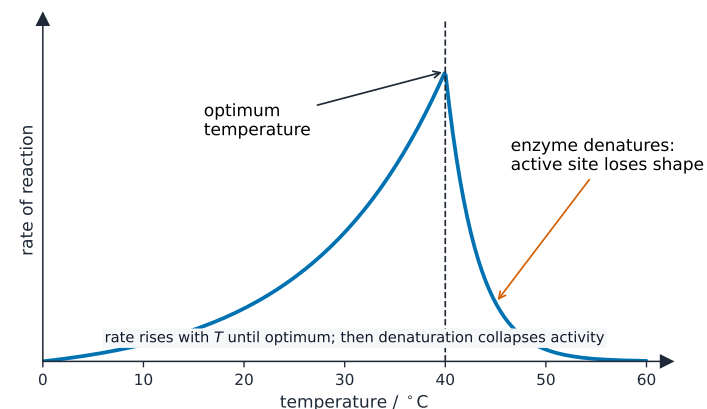
You must be able to:

- explain the effect of temperature, pH, enzyme concentration and substrate concentration on rate
- explain $V_{\max}$ and K_m and use K_m to compare affinity
- explain competitive and non-competitive inhibition, and state the advantages of immobilised enzymes

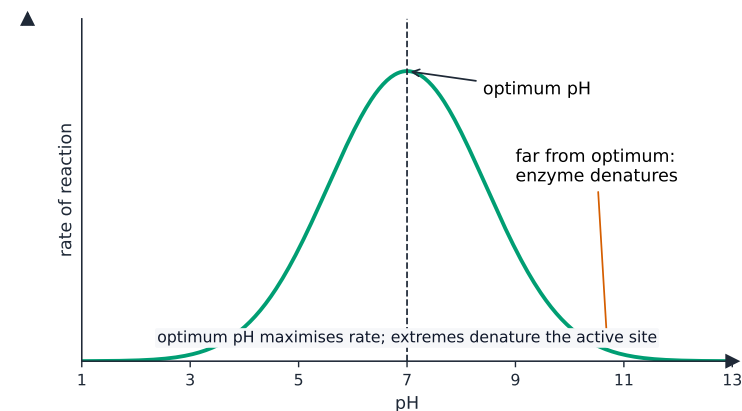
1 Worked examples

■ Temperature and pH

Rate rises with temperature (more **kinetic energy** 动能, more collisions) up to the **optimum**; above it the enzyme **denatures** 变性 — bonds holding its shape break, the active site changes, and rate falls fast. Each enzyme also has an optimum pH; far from it the enzyme denatures.



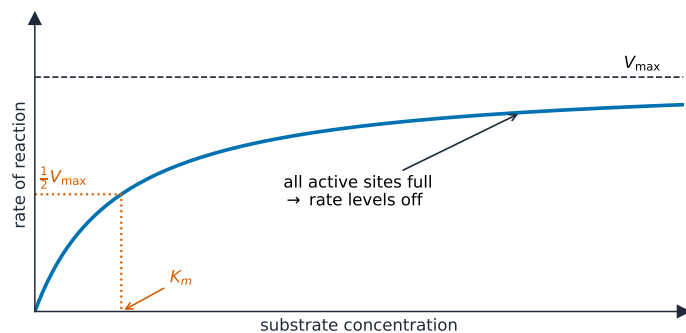
Rate rises to the optimum, then falls fast as the enzyme denatures



The rate peaks at the optimum pH and falls away on either side

■ Substrate concentration, $V_{\max}$ and K_m

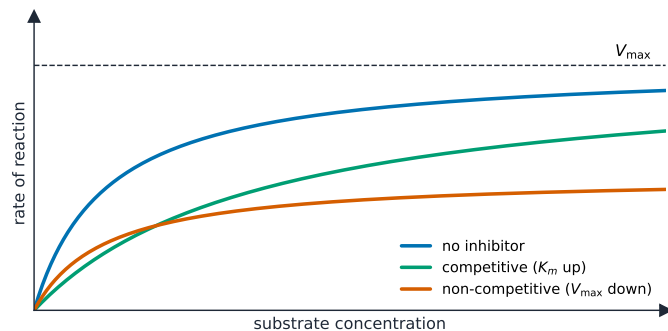
Adding substrate speeds the reaction until every active site is busy; then rate levels off at the maximum, $V_{\max}$. The **Michaelis-Menten constant** K_m is the substrate concentration that gives **half** of $V_{\max}$. A **low** K_m = **high affinity** 亲和力 (reaches half-speed at low substrate).



$$v = \frac{V_{\max}[S]}{K_m + [S]} \quad K_m = [S] \text{ at half } V_{\max}$$

K_m is the substrate concentration giving half $V_{\max}$ — a lower K_m means higher affinity

■ Reversible inhibitors



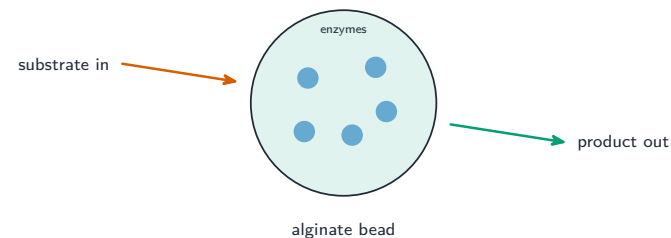
competitive: $\uparrow K_m$, same $V_{\max}$ · non-competitive: $\downarrow V_{\max}$

Competitive inhibition raises K_m (more substrate overcomes it); non-competitive lowers $V_{\max}$

- **competitive** 竞争性: binds the active site (similar shape to substrate); more substrate overcomes it $\rightarrow V_{\max}$ unchanged, K_m rises.
- **non-competitive** 非竞争性: binds elsewhere, changing the active site; more substrate does not help $\rightarrow V_{\max}$ falls.

■ Immobilised enzymes

An **immobilised enzyme** is fixed in place (e.g. trapped in **alginate** 海藻酸盐 beads).



Enzymes stay trapped while substrate flows in and product out — so they are not washed away

Advantages: re-used many times; pure product; more stable to temperature/pH; can run continuously.

2 Practice

2.1 Explain why the rate of an enzyme reaction falls above the optimum temperature.[3]

2.2 State **two** advantages of using an immobilised enzyme rather than a free one. [2]

3 Exam-style questions

3.1 A competitive inhibitor is added to an enzyme reaction. Compared with no inhibitor, what happens to $V_{\max}$ and K_m ? [1]

- **A** $V_{\max}$ unchanged; K_m increases
- **B** $V_{\max}$ decreases; K_m unchanged
- **C** both increase
- **D** both decrease

3.2 Enzyme Y has a lower K_m than enzyme Z for the same substrate. This means that enzyme Y: [1]

- **A** has a higher affinity for the substrate
- **B** has a higher $V_{\max}$
- **C** is denatured more easily
- **D** works at a higher optimum pH

3.3 A student investigates how temperature affects the rate at which catalase breaks down hydrogen peroxide. She measures the volume of oxygen produced in the first minute at five temperatures.

(a) State **two** variables she must control to make this a fair test. [2]

(b) Describe and explain the results she would expect between 10 °C and 60 °C. [4]

(c) Explain why a buffer solution would be used in a similar experiment that varied pH. [2]

3.4 The graph of rate against substrate concentration for an enzyme levels off at high substrate concentration. Explain why. [2]

Solutions

2.1 heat breaks the bonds (hydrogen/ionic) holding the enzyme's shape; the active site changes shape / denatures; so the substrate no longer fits and fewer enzyme-substrate complexes form.

2.2 Any two: re-used many times / product is pure (no enzyme) / more stable to temperature and pH / allows continuous flow.

3.1 A. A competitive inhibitor raises K_m but leaves V_{max} unchanged (more substrate overcomes it).

3.2 A. A lower K_m means half V_{max} is reached at lower substrate concentration — higher affinity.

3.3 (a) any two: substrate (hydrogen peroxide) concentration / volume / enzyme concentration / pH / time.

(b) rate rises from 10 °C towards the optimum as molecules gain kinetic energy and collide more often; above the optimum the enzyme denatures, so the rate falls.

(c) a buffer keeps pH constant so that pH is not an uncontrolled variable affecting the rate.

3.4 at high substrate concentration every active site is occupied / saturated, so adding more substrate cannot increase the rate further — it is at V_{max} .