3. Enzymes

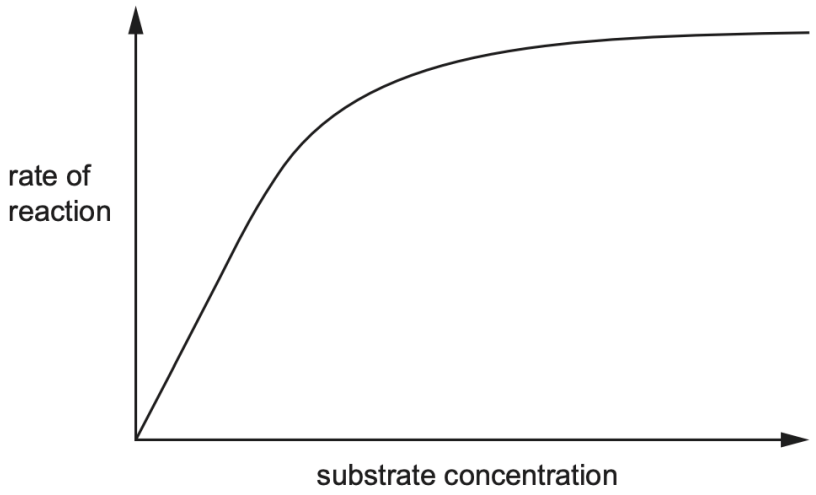
1. State the term used to describe enzymes that act outside the cells that synthesise them.
   - Extracellular enzymes

2.

Sulfonamide is a competitive inhibitor of carbonic anhydrase.

Fig. 3.2 shows the effect of increasing substrate concentration on the rate of the reaction catalysed by carbonic anhydrase.

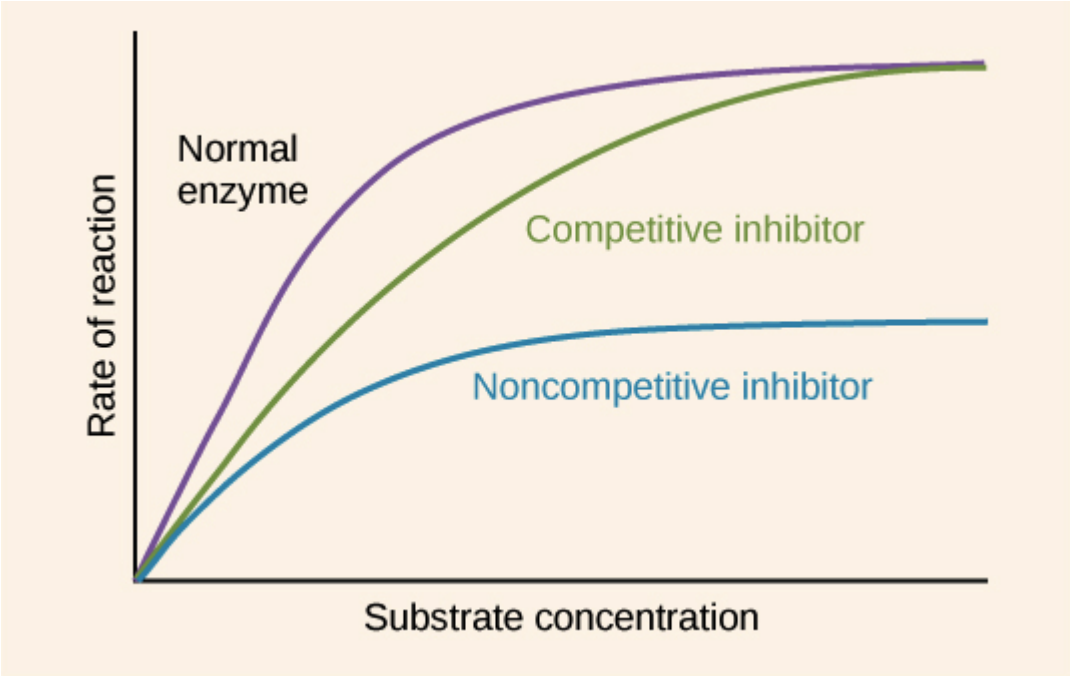
Sketch a curve **on Fig. 3.2** to show the effect of sulfonamide on the rate of reaction catalysed by carbonic anhydrase.



- curve below original line
- curve will merge with the original line after it begins to plateau

NOTE:
- V_{max} = maximum rate of reaction = when enzyme is saturated
- K_m = substrate concentration at ½V_{max}

	Competitive inhibitor	**Non-competitive inhibitor**
V_{max}	No change	Decreases
K_m	Increases	No change



3.

(b) RNA aptamers are short, single-stranded RNA molecules that can be used to study some infectious diseases.

Scientists studying an infectious disease in animals investigated the effect of RNA aptamers on the activity of RNA polymerase that is produced by the pathogen.

The aptamers bind to a specific region of the RNA polymerase.

Table 6.1 shows the effect of two aptamers, F47 and F52, on the activity of RNA polymerase produced by the pathogen.

Table 6.1

aptamer present	V_{max} of RNA polymerase / arbitrary units	K_m of RNA polymerase / arbitrary units
none	1664	346
F47	1072	508
F52	1467	523

With reference to Table 6.1, compare the effect of the aptamers on the affinity of RNA polymerase for its substrate.

- both increase Km, therefore both reduce the affinity of RNA polymerase for its substrate
- presence of F52 reduces the affinity for the substrate more than F47

With reference to Table 6.1, suggest explanations for the effect of the presence of an aptamer on the rate of transcription catalysed by the RNA polymerase.

- decreased maximum rate of reactions / rate of transcription because Vmax lower qualified
- binding of the aptamer changes the tertiary structure / shape of active site of RNA polymerase // binding changes shape of enzyme so active site less complementary to substrate
- so substrate / RNA nucleotides bind less easily to active site // fewer enzyme-substrate complexes form
- binding prevents RNA polymerase from binding to template strand
- binding prevents RNA polymerase from forming bond between RNA nucleotides

4. Suggest why it is possible for a protease to act on different types of protein.
   - area to be hydrolysed could be between same amino acids
   - active site has some flexibility for hydrolysing / binding to similar substrates
   - proteins / substrates similar shapes so fit active site
   - active site and substrates / proteins still have complementary shapes
   - AVP: e.g. large enzyme / enzyme complex with more than one active site

5. Suggest how modifying the R-group of an amino acid in an enzyme can reduce the catalytic activity of the enzyme.
   - ref. to effect on ionic bonding / hydrogen bonding / hydrophobic interactions with other R groups
   - changes the shape / conformation of the active site
   - active site no longer complementary to substrate
   - enzyme–substrate complex not formed / formed at reduced rate
   - ref. to effect of activation energy not being reduced: e.g. changed charges so no electron transfer
   - no longer provides hydrophobic regions for reaction to occur

6. P. jirovecii (a fungus) produces an enzyme known as 1,3-β-D-glucan synthase. The enzyme catalyses the synthesis of 1,3-β-D-glucan. The therapeutic drug caspofungin is a non-competitive inhibitor of 1,3-β-D-glucan synthase. With reference to the mechanism of action of caspofungin, explain how the drug may be useful to treat cases of pneumonia caused by P. jirovecii.
   - caspofungin binds to site on enzyme other than active site ; binds to allosteric site
   - changes shape of active site
   - substrates cannot bind to active site / active site no longer complementary to substrates / enzyme-substrate complexes do not form
   - less / no 1,3- -D glucans / products synthesised / produced
   - cell wall weakened / synthesis hindered
   - leads to osmotic lysis of, P. jirovecii / cell / fungus // A bursting / cytolysis

- reduces / prevents population growth // decrease number of P. jirovecii
- reduced number increases chance of immune system eliminating the fungus

7. The reaction catalysed by starch phosphorylase enzyme occurs at the ends of amylose molecules. Describe the sequence of events that occurs when starch phosphorylase catalyses the addition of a molecule of glucose to the end of an amylose molecule.
   - substrate / glucose binds to active site / forms enzyme-substrate complex
   - end of amylose / glucose residue at end of amylose molecule binds to active site / forms enzyme-substrate complex
   - AVP: detail of binding to active site – formation of hydrogen bonds
   - active site changes shape when substrates bind // induced fit
   - enzyme decreases the activation energy
   - (-1,4-)glycosidic bond forms
   - condensation reaction / water formed

8. Suggest the advantages to the cell of enzyme pathways being located in cell membranes, rather than in the cytosol of the cell (fluid portion of cytoplasm).
   - can organise / order enzymes / events in pathway
   - consequence in terms of distance: product of one reaction is close to next enzyme, where it acts as substrate // enzyme more likely to be closer to substrate // distance between enzymes shorter
   - so increases chance of successful collisions between substrate and enzymes // increased rate of formation of final product // decrease time for ES complex to occur
   - idea that products can pass to either side of the membrane
   - all reactions localised / occur in the same location within the cell

9. Trypsin is an enzyme which catalyses the hydrolysis of casein (a protein).
   Explain the results shown:

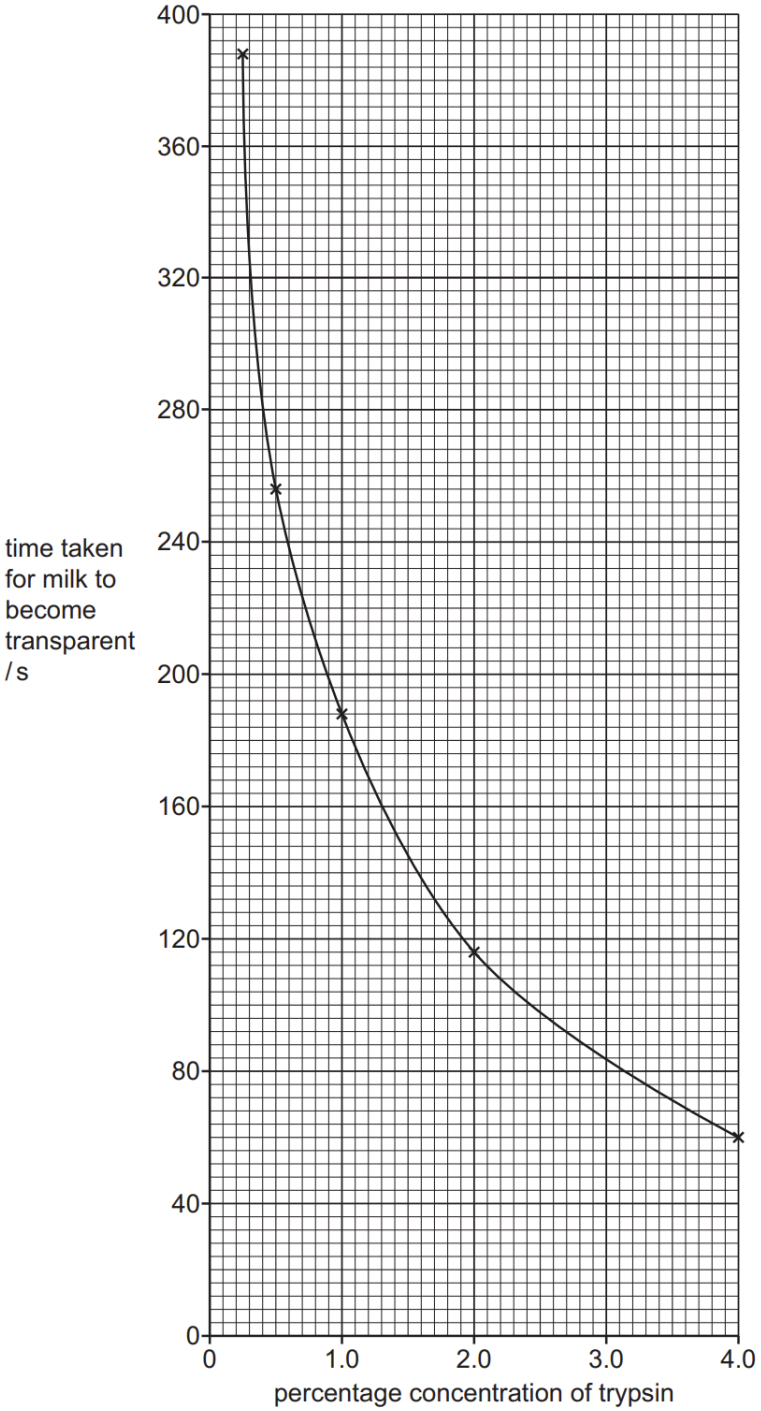


Fig. 5.1

- at lower concentrations there are not enough active sites / enzymes
- substrate molecules cannot enter active site until product released
- as concentration increases, increased number of active sites
- as concentration increases there are more successful collisions between enzyme and substrate / casein

- so allows increase in number of enzyme-substrate complexes formed per unit time
- increased rate of casein breakdown as enzyme concentration increases
- at high concentration substrate concentration is becoming the limiting factor

10.

temperature / °C	percentage of maximum activity of immobilised trypsin	percentage of maximum activity of trypsin free in solution
25	60	100
35	85	100
45	98	80
55	95	20
65	100	5

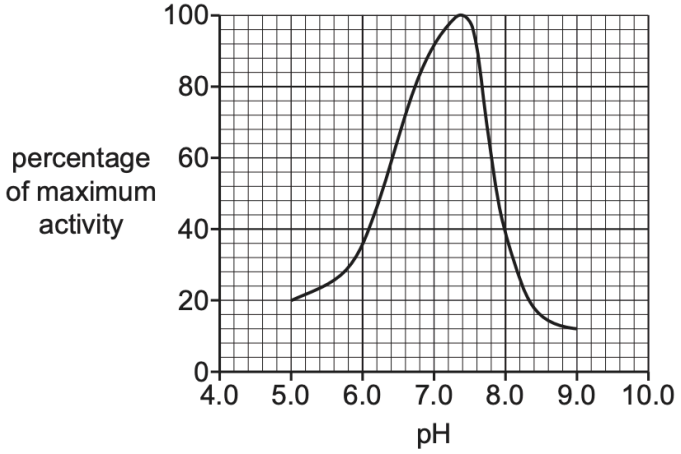
State a reason for the difference in percentage of maximum activity of immobilised trypsin and trypsin free in solution at 25°C.

- immobilising changes the optimum temperature of the enzyme
- trypsin free in solution has increased chance of collision with substrate
- immobilising the enzyme may have altered the tertiary structure of the enzyme / shape of the active site
- immobilising may have covered part of the active site / active site of some enzymes
- suggestion that material may not be inert and may have an inhibitory effect at lower temperatures

Suggest and explain why the percentage of maximum activity of immobilised trypsin at 55°C is higher than the percentage of maximum activity of trypsin free in solution at 55°C.

- immobilisation protects / stabilises trypsin
- from thermal denaturation
- maintains shape of active site
- immobilisation may result in lower kinetic energy / vibration of the enzyme molecules compared to free (so protects from denaturation)
- prevents the breaking of bonds holding the tertiary structure (of immobilised enzyme molecule)
- ref. to hydrogen / ionic bonds

1. Effect of pH on activity of collagenase (enzyme that breaks down collagen):

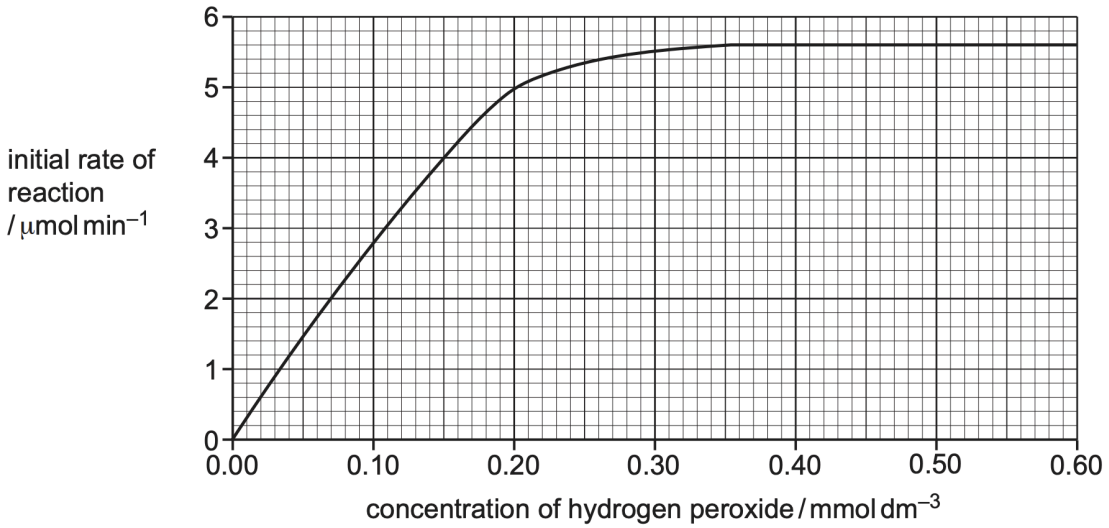


Explain why activity of collagenase is lower at pH 8.0 than at the optimum pH.

- at pH8.0, ionic / hydrogen bonds (between R groups) broken / altered
- active site shape altered
- (active site) no longer / less complementary to substrate / collagen OR fewer enzyme–substrate complexes formed
- AVP: the amino acids in the active site affected by the changing pH ref. to partial denaturation

2. Explain the induced-fit hypothesis.
   - active site is not (fully) complementary to substrate ;
   - active site changes shape / moulds around to fit the substrate ; conformational change
   - enzyme-substrate complex / ESC, forms ;
   - active site returns to original shape on release of product ;
   - AVP: change of shape (to give complementary fit) lowers activation energy / puts strain on bonds

3. Enzyme peroxidase catalyses the breakdown of hydrogen peroxide. Students investigated the effect of increasing the concentration of hydrogen peroxide on the activity of peroxidase:



a. Explain the effect of increasing the concentration of hydrogen peroxide on the initial rate of reaction as shown in Fig. 4.1.

- rate of reaction increases as substrate concentration increases to 0.33 – 0.35 mmol dm-3:
  - up to 0.33 to 0.35 mmol dm–3 some active sites are not occupied.
  - more collisions between enzyme / active site and substrate molecules, which leads to increase in formation of enzyme-substrate complexes.

- rate of reaction remains constant above 0.33–0.35 mmol dm–3:
  - all active sites are occupied / saturated ;
  - correct reference to limiting factor for slope ; substrate concentration is limiting at low substrate concentrations.
  - correct reference to limiting factor for plateau ; enzyme concentration is limiting at higher substrate concentrations.

b. The students determined the K_m for radish peroxidase as 0.10 mmol dm–3. With reference to the figure, describe how they determined the K_m

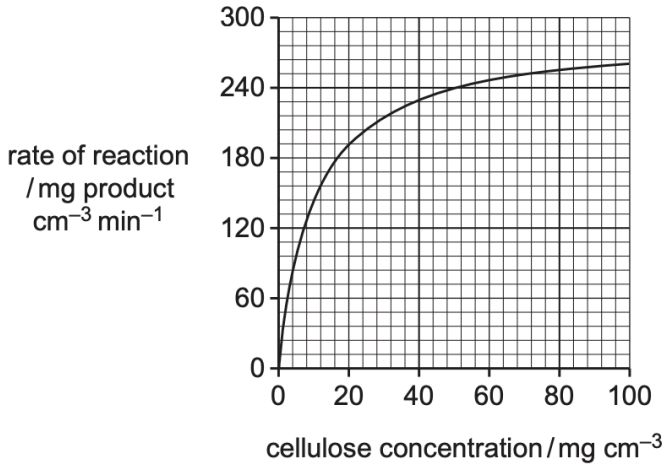
- determined Vmax / maximum rate as 5.6 (μmol min–1)
- K_m is the substrate concentration at half the Vmax

4.

Juices that are extracted commercially from fruits can be made less cloudy by the breakdown of the cell wall using the enzymes cellulase, pectinase and xylanase:

- cellulase hydrolyses cellulose
- pectinase hydrolyses pectin
- xylanase hydrolyses hemicellulose.

(c) Fig. 5.2 is a graph showing the effect of cellulose concentration on the activity of cellulase, which is used in making fruit juice less cloudy.



Describe and explain the graph.

- overall trend/ general description: as cellulose / substrate, concentration increases rate of reaction increases OR as cellulose / substrate, concentration increases, steep increase in rate of reaction then, less steep increase / (begins to) plateau

Any 2:

- increasing cellulose concentration increases number of collisions between enzyme and substrate / enzyme-substrate complexes / ESCs ;
- at low cellulose concentrations, (many) active sites, available / not all used / not saturated ;
- at higher cellulose concentrations, active sites becoming saturated / most active sites occupied / most active sites not available
- Named limiting factor: at low(er) cellulose concentrations, cellulose concentration limiting / enzyme concentration not limiting
- As cellulose concentration increases, change from cellulose concentration limiting to enzyme concentration as limiting factor.
- At higher cellulose concentrations, enzyme concentration limiting / cellulose concentration not limiting

5.

Ultrasound is one possible method that can be used to destroy microorganisms that contaminate fruit juices. Ultrasound is the term given to sound waves that are out of the range of human hearing.

An investigation was carried out into the effect of ultrasound on the activity of cellulase, pectinase and xylanase used in fruit juice manufacture.

For each enzyme, the effect of ultrasound was compared with no ultrasound on the:

- maximum rate of reaction (V_{max})
- Michaelis-Menten constant (K_m)
- catalytic efficiency (V_{max}/K_m)

Table 5.1 summarises the results. A higher V_{max}/K_m indicates a higher catalytic efficiency.

Table 5.1

enzyme	method	comparison of V_{max}	comparison of K_m	V_{max}/K_m / min^{-1}
cellulase	ultrasound	higher	higher	34
	no ultrasound	lower	lower	29
pectinase	ultrasound	same	lower	945
	no ultrasound	same	higher	759
xylanase	ultrasound	higher	same	146
	no ultrasound	lower	same	125

(i) In terms of changes in the interaction between enzyme and substrate when ultrasound is used, suggest explanations for the lower K_m for pectinase and the higher V_{max} for xylanase, as shown in Table 5.1.

lower Km for pectinase, any 2 from:

- Ultrasound increases affinity of enzyme for substrate ;
- Ultrasound makes shape of active site more complementary ;
- Ultrasound makes (position of) active site more accessible (to substrate) // makes it easier for substrate to enter (active site)
- Ultrasound breaks up pectin to expose more substrate for binding ;

higher Vmax for xylanase, any 2 from:

- Ultrasound increases rate of, collision between enzyme and substrate / more enzyme-substrate complexes per unit time ;
- Ultrasound increases rate of catalysis after binding ;
- Ultrasound may change substrate for easier hydrolysis ;
- Ultrasound may lower, more than normal, activation energy required for reaction ; increased ability to, break / form, bonds.

(ii) Explain whether the data shown in Table 5.1 supports the recommendation that ultrasound can be used in the manufacture of fruit juices.

- yes (as all have) increased, catalytic efficiency / productivity / Vmax /Km ;
- any two from:
    - pectin 24.5 / 25%
    - cellulase 17.25 / 17%
    - xylanase 16.8 / 17%

6.

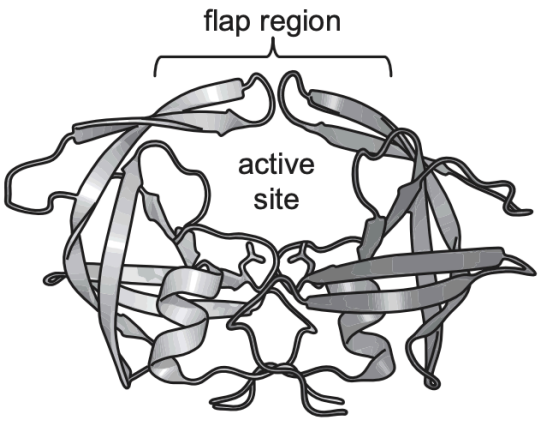


Fig. 3.1

The flap region of HIV protease is flexible.

With reference to figure, suggest and explain how the mechanism of action of HIV protease can be described as an induced fit.

- flap region opens to let substrate enter OR flap region closes round substrate.
- induced fit involves change in shape of active site / active site moulding around substrate.
- ref. to better fit / fully complementary
- ref. To formation of enzyme-substrate complex
- AVP:
    - induced fit substrate not fully complementary to (shape of) active site.
    - active site / enzyme returns to original shape on release of product
    - change of shape (to give complementary fit) lowers activation energy / puts strain on bonds

7. Hydrolytic enzymes, known as collagenases, are secreted by cells in an inactive form. Cells also secrete inhibitors of collagenases. The activity of the enzymes and inhibitors is regulated so that the development and maintenance of the extracellular matrix is controlled.

State and explain what the outcome will be for the composition of the extracellular matrix if collagenase inhibitor activity is needed.

- collagen, not broken down / not hydrolysed / breakdown prevented ;
- detail ; collagen does not fit into active site / active site changes shape; no / few ESC / enzyme substrate complexes form.
- collagen continues to be synthesised / released ;

8.

Synthetic inhibitors have been trialled as potential treatment for diseases caused by a lack of regulation of collagenase activity.

Research involves investigating the mechanism of action of an inhibitor.

State the effect that a **non-competitive** inhibitor will have on the maximum rate of reaction, V_{max}, and the Michaelis–Menten constant, K_m, of collagenase.

- V_{max}: decreases
- K_m: stays the same