3. Enzymes

3.2 Factors that Affect Enzyme Action

Paper 2

Question Paper

1 People who become infected with human immunodeficiency virus (HIV) are at risk of developing HIV/AIDs, particularly if antiretroviral therapy (ART) is not available.

(e) *P. jirovecii* 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*.

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2 Trypsin is an enzyme which catalyses the hydrolysis of casein, a protein found in milk.

Milk that contains casein has a cloudy, white appearance. As the casein is hydrolysed by trypsin, the milk changes in appearance to a clear (transparent), colourless solution.

A student carried out an experiment to investigate the effect of enzyme concentration on the rate at which trypsin hydrolyses casein.

The student added a solution of trypsin to a sample of milk and recorded the time taken for the milk to become transparent. The student repeated the experiment with different concentrations of trypsin. All other variables were kept constant.

Fig. 5.1 shows the results from the experiment.

(a) (i) When the concentration of trypsin increases from 2.0% to 4.0%, the time taken for the milk to become transparent decreases by 48%.

Calculate the percentage decrease in the time taken for milk to become transparent when the concentration of trypsin increases from 0.25% to 0.5%.

Write your answer to the nearest whole number.

percentage decrease ..... [1]

(ii) Explain the results shown in Fig. 5.1.

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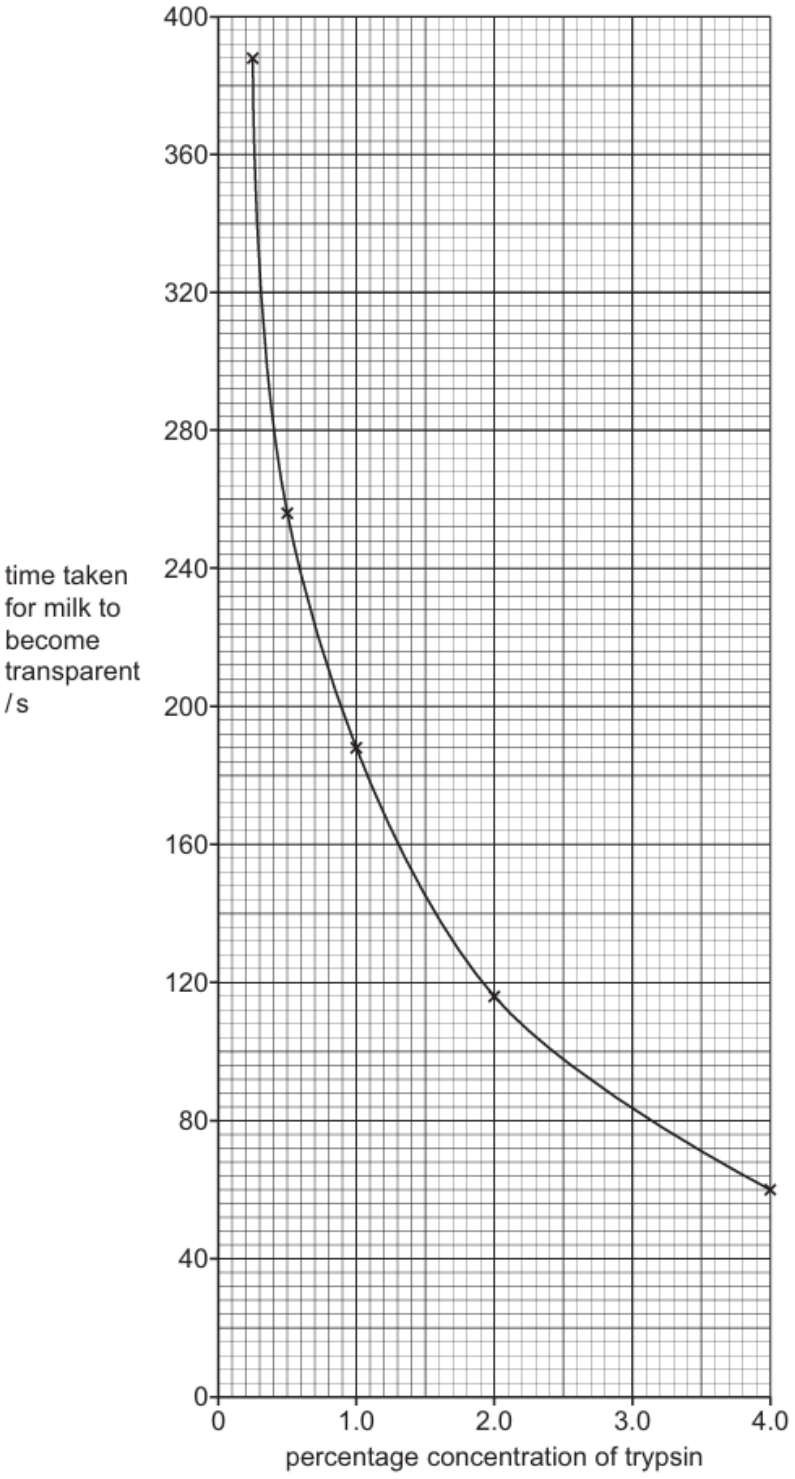

time taken for milk to become transparent / s
400
360
320
280
240
200
160
120
80
40
0
0
1.0
2.0
3.0
4.0
percentage concentration of trypsin

Fig. 5.1

(b) Trypsin has the potential to be used in a wide range of industrial processes.

The use of immobilised enzymes in industrial processes has many advantages.

Scientists investigated the effect of temperature on the activity of trypsin immobilised on the surface of a material and trypsin free in solution.

Table 5.1 shows the results of the investigation.

Table 5.1

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

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

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(ii) 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.

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3 Keratin is the structural protein in feathers of birds. Keratin polypeptides are composed of a high proportion of cysteine amino acids, which have sulfur-containing R groups.

Keratin polypeptides form filaments. The two main types of keratin in feathers are α-keratin, which consists of many α-helices, and β-keratin, consisting of many β-pleated sheets.

Keratinases are used to degrade the large quantities of waste feathers from chickens and turkeys that are processed in the food industry. The products of feather degradation can be used in animal feed.

Scientists investigated whether three different keratinases, K12, A22 and P3, were suitable as industrial enzymes. These enzymes were extracted from three different soil bacteria.

The effects of temperature and pH on the activity of each keratinase were investigated.

The results are shown in Fig. 2.1 and Fig. 2.2.

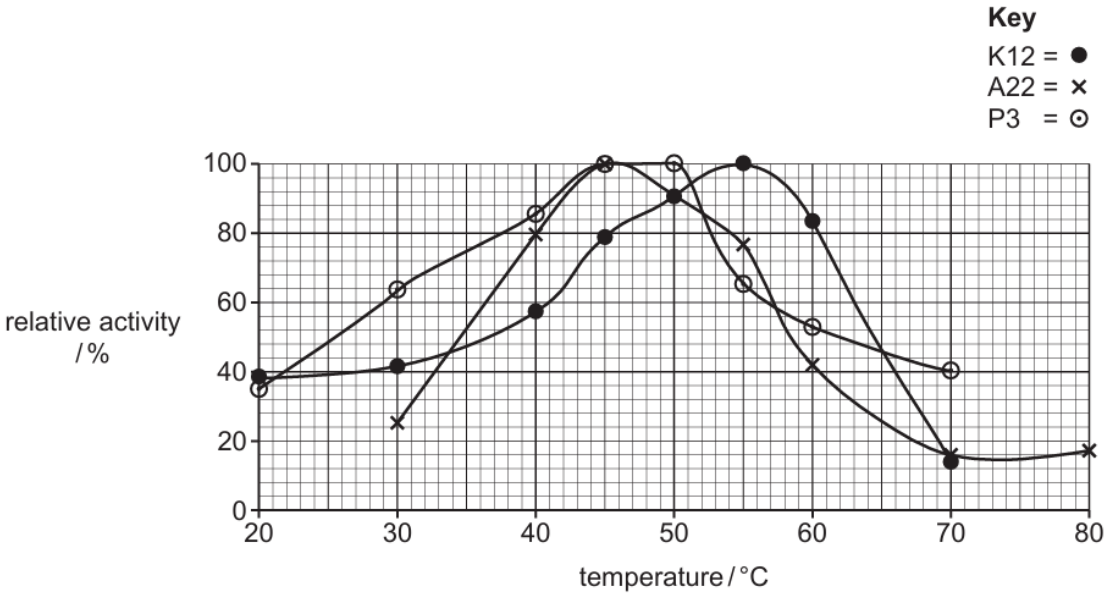


Fig. 2.1

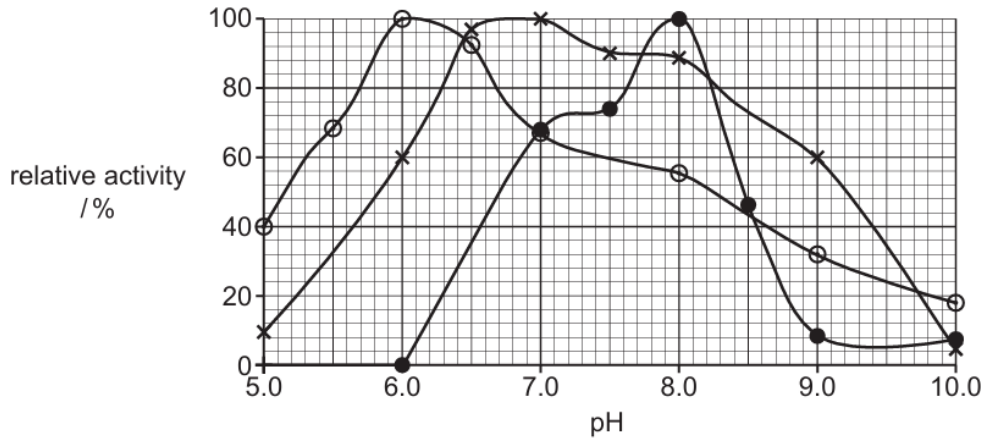


Fig. 2.2

(d) To degrade feather waste from industry, it is an advantage to use keratinases that show at least 60% relative activity in conditions where temperature and pH can vary widely.

Table 2.1 shows, for each keratinase, the working range of temperature and pH where at least 60% relative activity is obtained.

Use Fig. 2.1 and Fig. 2.2 to complete Table 2.1 **and** use the completed table to:

- name the keratinase that has the widest working range of temperature
- name the keratinase that has the widest working range of pH.

Table 2.1

keratinase	temperature range with at least 60% relative activity / °C	pH range with at least 60% relative activity
K12	41–63	
A22		6.0–9.0
P3	29–56	5.3–7.5

Keratinase with a relative activity of at least 60% that has:

- the widest working range of temperature .....
- the widest working range of pH .....

[4]

(e) Some detergents contain proteases to remove stains from clothes. These enzymes have a high relative activity in alkaline conditions.

The scientists reported that K12 and A22 could be suitable for use in the detergent industry.

With reference to Fig. 2.1 and Fig. 2.2, discuss the advantages **and** disadvantages of using K12 and A22 in the detergent industry.

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4 **(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

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

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(ii) 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.

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5 The enzyme carbonic anhydrase has been found in a wide range of organisms and acts as a catalyst in many tissues.

Studies have shown that there are differences in the protein structure of the enzyme and differences in the number and organisation of introns and exons of the gene coding for the enzyme.

All carbonic anhydrase enzymes catalyse the same reversible reaction, shown in Fig. 4.1.

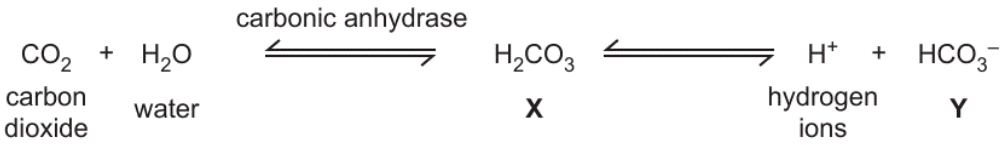


Fig. 4.1

(f) Human CA isoforms in some epithelial cells in the eye have a role in the formation of the clear fluid of the eye known as aqueous humour. Overactivity of the enzyme may lead to a harmful increase of pressure within the eye and cause a condition known as glaucoma.

Acetazolamide is a therapeutic drug that can be used in the treatment of glaucoma. It acts as a reversible non-competitive inhibitor.

Describe the mechanism of action of acetazolamide as a reversible non-competitive inhibitor of carbonic anhydrase.

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6 A tyrosine kinase receptor (TKR) is a protein complex found in the cell surface membrane of mammalian cells.

TKR has two components involved in the process of cell signalling:

- a receptor for the signalling molecule (ligand)
- an enzyme that catalyses the transfer of a phosphate group from ATP to an intracellular protein.

Fig. 3.1 is a diagram to show how TKR is involved in cell signalling.

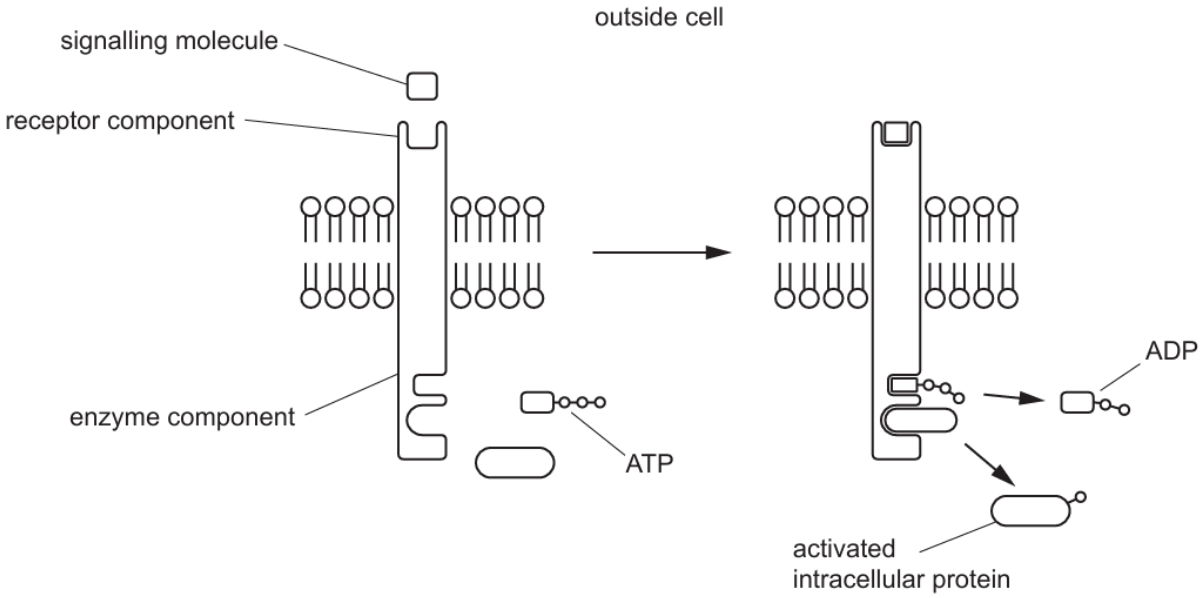


Fig. 3.1

The drug GNF-5 is used in the treatment of some cancers. GNF-5 affects the activity of TKR by binding to the enzyme component of the complex.

Researchers investigated the effect of GNF-5 on the activity of TKR using different concentrations of ATP solution. In an experiment the activity of TKR was measured with no GNF-5 **and** with GNF-5.

The results are shown in Fig. 3.2.

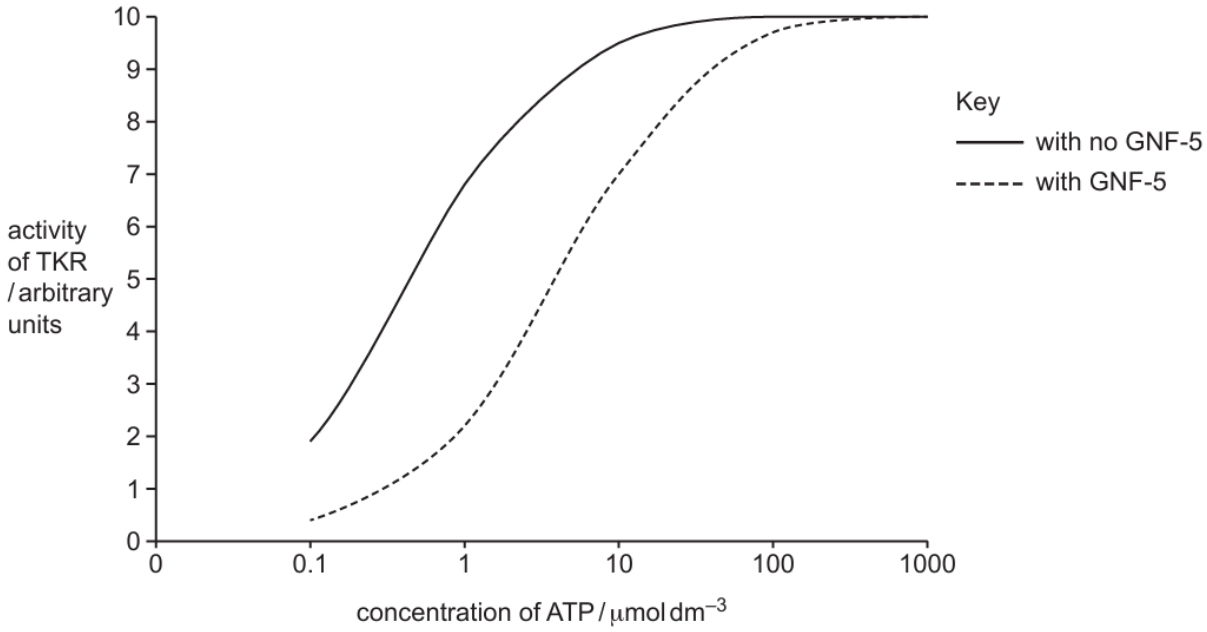


Fig. 3.2

(b) The researchers concluded that GNF-5 acts as an inhibitor of the enzyme component of TKR **and** that it is a competitive inhibitor.

Use Fig. 3.2 to provide evidence for these conclusions.

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(c) A mutation of the gene coding for TKR results in changes to the enzyme component of TKR. This altered form of TKR is known as T315L.

The effect of GNF-5 on the activity of T315L was also investigated.

The results of this investigation are shown in Fig. 3.3.

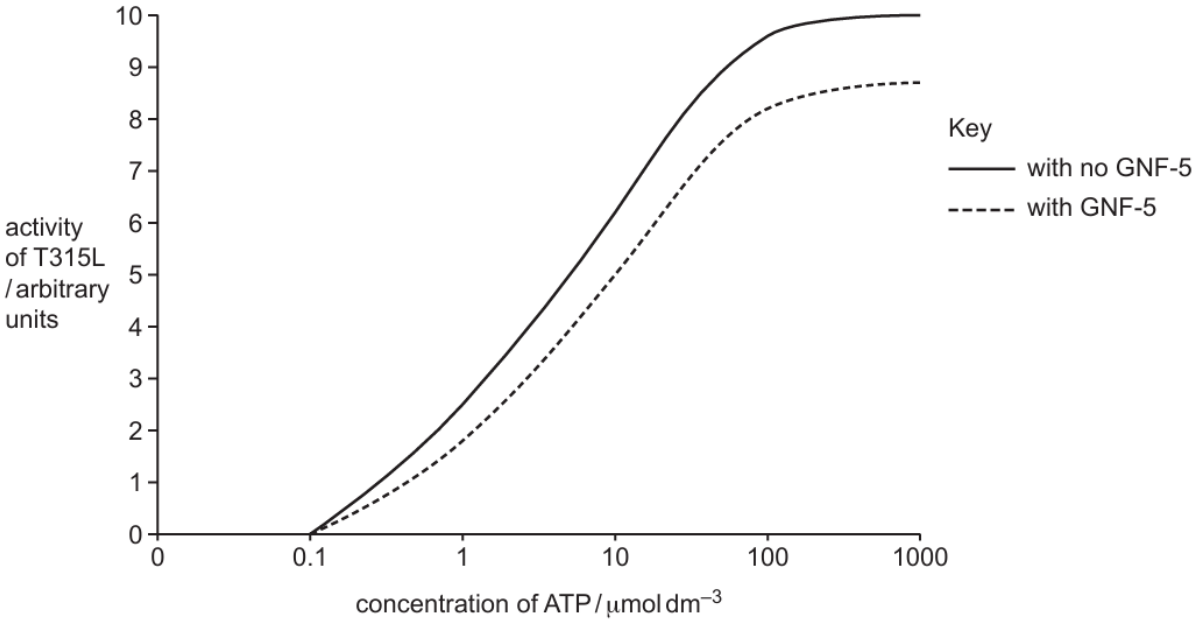


Fig. 3.3

Use Fig. 3.2 **and** Fig. 3.3 to:

(i) State how the activity of T315L differs from TKR when **no** GNF-5 was present.

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(ii) State how the effect of GNF-5 on T315L differs from the effect of GNF-5 on TKR.

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7 Fibroblasts are one of the cell types of connective tissue. The cells synthesise and secrete collagen, which forms part of the supporting external cellular environment, known as the extracellular matrix.

(c) 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.

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

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(ii) 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} .....

K_m .....

[2]

8 **(c)** Scientists have studied the process of cell death that occurs during the development of the cells that become mature xylem vessel elements.

During this development, the tonoplast ruptures (bursts) and releases hydrolytic enzymes contained in the vacuole into the cytoplasm.

(iii) During development of the xylem vessel elements the pH of the cytoplasm decreases. This change in pH activates enzymes in the cytoplasm that cause organelles to swell.

Suggest how a change in pH of the cytoplasm can activate enzymes.

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9 Fig. 5.1 is a transmission electron micrograph showing parts of two plant cells. The function of the middle lamella is cell-to-cell adhesion. The middle lamella is composed of a polysaccharide known as pectin.

Pectin interacts with the polysaccharides cellulose and hemicellulose in the cell walls of the plant cells so that the cell walls are held close together, as shown in Fig. 5.1.

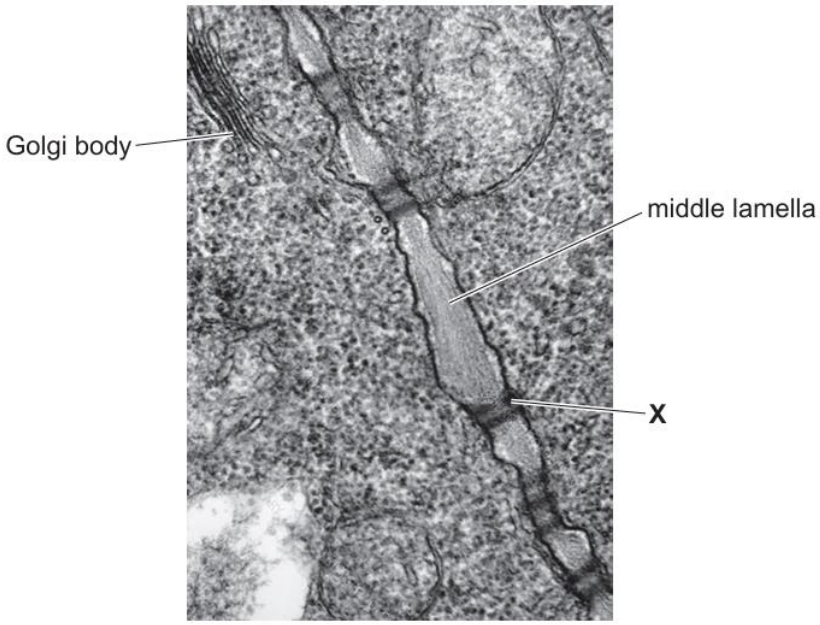


Fig. 5.1

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.

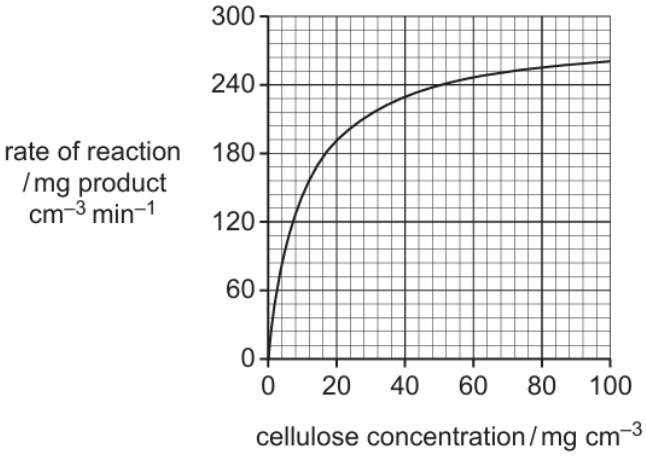


Fig. 5.2

Describe **and** explain the curve shown in Fig. 5.2.

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(d) 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.

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

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10 **(b)** Radish plants contain the enzyme peroxidase that catalyses the breakdown of hydrogen peroxide.

Students investigated the effect of increasing the concentration of hydrogen peroxide on the activity of peroxidase extracted from radish.

The results of their investigation are shown in Fig. 4.1.

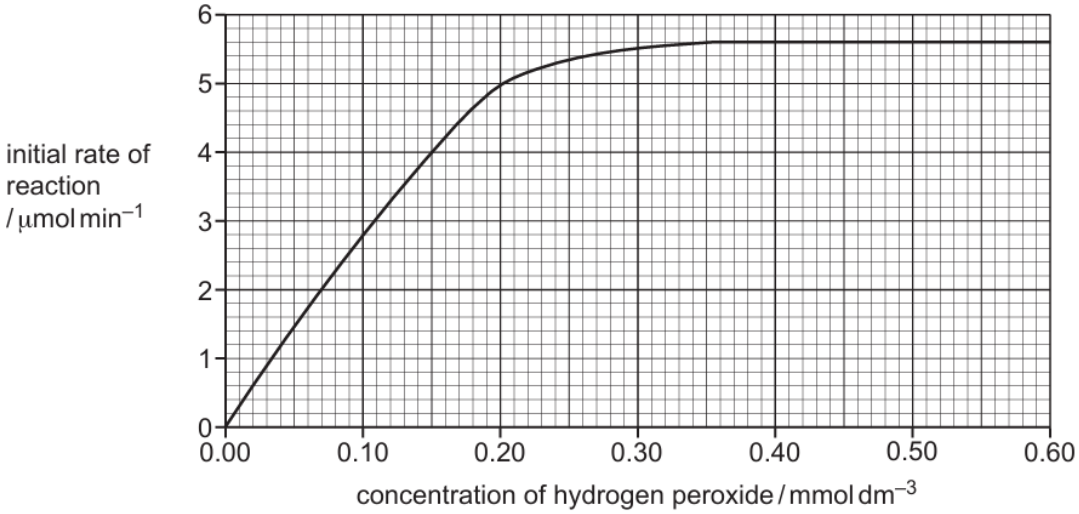


Fig. 4.1

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

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(ii) The students determined the K_m for radish peroxidase as 0.10 mmol dm^{-3}.

With reference to Fig. 4.1, describe how they determined the K_m.

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11 Baculovirus is a virus that kills some of the insect pests of major crops. When the virus is released to the outside of the insect body, it is contained within stable, protective structures known as polyhedrons.

The main component of the polyhedron is a protein molecule, polyhedrin.

Polyhedrons can be sprayed onto plants as a bioinsecticide. They are ingested by feeding insect larvae and once inside the insect gut they break down to release the virus.

(b) Suggest **and** explain the conditions present in the insect gut that can cause the breakdown of polyhedrons.

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12 Amylase is an enzyme that catalyses the hydrolysis of starch into reducing sugars.

A student carried out an experiment to investigate the hydrolysis of starch using immobilised amylase. Fig. 6.1 is a diagram of the apparatus that was used in the investigation.

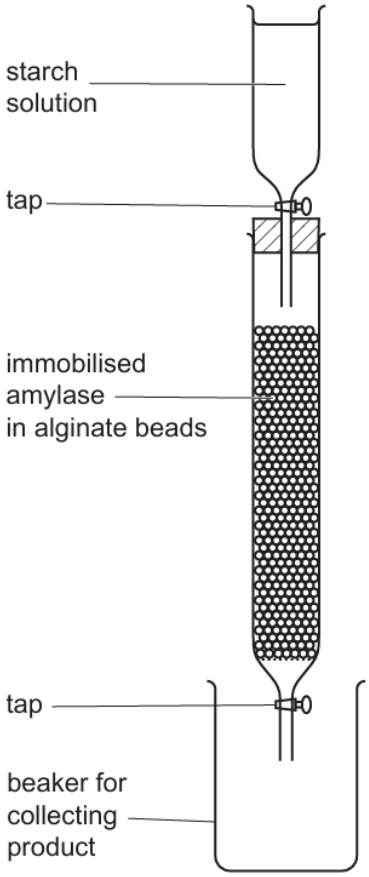


Fig. 6.1

The alginate beads were all the same size.

Both taps were opened to allow the starch solution to flow down the column and for the product to be collected.

The product was tested for the presence of reducing sugar and starch.

(a) The results of the investigation showed that the product collected in the beaker contained reducing sugar **and** starch.

With reference to Fig. 6.1, describe a method that would allow the student to use the immobilised amylase to collect a product that contains **only** reducing sugar.

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(b) One standardised variable in the investigation is the size of the alginate beads.

Suggest **one** reason why using larger or smaller alginate beads in the column would affect the results obtained.

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13 The enzyme glucose 6-phosphate dehydrogenase (G6PD) is composed of two identical polypeptide chains.

(a) Students investigated the activity of two forms of G6PD, **J** and **K**, at different concentrations of substrate. **K** is a form of the enzyme that results from a mutation that changes one amino acid in the polypeptide.

The results are shown in Fig. 3.1.

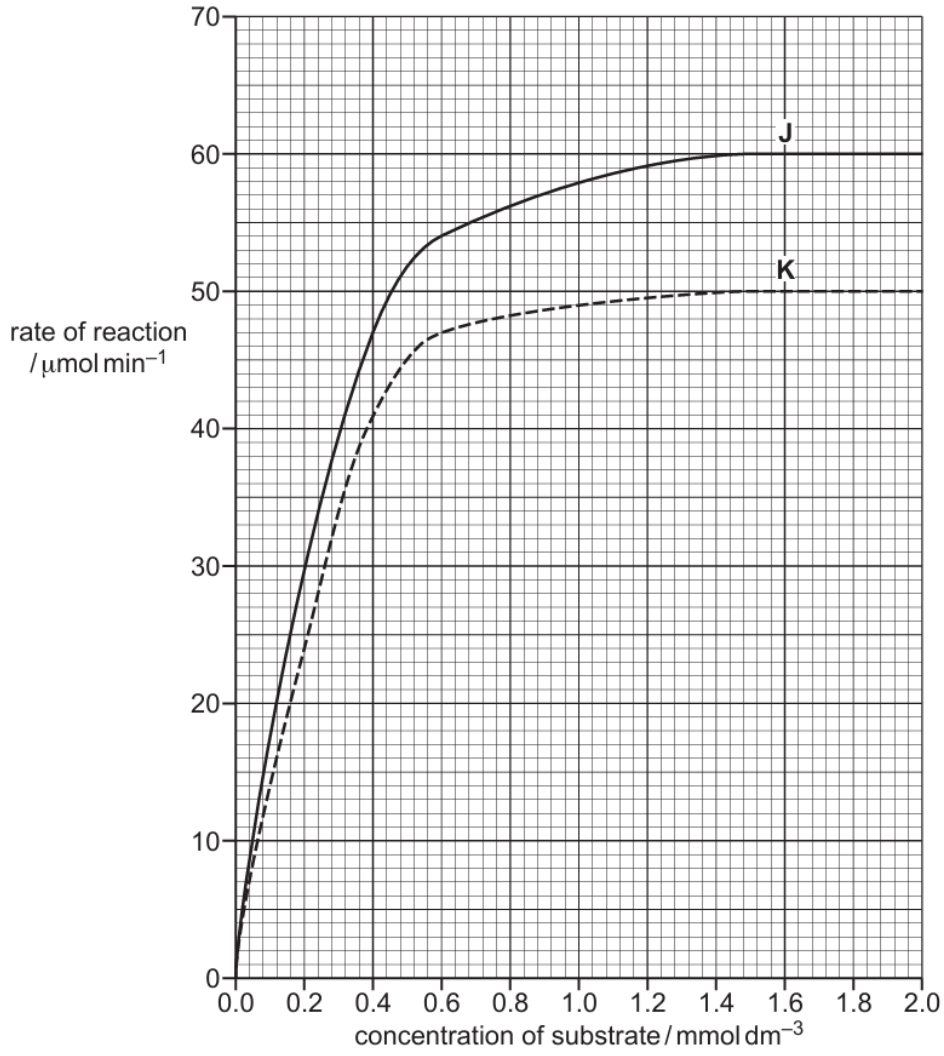


Fig. 3.1

(i) Use Fig. 3.1 to complete Table 3.1 by stating:

- the V_{max} and the K_m for enzymes **J** and **K**
- the units for V_{max} and K_m.

Table 3.1

	V_{max} /	K_m /
J		
K		

[3]

(ii) With reference to Fig. 3.1 and Table 3.1, describe the effect of the mutation on the activity of G6PD **and** suggest an explanation for this effect.

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14 **(b)** Lipases are enzymes that digest triglycerides in the lumen of the human intestine. These enzymes are released by exocytosis from intestinal epithelial cells.

Scientists have found that treating milk with lipase can improve its taste.

The scientists carried out an experiment to determine the effect of lipase activity on the triglycerides found in milk.

- Lipase was immobilised in alginate beads.
- The pH of a known volume of milk was adjusted to pH 8 by adding an alkali.
- The beads were then mixed with this milk in a beaker.
- The pH of the reaction mixture was recorded over a period of 24 hours.

The results are shown in Fig. 2.2.

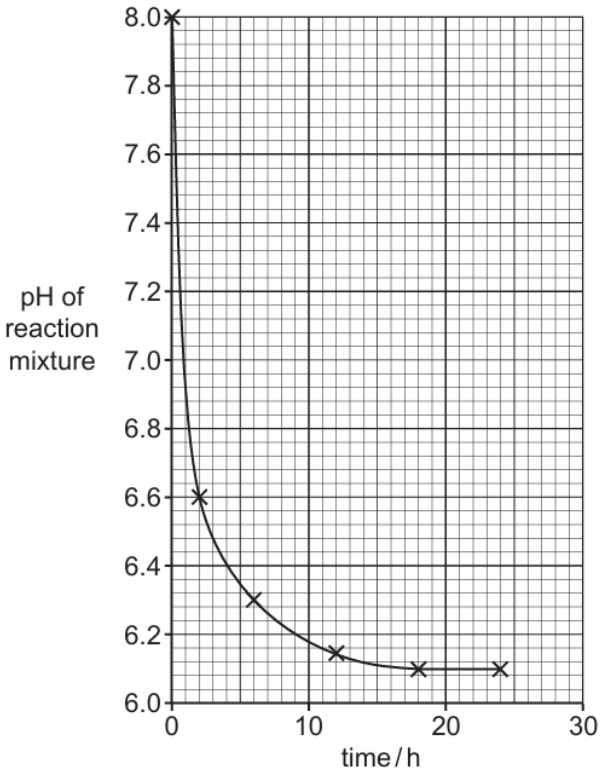


Fig. 2.2

Fig. 2.2 shows that the pH decreases steeply and then, after 18 hours, remains constant.

(ii) Calculate the time taken for the pH to decrease from pH 6.6 to pH 6.3.

time taken = ... h [1]

(iii) Explain the results shown in Fig. 2.2.

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(iv) The scientists repeated the experiment using a higher concentration of lipase. All other variables remained constant.

Predict how an increase in the concentration of the lipase would affect the results of the experiment.

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15 The treatment for people with active tuberculosis (TB) lasts six months and involves a combination of antibiotics. This is usually very effective if the person has a susceptible (non-resistant) strain of *Mycobacterium tuberculosis*, the causative organism of TB.

Table 2.1 summarises one recommended treatment strategy that involves a combination of antibiotics.

Table 2.1

antibiotic	length of treatment	mode of action of antibiotic
rifampicin (R)	6 months	enters bacterial cells and inhibits protein synthesis
isoniazid (H)	6 months	prevents the synthesis of cell wall components known as mycolic acids
ethambutol (E)	first two months	prevents mycolic acids from being added to the cell wall
pyrazinamide (Z)	first two months	prevents the synthesis of fatty acids

Rifampicin binds tightly to an RNA polymerase molecule close to its active site. This affects the activity of the enzyme.

(b) (ii) During the formation of RNA, a number of events occur that involve the action of RNA polymerase.

Suggest ways in which rifampicin can affect the activity of RNA polymerase.

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16 Sucrose phosphorylase is an enzyme found in some species of bacteria. One function of this enzyme is for the production of compounds that help to protect the cell from harmful osmotic changes in the external environment.

Fig. 5.1 shows the reversible reaction that takes place within the bacterial cell.

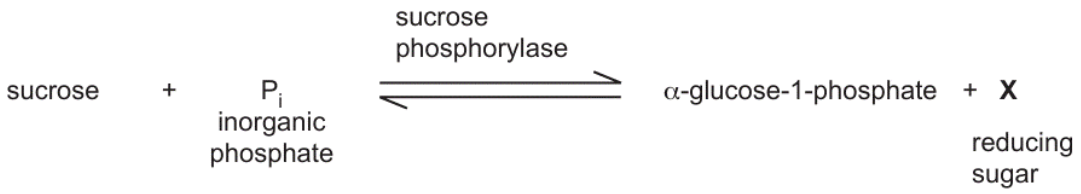


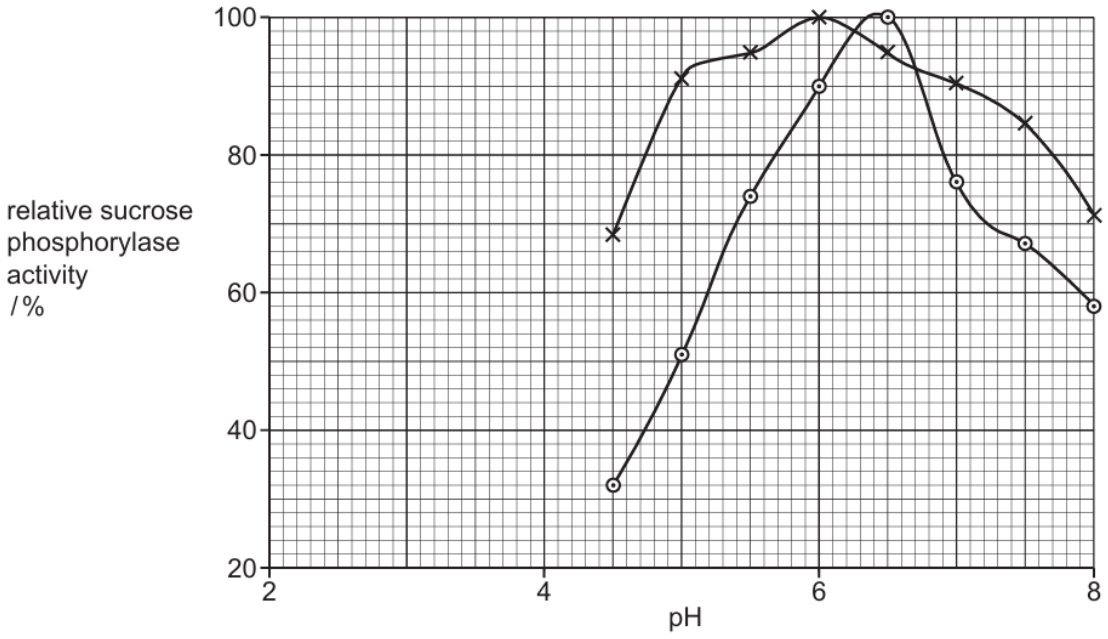
Fig. 5.1

(c) An enzyme that catalyses a reaction of commercial interest needs to be investigated to see if it is suitable for use in industry.
For example:

- immobilised enzymes may be used as they have a longer shelf-life than the enzyme free in solution
- many industrial reactions are carried out at higher temperatures to minimise contamination of products by microorganisms.

Fig. 5.2 shows the results of an investigation to compare the activity of sucrose phosphorylase free in solution (free enzyme) with immobilised sucrose phosphorylase (immobilised enzyme) at different pHs.

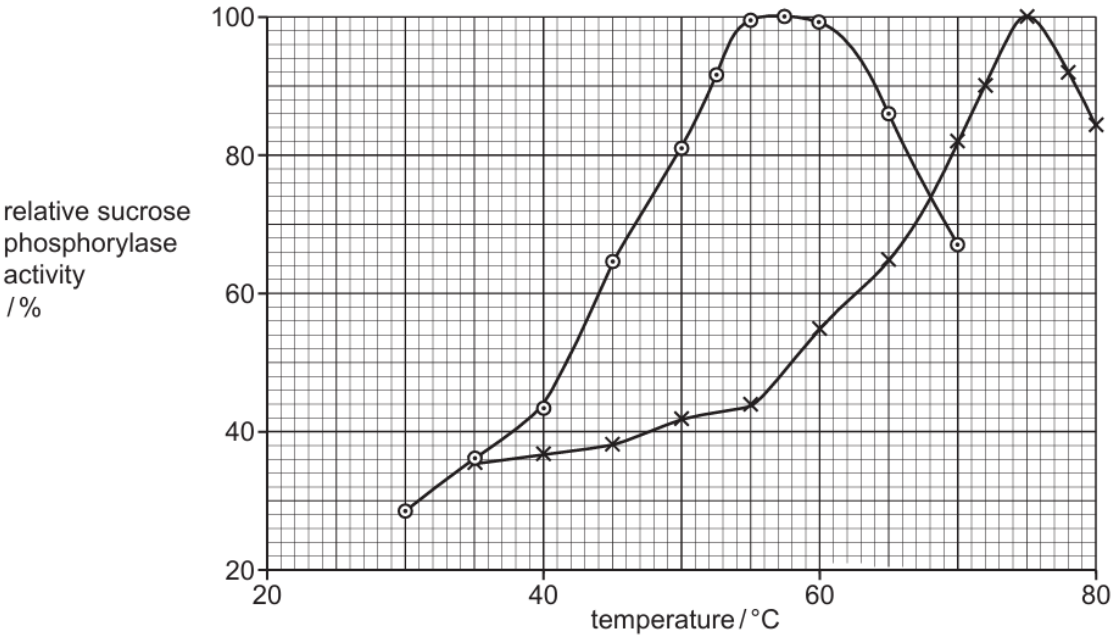
Fig. 5.3 shows the activity of the free enzyme and immobilised enzyme at different temperatures.



Key

⊙ free enzyme
× immobilised enzyme

Fig. 5.2



Key

⊙ free enzyme

× immobilised enzyme

Fig. 5.3

With reference to the results shown in Fig. 5.2 and Fig. 5.3, discuss which sucrose phosphorylase enzyme, free or immobilised, is better for use in industrial reactions.

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17 Fig. 2.1 is a transmission electron micrograph showing the bacterial pathogen that causes tuberculosis (TB).



Fig. 2.1

(b) Rifampicin is one of the antibiotics used to treat TB.

Rifampicin inhibits RNA polymerase in bacterial cells by binding to a site other than the active site. This prevents polypeptide synthesis.

(i) Suggest **and** explain how rifampicin prevents polypeptide synthesis in bacterial cells.

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18 Telomerase is an enzyme that is important in maintaining the telomeres that are located at the ends of chromosomes.

(d) An investigation was carried out into the effect of substrate concentration on the activity of telomerase.

The results are shown in Fig. 6.1.

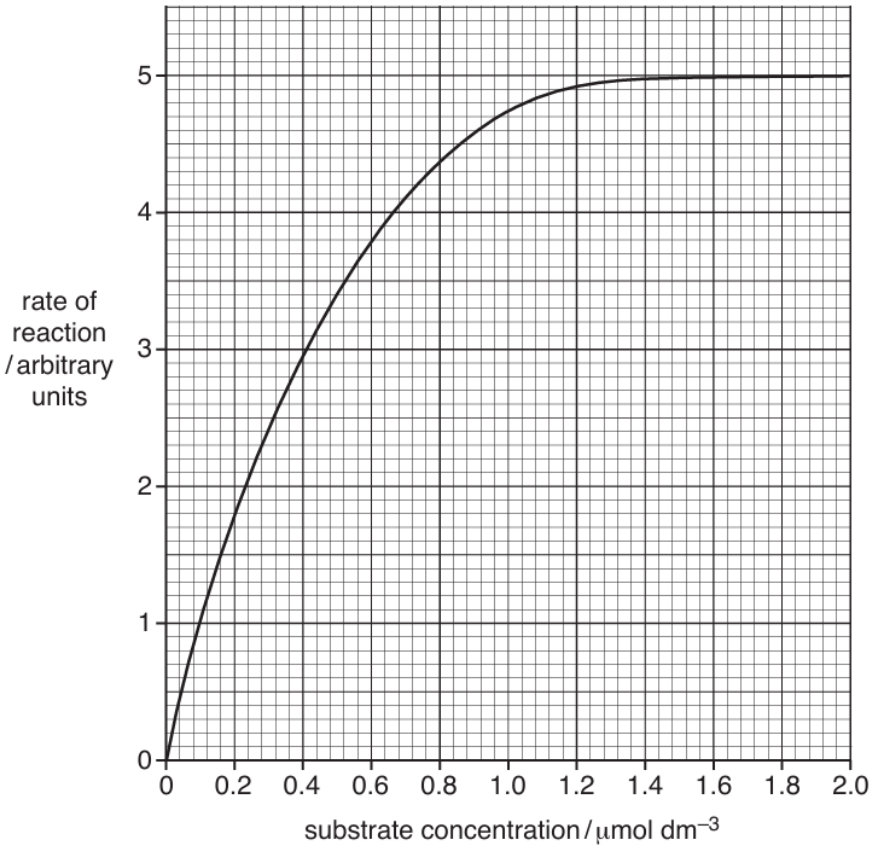


Fig. 6.1

With reference to Fig. 6.1, explain the difference in the rates of reaction obtained for telomerase at a substrate concentration of 0.2 μmol dm^{-3} compared with a substrate concentration of 1.6 μmol dm^{-3}.

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19 Neutrase® is an enzyme that is used to hydrolyse proteins in solution. When the enzyme is mixed with a 2% protein solution the reaction mixture changes from white to colourless.

A student carried out an experiment to find the effect of copper sulfate and potassium sulfate on the activity of Neutrase®.

The student made four reaction mixtures in test-tubes **A** to **D**. Test-tubes **A** to **C** contained equal volumes of protein solution and 0.1 cm^3 of solutions of copper sulfate or potassium sulfate. Test-tube **D** contained the same volume of protein solution and 0.1 cm^3 of water.

0.5 cm^3 of a 1% Neutrase® solution was added to test-tube **A** and immediately placed into a colorimeter. The colorimeter was used to measure the intensity of light that is absorbed by the solution (absorbance) over 100 seconds. The procedure was repeated with the other reaction mixtures, **B**, **C** and **D**.

The results are shown in Fig. 3.1.

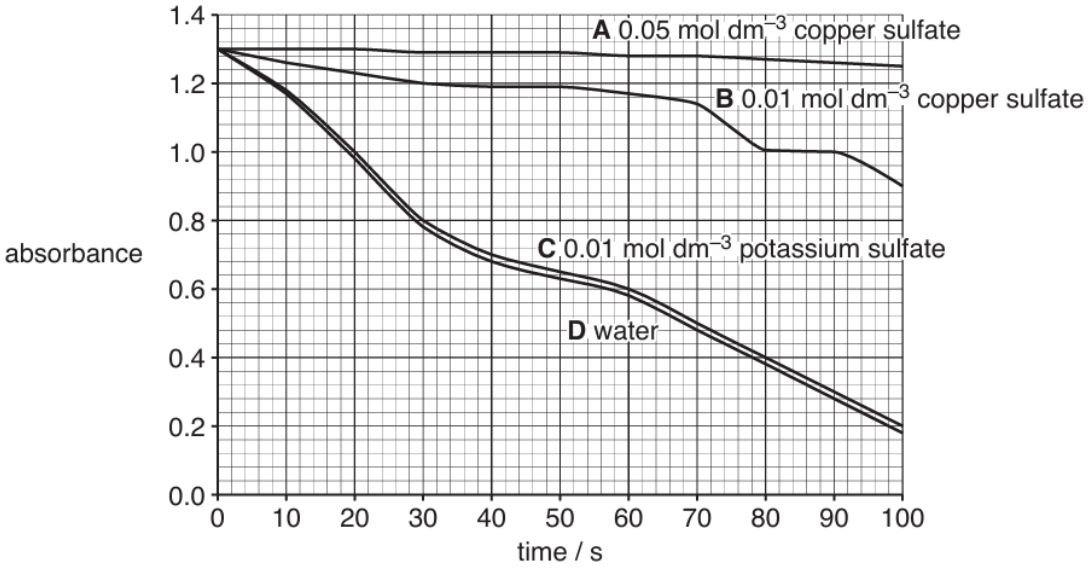


Fig. 3.1

(a) (ii) With reference to Fig. 3.1:

- describe the effects of copper sulfate solution and potassium sulfate solution on the activity of Neutrase®
- suggest explanations for the effects that you have described.

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(b) Neutrase® can be immobilised in alginate. Immobilised Neutrase® is used in the food industry to produce foods with high nutritional content.

Explain the advantages of using immobilised enzymes, such as Neutrase®, compared with using the same enzymes free in solution.

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20 Catalase is an enzyme that catalyses the breakdown of hydrogen peroxide, which is a waste product of cell metabolism.

The reaction catalysed by catalase is shown in Fig. 6.1.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Fig. 6.1

(a) A student carried out two experiments to investigate the progress of the reaction shown in Fig. 6.1. Potato tissue was used as the source of the enzyme.

Six pieces of potato were cut, each measuring 20 mm × 10 mm × 10 mm.

In the first experiment, hydrogen peroxide solution was added to three of the pieces of potato tissue and the progress of the reaction was measured.

Fig. 6.2 shows how the first experiment was set up.

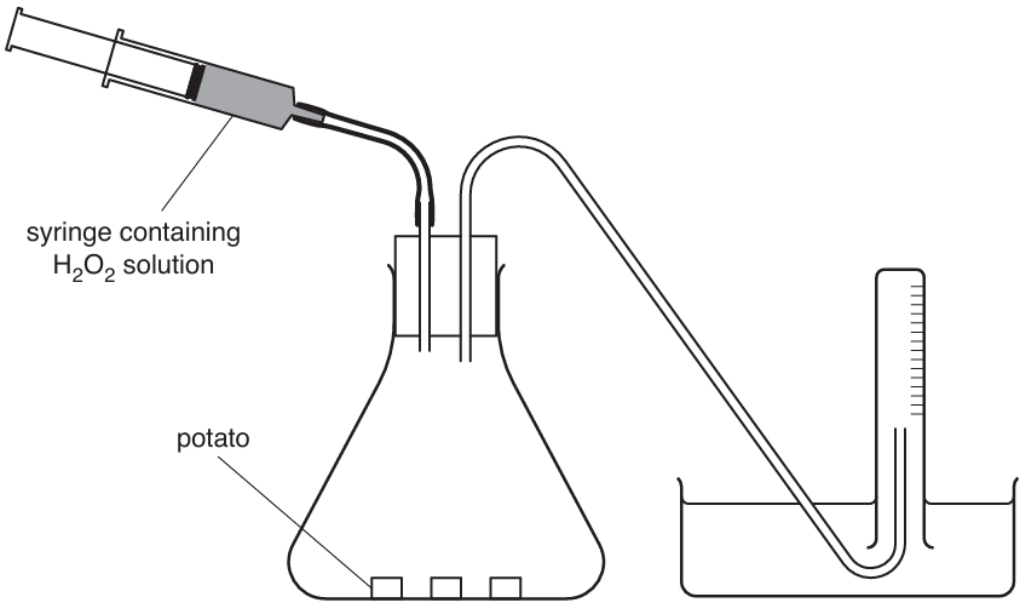


Fig. 6.2

(ii) In the second experiment, the student cut each of the three remaining pieces of potato to obtain six pieces, each measuring 10 mm × 10 mm × 10 mm.

Using exactly the same conditions, the student measured the progress of the reaction and obtained different results to the first experiment.

Explain why the results of the second experiment were different from the results of the first experiment.

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(b) The student then investigated the effect of temperature on the activity of catalase.

On Fig. 6.3, sketch a curve to show how temperature affects the activity of an enzyme such as catalase.

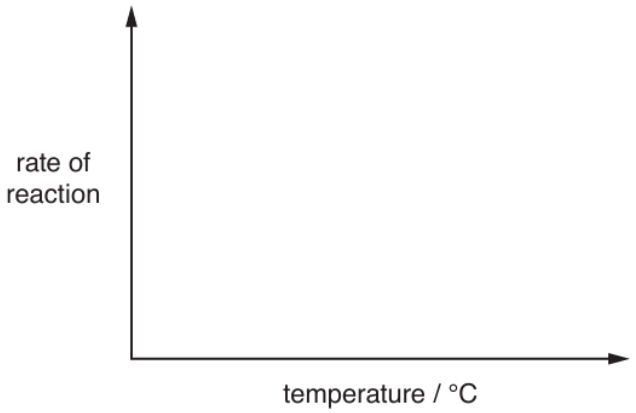


Fig. 6.3

[1]

21 Enzyme inhibitors and monoclonal antibodies can be used in the treatment of disease.

(a) Mevinolin is an enzyme inhibitor that can be prescribed as a drug to reduce the concentration of cholesterol in blood plasma.

High concentrations of cholesterol in the blood have been linked to an increased risk of cardiovascular disease.

Mevinolin acts as a competitive inhibitor of the enzyme HMG CoA reductase. This enzyme catalyses one of the first steps in the synthesis of cholesterol, as shown in Fig. 6.1.

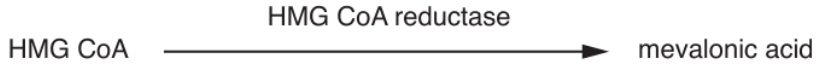


Fig. 6.1

Explain how mevinolin inhibits the enzyme HMG CoA reductase.

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22 Researchers isolated a sucrase enzyme from the bacterium *Bacillus subtilis*. They immobilised the enzyme in alginate beads.

The researchers investigated the effects of temperature on the activity of the immobilised sucrase compared with the activity of the same enzyme free in solution.

The results are shown in Fig. 3.1.

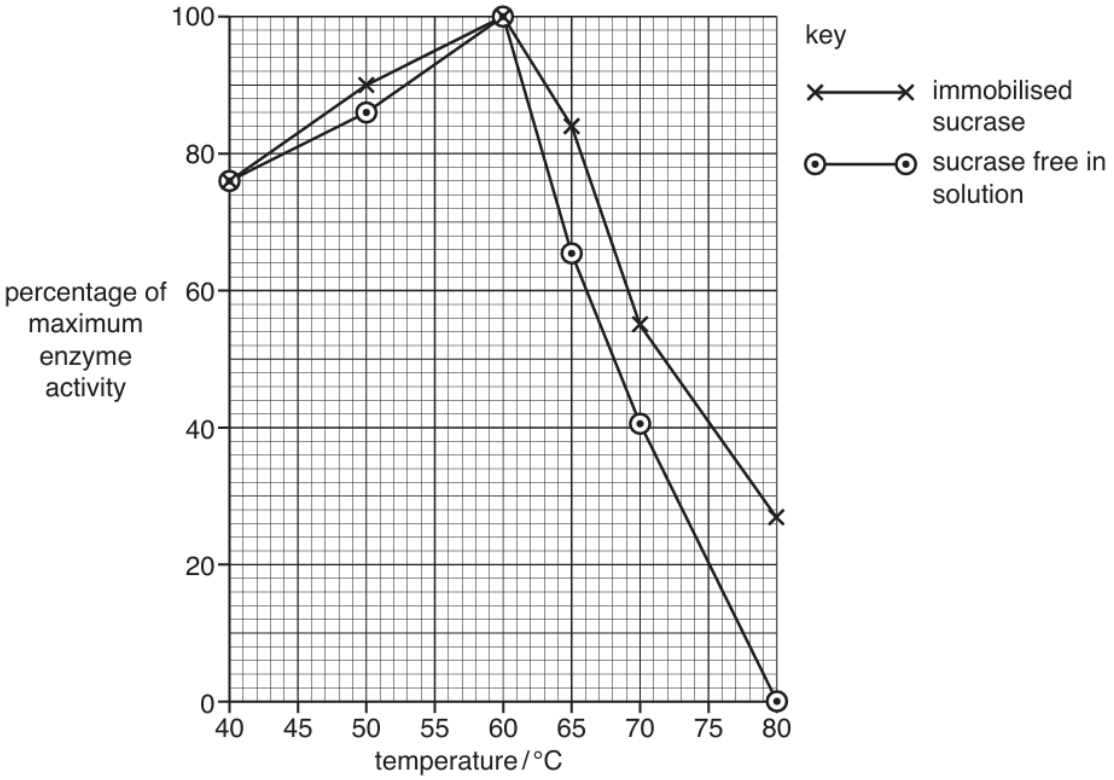


Fig. 3.1

(a) With reference to Fig. 3.1, **compare** the effects of temperature on the activity of immobilised sucrase with the activity of sucrase free in solution.

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The researchers also investigated the effects of pH on the activity of the immobilised sucrase compared with its activity free in solution.

The results are shown in Fig. 3.2.

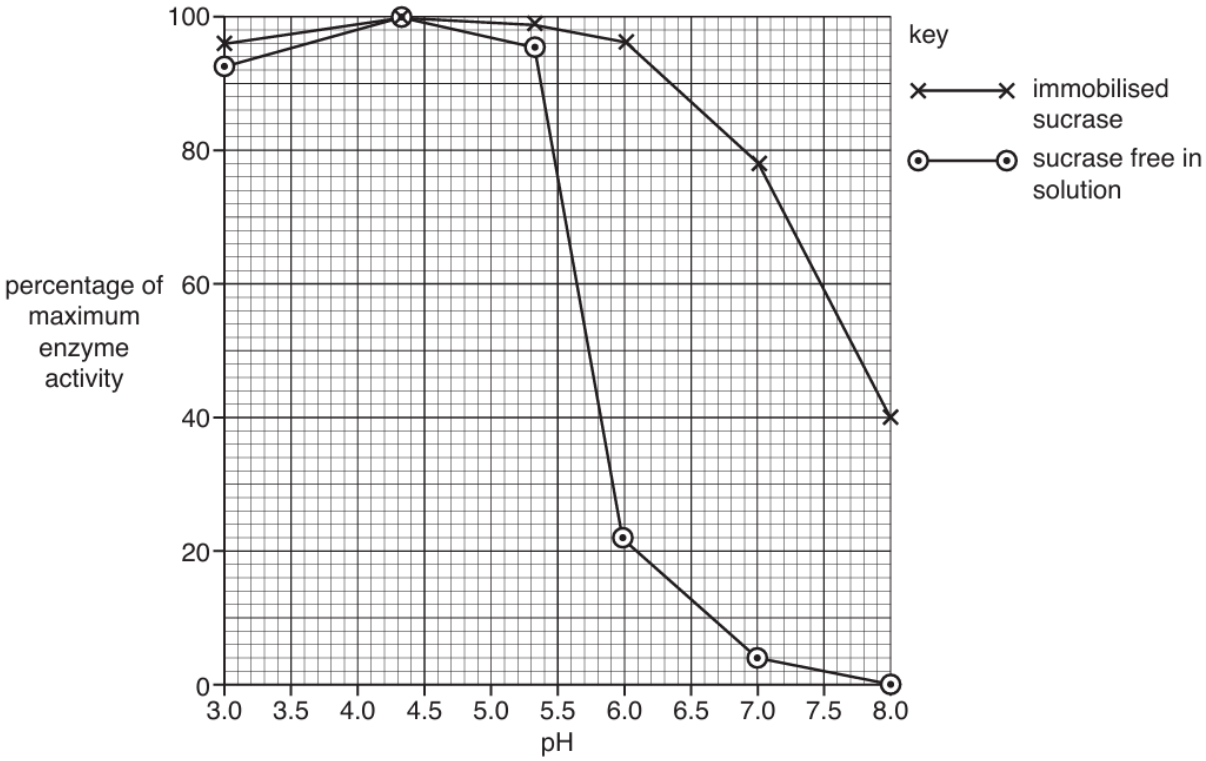


Fig. 3.2

(b) Fig. 3.2 shows that immobilised sucrase remains active over a wider range of pH compared with sucrase free in solution.

Suggest reasons for the higher activity of immobilised sucrase over the range of pH between 5.5 and 8.0.

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(c) State **one** variable that the researchers should keep constant in **both** investigations **and** explain your answer in terms of enzyme action.

variable .....

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(d) There are many advantages of using immobilised enzymes in industry.

Suggest **two** advantages of using immobilised enzymes in industry **other than** remaining active over a greater range of pH.

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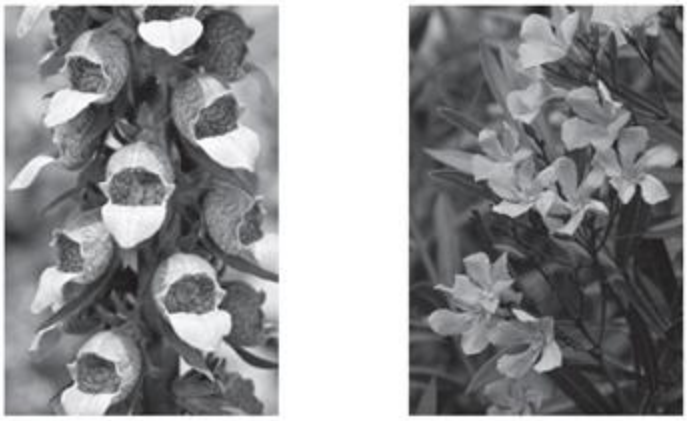
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23 Woolly foxglove, *Digitalis lanata*, shown in Fig. 2.1**A**, and common oleander, *Nerium oleander*, shown in Fig. 2.1**B**, are plants grown for the attractive flowers that they produce.

Both plants are poisonous, as their leaves produce toxic organic compounds known as cardiac glycosides. Cardiac glycosides have a powerful effect on the action of cardiac muscle.



A B

Fig. 2.1

Cardiac glycosides have an effect on the movement of ions into and out of cardiac muscle cells. The outcome is an increased ability for the cells to contract.

(c) Investigations into the action of the cardiac glycoside oleandrin, extracted from *N. oleander*, have shown that it acts to prevent the correct functioning of Na/K-ATPase, a membrane transport protein.

Na/K-ATPase has a role as an enzyme **and** as a transport molecule.

- ATPase is an enzyme that catalyses the hydrolysis of ATP to ADP and inorganic phosphate.
- Energy released from this hydrolysis is used to transport sodium ions (Na^+) out of cardiac muscle cells and potassium ions (K^+) into the cells.

(iii) Oleandrin is a non-competitive reversible inhibitor of ATPase.

Describe the mode of action of oleandrin **and** explain how this will affect ion movement through Na/K-ATPase transport proteins of the cell surface membranes of cardiac muscle cells.

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24 Fig. 2.1 shows the disaccharide lactose, which is found in milk.

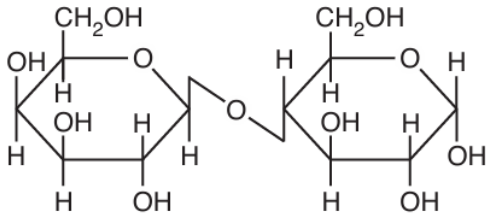


Fig. 2.1

(c) Enzymes, such as lactase, are often immobilised for use in the food industry.

A scientist carried out an investigation to determine the effects of temperature on the activity of lactase when it was immobilised and when it was free in solution.

The scientist produced alginate beads containing lactase for use in this investigation. The beads varied in size. The scientist selected small beads for the investigation and put them into a glass column.

(i) Suggest the advantage of using small beads rather than large beads.

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(ii) Fig. 2.2 shows the results of the investigation to determine the effects of temperature on the activity of lactase when it was immobilised, **I**, and when it was free in solution, **F**.

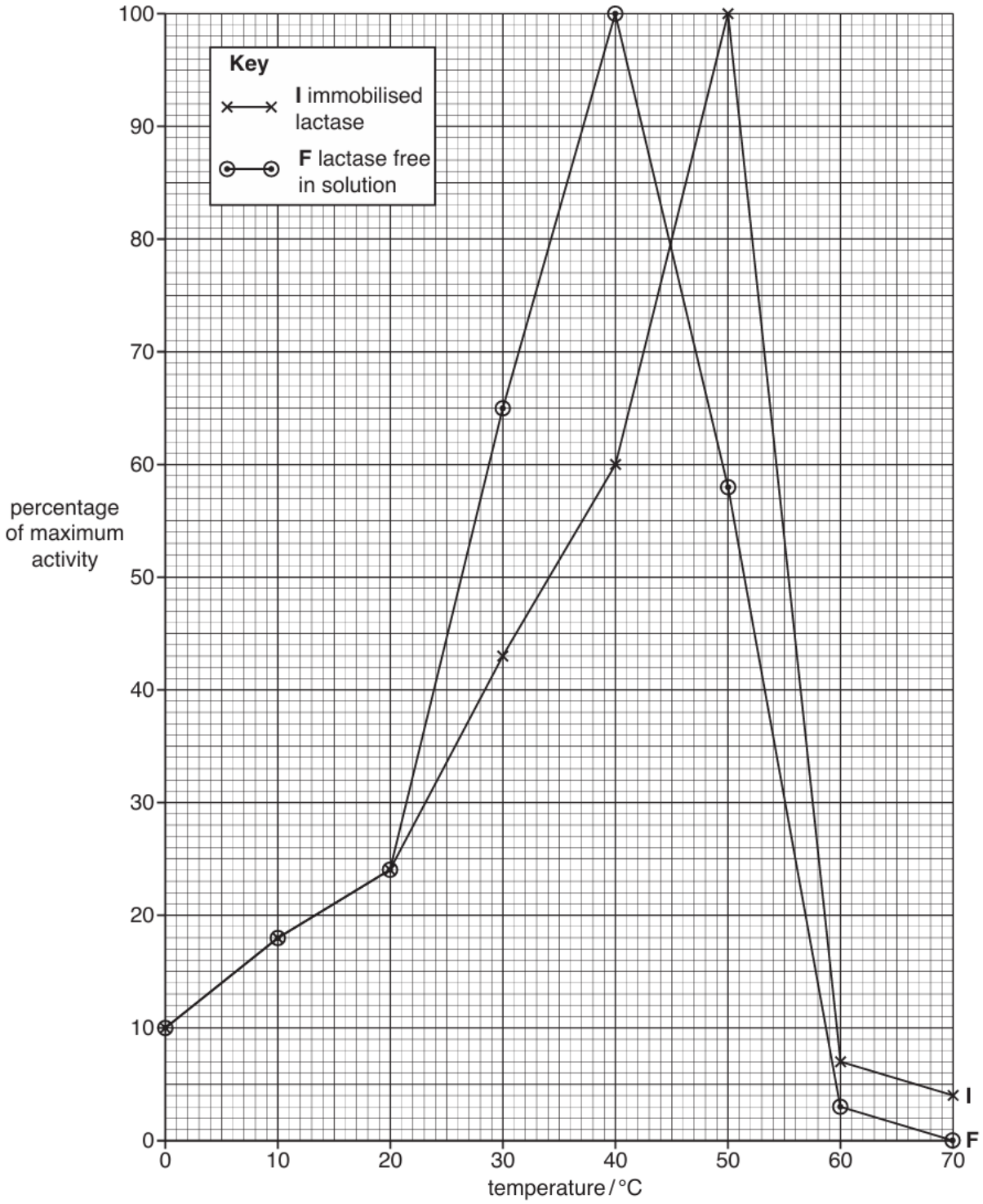


Fig. 2.2

With reference to Fig. 2.2, compare the effect of temperature on the activity of immobilised lactase, **I**, and lactase free in solution, **F**.

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