20(a). The student conducted a further investigation using the same enzyme and substrate.

- A range of substrate concentrations was used.
- The investigation was repeated in the presence of an inhibitor of amylase activity extracted from kidney beans.

Fig. 2.2 shows a sketch of the student's results.

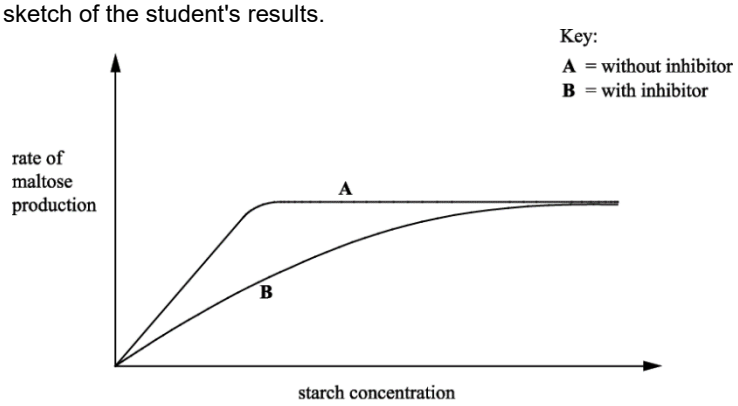


Fig. 2.2

i. Explain the mechanism by which the extract from the kidney bean inhibited the amylase.

---

[3]

ii. What evidence from the graph supports your answer to part **(i)**?

---

[1]

(b). Amylase is an enzyme that breaks down starch into maltose.

A student investigated the breakdown of starch into maltose. The results are shown in **Fig. 2.1**.

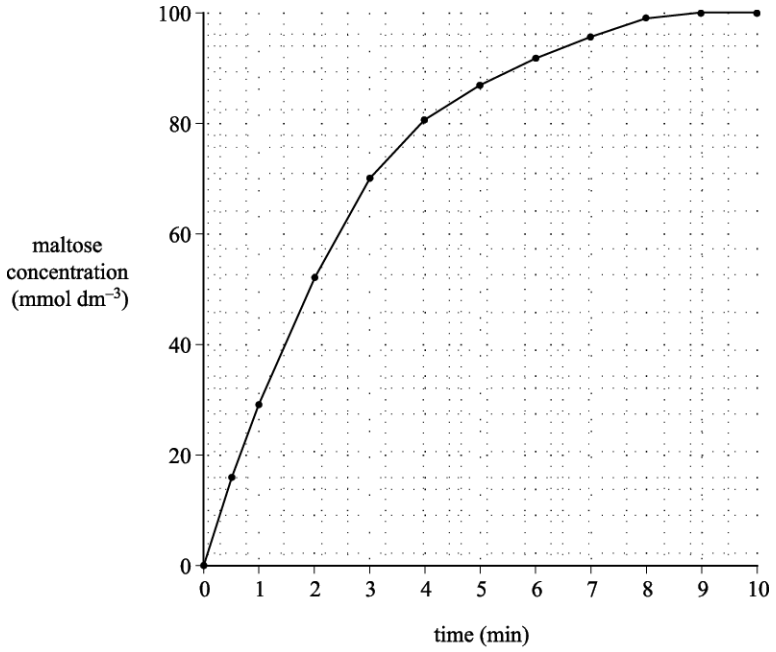


Fig. 2.1

i. Calculate the rate of maltose production over the first 30 s.

Show your working and use appropriate units.

Answer..... **[2]**

ii. How would this calculated rate differ from the 'true' initial rate of reaction?
Explain your answer.

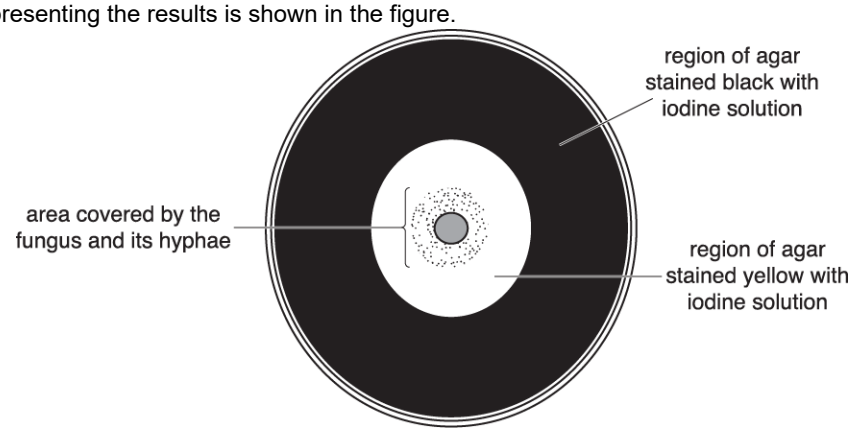
---

[3]

21. Fungi produce enzymes to digest complex food substances. Amylase is an enzyme that catalyses the conversion of starch to maltose.

- A sample of the fungus *Amanita citrina* was placed on agar in a petri dish.
- The agar contained starch.
- The dish was incubated until the thread-like hyphae had grown a few centimetres.
- Iodine solution was then poured onto the surface of the agar.

A diagram representing the results is shown in the figure.



i. To which genus does this fungus belong?

---

[1]

ii. The region of yellow staining shown in the figure includes part of the agar where the fungus had not yet grown.

What does this pattern indicate about the action of the fungal enzymes?

---

[1]

22. Liver is a rich source of the enzyme catalase.

The table below describes examples of three terms from the list below.

coenzyme **competitive inhibition** **enzyme-product complex**

induced-fit **lock and key** **non-competitive inhibition**

prosthetic group **secondary structure** **tertiary structure**

Complete the table by choosing a term from the list that matches each example.

Example	Term
The haem molecule bound to each polypeptide chain of catalase.	
Binding of the hydrogen peroxide substrate to catalase causes a change in the shape of the active site of catalase.	
Cyanide ions decrease the action of catalase even at high concentrations of hydrogen peroxide.	

[3]

23. Which of the statements about enzyme catalysed reactions is true?

A Binding of a substrate to the active site can weaken bonds in the substrate.

B Enzymes allow reactions to happen at a rate faster than the V_{max}.

C Enzymes increase the rate of reactions by increasing the activation energy.

D Every 10 °C increase in temperature will approximately double the rate of all enzyme controlled reactions.

Your answer ☐

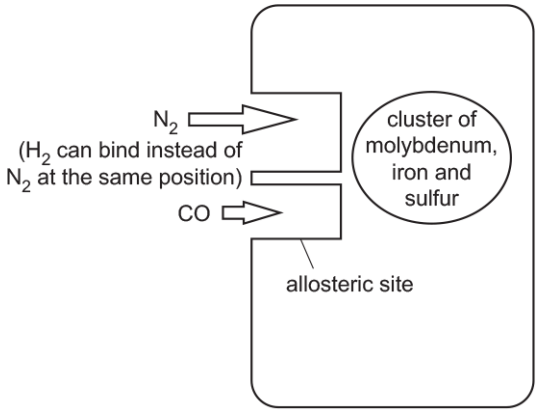
[1]

24. Nitrogen fixation is an important part of the nitrogen cycle.

The rate of nitrogen fixation is reduced by the presence of oxygen.

Rhizobium uses the enzyme nitrogenase to fix atmospheric nitrogen.

Fig. 4 shows a simplified representation of the structure of nitrogenase and the reaction that it catalyses.



Overall reaction: $N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$

Fig. 4

i. What can you conclude from Fig. 4 about the molecules or ions that affect the functioning of the nitrogenase enzyme?

---

[4]

ii. Leghaemoglobin is a molecule that improves the performance of nitrogenase. It has very similar properties to mammalian haemoglobin.

Suggest **two** ways in which leghaemoglobin improves the performance of the nitrogenase enzyme.

---

[2]

25. Icefish live in very cold water.

Icefish contain biological molecules that allow them to tolerate cold temperatures.

A group of students investigated the effect of temperature on the activity of two forms of trypsin: human trypsin and icefish trypsin.

Part of their method is shown below:

- use 10 cm^3 of 5% trypsin solution for all trials
- measure enzyme activity at 10, 20, 30, 40 and 50 °C for both enzymes
- carry out each trial in the same pH buffer
- repeat the experiment 5 times at each temperature
- measure enzyme activity by recording the area of gelatine on a photographic film that is broken down over a set time period
- calculate the rate of enzyme activity at each temperature.

i. Suggest **and** explain two improvements that would increase the validity of the students' investigation.

Improvement: ---

Explanation: ---

Improvement ---

Explanation ---

---

[4]

ii. Suggest appropriate units to use to represent the rate of enzyme activity in this investigation.

---

[1]

iii. The students recorded the temperature that produced the fastest reaction rate in each of the five replicates. These results are shown in Table 3.

Replicate	Temperature that produced the fastest reaction rate (°C)	
	Human trypsin	Icefish trypsin
1	40	20
2	10	10
3	30	20
4	40	30
5	40	30
Mean =	32.0	22.0
Mode =	40	20 and 30
Median =	40	20

Table 3

One of the students made the following statement:

I think the mean is a more accurate measure than the median or mode for these results.

Evaluate the student's statement.

---

[2]

iv. The students wanted to know whether there was a difference between the reaction rates of the two forms of trypsin at 30 °C.

They performed a statistical test on the mean of the five replicates for human trypsin and the five replicates for icefish trypsin.

Suggest the most appropriate statistical test for the students to use **and** explain why this test is appropriate.

---

[2]

26. The concept of molecules with complementary shapes can be used to explain many processes in living things.

Complete the following passage about the mechanism of enzyme action.

Enzymes are proteins which speed up the rate of biological reactions. They form an ..... by binding to their substrate at a site known as the..... . This site has a specific shape created by the..... structure of the protein molecule. This means that each enzyme can bind to only one type of substrate molecule.

This is explained by the lock and key hypothesis. In an alternative hypothesis, the binding site changes shape to fit more closely around the substrate molecule. This is called the ..... hypothesis. This hypothesis can help to explain how enzymes enable reactions to occur at lower temperatures by reducing the ..... required for the reaction to occur.

[5]

27. Enzymes are affected by temperature. Fig. 25.2 shows the time course of a mammalian enzyme reaction at different temperatures.

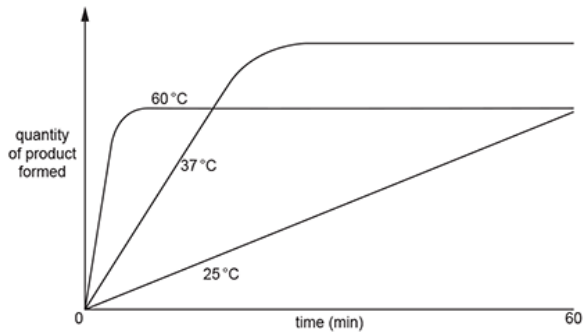


Fig. 25.2

i. Explain why there is a difference in the shapes of the curves at 37 °C and 60 °C.

_____

_____

_____

_____

[2]

ii. Explain why there is a difference in the shapes of the curves at 25 °C and 37 °C.

_____

_____

_____

_____

_____

_____

[2]

28(a). The protease enzyme bromelain can be extracted from pineapples. A student investigated the effect of changing the concentration of the enzyme and measured the time taken to break down the protein gelatine.

The data from the student's experiment is shown in Table 26.

Concentration of bromelain (%)	Rate of protein digestion (s^{-1})	Standard deviation
0.010	0.0037	0.00014
0.025	0.0090	0.00034
0.050	0.0155	0.00260
0.075	0.0184	0.00371
0.100	0.0198	0.00340

Table 26

i. Describe how the rate of reaction was calculated.

_____

_____

[1]

ii. Explain what the standard deviation shows in Table 26.

_____

_____

_____

_____

[2]

(b). Fig. 26 shows the results plotted on a graph with the standard deviations as error bars.

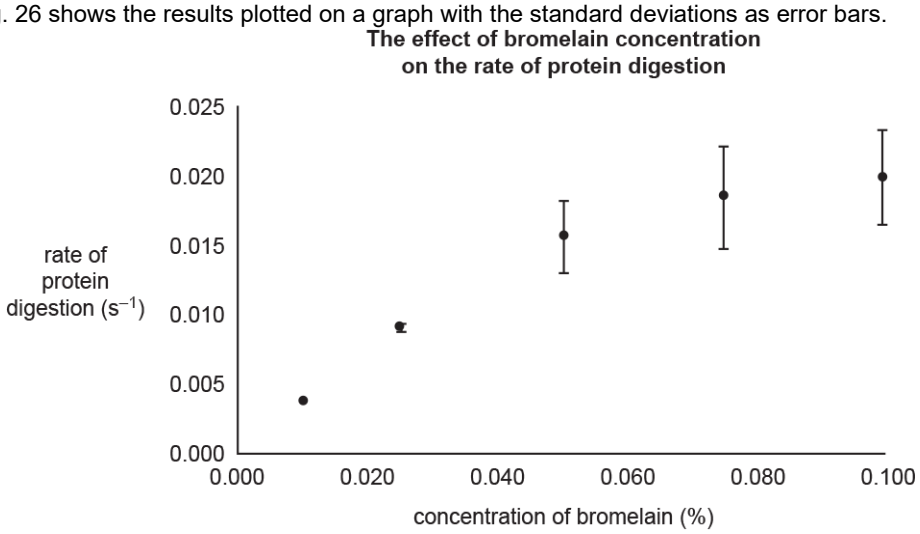


Fig. 26

Explain the pattern shown in the data using Table 26 and Fig. 26.

_____

_____

_____

_____

_____

_____

[3]

29(a). Lipase is an enzyme that catalyses the breakdown of lipids.
An investigation was carried out to see the effect of temperature on the activity of a lipase.

- 5 cm^3 of an alkaline solution of lipid was poured into a test tube.
- The test tube was placed into a water bath maintained at 20 °C and left to equilibrate.
- A few drops of an indicator were added to the wells of a white spotting tile. The indicator is pink above pH values of 8.3 and turns colourless at pH values below 8.3.
- Once the lipid solution had equilibrated, 1 cm^3 of 0.5% lipase solution at the same temperature was then added to the test tube.
- For five minutes, at 30 second intervals, the solution was stirred and a few drops were removed from the test tube and placed in a well on the white spotting tile.
- The time was recorded when the solution and indicator did not remain pink.
- The procedure was repeated four more times at 20 °C and then again at a further six temperatures.

The results are shown in Table 4.1 below.

Temperature (°C)	Time when solution did not remain pink				
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
20	210	270	240	300	270
25	90	120	210	180	120
30	60	60	90	90	60
35	60	60	60	90	60
40	210	120	210	180	210
45	240	300	300	–	270
50	–	–	–	–	–

Table 4.1

i. Why is pH **not** a controlled variable in this investigation?

---

[1]

ii. Identify **one** variable that has been controlled in this procedure.

---

[1]

iii. Identify **one** variable, other than pH, that has **not** been controlled in this procedure.

---

[1]

iv. The procedure required the solution to be stirred and then drops of solution to be placed on a white spotting tile. Suggest why this procedure was followed rather than simply adding indicator to the test tube, stirring the solution and looking for the colour change in the test tube.

---

[1]

v. What can be concluded from the results in Table 4.1 about the optimum temperature for lipase activity?

---

[1]

vi. Describe **two different** ways in which the procedure could be modified to obtain a more accurate value for the optimum temperature for lipase activity.

1)

---

2)

---

[4]

(b). There are two models for the mechanism of enzyme action. Outline how changes in temperature can affect these mechanisms of lipase action.

[6]

30(a). The student then investigated the effect of pH on the activity of the amylase.

This was the method used,

- Tubes containing starch and amylase were set up in a range of pH buffer solutions.
- The same concentration of starch and amylase were used each time.
- A small sample of the solution was removed and tested for the presence of starch at 20 s intervals.
- The procedure was repeated three times and a mean was calculated for each pH.

The student presented the results in **Table 2.1**.

pH	4	5	6	7	8	9
Mean amylase activity (% of maximum)	27	68	96	100	50	29

Table 2.1

i. Another student wanted to replicate the investigation.

Refine the method, by giving additional information, so that reproducible results would be obtained.

---

[3]

ii. Explain, with reference to bonding, why amylase activity is low at pH 4.

---

[4]

iii. The student concluded that the optimum pH for amylase was pH 7.

A teacher made the following statement:

*'The results in **Table 2.1** provide only weak support for the conclusion that the optimum pH for amylase is pH 7.0'*

Evaluate the statement **and** suggest an improvement to the student's procedure that would support the conclusion more strongly.

Evaluation

Improvement

---

[3]

(b). Amylase activity is increased in the presence of chloride ions.

State the name given to any inorganic ion that increases the activity of an enzyme.

---

[1]

31. Some inorganic ions have roles in enzyme-controlled reactions.

Which of the rows, **A** to **D**, in the table below is correct?

	Role of ion	
	Cofactor for amylase	**Prosthetic group for carbonic anhydrase**
A	Zn^{2+}	Cl^-
B	Zn^+	Cl^-
C	Cl^{2-}	Zn^+
D	Cl^-	Zn^{2+}

Your answer ☐

[1]

32. Catalase is an intracellular enzyme with an iron-containing haem group.

i. State the term used to describe an ion that is essential for the enzyme to function.

---

[1]

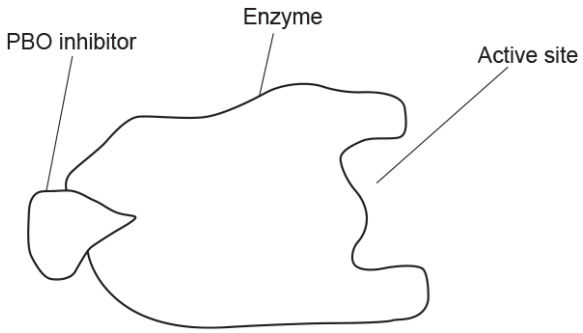
ii. Name another conjugated protein that contains a haem group.

---

[1]

33. Mosquito nets help to prevent the spread of malaria. They are often treated with an insecticide called permethrin. Some mosquitoes have developed resistance to the insecticide permethrin. Resistant mosquitoes produce an enzyme to detoxify the permethrin.

Scientists discovered that piperonyl butoxide (PBO) inhibits the activity of this enzyme in mosquitoes. The diagram shows how PBO acts on this enzyme.



With reference to the diagram, describe how PBO is acting as an enzyme inhibitor.

---

[3]

34. Fig. 18.2 shows the effect of concentration of hydrogen peroxide on the rate of reaction of catalase.

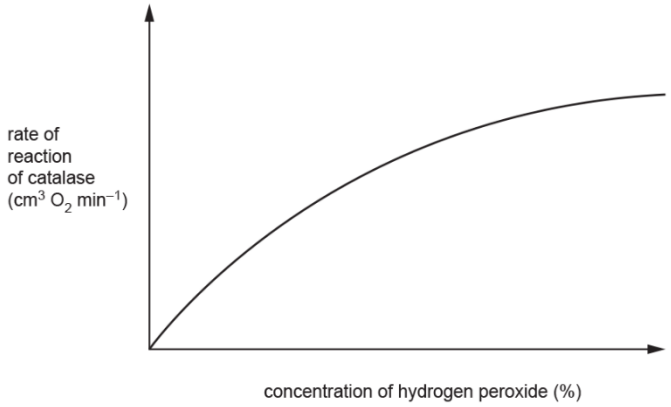


Fig. 18.2

Cyanide ions inhibit catalase, even at high concentrations of hydrogen peroxide.

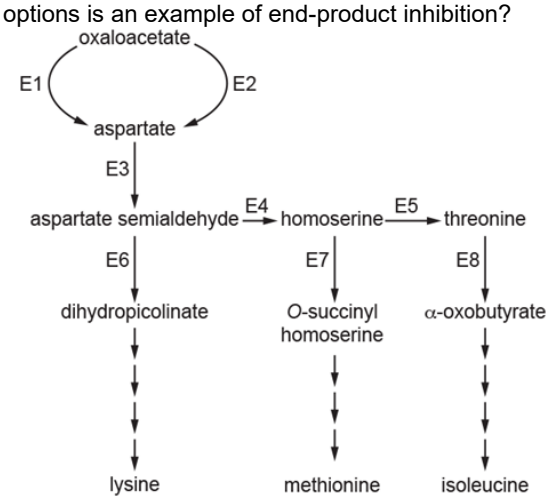
Sketch a line on **Fig. 18.2** to show the effect of repeating the experiment in the presence of cyanide ions.

[Answer on Fig 18.2] **[1]**

35. The figure shows the metabolic pathways leading to synthesis of five amino acids: aspartate, lysine, methionine, threonine and isoleucine.

E1 to E8 represent different enzymes involved in these pathways.

Which of the options is an example of end-product inhibition?



A aspartate inhibits E3 and E4

B isoleucine inhibits E7

C lysine inhibits E1 and E6

D methionine inhibits E8

Your answer ☐

[1]

36. Respiration is an important metabolic process that takes place in all living cells.

The black widow spider, *Latrodectus hesperus*, paralyses and kills its prey with venom. The venom contains a toxin known as latrotoxin. If a human is bitten, this toxin can cause serious harm by damaging heart tissue. Latrotoxin causes influx of Ca^{2+} ions, which disrupts normal cell function, including respiration.

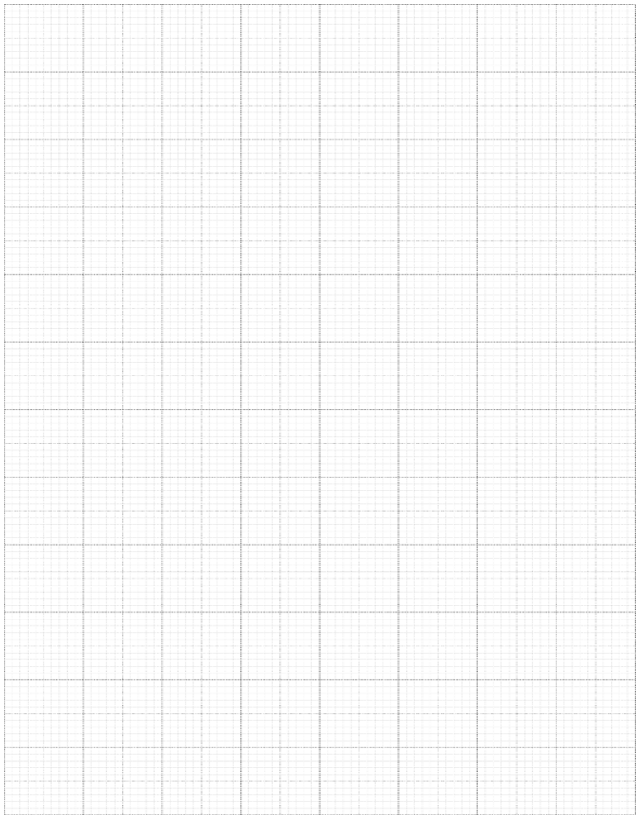
An investigation was carried out into the effect of latrotoxin on aerobic respiration in humans.

The rate of activity of malate dehydrogenase, a mitochondrial enzyme involved in aerobic respiration, was analysed at different substrate concentrations. The substrate concentrations used were within the normal range for a living cell.

The data is shown in Table 19.1.

Concentration of malate (mmol dm^{-3})	Rate of reaction of malate dehydrogenase (mmol dm^{-3} s^{-1})
0.0	0.0
1.0	37.7
2.0	55.2
3.0	66.0
4.0	74.8
6.0	83.1
8.0	88.9
10.0	92.3
14.0	96.9
18.0	99.0

i. Use the space provided to plot a suitable graph of these data.



ii. Calculate the mean increase in malate dehydrogenase activity for every 1 mmol dm^{-3} increase in malate concentration between 1.0 and 10.0 mmol dm^{-3}.

Give your answer to **two significant figures**.

Show your working.

Answer = ... **[3]**

iii. The normal maximum rate of malate dehydrogenase activity is 100 mmol dm^{-3} s^{-1}.

The data in Table 19.1, **on the Insert**, were obtained in the presence of latrotoxin.

What can be deduced from these results about latrotoxin's mode of action as a poison? Justify your answer.

_____

_____

_____

_____

_____

_____

[3]

37. Fig. 20.2 shows the effect of copper ions on the activity of catalase.

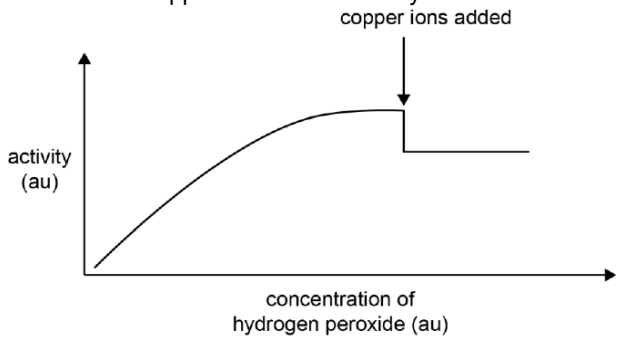


Fig. 20.2

What can you conclude from Fig. 20.2 about the type of inhibition shown? Explain your answer.

_____

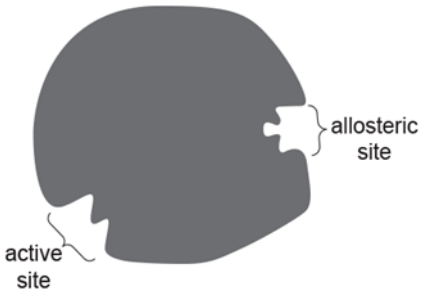
_____

_____

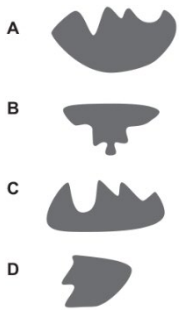
_____

[2]

38. The image below shows a diagram of an enzyme.



Which of the following could be a competitive inhibitor of this enzyme?



Your answer ☐ **[1]**

39. Copper (II) ions act as irreversible non-competitive inhibitors of the enzyme catalase.

i. Describe how a non-competitive inhibitor works to inhibit the activity of an enzyme.

---

[2]

ii. Catalase is found in all living things that are exposed to oxygen. It protects cells from oxidative damage by breaking down hydrogen peroxide to water and oxygen.

Catalase is a useful biomarker of oxidative stress in fish exposed to water contaminated with copper ions.

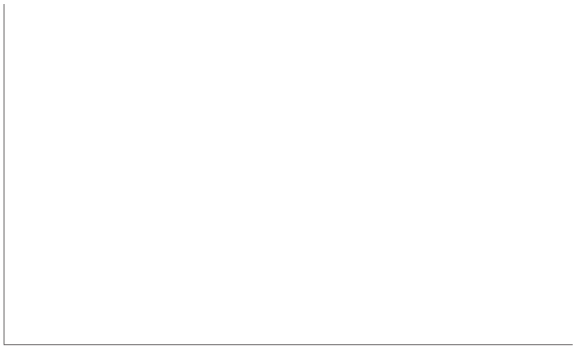
A group of students carried out an experiment to explore the effects of copper sulfate on the action of catalase. They measured the activity of catalase exposed to different concentrations of copper sulfate.

The results of their experiment are shown in Table 4.

Concentration of copper sulfate (moles dm^{-3})	**Volume of oxygen gas produced (cm^3)**
0.00	14.50
0.05	10.50
0.10	7.55
0.15	5.80
0.20	4.20

Table 4

In the space provided below, **sketch** a graph of the results in Table 4.



[2]

iii. What can the students conclude from their results?

_____

_____

_____

_____

_____

[2]

iv. Three rivers in the Himalayan foothills were polluted with copper, which affected the aquatic wildlife. Scientists were provided with one dead Indian Barb fish, *Esomus danricus*, from each of the rivers.

Scientists were unable to take a direct measurement of the copper ion concentration in the fish.

Using the information provided in (ii), suggest how the scientists could use the fish tissue to compare the copper ion pollution in the three rivers.

_____

_____

_____

_____

_____

_____

_____

[3]