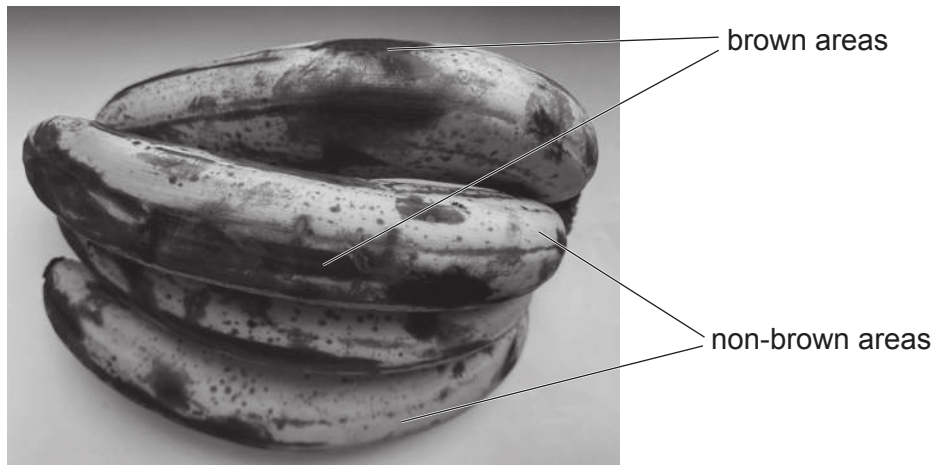




- 1 Polyphenol oxidase is an enzyme involved in the browning of fruit.

Fig. 1.1 shows browning of the skin of bananas.



**Fig. 1.1**

A student extracted polyphenol oxidase enzyme from ripened bananas. The student used this enzyme extract to investigate the factors affecting the activity of the enzyme.

The student initially found that the enzyme extract was too concentrated, so the student made a 1:10 dilution of the enzyme extract.

- (a) Describe how the student made a 1:10 dilution of the enzyme extract.

.....

.....

..... [1]

- (b) The student studied the effect of pH on the activity of polyphenol oxidase enzyme.

Dopamine hydrochloride solution was used as a substrate for the enzyme. The product of this reaction is red in colour.

A colorimeter can be used to follow the progress of this enzyme-catalysed reaction.

The student set up:

- 7 colorimeter tubes containing a solution of the substrate, dopamine hydrochloride
- 6 colorimeter tubes containing diluted polyphenol oxidase extract, each in a different buffered solution: pH 3.0, pH 4.0, pH 5.0, pH 6.0, pH 7.0, pH 8.0
- 1 colorimeter tube containing water in pH 7.0 buffer.

The colorimeter tubes were equilibrated at a set temperature.

A colorimeter was set up with a filter transmitting light at 470 nm.

At time 0 seconds, the substrate solution and the polyphenol oxidase solution at pH 3.0 were mixed in one colorimeter tube. The colorimeter tube was then immediately put into the colorimeter.

The absorbance was measured at 15 second intervals over the first three minutes of the reaction.

The procedure was repeated for the remaining buffered solutions and the water at pH 7.0.

(i) Identify the **independent** variable in this experiment.

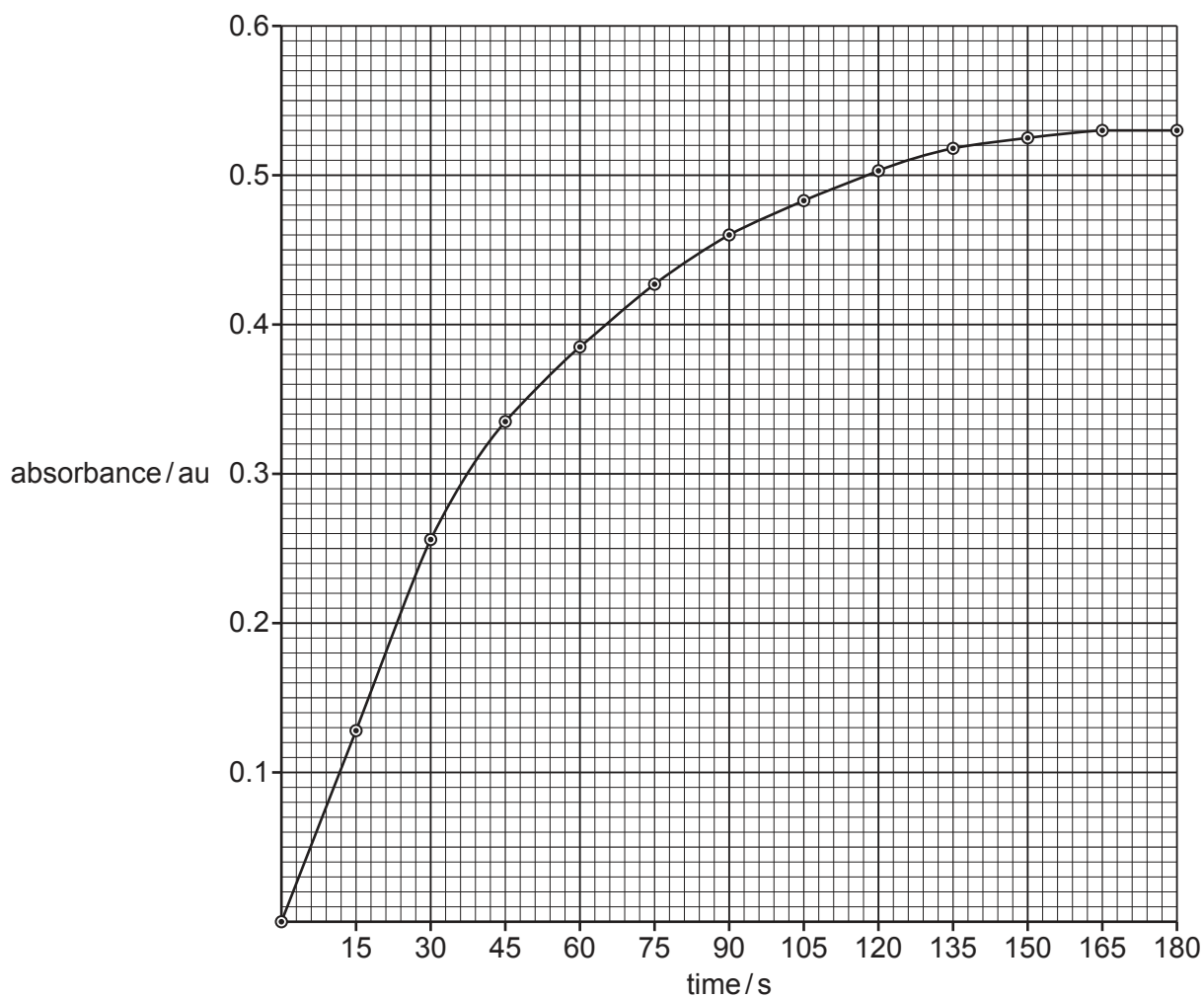
.....  
..... [1]

(ii) Identify **one** variable that the student has standardised.

.....  
.....  
..... [1]

- (c) The student then plotted a graph of the results, showing the increase in absorbance. Absorbance is measured in absorbance units (au).

Fig. 1.2 shows the results for the increase in absorbance at **pH 5.0**.



**Fig. 1.2**

- (i) Use Fig. 1.2 to calculate the initial rate of this reaction in absorbance units per second ( $\text{au s}^{-1}$ ).

Show your working.

initial rate of reaction = .....  $\text{au s}^{-1}$  [2]

- (ii) Using the internet, the student found that pH 7.0 is the optimum pH for polyphenol oxidase.

On Fig. 1.2, sketch the line you would expect to get for the rate of reaction at **pH 3.0**. [1]



- (e) The browning of fruit such as bananas can make them more difficult to sell. One way to prevent browning of bananas is to use an anti-browning agent.

The student searched the internet and found data from different scientific papers on the inhibitory effect of various anti-browning agents on polyphenol oxidase from different plants.

The student processed and ranked these data, as shown in Table 1.1.

**Table 1.1**

<b>anti-browning agent</b>	<b>percentage of maximum activity of polyphenol oxidase</b>	<b>plant source of polyphenol oxidase</b>
heated onion extract	17.0	potato
fresh onion extract	44.4	potato
ascorbic acid	60.0	sorrel
L-cysteine	70.0	sorrel
potassium sorbate	76.8	potato
citric acid	80.4	potato
EDTA	88.0	red poppy
no anti-browning agent (control)	100.0	potato

The student concluded that heated onion extract was the most effective anti-browning agent and that EDTA was the least effective.

Use the information to suggest why this conclusion may **not** be supported.

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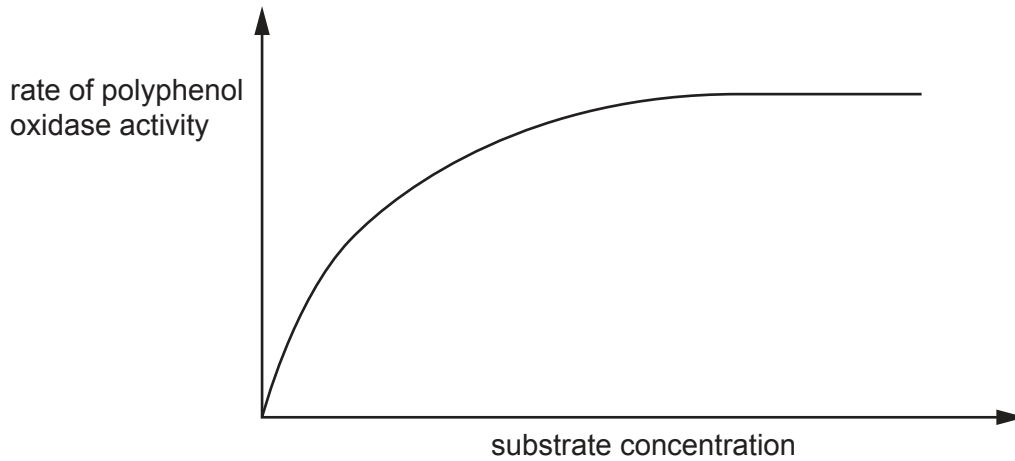
.....

..... [2]

- (f) The student found that a sulfur-containing molecule in the heated onion extract acted as a non-competitive inhibitor to the enzyme polyphenol oxidase.

Fig. 1.3 is a graph showing how the substrate concentration affects the rate of polyphenol oxidase activity.

On Fig. 1.3 sketch the curve that shows the rate of activity for polyphenol oxidase treated with the heated onion extract.



**Fig. 1.3**

[1]

[Total: 16]

- 2 Air pollution due to motor vehicles is an environmental issue that needs constant monitoring.

Lichens consist of protocists and fungi living in close association. The protocists and fungi each benefit from this association. Lichens are found in a large range of habitats, including on the surface of tree trunks (tree bark), as shown in Fig. 2.1.

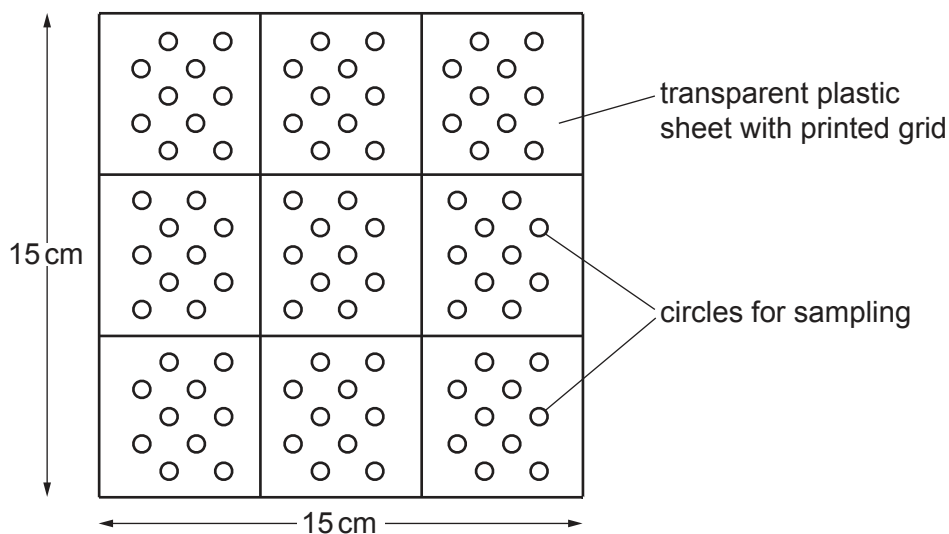


**Fig. 2.1**

Lichens are sensitive to air pollution. The abundance of lichens decreases with increasing levels of air pollution and so lichens can be used to monitor levels of air pollution.

Some scientists investigated the relationship between distance from a road and levels of air pollution. The scientists investigated this relationship by measuring the abundance of lichens on the tree trunks in a woodland beside a busy road.

The scientists used the grid shown in Fig. 2.2 to determine the abundance of lichens on the tree trunks.



**Fig. 2.2**



For each tree sampled, the scientists:

- placed the grid against the tree trunk
- counted the number of circles that contained lichen
- used the number of circles that contained lichen to calculate the percentage cover of lichen on the tree
- measured the distance from the tree to the road.

(a) (i) State **two** variables that the scientists should standardise in this investigation.

.....  
.....  
.....  
.....  
..... [2]

(ii) State **one** risk **and** the safety precaution that the scientists should take when measuring the abundance of the lichens on the tree trunks.

.....  
.....  
.....  
.....  
..... [2]

- (b) The scientists recorded the presence of lichens by shading in the circles on the grid where lichens were observed.

The results are shown in Fig. 2.3.

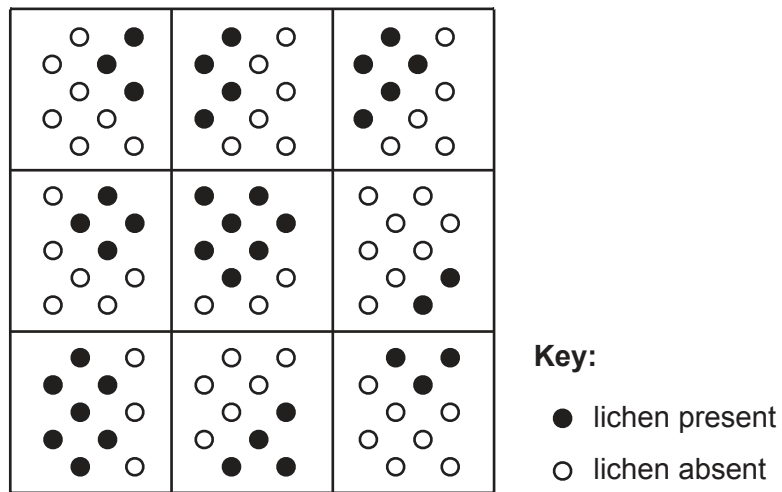


Fig. 2.3

Use the grid in Fig. 2.3 to calculate the percentage cover of the lichen on the tree trunk.

Show your working.

percentage cover of lichen = ..... % [2]

- (c) The scientists measured the percentage cover of lichens on 10 trees, each growing at a different distance from the road.

The scientists carried out a Spearman's rank correlation analysis to find out if there was a correlation between the distance from the road and the percentage cover of lichens on the tree trunks.

Table 2.1 shows part of the table used to determine the data needed for the Spearman's rank correlation equation.

- (i) Complete Table 2.1 for tree number 10.

Table 2.1

tree number	distance from road /m	rank for distance from road	percentage cover of lichen	rank for percentage cover of lichen	difference in rank ( $D$ )	$D^2$
10	5.6	7	74	5		

[1]

The formula for calculating Spearman's rank correlation coefficient ( $r_s$ ) is:

Key to symbols:

$$r_s = 1 - \left( \frac{6 \times \Sigma D^2}{n^3 - n} \right)$$

$D$  = difference in rank between each pair of measurements

$n$  = number of pairs of items in the sample

- (ii) The scientists calculated the value of  $\Sigma D^2$  as **39.75**.

Calculate the Spearman's rank correlation coefficient ( $r_s$ ) value to **three** significant figures.

Use this space for working.

Spearman's rank correlation coefficient ( $r_s$ ) = ..... [2]

(iii) State the conclusions that can be made from the calculated value of Spearman's rank correlation coefficient ( $r_s$ ).

.....

.....

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..... [3]

(iv) State how the scientists could use the calculated value of  $r_s$  to determine whether or not the correlation is due to chance.

.....

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..... [2]

[Total: 14]

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