1 You will investigate the water potential of potato cells.

When pieces of potato are put into a sucrose solution, water will move by osmosis into and out of the potato cells. The overall direction of water movement depends on the difference between the water potential of the potato cells and the water potential of the sucrose solution.

- If the overall movement of water is out of the potato cells, the sucrose solution will become less concentrated.

- If the overall movement of water is into the potato cells, the sucrose solution will become more concentrated.

Fig. 1.1 shows how the change in concentration of the sucrose solution after 15 minutes can be assessed. A blue dye is added to the sucrose solution around the potato pieces at the end of the 15 minutes. The blue dye does not affect the concentration of the sucrose solution. After mixing, a drop of the blue sucrose solution is added to the original sucrose solution in a separate test-tube.

The movement of the blue drop is then observed.

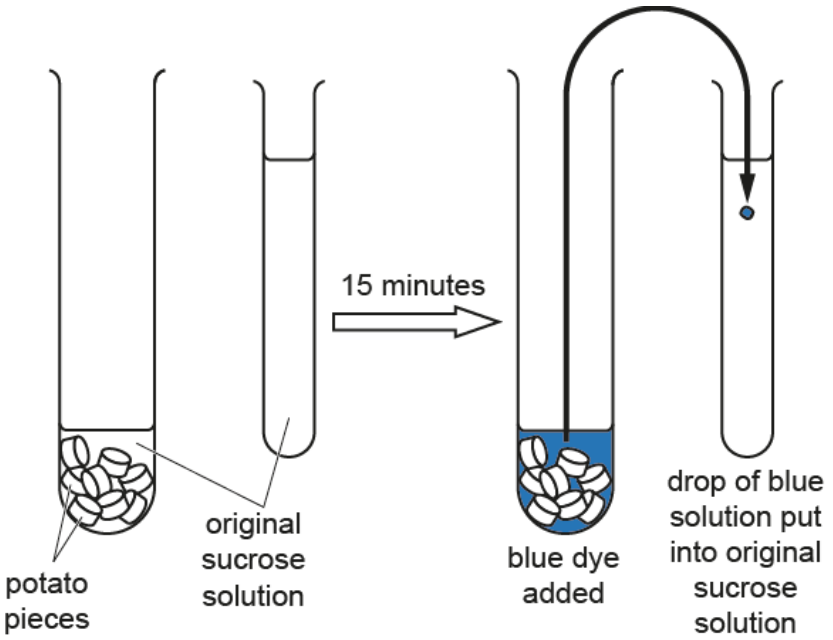


Fig. 1.1

- If the sucrose solution has become less concentrated, then the density of the sucrose solution will have decreased.

- If the sucrose solution has become more concentrated, then the density of the sucrose solution will have increased.

(a) (i) When two solutions of different density are added to one another without mixing, the denser solution will sink to the bottom and the less dense solution will rise to the top.

Complete Fig. 1.2 by drawing an arrow on each test-tube, as shown in the key, to predict how you expect the drop of blue solution to move.

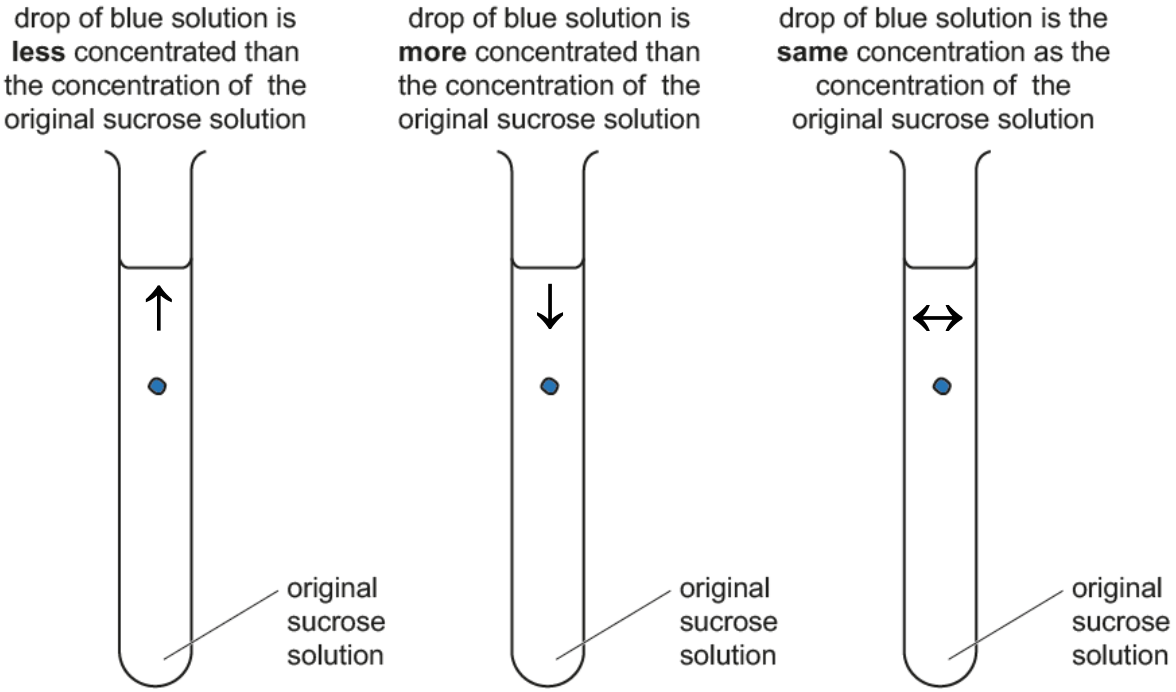


Fig. 1.2

1 All three directions correct ; in order from left to right ↑, ↓, ↔

[1]

You are provided with:

- 5 potato cylinders, in a Petri dish labelled **P**
- 150cm^3 1.00 mol dm^{-3} sucrose solution S, in a container labelled S
- 250cm^3 distilled water W, in a container labelled W
- 20cm^3 blue dye M, in a container labelled M.

If **M** comes into contact with your skin, wash it off immediately under cold water.

It is recommended that you wear suitable eye protection.

You will carry out a **serial** dilution of the 1.00 mol dm^{-3} sucrose solution, **S**, to reduce the concentration by **half** between each successive dilution.

You will need to prepare **four** concentrations of sucrose solution in addition to the 1.00 mol dm^{-3} sucrose solution, **S**.

After the serial dilution is completed, you will need to have 50.0 cm^3 of each concentration available to use.

(ii) Complete Table 1.1 to show how you will make the concentrations of the sucrose solutions needed.

……..…….[3]

Table 1.1

	sucrose solution				
concentration of sucrose solution / mol dm^{-3}	**1.00**	0.50	0.25	0.125	0.0625
concentration of sucrose solution to be diluted / mol dm^{-3}		1.00	0.50	0.25	0.125
volume of the sucrose solution to be diluted / cm^3		50.0	50.0	50.0	50.0
volume of distilled water to make the dilution /cm^3		50.0	50.0	50.0	50.0

1 Correct concentrations 0.50, 0.25, 0.125 and 0.0625 mol dm^{-3} ;
REJECT if not by serial dilution, as specified by question stem
2 Correct volumes of sucrose solution to be diluted (accuracy to 1 d.p if using measuring cylinder) to form total volume of 100.0cm^3 for each concentration ;
3 Correct volumes of distilled water W (accuracy to 1 d.p if using measuring cylinder) to form total volume of 100.0cm^3 for each concentration ;

Read steps **1-22.**

Proceed as follows.

1 Prepare the concentrations of sucrose solution, as decided in **(a)(ii)**, in the beakers provided.

2 Label five boiling tubes with the concentrations of sucrose solution prepared in step 1, including 1.00 mol dm^{-3}.

3 Put the five potato cylinders onto a white tile.

(iii) The potato cylinders all have the same diameter.

State the method that you will use to standardise the surface area of all five potato cylinders.

……[1]

1 Cut all potato cylinders to the same length (e.g. 6.00cm) ;

4 Carry out the method stated in **(a)(iii)** for each of the five potato cylinders.

5 Cut one of the potato cylinders into eight pieces of approximately the same length.

6 Put the eight **pieces** of potato into one of boiling tubes labelled in step 2.

Record the length of each potato **piece**.

length of potato piece = ……cm

Markers' guide :
Maximum length = 1.00cm (maximum length of potato cylinders provided to candidates is 8.00cm)

7 Repeat step 5 and step 6 for the four other potato cylinders so that there are eight pieces of potato in each of the boiling tubes labelled in step 2.

You will put sucrose solution into each boiling tube to just cover the eight pieces of potato.

You will need to standardise the volume of sucrose solution put into each of the boiling tubes.

(iv) State the volume of sucrose solution you will use to **just** cover the eight pieces of potato in each boiling tube.

volume = …… [1]

1 appropriate volume (range : 2.0cm^3 – 15.0cm^3) with units (**REJECT** ml) ;

Markers' guide :

0.20cm / piece – 2.0 (use of syringe) to 5.0cm^3
0.50cm / piece – 5.0cm^3 to 8.0cm^3
0.75cm / piece – 8.0cm^3 to 10.0cm^3
1.00cm / piece –10.0cm^3 to 15.0cm^3

8 Put each concentration of sucrose solution prepared in step 1, including 1.00 mol dm^{-3}, into the appropriately labelled boiling tube. For each boiling tube, use the volume of sucrose solution stated in **(a)(iv)** to cover the potato pieces.

9 Leave the pieces of potato in the sucrose solutions for 15 minutes. While you are waiting, continue with step 10 to step 13.

10 Label five test-tubes with the concentrations of sucrose solution that you have used in step 8.

11 Put a mark 5.0 cm from the bottom of each of the test-tubes, as shown in Fig. 1.4.

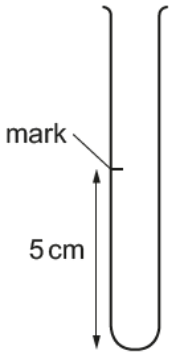


Fig. 1.4

12 Put 15.0cm^3 of 1.00 mol dm^{-3} sucrose solution into the appropriately labelled test-tube.

13 Repeat step 12 with each of the other concentrations of sucrose solution.

14 After leaving the pieces of potato for 15 minutes in step 9, put 1.0 cm^3 of the blue dye, **M**, into each of the boiling tubes containing eight pieces of potato in sucrose solution.

15 Swirl the contents of the boiling tubes to mix **M** with the sucrose solution. The blue dye may not mix in completely. This will not affect the results.

16 Use a pipette to remove a sample of the blue solution from around the pieces of potato in the boiling tube to which 1.00 mol dm^{-3} sucrose solution had been added.

Throughout step 17 to step 20, the pipette must be held still so that its position does not change.

Drops can then be released and observed without disturbing the sucrose solution.

17 Put the end of the pipette into the small test-tube containing 1.00 mol dm^{-3} sucrose solution.

The end of the pipette should be level with the mark on the small test-tube, as shown in Fig. 1.5.

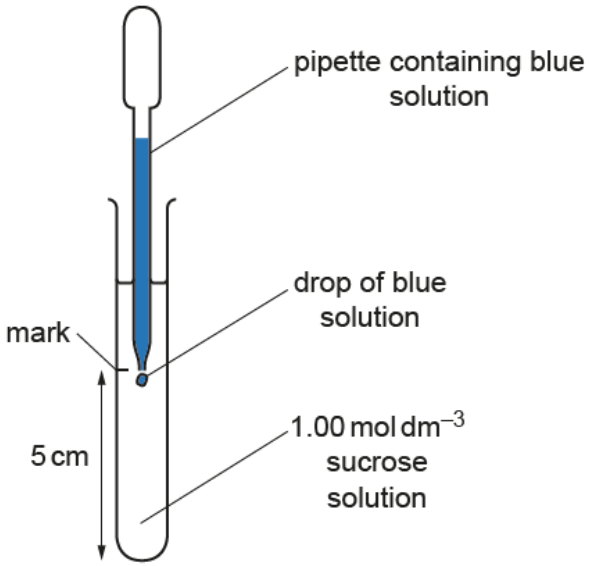


Fig. 1.5

18 Keeping the end of the pipette as still as possible, release a drop of the blue solution from the pipette.

19 Observe the direction **and** time taken for the drop of blue solution to reach the top or bottom of the test tube.

20 Repeat step 18 and step 19 two more times.

21 Record your observations in **(a)(v)**.

22 Repeat step 16 to step 21 for the other concentrations of sucrose solution. In step 17 and step 18, make sure that drops of the blue solution from each boiling tube are released into the small test-tubes labelled with the same concentration of sucrose.

(v) Record, in an appropriate table, your observations of the direction **and** the time taken for the drops to reach the top or the bottom of the test tube.

You may use the same symbols in **(a)(i)** to show the direction of movement of the drops.

If no movement occurs, record the time taken as ' – '.

……[6]

Table showing how concentration of sucrose solution affects direction of movement and time taken for movement of the drop of blue solution

Concentration of sucrose / mol dm^{-3}	Direction of movement	Time taken for the drops to reach the top or the bottom of the test tube / s			
		Replicate 1	Replicate 2	Replicate 3	Average
1.00	↑	15	16	14	15
0.50	↑	40	39	84	40
0.25	↔	-	-	-	-
0.125	↓	56	58	53	56
0.0625	↓	35	40	38	38

anomalous result

1 Independent variable: [Concentration of sucrose] heading presented on leftmost column with appropriate heading ; correct units mol dm^{-3} ;
2 Dependent variable: direction of movement ;
3 Time taken for the drops to reach the top or the bottom of the test tube / s recorded appropriately (to correct precision of whole number in s) ;
4 Observations: recorded for all concentrations + triplicates ;
(time recorded to correct precision of round number in s) ;
5 Calculation of average using triplicates data
(average time to correct precision of whole number in s) ;
E.g. for 0.0625, the calculated average was 37.6. The average presented within the table was 38 (rounded up).
6 Trend 1: direction of movement changing from movement up to movement down as sucrose concentration decreases ;
Markers' guide :
Do not penalize if students do not record any horizontal arrow

7 Trend 2: different timing recorded with fastest up at high sucrose concentrations and fastest down at low sucrose concentrations ;
[Max 6]

(vi) Using your results in **(a)(v)**, estimate the concentration of sucrose solution that has a water potential equal to the water potential of the potato cells.

concentration of sucrose solution = ... mol dm^{-3} [1]

1 Correct estimate from student's results of the concentration of sucrose with a water potential equal to the water potential of the potato tissue ;

Markers' guide :
Examiner's result = 0.25 mol dm^{-3}
Allow ecf from recorded results ;

(vii) Describe how you would modify the procedure to obtain a more accurate estimate in **(a)(vi)**.

……[2]

1 Increasing number of concentrations of sucrose tested ; (at dilution factor of 10, instead of 2)

2 **near the concentration / named range specific to candidates' results** where the estimated concentration in (vi) is to determine more accurately where there is no change in direction of movement of the drops ;

3 Increase the number of concentrations of sucrose tested ; (increase from 5 concentrations to 10 concentrations)

4 within the **same range** (0.0625 mol dm^{-3} to 1 mol dm^{-3}) to determine more accurately where there is no change in direction of movement of the drops ;

(viii) Describe the movement of water molecules when the water potential of the sucrose solution surrounding the piece of potato is the same as the water potential of the potato cells.

……[2]

1 Water molecules are moving in **both directions / no net** movement of water molecules ;
2 at the same rate in both directions ;

(ix) State **one** source of error in the procedure that you have carried out.

.....[1]

1 The droplet of blue solution does not keep shape ;
2 Difficult to hold pipette still ;
3 Difficulty in judging the time taken to reach the top or bottom of the test tube;
4 Pressure of release of the blue solution affects timing taken to reach the top or bottom of the test tube ;

Other accepted points :
5 Different potato cylinders may have different starting water potentials ;
6 Unequal incubation time of potato pieces in their respective sucrose solutions / removal of potato pieces from Petri dish did not occur at the same time

(b) Another student investigated the effect of different concentrations of sucrose solution by modifying the experiment and measuring the change in mass of potato tissues after being immersed in sucrose solutions instead.

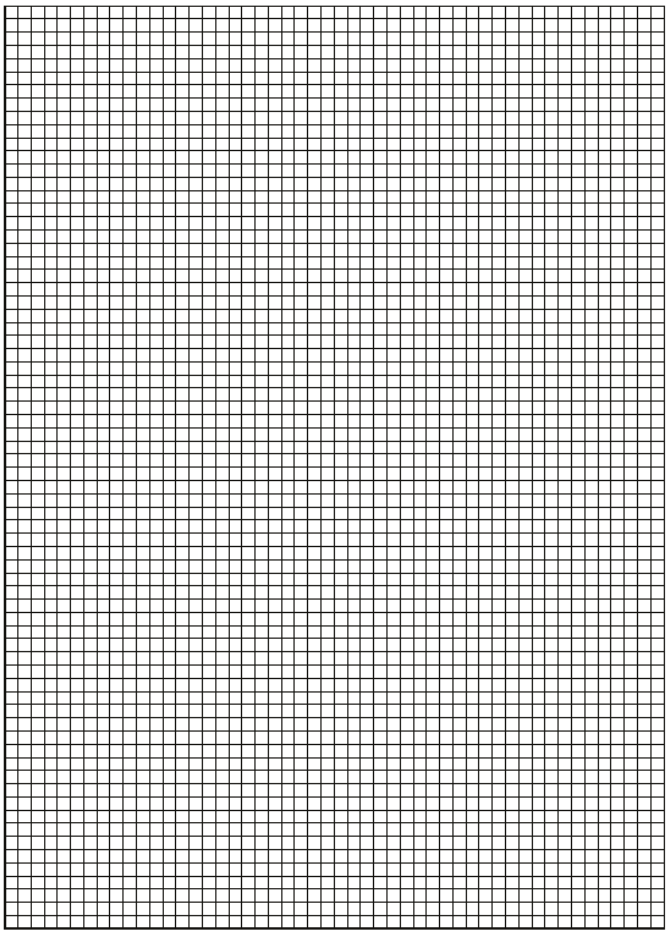
The mass of each piece of potato tissue was measured before and after soaking in different concentrations of sucrose for 1 hour and the change in mass calculated.

Table 1.2 shows the results of this investigation.

Table 1.2

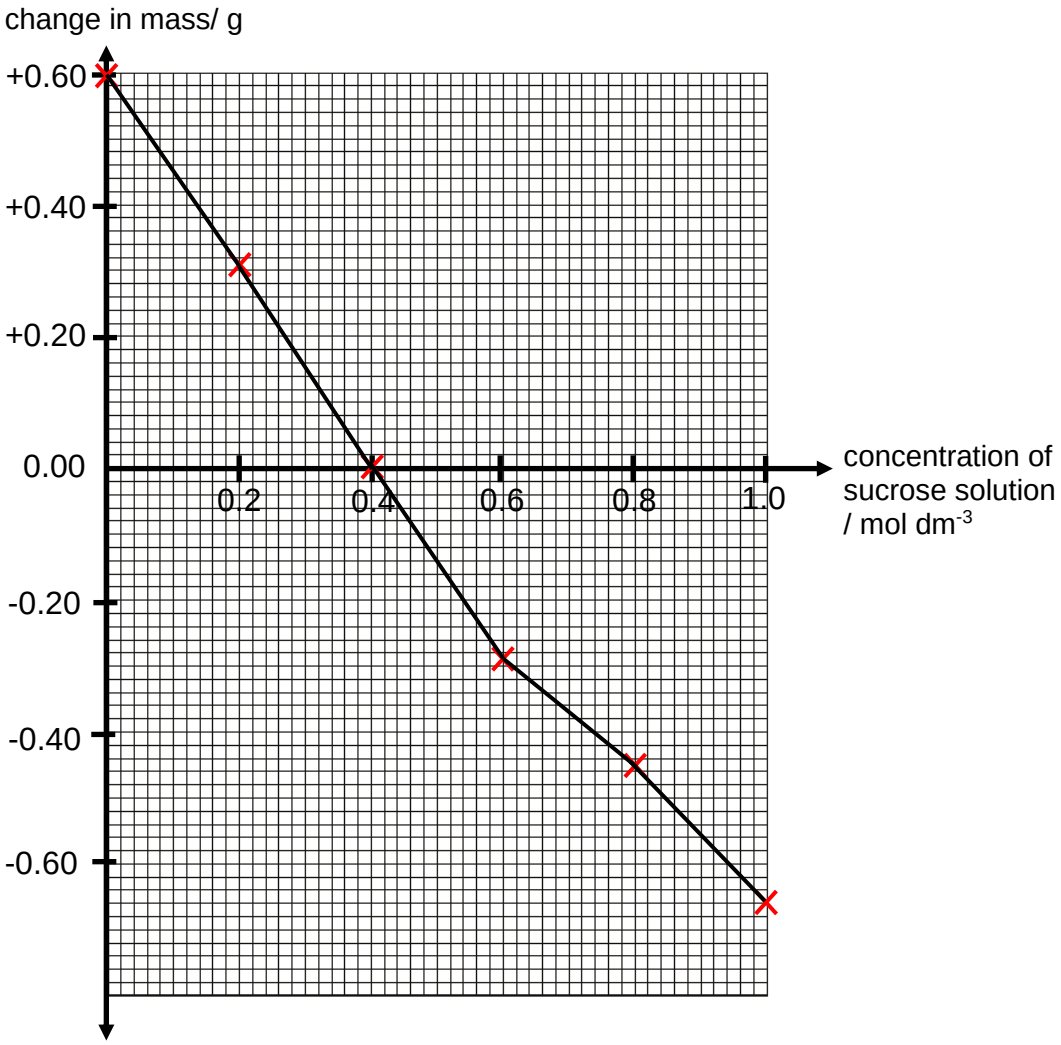
concentration of sucrose solution / mol dm^{-3}	change in mass / g
0	+0.60
0.2	+0.31
0.4	0.00
0.6	–0.29
0.8	–0.45
1.0	–0.66

(i) Use the grid provided to display the relationship between sucrose concentration and change in mass, as shown in Table 1.2.



[4]

Table showing effect of different concentrations of sucrose solution on change in mass in potato tissues



1 Scale: Sensible scale with graph occupying at least 1/2 the grid on both X- and Y- axes + equidistant divisions on axes ;
x-axis: 0.2 to 2cm, labelled at least every 2 cm
and
y-axis: 0.2 to 2cm, labelled at least every 2cm ;

2 Axes & Units:
x-axis : concentration of sucrose solution / mol dm^{-3} (1 d.p for x-axis)
and
y-axis: change in mass / g ; (2 d.p for y-axis)

3 Plot: accurate plotting of points / bar drawing to nearest half a small square using small crosses ;

4 Graph: point to point straight line drawn passing through all points; [Allow smooth curve passing through all points]

(ii) Using your knowledge of water potential, explain the difference in the change of mass in the potato piece immersed in 1.0 mol dm^{-3} sucrose solution.

……..[3]

1 [Quote] potato piece in 1.0 mol dm^{-3} lost mass -0.66g ; water potential in the cells were higher than the surrounding sucrose solution ;
2 ref water moved **out** of the cells ;
3 correct reference to osmosis / water moving from region of higher water potential to region of lower water potential, across the partially permeable membrane;

[Total : 25]

2 When bananas ripen, they turn brown in colour. This is due to the formation of a brown product called melanin.

The enzyme catechol oxidase acts on its substrate, catechol, leading to the formation of a brown product, melanin, as shown in Fig. 2.1.

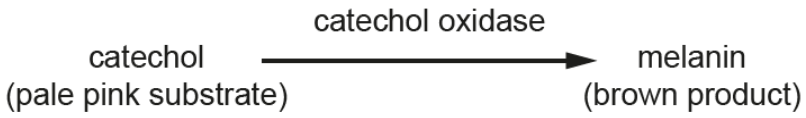


Fig. 2.1

As melanin is produced, the colour of the reaction mixture changes to brown. The intensity of the brown colour produced is proportional to the concentration of melanin. The more active the enzyme, the more intense the brown colour.

A colorimeter is used to measure the absorbance of the reaction mixture. Absorbance is a measure of the light absorbed by a coloured solution. With the reaction shown in Fig. 2.1, the more intense the brown colour, the higher the absorbance.

Catechol oxidase can be extracted from bananas.

(a) Design an experiment to investigate the effect of catechol concentration on the catechol oxidase-catalysed reaction shown in Fig. 2.1.

In your plan, you **must** use :

- An extract of catechol oxidase enzyme solution prepared and kept cold until needed
- A stock solution of 1.0% catechol solution
- A buffer solution of pH 7.0

You may select from the following apparatus and plan to use appropriate additional apparatus :

- normal laboratory glassware, e.g. test-tubes, boiling tubes, beakers, measuring cylinders, graduated pipettes, glass rods, etc
- colorimeter
- access to hot water and ice
- syringes
- timer, e.g. stopwatch

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- be illustrated by relevant diagrams, if necessary
- identify the dependent variable and the independent variable
- identify the variables you will need to control
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed.

Suggested answer based on Full planning Framework
[Green highlights are mandated by Q stem, exactly 13M]

Background knowledge: [A - 1M]

[A – any 2 for 1m]

- ✓ When substrate concentration increases, rate of enzymatic reaction increases due to increase in frequency of effective collisions between enzyme and substrate molecules ;
- ✓ increase in concentration of enzyme-substrate complexes formed per unit time , results in increase in concentration of products formed per unit time ;
- ✓ At high substrate concentration, all the active sites of all enzymes are saturated with substrate molecules ; no further increase in products formation

Rationale of setup: *[given in question]*

- ✓ The rates of reaction can be monitored by measuring the amount of melanin produced at the end of 5 minutes (i.e. a fixed duration of time)
- ✓ Colour intensity / absorbance value reflects the amount of melanin produced in the reaction
- ✓ More melanin produced results in a darker solution which is measured to give a higher absorbance value on the colorimeter

Hypothesis: [B - 1M]

[B – 2 for 1m]

- ✓ As concentration of catechol substrate increases, the absorbance of the reaction mixture increases linearly as melanin production increases
- ✓ No further increase in absorbance values at higher catechol substrate concentrations

Variables [C - 2M]

1M

- ✓ Independent variable: Concentration of catechol substrate / 1.0%, 0.8%,0.6%, 0.4%, 0.2%
- ✓ Dependent variable: Absorbance of reaction mixture measured by colorimeter (after 5 minutes) / AU

✓ Other variables to be kept constant: [Apparatus & quantity to be indicated]

1M (Min 2x.)

i. Volumes of the following to be kept constant using a 5.0cm^3 syringe:
   - Catechol susbtrate : 5.0cm^3
   - Extracted catechol oxidase enzyme solution : 1.0cm^3

   [Max 1]

ii. Time taken for reaction using a stopwatch e.g. 5 mins

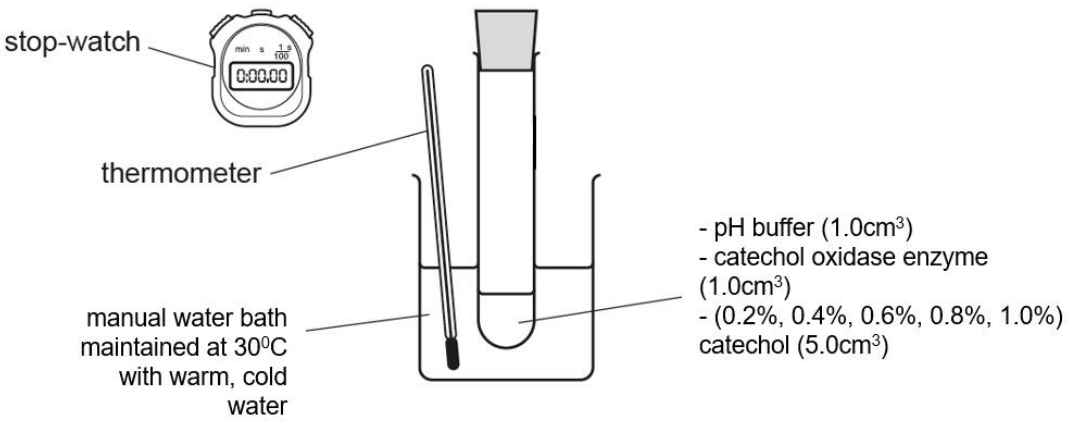
iii. Temperature of reaction using e.g. 30°C

iv. pH of reaction mixture using pH buffer ; e.g. pH 7; at a specific volume e.g. 1.0cm^3

Control [D - 1M]

- Set-up 1: Replace the catechol oxidase with 1.0cm^3 / equal volume of boiled and cooled catechol oxidase in the set-up ; and keep all other experimental conditions the same ;
- Set-up 2: Replace the catechol oxidase with 1.0cm^3 / equal volume of distilled water in the set-up ; and keep all other experimental conditions the same ;
- Purpose: To show that any change in the absorbance values is due to the action of catechol oxidase on catechol and not due to any other factors.

Diagram (with labels) [E- 1M]



[E – Any 2 for 1m]

- thermometer
- pH buffer + catechol oxidase enzyme + catechol in test-tube
- water bath **REJECT** thermostatically controlled water bath

Procedure [Apparatus and quantity stated] **[F - 4M]**

Examiner's comments :

Procedures should be clearly written as if you are writing a set of instructions for someone else to follow.

1 Using 50.0cm^3 measuring cylinders and 100.0cm^3 beakers, prepare 20.0cm^3 of 5 different concentrations of catechol solution using the 1.0% stock solution and distilled water, according to the volumes stated in the dilution table below.

Concentration of catechol solution / %	Volume of 1% catechol stock / cm^3	Volume of distilled water /cm^3
0.2	4.0	16.0
0.4	8.0	12.0
0.6	12.0	8.0
0.8	16.0	4.0
1.0	20.0	0.0

[F – dilution table]

[Accept: appropriate volume of catechol solution as given volume is not specified in question]

[Accept: serial dilution table]

2 Measure 5.0cm^3 of 1% catechol and 1.0cm^3 of pH 7.0 buffer into a test-tube using 10cm^3 and 1cm^3 syringes respectively.

3 In a separate test-tube, add 1.0cm^3 of chilled catechol oxidase extract using a syringe. Chill on ice until ready to use.

4 Equilibrate the two test-tubes separately in the 30°C water bath for 3 minutes. Ensure temperature is kept constant by monitoring with a thermometer and using warm or cold water to adjust the temperature when required.

[F – equilibration]

5 Add the catechol oxidase extract into the test-tube containing catechol and stir using a glass rod.

6 Immediately start the stop-watch and allow the reaction to proceed for 5 minutes.

7 After 5 minutes, place the test-tube in a boiling water bath for 1 minute to inactivate catechol oxidase.

[F – denature]

8 Transfer the reaction mixture into a cuvette. Calibrate the colorimeter first using a cuvette filled with distilled water. Then measure and record down the absorbance of the reaction mixture.

[F – colorimeter]

9 Carry out steps 2 – 9 for each of the other concentrations of catechol solutions.

10 To ensure **reliability** of results, repeat steps 2-8 of the experiment 2 more times for each concentration of catechol solution. Use fresh solutions for each replicate. Exclude any anomalous results and calculate the average absorbance value for each concentration of catechol solution.

11 To ensure **reproducibility** of results, repeat steps 2-10 two more times using freshly prepared solutions (catechol oxidase enzyme extract, catechol solution, pH buffer) and clean apparatus.

[F – replicates and repeats]

[Dilution procedure + values calculated in the table – Step 1 – 1M]

[Equilibration of solutions at constant temperature – Step 4 – 1M]

[Procedure on use of colorimeter – Step 8 – 1M]

[Replicates and Repeats - Steps 10,11 – 1M]

[Stopping the reaction by putting into boiling water bath – step 7]

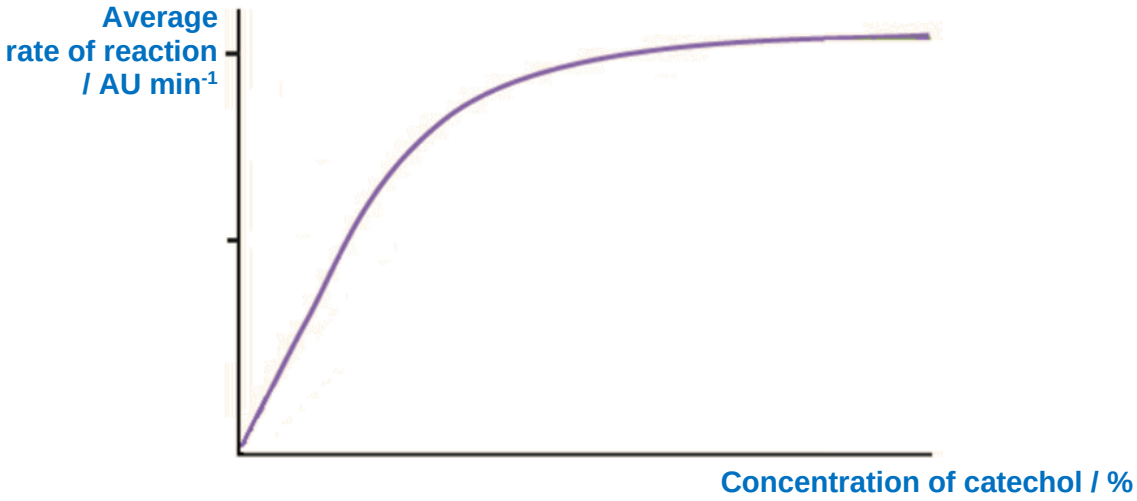
Table and Data [G- 1M]

Table showing absorbance of solution at different concentrations of catechol solution

Concentration of catechol solution /	Absorbance of solution / AU				Rate of reaction / AU min^{-1} or AU s^{-1}
	Replicate 1	Replicate 2	Replicate 3	Average	
0.2					
0.4					
0.6					
0.8					
1.0					

Markers' guide :
1m credited for table if students included columns for triplicates and the calculation of average absorbance.

Graph [H - 1M]



Risk & Precaution [Not needed]

Risk	Precaution
Risk of electrocution from power supply when handling the colorimeter	• Ensure hands are dry before using to prevent electric shock.

3 During this question you will require access to a microscope and slide M**1**.

M1 is a slide of a stained transverse section through a leaf of a land plant.

You are not expected to be familiar with this specimen.

(a) Use your microscope to observe the different tissues in the region of slide M1 shown by the shaded area in Fig. 3.1.

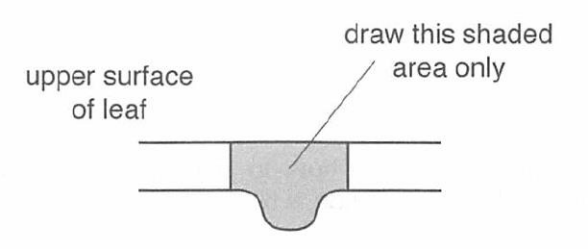
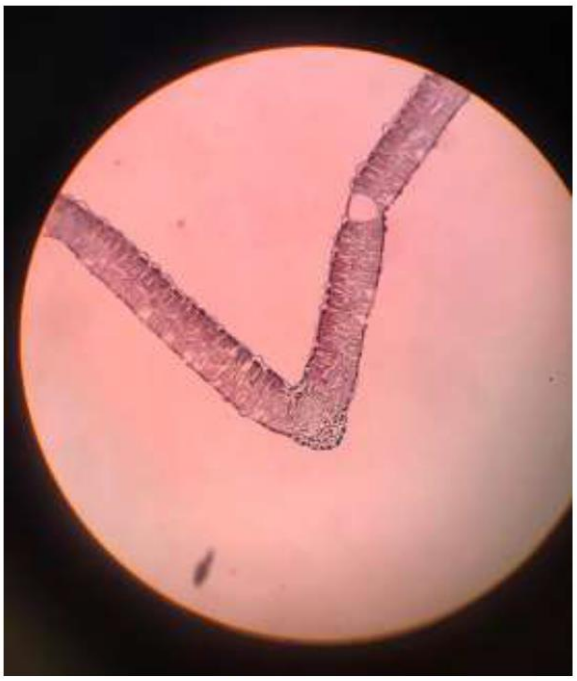


Fig. 3.1

(i) Use the space provided to draw a large plan diagram of the part of the leaf on slide **M1** shown by the shaded area in Fig. 3.1.

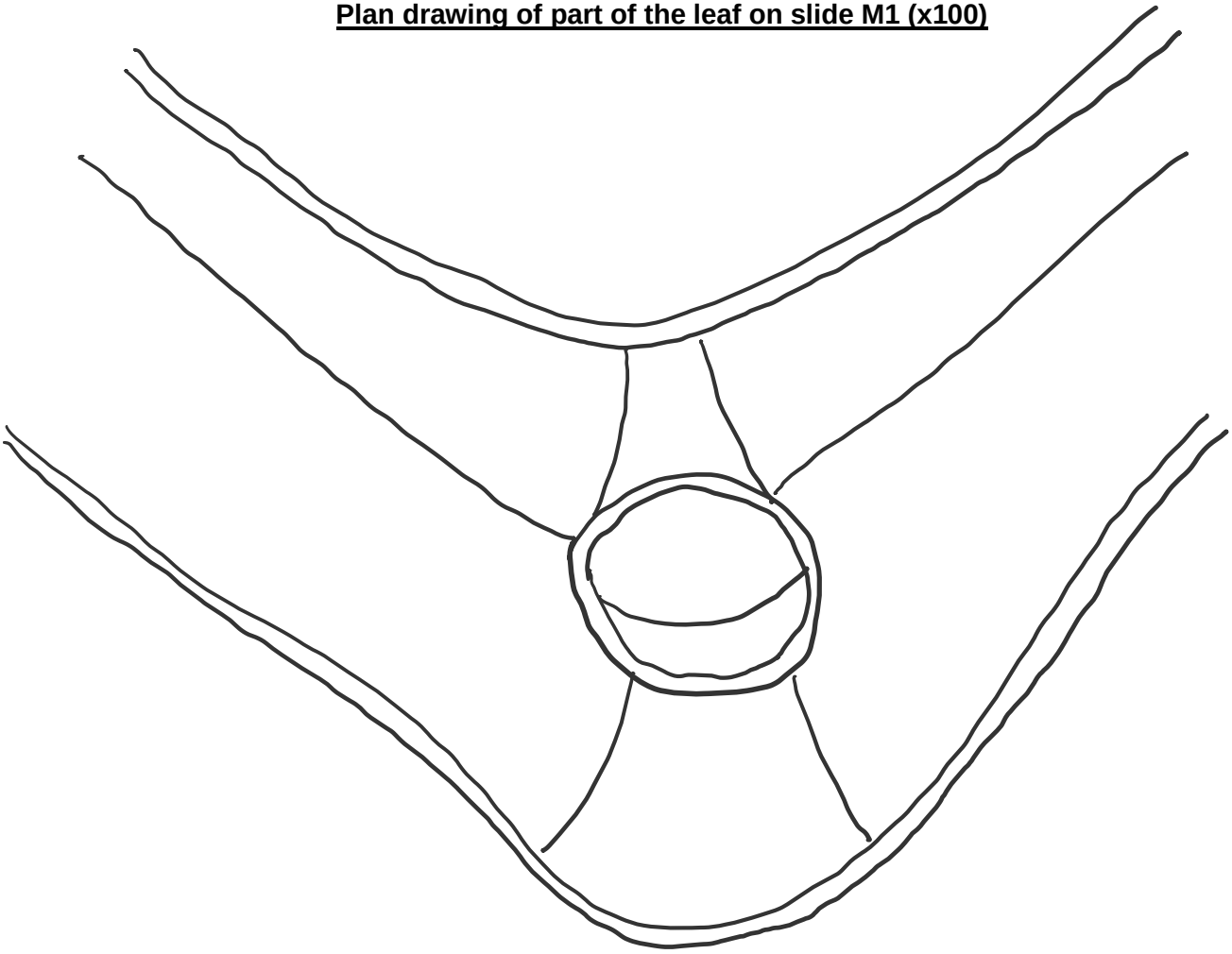
A plan diagram shows the arrangement of different tissues, including their correct shapes and proportions. No cells should be drawn.

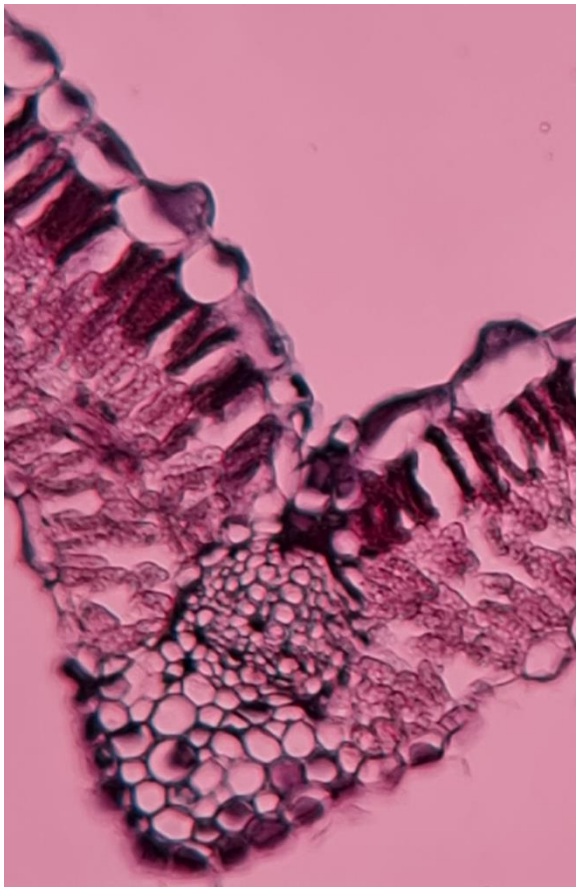
Labels are **not** required.



[4]

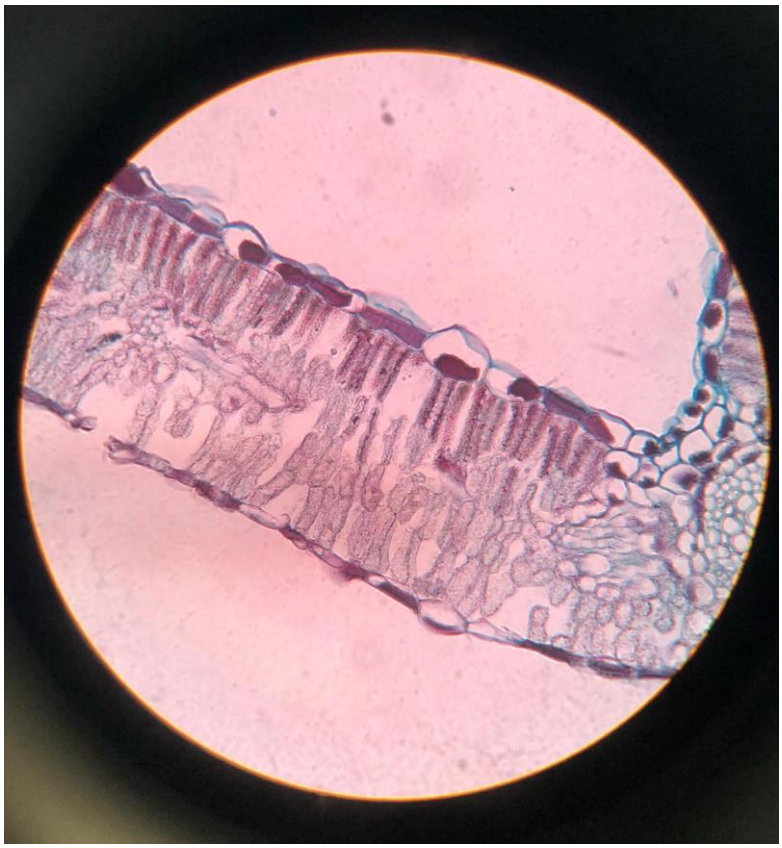
Plan drawing of part of the leaf on slide M1 (x100)





1 Size: size of drawing is sufficiently large (at least 2/3 of space provided) ;
Correct shape : 4 layers in the leaf blade + central vascular bundle is present + layer below vascular bundle ;
2 Correct proportion : epidermal cell layer thinnest + pronounced layer below vascular bundle relative to other layers + general proportion of different layers in leaf blade ;
3 Quality: no individual cells drawn + smooth lines + no shading

(ii) Observe the outer layer of cells on the upper surface of the leaf on slide **M1**. This outer layer of cells is called the upper epidermis.



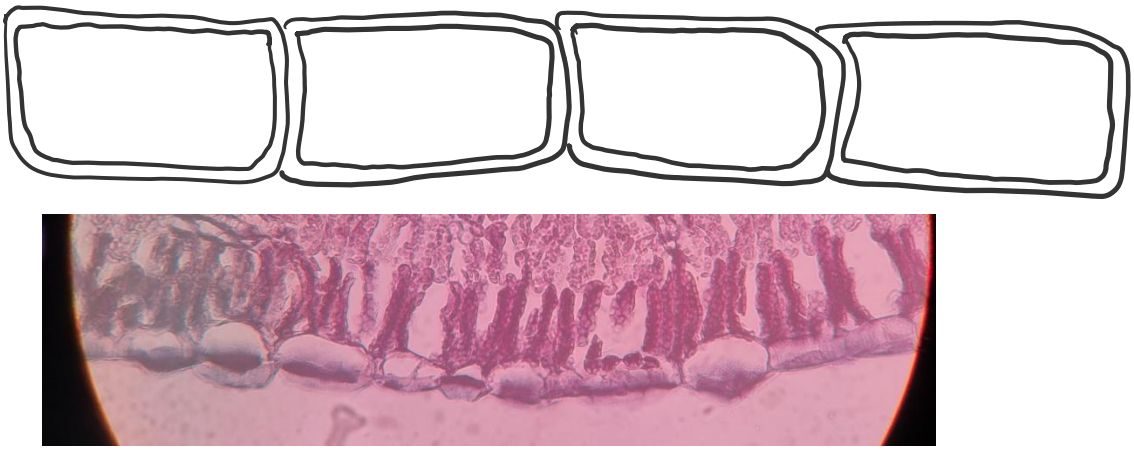
Select four cells from the upper epidermis that form a chain.

Make a large drawing of this chain of **four** cells.

Labels are not required.

[4]

High power drawing of chain of four cells from upper epidermis (x400)



1 Size : size of drawing is sufficiently large (at least 1/2 of space provided) ;
2 Quality: drawn with clear, continuous lines + no shading ;
3 Shape: Four whole cells drawn + elongated shape ;
Markers' guide :
Credit was given if at least 2 out of 4 cells showed an elongated shape
4 Details of cell wall : 3 lines where cells touch + cell wall remains as 2 lines ;

(iii) In land plants, one of the main functions of the epidermis is to reduce water loss.

From your observation of slide **M1**, suggest **one** way in which the upper epidermis of land plants is adapted to reduce water loss.

……[1]

1 Presence of thick cuticle / waxy covering and prevents the loss of water vapour through the epidermis via transpiration) ;

(b) Fig. 3.2 is a photomicrograph of a different part of the same leaf.

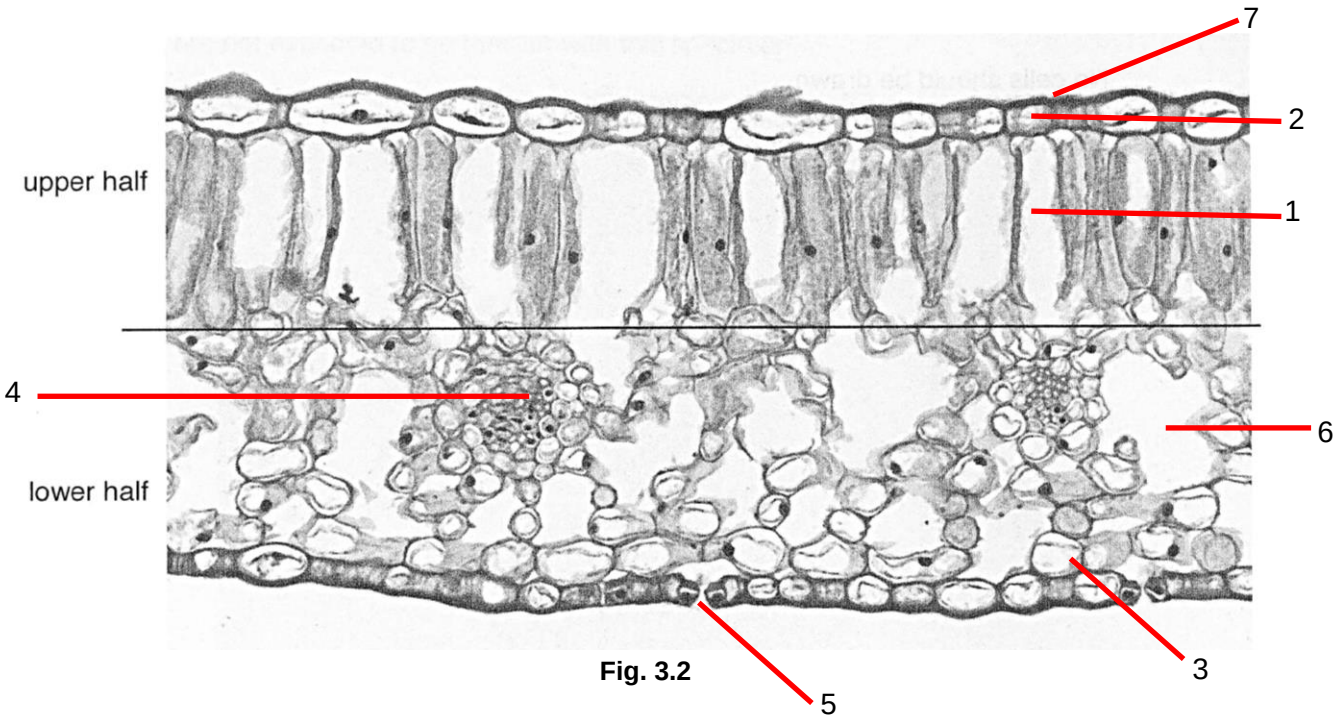


Fig. 3.2

(i) There are observable differences between the upper and lower halves of the leaf shown in Fig. 3.2. Identify three differences between the upper and lower halves of the leaf.

For each of the three differences, draw one label line to a feature in Fig. 3.2 that shows this difference. Label the three features **F, G** and **H**.

You may label each feature in either the upper half or the lower half of Fig. 3.2. Each labelled feature must relate to a separate difference.

Complete Table 3.1 to describe the differences between the upper and lower halves of the leaf for each of these **three** features.

Table 3.1

feature	upper half	lower half
F		
G		
H		

[4]

Feature	Upper half	Lower half
1 cell shape	Majority of cells are regularly shaped / rectangular (palisade mesophyll cells) ;	Majority of cells are irregularly shaped (spongy mesophyll cells) ;
2 cell size /epidermis size	Made up of larger cells ; Thick epidermis ;	Made up of smaller cells ; Thin epidermis ;
3 packing densities	Cells are densely packed ;	Cells are packed less closed together / loosely ;
4 vascular bundles	Absence of vascular bundles (containing xylem & phloem tissues) ;	Presence of vascular bundles (containing xylem & phloem tissues) ;
5 stomata	Absence of stomata / guard cells in upper epidermis layer ; **REJECT** less	Presence of stomata / guard cells in lower epidermis layer ;
6 air spaces	Intracellular air spaces absent ; **REJECT** less	Intracellular air spaces present ;
7 cuticle	Thicker layer of cuticle ;	Thinner layer of cuticle ;

(ii) Fig. 3.3 shows a diagram of a stage micrometer scale that is being used to calibrate an eyepiece graticule.

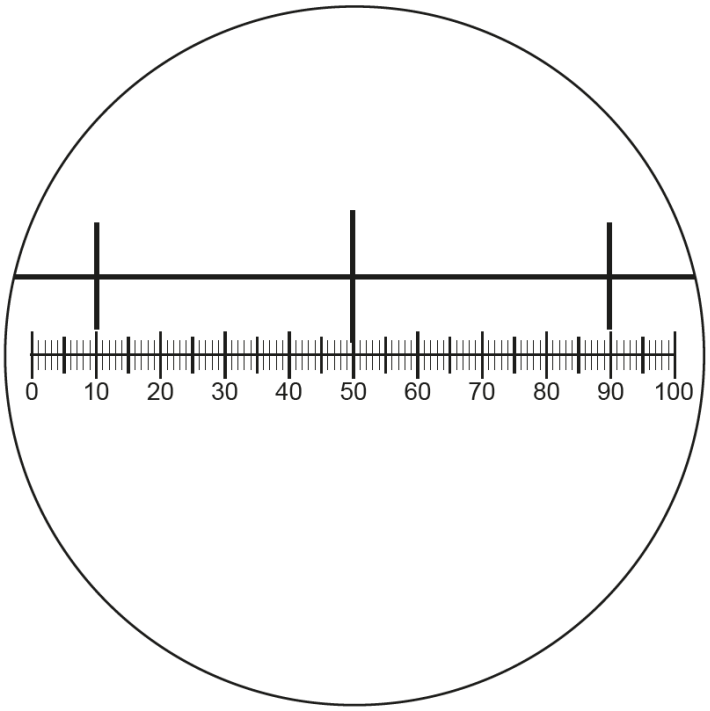


Fig. 3.3

The length of one division on this stage micrometer is **0.8 mm**.

Use Fig. 3.3 to calculate the actual length of one eyepiece graticule unit.

Show your working.

……[3]

1 1 stage micrometer unit corresponds to 40 eyepiece graticule units ;

2 40 eyepiece units = 0.8mm
1 eyepiece unit = 0.8mm / 40 x 1000 = 20μm (round)
= 0.02mm (2dp)

3 Correct answer with appropriate units ;

Fig. 3.4 shows the same specimen as Fig. 3.2 viewed through a microscope fitted with the eyepiece e graticule shown in Fig. 3.3.

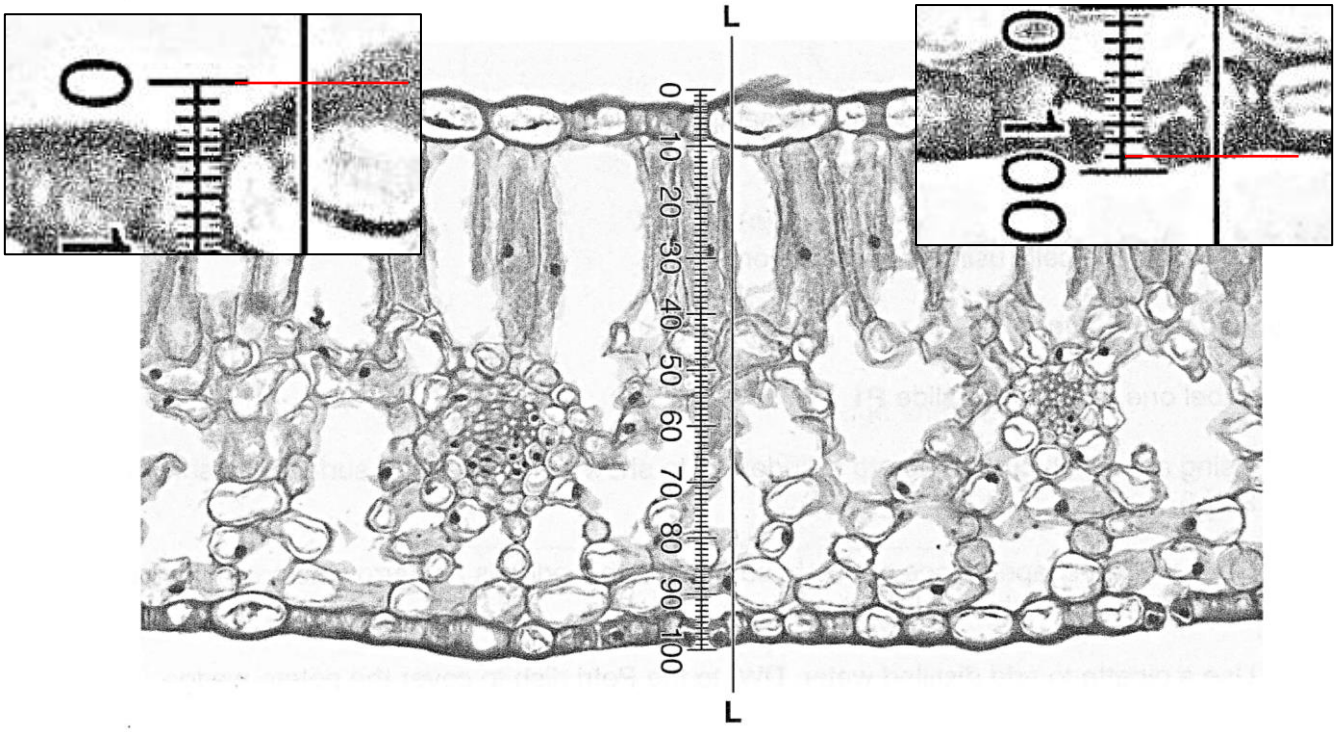


Fig. 3.4

(iii) Use your answer to (b)(ii) to find the actual thickness of the leaf at the position shown by the line L-L in Fig. 3.4.

Show your working.

[2]

1 Thickness of leaf at L-L = 99 eyepiece units
(Given that 1 E.P.U = 20µm) ;

2 Thickness of leaf = 99 x 20µm= 1980µm ;
Thickness of leaf = 99 x 0.02mm = 1.98mm ;

[Total : 18]