

RAFFLES INSTITUTION
2025 Year 6 Preliminary Examination

Higher 2

CANDIDATE
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BIOLOGY

Paper 4 Practical

9744/04

22nd August 2025

2 hours 30 minutes

Candidates answer on the Question Paper.

Additional Materials: As listed in the Confidential Instructions.

(a) Before you proceed, read carefully through **the whole** of Question **1(a)**.

You are provided with a blue solution, labelled **I**, which is alkaline.

I is blue because it contains an indicator.

Carbon dioxide reacts with **I** when bubbled into it. When enough carbon dioxide reacts with **I**, the indicator will change from blue to yellow (even if the solution is cloudy). This is the end-point.

Hydrochloric acid, **H**, can also turn indicator **I** yellow. If only a small volume of carbon dioxide is bubbled into **I** then the indicator will remain blue. Hydrochloric acid, **H**, can then be added slowly until the indicator turns from blue to yellow.

(i) What is the expected trend of volume of **H** that needs to be added to reach end point with increased volume of carbon dioxide that has reacted with indicator, **I**. [1]

The greater the volume of carbon dioxide that has reacted with **I**, the lower volume of **H** needed to be added to reach the end-point;

You are provided with:

labelled	contents
YA	yeast cell suspension
I	alkaline solution (blue)
H	hydrochloric acid

I and H are irritants. Suitable eye protection should be worn. If I or H comes into contact with your skin, wash it off immediately under tap water.

You are first required to find the volume of **H** needed to reach the end-point, when **no** carbon dioxide has been bubbled into **I**.

Proceed as follows:

1. Put 5 cm³ of **I** into a test-tube.
2. Use a syringe, containing 2 cm³ of **H**, to put drops of **H** into **I** as shown in Fig. 1.1.

Mix well as you add **H**, until the end-point is reached. You may need to fill the syringe again.

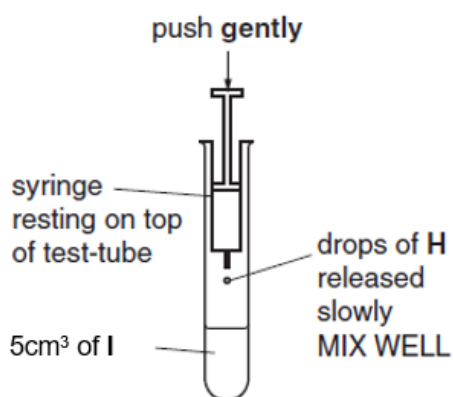


Fig 1.1

- (ii) Record the volume of **H** needed to reach the end-point.

volume of **H** 1.6 – 2.0 cm³ [1]
records volume of H to 1d.p or 2 d.p + cm³ + accuracy

Yeast cells release carbon dioxide from some of their metabolic reactions.

A student investigated the release of carbon dioxide from a yeast cell suspension, using the apparatus shown in Fig. 1.2.

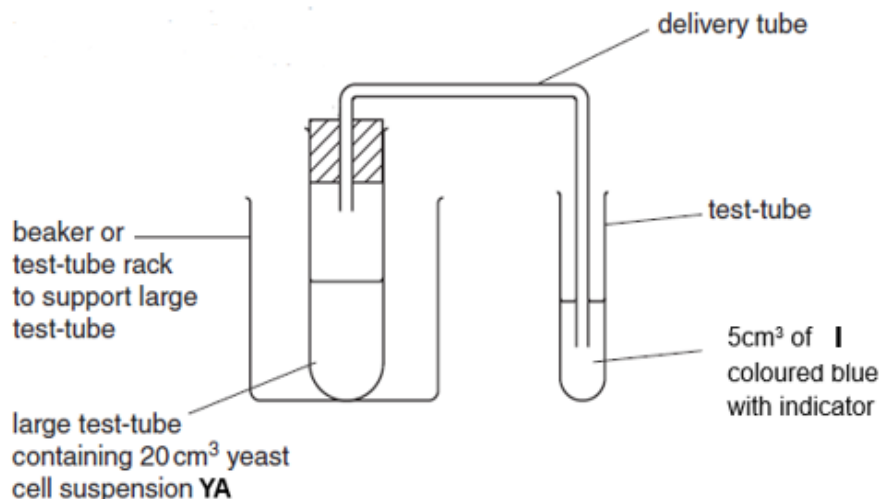


Fig 1.2

The student set up this apparatus and left it for 10 minutes.

The student observed that during the 10 minutes the bubbles of carbon dioxide were not released at a constant rate.

(iii) Suggest an explanation for the student's observation on the rate of release of carbon dioxide. [1]

1. pH / temperature was not kept constant;
2. Depletion of respiratory substrate over time, hence concentration of respiratory substrate not the same;
3. Yeast activity / numbers of yeast decreases / increases over time;
4. AVP e.g. O₂ depletion (Since boiling tube is airtight, concentration of O₂ will decrease as it is being used in respiration, so rate of respiration will decrease, which causes rate of CO₂ to decrease as well.)

You are required to investigate this observation using the apparatus set up as in Fig. 1.2.

You will move the delivery tube to different test-tubes containing I, at different times during the 10 minutes.

(iv) Decide the length of time you will leave the delivery tube in each test-tube containing I.

State the length of time.

time 2 minutes; [1]

[Marker to accept: every 2 min / at 2, 4, 6, 8 + 10 minutes]

(v) State **one** significant source of error that may occur when the delivery tube is moved from one test-tube to the next.

Describe how you will reduce this error. [2]

Source of error [1]

1. end of delivery tube different level in each test-tube;
2. time to transfer delivery tube different each time;

3. loss of CO₂ from delivery tube during transfer;
R : colour determination of end-point by naked eye / temp not maintained constant / lack of equilibration time for the setup;

Description [1]

1. constant level: mark delivery tube or test-tube/ mtds to ensure test-tubes place at constant level;
2. time in I constant: keep clock running, record time delivery tube in I / mtds to ensure time in I is constant (e.g. pause & reset the stopwatch during trf);
3. no loss of CO₂ during trf : seal end of delivery tube during transfer;

Read steps **3** to **10** before proceeding:

3. Put 5 cm³ of **I** into a test-tube. Repeat for the number of test-tubes you need to use for your times in **(a)(iv)**.
4. Obtain **YA** by placing the nozzle of a large syringe below any froth on the surface.
5. Put 20 cm³ of **YA** into the large test-tube.
6. Put the bung containing the delivery tube into the large test-tube. It **must** be airtight.
7. Put the end of the delivery tube into the first test-tube containing **I**. Immediately start timing.
8. After the time you decided in **(a)(iv)**, move the delivery tube to the next test-tube containing **I**.
9. Repeat step **8** for all the test-tubes which you set up in step **3**.
10. After removing the delivery tube from the last test-tube, repeat step **2** with any of the test tubes where there is still a blue colour.

Unplug the rubber bung from the large test tube after step 10 is completed.

Record your results in **(vi)**.

For any test-tubes where **I** is yellow (even if the solution is cloudy) record '0'.

- (vi)** Prepare the space below to record your results. [4]

Time / min	Volume of H required to reach end-point / cm ³
2	
4	
6	
8	
10	

1. Organised into table all columns separated by a line;
2. Headings (top or to left of data) time / minutes (A: seconds) + volume of H to reach end-point / cm³;
3. Records all volumes less than volume recorded in (a)(ii) and cannot be 0 cm³;
4. All recorded results to 1 d.p or 2 d.p;
I: processed data, number of bubbles etc.
A: trend with decreasing vol or same vol or no specific trend.

(vii) Explain whether your results in (vi) support the student's observation. [1]

Yes, it supports the student's observation because the volume of H across different timepoints was different / increasing / decreasing;

OR

No, it does not support student's observation because volume of H across different timepoints was the same/ (relatively) consistent / did not differ significantly ($\pm 0.1\text{cm}^3$);

(b) Before you proceed, read carefully through **the whole** of Question 1(b).

Yeast cells also contain enzymes that hydrolyse sucrose into reducing sugars, as shown in Fig. 1.3.



Fig.1.3

You will investigate the activity of the enzymes in yeast cells.

To immobilise the yeast cells in sodium alginate beads, yeast and alginate should be in equal proportions. (You will need no more than 20 cm^3 of the mixture).

Alginate will solidify when released into calcium chloride solution, and should be left in the solution for 5 min.

You are provided with the following which you **must** use:

labelled	contents
YB	15 cm^3 yeast cell suspension
A	15 cm^3 sodium alginate solution
C	20 cm^3 calcium chloride solution

Alginate and calcium chloride are irritants. Suitable eye protection should be worn. If A or C comes into contact with your skin, wash it off immediately under tap water.

You are also provided with the following which you may use:

Apparatus:

100 cm^3 beaker

5 cm^3 syringes

glass rod

(i) Describe the steps you would carry out to make 30 yeast alginate beads of similar size and shape. [2]

1. Using a syringe, add 10 cm^3 of A and 10 cm^3 of YB into a beaker and mix well with a glass rod;

A: volume of each $<10\text{ cm}^3$ if proportion of yeast to alginate is 1:1

2. Using a syringe / glass rod, add A and YB mixture drop by drop into another beaker with calcium chloride solution;

After 5 minutes, select 30 beads of similar size and shape.

Yeast will require 5 - 8 min to hydrolyse sucrose for the quantifiable amounts of products to be produced.

You are also provided with the following which you **must** use:

labelled	contents
B	10 cm ³ Benedict solution
S	50 cm ³ sucrose solution

You are also provided with the following which you may use:

Apparatus:

250 cm ³ beaker	Bunsen burner
100 cm ³ beaker	forceps
5 cm ³ syringes	test-tubes
10 cm ³ syringes	Thermometer

Care should be taken when using the Bunsen burner and hot water.

- (ii) Describe the steps to carry out a quantitative experiment to investigate the effect of enzyme concentration on the rate of hydrolysis of sucrose solution, **S**. You are required to use the yeast-alginate beads prepared in **(b)(i)**. You are not required to plan for replicates and safety precautions. [4]

Marks awarded

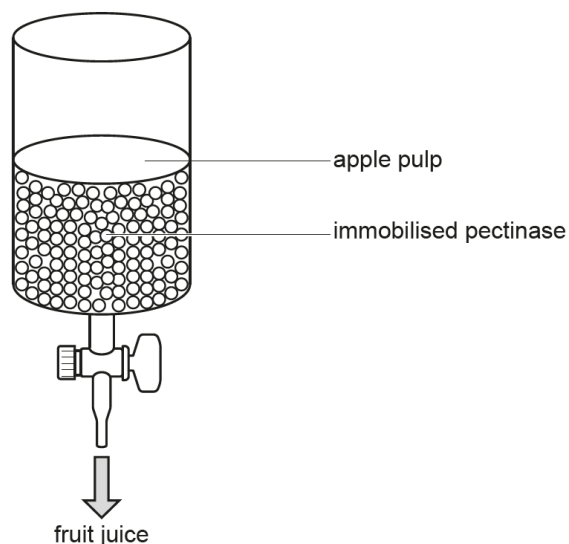
- 1m for correct numbers of beads 2, 4, 6, 8, 10 in each beaker (independent variable – 5 equal intervals): Nb Must use 30 beads
- 1m for 2 controlled variables (S must be fixed volume using syringe, reaction time of 5 – 8 mins using stopwatch, maintain water bath temperature with hot and cold water and monitor with thermometer); Penalise if B and S are added at the start
- 1m for correct description of Benedict test with correct 1:1 ratio of volumes (hydrolysed S : Benedict's solution) and place in boiling water bath
- 1m for quantitative measurement – must measure time taken for first colour change
- 1m for calculating rate (dependent variable)

- (iii) Carry out your planned procedure using 8 yeast-alginate beads. Calculate the relative rate of yeast activity. Show your working. [1]

Refer to teachers' trial results

Time taken for colour change max 200s(s) + calculate relative rate (1/time taken) + unit (s⁻¹) → result within range 20s to 200s

- (c) Pectinases are enzymes that are used to increase the volume of fruit juice extracted from apples. Pectinases can be immobilised and placed inside a container with apple pulp as shown in Fig. 1.4. The fruit juice is then collected.

**Fig. 1.4**

A scientist carried out an investigation to determine the effect of temperature on the activity of immobilised pectinase by measuring the volume of fruit juice extracted. All other variables were kept constant.

The results are shown in Table 1.1.

Table 1.1

immobilised pectinase	
temperature / °C	volume of fruit juice / cm³
30	38
40	54
50	79
60	98
70	62
80	4

The scientist repeated the investigation replacing the immobilised pectinase with the same amount of pectinase but not immobilised in beads (free pectinase).

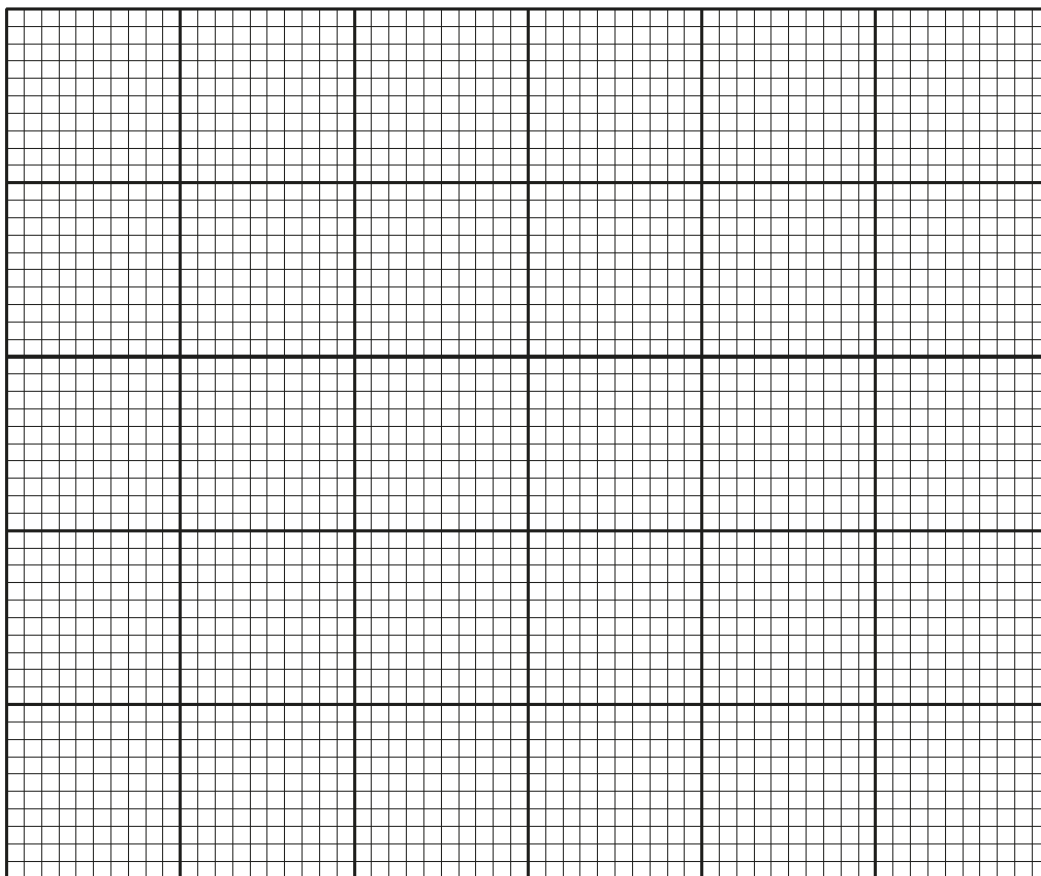
The results are shown in Table 1.2.

Table 1.2

free pectinase (not immobilised)	
temperature / °C	volume of fruit juice/cm³
30	52
40	85
50	98
60	41
70	12
80	4

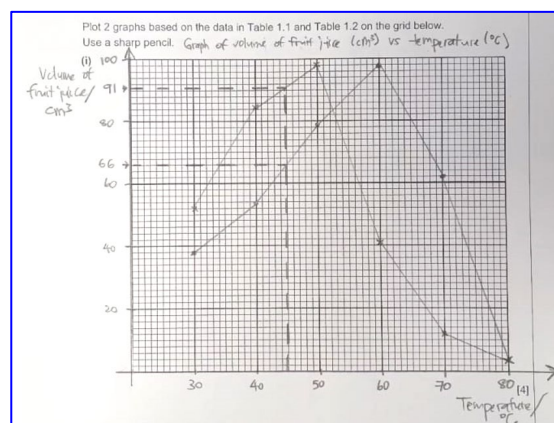
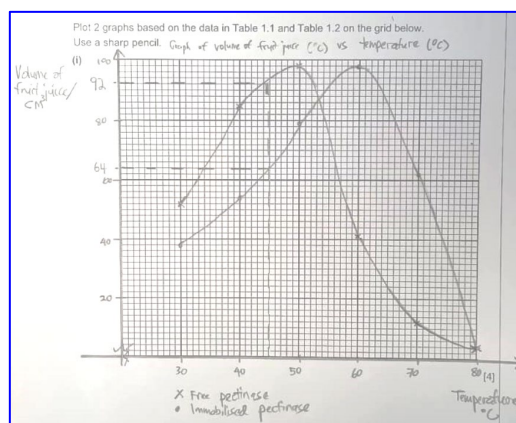
Plot 2 graphs based on the data in Table 1.1 and Table 1.2 on the grid below.
Use a sharp pencil.

(i)



[4]

Answers:



1. Label on x-axis: temperature (/) °C + label on y-axis: volume of fruit juice (/) cm³;
2. Size + scale (2/3 of graph paper for height and width);
3. For both graphs: correct plotting of all six points + 2 different symbols;
4. For both graphs: no extrapolation + all six plots joined with thin line passing through all points + line is either smooth curve or joined point to point;

(ii) Using your graph, estimate the volume of the fruit juice extracted at 45°C.
Show on your graph how you obtained your answer.

volume of fruit juice extracted from immobilized pectinase

.....[1]

volume of fruit juice extracted from free pectinase

.....[1]

1. working must be clearly shown on graph: use of ruler to draw lines that help find co ordinates from X and y axes + write those co-ordinates;

2. correct answer + units;

Note: vol. from immobilized pectinase is 64-68 cm³ vs vol from free pectinase is 91-96 cm³

- (iii) Suggest an explanation for the difference in volume of fruit juice obtained when using the free pectinase at 45°C and when using immobilised pectinase. [1]

free pectinase can more easily come into contact with apple pulp;

OR

less apple pulp can come into contact with pectinase in beads;

- (iv) Suggest why more fruit juice was obtained when using immobilized pectinase between the temperatures of 60°C and 70°C than when using the free pectinase. [1]

1. more thermostable; [R: stable alone]

2. immobilised enzymes only denature at higher temperatures;

- (d) More readings were taken at 50 °C of the immobilised pectinase and the free pectinase and the results are shown in Table 1.3.

Table 1.3

sample number	immobilised pectinase	free pectinase
1	78	99
2	73	97
3	72	96
4	77	98
5	75	96
6		100
7		100
Mean	75	98
Standard deviation	2.28	1.73

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

$$v = n_1 + n_2 - 2$$

The *t* test value is 17.49.

Table 1.4

degrees of freedom	probability, p				
	0.5	0.1	0.05	0.01	0.001
1	1.00	6.31	12.71	63.66	636.62
2	0.82	2.92	4.30	9.92	31.60
3	0.76	2.35	3.18	5.84	12.92
4	0.74	2.13	2.78	4.60	8.61
5	0.73	2.02	2.57	4.03	6.87
6	0.72	1.94	2.45	3.71	5.96
7	0.71	1.89	2.36	3.50	5.41
8	0.71	1.86	2.31	3.36	5.04
9	0.70	1.83	2.26	3.25	4.78
10	0.70	1.81	2.23	3.17	4.59
11	0.70	1.80	2.20	3.11	4.44
12	0.70	1.78	2.18	3.05	4.32
13	0.69	1.77	2.16	3.01	4.22
14	0.69	1.76	2.14	2.98	4.14
15	0.69	1.75	2.13	2.95	4.07
16	0.69	1.75	2.12	2.92	4.01

- (i) Use Table 1.4 and the t test value to decide whether the null hypothesis should be rejected.

Explain your answer. [2]

1. At degrees of freedom = 10, probability is less than 0.001;
2. At 5% level of significance, $p < 0.05$, null hypothesis is rejected. Hence, difference between mobilised and immobilised pectinase is significant and any difference is not due to chance (Compulsory point) R: $p < 0.001 < 0.05$

- (ii) Other than the effect of temperature, suggest an advantage of using immobilised enzymes in industrial processes. [1]

1. Immobilisation offers greater enzyme stability in variable conditions e.g pH. This increased stability helps maintain greater efficiency of the reaction process;
2. Using immobilised enzymes makes recovery and reuse of the enzymes far more straightforward. This is particularly advantageous where the enzyme may be hard (or expensive) to produce;
3. Immobilisation also ensures enzyme does not contaminate final product of reaction as the enzymes are physically trapped by matrix/ polymer;

[Total: 29]

Question 2

- (a) **R1** is a slide of a stained transverse section through a leaf.

Draw a large plan diagram of the region of the leaf on **R1** indicated by the shaded area in Fig. 2.1. This region must include **two** vascular bundles.

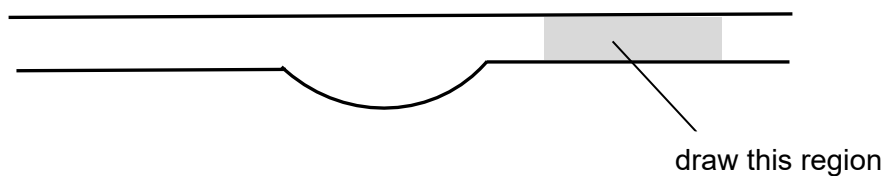
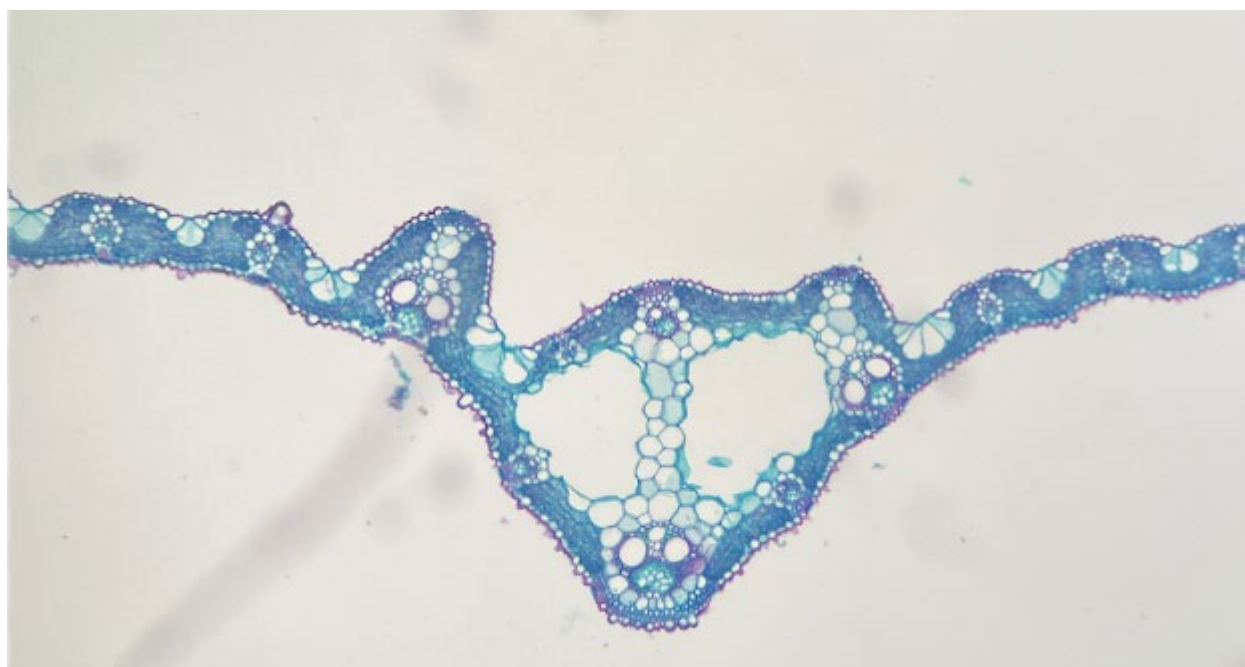
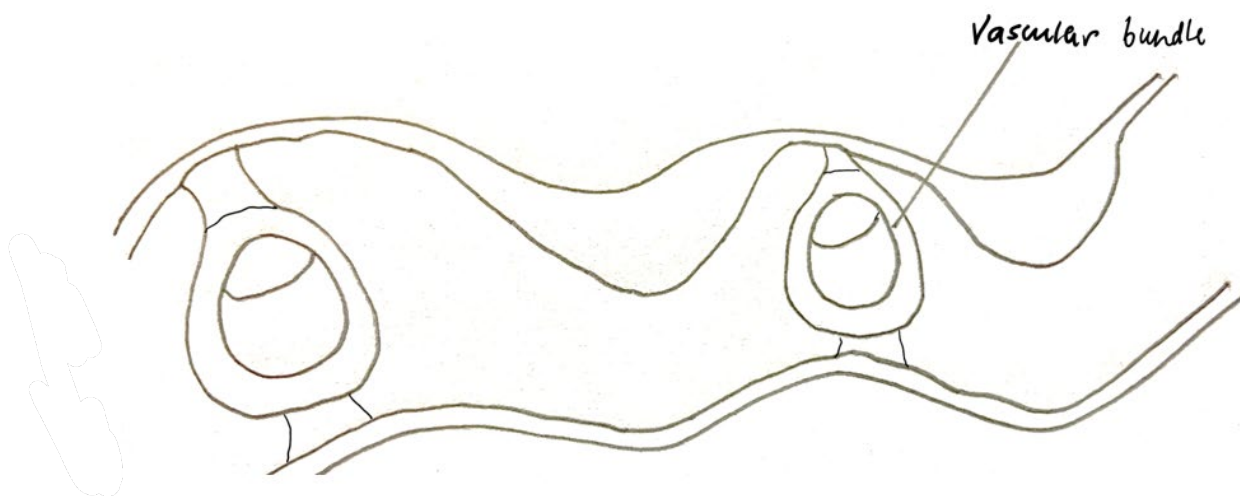
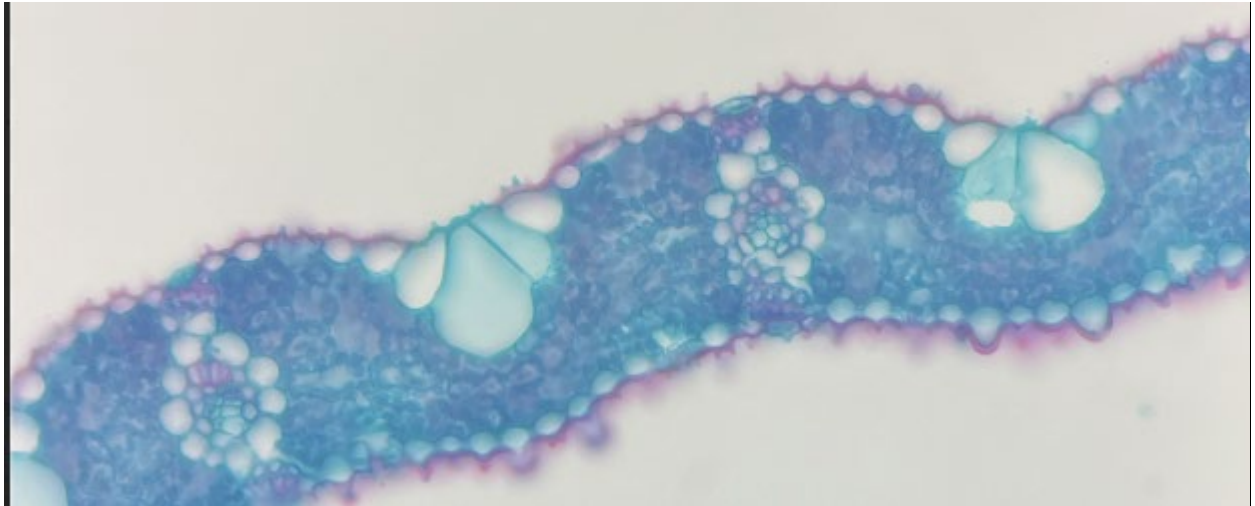


Fig. 2.1

Use a sharp pencil. No cells should be drawn.

Use **one** ruled label line and a label to identify **one** vascular bundle.





P

Scale: at least 2/3 of the space provided

1. **PL - Proportion & Lines**: thickness of each layer correctly represented, including bulliform cells AND smooth thin lines, (vascular bundle span the leaf if drawn);
2. **O - Observation**: draws 3 layers – should be wavy overall shape, upper epidermis, mesophyll cells, lower epidermis, with at least 2 vascular bundles with layer enclosing it; I: if additional layer of hair drawn over the epidermis
3. **P - Plan diagram** (no cells, no shading),
4. **VO** - label Vascular bundle correctly + correct Orientation of drawing (upright with bulliform cells region above).

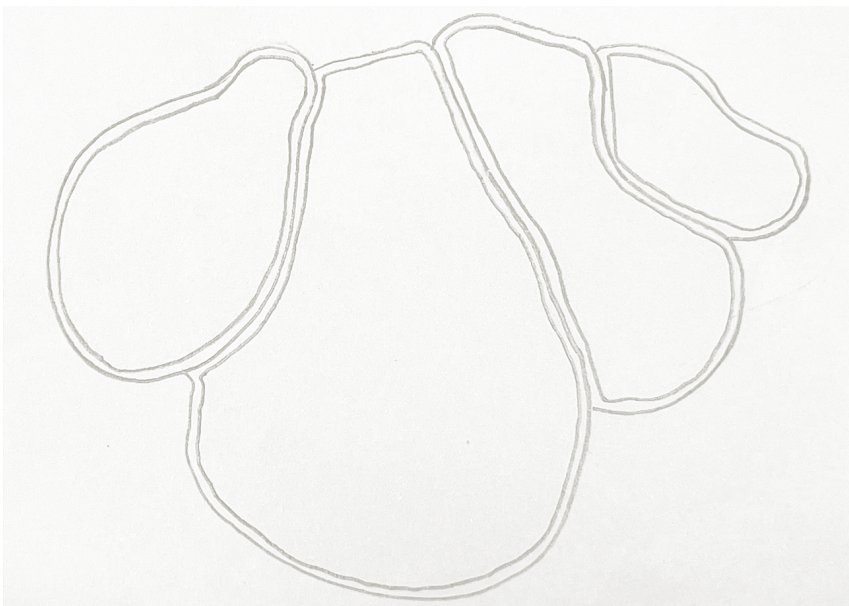
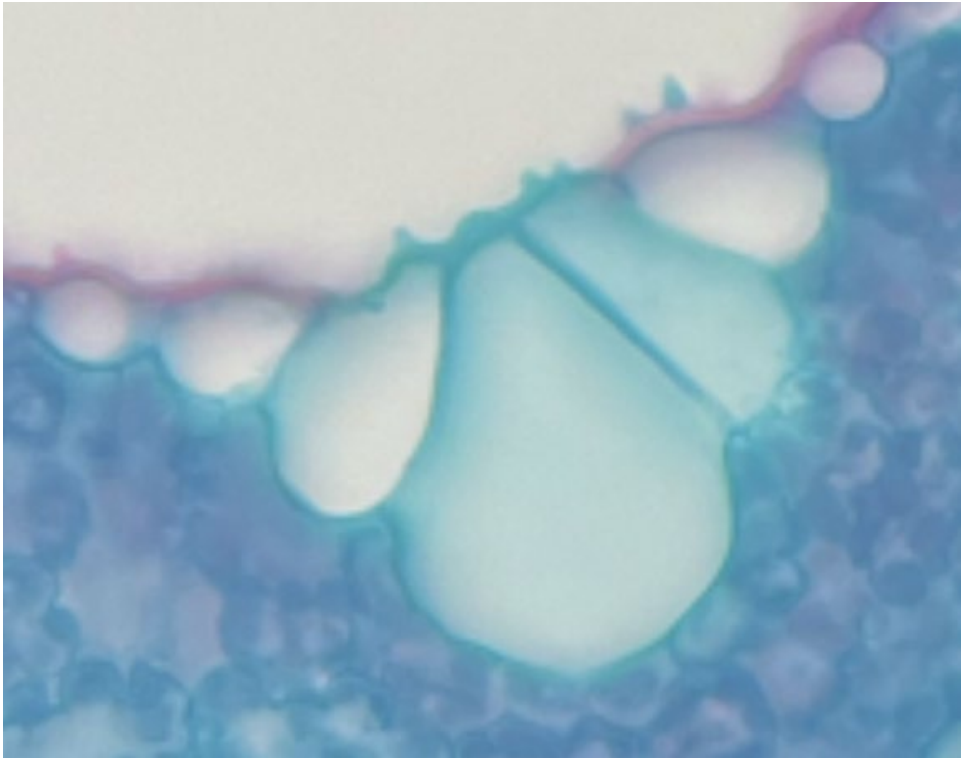
[4]

- (b) (i) Observe the upper epidermis of the slide **R1**. This layer is one-cell thick. There are specialised cells which are larger and bubble shaped, called bulliform cells. They are involved in the rolling and unrolling of leaves.

Select a group of four bulliform cells found in slide **R1**.

Make a large, detailed drawing of 4 adjacent cells. Cells must be touching each other.

Labels are **not** required. [3]



1. **Scale:** at least 2/3 of space provided;
2. **Proportion and quality of lines** - uniformly thin cell walls, no sharing of cell wall, smooth lines;
3. **Observation:** shape and 4 cells touching one another, presence of big and small cells (R: if all cells are same size);

- (ii) Suggest the type of environment this plant is adapted to and explain the role of bulliform cells in this adaptation. [2]

1. In a dry environment [compulsory]; R: Bright and hot
2. Loss of water from the bulliform cells cause it to become smaller will cause the leaf to roll;
3. Rolling of leaf will reduce water loss as less surface area of the leaf is exposed to the air and reduce water concentration gradient from stomata to external air;

- (c) (i) Fig. 2.2 is a photomicrograph of part of a stained transverse central section of a leaf from a different plant.

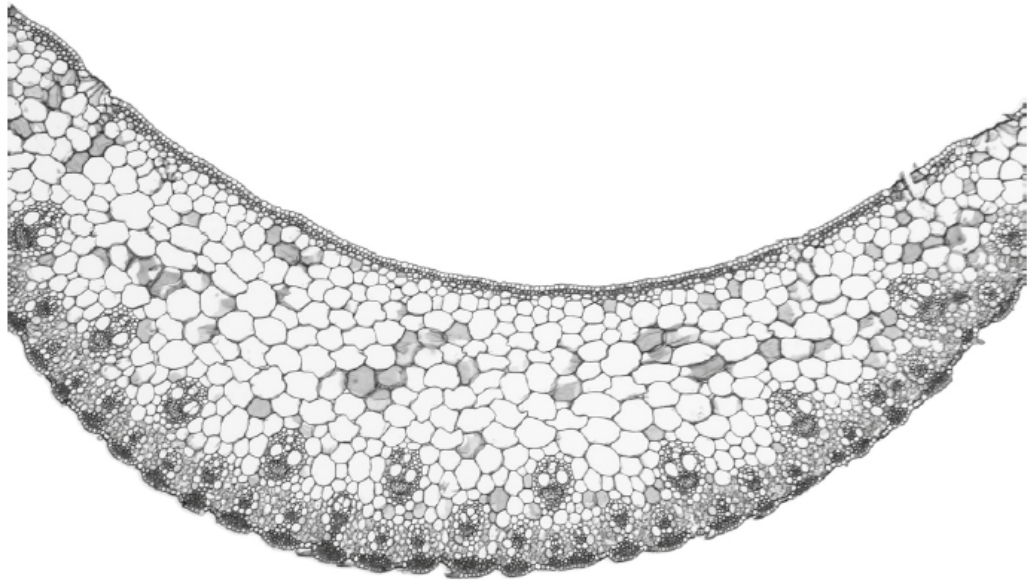


Fig. 2.2

Record in the space below **two** observable differences (other than size and reference to bulliform cells) between the specimen on slide **R1** and the leaf in Fig. 2.2.

Table with ruled lines; (penalize 1m if no table)

Any **two** from:

Pt.	Feature	slide R1	Fig. 2.2
1.	Arrangement of vascular bundle	Located centrally along the leaf section/ spans across width of leaf	Located at closer to the lower epidermis of leaf
2.	Number of rows of vacular bundles	One row	2 rows
3.	<u>Mid-rib/thicken middle portion</u>	Presence of mid rib	Absence of mid rib
4.	<u>AVP.</u> E.g. irregular vs smooth surface		

R: any reference to size including proportion, distance, width, area etc.

R: any reference to bulliform cells

R: any reference made to stomata – not clear in Fig 2.2

[3]

- (ii) Fig. 2.3 is the same photomicrograph as that shown in Fig. 2.2.

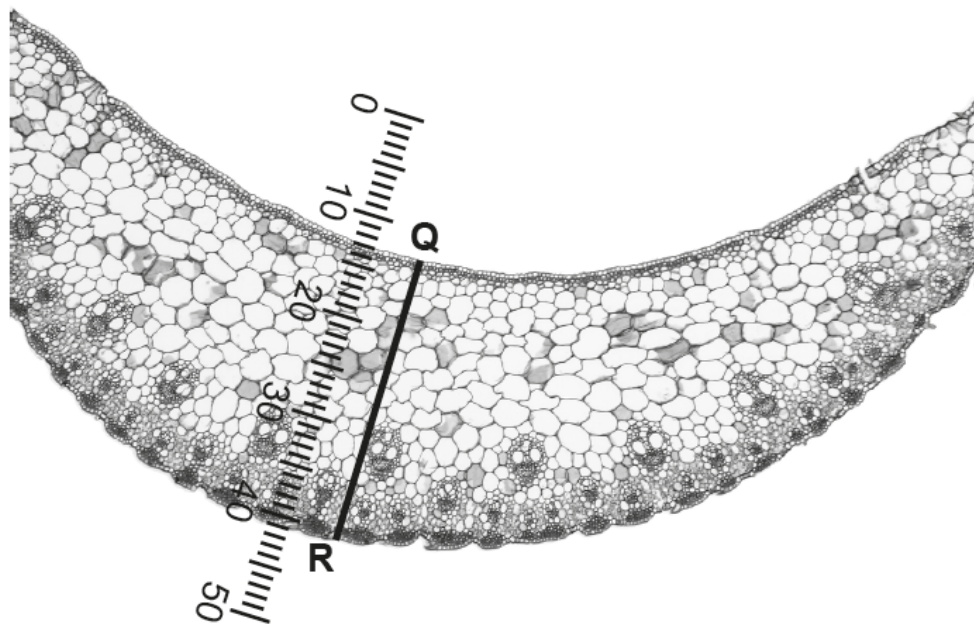


Fig. 2.3

An eyepiece graticule scale is shown in Fig. 2.3.

The calibration of the eyepiece graticule scale is:

$$1 \text{ eyepiece graticule division} = 34 \mu\text{m}$$

Use the calibration of the eyepiece graticule to calculate the actual width of the section in Fig. 2.3 shown by the line **Q–R**.

Show your working. [2]

1. Line **Q – R** = 28 eyepiece graticules;
2. The number of eyepiece graticules multiplied by 34 ie $28 \times 34\mu\text{m}$;
= 952 μm with the appropriate units;

[Total: 14]

Question 3

- (a) Duck weed grows on the surface of pond water.

The plant has simple structures. It has leaf-like photosynthetic tissue called a thallus. Each plant has a single underwater root and a thallus which floats on the water. Stems may not be present. It reproduces mainly through asexual reproduction by buds that grow and split off to form separate plants.

Fig. 3.1 shows the appearance of three of these plants.

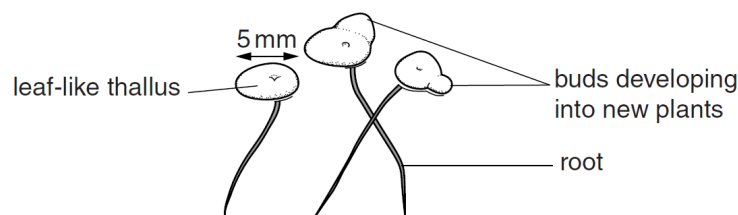


Fig. 3.1

Fig. 3.2 shows seven thalli of different sizes and stages of development.



Fig. 3.2

Fig. 3.3 is an enlarged drawing of the same structure that is in Fig. 3.2. Each square of the grid is 1 cm^2 .

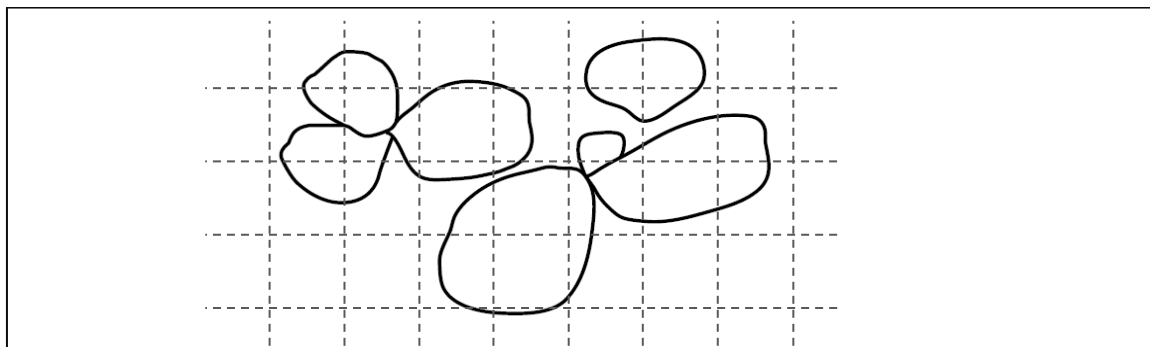


Fig. 3.3

- (i) Use the grid to estimate the total area of the thalli shown in Fig. 3.3. [1]

Answer = $13 \times 1\text{ cm}^2 = 13\text{ cm}^2$ (A : 11 – 14)

- (ii) Describe the method you used to decide which of these squares to include. [1]

Count squares that are half full, more than half and full as 1 square [9700 pre university answers];

A: Count squares that are more than half and full as 1 square;

- (b) Duck weed can occur on the surface of water polluted by sewage which contains high levels of cadmium and copper ions.

A student wants to compare the effect of different concentrations of cadmium and copper ions on the growth rate of duckweed. In addition to standard laboratory equipment, you are provided with:

- stock solutions of 10 mg cm^{-3} cadmium sulfate and 10 mg cm^{-3} copper sulfate, and
- plants were collected from a pond and those with a thallus without buds were selected.

- (i) The student used the stock solution provided to prepare the other concentrations of copper sulfate. Describe how this can be achieved using appropriate apparatus. [2]

1. using named apparatus, correct vol of copper sulfate to water to prepare a known conc of copper sulfate;

E.g.: to 8 mg cm^{-3} take 8ml of stock and add 2 ml of water;

2. references to 4 other stated dilutions given – equal dilution interval (A: serial dilution)
e.g.: 2, 4, 6 & 8 mg cm^{-3}

R: labelled diagram / dilution table

- (ii) Based on the information given in (a) and (b), plan an investigation to compare the minimum concentrations of cadmium and copper ions in solutions that may inhibit the growth rate of duck weed.

Do **not** repeat any details of how the different concentrations of cadmium and copper ions in solutions are prepared and the method described in (a)(ii). You are not required to plan for repeats and replicates. [4]

1. Independent variable: Three thalli were placed in beakers containing each of the 5 dilutions / state 5 conc of cadmium sulfate;
Note: only 1 type of ions must be investigated at any one time
2. Measurable variable: Total area of thalli in each beaker was counted every day (A: alternate days) over a specified period of time e.g. 10 days;
Note: Measurable variable must be taken after a period of time.
3. Dependent variable is rate of growth of thalli where rate = total area/number of days;
4. Control experiment: Keeping all variables constant, repeat the expt with thalli in water without cadmium and copper ions; R: pond water
5. Repeat experiment with copper sulfate; (idea of comparison)
6. Format of results: Using table/plot a graph/bar chart to determine the MIC of the copper and cadmium ions in any suitable format;
7. Determine MIC: Beyond minimum inhibitory concentration (MIC), there will not be any growth/death of thalli; COMPULSORY PT
8. Constant variables (find from above pt 1-7 eg Pt2)
[Any 1]
(a) Duration / time of investigation/ number of days experiment is conducted;
(b) Plants; A: similar total surface area of thalli, age of plants, source
(c) Temperature where experiment was conducted;
(d) Cadmium/copper-containing solutions should have same concentrations/ volumes at any one time;
(e) Aeration;
(f) Light used to expose thalli;
AVP

- (c) Fig. 3.4 shows a transmission electron micrograph of a cell from a closely-related aquatic plant, sago pondweed.

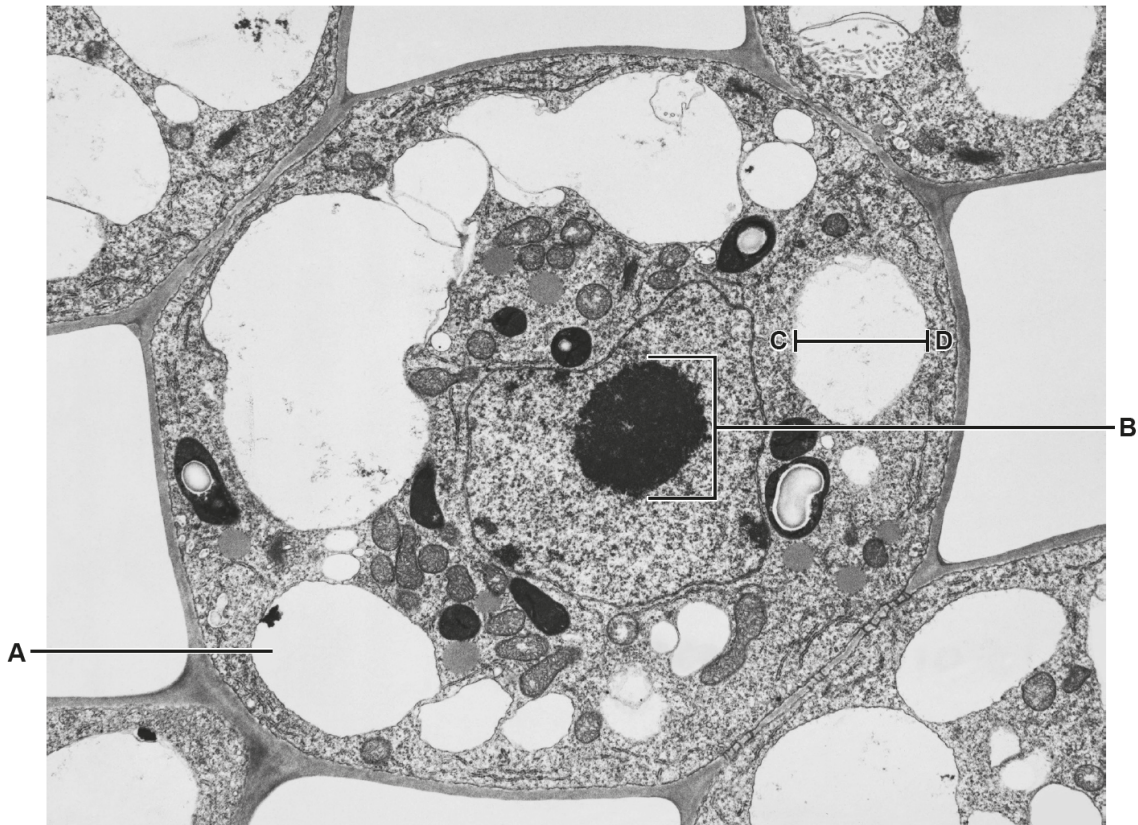


Fig. 3.4

- (i) Identify the cellular components shown at **A** and **B**. [2]
A: vacuole B: nucleolus
- (ii) The real size of the line between **C** and **D** is 1.4×10^{-6} m. Calculate the magnification that was used to produce the image in Fig. 3.4. Give your answer to 2 significant figures.

Show your working. [2]

Length of drawing of CD line
 = 18 mm (A: 17 – 17.5mm)

Magnification
 = length of drawing / actual length
 = $18 \times 10^{-3} / 1.4 \times 10^{-6}$
 = 12857
 = 13000 (2 s.f.)

[Total: 13]

End of Paper