VICTORIA JUNIOR COLLEGE
JC 2 PRELIMINARY EXAMINATION
2024
HIGHER 2

NAME: ……

CT CLASS: ……

BIOLOGY 9744 / 04

Paper 4 Practical

29/08/2024

2 hours 30 minutes

Candidates answer on the Question Paper.
Additional Materials: As listed in the Confidential Instructions.

READ THESE INSTRUCTIONS FIRST

Write your name and CT in all the work in hand in.
Give details of the practical shift and laboratory, where appropriate, in the boxes provided.
Write in dark blue or black pen.
You may use an HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together.
The number of marks is given in bracket [ ] at the end of each question or part question.

Shift
Laboratory

Question	Marks
1	
2	
Total	

Answer **all** questions.

Question 1

Researchers are exploring the development of plant-based, biodegradable plastics as an alternative to petroleum-based plastics.

Switchgrass is an example of a crop that synthesises polymers naturally and is used for biodegradable plastic production.

Switchgrass which are genetically modified with genes from another organism can result in the plants synthesising more polymers. These plants are termed as transgenic plants.

The ability to synthesise polymers is highly dependent on the growth rate of the plants, which in turn depends on the rate of photosynthesis.

(a) (i) For the same concentration of chloroplasts, predict how the rate of photosynthesis in the commercially-available switchgrass chloroplast extract would compare to that in the transgenic plant. [1]

- The chloroplast extract from the transgenic plant will have a higher rate of photosynthesis;

To test for the rate of photosynthesis, the blue dye, DCPIP, which acts as a substitute for $NADP^+$ can be used.

oxidised DCPIP (dark blue) $\xrightarrow{\text{photosynthesising tissues}}$ reduced DCPIP (colourless)

You are required to:

- investigate the rate of photosynthesis in different concentrations of commercially available switchgrass chloroplast extract
- obtain a chloroplast extract from the transgenic plant
- estimate the concentration of chloroplasts in the transgenic plant.

You are provided with:

- 15 cm^3 commercially available 10% switchgrass chloroplast extract, in a small vial labelled **SE1**
- A leaf from transgenic plant labelled **T**
- cold buffer solution, in a large vial labelled **B**
- distilled water, in a large vial labelled **W**
- a block of agar stained with DCPIP.

The DCPIP agar may stain your skin. You are advised to wear gloves when handling the agar.

The agar must be covered using aluminium foil when not in use to prevent decolourisation.

Assume that the temperature in the lab remains relatively constant. It is not necessary to use a water bath maintained at room temperature.

Proceed as follows.

1 You are required to carry out a serial dilution of **SE1** to reduce the concentration of the chloroplast extract by **a factor of ten** between each of three successive dilutions, **SE2**, **SE3** and **SE4**.

You will need to make up 8.0 cm^3 of each solution. Some of this will be used to make the next solution.

(ii) Describe how you would carry out serial dilution to obtain **SE2**, **SE3** and **SE4**. [3]

1. Correct switchgrass chloroplast extract used for each dilution;
2. Correct volumes of switchgrass chloroplast extract and distilled water used to make up 8.0 cm^3 of each solution;
3. Ref to appropriate and logical steps in description;

concentration switchgrass chloroplast extract / %	switchgrass chloroplast extract used for dilution	volume of switchgrass chloroplast extract used for dilution / cm^3	volume of distilled water used / cm^3
1.00 (SE2)	SE1	0.8	7.2
0.10 (SE3)	SE2	0.8	7.2
0.01 (SE4)	SE3	0.8	7.2

(iii) Suggest an advantage of carrying out serial dilution. [1]

- Ref. to ability to obtain small concentrations – not possible with simple dilution from stock as volumes of stock solution needed to make small concentrations are too small to be measured accurately with the apparatus provided;
- Ref. to ability to test a bigger range of concentrations;

2 Label three small vials, **SE2**, **SE3** and **SE4**. Use them to prepare the different concentrations of **SE2**, **SE3** and **SE4**, as described in **(a)(ii)**.

3 Place the block of DCPIP stained agar onto a white tile. Using a ruler and a knife, cut the agar block into identical cubes of about 1.5 cm × 1.5 cm × 1.5 cm, as shown in Fig. 1.1.

It may be difficult to cut them to exactly the same size. You will use only **five** agar cubes for this part of the experiment. Choose five that are the most identical to one another. Set aside the rest of the agar cubes for backup.

Place all the agar cubes onto the Petri dish provided and cover the Petri dish with aluminium foil.

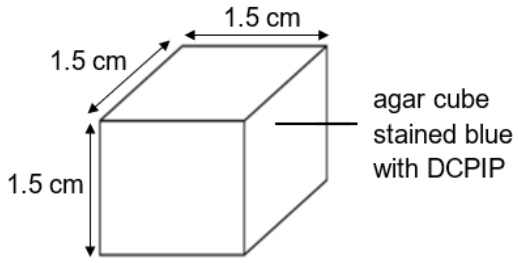


Fig. 1.1

4 Using a white tile and knife, chop up 3.0 cm by 3.0 cm of the fresh leaf **T** into smaller pieces and put them into the mortar.

5 Slowly add approximately 15.0 cm^3 of cold buffer solution **B** to the mortar. Grind the leaf pieces to obtain a chloroplast extract of **T**.

(iv) Outline the importance of using cold buffer for chloroplast extraction. [1]

Any one:
- Cold – Lower activity of hydrolytic enzyme / Make hydrolytic enzyme inactive to prevent damages to chloroplasts;
- Contains pH buffer – Maintains pH to prevent denaturation of enzymes in chloroplasts;

6 Place a sieve on top of the opening of a large plastic vial. Filter the contents of the mortar to remove the plant debris, keeping filtrate **TE**.

7 Use a piece of aluminium foil to cover the vial containing **TE** to protect it from light.

8 Label a small vial **TE** and put 7.0 cm^3 of **TE** into the vial.

9 You will only need 7.0 cm^3 of **SE1**. Use a syringe to discard any excess **SE1** from the small vial into the sink. Put a piece of agar cube into the small vial containing **SE1** and immediately start the stopwatch. Incubate for 10 minutes.

10 Repeat step **9** for **SE2**, **SE3**, **SE4** and **TE**. You may stagger the start times for incubation of each vial.

11 Gently swirl the contents of the vials at regular intervals.While you are incubating the agar cubes, continue to read through this Question Paper up to step **15** and complete **(a)(vi)**.

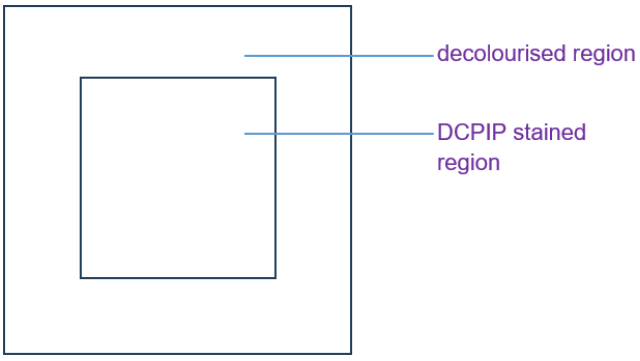
12 Fill a large vial with tap water. This will be used to rinse the agar cubes in step **13** to **stop** the reaction.

13 After 10 minutes, use a pair of forceps to remove the agar cubes from each solution and rinse the cubes in the vial.

14 Put each agar cube on a white tile and cut it into half.

(v) In the square below, draw the decolourised region and DCPIP stained region of the cut end of the agar cube that has been immersed in **SE1**.

Add a ruled line to label the “decolourised region” and a second ruled line to label “DCPIP stained region”. [1]



15 Measure the length of the DCPIP stained region for **all** the agar cubes, to the nearest mm. Record these measurements in the table prepared in **(a)(vi)**.

*Ensure that these measurements are done **immediately** as the agar cubes will continue to decolourise when exposed to light.*

(vi) Use this space to record your results for step **15** in a suitable format. [3]

concentration of SE / %	length of DCPIP stained region / mm
10.00	9
1.00	10
0.10	11
0.01	13
Unknown (TE)	10

1. Correct trend;
2. Correct headings and units;
3. Data to nearest whole number (mm);

(vii) Using the data obtained in **(a)(vi)**, estimate the concentration of chloroplasts in **TE**. [1]

- 1.00% (A: 0.10%);

(viii) Identify **one** significant source of error in the procedure and describe how you would make an improvement to reduce this source of error. [2]

1. Light source is ambient light which is not constant for all vials;
2. Use a lamp placed at a set distance e.g. 10 cm, for each vial, conduct experiment in dark room;

(ix) The percentage decolourisation of an agar cube can be taken as a measure of photosynthetic efficiency. Suppose an agar cube in **(a)(vi)** had 10 mm of its length stained with DCPIP, calculate its percentage decolourisation. Show all your working clearly. [3]

1. Correct calculation of length / area / voume of decolourisation;
2. Correct calculation of percentage of decolourisation;
3. Answers correctly calculated to 1 d.p;

(Clear working statements expected)

Using fresh DCPIP stained agar cubes and **TE**, another experiment was conducted to investigate the effect of different light intensities on the rate of photosynthesis of the transgenic plant.

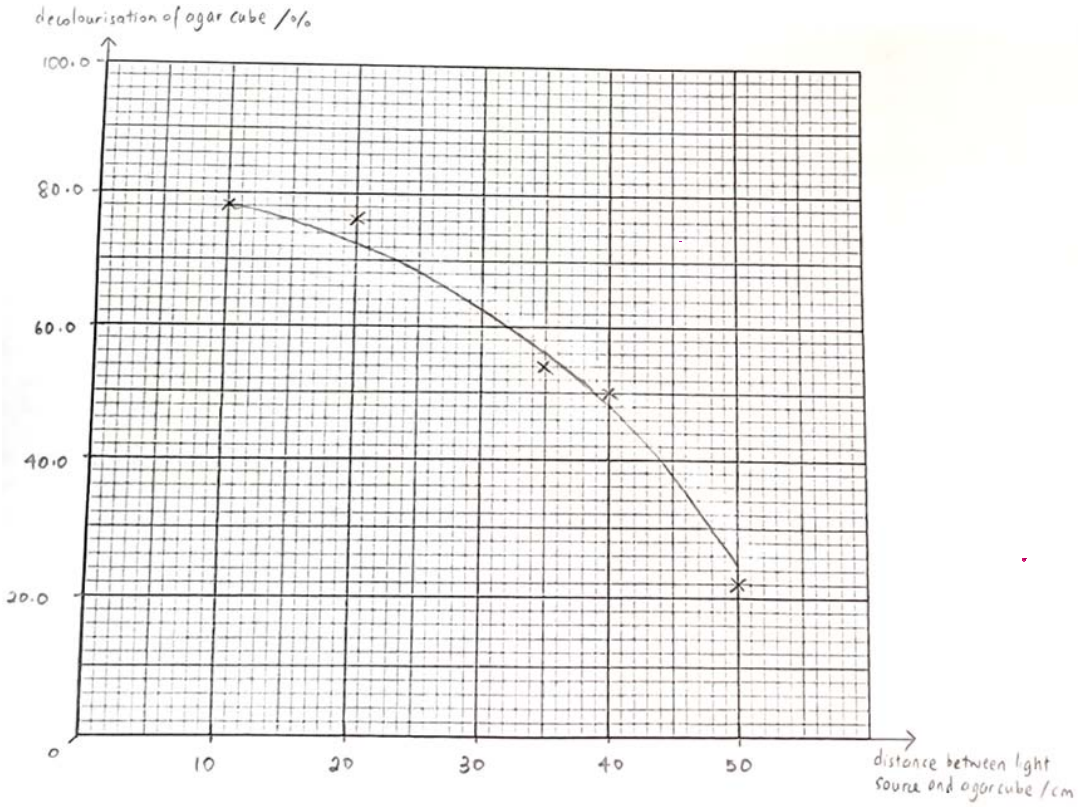
(b) Table 1.1 shows the percentage decolourisation of each agar cube at five different intensities of light.

Table 1.1

Distance between light source and agar cube / cm	Decolourisation of agar cube / %
10	78.0

20	76.0
35	54.0
40	50.0

Use the grid provided to draw a line of best-fit for the results shown in Table 1.1. [4]



[4]

[Total: 20]

Question 2

Hydrogencarbonate indicator is a solution that changes colour depending on the concentration of carbon dioxide in the solution. These colours are related to different pH values, as shown in Table 2.1.

Table 2.1

colour of hydrogencarbonate indicator solution	pH	concentration of carbon dioxide in the solution
yellow	7.6	↑ increasing carbon dioxide concentration
yellow-orange	7.8	
orange	8.0	

orange-red	8.2	
red	8.4	atmospheric concentration
red-magenta	8.6	↓ decreasing carbon dioxide concentration
magenta	8.8	
magenta-purple	9.0	
purple	9.2	

Chlorella vulgaris is a protoctist that is single-celled, aquatic and photosynthetic. It can be immobilised in alginate beads.
Alginate beads with immobilised *C. vulgaris* can be used to measure the rate of photosynthesis. Sodium hydrogen carbonate solution ($NaHCO_3$) can be used as a source of carbon dioxide.

A student used alginate beads with immobilised *C. vulgaris* in hydrogencarbonate indicator solution and 0.1M $NaHCO_3$ to investigate the rate of photosynthesis in different light intensities.

Fig. 2.1 shows some of the apparatus and reagents the student used.

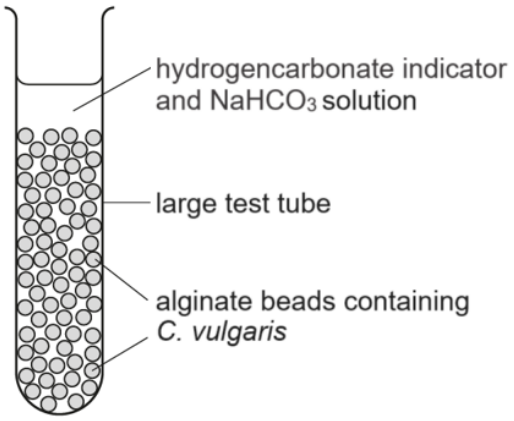


Fig. 2.1

(a) The student noticed that the hydrogencarbonate indicator solution changed from red to magenta, when the alginate beads with immobilised *C. vulgaris* in Fig. 2.1 was exposed to light.

Explain why the colour of the indicator started off as red and later changed to magenta. [2]

1. Started off are as red because it was exposed to atmospheric carbon dioxide / initial concentration of carbon dioxide in the solution was the same as atmospheric concentration, hence pH 8.4;
2. When exposed to light, *C. vulgaris* photosynthesised, using up CO_2, reducing concentration of CO_2 in the solution, increasing pH to 8.6;

(b) The student wanted to reuse the setup in Fig. 2.1 and colour chart in Table 2.1 to investigate the rate of photosynthesis in different concentrations of carbon dioxide. He has run out of 0.1M $NaHCO_3$. Instead, he has access to the following materials and apparatus:

- 0.5M $NaHCO_3$
- hydrogencarbonate indicator solution
- alginate beads with immobilised *C. vulgaris*
- distilled water
- table lamp
- standard lab apparatus
- access to hot and cold water

Describe a procedure the student could use to carry out this investigation.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- identify the dependent variable and the independent variable
- identify the variables you will need to control
- include a suitable control
- a safety precaution
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed
- include a prediction and the justification for the prediction.

You do **not** need to include a diagram of the experimental setup. [9]

1. Independent variable: concentration of carbon dioxide / $NaHCO_3$, dependent variable: extent of change in colour (degree of pH change)
2. Preparing independent variables:
   (i) Specified 5 different $NaHCO_3$ concentrations to prepare;
   (ii) Accurate description how to prepare / dilution table shown;
3. Keeping other variables constant: (max 2)
   (i) Light intensity: Set distance of table lamp specified;
   (ii) Temperature: Set temperature specified + Accurate description of how to prepare water bath with hot and cold water, using thermometer to monitor and how to maintain;
   (iii) Concentration of enzymes: specified fixed number of alginate beads
   (iv) Voulme of substrate: specified fixed volume of $NaHCO_3$
4. Control: Accurate description of repeat set-up in the dark;
5. Data recording:
   (i) With refer to Table 2.1, record initial and final colour and pH + fixed reaction time / Record time for initial color to change to magenta (pH 8.8.);
6. Repeat for all concentrations and repeat whole experiment at least twice;
7. Safety precautions (any 1);
   (i) Indicator / alginate may be irritants/cause allergies, so wear gloves to protect skin
   (ii) Hot bulb may cause burns, so do not touch with bare hands
   (iii) Danger of electrocution, so do not touch power socket with wet hands
8. Prediction: The higher the concentration of carbon dioxide, the higher the degree/extent of colour change in a fixed time / faster time for initial colour to change to magenta;
9. Justification: The higher the substrate (CO_2) concentration, the higher the rate of photosynthesis, i.e. the higher rate of CO_2 usage, hence the greater the pH change in a fixed time / faster time for initial pH to change to fixed pH e.g. pH 8.8;

(c) The student set up a large test-tube containing alginate beads with immobilised *C. vulgaris* in hydrogencarbonate indicator solution at **pH 8.4** (red).

The student kept this set-up in the dark for 12 hours.

Predict and explain the results that will be observed after 12 hours in the dark. [2]

1. Indicator solution will turn yellow/orange due to decrease in pH;
2. Without light, *C. vulgaris* stops photosynthesising, but continues to respire, producing CO_2, increasing concentration of CO_2 in the solution;

(d) Some scientists wanted to culture cells of *C. vulgaris* on a large scale for use as a biofuel.

To determine the optimal growing conditions for *C. vulgaris*, the scientists needed to determine the number of cells per cm^3 of suspension to monitor the population growth.

They tried two methods to determine the number of cells per cm^3 of suspension. The first method used a Secchi stick, as shown in Fig. 2.2.

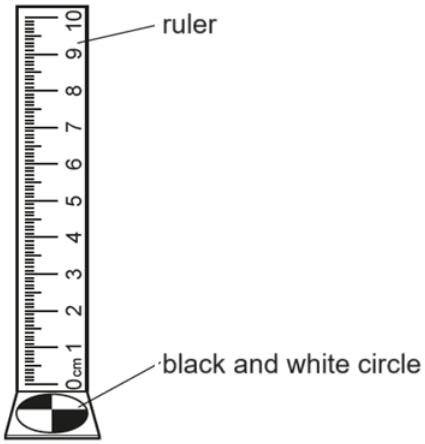


Fig. 2.2

The Secchi stick is lowered into the suspension of cells until the black and white circle is not able to be seen from above.The depth in cm is recorded from the ruler, as shown in Fig 2.3.

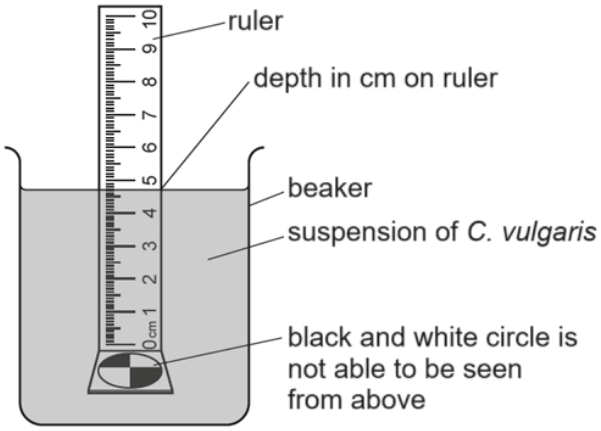


Fig. 2.3

The $\log_{10}$ of the number of cells is determined from a graph of $\log_{10}$ of cells counted per cm^3 suspension against Secchi depth (cm), as shown in Fig 2.4.

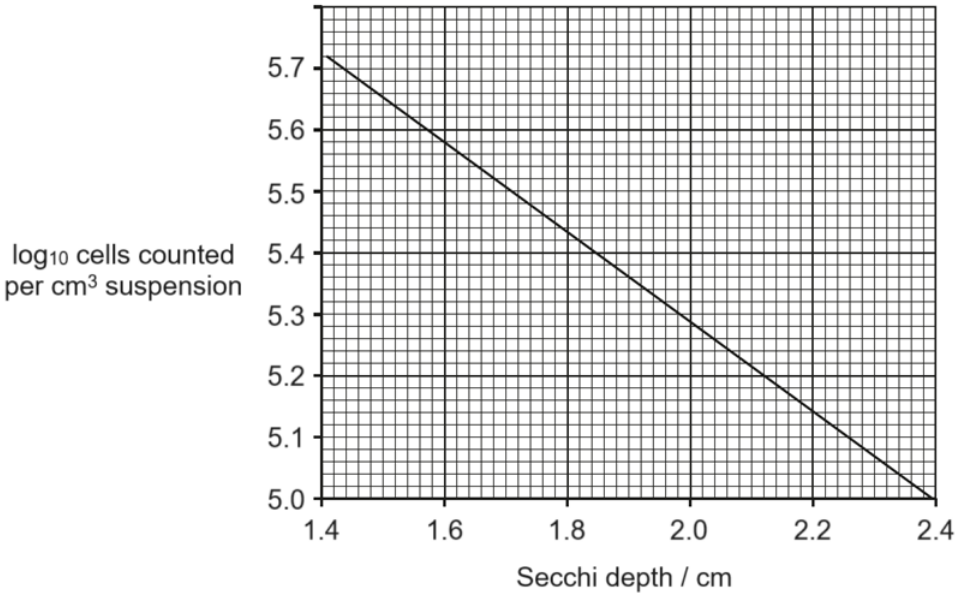


Fig. 2.4

(i) When the scientists inserted the Secchi stick into a sample from their cell suspension, the circle (on the Secchi stick) was not able to be seen at a depth of **1.9 cm**.

Using the graph in Fig. 2.4, state the $\log_{10}$ number of cells per cm^3 of suspension. Show how you derive the value on the graph and record your answer below. [1]

- Correct answer: 5.36 + show on the graph;

The second method used a counting chamber to determine the number of cells per cm^3 of suspension. Fig. 2.5 shows a section of a counting chamber with cells present, as viewed using the high power of a light microscope.

The depth of the 1 mm × 1 mm counting chamber is **0.1 mm**.

The scientists counted the number of cells in several sections of a counting chamber.

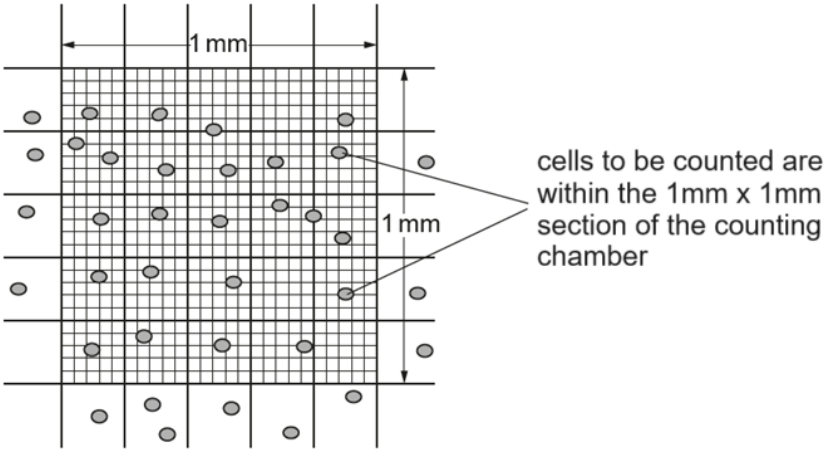


Fig. 2.5

(ii) Count the number of cells in the 1 mm × 1 mm section of the counting chamber shown in Fig. 2.5.

Use your answer to calculate the number of cells per cm^3 of the suspension. Show all your working. [3]

1. correct number of cells counted: 24**;**
2. correct working:

$1cm^3 = 10 \times 10 \times 10 = 1\,000\ mm^3$

Number of cells counted per 0.1 mm^3 = 24

Number of cells per cm^3 = 24 / 0.1 x 1 000;

3. correct calculation of number of cells per cm^3: 240 000**;**

(Clear working statements expected)

(iii) Suggest one reason why using the Sechhi stick may be less accurate then using the counting chamber. [1]

Any 1:

1. subjective i.e. difficult to determine when circle is no longer visible;
2. error of 0.5 mm in the ruler;
3. *idea that* inserting Secchi stick, mixes / disturbs suspension;

[Total: 18]

Question 3

Photosynthesis occurs in the leaves of plants. **M1** is a slide of a stained transverse section through a plant leaf.

(a) **(i)** Draw a large plan diagram of the region of the leaf on **M1** indicated by the shaded region in Fig. 3.1. Use a sharp pencil.

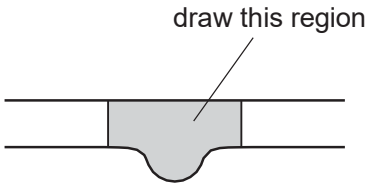


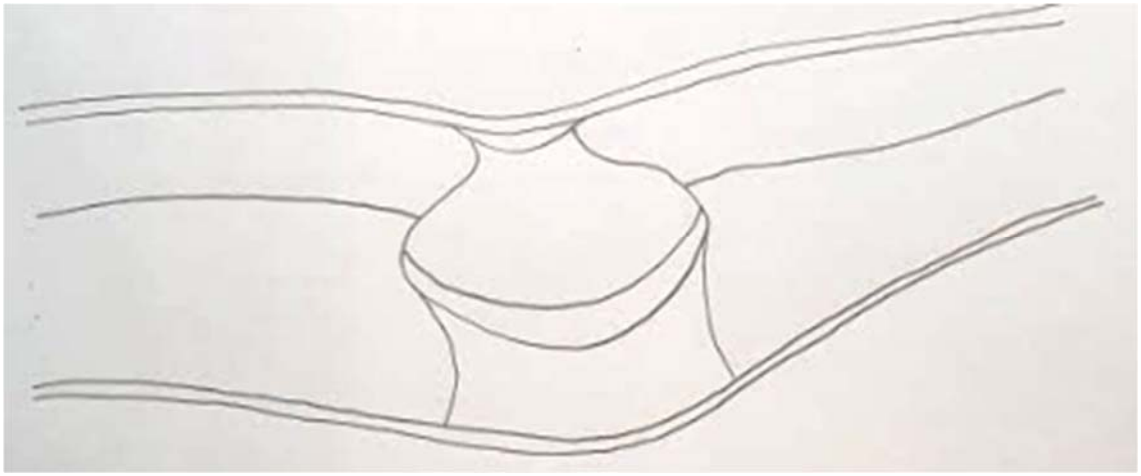
Fig. 3.1

A plan diagram shows the arrangement of different tissues. Your drawing should show the correct shapes and proportions of the different tissues.

No cells should be drawn.

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

1. Large drawing (occupy at least half space), with clean, smooth continuous lines;
2. Correct number of layers, no cells drawn;
3. Correct shapes and proportion of tissues;



(ii) Observe the **unshaded** region on slide **M1.** The bottom-most layer of cells is called the lower epidermis and is one cell thick.

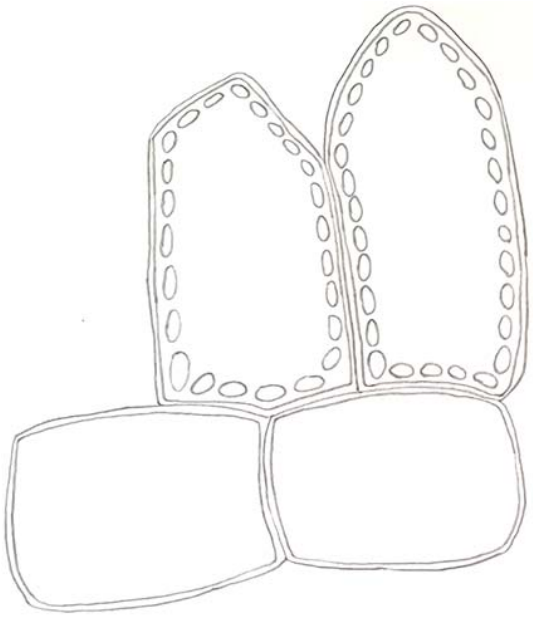
Select a group of four touching cells comprising two cells from the lower epidermis and two cells from the layer above the lower epidermis.

Each cell must touch at least two other cells in the group.

Make a large drawing of this group of four cells. Include any internal structures that you can see.

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

1. Large drawing (occupy at least half space), with clean, smooth continuous lines;
2. four touching cells comprising two cells from the lower epidermis and two cells from the layer above the lower epidermis (spongy mesophyll cells);
3. Correct shape for epidermal cells (most are wider but shorter, and more oblong-shaped) and spongy mesophyll cells (some are more irregular-shaped, most are longer and narrower compared to epidermal cells);
4. Cell wall for all cells, chloroplasts for cells in the layer above epidermis;



(iii) A student hypothesised that the ratio of the number of cells in the lower epidermis to the number cells in the layer above is 1:3. He took many sample counts and wanted to determine if the ratio he observed followed the expected. Name the statistical test that he should use and explain the conclusion he should make if the p value is found to be 0.49. [3]

Statistical test: chi-square test;

Conclusion:
1. Since p is more than 5%, the differences between observed and expected numbers are insignificant and due to chance;
2. Do not reject null hypothesis, i.e. the observed ratio follow the expected ratio;

(b) Fig. 3.2 shows a photomicrograph of a stage micrometer scale that is being used to calibrate an eyepiece graticule.

The length of one division on this stage micrometer is 1 mm.

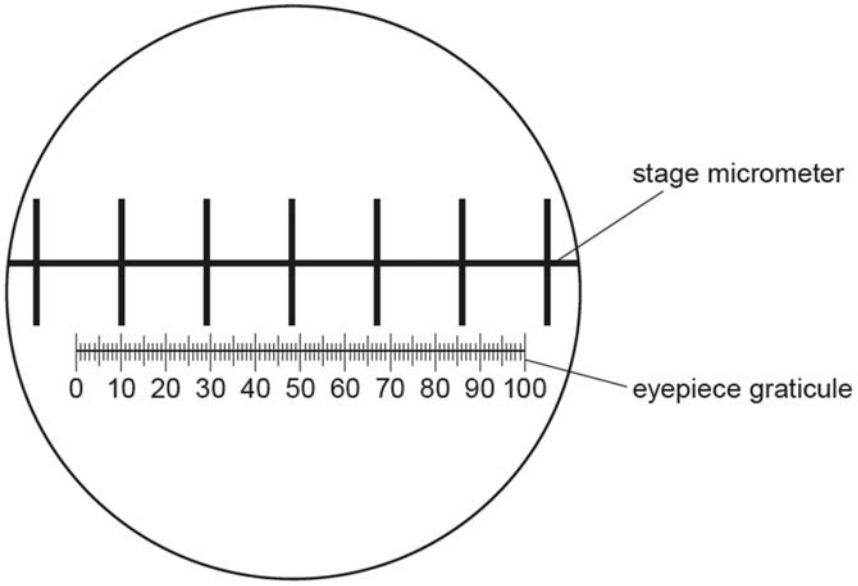


Fig. 3.2

(i) Use Fig. 3.2 to calculate the actual length of one eyepiece graticule unit.

Show your working and include appropriate units. [3]

19 eyepiece graticule units = 1 stage micromter unit = 1mm;
1 eyepiece graticule unit = 1 / 19 x 1000; = 52.6 um (correct to 1 d.p.);

1. Correct calibration;
2. Correct working;
3. Correct answer to 1 or 2 d.p. in um;

Fig. 3.3 shows a photomicrograph of a transverse section through a different leaf. This was taken with the same microscope and lens used to take the photomicrograph in Fig. 3.2.

The eyepiece graticule has been placed across the leaf section to measure its thickness.

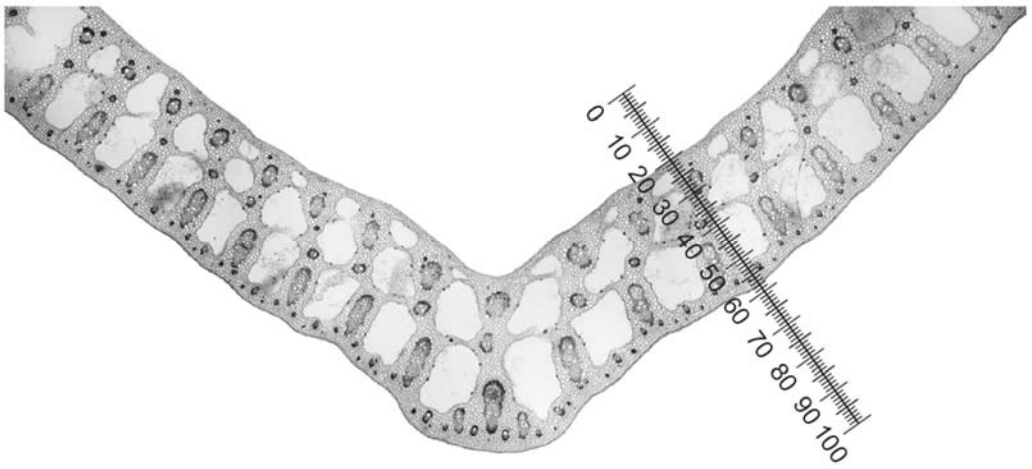


Fig. 3.3

(ii) Use the calibration of the eyepiece graticule from **3(b)(i)** to calculate the actual thickness of the leaf section in Fig. 3.3.

Show your working and write your answer to **two** decimal places. [2]

Actual thickness = 58 – 20 = 38 eyepiece graticule units;
= 38 x 52.6
= 1998.80 um;

1. Correct measurement in eyepiece graticule units;
2. Correct working and final answer to 2 d.p;

(iii) Identify **three** observable differences, other than colour and the presence of air spaces, between the leaf section on **M1** and the leaf section in Fig. 3.3.

Record these observable differences in Table 3.1. [3]

Table 3.1

feature	M1	Fig. 3.3
Vascular bundle	Presence of central vascular bundle	Absence of central vascular bundle / vascular bundles
Number of vascular bundles	Fewer vascular bundles	More vascular bundles
Mesophyll layers / Cells in between epidermis	Distinctive layers of cells (of palisade and spongy mesophyll cells)	No distinct layers / cell look uniform
Stomata / guard cells	Presence of stomata / guard cells	Absence of stomata / guard cells
Measurements e.g. thickness of leaf?	Need to measure M1	

[Total: 17]