Class | Adm No

Candidate Name: _____

H

millennia institute

2023 End-of-Year Examination

Pre-university 3

BIOLOGY HIGHER 2 **9744/04**

Paper 4 Practical **29 August 2023**

Candidates answer on the Question Paper **2 hour 30 minutes**

READ THESE INSTRUCTIONS FIRST

Do not open this booklet until you are told to do so.
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 and graphs.
Do not use staples, paper clips, highlighters, glue or correction fluid/tape.

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

The use of 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 brackets [ ] at the end of each question or part question.

Shift
Laboratory

For Examiner's Use	
1	/20
2	/21
3	/14
Total	/55

Candidates with access to microscope at the start of the paper are given the **first 1 hour 15 mins** to use it. Please answer **Question 3,** within this time frame.

Candidates with no access to microscope at the start of the paper will be given access 1 hour 15 minutes after the start of the paper. You may proceed with **Question 1** first.

Answer **all** questions.

Question 1

You are provided with a solution labelled **E** containing an enzyme which coagulates (clots) milk. Enzyme **E** hydrolyse peptide bonds between certain amino acids in a protein found in milk and this results in the coagulation of the milk. Calcium ions are needed for this coagulation.

When a mixture of milk, calcium chloride solution and **E** is gently turned in a test-tube, the coagulation goes through the stages shown in Fig. 1.1.

Stage **3** is the end-point of the enzyme-catalysed coagulation.

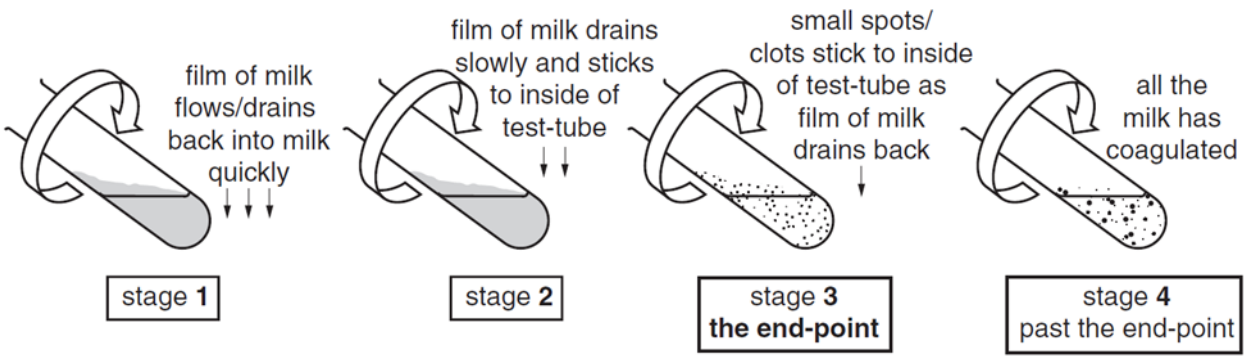

Fig. 1.1

You will investigate the effect of temperature on the time taken to reach the end-point. You will test the activity of enzyme **E** at 30°C and other temperatures up to a maximum of 50°C.

You are provided with:

- 70 cm^3 of 100% milk, labelled **M**
- 20 cm^3 of enzyme solution, labelled **E**
- 20 cm^3 of calcium chloride solution, labelled **C**

If **C** or **E** comes into contact with your skin, wash off immediately under water.

It is recommended that you wear suitable eye protection.

(a)

(i) List the temperatures that you will be using to investigate the effect of temperature on the enzyme of enzyme **E**. You must include 30°C and 50°C in the range of temperatures.

…… [1]

Read steps 1 to 12.

Proceeds as follows.

1 Set up a water bath at 30ºC to be used later in step **5**.

2 Put 10 cm^3 of **M** into a test-tube.

3 Put 1 cm^3 of **C** into the test-tube.

4 Gently shake the test-tubes to mix **M** and **C**.

5 Put the test-tubes into the water bath and leave for 3 minutes.

(ii) Explain why the test-tubes are left in the water bath for 3 minutes in step **5**.

……

…… [1]

6 Remove the test-tubes from the water bath.

The process of clotting will start when **E** is added to the test-tube.

7 Put 1 cm^3 of **E** into the test-tube, so that it runs down the side of the test-tube and forms a layer on the surface of the mixture, as shown in Fig. 1.2 .

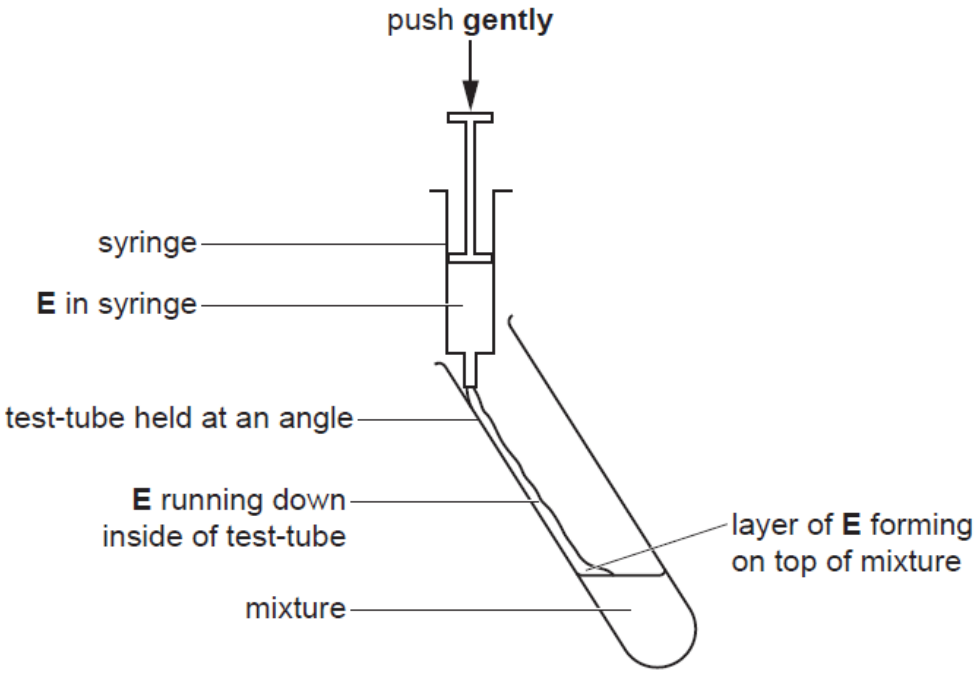

Fig. 1.2

8 Gently shake the test-tube to mix the solutions and start timing with a stop-watch.

9 **Rotate** the test-tube as shown in Fig. 1.3.

Continue to rotate it while observing the mixture until the end-point shown in stage 3 of Fig. 1.1.

Stop timing when small clots are observed. If stage 3 is not observed by 180 seconds, stop timing and record this as 'more than 180'.

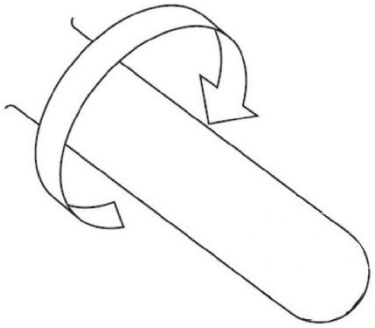

Fig. 1.3

10 Record in **(a)(iii)** the time to reach the end-point.

11 Set up the water-bath at the next temperature after 30°C stated in **(a)(i)**.

12 Repeat step **2** to step **11** for all the temperatures stated in **(a)(i)**.

(iii) Record your results in an appropriate table.

Calculate the rate of reaction for each temperature and show your answer clearly in the table.

[5]

In order to deduce the optimal temperature of the enzyme involved in this enzyme-catalysed reaction, the errors in the experimental procedure need to be reduced.

(iv) Describe 2 significant sources of error in the procedure that reduce confidence in your result.

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…… [2]

(v) Describe two improvements to this procedure that would enable the deduction of the optimal temperature of this enzyme involved.

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…… [2]

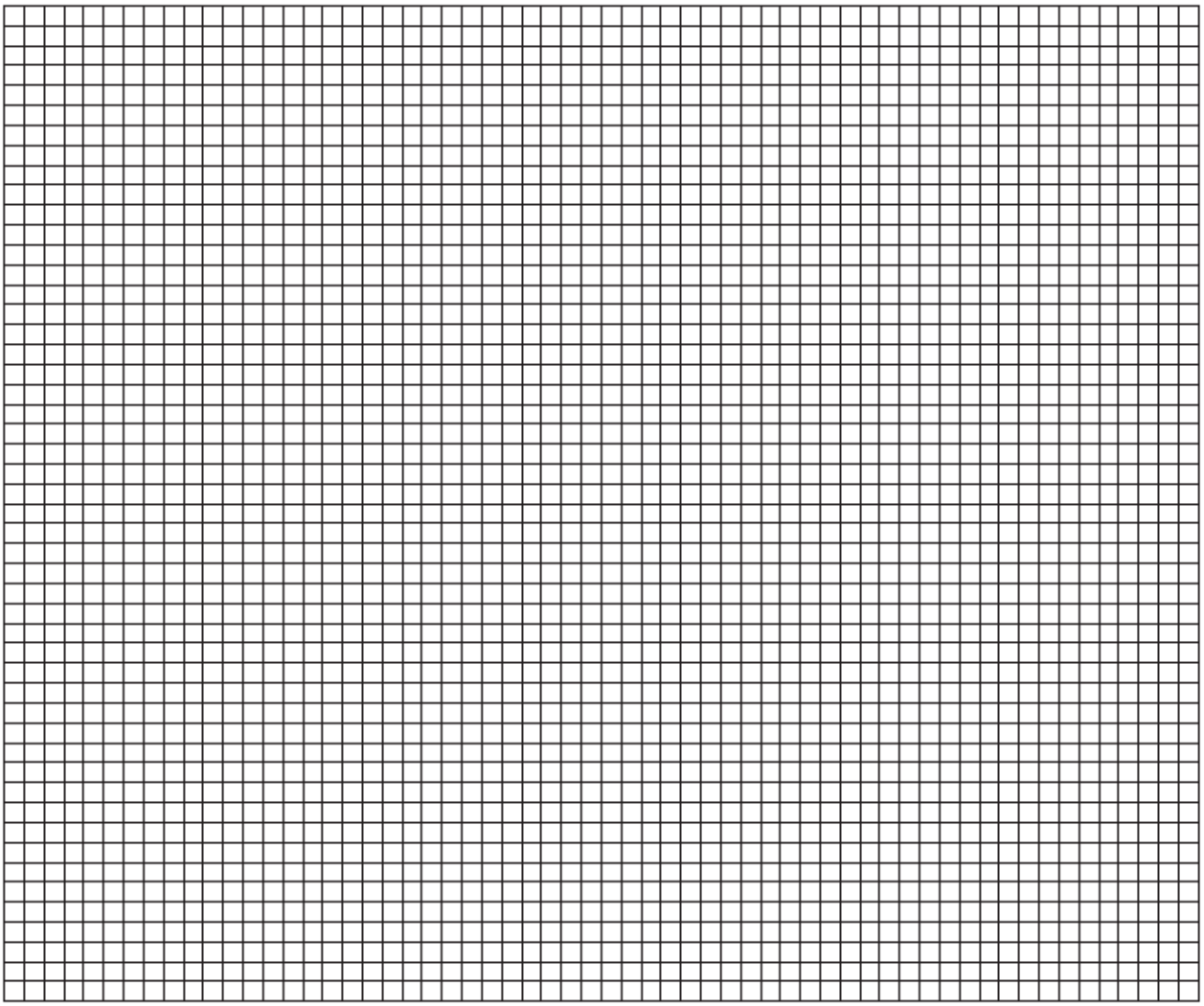
(b) A student carried out an investigation into the effect of enzyme concentration on the clotting of milk. The student calculated the activity of the enzyme for each concentration of enzyme.

The results are shown in Table 1.1.

Table 1.1

enzyme concentration (%)	activity of enzyme (A.U.)
0.05	19
0.10	34
0.15	50
0.20	65
0.30	96

(i) Plot a graph of the data in Table 1.1 on the grid provided.



[4]

(ii) Use your graph to determine the activity of the enzyme when the enzyme concentration is 0.25%.

Show on your graph how you obtain the answer.

activity of enzyme: …… A.U.[2]

(iii) Describe and explain the trend shown in your graph.

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…… [3]

[Total: 20]

Question 2

In this question, you will investigate the water potential of potato tuber cells. In your investigation, you will be given known concentrations of sucrose and distilled water (**W**). The concentration of sucrose in each solution is shown in Table 2.1.

Table 2.1

Solution	Concentration of sucrose solution (mol dm^{-3})
W	0.0
S1	0.3
S2	0.6
S3	0.9

You are provided with:

- four pieces of 5 cm potato cylinders
- distilled water labelled **W**
- sucrose solutions, labelled **S1**, **S2**, and **S3** (see Table 2.1)
- test tubes, Pasteur pipettes, beakers and 5 cm^3 syringes
- petri dish and paper towels
- stop-watch
- methylene blue solution

Proceed as follows:

1 Using clean syringes, place 6 cm^3 of distilled water (**W**) into a small beaker and label the beaker "**W'**.

2 Repeat step **1** for **S1**, **S2** and **S3** and label the beakers accordingly.

3 Place another 6 cm^3 of distilled water (**W**) in a test tube and label the test-tube **"W-blue"**. Add two drops of methylene blue into test tube **W-blue** and mix. This would colour the distilled water blue without significant alteration of the water potential.

4 Repeat step **3** to dispense sucrose solutions **S1**, **S2** and **S3** into appropriately labelled test tubes.

The test-tubes containing **S1** and two drops of methylene blue should be labelled as **S1-blue**. Label appropriately for **S2** and **S3**.

5 Use a scalpel, cut each potato cylinder into 10 discs of approximately equal thickness. You will need a total of 40 discs.

6 Place 10 potato discs into the beaker **W**, containing 6 cm^3 of distilled water. Ensure that the discs are completely soaked in the liquid. Using a stopwatch, incubate the potato discs for 25 minutes.

7 After 25 minutes, decant the liquid in beaker **W** into a suitably labelled clean test tube.

8 With a Pasteur pipette, collect a small amount of the coloured solution from test-tube **W-blue**.

9 Very gently, by squeezing on the Pasteur pipette, introduce one drop of the coloured liquid into the centre of the decanted liquid prepared in step **7** (Fig. 2.1). Be careful not to disperse the coloured liquid with any sudden squeezing of the Pasteur pipette. Withdraw the pipette slowly.

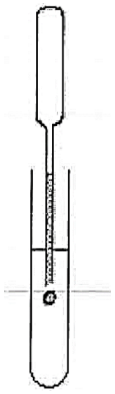

Fig. 2.1

10 Observe whether the drop of coloured liquid remains in the same position, floats, or sinks, **and** how fast it occurred. Release another drop of coloured liquid and continue until you are certain you have made the correct observation about the behaviour of the drop of coloured liquid.

11 Using clean pipettes, beakers and test tubes, repeat **steps 6 to 10** with solutions **S1**, **S2** and **S3**.

In a similar manner, introduce one drop of coloured liquid from **S1-blue**, **S2-blue** and **S3-blue** into the decanted liquids of **S1**, **S2** and **S3** respectively, after incubating the potato discs for 25 minutes.

(a) Record your observations in the space below.

[4]

(b) During the incubation of potato discs in the various solution, movement of water molecules affects the density of the incubating solution. Volume of the incubating solution changes but mass of sucrose in the solution remains unchanged.

(i) Explain what will happen to the density of the incubating solution for **S3**.

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…… [3]

(ii) Using your answer in **(b)(i)**, explain your results for **W** and **S3**.

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…… [2]

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(c) Potato contains starch which can be hydrolysed by enzyme, such as amyloglucosidase to release glucose. Copper sulfate binds to a site on amyloglucosidase other than the active site and changes the conformation of the active site.

1% amyloglucosidase is immobilised in sodium alginate beads forming amyloglucosidase-alginate (AGA) beads. A fixed number of AGA beads of uniform shape and size are then packed in a column as shown in Fig. 2.2.

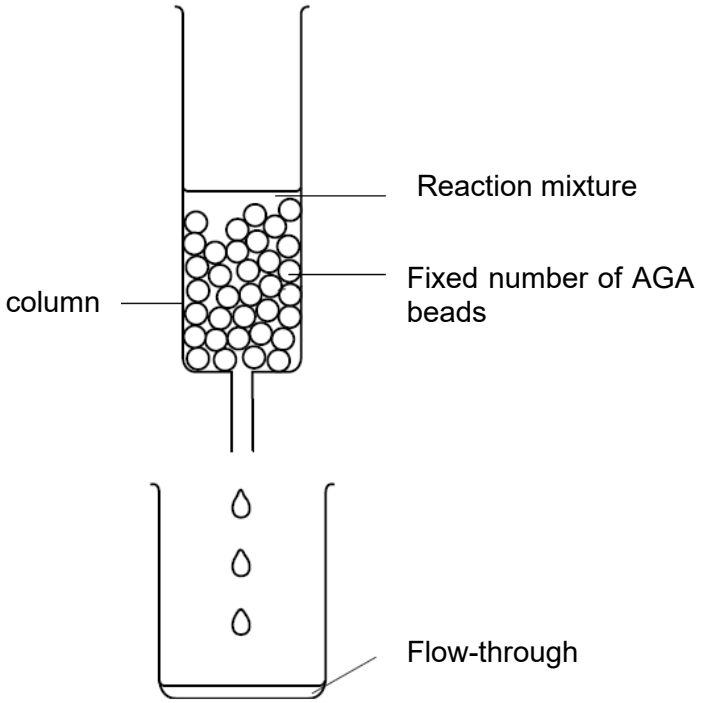

Fig. 2.2

In each run of the experiment, 10 cm^3 of reaction mixture is poured through the column. Glucose solution that is immediately collected is known as flow-through.

It takes five minutes for all the flow-through to be collected.

Using this information and your own knowledge, design an experiment to investigate the effect of increasing concentrations of copper sulfate on the rate of hydrolysis of starch by amyloglucosidase.

You must use:

- 0.3% copper sulfate solution,
- 5% starch,
- AGA beads,
- distilled water,
- column,
- Benedict's solution,
- Bunsen burner with tripod, gauze and bench mat,
- funnel and filter paper
- pH 7.0 buffer,
- drying oven / desiccator,
- retort stand with clamps,
- weighing balance.

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.
- syringes.

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 independent and dependent variables,
- identify the variables you will need to control,
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and reliable as possible,
- show how you will record your results and the proposed layout of results table and graph,
- use the correct technical and scientific terms.

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.... [12]

[Total: 21]

Question 3

L1 is a slide of a stained transverse section of a leaf. You are not expected to be familiar with this specimen.

(a) Use your microscope to observe the different tissues in the region of slide **L1** shown by the shaded area in Fig. 3.1.

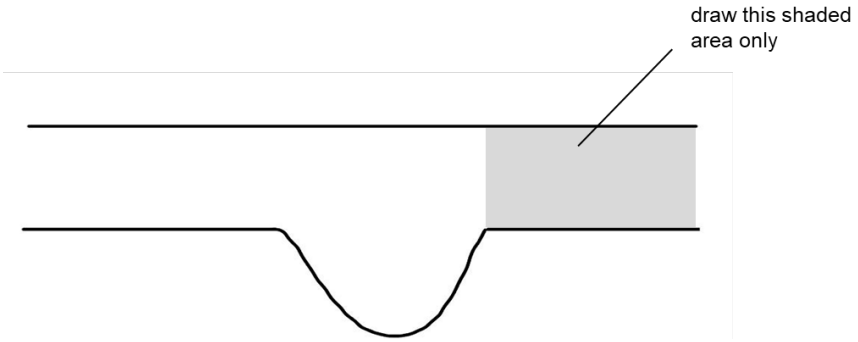

Fig. 3.1

(i) Use the space provided to draw a large plan diagram of the part of the leaf on slide **L1** 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 could be drawn.

Labels are **not** required.

[5]

(ii) Using the stage micrometer, calculate the average length of at least 3 vascular bundles in **L1**.

Show your working clearly in the space below.

Average length of vascular bundles: …….. μm
[3]

(b) Fig. 3.2 is a photomicrograph of a stained transverse section of part of a leaf from another plant. A grid has been placed over the photomicrograph to help you answer the question.

Each square is 1 cm^2.

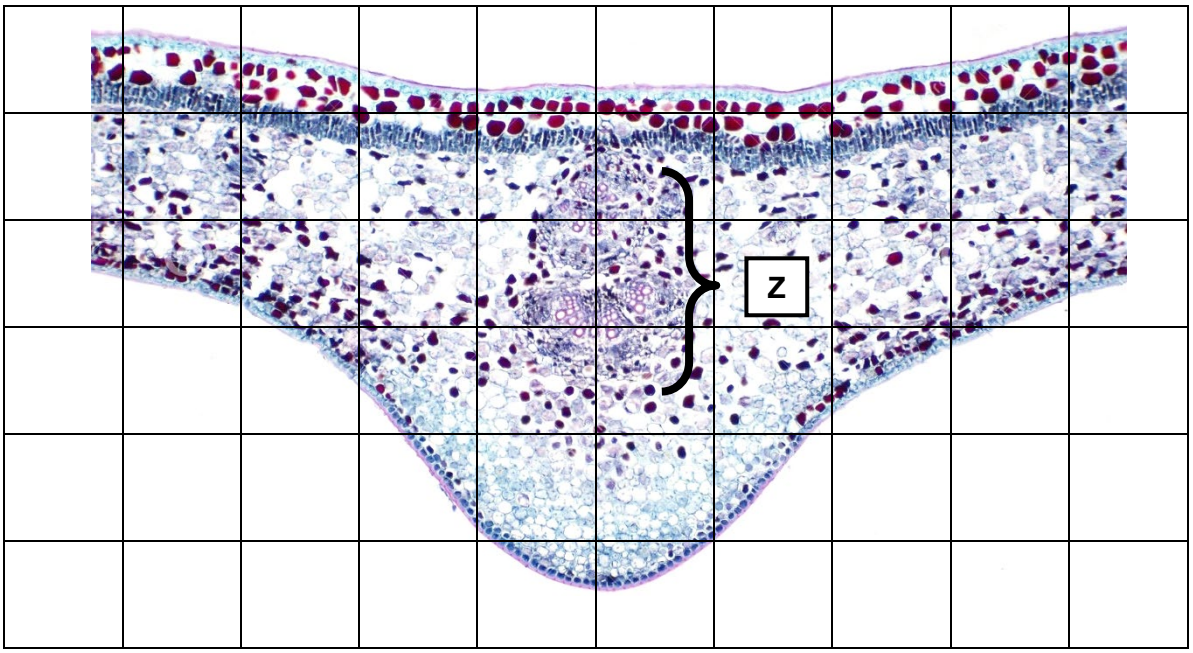

Fig. 3.2

(i) Describe how you will use the grid to find the total area of the leaf.

……..

…… [1]

(ii) Using the method you described in **(b)(i)**, find the total area of the leaf and area of the leaf section occupied by vascular bundle labelled **Z**.

total area of the leaf: …… cm^2
area of the leaf section occupied by vascular bundle labelled **Z:** …… cm^2
[2]

(iii) Calculate the percentage of the part of the leaf shown in Fig. 3.2 that is occupied by the vascular bundle, labelled **Z**.

Show all the steps of your working.

percentage of leaf occupied by **Z**: …… %
[2]

(iv) Suggest how you could modify the procedure you have used in **(b)(i)** to give a more accurate estimate of the area of the leaf.

……..

…… [1]

[Total: 14]

End of Paper