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.

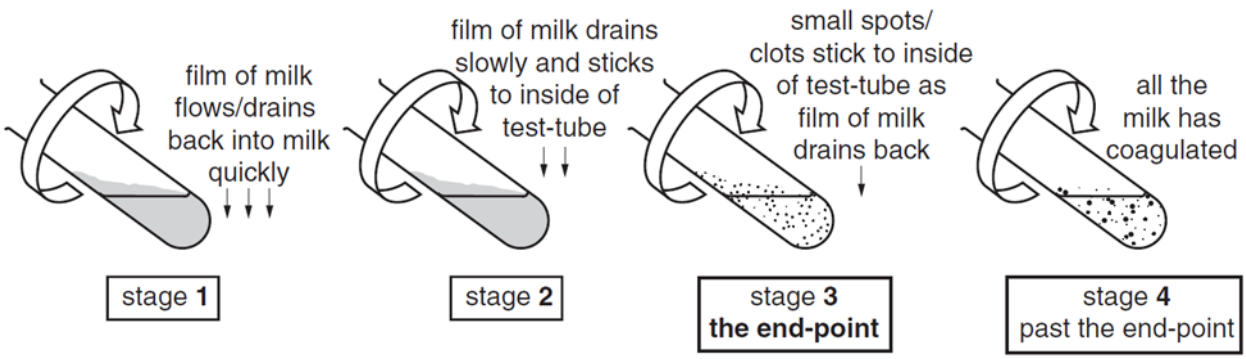


Fig. 1.1

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.

30°C, 35°C, 40 °C, 45 °C, 50°C; .. [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**.

To ensure temperature of milk and calcium are the same/equilibrated to prevent significant change in temperature upon adding E;

..

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. 1.1.

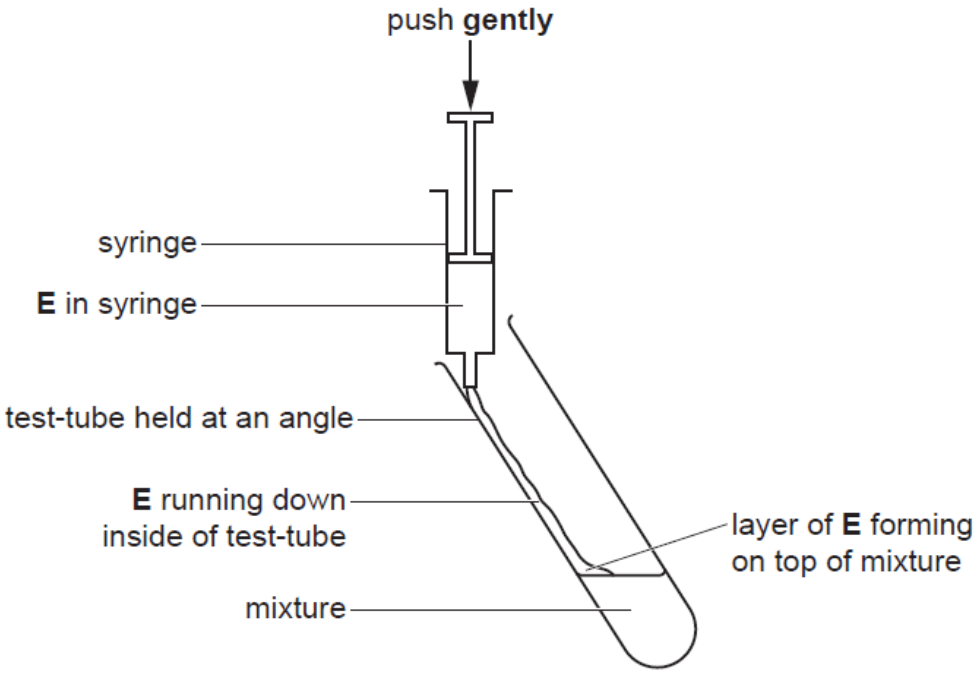


Fig. 1.2

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'.

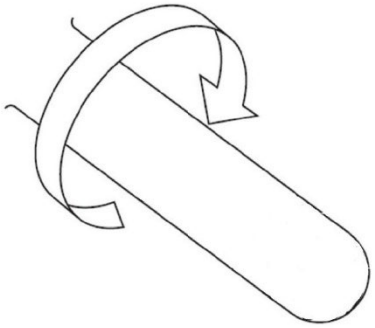


Fig. 1.3

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.

Temperature (°C)	Time taken for clotting (s)	Rate of reaction (s^{-1})
30	68	0.0147
35	40	0.0250
40	27	0.0370
45	20	0.0500
50	25	0.0400

1. Headings with appropriate units;
2. Record time for all temperature;
3. Highest temperature the fastest time;
4. Time taken recorded to nearest second;
5. Rate calculated and shown in same precision (3 s.f)

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.

Reduce error

1. Did not equilibrate temperature of enzyme, so temperature of enzyme may not be same as milk;
2. Different speed of rotating test tubes and this affect rate of blood clotting;
3. Lack of replicates to ensure results is reliable;
4. Subjective/difficult to assess if end point is reached
5. Temperature not maintained throughout the incubation

[2]

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

1. Difficult to assess if end point is reached, so either take video of all experiment and decide end-point clearly, or compare results with an image of end-point;
2. Measure mass of clotting after a fixed duration instead of measuring end-point;
3. Greater range of temperature , such as 0 to 100oC instead of 30 to 50oC;
4. Greater number of temperature, such as 10 temperatures instead of 5 temperatures)
5. Draw results on graph to derive highest rate of reaction;

[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.

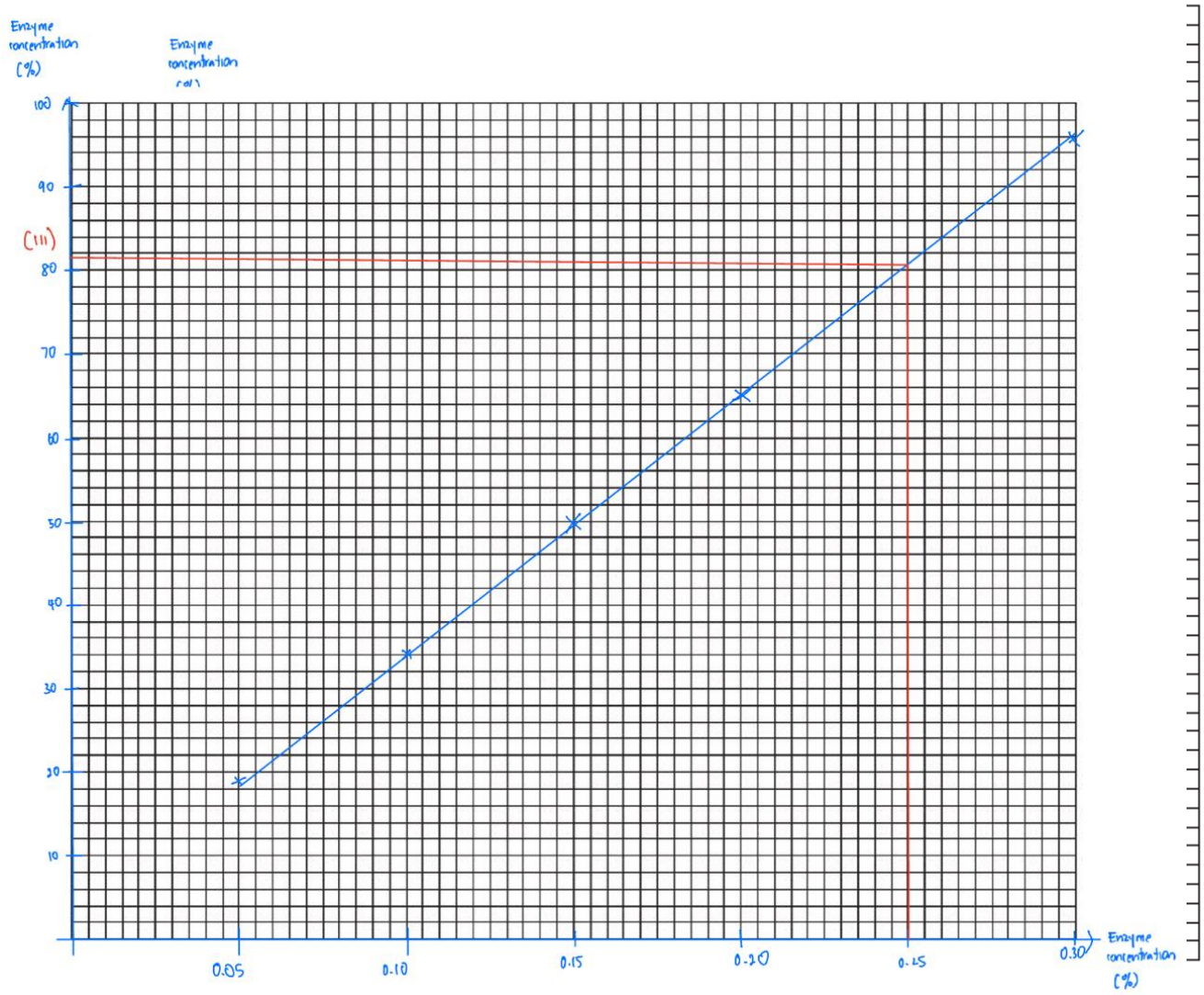
The results are shown in Table 1.1.

Table 1.1

enzyme concentration (%)	activity of enzyme (A.U.)
0.30	96

1. x-axis: % enzyme concentration and y-axis: activity of enzyme (/) au ;
2. scale on x-axis: 0.05 to 2 cm, labelled at least every 2 cm and scale on y-axis: 20 to 2 cm, labelled at least every 2 cm ;
3. correct plotting of all five points using small crosses or dots in circles ;
4. five plots joined with thin line passing through all points (line is either smooth curve or joined plot to plot) ;

(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.

Show working on graph;
80-82a.u.;

nzyme: ..... a.u.[2]

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

1. as enzyme concentration increases from 0.05% to 0.30%, the enzyme activity increases linearly from 19-96 a.u;
2. as more enzymes are present, there will be more available active sites, increasing frequency of successful collisions between enzyme and substrates;
3. more enzyme substrate complexes form per unit time/ more products form per unit time;

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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 (**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.

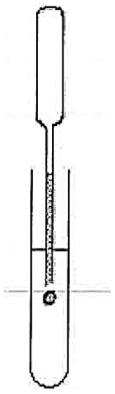

Fig. 2.1

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.

Table showing the observation of coloured liquid in different samples

solutions	observations
W	Float immediately/OWTTE
S1	Sink, then float in 30s/remains the same
S2	sink quickly/ in 5 seconds
S3	sink quickly / in 2 seconds

1. **[table]** Correct heading: (independent variable) name of solutions/concentration of sucrose solution with units. (dependent variable) observations;
2. **[all]** record all four observations using given keywords;
3. **[accuracy]** record **W** as **float** and **S2, S3 as sink**;
4. **[rate]** record relative rate of movement in S2 (slower/OWTTE) and S3 (quicker/OWTTE);

[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.

1. Potato cells contain higher water potential than surrounding sucrose solution;
2. Water molecules moves from potato cell from a higher water potential to a surrounding sucrose solution of lower water potential/alternate phrasing;
3. Across a partially permeable membrane via osmosis;

Max 2

4. Decanted/incubating solution S3 became less dense due to increase in volume;
5. Density is mass over volume/density and volume have inverse relationship;

..... [3]

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

1. At low concentration of sucrose/high water potential of **W**, decanted solution is denser that then coloured drop/coloured drop is less dense than the decanted solution;
2. At high concentration of sucrose (S3)/low water potential, decanted solution is less dense that then coloured drop/coloured drop is denser than the decanted solution;

..... [2]

Blank Page

(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.

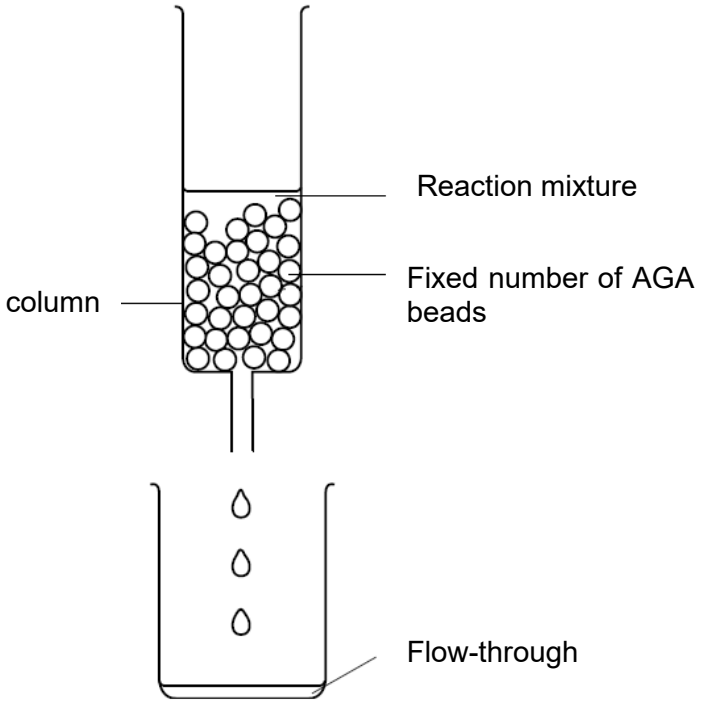

Fig. 2.2

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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Theory [3 marks]

1. Copper sulfate is a non-competitive inhibitor of amyloglucosidase thus binding of copper sulfate causes a conformational change to amyloglucosidase, preventing amyloglucosidase-starch complex formation;
2. As concentration of copper sulfate increases, (idea of)more inhibitor-enzyme complexes are formed and lesser enzyme-substrate complexes are formed per unit time;
3. Rate of starch hydrolysis into glucose decreases per unit time, mass of brick red precipitate formed from Benedict's test decreases;
4. Independent variable: concentrations of copper sulfate
   Dependent variable: rate of hydrolysis of starch by amyloglucosidase.
5. Constant variables:
   - Size and shapes of AGA beads to be kept constant by discarding beads of abnormal sizes and shapes.
   - Equilibrate temperature of enzyme, substrate and copper sulfate before mixing them together by incubating them separately in room temperature/fixed temperature;
   - Volume of enzyme, substrate and copper sulfate used by standardising using 5 cm^3 syringes;
   - pH of reaction mixture to be kept constant by adding pH 7.0 buffer into reaction mixture;
   - Amount of time conducted for Benedict's test to be kept constant by using stopwatch to monitor 3 minutes;

Procedure [7 marks]

1. Conduct a pilot experiment to determine suitability of the apparatus, optimum conditions and amount of materials used;
2. Prepare serial dilution of 0.3% copper sulfate solution by using distilled water as shown in table below;
3. Labelled diagram of column with retort stand and beaker to collect flowthrough;
4. (idea of) Equilibrate copper sulfate solution, starch solution, AGA beads and pH 7.0 buffer separately at room temperature for 10 minutes to ensure temperature of these solution and AGA beads are the same before adding them together;
5. Prepare column with retort stand and beaker;
6. Collect 30 AGA beads of similar size and add into the column
7. **(showing fixed volumes and apparatus)** Using 5 cm^3 syringes, draw out 6 cm^3 of starch, 2 cm^3 of 0.3% copper sulfate solution and 2 cm^3 of pH 7.0 buffer (e.c.f) and add them into the column;
8. **(idea of)** Allow reaction to run for 15 minutes at room temperature, time using stopwatch;
9. After 15 minutes, collect flow-through in the beaker for at least 5 minutes, into a test-tube labelled 0.3%;
10. Conduct **Benedict's test** using (same volume) 3 cm^3 of flow-through solution and 3 cm^3 of Benedict's solution;
11. Using Bunsen burner with tripod, gauze and bench mat, boil water in beaker;
12. Place test-tube with 3 cm^3 of flow-through solution and 3 cm^3 of Benedict's solution in boiling water for 3 minutes;
13. Using funnel and filter paper, **filter** brick-red precipitate from test-tube;
14. **Dry** precipitate using oven;

14. Using a weighing balance, weigh and record the mass of precipitate;

15. Repeat step 5 to 14 with the other concentrations of copper sulfate solution;
16. Conduct replicates twice to ensure no anomalies and repeat the entire experiment with fresh batch of reagents once to ensure reproducibility.

17. Prepare a control set up with no copper sulfate solution to show that the result is due to the effect of copper sulfate solution;

Results [2 marks]

Table; (e.c.f once)

Concentration of copper sulfate (%)	mass of precipitate (g)				Rate of starch hydrolysis (g min^{-1})
	Reading 1	Reading 2	Reading 3	Average	

Graph;

……

……

……

……

……

……

…… [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.

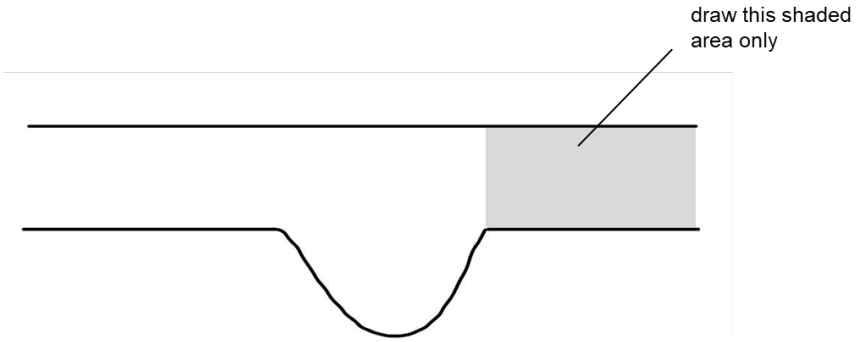


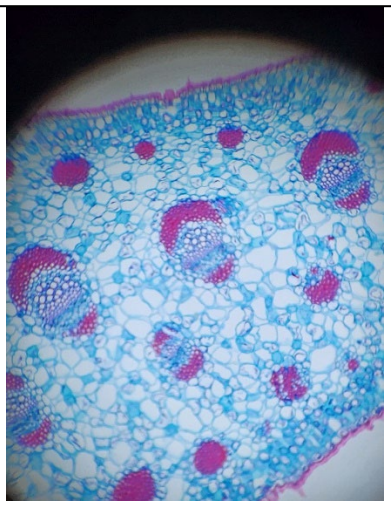
Fig. 3.1

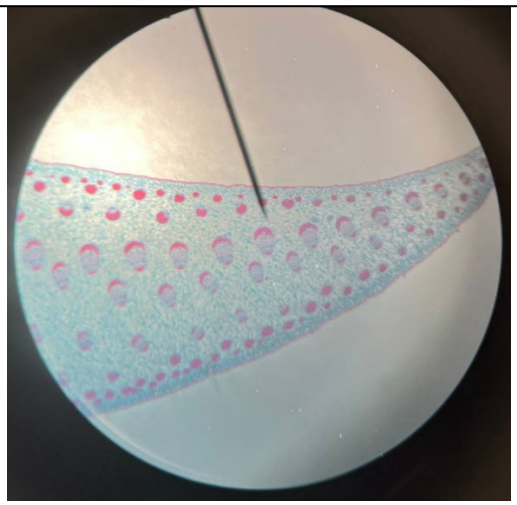
(i) Use the space provided to draw a large plan diagram of the part of the leaf on slide **L1** shown by the shaded area in Fig. 3.1.

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

Labels are **not** required.

1. Drawing with smooth and clear lines;
2. no cell drawn and occupied ¾ of space given;
3. At least 2 tissues + vascular bundle drawn in correct **shapes**;
4. At least 2 tissues + vascular bundle drawn in correct **proportion** (thickness of each tissue layer);
5. at least 4 correct boundaries drawn;





[5]

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

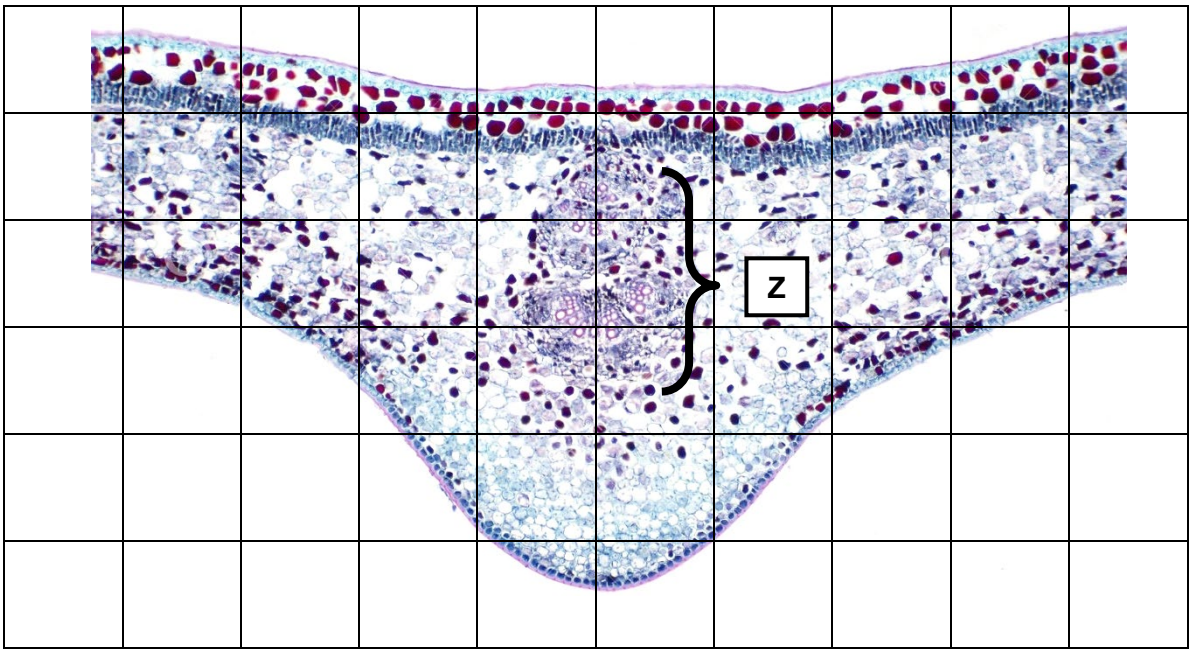
Show your working clearly in the space below.

1. Eyepiece divisions of 3 vascular bundles;
2. Using calibration to calculate actual length of vascular bundles;
3. Average length;

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.



b

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

Count the number of squares with half or more than its area covered by leaf region; ……

. [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**.

22 to 26

total area of the leaf: …… cm^2

area of the leaf section occupied by vascular bundle labelled **Z:** … 2 .. 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 = 2/24 x 100% = 8.3%

1. Correct calculation;
2. Answer to 1 significant figure / 2 sf / whole number;

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.

Use a grid with squares of area less than 1 cm^2

[1]

[Total: 14]

End of Paper