

TEMASEK JUNIOR COLLEGE
2024 JC2 PRELIMINARY EXAMINATION
Higher 2



BIOLOGY

9744/04

Paper 4 Practical

Answers

READ THESE INSTRUCTIONS FIRST

Write your name, CG, Centre number, index number on all the work you 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 work 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	/29
2	/10
3	/16
Total	/55

Answer **all** questions.

- 1 You are provided with a solution, labelled **E**, containing an enzyme which coagulates (clots) milk. **Calcium ions are required for this coagulation.**

You are required to:

- carry out a trial test to think about sources of error
- investigate the effect of substrate concentration on this enzyme-catalysed coagulation.

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

Stage 4 is the end-point of the enzyme-catalysed coagulation as shown in Fig. 1.1.

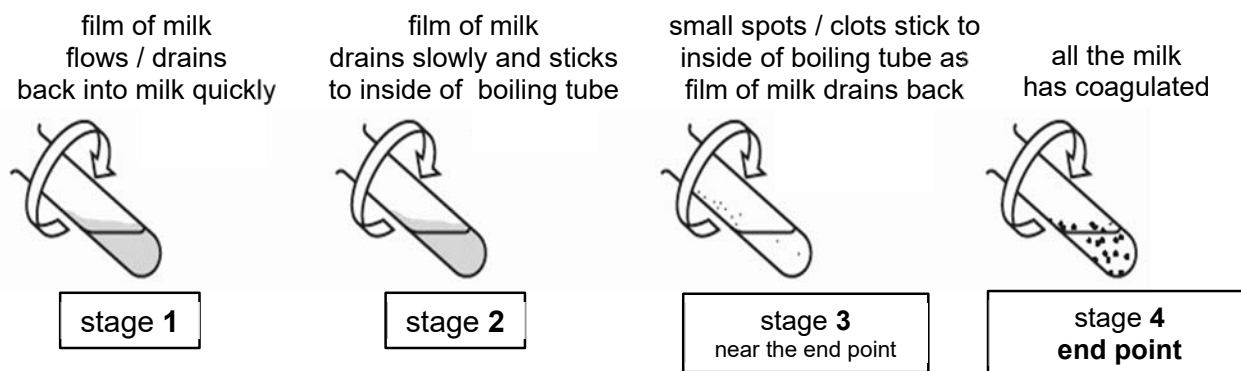


Fig. 1.1

You are provided with:

- 20% calcium chloride solution **C**, in a container labelled **C**
- 1% enzyme solution **E**, in a container labelled **E**
- Milk **M**, in a container labelled **M**
- distilled water **W**, in a container labelled **W**.

Both calcium chloride solution C and renin solution E are irritants. Suitable eye protection should be worn. If C or E comes into contact with your skin, wash off immediately under a tap.

You are required to carry out a trial test (step 1 to step 16) before you start your investigation.

Read step 1 to step 16 before proceeding.

Proceed as follows.

- 1 You are provided with a beaker labelled **water-bath**. Use the hot and cold water to set up a water-bath in this beaker. The starting temperature of the water-bath should be between 35 °C and 40 °C.

You will **not** need to maintain this temperature during steps 2 to 15.

- 2 Put 10.0 cm³ of **M** into a boiling tube.
- 3 Repeat step 2 so that you have two boiling tubes containing **M**.

- 4 Put 1.0 cm³ of **C** into each boiling tube.
- 5 Gently shake each of the boiling tubes to mix **M** and **C**.
- 6 Take the temperature of the water-bath and record this temperature in (a)(ii) on page 4.
- 7 Put the boiling tubes into the water-bath and leave for at least 3 minutes.

- (a) (i) Explain why the boiling tubes are left in the water-bath for at least 3 minutes in step 7. [1]

To allow contents of the test-tubes to reach the temperature of the water-bath.
 (@ to allow contents/solutions/mixture to equilibrate to temperature of the water bath)

Reject if

1. students stated a range of temperature
2. refer to enzymes (Note: enzymes have not been added yet)
3. refer to maintaining optimum temperature
4. refer to temperature fluctuating

- 8 Remove one of the boiling tubes from the water-bath.

The process of coagulation will start when **E** is added to the boiling tube.

- 9 Put 1.0 cm³ of **E** into the boiling tube, so that it runs down the side of the boiling tube and forms a layer on the surface of the mixture, as shown in Fig. 1.2.

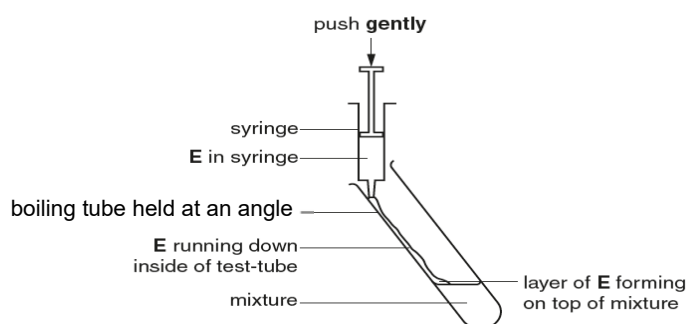
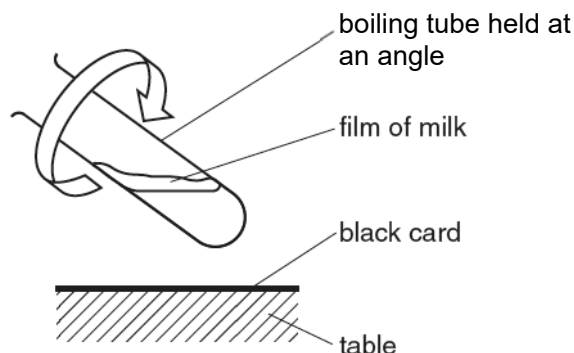


Fig. 1.2

- 10 Gently shake the boiling tube to mix the solutions and start timing.
- 11 Hold the boiling tube over a piece of black card on the table as shown in Fig. 1.3.
- 12 Gently rotate the boiling tube to form a film of milk on the inside of the boiling tube.



[TURN OVER

Fig. 1.3

13 Observe the film until the end-point is reached (stage 4 in Fig. 1.1). Ignore any small bubbles on the inside of the boiling tube. Stop timing.

14 Record in (a)(iii) the time taken to reach the end-point.

If the end-point has not been reached in 3 minutes, stop the experiment and record 'more than 180'.

15 Repeat step 8 to step 14 with the other boiling tube in the water-bath.

16 Take the temperature of the water-bath when the final boiling tube has been removed and record this in (a)(ii).

(ii) Temperature may be a source of error in this investigation.

State the temperatures of the water-bath.

temperature of water-bath taken in step 6: 40 °C

temperature of water-bath taken in step 16: 36 °C

Explain whether the temperature of the water-bath is a significant source of error in this investigation. [1]

Temperature of water-bath is a significant source of error because the temperature dropped from 40°C to 36°C when the final tube was removed.

Note: the reason for recording the temperature in step 6 and in step 16 is to alert student to fact that the temp was not maintained.

(iii) Record your results in an appropriate table. [3]

Solution	Time taken to reach end-point / s		
M	Reading 1	Reading 2	Average
	19	23	21

CH: Column heading with UNITS – 1 mark

D : Records 2 readings – 1 mark

Pr : data recorded whole number for second – 1 mark

(iv) A significant source of error for this investigation is deciding when the end-point is reached.

Suggest **one** advantage of carrying out this trial test **before** investigating the effect of substrate concentration on this enzyme-catalysed reaction. [1]

- To learn how to identify when the end-point reached

- (v) Complete Table 1.1 by stating:
- two **other** possible sources of error that may have affected your results
 - an improvement to reduce the effect of each error.

Table 1.1

error	improvement
1. The reaction was not carried out at a constant temperature. which can affect rate of coagulation.	Keep boiling tube in water-bath while observing clotting.
2. Solution E /Enzyme solution is not equilibrated to same temperature before adding to mixture. which can affect rate of coagulation.	Prepare 5.0 ml of solution E into a clean test tube and place it in the same water-bath.
3. Rate of rotation is not standardized, which can affect rate of coagulation.	Use a rotating machine / magnetic stirrer/ shaker to standardize the rotation rate.
4. Some solution E / enzyme is stuck at the side of the boiling tube (did not flow down into the mixture), resulting in differences in the volume of E used in each set-up.	<ul style="list-style-type: none"> ▪ Add solution E to the mixture directly. Or ▪ Use a longer syringe to release solution E at the side of the boiling tube nearer to the mixture / deeper into the boiling tube .

[4]

- vi) You are required to make up a final volume of 10.0 cm³ of five different concentrations of calcium chloride solution by **proportional dilution** = simple dilution.

These must include 20% calcium chloride and 0% calcium chloride solutions.

Show, in a table, how you will make up the calcium chloride solution using the 20% calcium chloride solution, **C**, and distilled water, **W**. [3]

concentration of calcium chloride solution / %	volume of calcium chloride solution / cm ³	volume of distilled water/ cm ³
20	10.0	0.0
15	7.5	2.5
10	5.0	5.0
5	2.5	7.5
0	0.0	10.0

Marking points:

1. C: DRAW LINES FOR TABLE + Correct Concentrations
2. V : Correct volumes for both protein and distilled water
3. P : ALL volume to 1 d.p.

[TURN OVER

- 17 Prepare all the concentrations of calcium chloride solution as shown in your table in **(a)(vi)**.
- 18 Adjust the temperature of the water-bath so that it is between 35 °C and 40 °C. You will **not** need to maintain this temperature during step 19 to step 24.
- 19 Put 1.0 cm³ of 20% calcium chloride, **C** into a boiling tube.
- 20 Put 10.0 cm³ of **M** into a boiling tube.
- 21 Repeat step 19 with each of the other concentrations of calcium chloride solutions that you have prepared.
- 22 Gently shake each of the boiling tubes to mix the milk and **C**.
- 23 Put the boiling tubes in the water-bath and leave for at least 3 minutes.

While you are waiting read step 8 to step 13.

- 24 After 3 minutes remove one of the boiling tubes from the water-bath. Add 1.0 cm³ of **E** as in step 9, then repeat step 10 to step 13 and record in **(a)(vii)** the time taken to reach the end-point.
- 25 Repeat step 24 with each of the other boiling tubes.

(vii) Record your results in an appropriate table. [4]

Concentration of calcium chloride / %	Time taken to reach end-point / s
20	23
15	44
10	58
5	62
0	120

1 **CH** :

- **table drawn [all the boundary lines drawn]**
- **concentration of calcium chloride / %**
- **time to reach the end-point / s ;**

2 **D** : **timing must be different for each substrate conc.**

3 **Tr** : records the fastest time for the highest concentration of **C** and slowest time for the lowest conc of **C** ;

4 **P** : records time as whole seconds ;

(viii) Describe a control that could be carried out as part of your investigation. [1]

- uses same volume of boiled and cooled enzyme, E subjected to the same conditions as the other tubes;
- Replace enzyme with same volume of distilled water, subjected to the same conditions as the other tubes;

(b) In a different investigation, a student studied the effect of the independent variable pH on the coagulation of milk.

The results are shown in Table 1.2.

Table 1.2

pH of milk	activity of milk clotting enzyme / arbitrary units				
	trial 1	trial 2	trial 3	trial 4	trial 5
6.02	8.8	8.7	8.9	8.2	8.7
6.22	6.8	6.8	6.8	6.7	6.9
6.40	4.9	4.3	4.4	4.3	4.3
6.64	1.1	1.0	1.0	0.9	0.9
6.70	0.7	0.6	1.1	0.5	0.5

- (i) Three of the values in Table 1.2 are anomalous. Draw a circle around each of these values. [1]
- (ii) Process the raw data in Table 1.2 and present it clearly in the space below. Show clearly any working. [3]

pH of milk	Average activity of milk clotting enzyme / arbitrary units
6.02	$\frac{8.8 + 8.7 + 8.9 + 8.7}{4} = 8.8$
6.22	$\frac{6.8 + 6.8 + 6.8 + 6.7 + 6.9}{5} = 6.8$
6.40	$\frac{4.3 + 4.4 + 4.3 + 4.3}{4} = 4.3$
6.64	$\frac{1.1 + 1.0 + 1.0 + 0.9 + 0.9}{5} = 1.0$
6.70	$\frac{0.7 + 0.6 + 0.5 + 0.5}{4} = 0.6$

C – column heading, word “average” in the column heading, data presented in Table form, units in column heading.

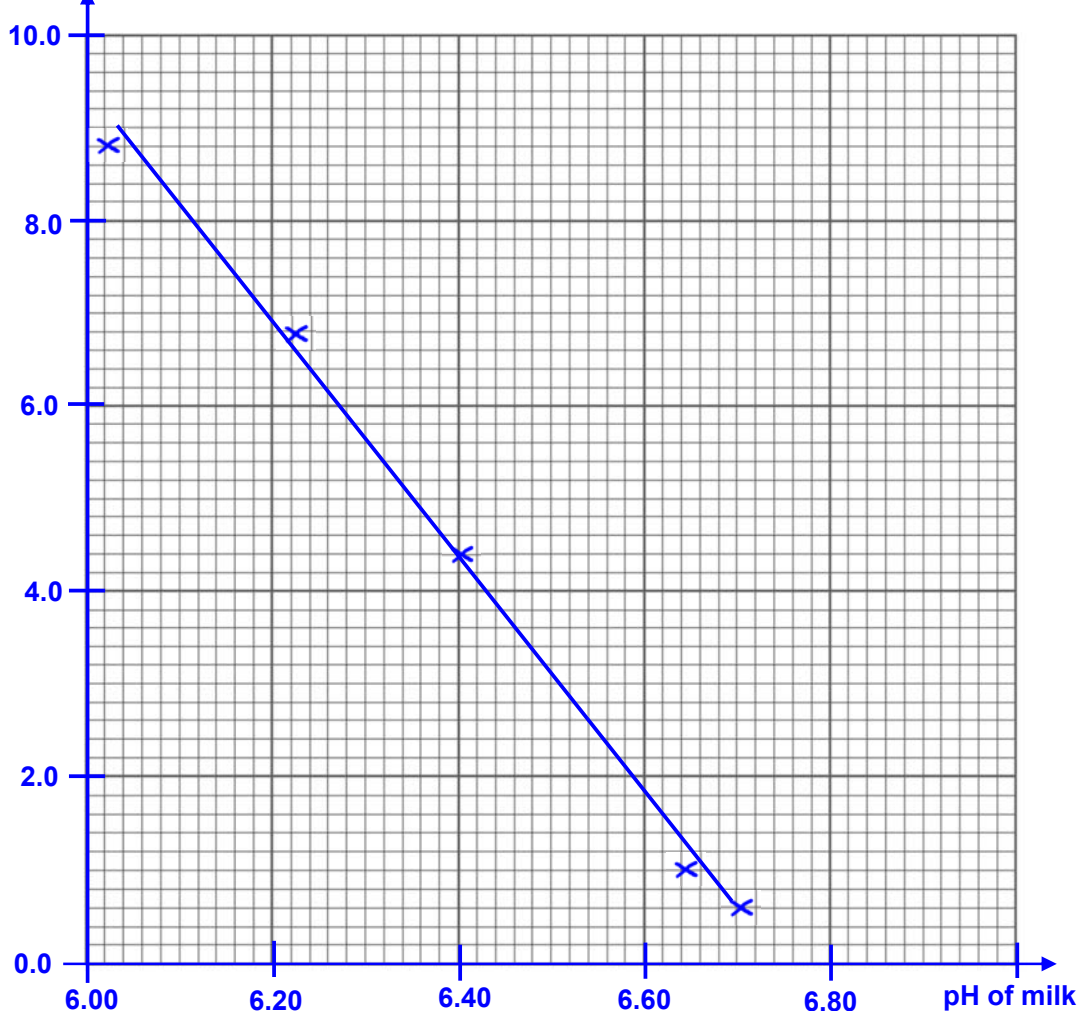
A – correct average working and values

P – correct precision to 1 d.p

[TURN OVER

- (iii) Use the grid to draw the graph of the data you have processed in (b)(ii). Draw the **line of best fit**.

Average activity of milk clotting enzyme / arbitrary units



[4]

- (iv) Explain the **relationship** between pH and the enzyme shown in the data. [3]

1. pH 6.02, the conformation of active site of the enzyme is most ideal for substrate binding.
2. Frequency of successful collision between enzyme and substrate is highest, with greatest number of enzyme-substrate complexes formed.
3. pH greater than 6.02, a change in H⁺ concentration
4. This changes the ionic charges on the basic and acidic R groups of amino acid residues.
5. The ionic bonds are disrupted and substrate binding is affected.
6. The shape of active site is changed and is less complementary to the shape of substrate.
7. Rate of effective collision decreases and the rate of enzyme-substrate complex formation decreases. Less products are formed per unit time.

[Total: 29]

A business has been buying milk from the same supplier for a number of months. Recently, the business has found that the milk has been diluted with water.

How much water has been added can be determined by measuring the density of the milk.

The density of milk can be measured using a copper sulfate solution of standard concentration. Milk of different concentration will have different density.

When a small drop of milk is placed in copper sulfate solution in a measuring cylinder, a layer of copper proteinate forms around the milk and this prevents the milk and copper sulfate solution mixing. Since milk is denser than the standard copper sulfate solution, the drop of milk sinks to the bottom.

Fig. 2.1 shows the movement of the drop of milk through the copper sulfate solution.

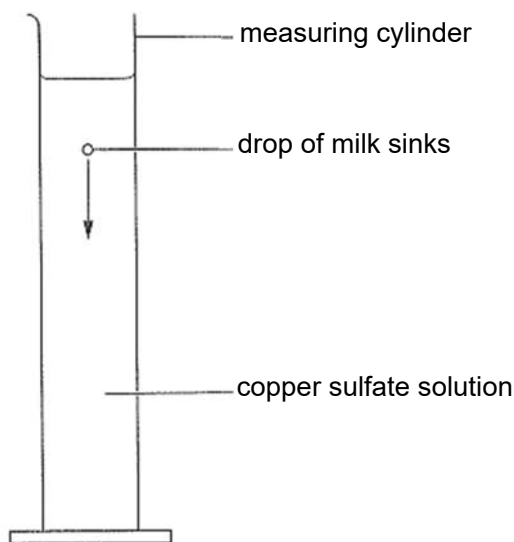


Fig. 2.1

The density of milk decreases as more water is added. Milk of higher concentration has higher density. The more dense the milk, the faster the drop will sink.

Using this information and your own knowledge, design an experiment to estimate the percentage of water added to the milk supplied to the business.

You must use:

- 10.0 cm³ sample of the milk supplied to the business,
- 100.0 cm³ undiluted 100% milk,
- 100.0 cm³ distilled water,
- 1 dm³ 0.1 mol dm⁻³ copper sulfate solution,
- 100.0 cm³ measuring cylinder,
- 1.0 cm³ syringe with needle attached,
- timer, e.g. stopwatch

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

- normal laboratory glassware e.g. test-tubes, beakers, measuring cylinders, graduated pipettes, glass rods, etc.
- syringes.

[TURN OVER

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 diagram(s), if necessary
- identify the independent and dependent variables
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and repeatable as possible
- include layout of results tables and graphs with clear headings and labels
- use the correct technical and scientific terms
- include reference to safety measures to minimise any risks associated with the proposed experiment.

[10 marks]

[Independent variable] – ½ mark each

Independent variable – Concentration of milk

Note: Do NOT write “percentage of water added”. You show the calculation in your plan.

Dependent variable – Time taken for a drop of milk to sink to the bottom

Note: MUST mention “bottom” to get the mark so that it is a precise position to stop the stopwatch

[Hypothesis/ trend/ theory] – ½ mark each

T1. When more water is added to milk, concentration of milk is lower and density of the milk decreases [follow given instructions],

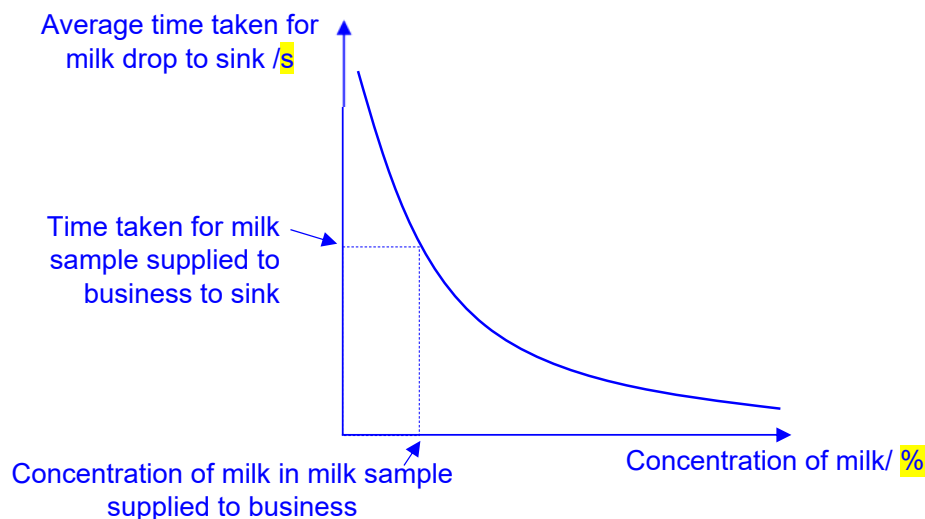
T2. hence it will sink more slowly in copper sulfate solution.

[Table of data] – ½ mark each

Concentration of milk/ %	Time taken for milk drop to sink to bottom / s			
	Reading 1	Reading 2	Reading 3	Average
100				
50				
25				
12.5				
6.25				

Note: MUST separate the data for milk sample (MS) since it is an unknown concentration.

Solution	Time taken for milk drop to sink to bottom / s			
	Reading 1	Reading 2	Reading 3	Average
Milk sample				

[Graph] - [1]**METHOD:****[Dilution table; Use of undiluted milk] - [1]**

DT: Using 10cm³ syringe, prepare 5 concentrations of milk, using the undiluted milk and perform serial dilution by a factor of 2.

Note: Many students did not write this line to indicate purpose of table and whether it is simple or serial dilution.

Concentration of milk / %	Volume of milk from <u>preceding concentration</u> / cm ³	Volume of <u>distilled water</u> / cm ³
100	20.0	0.0
50	10.0	10.0
25	10.0	10.0
12.5	10.0	10.0
6.25	10.0	10.0

OR

Using 10cm³ syringe, prepare 5 concentrations of milk, using the undiluted milk to perform simple dilution.

Concentration of milk / %	Volume of undiluted milk / cm ³	Volume of <u>distilled water</u> / cm ³
100	20.0	0.0
80	16.0	4.0
60	12.0	8.0
40	8.0	12.0
20	4.0	16.0

[TURN OVER]

[Constant variable: Volume of copper sulfate] - [1/2]

1. Add 100cm³ of 0.1 mol dm⁻³ copper sulfate solution into a 100cm³ measuring cylinder.

[Set-up: Use of syringe, Volume of milk released, Height released] – ½ mark each

2. Fill the 1 cm³ syringe with needle attached with 1 cm³ of 100% milk.
3. Gently release 0.5cm³ of milk into the copper sulfate solution
4. from the 90cm³ marking (tip of needle) of measuring cylinder (filled with copper sulfate).

[Data collection: Time taken for milk to sink] – [1]; [1/2] if did not state “stop stopwatch”

5. Immediately start the stopwatch and stop stopwatch when drop reaches bottom
6. Record the time taken for the milk to sink to the bottom of measuring cylinder.

[Repeat for other concentrations] – ½ mark

7. Repeat steps 1 to 5 for other concentrations of milk (50%, 25%, 12.5%, 6.25%).

[Testing business milk sample] - [1]

8. Repeat steps 1 to 6 with milk sample that is supplied to business.

[Repeats/ Triplicates] – [1]

9. Repeat steps 1 to 6 to obtain three readings / triplicates using fresh prepared milk and copper sulphate solution

Note: many students did not include “fresh samples of copper sulphate”

[Determine percentage of water]

10. Using the data collected, plot a curve of milk standards. [plotting a standards curve]
11. The concentration of the milk sample can be obtained as shown from the graph.

12. The percentage of water added to the milk supplied to the business can be obtained by 100% minus concentration of milk. – [1]

[Narrower range for better estimate] – [1]

13. Repeat the experiment with more concentrations / narrow range of milk concentration to obtain more accurate estimation of the percentage of water added to the milk.

[Safety precautions] – ½ mark each

14. Syringe with needle is sharp (high risk), handle with care to avoid cuts / injuries.
15. Copper sulfate is an irritant (medium risk), wear gloves and goggles when handling to avoid contact with skin and eyes respectively.

- 3 **J1** is a slide of a stained transverse section through a plant leaf. This plant grows in water and the leaf contains many large air spaces.

You are not expected to be familiar with this specimen.

You are required to:

- use the eyepiece graticule to measure the depth of the leaf lamina, the length of a vascular bundle and the length of an air space.
- use these measurements to draw a plan diagram of part of the leaf.

The eyepiece graticule in the microscope can be used to measure different tissues.

- (a) Select the part of the leaf (lamina) on **J1**, shown in Fig. 3.1.

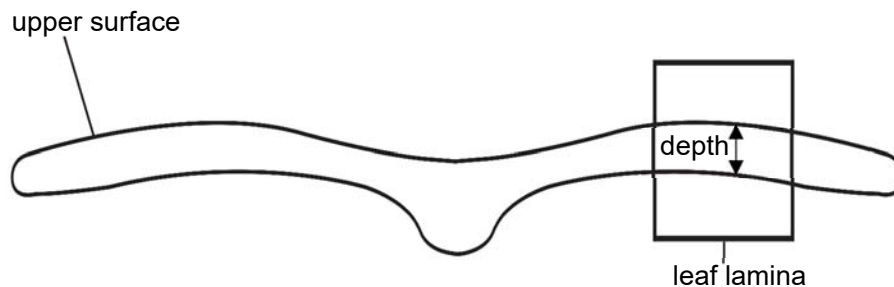


Fig. 3.1

- (i) Use the eyepiece graticule in the microscope to measure:
- the depth of the lamina
 - the length of a vascular bundle in the lamina
 - the length of an air space

depth of leaf lamina **68** eyepiece graticule units

length of vascular bundle **12** eyepiece graticule units

length of an air space **15** eyepiece graticule units

[1]

- (ii) Use the measurements from **(a)(i)** to help you to draw a large plan diagram to show the distribution of the tissues in the section indicated in Fig. 3.2.

You are required to use a sharp pencil for drawings.

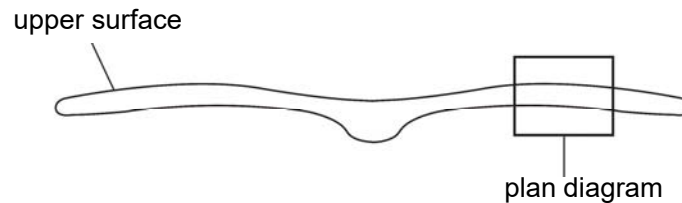
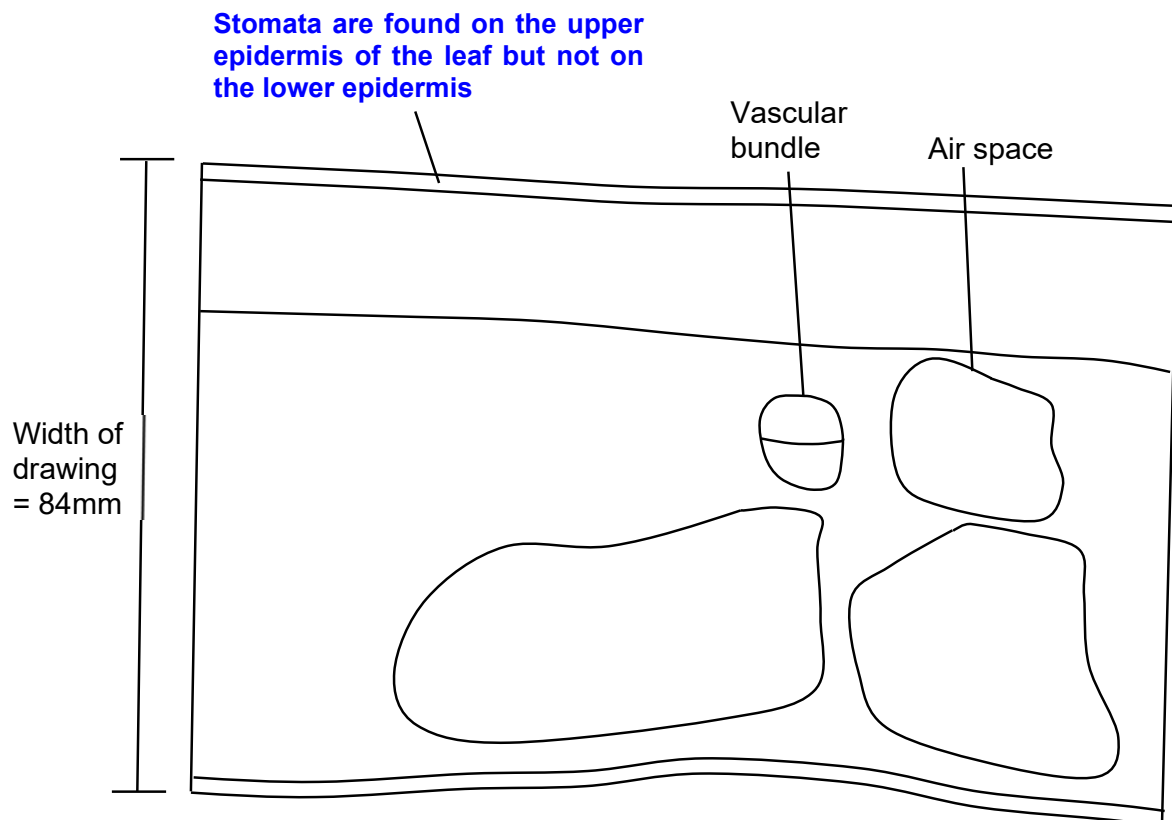


Fig. 3.2

Within this part of the leaf there will be a number of large air spaces.

You should only draw **three** of these air spaces. Your drawing should show the correct shape and proportion of the tissues, three air spaces and **one** vascular bundle.

Using ruled lines, label **one** of the air space and the vascular bundle. [4]



[4]

M1	<ol style="list-style-type: none"> clear, sharp, unbroken lines no shading Must not use ruler for any part of the drawing (except labelling lines AND border lines) Draw the correct section – leaf lamina not the mid-rib. Must not draw the box as shown in fig. 3,2. 	Reject <ul style="list-style-type: none"> - if drawn over the print of question - feathery lines - overlaps or gaps - any lines thicker than 1mm - BORDER LINE cannot project beyond epidermis
M 2	<ol style="list-style-type: none"> no cells drawn AND <ol style="list-style-type: none"> use 5 horizontal lines to show 4 different layers of tissue AND <ol style="list-style-type: none"> Must have 2 vertical border lines drawn with ruler AND <ol style="list-style-type: none"> Correct thickness of upper and lower epidermis AND <ol style="list-style-type: none"> Thickness of palisade mesophyll is $\frac{1}{3}$ the thickness of spongy mesophyll 	Reject if student did not start from the end of boundary lines given
M 3	<ol style="list-style-type: none"> 1 vascular bundle of correct shape, correct proportion and position AND <ol style="list-style-type: none"> Must show 2 regions WITHIN vascular bundle AND <ol style="list-style-type: none"> Xylem tissue about the same proportion as phloem tissue or slightly more AND <ol style="list-style-type: none"> 3 complete air space Air space cannot be fused together 	Reject of vascular bundle not proportional to epidermis & spongy mesophyll layer
M 4	<ol style="list-style-type: none"> correct label with label line to vascular bundle AND <ol style="list-style-type: none"> correct label with label line to air space 	Reject if label within drawn area

- (iii) Annotate (brief notes with label line) on your drawing in **(a)(ii)** to describe one difference between the cells in the upper epidermis and the lower epidermis. [1]

- (b)** To calculate the magnification of your drawing, you will need to calibrate your eyepiece graticule.

You are provided with a stage micrometer. The 1 cm stage scale is divided into 100 divisions.
The length of each division is 0.1 mm.

- (i)** Calculate the length of each division in micrometers.

Show your working.

[TURN OVER]

$$\text{Length in micrometres} = 0.1 \times 1000 \text{ [1/2]} = 100 \mu\text{m}$$

$$100 \text{ [1/2]} \mu\text{m [1]}$$

- (ii) Using the **low-power objective lens (x10)**, adjust the focus until you can see the eyepiece graticule on top of the stage scale.

Count the number of eyepiece graticule divisions that match an exact number of stage scale divisions.

number of eyepiece graticule divisions 100

number of stage micrometer scale divisions 10

Use this information to calculate the actual width between each division on the eyepiece graticule.

Show your working. [2]

$$100 \text{ eyepiece graticule divisions} = 10 \text{ stage micrometer division}$$

$$= 10 \times 100 \mu\text{m} = 1000 \mu\text{m [1/2]}$$

$$\begin{aligned} 1 \text{ division on the eyepiece graticule} &= (1 \div 100) \times 1000 \mu\text{m [1/2]} \\ &= 10 \mu\text{m} \end{aligned}$$

$$10 \mu\text{m [1]}$$

- (iii) Use the measurement in (a)(i) and (b)(ii) to calculate the magnification of your drawing in (a) (ii).

Show all your working clearly. [2]

$$\text{Actual width of leaf} = 68^* \times 10 \mu\text{m} = 680 \mu\text{m [1/2]}$$

$$\text{Drawing size} = 84^* \text{ mm} \times 1000 = 84 \times 10^3 \mu\text{m [1/2]}$$

$$\begin{aligned} \text{The magnification of drawing} &= \frac{\text{Drawing size}}{\text{Actual size}} \quad \left. \vphantom{\frac{\text{Drawing size}}{\text{Actual size}}} \right\} \text{ [1/2]} \\ &= 84 \times 10^3 \mu\text{m} \div 680 \mu\text{m} \\ &= 123.5 \times \end{aligned}$$

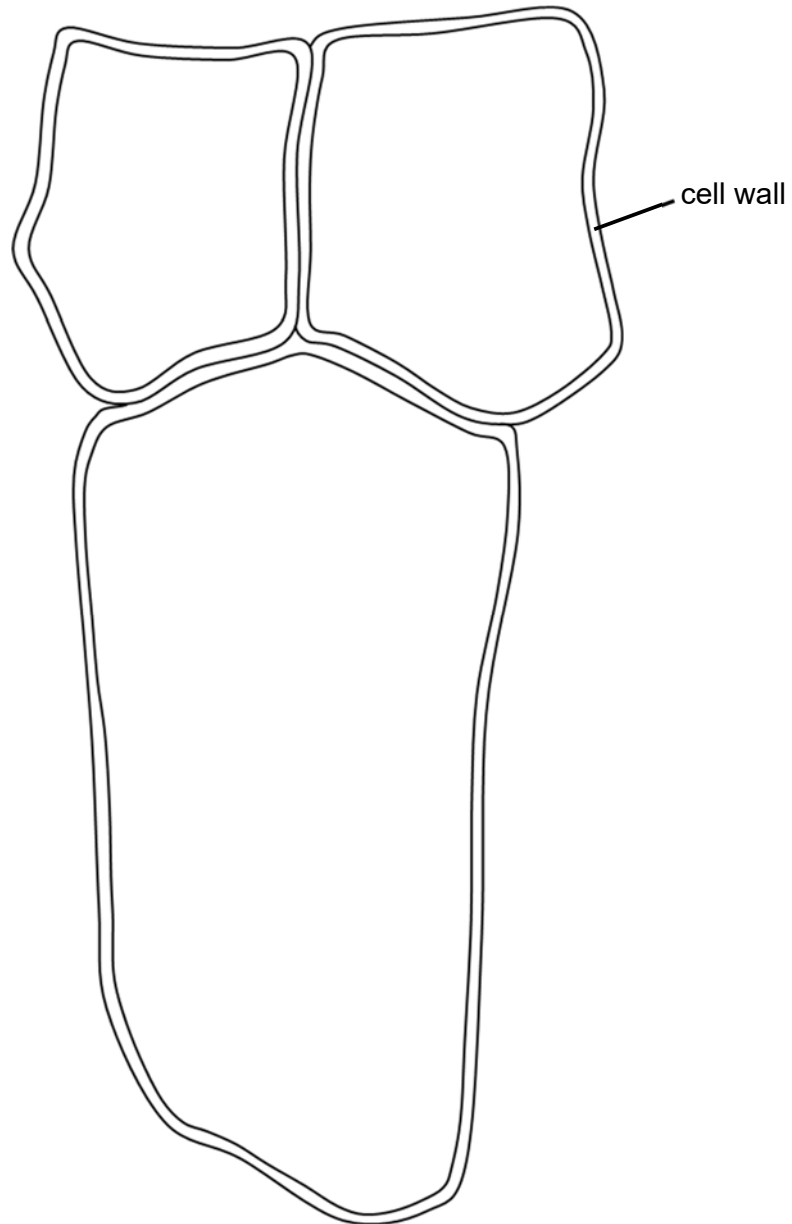
$$\text{Magnification } 123.5 \times \text{ OR } \times 123.5 \text{ [1/2]}$$

- (c) Observe the upper epidermis at the top of the leaf on J1.
Select one group of three cells with:
- two cells from the upper epidermis
 - one adjacent (touching) cell from the tissue below.

Each cell of the group must touch at least one of the other cells.

Make a large drawing of this group of **three** cells.

Use **one** ruled label line and label to identify the cell wall of one cell. [4]

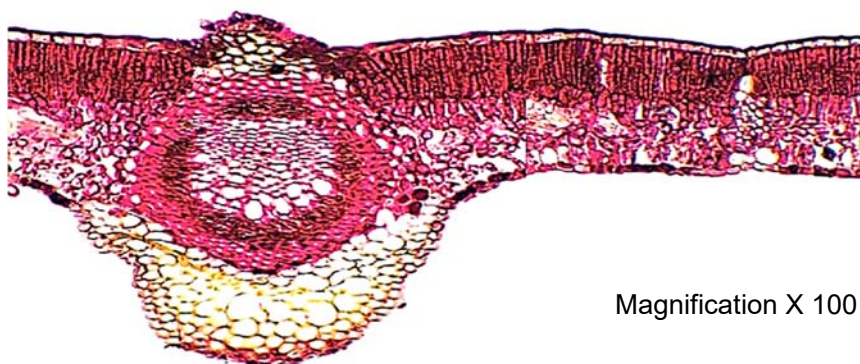


M 1	1. clear, sharp, unbroken lines AND 2. no shading AND 3. minimum size of at least 50mm for long palisade mesophyll	Reject - if drawn over the print of question - feathery lines - overlaps or gaps
M 2	1. only 3 cells drawn AND 2. 2 epidermal cells touching each other and touching 1 palisade mesophyll cell below. AND	Reject - if more than 3 cells - if cells are wrong shapes

	3. <u>no space between</u> all the cells AND 4. <u>epidermal cells</u> are <u>square-shape</u> but <u>palisade mesophyll</u> is <u>elongated</u>	
M 3	1. <u>cell wall</u> drawn with two lines <u>more</u> <u>than 2 mm</u>	Reject - if wall is thick
M 4	1. correct label with <u>label line</u> to a cell wall 2. label cell wall	Reject - if did not draw label line with ruler.

- (d) Fig. 3.3 and Fig. 3.4 are photomicrographs of transverse sections (TS) of leaves from the same plant.

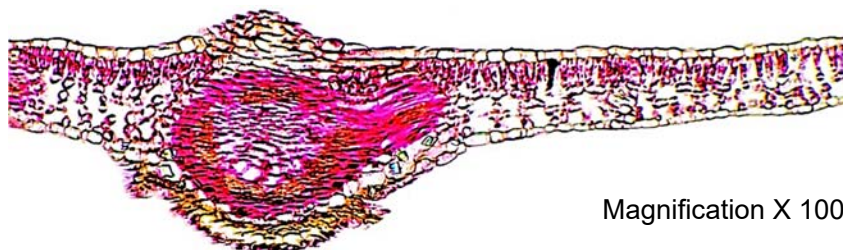
TS of leaf grown in sunlight.



Magnification X 100

Fig. 3.3

TS of leaf grown in shade



Magnification X 100

Fig. 3.4

Record **one** observable differences between Fig. 3.3 and Fig. 3.4. in Table 3.1.
Do not include difference in the overall thickness of the leaves. [1]

Table 3.1

Any one:

feature	Fig. 3.3	Fig. 3.4
Cells of the palisade layer	The cells are closely packed together	The cells are loosely packed together

Arrangement of palisade mesophyll cells	Palisade mesophyll are arranged in two layers	Palisade mesophyll are arranged in one layer
Amount of air spaces in mesophyll layer	Less air spaces between cells	More air spaces between cells

[Total: 16]