EUNOIA JUNIOR COLLEGE
JC2 Preliminary Examinations 2023
General Certificate of Education Advanced Level
Higher 2

CANDIDATE NAME								
CIVICS GROUP	**2**	**2**	**-**			REGISTRATION NUMBER		

BIOLOGY

9744/04

Paper 4 Practical

24 August 2023

2 hours 30 minutes

Candidates answer on the Question Paper.

READ THESE INSTRUCTIONS FIRST

Write your name, civics group and registration 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 stapler, paper clips, highlighters, glue or correction fluid/tape.

Shift
Laboratory

Answer **all** questions in the spaces provided in 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.

The number of marks is given in brackets [ ] at the end of each question or part question.

For Examiner's Use	
1	
2	
3	
Total	**55**

This document consists of **18** printed pages and **2** blank pages.

BLANK PAGE

Answer **all** questions.

1 Yeast cells contain enzymes that catalyse metabolic reactions. Some of these reactions release carbon dioxide.

You will investigate the release of carbon dioxide from a mixture of yeast and carbohydrate. The mixture is put into dialysis (Visking) tubing.

The dialysis tubing acts as a partially permeable membrane, allowing the carbon dioxide to diffuse out of the dialysis tubing.

You are provided with the materials shown in Table 1.1.

Table 1.1

labelled	contents	hazard	volume / cm^3
Y	1g dried yeast in boiling tube	none	-
G	10.0% warm glucose solution	none	20
W	distilled water	none	50
B	bromothymol blue indicator solution	harmful	10
V	20 cm length dialysis tubing in a beaker of distilled water	none	-

If any solution comes into contact with your skin, wash off immediately under cold water.

It is recommended that you wear suitable eye protection.

To test for the release of carbon dioxide, a sample of the water surrounding the dialysis tubing is added to drops of an indicator, **B**.

Fig. 1.1 shows the effect of increasing concentration of carbon dioxide on the colour of **B**. Yellow is the end-point.

Fig. 1.1

Carry out step 1 to step 21.

step 1 Using the beakers labelled **hot water** and **cold water**, adjust the water in the beaker labelled **water-bath** to 45 °C. You will **not** need to maintain this temperature.

step 2 Put 15 cm^3 of **G** into the boiling tube labelled **Y**. Mix well.

Between step 3 and step 4, you will be leaving the apparatus for 15 minutes. Use this time to continue with other parts of Question 1.

step 3 Put boiling tube **Y** into the water-bath for 15 minutes.

step 4 After 15 minutes, remove boiling tube **Y** from the water-bath.

step 5 Stir the mixture in boiling tube **Y** and pour it into a beaker.

step 6 Label the spotting tile with the sample times in minutes, as shown in Fig. 1.2.

step 7 Put 3 drops of **B** onto the spotting tile at each sample time, as shown in Fig. 1.2.

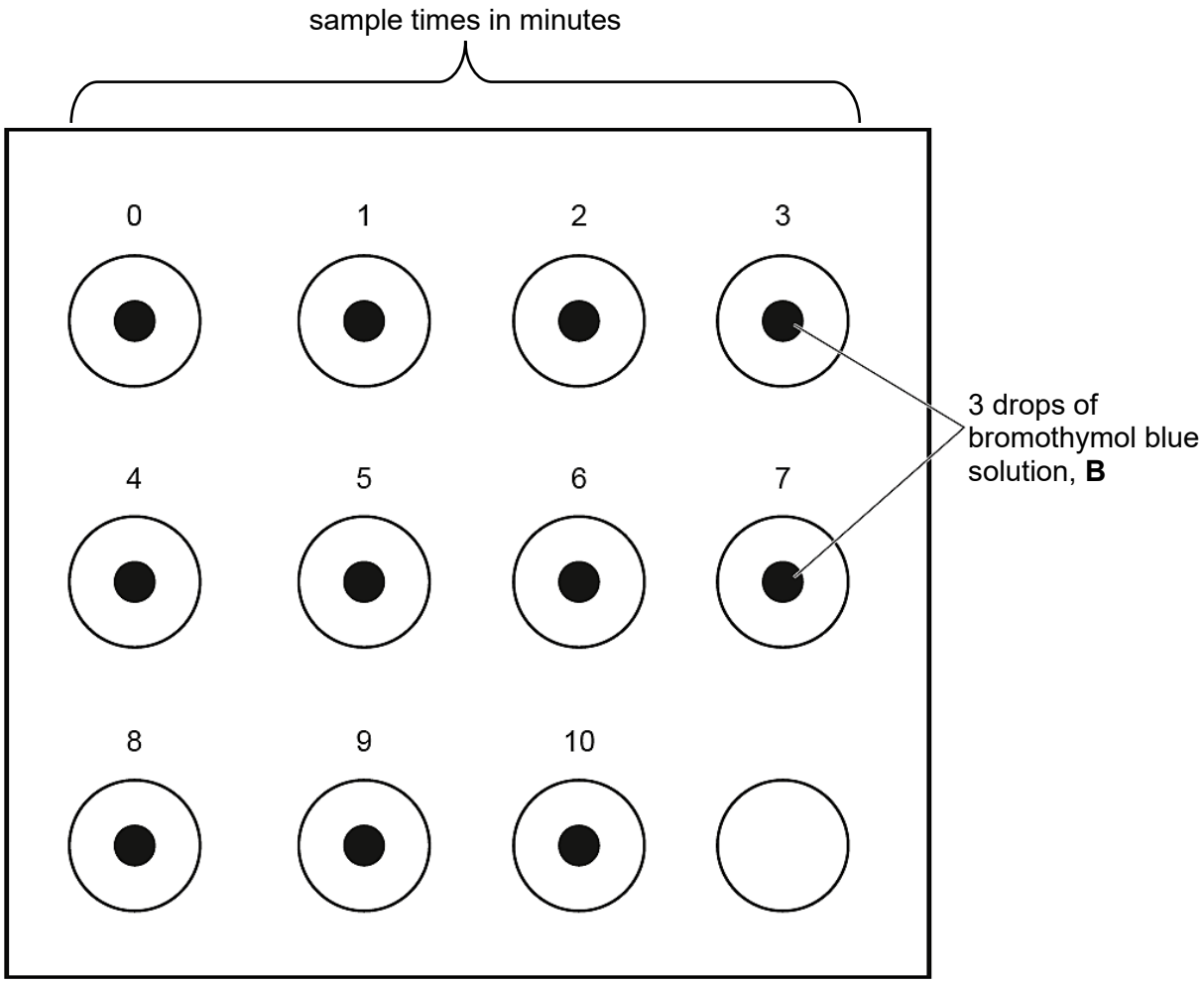

Fig. 1.2

Fig. 1.3 shows the apparatus you will set up for this investigation.

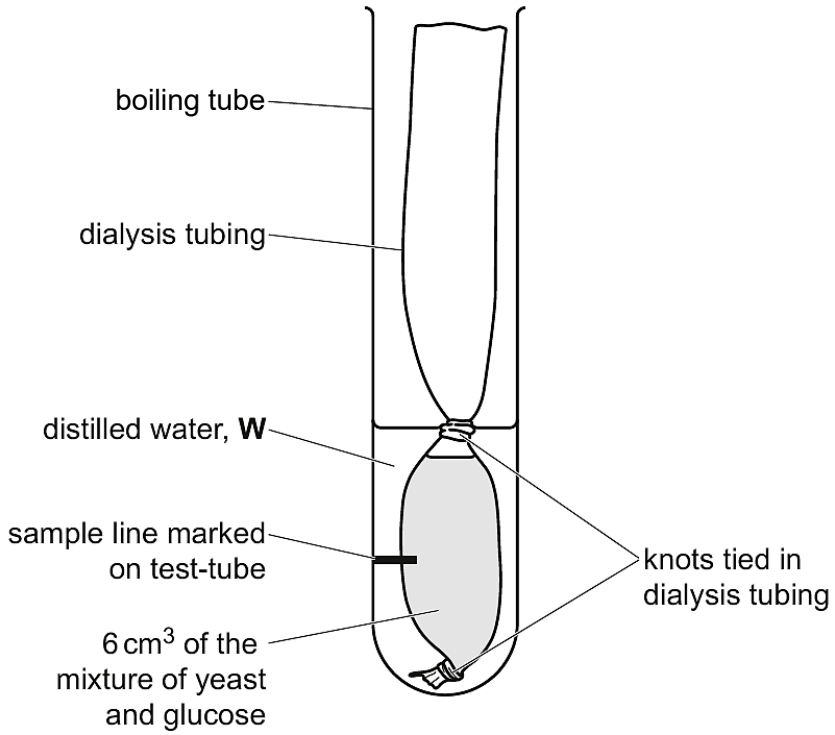

Fig. 1.3

step 8 Tie a knot in the dialysis tubing as close as possible to one end, so that the end is sealed.

step 9 To open the other end, rub the tubing gently between your fingers and thumb.

step 10 Stir the mixture in the beaker from step 5 and put 6.0 cm^3 of this mixture into a syringe.

step 11 Wipe the outside of the syringe and put the mixture from the syringe into the dialysis tubing.

step 12 Rinse the outside of the dialysis tubing by dipping it into the water in the container labelled **V**.

Look carefully at Fig. 1.3 to help you with step 13 to step 15.

step 13 Tie a knot just above the level of the mixture in the dialysis tubing, as shown in Fig. 1.3.

step 14 Put the dialysis tubing into a clean boiling tube so that it is resting on the bottom of the boiling tube, as shown in Fig. 1.3.

step 15 Draw a line on the boiling tube so that it is half-way between the two knots, as shown in Fig. 1.3. This is where you will take your samples from.

step 16 In this step, you will use a syringe to measure the volume of distilled water, **W**, needed to cover the section of dialysis tubing containing the mixture.

Use a syringe to put **W** into the boiling tube to cover the section of dialysis tubing containing the mixture.

(a) (i) State the volume of **W** that you added to the boiling tube in step 16.

volume of **W** = ... cm^3 [1]

step 17 Take a sample of **W** from the boiling tube at the point you marked in step 15, using a pipette.

step 18 Put 3 drops of **W** onto **B** at time 0 on the white tile. Put the remaining **W** in the pipette back into the boiling tube.

step 19 Start timing and put the boiling tube containing the dialysis tubing into the beaker labelled **water-bath**.

step 20 Mix the sample of **W** and **B** on the white tile and immediately record the colour in **(a)(ii)**, using the colours stated in Fig. 1.1.

step 21 Repeat step 17, step 18 and step 20 for each of the sampling times until the end-point (yellow) is reached for **two** consecutive samples. If the end-point is not reached at 10 minutes, stop timing.

(ii) Record your results in an appropriate table.

[3]

(iii) This investigation used colour to indicate the concentration of carbon dioxide in the sample.

Suggest **three** improvements to this investigation that would increase the accuracy of the results.

1 ……

……

2 ……

……

3 ……

……

[3]

(iv) A student wishes to repeat the investigation using the same procedure but with different concentrations of glucose. Using a table, show how the different concentrations of glucose can be made. You need to ensure that a sufficient volume is made for each glucose concentration.

[4]

(v) A student repeated the investigation using the same procedure but with starch as the substrate instead of glucose.

Suggest why it took much longer to reach the end-point when starch was used as the substrate.

……

……

……

……

……

……

……

……

[4]

[Total: 15]

2 **(a)** Many people are intolerant to the disaccharide lactose, which is found in milk. Commercially, the enzyme lactase can be immobilised and used to catalyse the breakdown of lactose to the monosaccharides glucose and galactose. These sugars taste sweeter and are easier to digest than lactose.

One way of immobilising lactase is using alginate beads, where lactase is contained within the beads. A student investigated the effect of alginate bead diameter on the hydrolysis of lactose.

The student:

- put beads with a diameter of 2mm into a syringe, up to the 5 cm^3 line
- put of 5 cm^3 lactose into this syringe
- left the syringe for 5 minutes
- measured the concentration of lactose after 5 minutes

The student used this method with the bead diameters shown in Fig. 2.1.

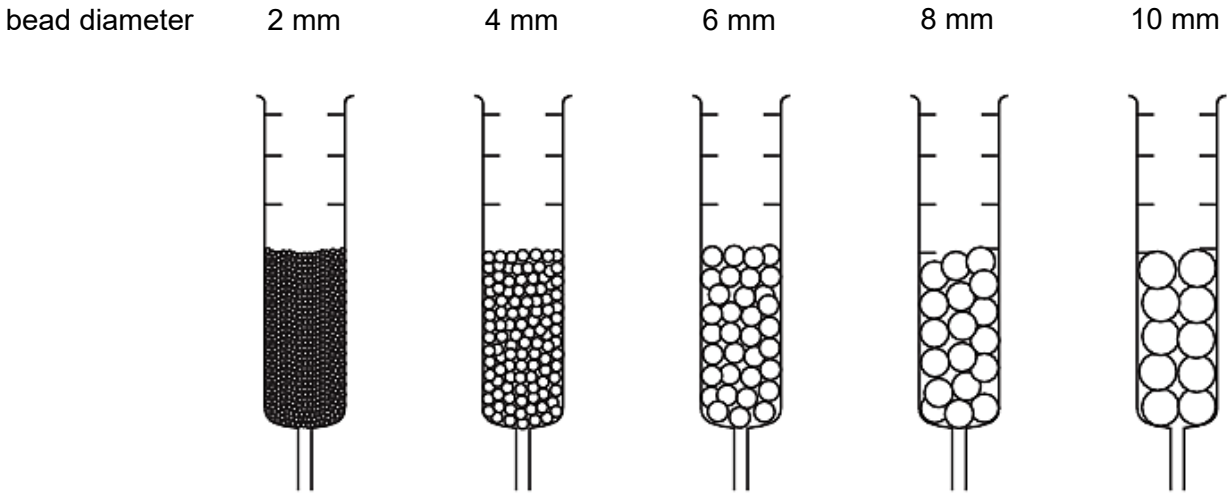

Fig. 2.1

Table 2.1 shows the results of this investigation.

Table 2.1

bead diameter / mm	percentage concentration of lactose after 5 minutes / %
2	20.5
4	21.0
6	29.5
8	40.5
10	69.0

(i) Plot a graph of the data shown in Table 2.1 on the grid in Fig. 2.2.

Use a sharp pencil.

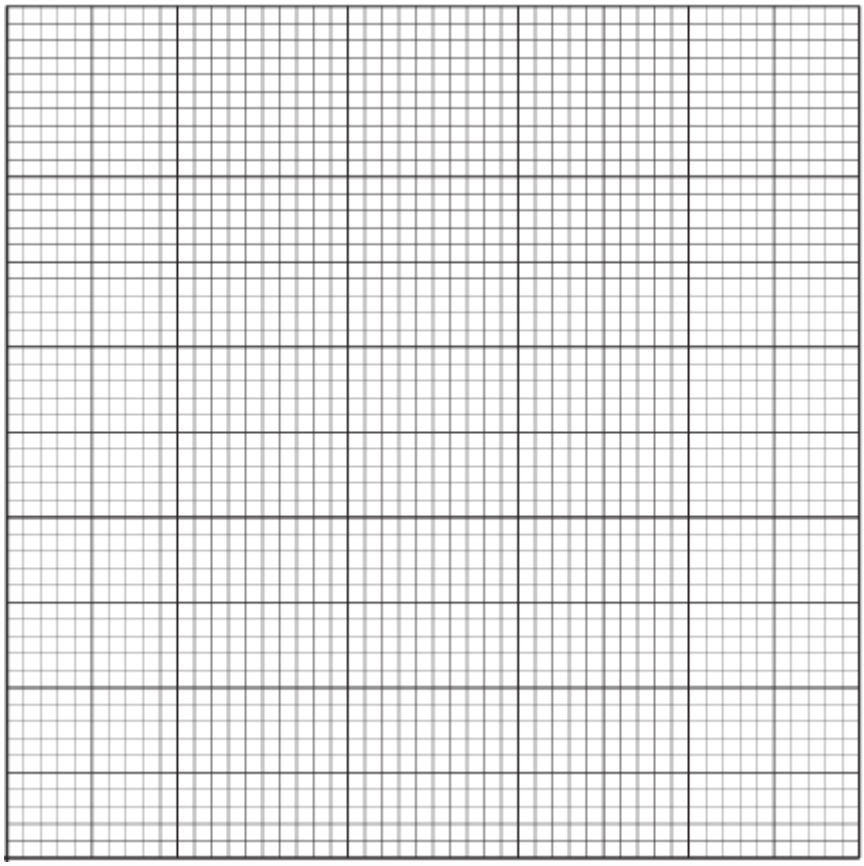

Fig. 2.2

[4]

(ii) Use your graph to find the concentration of lactose in the milk after 5 minutes, when the bead diameter was 5 mm. Show clearly on your graph how the concentration is obtained.

concentration of lactose = ……% [1]

Enzymes can be immobilised in a number of different ways, using different materials.

Fig. 2.3 shows three ways of immobilisation of enzymes.

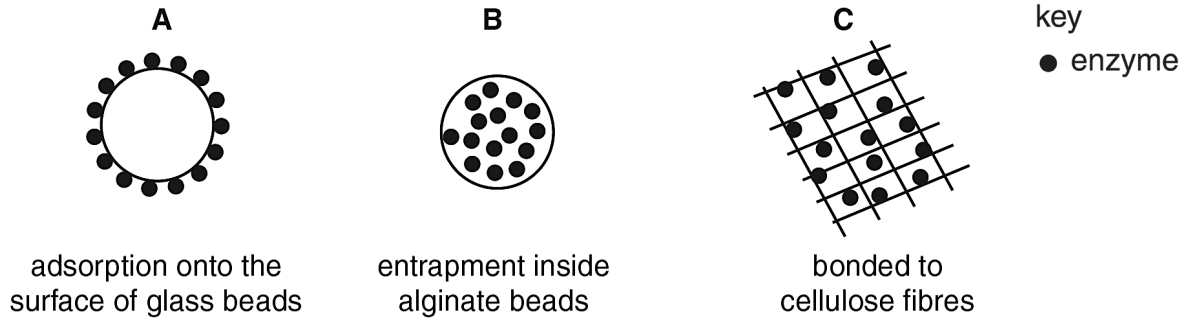

Fig. 2.3

A student carried out an investigation to compare the activity of the enzyme lactase that had been immobilised in the three different ways shown in Fig. 2.3.

- A solution containing 20 mg cm^{-3} of lactose was poured through a column containing the immobilised enzyme.
- The solution containing the products was collected and the concentration of glucose measured using a biosensor.

(b) State a null hypothesis that the student could make for this investigation.

……..…..

……..……

[1]

Table 2.2 shows the student's results.

Table 2.2

	way of immobilisation		
	A: adsorption onto the surface of glass beads	**B**: entrapment inside alginate beads	**C**: bonded to cellulose fibres
mean volume of solution containing product / cm^3	21	25	20
mean glucose concentration / mg cm^{-3}	15	10	12
mean total glucose collected / mg		250	

(c) Complete Table 2.1 by calculating the mean total glucose collected for **A** and **C**. [1]

(d) Without a biosensor, it will not be possible to determine the exact concentration of glucose produced by the three ways of immobilising lactase.

However, it is still possible to determine if there is any difference in the concentration of glucose produced using the following apparatus and reagents:

- lactase immobilised via the three methods shown in Fig. 2.3
- 20 mg cm^{-3} lactose solution
- Benedict's solution
- Bunsen burner
- wire gauze
- tripod stand
- beaker
- test tubes
- 5 cm^3 syringes
- stopwatch

To determine how the method of enzyme immobilisation affects the rate of lactase activity, you are to plan an investigation using the above apparatus and reagents.

(i) State the independent variable.

……..…….

……..……

[1]

(ii) State the dependent variable and how it can be determined using the above apparatus and reagents.

……..…….

……..……

……..…….

……..……

[2]

(iii) Describe and explain a control that should be included in the investigation.

……..…….

……..……

……..…….

……..……

[2]

(iv) Other than temperature and pH, identify **two** other variables that should be kept the same (standardised) in the investigation.

……..…….

……..……

……..…….

……..……

[2]

(v) State a risk and precaution.

……..…….

……..……

[1]

(vi) You are provided with vials G1, G2 and G3.

Each of these vials contain the glucose collected from one of the three ways of immobilising the lactase. Using Table 2.2 and your answer in **(d)(ii)**, deduce which vial contains glucose collected from **A**. Explain your answer.

……..…….

……..……

……..…….

……..……

[2]

(e) Table 2.3 shows the results of a number of statistical tests to find out if the difference in the rates of reaction were significant.

Table 2.3

statistical tests carried out between different ways of immobilisation		
A and **B**	**A** and **C**	**B** and **C**
$p < 0.05$	$p < 0.05$	$p > 0.05$

(i) State the statistical test that was carried out.

……

[1]

(ii) Explain the results shown in Table 2.3.

……

……

……

……

……

……

……

……

[4]

[Total: 22]

3 **K1** is a slide of a stained transverse section through a plant stem.

You are not expected to have seen **K1**.

(a) **(i)** Draw a large plan diagram of the region of the stem on **K1** indicated by the shaded region in Fig. 3.1. Your plan drawing should include at least 1 large vascular bundle and 2 smaller vascular bundles.

Use a sharp pencil.

Use one ruled label line and label to identify the epidermis.

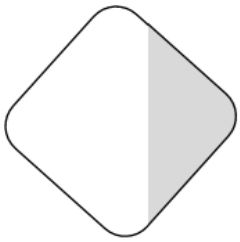

Fig. 3.1

[5]

(ii) Observe one of the larger vascular bundles on **K1**. The cell walls of a xylem vessel element in the vascular bundle are thickened with lignin.

Select a group of **four** large adjacent xylem vessel elements.

Each xylem vessel must touch at least **one** other xylem vessel element.

- Make a large drawing of this group of four xylem vessel elements.
- Use **one** ruled label line and label to identify the wall of **one** xylem vessel element.
- Include the actual dimension of one xylem vessel element.
- Include the magnification of your drawing and show how this magnification was obtained.

[5]

(b) Fig. 3.2 shows a diagram 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.

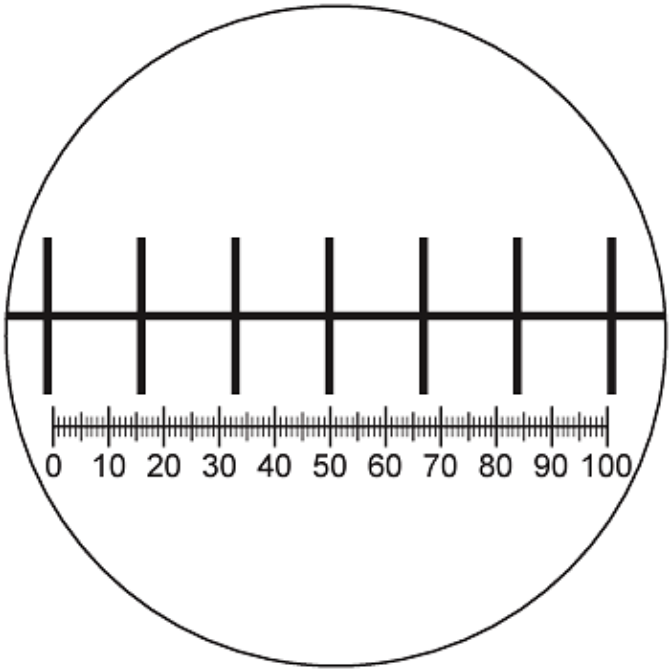

Fig. 3.2

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

Show your working.

Actual length = …….. [3]

Fig. 3.3 shows a photomicrograph of a transverse section of the stem of a different plant to **K1**.
The same microscope and lenses in Fig. 3.2 were used to view this transverse section.
The eyepiece graticule has been placed across the diameter of the stem section.

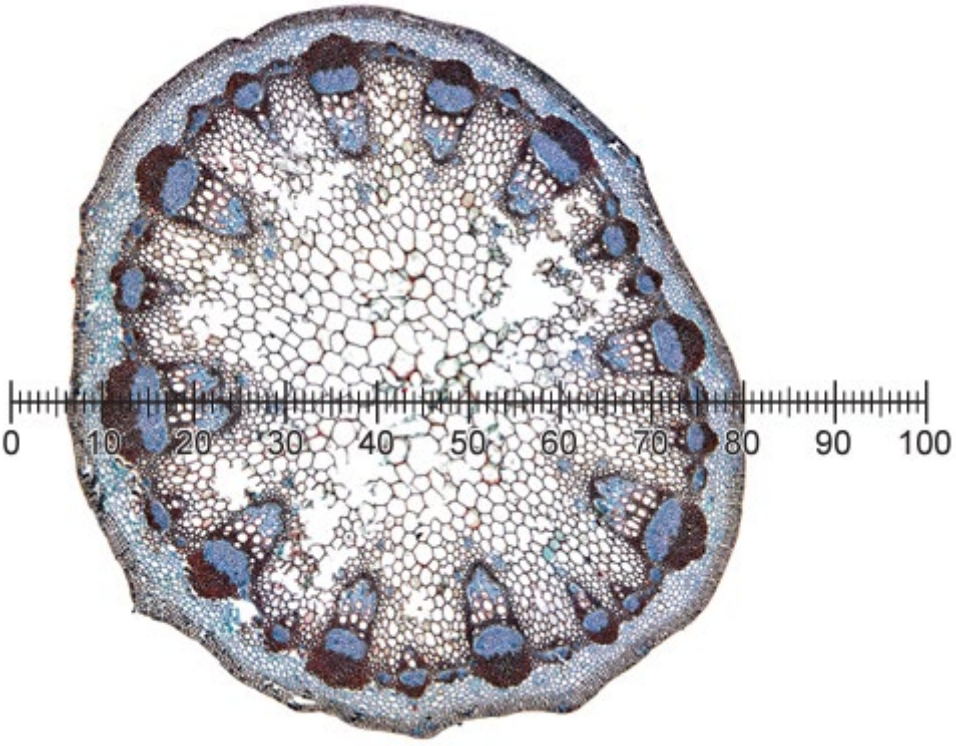

Fig. 3.3

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

Show your working.

Actual diameter = ... [2]

(iii) Identify **three** observable differences, other than size and colour, between the stem section on **K1** and the stem section in Fig. 3.3.

Record **three** observable differences in Table 3.1.

Table 3.1

feature	K1	Fig. 3.3

[3]

[Total: 18]

BLANK PAGE