Civics Group Index Number Name (use BLOCK LETTERS)



ST. ANDREW'S JUNIOR COLLEGE 2024 JC2 PRELIMINARY EXAMINATION

H2 BIOLOGY

9744/04

Paper 4: Practical Exam

Tuesday

20th August 2024

2 hours 30 minutes

READ THESE INSTRUCTIONS FIRST

Write your name, civics group and 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 a HB pencil for any diagram, graph or rough working. Do not use staples, paper clips, highlighters, glue or correction fluid.

Answer all questions in 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.

Shift Laboratory

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

IMPORTANT INFORMATION TO CANDIDATES:

Candidates with access to **microscope** at the start of the paper are given the **first 1h 15 min** to use them. Please answer **QUESTION 3** within this time frame. Once you have finished working with the microscope, you can move on to **QUESTION 1 or 2**.

Candidates with no access to microscope at the start of the paper should proceed with QUESTION 1 or 2 first. You will be given access to the microscope at 1h 15 min after the start of the paper.

| For Exar | miner's Use |
|----------|-------------|
| 1 | / 23 |
| 2 | / 12 |
| 3 | / 20 |
| Total | / 55 |

This document consists of 21 printed pages.

Turn over

Question 1

Agar stained with universal indicator can be used to investigate diffusion.

When hydrochloric acid diffuses into the agar it changes the colour from green to pink.

You will investigate the diffusion of different concentrations of hydrochloric acid in agar.

You are provided with the materials shown in Table 1.1.

Table 1.1

| labelled | contents | hazard | volume/ cm ³ |
|----------|---|----------|-------------------------|
| н | 2.0 mol dm ⁻³ hydrochloric acid | irritant | 20.0 |
| W | distilled water | none | 40.0 |
| | 2 Petri dishes containing agar | none | - |
| sheet T | sheet T | - | - |

If H comes into contact with your skin, wash off immediately with cold water.

It is recommended that you wear suitable eye protection.

You will need to:

- prepare different concentrations of hydrochloric acid, H
- measure the diffusion distance for each concentration of hydrochloric acid.

You will need to carry out a serial dilution of the 2.0 mol dm⁻³ hydrochloric acid, **H**, to reduce the concentration by half between each successive dilution.

You will need to prepare four concentrations of hydrochloric acid in addition to 2.0 mol dm⁻³ hydrochloric acid, **H**.

After the serial dilution is completed, you will need to have 5.0 cm³ of each concentration available to use.

(a) (i) Complete Fig. 1.1 to show how you will prepare your serial dilution.

Fig. 1.1 shows the beakers you will use.

For each beaker, add labelled arrows to show:

- the volume of hydrochloric acid transferred,
- the volume of distilled water, W, added.

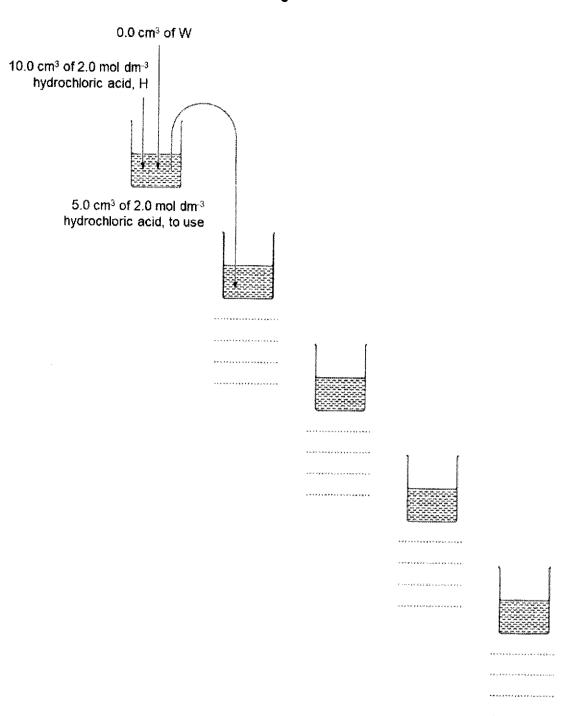


Fig. 1.1

[3]

Proceed as follows.

1 Prepare the concentrations of hydrochloric acid, as decided in (a)(i), in the beakers provided.

The different concentrations of hydrochloric acid will be put into wells cut into the agar. The position of one well is shown in Fig. 1.2.

You need to decide where to put **four** more wells in the agar in each Petri dish so that the wells are positioned away from each other and away from the edge of the Petri dish. Hydrochloric acid will diffuse into the agar around each well.

(ii) Complete Fig. 1.2 by:

- drawing four small circles to show where you have decided the wells should be positioned in the agar
- labelling the **five** small circles in Fig. 1.2 with the concentrations of hydrochloric acid you prepared in step 1.

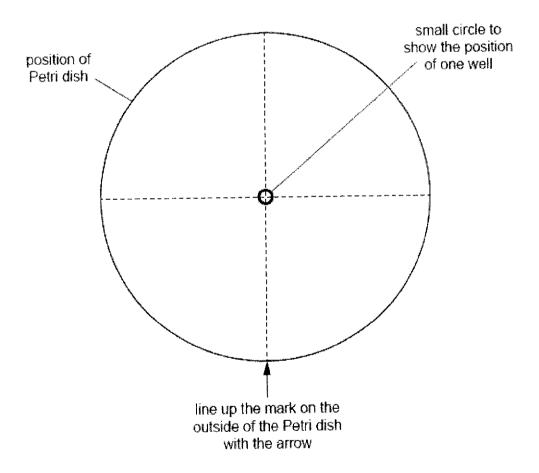


Fig. 1.2

Carry out steps 2 - 14.

- 2 Draw a mark on the outside edge of one of the Petri dishes containing agar.
- 3 Put the Petri dish on Fig. 1.2 so that the mark on the edge of the Petri dish lines up with the arrow in Fig. 1.2. Keep the Petri dish in this position over Fig. 1.2 for the whole of Step 4.
- 4 Use a straw to cut wells in the agar at the positions of the small circles on Fig. 1.2:
 - put the end of a straw on the surface of the agar over the centre small circle
 - carefully push the straw into the agar
 - lift up the straw to leave a well in the agar
 - hold the straw over the container labelled For waste
 - blow gently through the straw to remove the agar from the end of the straw
 - if the small circle of agar is **not** lifted by the straw, use a scalpel or mounted needle to carefully remove the agar and put it in the container labelled **For waste**.
- 5 Remove the Petri dish from Fig. 1.2 and leave it for use in step 7.
- Repeat step 2 to step 4 with the other Petri dish containing agar. You are provided with **sheet** T for use in step 7 and step 8.
- 7 Put one of the Petri dishes on circle A on sheet T so that the mark on the edge of the Petri dish lines up with the arrow.
- 8 Put the other Petri dish on circle **B** on **sheet T** so that the mark on the edge of the Petri dish lines up with the arrow.
- 9 For each Petri dish, **A** and **B**, use a Pasteur pipette to carefully put 2.0 mol dm⁻³ hydrochloric acid, **H**, into the appropriate well in the agar, as labelled in Fig. 1.2.
- 10 Repeat step 9 for the other concentrations of hydrochloric acid labelled in Fig. 1.2. Do not move the Petri dishes after the wells have been filled with the hydrochloric acid.
- 11 Start timing.

Between step 11 and step 12, you will be leaving the Petri dishes on **sheet T** for 20 minutes. Use this time to continue with other parts of Question 1.

12 After leaving the Petri dishes for 20 minutes, use a Pasteur pipette to remove the hydrochloric acid from the wells in both Petri dishes. Put this hydrochloric acid into the beaker labelled For waste.

13 Measure the diffusion distance, as shown in Fig. 1.3, for each concentration of hydrochloric acid in both Petri dishes.

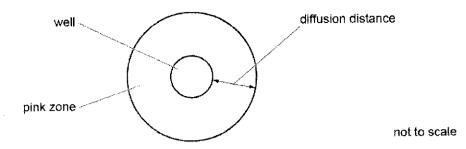


Fig. 1.3

| 14 Record Your results in tarting | 14 Record your results in | (a)(iii) |
|-----------------------------------|---------------------------|----------|
|-----------------------------------|---------------------------|----------|

(iii) Record your results in an appropriate table.

| (iv) Calculate the rate | of diffusion for | 2.0 mal dm ⁻³ | 3 hydrochloric acid | H h |
|---------------------------|------------------|--------------------------|----------------------|--------------------|
| rivi Calculate the rate (| alliusion ioi | Z.O IIIOI airi | Try droom nor to don | ∽ , - · · · |

Show your working and use appropriate units.

rate =[2]

[4]

| [3] (vi) Identify one significant source of error in this experiment. Suggest an improvement to eliminate this error. | (V | A student observed that the rate of diffusion was not constant during the investigation. Suggest how the student could modify the procedure to investigate the change in the rate of diffusion. |
|--|-----|---|
| [3] (vi) Identify one significant source of error in this experiment. Suggest an improvement to | | |
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[2]

(b) A scientist studied the rate of absorption of the amino acid alanine through the wall of the small intestine.

A concentration gradient was maintained throughout the investigation and all other variables were kept constant.

The results are shown in Table 1.2.

Table 1.2

| percentage concentration of alanine | rate of absorption /μm h ⁻¹ |
|--|---|
| 5 | 300 |
| 14 | 950 |
| 30 | 1625 |
| 50 | 1750 |
| 70 | 1775 |

(i) Plot a graph of the data shown in Table 1.2 on the grid in Fig. 1.4. Use a sharp pencil.

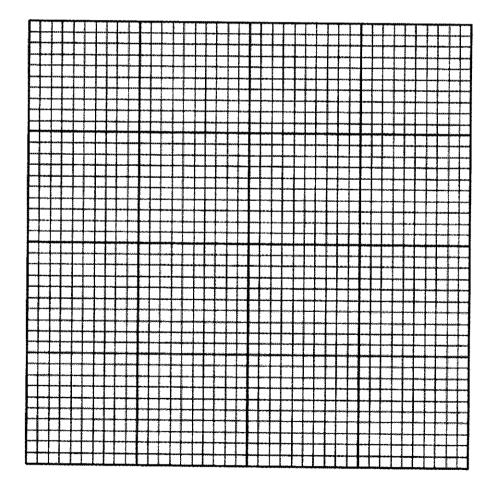


Fig. 1.4

|) A E | amino acids are transported into cells by facilitated diffusion. explain the shape of your graph in Fig. 1.4. |
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[Total: 23]

Question 2

The enzyme urease catalyses the breakdown of urea into carbonate ions and ammonium ions as shown in Fig. 2.1.

urea water
$$CO(NH_2)_2$$
 + $2H_2O$ CO_3^{2-} + $2NH_4^+$

A solution of urea does not conduct electricity, but a solution of the two ions does conduct electricity.

An increase in ions increases conductivity, so the rate of reaction is proportional to the conductivity.

A meter measures conductivity in microsiemens per centimetre (µScm⁻¹). The conductivity meter also records temperature.

Fig. 2.2 shows the experimental set-up during the course of a reaction.

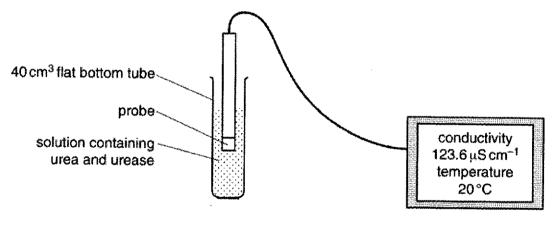


Fig. 2.2

Design an experiment, based on measuring the conductivity, to test whether or not the inhibition of urease by acetic acid is competitive.

In your plan you must use:

- 0.25 moldm⁻³ solution of urea
- 0.05 moldm⁻³ acetic acid
- 1 g per 10 cm³ urease solution
- a conductivity meter to measure the initial rate of reaction at each temperature.

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 dependent variable and the independent variable
- identify the variables you will need to control
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed
- include reference to safety measures to minimise any risks associated with the proposed experiment.

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[Total: 12]

Question 3

During this question you will require access to a microscope and a stage micrometer.

| You are provided with | two pieces of | cherry tomato is | n a petri dish | labelled K1 |
|-----------------------|---------------|------------------|----------------|-------------|
|-----------------------|---------------|------------------|----------------|-------------|

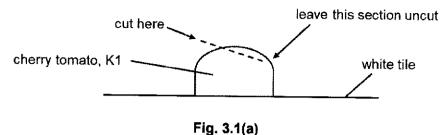
| Pro | oceed as follows. |
|-----|---|
| 1 | Place the cherry tomato cut-side up on a white tile so that you are able to observe the cross-section of the cherry tomato. |
| | (a) (i) Draw the cross section of the cherry tomato in the space provided. |
| | Your drawing should show the arrangement of the different regions and their correct shapes and proportions. |
| | No labels are required. |
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| | (ii) Calculate the magnification of your drawing in (a)(i). Show all your working. |
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St Andrew's Junior College 2024

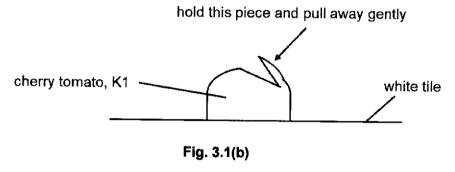
2 Place the cherry tomato cut-side down on a white tile.

Magnification = [2]

3 Using a scalpel, cut the tomato as close to the skin as possible, leaving a small section uncut, as shown in Fig. 3.1(a).



4 Holding the cut piece, gently pull the specimen away as shown in Fig. 3.1(b). You should obtain a single layer of the skin.



- 5 Using the scalpel and white tile, cut a small piece of the single layer skin and place it on a microscope slide.
- **6** Put one to two drops of distilled water on the thin piece of skin on the slide. You need to ensure that the skin is not folded.
- 7 Add a coverslip to the slide and use a paper towel to absorb any excess fluid.
- 8 Observe the slide with a microscope.

(b) (i) Using an appropriate objective lens, make a high power drawing of two touching cells.

At least one of these cells should have a nucleus. Label the nucleus.

[5]

(ii) A rough estimate of the area occupied by a cell observed in (b)(i) is given by X × Y as seen in Fig. 3.2.

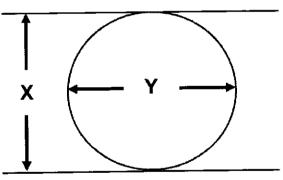


Fig. 3.2

Measure and record the dimensions of **X** and **Y** of one of the cell in **(b)(i)** in eyepiece graticule units.

X = eyepiece graticule units

Y = eyepiece graticule units

[1]

| (iii) Using the same objective lens you used in (b)(i), calibrate your eyepiece graticule using the 1 mm stage micrometer provided. |
|--|
| You should show all your working and use appropriate units. |
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| 1 eyepiece graticule unit =[2] |
| (iv) Using the measurement of length in eyepiece graticule units recorded in (b)(ii), calculate the estimated area of the cell. |
| You should show all your working. |
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| Area of the cell µm² [2] |
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The **concentration** of ascorbic acid can be estimated in a fruit extract by carrying out a test using starch solution and iodine solution.

lodine solution will be added one drop at a time using a syringe.

To practise releasing drops from a syringe:

- 1 Fill a syringe with 1.0 cm³ distilled water from beaker labelled W (from Question 1).
- 2 Hold the syringe over an empty test-tube as shown in Fig. 3.3 and push the plunger slowly to release one drop.
- 3 Repeat this until you can release one drop at a time.

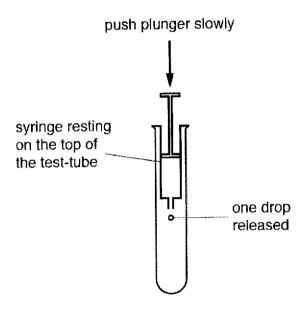


Fig. 3.3

You are provided with the materials shown in Table 3.1.

Table 3.1

| labelled | contents | hazard | volume/ cm³ |
|----------|-----------------|--------|-------------|
| iodine | iodine solution | none | 20.0 |
| S | starch solution | none | 20.0 |
| X | tomato extract | none | 20.0 |

If any solutions come into contact with your skin, wash off immediately under cold water.

It is recommended that you wear suitable eye protection.

You will now carry out a test on ${\bf X}$ to find the volume of iodine solution that needs to be added for the end-point to be reached.

The end-point is when the blue colour remains after 10 seconds.

- 4 Put 1.0 cm³ of **S** into a test-tube.
- 5 Put 5.0 cm³ of **X** into the same test-tube.
- 6 Shake the test-tube gently to mix the contents.
- 7 Fill a 1.0 cm³ syringe with exactly 1.0 cm³ of iodine.
- 8 Wipe off any iodine from the outside of the syringe with a paper towel.
- 9 Put one drop of iodine, as shown in Fig. 3.3, into the mixture of S and X in the test-tube.
- 10 Mix gently and observe any colour change.
- 11 Repeat step 9 and step 10 until a blue colour appears. You may need to refill the syringe with iodine as in step 7.
- 12 When the blue colour appears, shake the test-tube gently for 10 seconds and see if the end-point has been reached.
- 13 If the blue colour disappears then repeat step 9 to step 12 until the mixture stays blue for at least 10 seconds.
- 14 Record in (c)(i) the volume of iodine solution added.
- 15 Repeat steps 4 to 14 two more times and calculate average volume of iodine solution added.
- (c) (i) Record your results in an appropriate table below.

Table 3.2 below shows the volume of iodine solution needed to reach the end-point at various concentration of ascorbic acid.

Table 3.2

| Concentration of ascorbic acid/ % | Volume of iodine solution needed to reach end-point/ cm³ | |
|-----------------------------------|--|--|
| 0.10 | 2.15 | |
| 0.08 | 1.60 | |
| 0.06 | 1.25 | |
| 0.04 | 0.75 | |
| 0.02 | 0.35 | |

| (ii) Using your result in (c)(i), estimate the concentration of ascorbic acid in X. | |
|---|-------|
| Concentration of ascorbic acid = | [1] |
| Пotal | : 20m |

Civics Group Index Number Name (use BLOCK LETTERS)



ST. ANDREW'S JUNIOR COLLEGE 2024 JC2 PRELIMINARY EXAMINATION

H2 BIOLOGY

9744/04

Paper 4: Practical Exam

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Write in dark blue or black pen.

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Answer all questions in 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.

Shift Laboratory

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

IMPORTANT INFORMATION TO CANDIDATES:

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| For Examiner's Use | | | |
|--------------------|------|--|--|
| 1 | / 23 | | |
| 2 | / 12 | | |
| 3 | / 20 | | |
| Total | / 55 | | |

This document consists of 21 printed pages.

Turn over

Marker's comments:

Please remember to write down your shift and lab no. in the provided box when asked to do so.

Question 1

Agar stained with universal indicator can be used to investigate diffusion.

When hydrochloric acid diffuses into the agar it changes the colour from green to pink.

You will investigate the diffusion of different concentrations of hydrochloric acid in agar.

You are provided with the materials shown in Table 1.1.

Table 1.1

| labelled | contents | hazard | volume/ cm ³ |
|----------|---|----------|-------------------------|
| н | 2.0 mol dm ⁻³ hydrochloric acid | irritant | 20.0 |
| W | distilled water | none | 40.0 |
| | 2 Petri dishes containing agar | none | - |
| sheet T | sheet T | - | <u>-</u> |

If H comes into contact with your skin, wash off immediately with cold water.

It is recommended that you wear suitable eye protection.

You will need to:

- prepare different concentrations of hydrochloric acid, H
- measure the diffusion distance for each concentration of hydrochloric acid.

You will need to carry out a **serial** dilution of the 2.0 mol dm^{-3} hydrochloric acid, **H**, to reduce the concentration by **half** between each successive dilution.

You will need to prepare **four** concentrations of hydrochloric acid in addition to 2.0 mol dm⁻³ hydrochloric acid, H.

After the serial dilution is completed, you will need to have 5.0 cm³ of each concentration available to use.

(a) (i) Complete Fig. 1.1 to show how you will prepare your serial dilution.

Fig. 1.1 shows the beakers you will use.

For each beaker, add labelled arrows to show:

- the volume of hydrochloric acid transferred,
- the volume of distilled water, W, added.

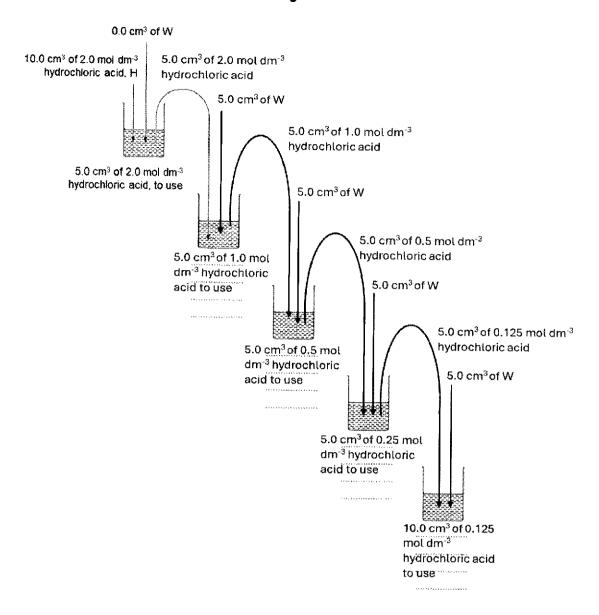


Fig. 1.1

- 1 states five concentrations: 2.0, 1.0, 0.5, 0.25, 0.125 mol dm_{-3} ;
- 2 states four transfers of 5.0 cm³ to each beaker from the previous beaker + arrow drawn;
- 3 shows four additions of 5.0 cm³ of W to each beaker + arrow drawn;

[3]

Proceed as follows.

1 Prepare the concentrations of hydrochloric acid, as decided in (a)(i), in the beakers provided.

The different concentrations of hydrochloric acid will be put into wells cut into the agar. The position of one well is shown in Fig. 1.2.

You need to decide where to put **four** more wells in the agar in each Petri dish so that the wells are positioned away from each other and away from the edge of the Petri dish. Hydrochloric acid will diffuse into the agar around each well.

(ii) Complete Fig. 1.2 by:

- drawing four small circles to show where you have decided the wells should be positioned in the agar
- labelling the **five** small circles in Fig. 1.2 with the concentrations of hydrochloric acid you prepared in step 1.

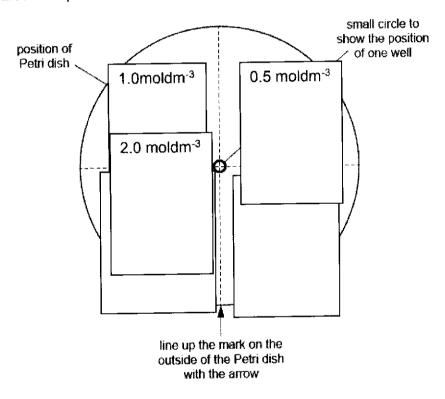


Fig. 1.2

1 draws four circles with one in each quadrant and labels each with the concentration of acid;

[1]

Carry out steps 2 - 14.

- 2 Draw a mark on the outside edge of one of the Petri dishes containing agar.
- 3 Put the Petri dish on Fig. 1.2 so that the mark on the edge of the Petri dish lines up with the arrow in Fig. 1.2. Keep the Petri dish in this position over Fig. 1.2 for the whole of Step 4.
- 4 Use a straw to cut wells in the agar at the positions of the small circles on Fig. 1.2:
 - put the end of a straw on the surface of the agar over the centre small circle.
 - · carefully push the straw into the agar.
 - lift up the straw to leave a well in the agar.
 - hold the straw over the container labelled For waste.
 - blow gently through the straw to remove the agar from the end of the straw.
 - if the small circle of agar is **not** lifted by the straw, use a scalpel or mounted needle.
 - to carefully remove the agar and put it in the container labelled For waste.
- 5 Remove the Petri dish from Fig. 1.2 and leave it for use in step 7.
- Repeat step 2 to step 4 with the other Petri dish containing agar. You are provided with **sheet** T for use in step 7 and step 8.
- 7 Put one of the Petri dishes on circle A on sheet T so that the mark on the edge of the Petri dish lines up with the arrow.
- 8 Put the other Petri dish on circle **B** on **sheet T** so that the mark on the edge of the Petri dish lines up with the arrow.
- 9 For each Petri dish, **A** and **B**, use a Pasteur pipette to carefully put 2.0 mol dm⁻³ hydrochloric acid, H, into the appropriate well in the agar, as labelled in Fig. 1.2.
- 10 Repeat step 9 for the other concentrations of hydrochloric acid labelled in Fig. 1.2. Do **not** move the Petri dishes after the wells have been filled with the hydrochloric acid.
- 11 Start timing.

Between step 11 and step 12, you will be leaving the Petri dishes on **sheet T** for 20 minutes. Use this time to continue with other parts of Question 1.

12 After leaving the Petri dishes for 20 minutes, use a Pasteur pipette to remove the hydrochloric acid from the wells in both Petri dishes. Put this hydrochloric acid into the beaker labelled For waste.

13 Measure the diffusion distance, as shown in Fig. 1.3, for each concentration of hydrochloric acid in both Petri dishes.

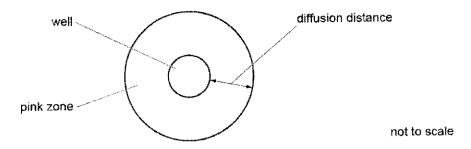


Fig. 1.3

- 14 Record your results in (a)(iii).
 - (iii) Record your results in an appropriate table.

Table showing diffusion distance at various concentration of hydrochloric acid

| Concentration | diffusion distance / mm | | |
|---|-------------------------|--------------|---------|
| of hydrochloric acid / mol dm ⁻³ | Petri dish A | Petri dish B | Average |
| 2.000 | 9.0 | 9.0 | 9.0 |
| 1.000 | 0.8 | 7.0 | 7.5 |
| 0.500 | 8.0 | 7.0 | 7.5 |
| 0.250 | 7.0 | 5.0 | 6.0 |
| 0.125 | 5.0 | 4.0 | 4.5 |

Title

- 1 heading for independent variable: concentration of hydrochloric acid / mol dm-3 + heading for dependent variables: diffusion distance / mm
- 2 records a distance for stated concentrations in (a)(i) and for both Petri dishes;
- 3 Average calculated and correct trend + ≤ 15 mm;
- 4 records distance in millimeters; (accept whole mm or 1 d.p.)

[4]

7 (iv) Calculate the rate of diffusion for 2.0 mol dm⁻³ hydrochloric acid, H. Show your working and use appropriate units. shows distance for 2 mol dm-3 divided by 20; 2 correct answer and appropriate units : Distance for 2 mol $dm^{-3} = 9.0 \text{ mm}$ Rate = 9.0 mm / 20 mins = 0.45 mm/60 s= $7.5 \times 10^{-3} \text{ mm s}^{-1}$ (answer to 1 dp) rate = 7.5 x10⁻³ mm s⁻¹..... [2] (v) A student observed that the rate of diffusion was not constant during the investigation. Suggest how the student could modify the procedure to investigate the change in the rate of diffusion.[3] use one concentration of hydrochloric acid e.g. 2.0 mol dm⁻³; 2 measure measuring the diffusion distance for fixed time intervals e.g. 4 , 8, 12 ,16, 20 mins (list out the time intervals); 3 calculate the rate of diffusion of the acid for each time interval (by measuring the distance divide by time interval): (vi) Identify one significant source of error in this experiment. Suggest an improvement to eliminate this error. [2] 1 [Error] Volume of HCl added is inconsistent as no fixed volume stated: 2 [Improvement] Fixed the volume of HCl for each concentration in each well at 0.5 cm³ using a 1cm3 syringe or 100-200µl micropipette to ensure accuracy of volume; OR

- 1 [Error] Unequal incubation time due to lag time between addition of hydrochloric acid to first well and last well/ ref. to different time for diffusion to take place
- 2 [Improvement] Stagger the start time to add hydrochloric acid (by 1 min).

AVP

- 1 [Error] Inconsistent size of wells due to soft straw/soft agar resulting in differing volume of HCl added/ SA for diffusion
- 2 [Improvement] use a cork borer to cut the well

A scientist studied the rate of absorption of the amino acid alanine through the wall of the small intestine.

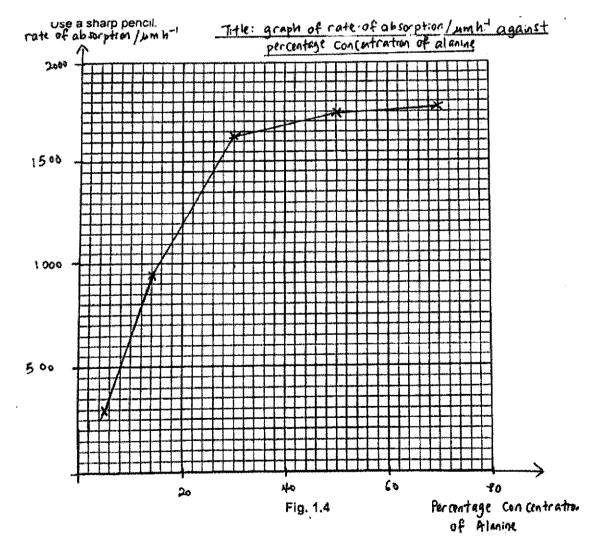
A concentration gradient was maintained throughout the investigation and all other variables were kept constant.

The results are shown in Table 1.2.

Table 1.2

| percentage concentration of alanine | rate of absorption /μm h ⁻¹ |
|-------------------------------------|---|
| 5 | 300 |
| 14 | 950 |
| 30 | 1625 |
| 50 | 1750 |
| 70 | 1775 |

(i) Plot a graph of the data shown in Table 1.2 on the grid in Fig. 1.4. Use a sharp pencil.



- label on x-axis: percentage concentration of alanine and label on y-axis: rate of absorption/ $m h^{-1}$;
- 2 scale on x-axis: 20 to 2 cm, labelled at least every 2 cm and scale on y-axis: 500 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 and line is either a smooth curve or joined plot to plot;

[4]

(ii) Amino acids are transported into cells by facilitated diffusion.

Explain the shape of your graph in Fig. 1.4.

- 1 As the concentration of alanine increases from 5 to 30% the rate of absorption increases from 300-1625µm h⁻¹;
 - 2 As the concentration of alanine increases from 30 to 70% the rate of absorption increases at a decreasing rate from 1625-1175µm h⁻¹ and is starting to plateau
 - 3 Alanine is transported into the cell by transport proteins/channel proteins/carrier proteins;
 - Transport proteins/channel proteins/carrier proteins are **reaching saturation** at 50% to 70% concentration of alanine as seen from the lower increase in rate of absorption;

[Total: 23]

Question 2

The enzyme urease catalyses the breakdown of urea into carbonate ions and ammonium ions as shown in Fig. 2.1.

urea water
$$CO(NH_2)_2$$
 + $2H_2O$ CO_3^{2-} + $2NH_4^+$ Fig. 2.1

A solution of urea does not conduct electricity, but a solution of the two ions does conduct electricity.

An increase in ions increases conductivity, so the rate of reaction is proportional to the conductivity.

A meter measures conductivity in microsiemens per centimetre (µScm⁻¹). The conductivity meter also records temperature.

Fig. 2.2 shows the experimental set-up during the course of a reaction.

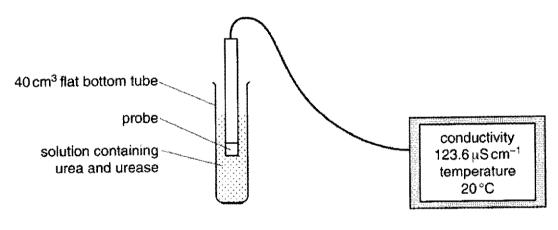


Fig. 2.2

Design an experiment, based on measuring the conductivity, to test whether or not the inhibition of urease by acetic acid is competitive.

In your plan you must use:

- 0.25 moldm⁻³ solution of urea
- 0.05 moldm⁻³ acetic acid
- 1 g per 10 cm3 urease solution
- a conductivity meter to measure the initial rate of reaction at each temperature.

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 dependent variable and the independent variable
- identify the variables you will need to control
- use the correct technical and scientific terms
- indicate how the results will be recorded and analysed

 include reference to safety measures to minimise any risks associated with the proposed experiment.

Answer

Variables [C - 2M; 1M I.V + D.V + 1M Controlled]

- ✓ <u>Independent</u> variable: Concentration of urea solution (i.e. 0.00, 0.05, 0.10, 0.15, 0.20 , 0.25 moldm⁻³);
- ✓ <u>Dependent variable</u>: Rate of urease reaction as determined by the <u>conductivity</u> in 1 minute
 / <u>uScm⁻¹ min⁻¹</u>
- ✓ Cither variables to be kept constant: [Apparatus & quantity to be indicated] (Any 2)
 - i. Volume of the following to be kept constant using a 5 cm³ syringe:
 - o Acetic acid used, 1 cm3
 - Urea solution, 5 cm³
 - o 0.1 g/cm³ urease solution, 1 cm³
 - ii. Time taken for reaction using a stopwatch, 1 min
 - iii. Temperature of reaction using thermostatically controlled water bath at 20°C
 - iv. **pH of reaction mixture** using **pH buffer** (pH 7)
 - v. same conductivity meter and probe used

√ Control [C - 1M]

 Set-up: Replace the urea solution with an equal volume (5 cm³) of distilled water, with all other experimental conditions kept the same.

(Expected results: No conductivity detected in minute)

OR

Replace the **urease** with an <u>equal volume (5 cm³) of boiled and cooled urease</u>, with <u>all other experimental conditions kept the same</u>.

• **Purpose**: To show that any conductivity is due to the breakdown of urea by urease and not due to any other factors.

40 cm³ flat bottom tube probe solution containing urea and urease conductivity 123.6 µS cm⁻¹ temperature

St Andrew's Junior College 2024

Thermostatically controlled water bath

9744/04/Preliminary Examinations

20°C

[12]

Procedure [Apparatus and quantity stated] - 5M

Set-Up:

1. Using 100 cm³ measuring cylinders and 100 cm³ beakers, prepare 100 cm³ of 5 different concentrations of urea using the 0.25 moldm⁻³ stock solution and distilled water, according to the volumes stated in the dilution table below.

| Concentration of urea / moldm ⁻³ | Volume of 0.025 moldm ⁻³ stock urea/ cm ³ | Volume of distilled water /cm³ | Total volume/ cm ³ | |
|---|---|--------------------------------|-------------------------------|--|
| 0.00 | 0.0 | 100.0 | 100.0 | |
| 0.05 | | | 100.0 | |
| 0.10 | 40.0 | 60.0 | 100.0 | |
| 0.15 | | | 100.0 100.0 | |
| 0.20 80.0 | | 20.0 | | |
| 0.25 | 100.0 | 0.0 | 100.0 | |

- Measure 10.0 cm³ of urea solution into a flat bottom tube 10 cm³ syringe
- 3. Measure **1.0 cm³** of urease solution and 1.0 cm³ of acetic acid into a separate test tubes using 10 cm³ and 1cm³ **syringes** respectively.
- Incubate the urea solution, urease and acetic acid separately in the 20°C thermostatically controlled water bath for 5 minutes using a stopwatch.
- 5. Add the urease and acetic acid solution into the <u>flat bottom tube</u> containing urea solution and stir using a glass rod.
- 6. Immediately place the probe into the flat bottom tube as shown in the diagram.
- 7. Start the stopwatch.
- 8. After 1 minute, record the conductivity in a table.
- 9. Repeat steps 2 8 for the other concentrations of urea solution.
- 10. To ensure <u>reliability</u> of results, repeat steps 2 to 8 **twice** for each concentration and <u>calculate</u> the average.
- 11. Repeat steps 2-10 using 1 cm³ of distilled water in place of the acetic acid added to the urease solution.
- 12. To ensure <u>reproducibility</u> of data, repeat the entire experiment twice using **freshly prepared reagents** (any one e.g. urease solution/ urea solution/ acetic acid) and <u>new apparatus</u>.
 - 1 [Dilution procedure + table] Step 1 + table
 - 2 [Equilibration of solutions at constant temperature] Steps 2 -
 - 3 [Data collection & Logical Steps] Steps 5 9
 - 4 [Procedure for uninhibited and inhibited enzymatic reactions] Steps 11
 - 5 [Replicates and Repeats] Steps 10 & 12
- ✓ Table showing the rates of reaction at different urea concentrations (uninhibited reaction)

| concentration of urea | Conductivity of solution without acetic acid/ µScm ⁻¹ (min ⁻¹) | | | | | |
|----------------------------------|---|-------------|-------------|---------|--|--|
| solution/ moldm ⁻³ | Replicate 1 | Replicate 2 | Replicate 3 | Average | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

Table showing the rates of reaction at different urea concentrations (in presence of inhibitor)

| concentration of urea | Conductivity of solution with acetic acid / µScm ⁻¹ (min ⁻¹) | | | | | |
|----------------------------------|---|-------------|-------------|---------|--|--|
| solution/ moldm ⁻³ | Replicate 1 | Replicate 2 | Replicate 3 | Average | | |
| | | | | | | |
| | | | | | | |
| " | | | | | | |
| | | | | | | |

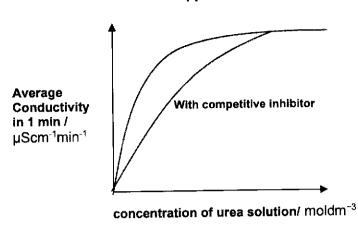
OR <u>Table showing the rates of reaction at different urea concentrations (in absence and presence of inhibitor)</u>

| concentratio n of urea | Conductivity of solution without acetic acid / µScm ⁻¹ min ⁻¹ | | | Conductivity of solution with acetic acid / µScm ⁻¹ min ⁻¹ | | | | |
|----------------------------------|---|----------------|----------------|--|----------------|----------------|----------------|---------|
| solution/ moldm ⁻³ | Replicate 1 | Replicate 2 | Replicate 3 | Average | Replicate 1 | Replicate 2 | Replicate 3 | Average |
| | | | | | | | | |
| | | | | | | | | - |
| | | | | | | | | |
| | | | | | | | | |

√ **State** [L - 1M]

Absence of competitive inhibitor





✓ Analysis of results (1 M)

If rate of reaction in the presence of acetic acid is the same as in the absence of the acetic acid (i.e. reach Vmax) under **high urea concentrations**, the acetic acid is a competitive inhibitor. **Accept**: Contrasting point for non-competitive inhibitor;

✓ Risk & Precaution [must provide two] - 1M]

| Risk | Precaution | | |
|---|--|--|--|
| Urea solution/urease/ Acetic acid is an irritant. | Do not allow chemical to come into contact with eyes - wear gloves and safety goggles/ if in contact, wash off with water immediately. | | |
| Glassware is fragile and may break. Can get <u>cut</u> / injured by broken glassware. | Handle with care/ Dispose of broken glassware promptly and safely. | | |
| Risk of electrocution when using conductivity meter/ thermostatically controlled bath | Ensure hands are dry before using the apparatus/ ensure table is dry | | |

Reject:

Risk of scalding when using hot water bath (water is only 20°C - 30 °C)

[Total: 12]

Question 3

During this question you will require access to a microscope and a stage micrometer.

You are provided with two pieces of cherry tomato in a petri dish labelled K1.

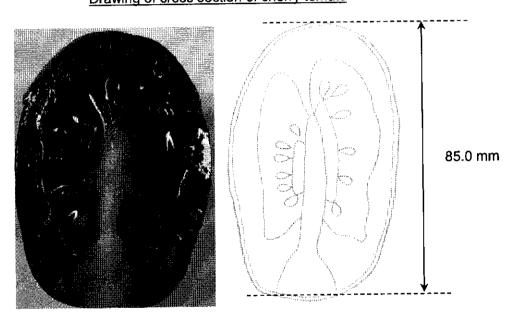
Proceed as follows.

- 1 Place the cherry tomato cut-side up on a white tile so that you are able to observe the crosssection of the cherry tomato.
 - (a) (i) Draw the cross section of the cherry tomato in the space provided.

Your drawing should show the arrangement of the different regions and their correct shapes and proportions.

No labels are required.

Drawing of cross section of cherry tomato



- 1 Title: Correct title (Drawing of cross section of cherry tomato)
- 2 Size: Size of drawing is sufficiently large (at least % of space provided);
- 3 **Proportion:** outermost layer thinnest OR comparable to 2nd outermost layer (if seeds are drawn, layer of locule surrounding seeds should be drawn) + Shape: oval shaped + at least 4 layers
- 4 Quality: Cells drawn with clear, continuous lines + No shading

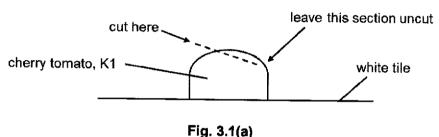
[4]

- (ii) Calculate the magnification of your drawing in (a)(i). Show all your working.
 - Show size of drawing (accept of size of drawing shown in (a)(i))+ specimen size in mm)
 - 2. Show calculation of magnification (image size/ specimen size) + answer in whole number with 'x'

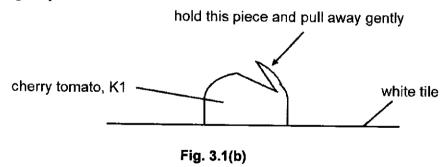
Size of drawing = 85.0 mm
Size of specimen = 32.0 mm
Magnification = size of drawing/actual size
= 85/32
= 2.6 = 3x

Magnification =3x.....[2]

- 2 Place the cherry tomato cut-side down on a white tile.
- 3 Using a scalpel, cut the tomato as close to the skin as possible, leaving a small section uncut, as shown in Fig. 3.1(a).



4 Holding the cut piece, gently pull the specimen away as shown in Fig. 3.1(b). You should obtain a single layer of the skin.

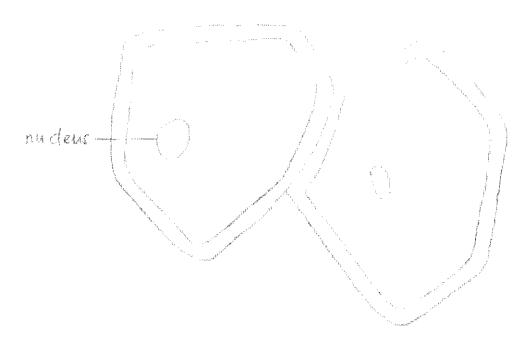


- 5 Using the scalpel and white tile, cut a small piece of the single layer skin and place it on a microscope slide.
- 6 Put one to two drops of distilled water on the thin piece of skin on the slide. You need to ensure that the skin is not folded.
- 7 Add a coverslip to the slide and use a paper towel to absorb any excess fluid.
- 8 Observe the slides with a microscope.

(b) (i) Using an appropriate objective lens, make a high power drawing of two touching cells.

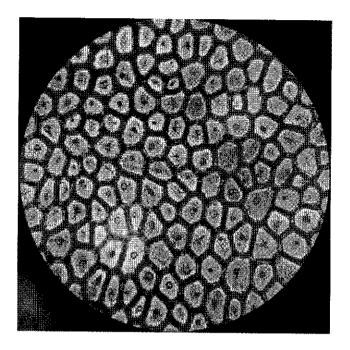
At least one of these cells should have a nucleus. Label the nucleus.

Detailed/ High power drawing of two touching cherry tomato skin cells (400x)



- 1. Title: High power drawing of two touching cherry tomato skin cells
- 2. Size: Size of drawing is sufficiently large (at least 3/4 of space provided);
- 3. Shape & proportion: 2 polygonal cells + adjacent cells + at least one cell with nucleus (occupies less than 40% of cell) + 2 lines for cell wall + uniform thickness of cell wall + cell wall sufficiently thick (4 -5 mm))
- 4. Quality: Cells drawn with clear, continuous lines + No shading
- 5. Label: nucleus labelled with label line drawn with a ruler and label written horizontally

[5]



(ii) A rough estimate of the area occupied by a cell observed in (b)(i) is given by X × Y as seen in Fig. 3.2.

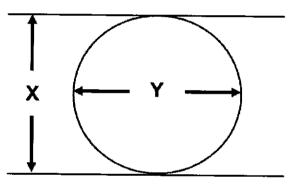


Fig. 3.2

Measure and record the dimensions of ${\bf X}$ and ${\bf Y}$ of one of the the cell in (b)(i) in eyepiece graticule units.

1. Answer within acceptable range

X: 4 – 30 epu Y: 3 – 28 epu

X = eyepiece graticule units
Y = eyepiece graticule units

[1]

(iii) Using the same objective lens you used in (b)(i), calibrate your eyepiece graticule using the 1 mm stage micrometer provided.

You should show all your working and use appropriate units.

- Show no. of epu corresponding to no. of division on stage micrometer under 40x objective lens
- 2. Show length of micrometer divided by no. of epu + answer 2.5 μm
 - 1 stage micrometer division = 4 eyepiece units
 - 1 eyepiece unit = $\frac{0.01}{4}$ mm = 0.0025 mm = 2.5 μ m

Reject: answers to 3 sf + answers in mm

1 eyepiece graticule unit =[2]

(iv) Using the measurement of length in eyepiece graticule units recorded in (b)(ii), calculate estimated area of the cell.

You should show all your working.

- 1. X + Y show working of number of epu x length of 1 epu
- 2. X x Y + correct calculation + precision

Area of the cell µm² [2]

The **concentration** of ascorbic acid can be estimated in a fruit extract by carrying out a test using starch solution and iodine solution.

lodine solution will be added one drop at a time using a syringe.

To practise releasing drops from a syringe:

- 1 Fill a syringe with 1.0 cm³ distilled water from beaker labelled **W** (from Question 1).
- 2 Hold the syringe over an empty test-tube as shown in Fig. 3.3 and push the plunger slowly to release one drop.
- 3 Repeat this until you can release one drop at a time.

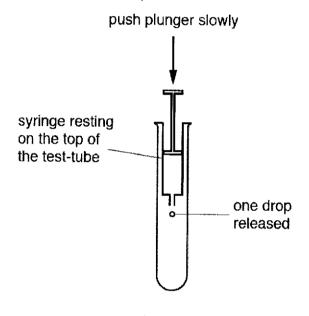


Fig. 3.3

You are provided with the materials shown in Table 3.1.

Table 3.1

| labelled | contents | hazard | volume/ cm ³ |
|----------|-----------------|--------|-------------------------|
| iodine | iodine solution | none | 20.0 |
| s | starch solution | none | 20.0 |
| X | tomato extract | none | 20.0 |

If any solutions come into contact with your skin, wash off immediately under cold water.

It is recommended that you wear suitable eye protection.

You will now carry out a test on **X** to find the volume of iodine solution that needs to be added for the end-point to be reached.

The end-point is when the blue colour remains after 10 seconds.

- 4 Put 1.0 cm³ of **S** into a test-tube.
- 5 Put 5.0 cm³ of X into the same test-tube.
- 6 Shake the test-tube gently to mix the contents.
- 7 Fill a 1.0 cm³ syringe with exactly 1.0 cm³ of iodine.
- 8 Wipe off any iodine from the outside of the syringe with a paper towel.
- 9 Put one drop of iodine, as shown in Fig. 3.3, into the mixture of S and X in the test-tube.
- 10 Mix gently and observe any colour change.
- 11 Repeat step 9 and step 10 until a blue colour appears. You may need to refill the syringe with iodine as in step 7.
- 12 When the blue colour appears, shake the test-tube gently for 10 seconds and see if the endpoint has been reached.
- 13 If the blue colour disappears then repeat step 9 to step 12 until the mixture stays blue for at least 10 seconds.
- 14 Record in (c)(i) the volume of iodine solution added.
- 15 Repeat steps 4 to 14 two more times and calculate average volume of iodine solution added.
- (i) Record your results in an appropriate table below.

| Type of solution | Volume of iodine needed to reach end-point/ cm³ | | | |
|------------------|---|-------------|-------------|---------|
| | Replicate 1 | Replicate 2 | Replicate 3 | Average |
| X | 0.900 | 0.900 | 0.900 | 0.900 |

- 1 heading for independent variable + left most column: Type of solution/ X + heading for dependent variables: volume of iodine solution added / cm³;
- 2 correct range (0.75 < X < 1.25) + 3 replicates/readings & average recorded;</p>
- 3 record to 2 or 3 d.p;

St Andrew's Junior College 2024

[3]

9744/04/Preliminary Examinations

Table 3.2 below shows the volume of iodine solution needed to reach the end-point at various concentration of ascorbic acid.

Table 3.2

| Concentration of ascorbic acid/ % | Volume of iodine solution needed to reach end-point/ cm³ | | |
|-----------------------------------|--|--|--|
| 0.10 | 2.15 | | |
| 0.08 | 1.60 | | |
| 0.06 | 1.25 | | |
| 0.04 | 0.75 | | |
| 0.02 | 0.35 | | |

- - According to student's answer in (c)(i).
 - Exact value given only if volume matches one of the concentration.

[Total: 20m]

