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Lab Book

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CONTENTS

INTRODUCTION	2
CORE PRACTICALS OVERVIEW	3
PAPER 3 PRACTICAL SKILLS	4
PAPER 6 PRACTICAL SKILLS	5
1 USE A SEMI-QUANTITATIVE METHOD WITH BENEDICT'S REAGENT TO ESTIMATE THE CONCENTRATIONS OF REDUCING SUGARS AND WITH IODINE SOLUTION TO ESTIMATE THE CONCENTRATIONS OF STARCH, USING COLOUR STANDARDS	6
2 INVESTIGATE THE VITAMIN C CONTENT OF FOOD AND DRINK	10
3 INVESTIGATE MEMBRANE PROPERTIES INCLUDING THE EFFECT OF ALCOHOL AND TEMPERATURE ON MEMBRANE PERMEABILITY	13
4 INVESTIGATE THE EFFECT OF TEMPERATURE, pH, ENZYME CONCENTRATION AND SUBSTRATE CONCENTRATION ON THE INITIAL RATE OF ENZYME-CATALYSED REACTIONS	18
5 (i) USE A LIGHT MICROSCOPE TO MAKE OBSERVATIONS AND LABELLED DRAWINGS OF SUITABLE ANIMAL CELLS; (ii) USE A GRATICULE WITH A MICROSCOPE TO MAKE MEASUREMENTS AND UNDERSTAND THE CONCEPT OF SCALE	27
6 PREPARE AND STAIN A ROOT TIP SQUASH TO OBSERVE THE STAGES OF MITOSIS	31
7 USE A LIGHT MICROSCOPE TO: MAKE OBSERVATIONS OF (i) TRANSVERSE SECTIONS OF ROOTS, STEMS, LEAVES; (ii) PLANT TISSUES; (iii) IDENTIFY SCLERENCHYMA FIBRES, PHLOEM, SIEVE TUBES, XYLEM VESSELS AND THEIR LOCATION	37
8 DETERMINE THE TENSILE STRENGTH OF PLANT FIBRES	41
9 INVESTIGATE THE ANTIMICROBIAL PROPERTIES OF PLANTS, INCLUDING ASEPTIC TECHNIQUES FOR THE SAFE HANDLING OF BACTERIA	45
10 INVESTIGATE THE EFFECTS OF LIGHT INTENSITY, LIGHT WAVELENGTH, TEMPERATURE AND AVAILABILITY OF CARBON DIOXIDE ON THE RATE OF PHOTOSYNTHESIS	51
11 CARRY OUT A STUDY OF THE ECOLOGY OF A HABITAT, SUCH AS USING QUADRATS AND TRANSECTS TO DETERMINE THE DISTRIBUTION AND ABUNDANCE OF ORGANISMS, AND MEASURING ABIOTIC FACTORS APPROPRIATE TO THE HABITAT	57
12 INVESTIGATE THE EFFECTS OF TEMPERATURE ON THE DEVELOPMENT OF ORGANISMS	62
13 INVESTIGATE THE RATE OF GROWTH OF MICROORGANISMS IN A LIQUID CULTURE, TAKING INTO ACCOUNT THE SAFE AND ETHICAL USE OF ORGANISMS	67
14 INVESTIGATE THE EFFECT OF DIFFERENT ANTIBIOTICS ON BACTERIA	74
15 USE AN ARTIFICIAL HYDROGEN CARRIER (REDOX INDICATOR) TO INVESTIGATE RESPIRATION IN YEAST	79
16 USE A SIMPLE RESPIROMETER TO DETERMINE THE RATE OF RESPIRATION AND RQ OF A SUITABLE MATERIAL (SUCH AS GERMINATING SEEDS OR SMALL INVERTEBRATES)	83
17 INVESTIGATE THE EFFECTS OF EXERCISE ON TIDAL VOLUME, BREATHING RATE, RESPIRATORY MINUTE VENTILATION, AND OXYGEN CONSUMPTION USING DATA FROM SPIROMETER TRACES	88
18 INVESTIGATE THE PRODUCTION OF AMYLASE IN GERMINATING CEREAL GRAINS	92
MATHS SKILLS	99
ANSWERS	101

INTRODUCTION

Practical work is central to the study of biology. The International Advanced Subsidiary/Advanced Level (IAS/IAL) specification includes 18 core practical activities that link theoretical knowledge and understanding to practical scenarios. By completing the core practical activities, you will learn to:

- follow and interpret experimental instructions, covering the full range of laboratory exercises set throughout the course, with minimal help from your teacher
- always work with interest and enthusiasm in the laboratory, completing most laboratory exercises in the time allocated
- manipulate apparatus, use chemicals, carry out all common laboratory procedures and use datalogging (where appropriate)
- work sensibly and safely in the laboratory, paying due regard to health and safety requirements without the need for reminders from your teacher
- gain accurate and consistent results in quantitative exercises, and make the most of the expected observations in qualitative exercises.

By the end of this course, you should be able to use a variety of apparatus and techniques to:

- design and carry out both the core practical activities and your own investigations
- collect data that can be analysed
- use data to draw valid conclusions.

Your knowledge and understanding of practical skills and activities will be assessed in all examination papers.

- Papers 1 and 2 (IAS) and 4 and 5 (IAL) will include questions based on practical activities, including novel scenarios.
- Paper 3 (IAS) and Paper 6 (IAL) will test your ability to plan practical work, including risk management and selection of apparatus.

Assessment of your practical knowledge and skills will focus on three main areas:

- **Planning:** You will be expected to plan an experiment set by Pearson (but you will not need to carry it out).
- **Implementation and measurements:** You will be given details of an experiment carried out by an inexperienced student, and asked to comment on the investigation.
- **Processing results/Analysing:** You will need to analyse a set of experimental results.

The ways in which you can demonstrate these practical skills are outlined in the tables on pages 4 and 5. You may wish to tick off each element as you gain confidence.

You will find answers and maths skills required for the practicals in the back of the book.

CORE PRACTICALS OVERVIEW

UNIT 1 (IAS): MOLECULES, DIET, TRANSPORT AND HEALTH

- 1 Use a semi-quantitative method with Benedict's reagent to estimate the concentrations of reducing sugars and with iodine solution to estimate the concentrations of starch, using colour standards
- 2 Investigate the vitamin c content of food and drink
- 3 Investigate membrane properties including the effect of alcohol and temperature on membrane permeability
- 4 Investigate the effect of temperature, pH, enzyme concentration and substrate concentration on the initial rate of enzyme-catalysed reactions

UNIT 2 (IAS): CELLS, DEVELOPMENT, BIODIVERSITY AND CONSERVATION

- 5 (i) Use a light microscope to make observations and labelled drawings of suitable animal cells; (ii) use a graticule with a microscope to make measurements and understand the concept of scale
- 6 Prepare and stain a root tip squash to observe the stages of mitosis
- 7 Use a light microscope to: (i) make observations, draw and label plan diagrams of transverse sections of roots, stems and leaves; (ii) make observations, draw and label cells of plant tissues; (iii) identify sclerenchyma fibres, phloem, sieve tubes and xylem vessels and their location
- 8 Determine the tensile strength of plant fibres
- 9 Investigate the antimicrobial properties of plants, including aseptic techniques for the safe handling of bacteria

UNIT 4 (IAL): ENERGY, ENVIRONMENT, MICROBIOLOGY AND IMMUNITY

- 10 Investigate the effects of light intensity, light wavelength, temperature and availability of carbon dioxide on the rate of photosynthesis using a suitable aquatic plant
- 11 Carry out a study of the ecology of a habitat, such as using quadrats and transects to determine the distribution and abundance of organisms, and measuring abiotic factors appropriate to the habitat
- 12 Investigate the effects of temperature on the development of organisms (such as seedling growth rate or brine shrimp hatch rates), taking into account the ethical use of organisms
- 13 Investigate the rate of growth of microorganisms in a liquid culture, taking into account the safe and ethical use of organisms
- 14 Investigate the effect of different antibiotics on bacteria

UNIT 5 (IAL): RESPIRATION, INTERNAL ENVIRONMENT, COORDINATION AND GENE TECHNOLOGY

- 15 Use an artificial hydrogen carrier (redox indicator) to investigate respiration in yeast
- 16 Use a simple respirometer to determine the rate of respiration and RQ of a suitable material
- 17 Investigate the effects of exercise on tidal volume, breathing rate, respiratory minute ventilation, and oxygen consumption using data from spirometer traces
- 18 Investigate the production of amylase in germinating cereal grains

PAPER 3 PRACTICAL SKILLS

Practical skills	Core practical								
	1	2	3	4	5	6	7	8	9
Planning									
Identify the apparatus required									
Identify the dependent and independent variables, standardised or controlled variables									
Describe how to measure relevant variables using the most appropriate instrument and correct measuring techniques									
Identify and state how to control all other relevant variables so that the investigation is a fair test									
Discuss whether repeat readings are appropriate									
Identify health and safety issues and discuss how these may be dealt with									
Discuss how the data collected will be used									
Identify possible sources of uncertainty and/or systematic error and explain how these may be reduced or eliminated									
Comment on the implications of biology (for example, benefits/risks) and on its context (for example, social/environmental/historical)									
Implementation and measurements	1	2	3	4	5	6	7	8	9
Comment on the number of readings taken									
Comment on the range of measurements taken									
Comment on significant figures									
Check a reading that is inconsistent with other readings (for example, a point that is not on the line of a graph)									
Comment on how the experiment may be improved, possibly by using additional apparatus (for example, to reduce errors)									
Processing results	1	2	3	4	5	6	7	8	9
Perform calculations, using the correct number of significant figures									
Plot results on a graph using an appropriate scale									
Use the correct units throughout									
Comment on the relationship obtained from the graph									
Determine the relationship between two variables or determine a constant with the aid of a graph (for example, by determining the gradient using a large triangle)									
Suggest realistic modifications to reduce errors									
Suggest realistic modifications to improve the experiment									
Discuss uncertainties, qualitatively and quantitatively									

PAPER 6 PRACTICAL SKILLS

Practical skills	Core practical								
	10	11	12	13	14	15	16	17	18
Planning									
Identify the most appropriate apparatus, giving details. Apparatus may include the range and resolution of instruments and/or relevant dimensions of apparatus (for example, the area of a quadrat used for an ecological investigation)									
Formulate a null hypothesis									
Identify the dependent and independent variables, standardised or controlled variables									
Discuss calibration of instruments (for example, whether a meter reads zero before measurements are made)									
Describe how to measure relevant variables using the most appropriate instrument(s) and techniques									
Identify and state how to control all other relevant variables so that the investigation is a fair test									
Discuss whether repeat readings are appropriate									
Identify health and safety issues and discuss how they may be dealt with									
Identify any ethical issues involved with the use of living organisms									
Discuss how the data collected will be used									
Implementation and measurements	10	11	12	13	14	15	16	17	18
Comment on how the experiment could have been improved, possibly by using additional apparatus (for example, to reduce errors)									
Comment on the number of readings taken									
Comment on the range of measurements taken									
Comment on significant figures – students may be required to identify and/or round up any incorrect figures in a table of results									
Identify and/or amend units that are incorrect									
Identify and check a reading that is inconsistent with other readings, (for example, a point that is not on the line of a graph)									
Analysing data	10	11	12	13	14	15	16	17	18
Explain how data should be tabulated, with appropriate units									
Perform calculations, using the correct number of significant figures									
Plot results on a graph using an appropriate scale and units – the graph could be logarithmic in nature									
Use the correct units throughout									
Comment on the trend/pattern obtained									
Determine the relationship between two variables or determine a constant with the aid of a graph (for example, by determining the gradient using a large triangle)									
Suggest realistic modifications to reduce errors									
Suggest realistic modifications to improve the experiment									

CORE PRACTICAL 1: USE A SEMI-QUANTITATIVE METHOD WITH BENEDICT'S REAGENT TO ESTIMATE THE CONCENTRATIONS OF REDUCING SUGARS AND WITH IODINE SOLUTION TO ESTIMATE THE CONCENTRATIONS OF STARCH, USING COLOUR STANDARDS

SPECIFICATION
REFERENCE

1.3

Procedure – Part 1: Benedict's reagent method

- 1 Plan how you will use the stock 2% glucose solution to make the following five concentrations of glucose solution: 2%, 1%, 0.5%, 1.5% and 0.25%. Write your plan in the space below.
- 2 Use the waterproof pen to label six test tubes and five small beakers with the different glucose concentrations they will contain. Label the sixth test tube 'fruit juice'.
- 3 Use the syringes, the distilled water and the 2% glucose solution to create 5 cm³ of each solution in the corresponding labelled beaker.
- 4 Use a clean syringe to add 2 cm³ of Benedict's reagent to each of the six labelled test tubes.
- 5 Using a clean syringe each time, add 1 cm³ of each glucose solution to the corresponding labelled test tubes.
- 6 Add 1 cm³ of fruit juice to the last labelled test tube.
- 7 Give each tube a gentle shake to ensure the contents are mixed.
- 8 Place all six test tubes in the water bath and start the timer.
- 9 After 2 minutes, use tongs to remove the test tubes from the water bath and place them in the test tube rack. Take care: the tubes will be hot.
- 10 Observe the tubes and record the results in a suitable table.

Dilutions planning (Use this space to plan how you will carry out your dilutions.)

Objectives

- To understand what is meant by a semi-quantitative test
- To be able to estimate concentrations of reducing sugars using Benedict's reagent
- To be able to estimate concentrations of starch using iodine solution
- To develop the skills needed to plan an investigation using dilutions

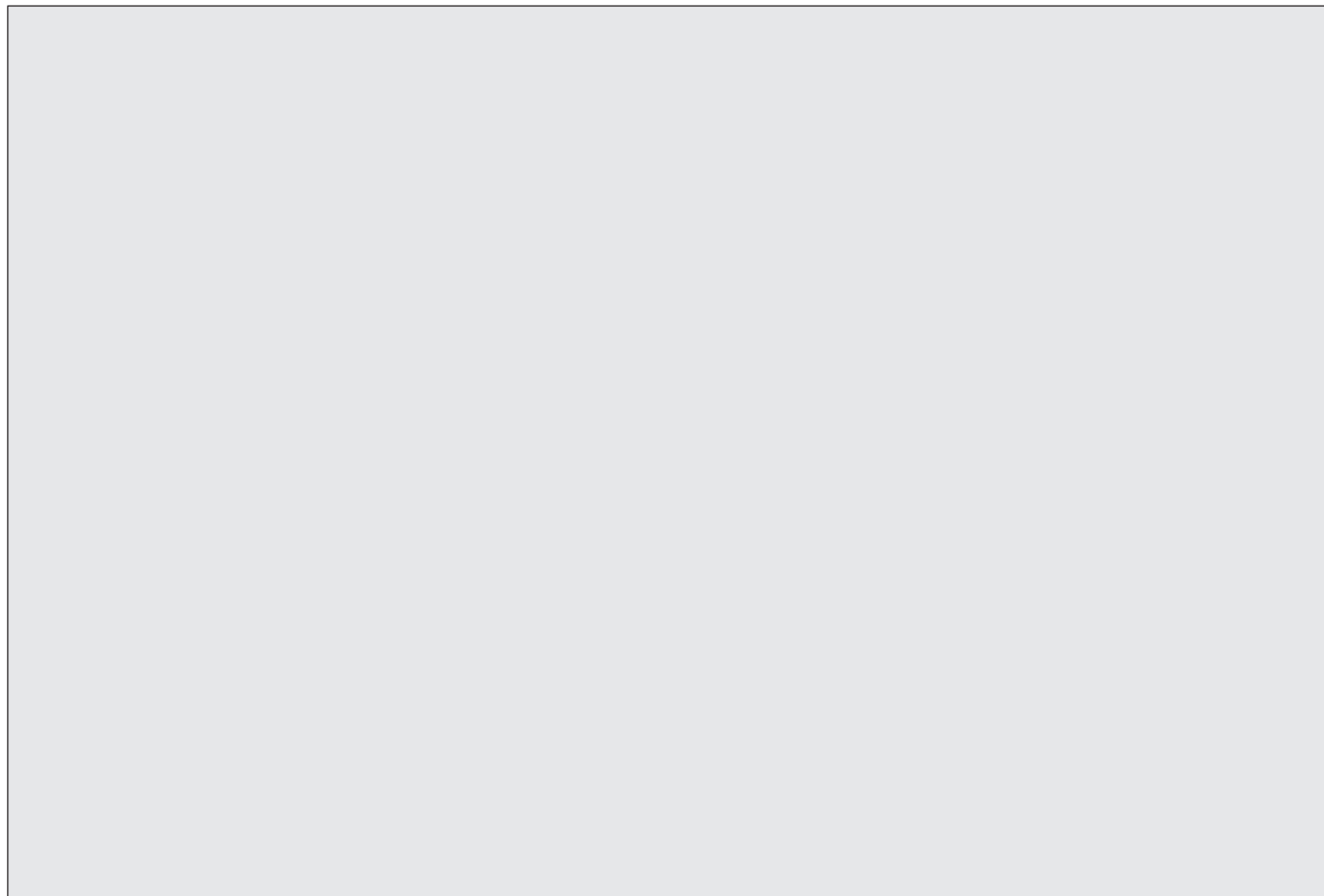
Equipment

- eye protection
- test tubes, tongs and test tube rack
- small beakers
- Benedict's reagent
- iodine solution
- 2% glucose solution
- 2% starch solution
- 'unknown' starch solution
- 1/10 dilution of fruit juice
- 1 cm³ and 5 cm³ syringes
- 3 cm³ pipettes
- distilled water
- waterproof marker pen
- water bath at 60–80 °C or a large beaker of recently boiled water
- timer or stop clock

Safety

- Wear eye protection.
- Avoid skin contact with Benedict's reagent, iodine and hot solutions.
- Handle the test tubes with tongs to avoid burns.
- Do not taste the fruit juice.

Results (Use this space to record your results.)

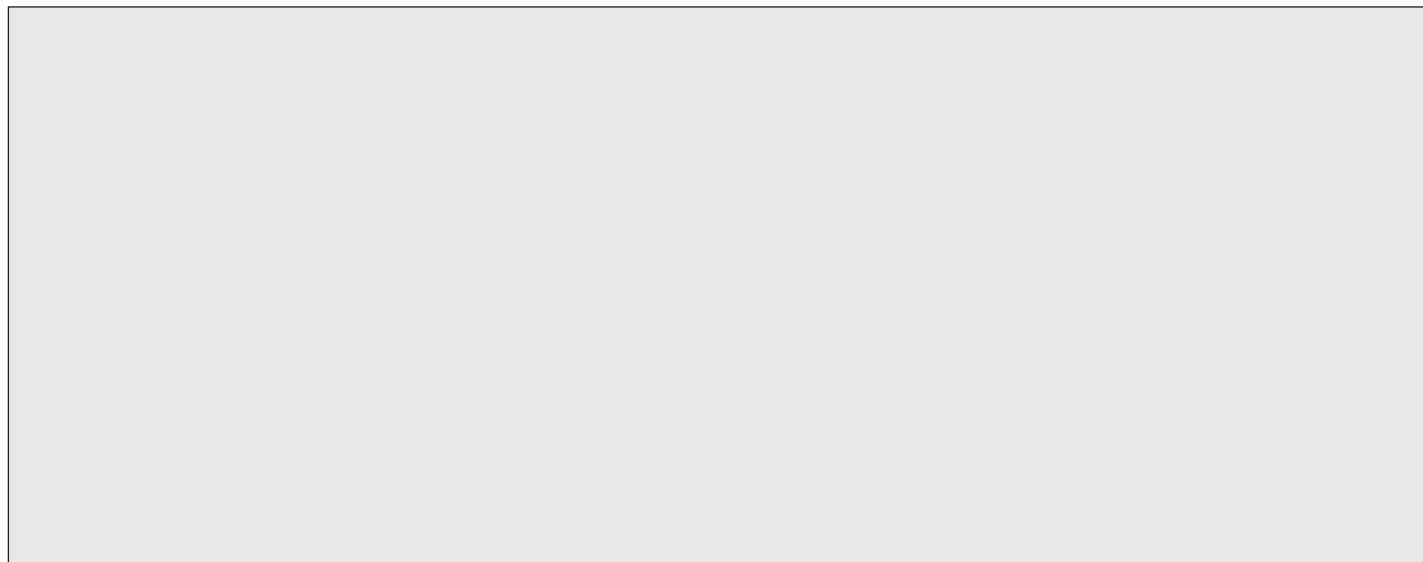


Learning tips

- When recording the results of the Benedict's test, use the correct descriptive language. Simply stating 'green' or 'blue' does not provide enough detail to allow comparison.
- One of the reasons for making the test semi-quantitative is to allow easier comparison, which may be less subjective and therefore more accurate. You will be expected to know the steps needed in the procedure and the expected results.

Analysis of results

Record which of the samples of glucose solution was closest to the colour of the sample in the fruit juice tube.



Procedure – Part 2: Iodine solution method

- 1 Plan how you will use the stock 2% starch solution to make the following five concentrations of starch solution: 2%, 1%, 0.5%, 0.2% and 0.1%. Write your plan in the space below.
- 2 Use the waterproof pen to label five test tubes and five small beakers with the different starch concentrations they will contain. Label the sixth test tube 'unknown'.
- 3 Use the syringes, the distilled water and the 2% starch solution to create 5 cm³ of each solution in the corresponding labelled beaker.
- 4 Use a clean syringe to add 0.5 cm³ of iodine to each of the six labelled test tubes. Add a further 10 cm³ of distilled water to each tube.
- 5 Using a clean syringe each time, add 5 cm³ of each starch solution to the corresponding labelled test tube.
- 6 Add 5 cm³ of the 'unknown' starch solution to the sixth test tube. Compare the colour produced in this tube with the colour of the diluted test tubes.
- 7 Record your results in a suitable table.

Dilutions planning (Use this space to show how you will carry out your dilutions.)

Results (Use this space to record your results.)

Analysis of results

Record which of the samples of starch solution was closest to the colour of the sample in the 'unknown' tube.

Questions

1 How could these investigations have been adapted to produce quantitative results?

.....
.....

2 Explain why the Benedict's test is not a suitable test for measuring the total sugar content of fruit juice.

.....
.....

3 Suggest one way in which the results of the Benedict's reagent investigation could have been made more reliable.

.....
.....
.....

4 How could you evaluate the accuracy of your experimental value for the concentration of glucose in the fruit juice?

.....
.....
.....

5 Name a variable that you controlled in the iodine investigation, explain how you controlled it and describe the possible effect on the result if it had not been controlled.

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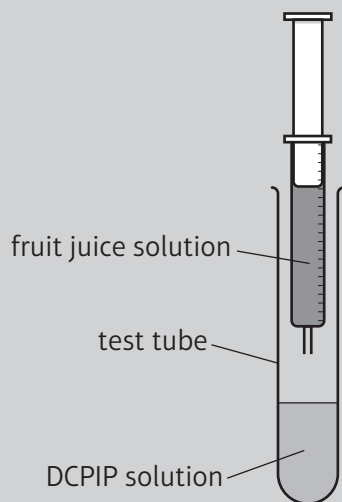


figure A Dropping fruit juice solution into DCPIP

Procedure

It is possible to determine the concentration of vitamin C in a solution by using dichlorophenolindophenol (DCPIP). Vitamin C is an antioxidant, so it reduces the DCPIP causing a colour change. By using a solution of vitamin C with a known concentration, it is possible to calculate the concentration of vitamin C in other solutions, for example, in fruit juices (see **figure A**).

- 1** Use the 5 cm³ syringe to draw up 5 cm³ of 1% DCPIP. Shake the syringe to expel any air bubbles.
- 2** Add 1 cm³ of DCPIP to a test tube.
- 3** Use a clean 5 cm³ syringe to draw up 5 cm³ of the 1% vitamin C solution.
- 4** Add the vitamin C solution to the test tube containing the DCPIP, one drop at a time. After each drop, shake the test tube slightly to ensure the solutions have mixed.
- 5** Continue to add vitamin C solution until the blue colour of the DCPIP disappears.
- 6** Record the volume of vitamin C solution added. You can find this volume by subtracting the value on the syringe from the original 5 cm³ in the syringe.
- 7** Repeat steps 2–6 twice more and calculate a mean value for the volume of 1% vitamin C solution needed to decolorise 1 cm³ of DCPIP.
- 8** Add 1 cm³ of DCPIP to a clean test tube.
- 9** Use a clean syringe to draw up 5 cm³ of a fruit juice. Add the fruit juice to the DCPIP one drop at a time. Record the volume of juice needed to cause the blue colour to disappear.
- 10** Repeat steps 8 and 9 twice more, then calculate the volume of fruit juice needed to decolorise 1 cm³ of DCPIP.
- 11** Repeat steps 8–10 with the other fruit juices.

Learning tips

- Ensure that the drops of vitamin C solution or fruit juice land directly in the DCPIP and do not stick to the side of the test tube. Otherwise, your results will not be accurate.
- Acidic fruit juices will not completely decolorise the DCPIP; instead, the solution will turn pink. This should be taken into consideration.

Objectives

- To be able to calculate the vitamin C concentration of fruit juices using the titration method
- To solve problems set in practical contexts
- To process and analyse data using appropriate mathematical skills

Equipment

- eye protection
- 1% DCPIP solution
- 1% vitamin C solution
- a selection of fruit juices
- test tubes
- test tube rack
- small beakers
- small syringes 1 cm³ and 5 cm³

! Safety

- Wear eye protection.
- Avoid skin contact with the DCPIP and test tube solutions.
- Do not taste the fruit juice.

Results (Use this space to record your results.)

Analysis of results

- 1 Write down the average volume of vitamin C solution needed to decolorise DCPIP (based on your results).

- 2 1 cm³ of the vitamin C solution contains 10 mg of vitamin C. You can use this information and your calculation of the average volume of 1% vitamin C solution required to decolorise the DCPIP to calculate the concentration of vitamin C in the fruit juices. Use the formula:

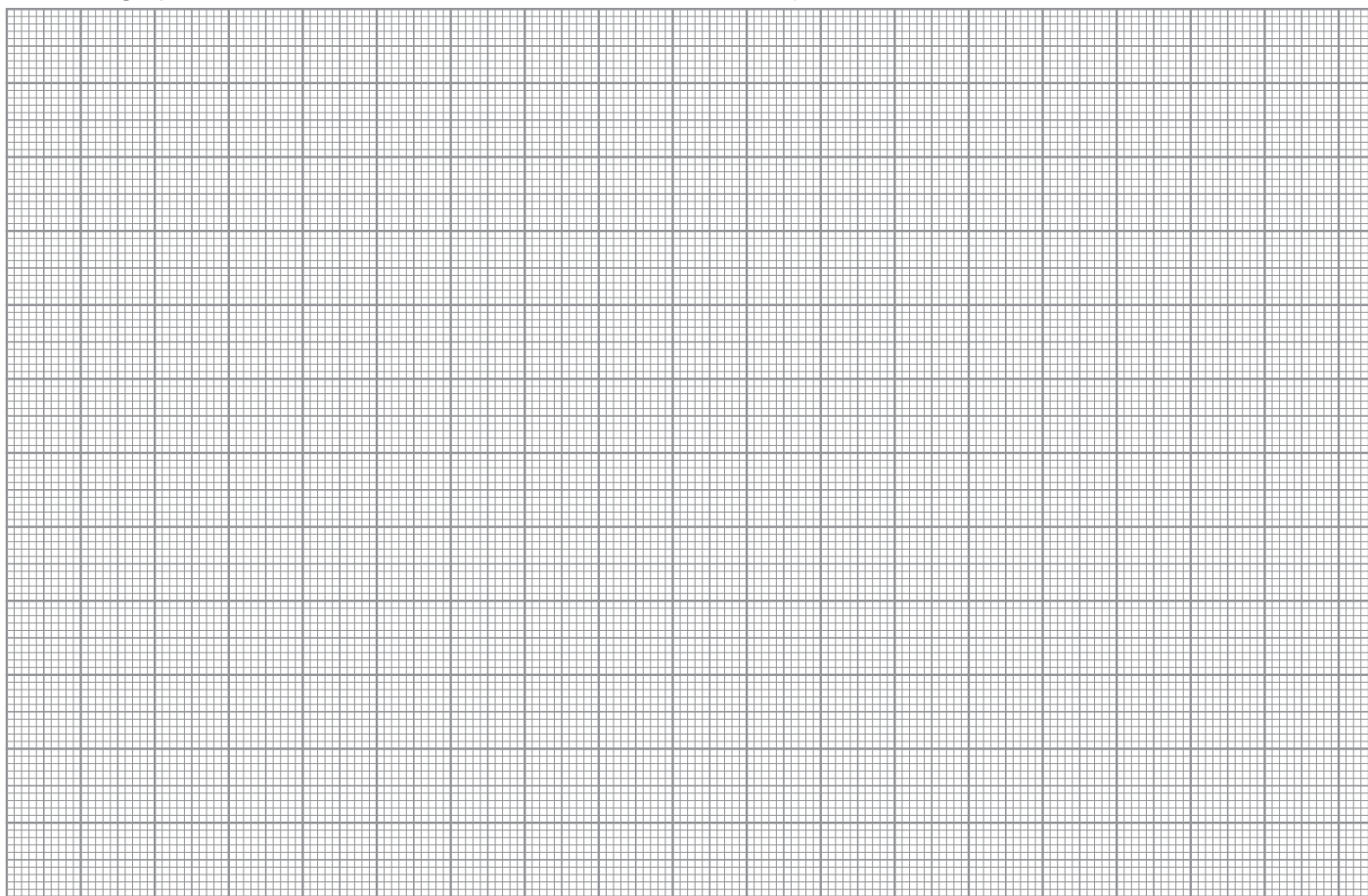
$$\text{concentration of vitamin C in fruit juice} = \frac{\text{volume of standard solution}}{\text{volume of fruit juice}} \times \text{concentration of standard solution}$$

For example, if the volume of 1% vitamin C solution needed to decolorise the DCPIP was 1.4 cm³ and the volume of fruit juice used was 2.6 cm³, the calculation would be:

$$\begin{aligned} \text{concentration of vitamin C in fruit juice} &= \frac{1.4}{2.6} \times 10 \\ &= 0.5 \times 10 \\ &= 5.4 \text{ mg} \end{aligned}$$

Use the formula to calculate the concentration of vitamin C in each of the fruit juices used.

3 Plot a graph to show the results obtained for the different fruit juices.



Questions

1 What were the independent and dependent variables in this investigation?

.....
.....

2 Why was each titration completed three times to calculate a mean?

.....
.....

3 Suggest one reason why syringes were used in this investigation rather than burettes.

.....
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.....
.....

4 Which of the fruit juices tested contained the highest concentration of vitamin C?

.....
.....

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Research

Find out why beetroots appear red, what causes the red pigment to escape from cells and why this happens.

Use this space to record your findings, citing all your sources using an appropriate format.

Learning tips

- Make sure you know how to cite references from scientific journals correctly. For example:
Butler, K.G. (2000) Pollen germination across the seasons. *School Science Review*, 82 (298), 93–94.
This format should be followed for all references to scientific journals, even if articles are available online. Do not provide only the web address.
- Make sure you draw tables and graphs correctly. The independent variable should always go in the left-hand column of a results table and on the horizontal axis of a graph. Numbers in tables, including calculations, should only be reported to the limits of the least accurate measurement. Data on graphs should be scaled so that the graph fills more than half of the available space.

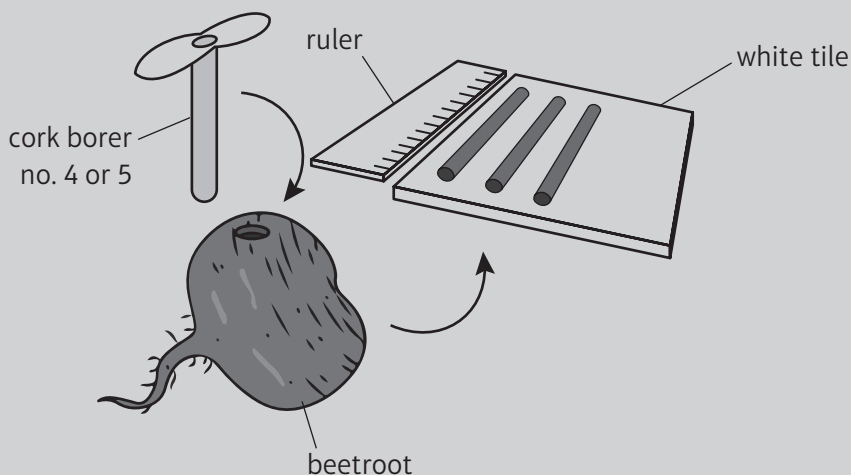


figure A Use a cork border to cut cylinders of beetroot.

Objectives

- To know how the effect of temperature and alcohol on membranes can be determined
- To be able to recognise quantitative variables that should be controlled in an investigation

Equipment

- eye protection
- water baths pre-set at required temperatures
- thermometer
- distilled water
- syringe
- beetroot
- cork borer
- ruler
- white tile
- knife
- 10 cm³ syringe
- pipette
- test tubes
- colorimeter
- cuvettes
- labels or pens for labelling
- forceps
- crushed ice
- ethanol (at 10%, 20%, 30%, 40% concentrations)

! Safety

- Wear eye protection.
- Water baths at temperatures above 50 °C may scald. Take care when removing lids to allow steam to escape away from the face or body. If you are splashed by hot water, or scalded by steam, cool under cold running water immediately.
- Take care with sharp items such as the cork borer and knife. Always cut or push downwards onto the tile.

(continued)

Procedure

Beetroots are root vegetables that appear red because the vacuoles in their cells contain a water soluble red pigment called betalain. These pigment molecules are too large to pass through cell membranes.

The effect of either temperature or alcohol on membranes can be investigated.

Part 1: Effect of temperature

- 1 Prepare eight water baths pre-set to a range of temperatures between 0 and 70 °C.
- 2 Take eight test tubes and label each one with the temperature of one of the water baths. Use a syringe to add 10 cm³ of distilled water to each test tube.
- 3 Place each tube in the water bath set to the corresponding temperature and leave for 5 minutes.
- 4 Check the temperature of each bath using a thermometer. The temperatures are unlikely to be exactly right, so record the actual temperatures.
- 5 Use a cork borer to cut eight beetroot cylinders (see **figure A**, page 13). Use a knife, ruler and white tile to trim them all to the same length (1 cm is sufficient). Wash the cylinders thoroughly with water until the water runs clear, then gently pat dry with a paper towel.
- 6 Add one beetroot cylinder to each test tube and leave in the water bath for 15 minutes.
- 7 Shake the tubes once. Then, working quickly and carefully, use forceps to remove the cylinder from each tube. Discard the cylinders but keep the supernatant liquid (the clear liquid above the solid). It may be easier to decant this liquid into clean test tubes.
- 8 Set the colorimeter to a blue/green filter and percentage transmission. Zero the colorimeter using a blank cuvette filled with distilled water.
- 9 Transfer liquid from each test tube in turn into a colorimeter cuvette, place in the colorimeter and take the percentage transmission reading. Record your results in a suitable table.

Results

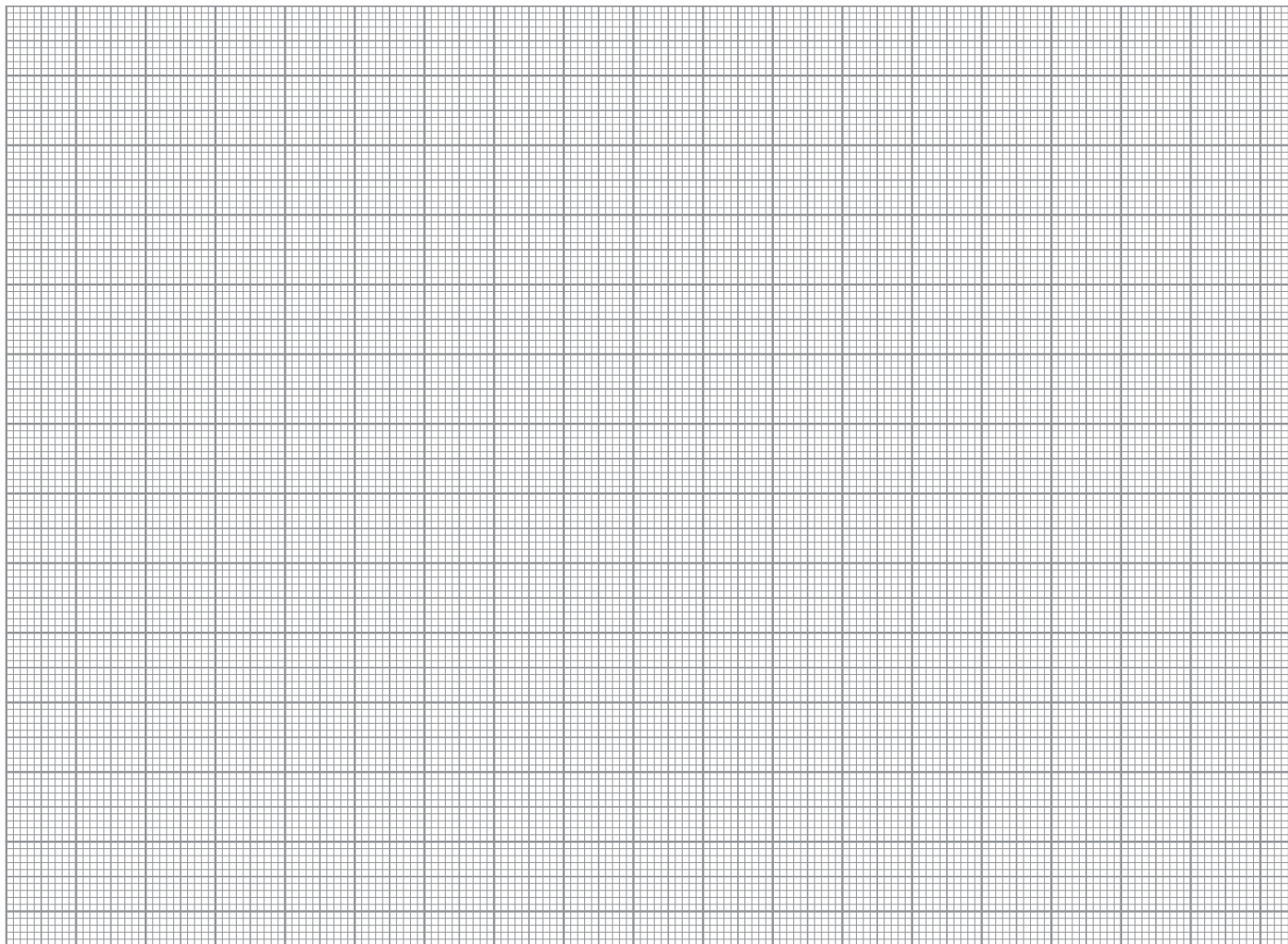
Using pooled class results if necessary, record your results in a suitable table showing repeats and a mean for each temperature.

! Safety (continued)

- Ethanol is highly flammable so keep away from naked flames and keep the stoppers on bottles.
- Do not handle electric plugs, sockets or switches with wet hands.
- Wash hands with soap and water when finished.

Analysis of results

- 1 Highlight the maximum and minimum values at each temperature and decide if any values are anomalies (i.e. results that show a substantial deviation from the general pattern of results). If possible, repeat the tests that produced these results and calculate a new mean.
- 2 Plot a graph of mean percentage transmission against temperature. Do not forget to use the actual temperature of each water bath. Add error bars to show the range of transmission values at each temperature.

**Part 2: Effect of alcohol**

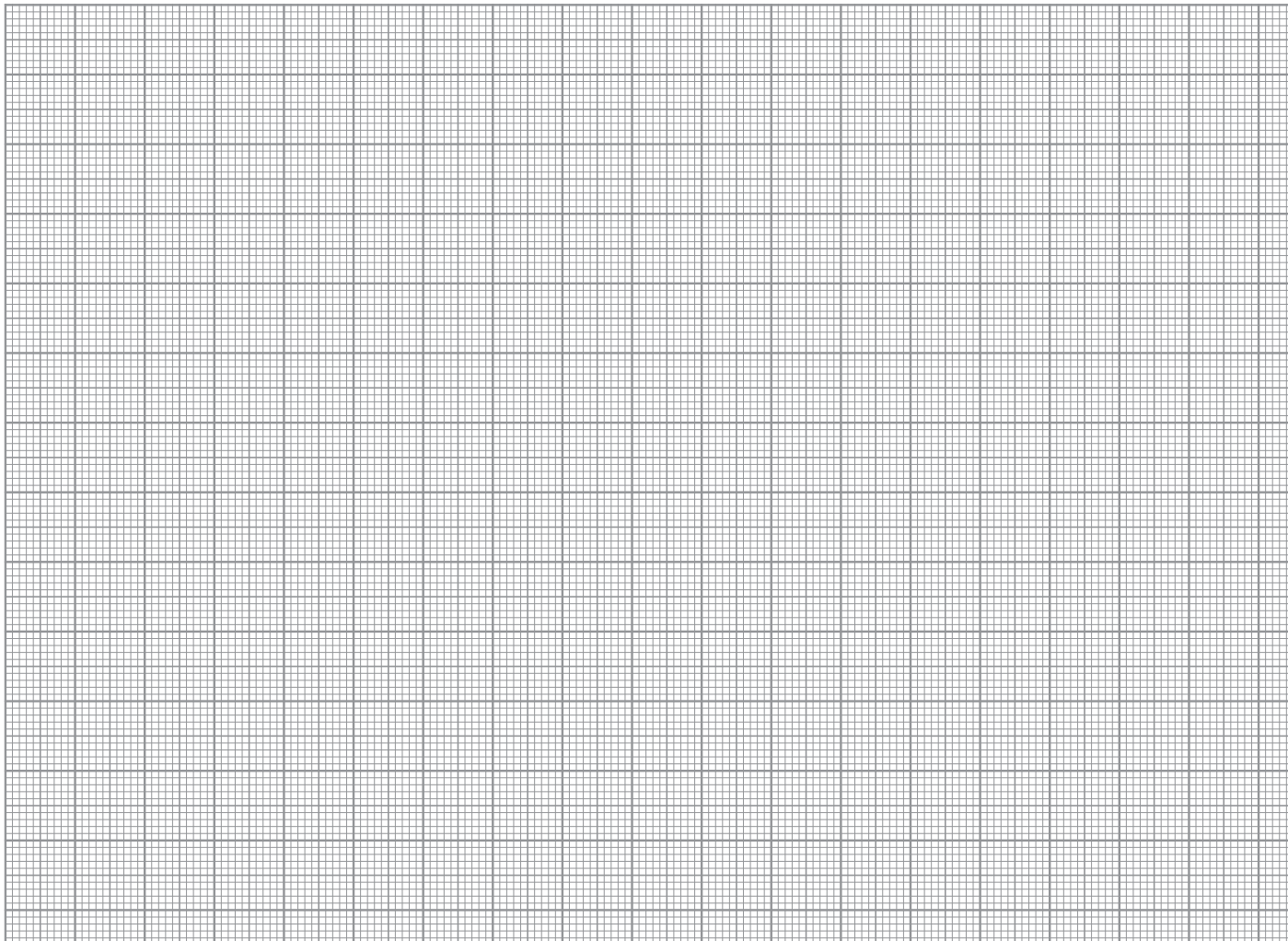
- 1 Take five test tubes and add 10 cm³ of ethanol to each one. Use a different concentration of ethanol in each tube (distilled water can be used for a 0% concentration).
- 2 Use a cork borer to cut five beetroot cylinders. Use a knife, ruler and white tile to trim them all to the same length (1 cm is sufficient). Wash the cylinders thoroughly with water until the water runs clear, then gently pat dry with a paper towel.
- 3 Add one beetroot cylinder to each of the five tubes and leave for 15 minutes.
- 4 Shake the tubes once. Then, working quickly and carefully, use forceps to remove the cylinder from each tube. Discard the cylinders but keep the supernatant liquid (the clear liquid above the solid). It may be easier to decant this liquid into clean test tubes.
- 5 Set the colorimeter to a blue/green filter and percentage transmission. Zero the colorimeter using a blank cuvette filled with distilled water.
- 6 Transfer liquid from each test tube in turn into a colorimeter cuvette, place in the colorimeter and take the percentage transmission reading. Record your results in a suitable table.

Results

Using pooled class results if necessary, record your results in a suitable table showing repeats and a mean for each concentration of alcohol.

Analysis of results

- 1** Highlight the maximum and minimum values at each concentration and decide if any values are anomalies (results that show a substantial deviation from the general pattern of results). If possible, repeat the tests that produced these results and calculate a new mean.
- 2** Plot a graph of mean percentage transmission against concentration. Do not forget to use the actual temperature of each water bath. Add error bars to show the range of transmission values at each concentration of alcohol.



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Questions

1 List the variables that were controlled during the experiment and state how they were controlled. This could be done using a table.

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2 Suggest why the tubes were placed in the water baths for 5 minutes before the cylinders were added.

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3 Why were the beetroot cylinders washed with distilled water and dried before the experiment began?

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4 Use the trend line of one of your graphs to describe the effect of temperature or alcohol concentration on the percentage transmission.

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5 Explain your results in detail in terms of what is happening to the beetroot membrane.

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Procedure – Planning

Milk protein (casein) is broken down by protease enzymes such as trypsin. The opaque white colour of the milk is replaced by a clear solution. Light passes more easily through the final solution, so the reaction can be monitored using a colorimeter (see **figure A**).

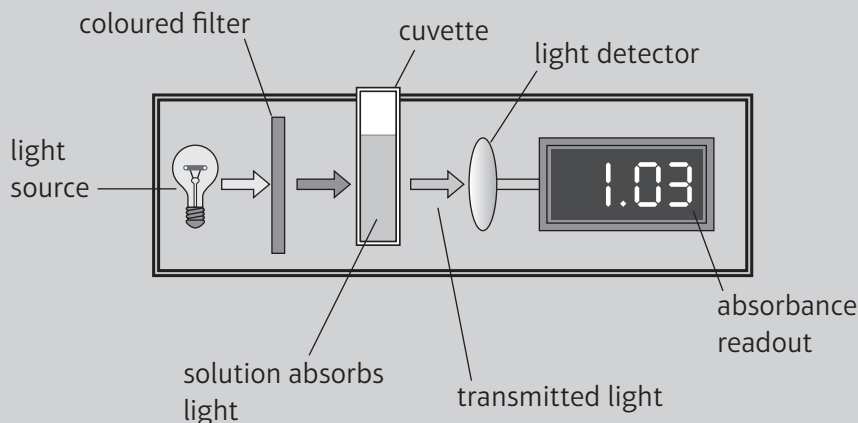


figure A Using a colorimeter

There are four variables which can change in this investigation: temperature, enzyme concentration, pH or substrate concentration. The procedures for each variable are given below. Although you may not complete all of these investigations, you are expected to have an understanding of the method for each one.

Choosing a range of values

Before you begin, you need to select a range of five values for the independent variable being investigated. When choosing the range of values for your independent variable, it is important to consider safety and validity. Values that are very close together are likely to produce very similar results, which may make it difficult to see a pattern. However, if the intervals between values are too large, this can also make it difficult to identify a pattern. If you use the same interval between the values tested, it will be easier to make a comparison between values. It is also essential to think about safety: consider whether any of the values chosen could present a hazard during the experiment.

Record your values in the spaces below.

Temperature (Use this space to record your selected temperatures.)

Objectives

- To be able to measure the initial rate of enzyme activity
- To understand why measuring the initial rate is important
- To understand the variables that can affect the rate of an enzyme-catalysed reaction and the result of changing each variable
- To be able to calculate the rate of a reaction using the gradient of a line

Equipment

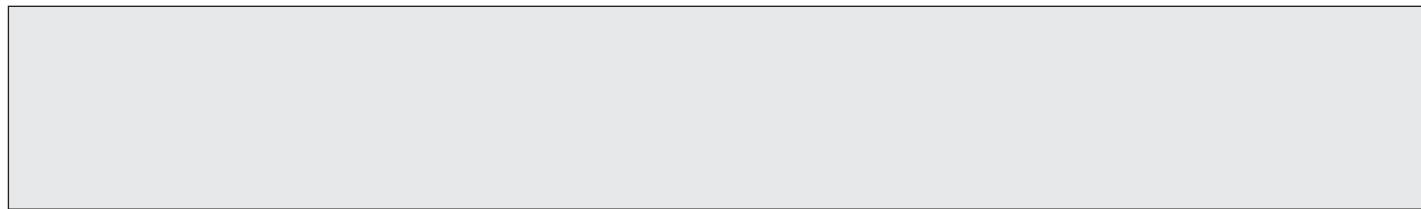
- eye protection
- skimmed milk powder suspension (2%)
- standard protease (trypsin) solution (1%)
- test tubes and holder
- pipettes
- access to colorimeter (or light meter with datalogger)
- two cuvettes
- distilled water
- water baths or beakers kept at set temperatures
- thermometers
- pH buffers at pH 5, 6, 7, 8, 9, 10

Safety

- Wear eye protection.
- Avoid skin contact with the trypsin solution. If you get it on your skin, wash it off with cold running water and tell your teacher.
- Hot water from the water baths may be a hazard. If a water bath is above 50°C, take care not to scald yourself.

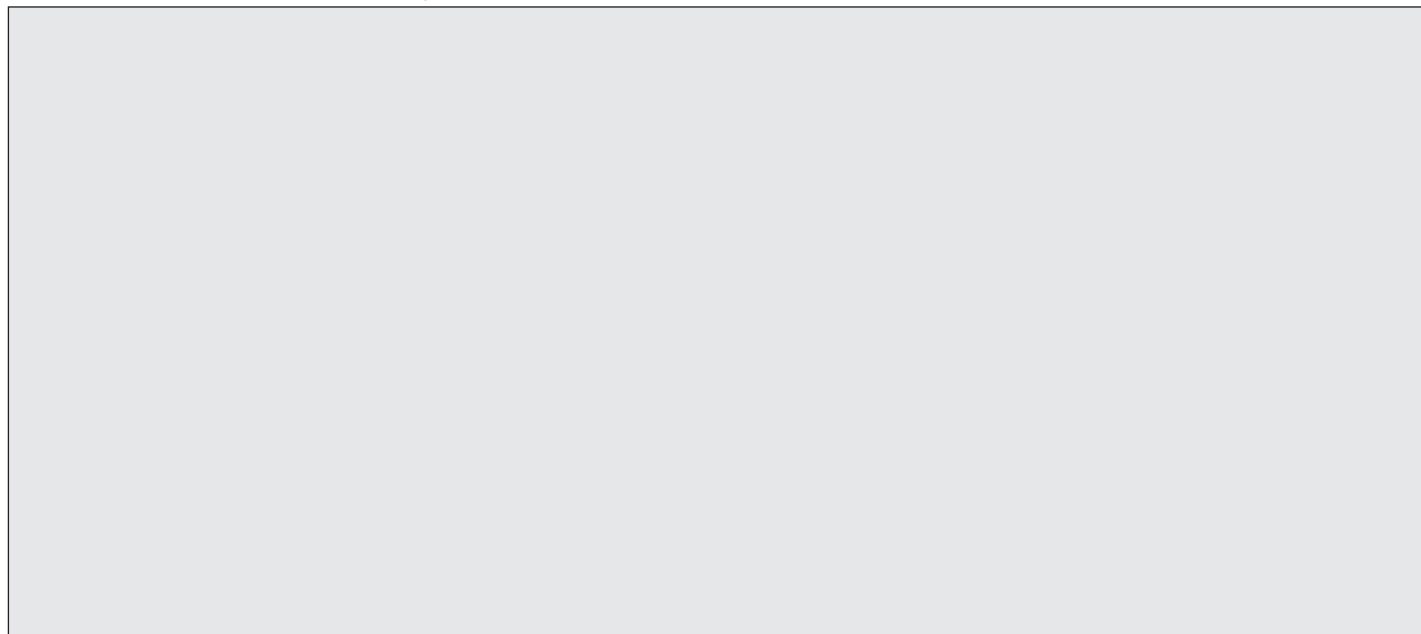
pH (Use this space to record your selected pH values.)

Choose a range of buffers so you can test different pH values for the solution. You should consider which buffers to use and the intervals between them. Also consider safety and the risks associated with using very high or very low pH solutions.



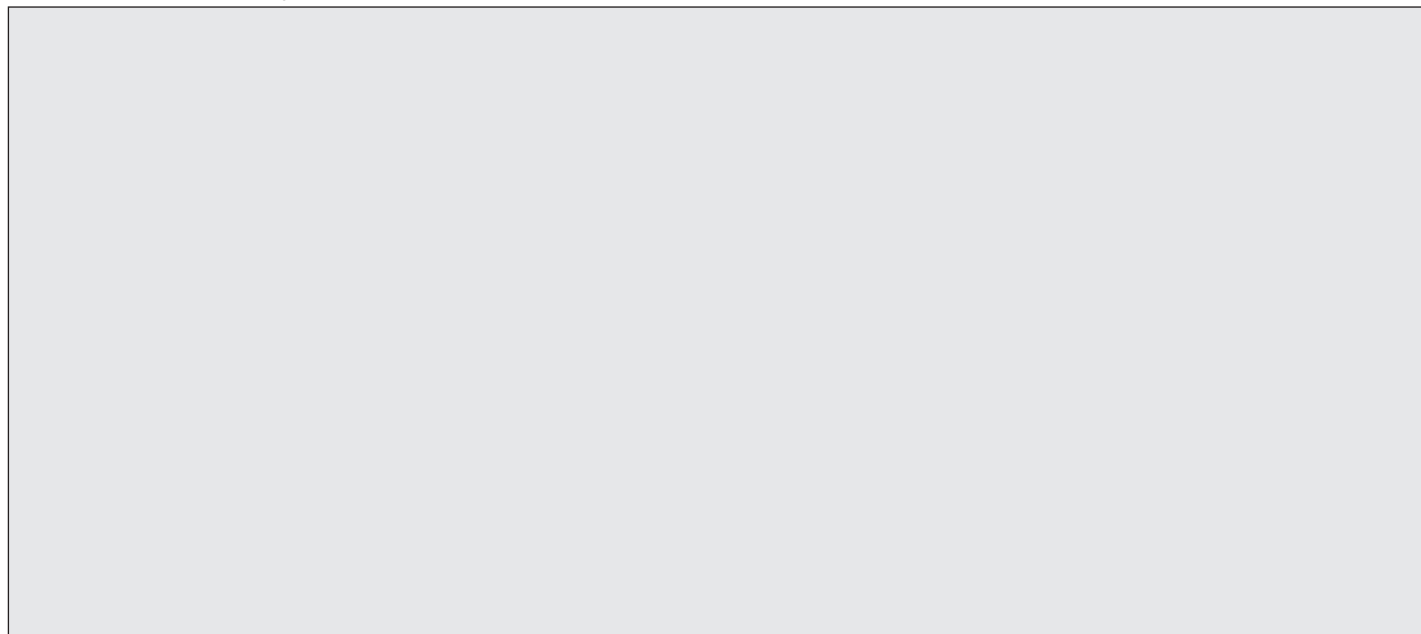
Enzyme concentration (Use this space to record your dilutions.)

Plan how you will dilute the 1% trypsin stock solution with distilled water to produce additional test solutions of 0.2%, 0.4%, 0.6% and 0.8%. Aim to produce 10 cm³ of each concentration.



Substrate concentration (Use this space to record your dilutions.)

Plan how you will dilute the 2% milk solution with distilled water to produce additional test solutions of 0.2%, 0.4%, 0.6% and 0.8%. Aim to produce 10 cm³ of each concentration.



Procedure – Part 1: Temperature

- 1 Prepare water baths for your chosen range of temperatures. Use thermometers to ensure each water bath stays at the correct temperature.
- 2 Take five test tubes and add 2 cm³ of 1% trypsin solution to each one. Label the tubes with the temperatures and place each one in the corresponding water bath.
- 3 Take five clean test tubes and add 2 cm³ of milk to each one. Label the tubes with the temperatures and place each one in the corresponding water bath.
- 4 Leave the test tubes for 5 minutes so they reach the required temperature.
- 5 Place 2 cm³ of trypsin solution and 2 cm³ of distilled water in a cuvette. Use this as a reference cuvette to set the colorimeter absorbance to zero.
- 6 Pour 2 cm³ of milk suspension at the first temperature being tested into the second cuvette.
- 7 Add 2 cm³ of trypsin solution from the same water bath to the milk in the cuvette. Working quickly, mix the trypsin solution and the milk by shaking gently, then place the solution into the colorimeter and start the datalogger.
- 8 Measure the absorbance immediately and then at 15-second intervals (more frequently if recording electronically) for 5 minutes, or until there is little change in absorbance.
- 9 Rinse the cuvette with distilled water and repeat steps 6–8 for each of the other four temperatures. Remember to use the reference cuvette to zero the colorimeter before each new set of readings. Record the data collected in a results table in the space on page 22.

Procedure – Part 2: pH

- 1 Select the buffer solutions for the pH values being investigated. Place 1 cm³ of trypsin solution, 1 cm³ of buffer solution and 2 cm³ of distilled water into a cuvette. Use this as a reference cuvette to set the colorimeter absorbance to zero.
- 2 Add 1 cm³ of trypsin solution and 1 cm³ of your first buffer solution to a cuvette.
- 3 Measure 2 cm³ of milk suspension into a second cuvette.
- 4 Add the mixture made in step 2 to the milk in the cuvette. Working quickly, mix the solution and the milk by shaking gently, then place the solution into the colorimeter and start the datalogger.
- 5 Measure absorbance immediately and then at 15-second intervals (more frequently if recording electronically) for 5 minutes, or until there is little change in absorbance.
- 6 Rinse the cuvette with distilled water and repeat steps 2–5 for each of the other four pH values. Remember to use the reference cuvette to zero the colorimeter before each new set of readings. Record the data collected in a results table in the space on page 22.

Procedure – Part 3: Enzyme concentration

- 1 Check your dilution calculations, then make up the solutions as planned.
- 2 Place 2 cm³ of trypsin solution and 2 cm³ of distilled water in a cuvette. Use this as a reference cuvette to set the colorimeter absorbance to zero.
- 3 Measure 2 cm³ of milk suspension into a second cuvette.
- 4 Add 2 cm³ of your first trypsin solution to the milk in the cuvette. Working quickly, mix the trypsin solution and the milk, then place the cuvette into the colorimeter and start the datalogger.
- 5 Measure absorbance immediately and then at 15-second intervals (more frequently if recording electronically) for 5 minutes, or until there is little change in absorbance.
- 6 Rinse the cuvette with distilled water and repeat steps 3–5 for each of the other four concentrations. Remember to use the reference cuvette to zero the colorimeter before each new set of readings. Record the data collected in a results table in the space on page 22.

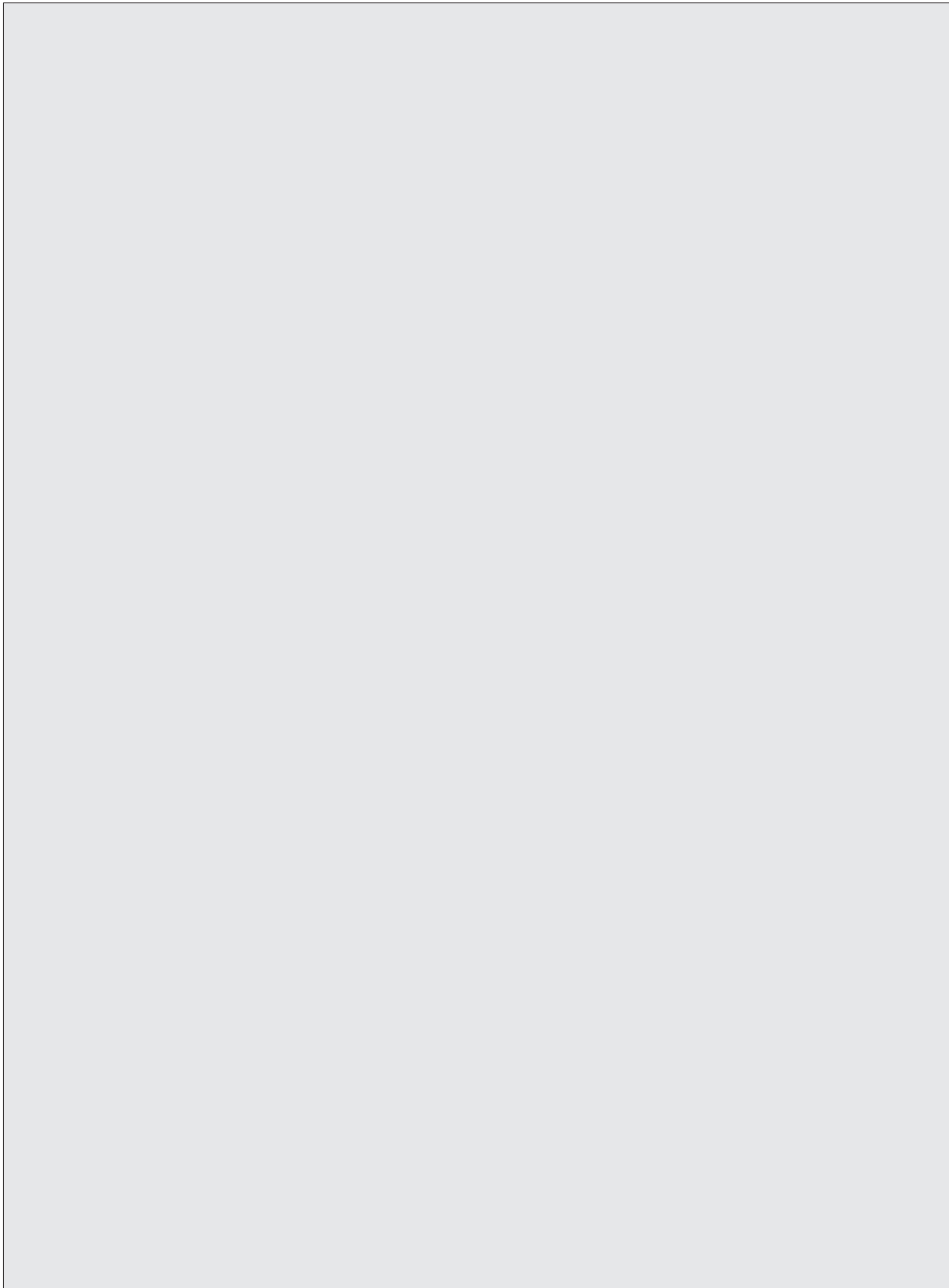
Procedure – Part 4: Substrate concentration

- 1 Check your dilution calculations, then make up the solutions as planned.
- 2 Place 2 cm³ of 1% trypsin solution and 2 cm³ of distilled water in a cuvette. Use this as a reference cuvette to set the colorimeter absorbance to zero.
- 3 Measure 2 cm³ of the 0.2% milk suspension into a second cuvette.
- 4 Add 2 cm³ of trypsin solution to the milk in the cuvette. Working quickly, mix the trypsin solution and the milk, place the solution into the colorimeter and start the datalogger.
- 5 Measure absorbance immediately and then at 15-second intervals (more frequently if recording electronically) for 5 minutes, or until there is little change in absorbance.
- 6 Rinse the cuvette with distilled water and repeat steps 3–5 for each of the other four milk concentrations. Remember to use the reference cuvette to zero the colorimeter before each new set of readings. Record the data collected in a results table in the space on page 22.

Learning tips

- Use a sharp pencil when drawing graphs. Use different symbols around plotted points to distinguish lines when several concentrations are plotted on one set of axes. Remember to include a key.
- Keep graph scales simple. Use one large square to represent 5, 10 or 20 (or perhaps 0.05, 0.1 or 0.2) when plotting intermediate points because then the smaller squares will have values that are easy to work with.
- To calculate the initial rate of a reaction, draw a tangent to the line at the steepest point. This line can then be turned into a right-angled triangle. To calculate the gradient of the line, divide the change in the value on the y-axis by the change in the value on the x-axis:
$$\text{gradient} = \frac{\text{change in y-axis}}{\text{change in x-axis}}$$
- It is unlikely that you will have time to investigate all four factors. However, you should be aware of the methods used.

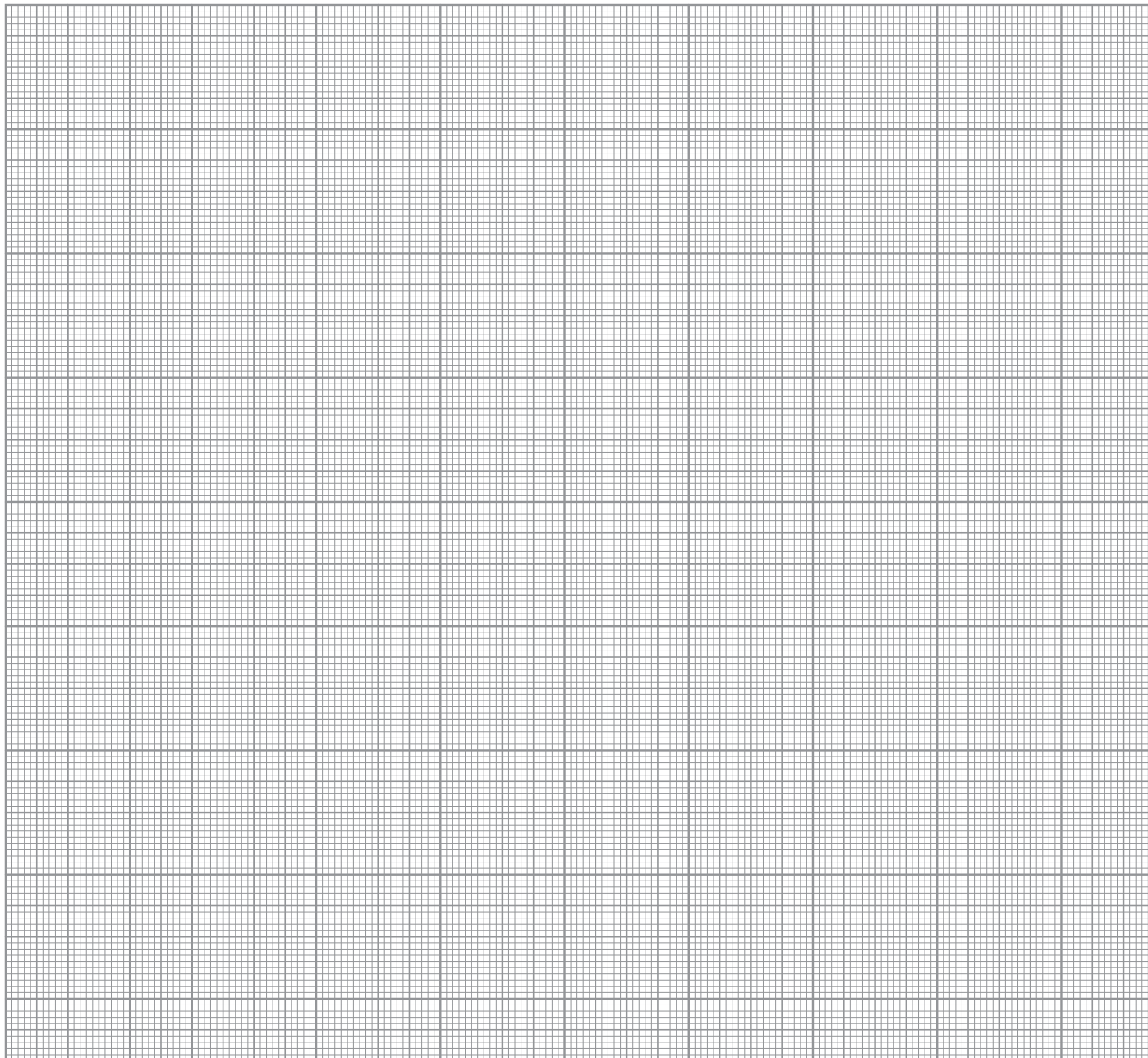
Results (Use this space to record your results.)



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Analysis of results

- 1 Plot a graph of absorbance against time. It should be possible to plot each variable as a different line on the same axes.



- 2 Use your graph to determine the initial rate of reaction for each factor. Do this by drawing a tangent to the initial part of each curve and calculating the gradient of each line.

3 Draw a second graph to show the initial rate of reaction against each factor you changed.



4 Write a short conclusion to describe and explain the results of this investigation

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Questions

1 What were the independent and dependent variables in this investigation?

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2 Ideally, you would repeat the procedure for each factor several times. Explain why it is important to measure the initial rate of the reaction rather than an average rate over a longer time period.

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3 If the surface of the cuvette is scratched, this can result in a greater absorbance of light. If the cuvette used for the reaction was scratched (but the reference cuvette was not), would this give a random or a systematic error? Explain your answer.

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- 4 Suggest one variable that would normally be controlled in an enzyme-catalysed reaction but which has not specifically been controlled in your investigation. Explain why this variable would usually be controlled carefully and suggest how this could be done.

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Learning tips

- Before starting this core practical, familiarise yourself with the parts of the microscope and how to focus it.
- Check that the lens and eyepiece are clean. Use lens tissue to clean them if necessary, as normal tissues or cloth can scratch lenses.
- If present, the condenser will need to be adjusted for each magnification. To focus the condenser correctly, place a slide on the stage and a pencil point on the light source, and adjust the condenser until both are in focus.
- If you use a mirror for illumination, take care not to use direct sunlight, as this can cause severe damage to eyes. Instead, use a bench lamp where possible.

Procedure – Part 1: Calibration

First, you will need to calibrate the eyepiece graticule (see **figure A**).

- 1 Place a micrometer slide on the stage of the microscope and focus on the micrometer scale, using the low-power objective. The smallest division of the micrometer scale is usually 100 μm.
- 2 Move the slide and rotate the eyepiece to align the scales of the eyepiece graticule and the stage micrometer in the field of view.
- 3 Count the number of divisions (eyepiece units or epu) on the eyepiece graticule that are equivalent to a known length on the micrometer slide and work out the length of one eyepiece unit. For example, if 100 μm is equivalent to 2 epu, then $1 \text{ epu} = \frac{100}{2} = 50 \mu\text{m}$ at this magnification.
- 4 Repeat steps 1–3 with the medium- and high-power objectives.

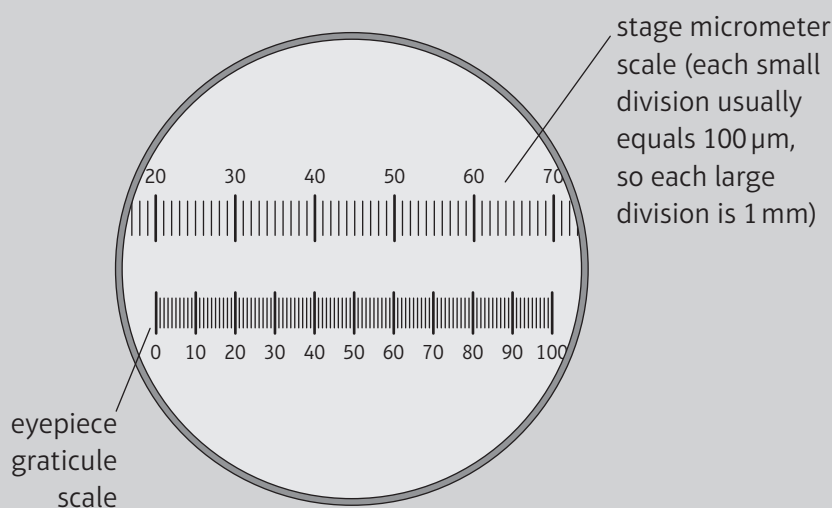


figure A Calibrating the stage micrometer using an eyepiece graticule

Objectives

- To be competent in the use of a microscope at high and low power, including the use of a graticule (eyepiece micrometer) to make measurements
- To know how to record observations using appropriate biological drawings
- To understand the importance of staining specimens in microscopy

Equipment

- eye protection
- cotton buds
- microscope with eyepiece graticule
- stage micrometer slide
- methylene blue
- glass microscope slides
- coverslip
- dropping pipette
- lens tissue
- absorbent paper (e.g. paper towel)
- large beaker of disinfectant solution

! Safety

- Methylene blue may be harmful if swallowed but is not otherwise classified as hazardous. However, there is always the possibility of unknown effects, so stains should be used with caution.
- Avoid skin contact with iodine and methylene blue stains. Wear gloves and eye protection when handling the stain. Clean up any spills immediately.
- Make sure all biohazardous material is placed in disinfectant solution as soon as possible. Cotton buds should only be used once, by one individual.

(continued)

Procedure – Part 2: Making observations

- 1 Wash your hands with soap and water.
- 2 Take a cotton bud and gently rub it on the inside of your cheek, then rub the cotton bud in a small circle in the centre of a glass slide. Immediately place the cotton bud in a beaker of disinfectant solution.
- 3 Add a few drops of methylene blue to the sample, then cover with a cover slip.
- 4 Turn the objective lens to low power and examine the stained slide under the microscope. To do this, bring the lens as close to the slide as possible while watching it from the side of the instrument. Then, looking through the eyepiece, use the coarse focusing knob to focus, moving the lens away from the stage. This avoids damage to the slide or lens. Finally, use the fine focus until a clear view of the cells is established.
- 5 Carefully sketch a few of the cells. Use **figure B** to help you identify the parts of the cell.

! Safety (continued)

- If you are using a microscope with daylight illumination (a mirror) do not place it where sunlight might strike the mirror as this will damage your retina and may cause blindness.

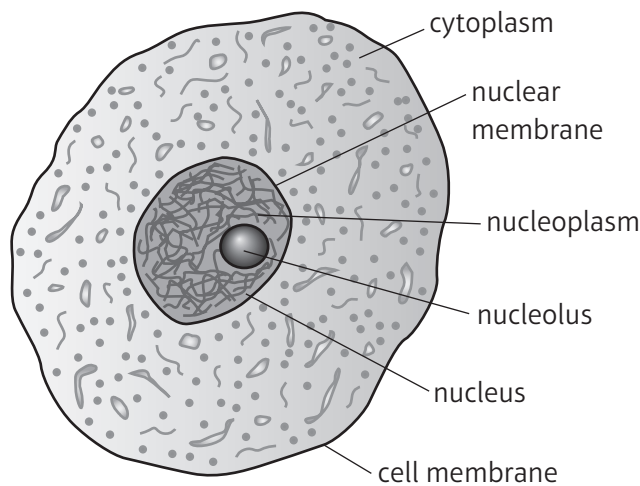


figure B Section of an animal cell as seen through a microscope

- 6 Use the eyepiece graticule to measure a cell's diameter. Add a scale bar to your diagram. Add a title and include the magnification at which you made your observations. For example, with an eyepiece lens magnification of $\times 10$ and an objective of $\times 10$, the total magnification will be $\times 100$. Remember, this is not the same as the magnification of the drawing.
- 7 Now, turn the objective disc to the medium-power lens, and focus until the cells are clear and distinct. Identify as many details of the cells as you can.
- 8 Finally, turn the objective disc to the high-power lens and focus using the fine-focusing knob only. Draw and label the detail of the cells as accurately as you can.
- 9 Measure the length and breadth of two cells. Record these measurements in your diagram.
- 10 Place the glass slide in the beaker of disinfectant solution.

Learning tips

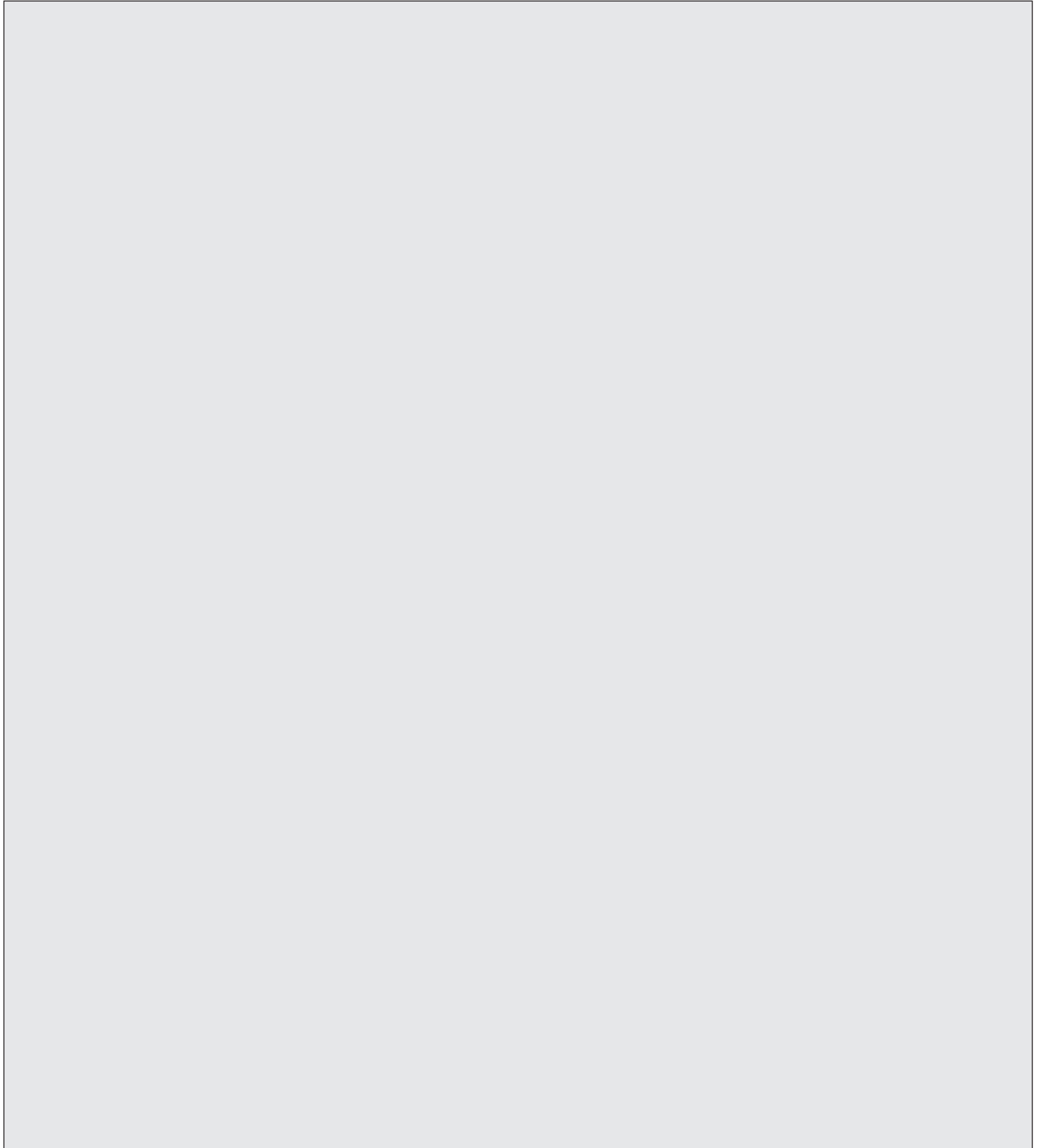
- To be able to answer examination questions about the magnification of images, make sure you learn the following equation:

$$\text{magnification} = \frac{\text{size of image}}{\text{size of real object}}$$
- You should know how to rearrange the magnification formula to calculate any of the values. Remember to convert all lengths to the same units, usually μm .

Tips for good biological drawings

- Use a sharp HB pencil. Keep lines clear and continuous, not feathery or sketched.
- Draw only what you see. Do not draw stylised patterns, and do not make it up.
- Start with an outline. Keep it large and think about proportions.
- Do not use shading or colour.
- Draw label lines in pencil with a ruler. Lines should not have arrowheads and should just touch the item to be labelled.

Diagrams (Produce detailed diagrams as described in the procedure.)



Questions

1 Use the scale bar on your diagram to calculate the magnification of the image you have drawn.

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2 Explain briefly how cells can be measured using a microscope.

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3 Suggest improvements to the method that was used to describe the size of cells in a tissue.

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Learning tip

- Before starting this practical, you may need to remind yourself about parts of the microscope and how to use the instrument as detailed in the learning tips on page 27.

Procedure

To see mitosis in action, you need to look at living cells. Garlic bulbs grow roots that have actively dividing cells in their tips, in a region called the meristem (see **figure A**). Each cell has only eight chromosomes, so it is relatively easy to see the chromosomes once they have condensed. In order to see the chromosomes inside the cells, you need to separate the cells and spread them out into a layer that is ideally just one cell thick. Plant cells are glued together by a middle lamella of pectins. Hydrochloric acid will break down the pectins that hold the cell together. Acetic orcein will stain the chromosomes dark red and fix the cells, stopping mitosis.

You should examine your preparation carefully, looking for cells undergoing different stages of mitosis. Identify the different stages by comparing your preparation with labelled pictures or photographs of cells during mitosis. Bear in mind that mitosis is a dynamic process, so cells may have been fixed in transition from one stage to the next. You will have to interpret what you see.

Make sure you follow all necessary safety precautions. You should also complete your own risk assessment before starting this practical. There is a risk assessment template for you to fill in on page 33.

- 1 This first step may have been done for you. Fill a small bottle with 1 mol dm^{-3} hydrochloric acid and place it in a thermostatically controlled water bath set at 55°C . Leave the bottle for 15 minutes to allow the acid to warm to the temperature of the water bath.
- 2 Place a garlic clove in the top of the bottle so that the roots are submerged in the hydrochloric acid at 55°C . Leave the roots in the acid for 5 minutes.
- 3 After 5 minutes, take out the garlic clove and rinse the roots thoroughly in tap water. Use a pair of sharp scissors to cut off several root tips of 5–10 mm in length. Let them fall into a small vial of acetic orcein standing on a white tile. Use the scissors to make sure the root tips are immersed in the stain. Place a lid or laboratory stretch film over the vial. Lids should have a pin-prick hole, or be slightly loose if they are screw caps, to prevent the ejection of liquid during heating.
- 4 Place the vial containing the root tips in acetic orcein in the 55°C water bath for 5 minutes to intensify the staining.
- 5 After 5 minutes, use forceps to remove the root tips from the vial and place them on a microscope slide. Add a drop of water to the root tips on the slide. Tease each root tip apart with needles (maceration), to spread out the cells a little. Cover with a coverslip. Replace the lid on the vial of

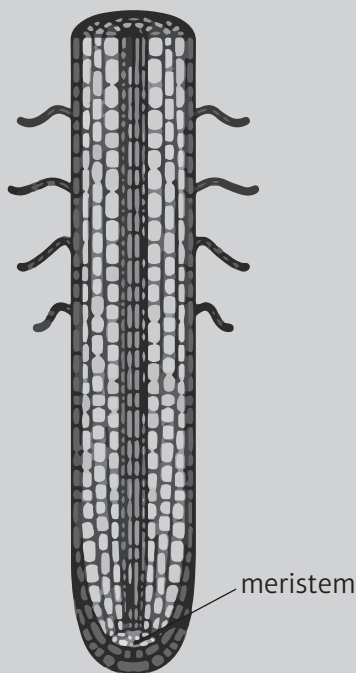


figure A Section of a root tip showing meristem

Objectives

- To know how to prepare a temporary slide of a root tip to observe mitosis
- To recognise the stages of mitosis in dividing cells
- To identify hazards, associated risks and control measures for the procedure

Equipment

- eye protection
- garlic clove with growing root tip
- glass slide and coverslip
- scissors
- water bath at 60°C
- small bottle with lid or laboratory stretch film
- hydrochloric acid 1 mol dm^{-3}
- acetic orcein stain in a small bottle or vial
- two dissecting needles
- paper towels
- microscope
- white tile
- fine forceps
- stop clock
- safety information sheet

! Safety

- Eye protection must be worn.
- Take care with glassware and scissors.
- Acetic orcein stain is corrosive, causes burns, has an irritating vapour and will stain. Wear eye protection and avoid contact with skin. If contact does occur, wash the area thoroughly with water for 10 minutes and tell your teacher. Avoid inhaling the vapour. If you spill the stain, do not attempt to mop it up; tell your teacher instead.
- Avoid skin contact with the hydrochloric acid.

(continued)

stain and return it to the teacher as instructed.

- 6 Wrap the slide in several layers of paper towel and press gently on the paper to squash the tissues. Take care not to twist the slide as you press down, or the coverslip will break.
- 7 Examine under the microscope on low power to identify the area of dividing cells or meristem (see **figure A**). Position the cells in the centre of the field of view. Meristem cells are small and square, have no obvious vacuoles and are usually found in rows.
- 8 Move to high power ($\times 400$). Identify as many stages of the cell cycle as you can in your field of view.
- 9 Count the number of cells in each of the stages of mitosis, plus interphase, in the field of view. Record your results in a table.
- 10 Draw and annotate one cell from each of the stages you have identified. Your drawings will be simple outlines of the cells and the groups of chromosomes in them; few other structures will be visible. Aim to show the relative sizes and positions of the chromosomes and the cell accurately. Annotate your drawings to describe what is happening.

Learning tip

- Cell counts for each stage of mitosis in the field of view should indicate the duration of each of the stages. This will be a relative value – the more cells you can see in one stage, the longer the duration of that stage in the cell cycle.



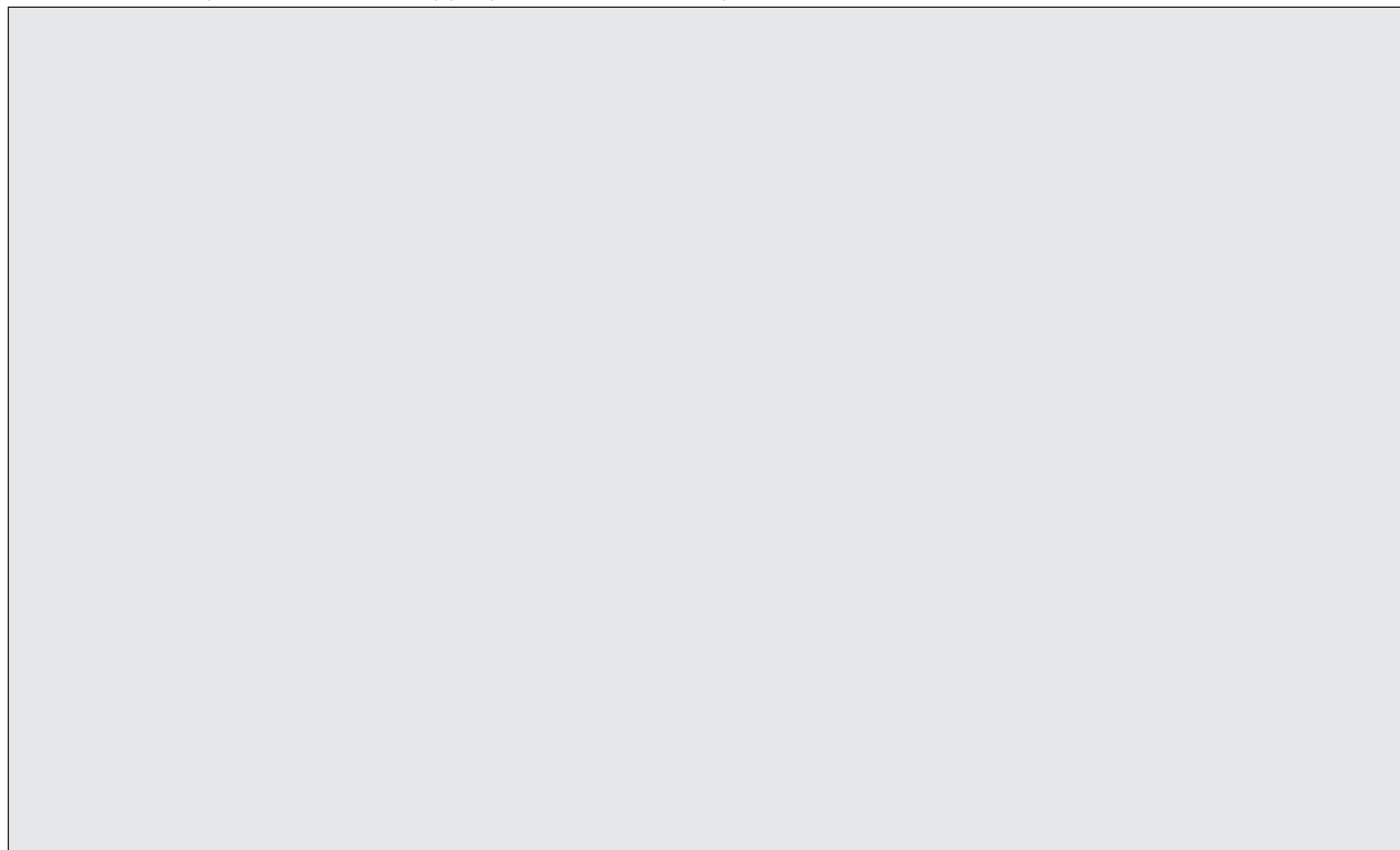
Safety (continued)

- If your microscope uses daylight illumination, be careful not to use it where sunlight could strike the mirror.
- The water bath at 60°C will scald your skin; cool under cold running water if you get splashed.
- Do not handle electric plugs, sockets or switches with wet hands.

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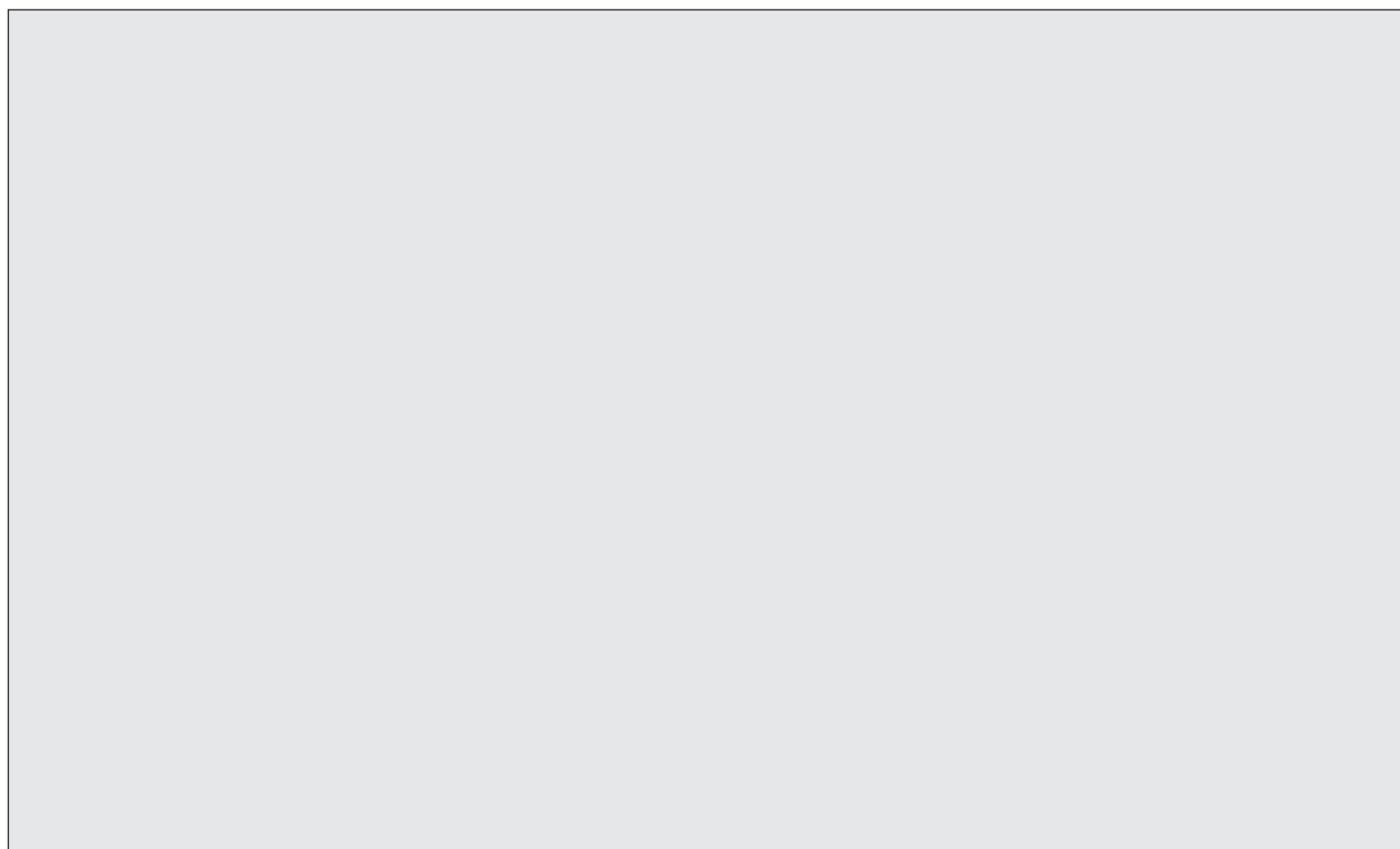
Risk assessment						
Hazard (What could cause an injury?)	People (Who might be harmed?)	Severity of harm (How bad could the injury be? On a 1–5 scale: 1 = irritation 5 = death)	Likelihood (How likely is it to happen? On a 1–5 scale: 1 = not very likely 5 = very likely)	Risk value (Severity × likelihood)	Controls (What measures can be put in place to prevent the hazard?)	Further action (What extra action may be needed if the hazard occurs?)

Results (Record your results in an appropriate table in the space below.)

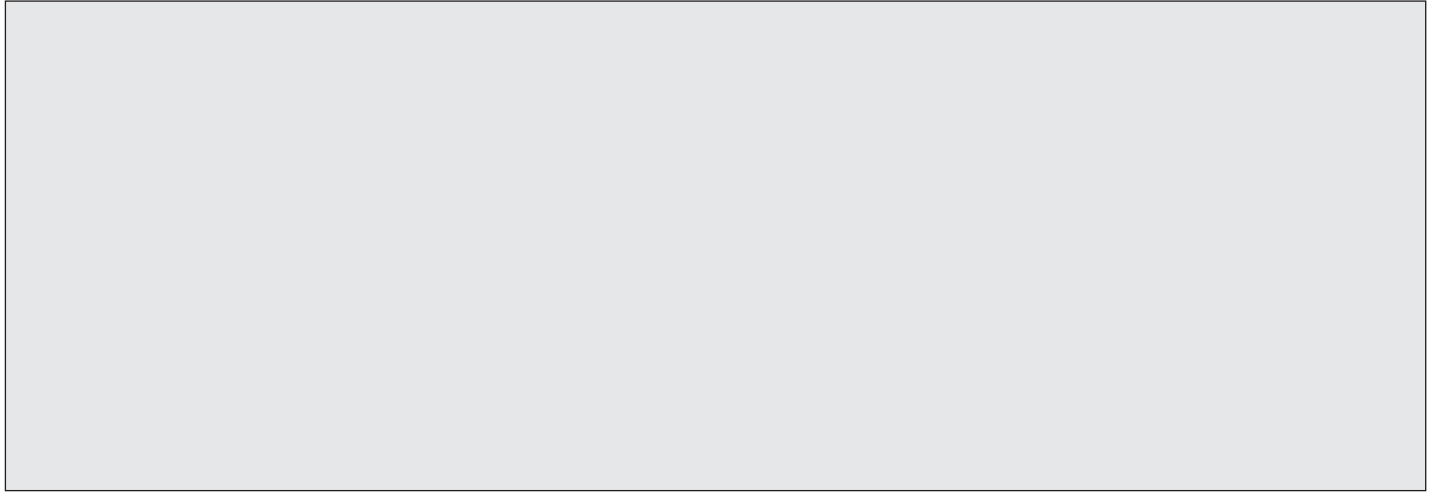


Analysis of results

- 1 Calculate the percentage of the cells in each stage by dividing the number of cells in the phase by the total number of cells and multiplying by 100. Add the percentages to your table. You could use a spreadsheet to calculate and record these values.

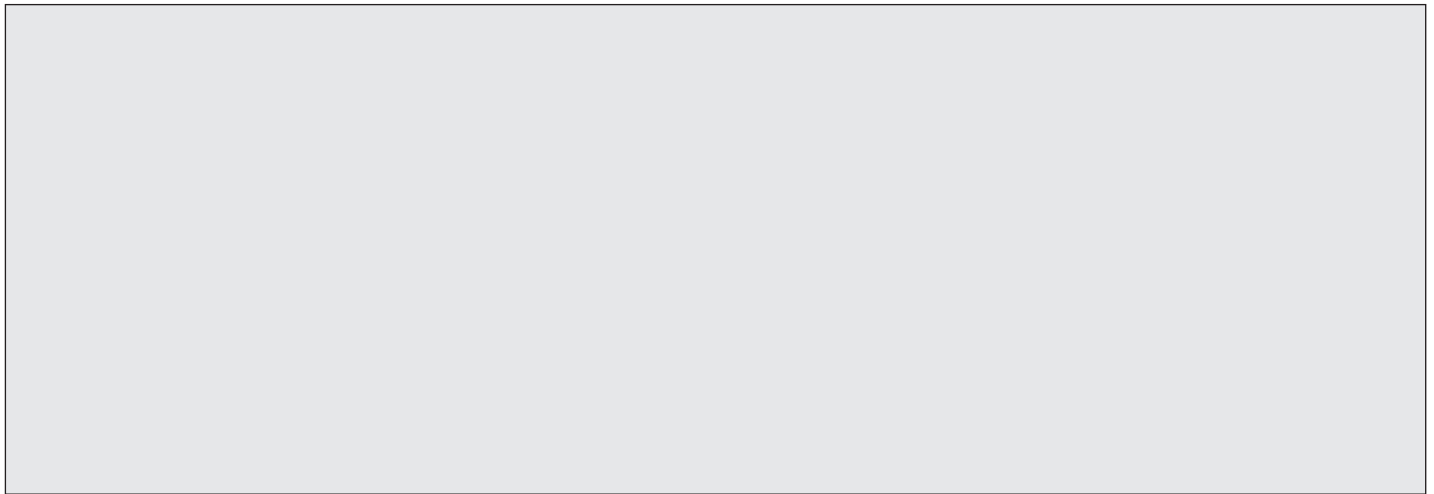


- 2 Given that your preparation freezes the process of mitosis at one point in time, what do the values calculated in analysis question 1 suggest to you about the length of time a cell spends in each stage of mitosis?

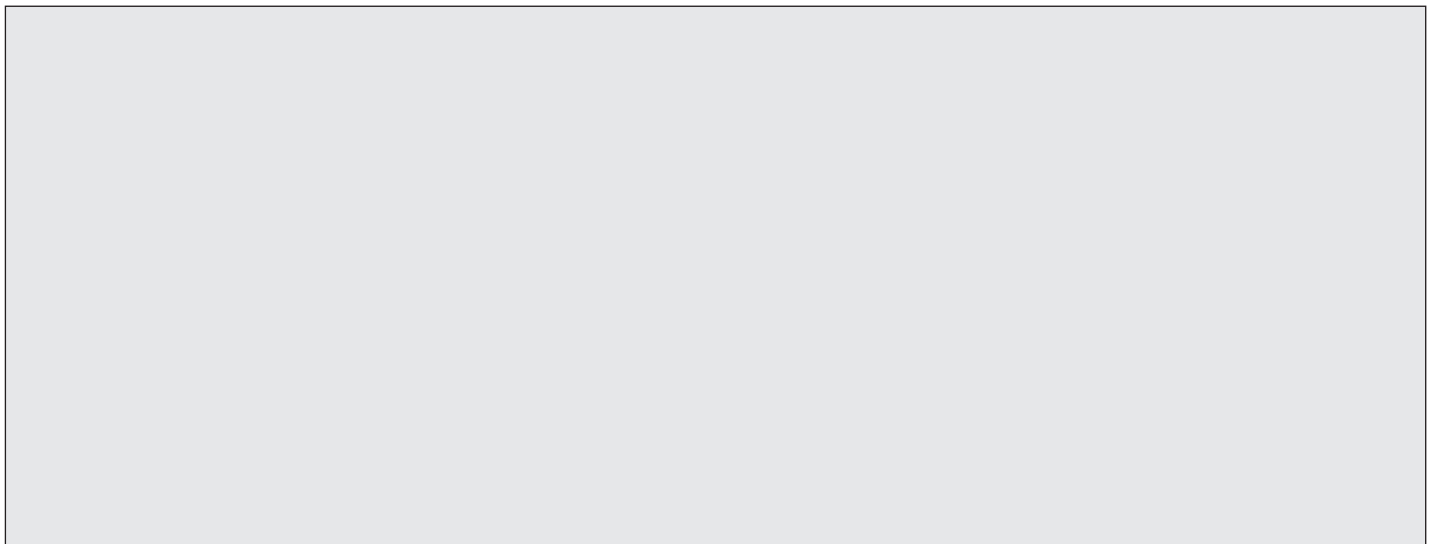


- 3 If a group of cells is dividing rapidly, a high proportion of the cells will be undergoing mitosis. A group of cells that is not dividing will have all cells in interphase of the cell cycle (chromosomes will not be clearly visible). The amount of cell division occurring in a tissue can be quantified using the mitotic index. The mitotic index is used to study tumour growth in cancer patients. Use the formula below to calculate the mitotic index for the root tip.

$$\text{mitotic index} = \frac{\text{number of cells containing visible chromosomes}}{\text{total cells in field of view}}$$



Diagrams (Draw and annotate one cell from each stage identified.)



Questions

1 Explain why the root tip is heated with acid.

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2 State the effect of maceration and pressing the slide preparation on the dividing cells.

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3 Describe what information the cell counts give about each stage of mitosis.

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4 Describe the role of mitosis in the life of an organism.

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CORE PRACTICAL 7: USE A LIGHT MICROSCOPE TO: MAKE OBSERVATIONS OF (i) TRANSVERSE SECTIONS OF ROOTS, STEMS, LEAVES; (ii) PLANT TISSUES; (iii) IDENTIFY SCLERENCHYMA FIBRES, PHLOEM, SIEVE TUBES, XYLEM VESSELS AND THEIR LOCATION

SPECIFICATION
REFERENCE

4.6

Learning tip

- Before starting this practical, you may need to remind yourself about parts of the microscope and how to use the instrument as detailed in the learning tips on page 27.

Procedure

- 1 Collect a piece of plant stem. Add a few drops of water to the centre of the white tile and wet the razor to reduce friction. Hold the plant stem firmly, keeping your fingers away from the edge of the razor. Cut several transverse sections (across the stem), keeping them as thin as possible. Incomplete thin sections may sometimes be better than thicker complete ones. Use a brush to transfer the sections to water in a watch glass.
- 2 Select the thinnest section and place it on a slide. Add a drop of water. Remove excess water by carefully touching the edge with absorbent paper.
- 3 Wearing gloves and eye protection, add two drops of toluidine blue O stain and leave for 2–4 minutes. Then add a coverslip and gently remove excess stain with a paper towel.
- 4 Turn the objective lens to low power. Examine the slide under the microscope. To do this, bring the lens as close to the slide as possible while watching it from the side. Then, looking through the eyepiece, focus using the coarse focusing knob, moving the lens away from the stage. This avoids damage to the slide and lens. Use the fine focus until a clear view of the section is established.
- 5 Still on low or medium power, draw and annotate a simple outline plan of the section. Show the arrangement of tissues within the stem but do not include any cell details. Use **figure A** to help you identify the different tissues and label them.

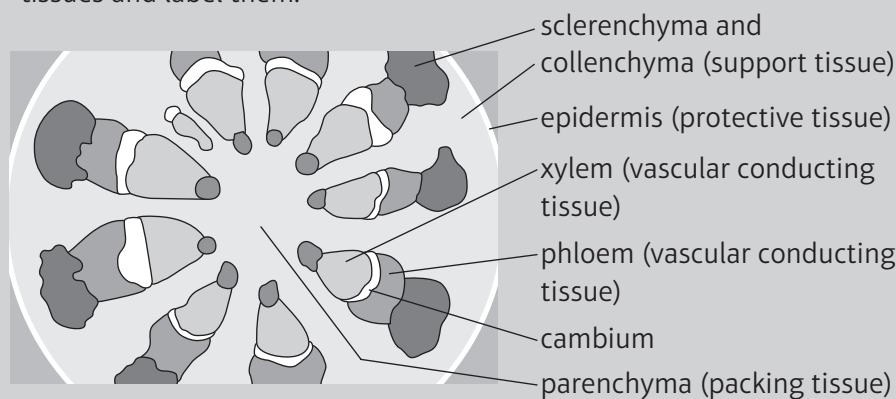


figure A Transverse section of a stem as seen through a microscope at low power

- 6 Repeat steps 1–5 using a piece of root.
- 7 To make a leaf cross section, place the leaf between two thin pieces of polystyrene. Gently stroke the razor blade across the top of the leaf and polystyrene to shave off four or five thin sections.
- 8 Select a thin section and place it on a glass slide with one or two drops of water. Cover with a cover slip.
- 9 Repeat steps 4 and 5 with the leaf section.

Note: You may not need to prepare your own specimens. You may instead be given pre-prepared slides to use. In this case, you will only need to follow steps 4 and 5 for the specimens provided.

Objectives

- To be able to use a microscope competently to observe biological specimens
- To learn how to draw and label plan diagrams accurately
- To be able to identify sclerenchyma fibre, phloem, sieve tubes and xylem vessels

Equipment

- eye protection
- protective gloves
- plant stem, at least 5 cm long
- root section, at least 3 cm long
- large plant leaf
- microscope
- toluidine blue O stain
- two glass microscope slides
- two coverslips
- small paintbrush or tweezers
- single-sided razor blade
- white tile
- watch glass
- dropping pipette
- mounted needle
- lens tissue
- polystyrene strips
- absorbent paper (e.g. paper towel)
- stop clock

! Safety

- Wear eye protection.
- Avoid all skin contact with the toluidine blue stain; disposable gloves may be worn.
- Tell your teacher if you spill the stain on the bench or on your skin.
- Take care when using razor blades and mounted needles and ensure you hand them back to your teacher when you have finished with them.

(continued)

CORE PRACTICAL 7: USE A LIGHT MICROSCOPE TO: MAKE OBSERVATIONS OF (i) TRANSVERSE SECTIONS OF ROOTS, STEMS, LEAVES; (ii) PLANT TISSUES; (iii) IDENTIFY SCLERENCHYMA FIBRES, PHLOEM, SIEVE TUBES, XYLEM VESSELS AND THEIR LOCATION

Learning tips

- Whole sections are not needed to make the plan drawings. A thin partial section is better than a thick whole section.
- Toluidine blue O is a metachromatic stain, which means it reacts with different chemical components of cells to produce a variety of colours. This can provide information about the nature of the cell. Toluidine blue O stains lignin and tannins green to blue, pectins pinkish purple and nucleic acids purplish or greenish blue.
- Use a sharp HB pencil. Keep lines clear and continuous, not feathery or sketched.
- Draw only what you see. Do not draw stylised patterns and do not make it up.
- Start with an outline. Keep it large and think about proportions.
- Do not use shading or colour.
- Draw label lines in pencil with a ruler. Lines should not have arrowheads and should just touch the item to be labelled.

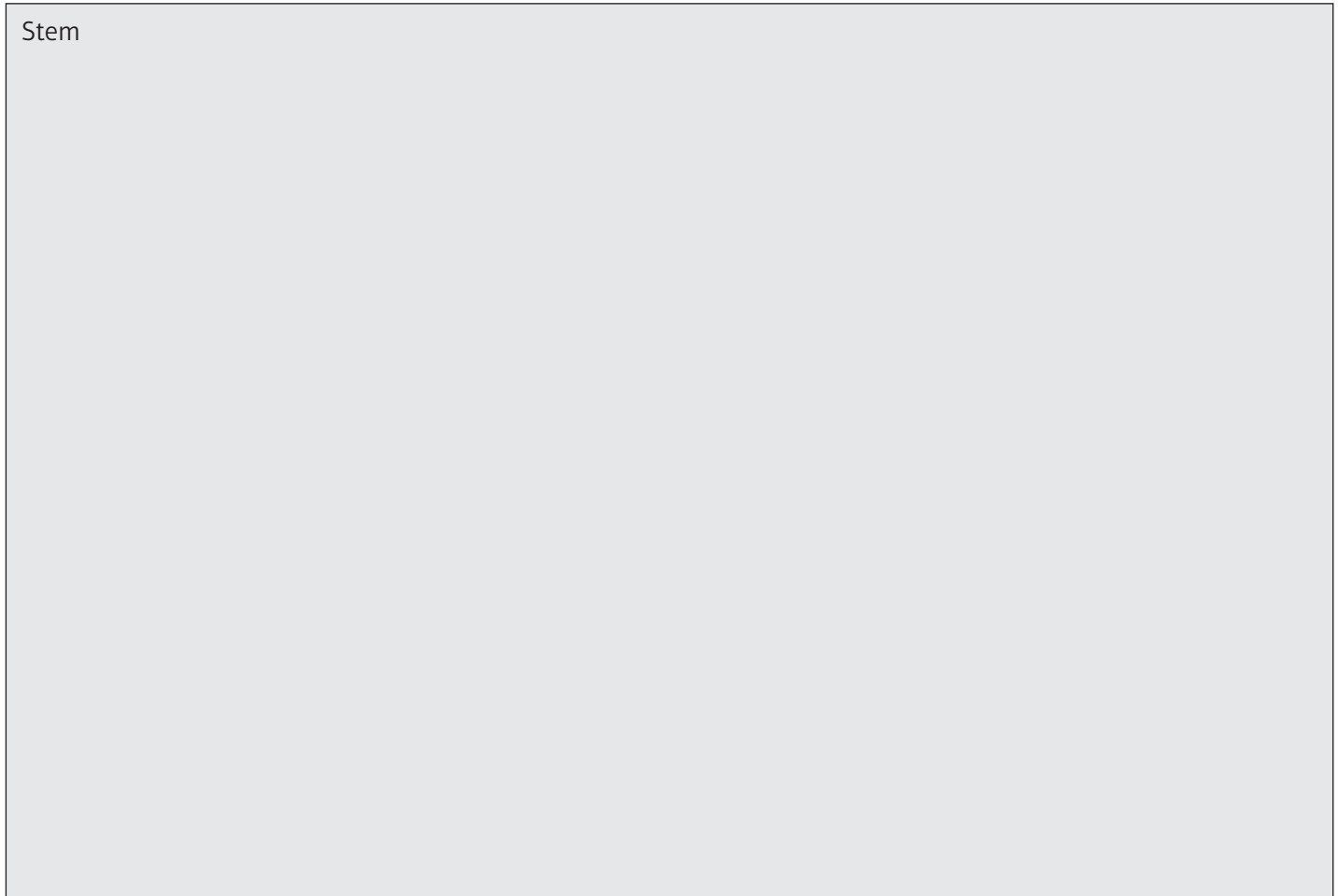
Diagrams (Draw detailed diagrams as described in the procedure.)



Safety (continued)

- If you are using a microscope with daylight illumination – a mirror – you must not put the microscope in a position where direct sunlight might strike the mirror and be reflected into the eyes through the microscope. This could cause permanent retinal damage or blindness.

Stem



**CORE PRACTICAL 7: USE A LIGHT MICROSCOPE TO: MAKE OBSERVATIONS OF
(i) TRANSVERSE SECTIONS OF ROOTS, STEMS, LEAVES; (ii) PLANT TISSUES; (iii) IDENTIFY
SCLERENCHYMA FIBRES, PHLOEM, SIEVE TUBES, XYLEM VESSELS AND THEIR LOCATION**

SPECIFICATION
REFERENCE

4.6

Root

Leaf

Questions

1 Suggest why a stain was used on the stem and root cells.

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2 Explain why it was important not to use too much stain when preparing the slide.

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3 Describe a method for comparing the size of the xylem tissue in the root and stem samples.

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Research

Research the retting process. Summarise the information in the space below.

Objectives

- To know how to determine the tensile strength of plant fibres
- To be able to comment on experimental design and evaluate scientific methods

Equipment

- celery (or other suitable plant stems)
- two retort stands with clamps
- knife
- white tile
- 10 g and 50 g masses
- plastic tray with cotton wool or other suitable cushioning

! Safety

- Use a sharp knife, place the celery on the tile and cut down onto the tile.
- Wash hands with soap and water after the practical work is over.
- Take care when using the knife to cut the celery.

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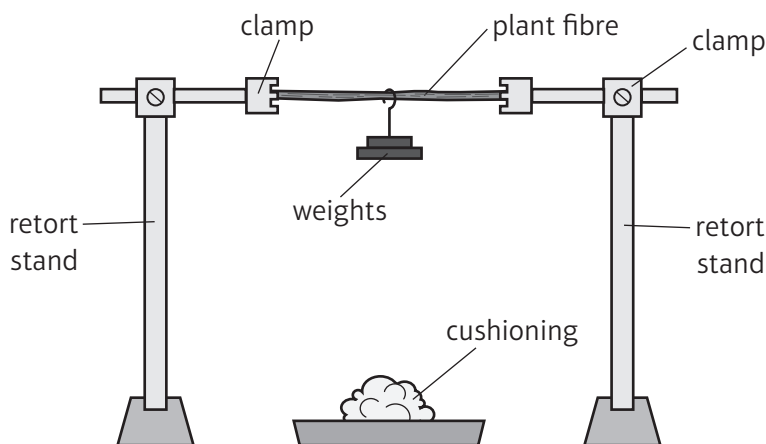


figure A Adding masses to the plant fibre string

Procedure

Although the retting process can be used to produce plant fibres, it is time consuming. Therefore, a simpler process of extracting plant fibres will be used in this investigation.

- 1 Carefully remove nine fibrous strings from the celery stalks, using the knife. Use a white tile as a cutting surface.
- 2 Carefully inspect the strings to ensure there are no breaks or cuts. Each string should have a constant diameter along its whole length.
- 3 Cut the strings so you have three that are 10 cm long, three 15 cm long and three 20 cm long.
- 4 Clamp one of the 10 cm strings between two retort stands. Ensure it is held securely.
- 5 Place the cushioning underneath the string. Ensure the tray is kept beneath the masses, so they do not strike the bench when the string breaks.
- 6 Add masses 10 g at a time until the string breaks (see **figure A**). Record the mass in the results table.
- 7 Repeat steps 4–6 with each of the other strings.

Learning tips

- The plant fibres being used are the xylem tissues. These can be identified by adding coloured dye to the water the celery stem is placed in.
- These fibres are non-living, so they are not easily digested by bacteria in the retting process and are therefore left behind. The retting process is used with different plants when they are processed to produce fibres.

Results (Use this space to record your observations in a suitable table.)

Analysis of results

1 Draw a graph to show the pattern you found in your results.



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2 Describe the pattern shown by your results.

Questions

1 Use your graph to predict the tensile strength of a celery string that is 12.5 cm long.

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2 Suggest one way in which this procedure could be modified to provide more accurate data.

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3 Identify one variable in this investigation that was not controlled and describe how this may have affected the data obtained.

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4 Suggest one further investigation that could be undertaken to determine the validity of the conclusions drawn from this investigation.

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Risk assessment

Before this activity you should complete a risk assessment. This should detail the hazards, risks and precautionary measures that you will take during the procedure. A large part of this will record how you will avoid contamination of your cultures, yourself and the surrounding environment using aseptic techniques. The procedure detailed below includes some such precautions, but they are not exhaustive. Record all identified risks and control measures in the table on the following page.

Procedure**Day 1**

- 1 Wash your hands with soap and water and disinfect your bench area, leaving it to soak for 10 minutes before wiping it down.
- 2 Place a piece of garlic in the mortar and grind it into a paste with the pestle. Add 10 cm³ of alcohol to the garlic paste.
- 3 Use the forceps to pick up a paper disc and place it in the mortar to soak up the garlic and alcohol solution.
- 4 Use the forceps to place the disc on the sterile agar plate to dry.
- 5 Use a marker pen to mark four quarters on the bottom of the agar plate that has been seeded with bacteria; label one garlic, one mint and two control.
- 6 Open the lid of the Petri dish containing the bacteria-seeded agar. Open it away from yourself and only open it slightly. Use the forceps to place the dry disc in the centre of the quarter labelled garlic.
- 7 Close the Petri dish.
- 8 Clean the pestle and mortar, then repeat steps 2–7 with the mint.
- 9 Add a small amount of alcohol to the small beaker.
- 10 Using clean forceps, add two paper discs to the beaker. Remove them and place them on the sterile agar plate dish to dry.
- 11 Open the lid of the seeded Petri dish, making sure you open it away from yourself and only open it slightly. Add one disc to each of the quarters labelled control.
- 12 Place one small piece of tape on each side of the Petri dish lid to hold it down. Do not tape it all the way around.
- 13 Invert the plate and incubate at a temperature no higher than 30 °C for 24 hours; alternatively, leave at room temperature for 48 hours.

Day 2

- 14 Use a ruler to measure the diameter of the clear zone around each disc. (It is also possible to measure the area of the clear zone using squared paper.) Record your results in a suitable table.
- 15 Clear away all the equipment you have used. Petri dishes should be returned for sterilisation.

Wash your hands and disinfect surfaces before leaving the laboratory.

Objectives

- To successfully compare the effect of plants on microbial growth
- To understand the safety issues of microbiological work and how to apply good aseptic techniques

Equipment

- eye protection
- bench spray of disinfectant
- paper towels or cloth
- agar plate seeded with bacteria
- sterile agar plate
- marker pen
- adhesive tape
- incubator
- forceps
- pestle and mortar
- mint
- garlic
- ethanol or denatured alcohol
- small filter paper discs
- 50 cm³ beaker

! Safety

- Wear eye protection.
- The microorganisms are a potential biological hazard.
- Wash your hands with soap and water before and after the activity.
- Do not open your inoculated plate. Instead, use the alternative plate provided by your teacher.
- Wear gloves when handling mint and garlic.
- Avoid skin contact with the disinfectant.
- Do not decant ethanol near a naked flame and stopper the bottle after use.
- Use aseptic techniques when transferring the bacteria to the Petri dishes.

(continued on page 47)

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Risk assessment						
Hazard (What could cause an injury?)	People (Who might be harmed?)	Severity of harm (How bad could the injury be? On a 1–5 scale: 1 = irritation 5 = death)	Likelihood (How likely is it to happen? On a 1–5 scale: 1 = not very likely 5 = very likely)	Risk value (Severity × likelihood)	Controls (What measures can be put in place to prevent the hazard?)	Further action (What extra action may be needed if the hazard occurs?)

Learning tip

- Most microorganisms require a good source of carbon and nitrogen as well as specific minerals. These nutrients can be supplied in a solid form. The solid medium used in the Petri dishes in this investigation was nutrient agar (agar with some nutrients added), but many bacteria will not grow effectively on this simple medium. Agar is a jelly extracted from seaweed.

Results (Use the space below to record your results. It may be helpful to include annotated sketches.)

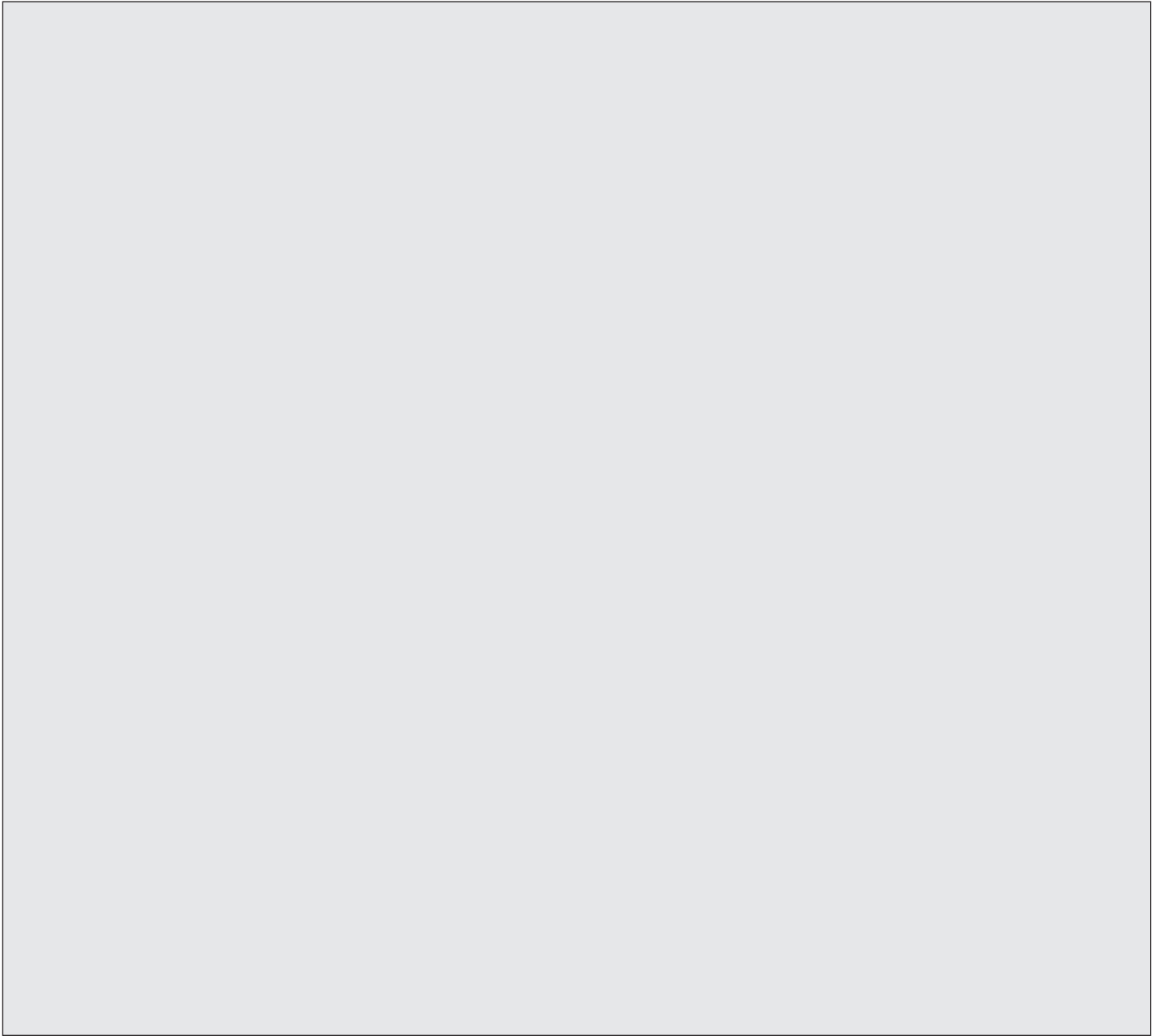
**Safety (continued)**

- Only open the Petri dish slightly when adding the discs with forceps (not fingers) and, once it is closed with some strips of tape, do not open it again.
- Disinfect the bench before and after working. Leave the disinfectant on the bench for about 10 minutes.
- If the agar plate shows signs of contaminating growth, do not open the plate. A technician will destroy it by autoclaving.

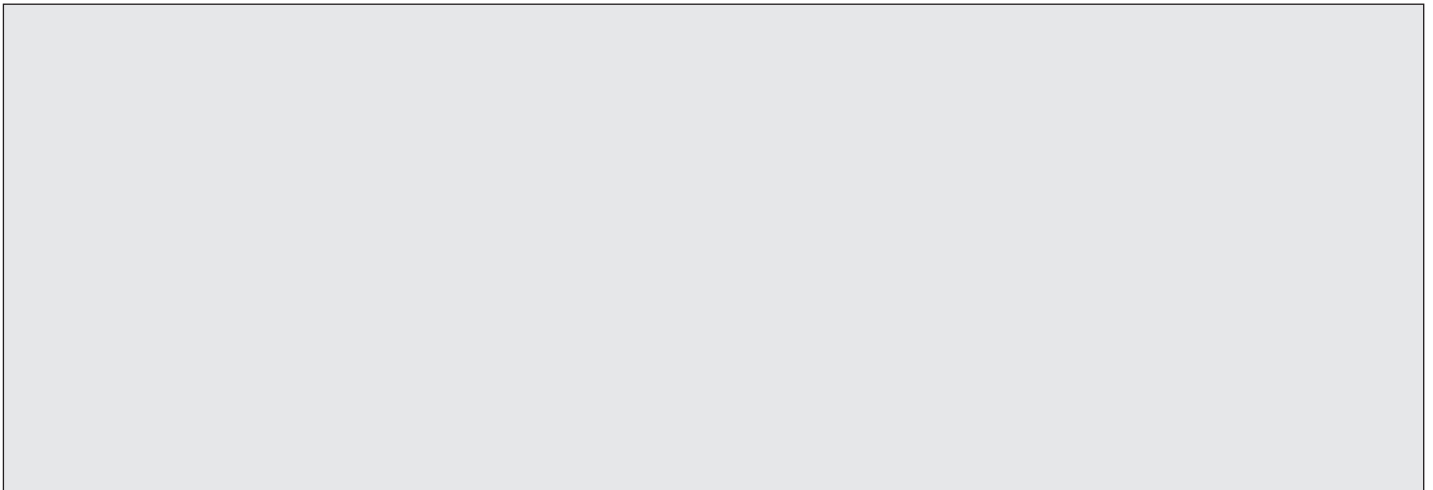
Large empty rectangular area for recording results and annotated sketches.

Analysis of results

- 1 Collate the results from the whole class. Calculate the mean area of the clear zone for each treatment.



- 2 Carry out some research to find out about one other plant material that can be used for its antimicrobial properties.



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Questions

1 Suggest a reason for including the control discs in this investigation.

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2 Explain why the plates should be incubated at a temperature lower than 30°C.

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3 Sterile (aseptic) technique is important in microbiology. Explain why this is the case even in this activity, where the bacterial species have been selected because they are harmless to humans.

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4 Based on the class data, suggest which of the two types of plant material had the stronger effect on the growth of bacteria.

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- 5 Explain why measuring the area of the clear zone around each disc is more accurate than measuring the diameter of the clear zone

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