CAMBRIDGE UNIVERSITY PRESS

Cambridge Assessment International Education
Endorsed for learner support

Cambridge International AS & A Level

Biology

Practical Workbook

Mary Jones and Matthew Parkin

Mary Jones and Matthew Parkin

Cambridge International
AS & A Level

Biology

Practical Workbook

CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE
UNIVERSITY PRESS

University Printing House, Cambridge CB2 8BS, United Kingdom

One Liberty Plaza, 20th Floor, New York, NY 10006, USA

477 Williamstown Road, Port Melbourne, VIC 3207, Australia

314–321, 3rd Floor, Plot 3, Splendor Forum, Jasola District Centre, New Delhi–110025, India

79 Anson Road, #06–04/06, Singapore 079906

Cambridge University Press is part of the University of Cambridge.

It furthers the University's mission by disseminating knowledge in the pursuit of education, learning and research at the highest international levels of excellence.

Information on this title: www.cambridge.org/ 9781108436816

First published 2018

20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

Printed in Spain by GraphyCems

A catalogue record for this publication is available from the British Library

ISBN 978-1-108-43681-6 Paperback

Acknowledgements

Thanks to the following for permission to reproduce images:

Cover Colin Varndell/SPL; *Inside* Fig 1.3 Ed Reschke/Getty Images; Fig 1.7 BSIP/Getty Images; Fig 3.5 & Fig 3.6 Greenhillwood Studios; Fig 6.1 & Fig 11.2 Biodisc, VISUALS UNLIMITED / SPL; Fig 6.3 DR KEITH WHEELER/SPL; Figures 6.10, 7.1, 7.9, 7.10, 7.11, 7.12, 7.13 BIOPHOTO ASSOCIATES/SPL; Fig 7.2 & Fig 10.3 Alvin Telser/SPL; Fig 7.3 toeytoey2530/Getty Images; Fig 7.4 BIOLOGY MEDIA/SPL; Fig 7.6 STEVE GSCHMEISSNER/SPL; Fig 10.2 JOSE CALVO/SPL; Fig 11.1 M. I. Walker/SPL; Fig 11.3 age fotostock/Alamy Stock Photo; Fig 11.5 Dorling Kindersley/ Getty Images; Fig 11.6 GEOFF KIDD/SPL; Fig 12.2 SPL

SPL = SCIENCE PHOTO LIBRARY

Contents

12 Ecology

Introduction

The science of biology is often defined as the study of the structure and behaviour of the natural world through observation and experimentation. Practical work is a very important part of any biology course. Carrying out practical work helps to give you a better understanding of how scientists think and a better understanding of the theory in your course.

This book has several aims:

- to guide you through all the specific experiments that are listed in the Cambridge Assessment International Education AS & A Level syllabus. These are experiments that you need to know both for the written and the practical examination papers.
- to help you develop an understanding of all the practical techniques that you are expected to know, such as how to perform serial dilutions, calculate magnifications and draw cells and tissues
- to help you learn how to plan valid, safe, reliable experiments, record your results and analyse your data correctly
- to help you to understand the topic areas better by carrying out your own observations

Practical work in biology can often be a little different to practical work in the other sciences. Living things, by definition, show great variation, and sometimes experiments may not yield exactly the results you expect. Always remember that science is about observation and a search for truth. Even though results may not be what you expected, you should try to explain them according to what they suggest and not what you think they should have been. If results are unexpected, it is perfectly acceptable to comment on why you think this may be and try to think of ways to extend or alter your experiment to improve it. There may be some experiments listed in the book that you are not be able to perform due to not having a piece of equipment. To help with this, your teacher will have sets of results for you to analyse, and there are many demonstrations of particular techniques that you can watch on the internet.

Working through all the experiments in this book will give you an excellent appreciation of all the practicals listed in the syllabus. Try not to see the work as a means to an end, though. Studying biology should be fun and fulfilling in its own right. Living things surround us, and we are also part of biology. Try to enjoy each experiment and use them as a launch pad for your own research, investigations, creativity and further experiments.

Safety

Laboratories are generally one of the places in the school where accidents are least likely to happen. (The most dangerous place, as far as the frequency of accidents is concerned, is outside the buildings.) This is because teachers and learners follow sets of rules in laboratories that are designed to keep everyone safe. It is essential that you always, without exception, follow all of the rules set out and displayed in the laboratories where you are working.

Making an assessment of the degree of risk associated with biology investigations is one of the skills that is assessed in Paper 3 and Paper 5. You should learn to think about risk every time you carry out an investigation. Once any risks have been identified, you then need to consider how you will reduce the level of risk. Most biology investigations are low risk, but some may involve a medium level of risk.

General rules for good practice

- You should **always** wear safety glasses when using liquids of any kind.
- If you have long hair, keep it tied back.
- You may also like to wear a protective laboratory coat, so that any spills do not get onto your clothes.
- Make sure that you understand any specific risks associated with the experiment, as explained by your teacher (see Table S1).

Table S1 lists some of the common sources of risk associated with biology investigations.

Source of risk	How to reduce risk	Comments
Glassware and sharp blades	Keep glassware on a laboratory surface – do not carry it around unnecessarily. Treat sharp blades, for example scalpel blades, knives or razor blades, with care. Avoid allowing reagents or liquids derived from specimens to come into contact with cuts or grazes on your skin.	Pushing glass tubing into a tight hole in a rubber bung is risky – it is best if the laboratory technician or your teacher does this, rather than doing it yourself.
Hot liquids (e.g. hot water in a water bath)	Keep hot liquids safely on a bench – do not carry them around. Use tongs when moving tubes into or out of very hot water baths. Do not sit down when using hot liquids – you can move faster to avoid spills if you are standing up.	
Chemicals	Keep all chemicals you are using in their labelled bottles. If you transfer a potentially hazardous chemical to another container, label the container first. When you remove the top from a container, place it upside down on the bench, so that its lower surface does not transfer any chemical to the bench surface.	Your teacher will know the precise level and type of risk posed by each chemical that you are using. Follow all safety instructions provided by your teacher.

(*Continued*)

Working with living organisms or material derived from them	Be aware of any allergies that you may have (e.g. to nuts, eggs or enzymes), and make sure that your teacher also knows about them. If collecting plants or animals from their habitats, make sure that you are able to recognise anything that might be poisonous, or that can sting or bite.	You may like to wear nitrile or latex gloves when working with biological specimens or substances derived from them. Boots or long socks are a good idea when working outdoors, particularly in long vegetation.
Working outdoors	Always work with a partner when outside. If one of you gets into difficulties, the other can call or search for help.	

Table S1

Skills chapter

Planning

- Always identify the independent and dependent variables.
- State the range and interval for your independent variable, and describe how you will achieve this.
- If possible, use at least five different values for the independent variable, with equal intervals.
- List the genuinely important variables that you should control and how you will do this.

Recording data

- Use a pencil and ruler to draw a results chart.
- Independent variable goes into the first column (or row), followed by the dependent variable.
- Headings include units; no units in the body of the table.
- Record all values to the appropriate number of significant figures. This is significantly important in tables where the number of significant figures should be consistent throughout the table.

Recording observations

- If recording qualitative results in a table, use short, clear descriptions.
- Draw using a medium–hard (HB) pencil, with a good quality eraser to hand.
- Draw what you can see, not what you think you ought to see.
- All lines on a drawing must be clean and clear, with no breaks or overlaps.
- Never use shading on a drawing.
- Take time to get proportions of different structures or areas correct on your drawings; for example, you can use an eyepiece graticule to make measurements of the relative widths of different tissues.
- Draw label lines with a ruler; no arrowheads; ensure the line touches the structure it is labelling.
- Use most of the space allocated for your drawing, with enough space around it for labels; labels should not be written on top of the drawing.
- Low-power plans show tissue outlines only, with no individual cells. High-power drawings show internal detail of individual cells, where visible.

Calculations

- Show every step in your calculation clearly.
- Calculated values (including means) must have the same number of significant figures as raw results, or one more.
- Do not include anomalous results in calculations; identify the result and state that you have not included it and why. You can identify anomalous data using standard deviation (mean ± 2SD to create a range of values - any raw data outside this is anomalous.)

Graphs and charts

- Construct all graphs and charts in pencil.
- Use a line graph where the variables on both axes are continuous.
- Use a bar chart where the *x*-axis variable is discontinuous. Bars do not touch and should not be shaded.
- Independent variable goes on the *x*-axis and dependent variable on the *y*-axis.
- Scales should be chosen to make as much use of the width and height of the grid provided as possible.
- Scales should go up in equal intervals. Intervals should be chosen so that it is easy to read intermediate points, for example going up in 2, 10, 50 s and so on.
- Label both axes fully (you can often copy the headings from the results chart) and include units.
- Plot points on a line graph as small, neat crosses that intersect exactly on the required point. (Alternatively, use a dot with a circle around it.)
- Check if there are instructions telling you whether to draw a best-fit line or a set of ruled straight lines joining points.
- In general, we only draw a best fit line when the points clearly follow a definite pattern. If you are unsure of this, then join points with ruled straight lines between the points.
- Do not extrapolate (extend the line beyond your data points) unless you are told to do so, or unless you are absolutely certain that it is reasonable to do so.

Chapter 1:
Microscopy

Chapter outline

This chapter relates to Chapter 1: Cell structure, in the coursebook.

In this chapter, you will complete practical investigations on:

- 1.1 Making a temporary slide and drawing cells
- 1.2 Measuring cells, using an eyepiece graticule and stage micrometer
- 1.3 Comparing animal cells and plant cells

Practical investigation 1.1:
Making a temporary slide and drawing cells

Introduction

In this activity, you will practise using a light microscope. You will also make a temporary mount of plant tissue, observe it using the microscope and make a drawing of some of the cells.

Equipment

You will need:

• a light microscope • a source of light (this could be built into the microscope, or a lamp, or bright light from a window) • two or three microscope slides • two or three coverslips • a dropper pipette • a mounted needle or seeker • forceps (tweezers) • sharp scissors or a blade (safety razor or scalpel) • filter paper or paper towel • tile • some pieces cut from an onion bulb • a medium–hard (HB) pencil • a good quality eraser

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- Take care when using a sharp blade to cut the onion epidermis.

Method

Part 1: Making a temporary slide and viewing it through a microscope

Figure 1.1 shows the parts of a microscope.

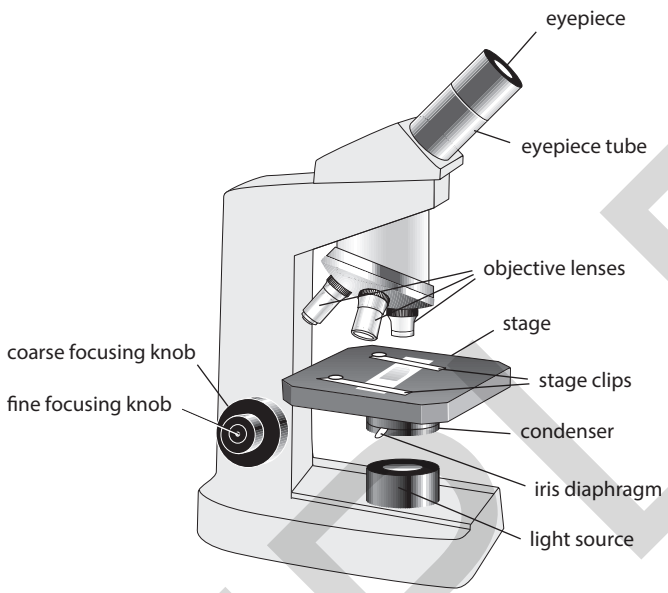


Figure 1.1

HINT
Your microscope will almost certainly not be the same as the one in Figure 1.1. For example, it may have a mirror instead of a light source.

1 Set up your microscope on the bench. Look for each of the parts that are labelled on the diagram.

2 You are now going to make a slide that you can view through your microscope.

- Take a piece of one of the layers from inside an onion bulb. Using scissors or a sharp blade, cut out one piece measuring approximately 1 cm × 1 cm.
- Using a dropper pipette, place a drop of water onto the centre of a clean microscope slide.
- Using forceps, gently peel away the very thin layer of epidermis on the inside surface of the piece of onion. **Immediately** place the epidermis into the drop of water on the slide. Use a mounted needle or seeker to gently spread out the epidermis, so that it is not folded over and is covered by water. You may need to add another drop of water to it.
- Gently lower a coverslip onto the slide, to cover the onion epidermis. It's a good idea to use a mounted needle (see Figure 1.2) as this helps to avoid trapping any air bubbles.

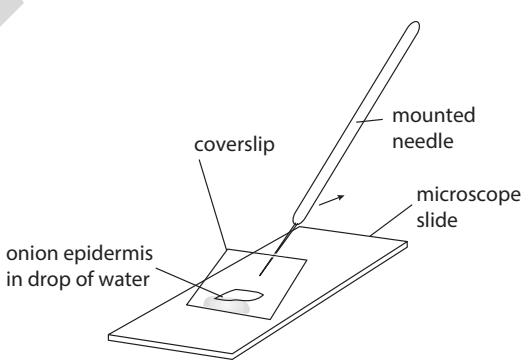


Figure 1.2

HINT
If you leave water on the surface of the slide, this may get onto the objective lens. Over time, deposits may form on the lens.

- Use filter paper to gently remove any water from the top of the coverslip or on the surface of the slide.

3 Now you can look at your slide through the microscope.

- Turn the objective lenses so that the smallest one is over the hole in the stage.
- Look down through the eyepiece and make sure that you can see light. If you cannot see the light, adjust the light source or the mirror.
- Place your slide on the microscope stage, with the epidermis over the hole.
- Looking from the side of the microscope, turn the coarse focusing knob to lower the objective lens, until the objective lens is almost touching the slide.
- Look down the eyepiece again. Slowly turn the coarse focusing knob the other way, to raise the objective lens. Stop when you can see the epidermis. It will probably not look clear.
- Now turn the fine focusing knob until you can see the epidermis clearly. You should be able to see something similar to Figure 1.3.

HINT
With some microscopes, it is possible to lower the objective lens so much that you can crash into the slide and break the coverslip. If you look from the side, it is less likely you will do this.

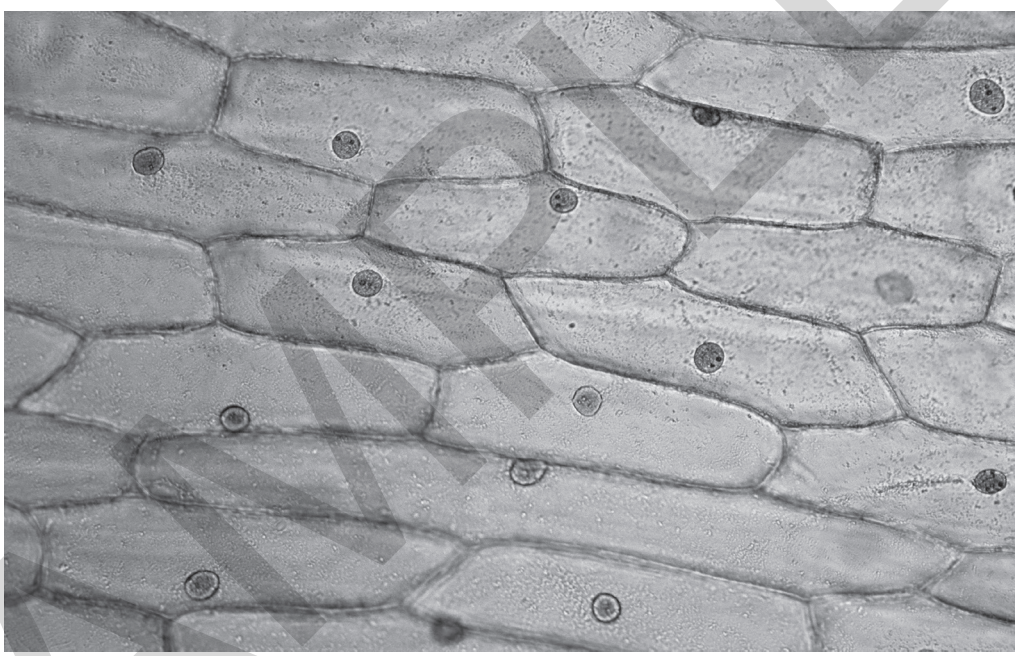


Figure 1.3

HINT
It is sometimes a good idea to keep changing the objective lens as you do your drawing. For example, you may decide to use the lowest power lens, but occasionally change up to the higher power lenses to check on the detail.

Part 2: Making a high-power drawing of onion epidermis

1 Focus on the onion epidermis using the lowest power objective, as described previously. Carefully swing the objective lenses around until the next largest one is over the slide. Focus using the fine focusing knob.

2 Decide which objective provides the best view of the epidermis. If you have an even higher power objective lens, you could try that one as well.

3 Make a drawing of the epidermis in the space that follows.

- Use a medium–hard, (HB) sharp pencil.
- Use a high quality eraser, so that you can completely remove any mistakes in your drawing.
- Your drawing should be large, using at least 50% of the space available – but make sure you leave enough space around it for your labels.
- Take care to get the shapes and proportions of the cells correct.

- All lines should be single and clear. Do not leave any gaps, however small, in the lines.
- Always show the cell walls with two lines – cell walls have thickness.
- Do not shade anything at all in your drawing.
- Draw what you can see, not what you think you should see.

4 Label the cytoplasm, nucleus and cell wall on your drawing.

- Use a pencil for the label lines. You may also like to use a pencil to write the names.
- Use a ruler to draw the label lines. Make sure that the end of the label line touches the part that you are labelling.
- Keep label lines separate from each other.
- The label lines can go in any direction, but the written labels should be horizontal.

Part 3: Adding a stain to a temporary slide

You are going to add some iodine in potassium iodide solution to your onion epidermis slide. This will colour any starch grains in the onion cells blue–black.

1 Place a small drop of iodine solution on the microscope slide, touching the edge of the coverslip.

2 Very carefully place one edge of a piece of a filter paper against the **opposite** side of the coverslip, as shown in Figure 1.4. The water underneath the coverslip will soak into the filter paper, bringing through the iodine solution.

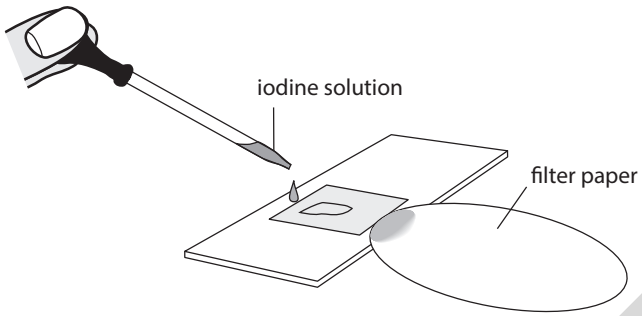


Figure 1.4

3 Clean the slide, and then observe the stained onion epidermis through the microscope. Describe any differences you can see in the stained cells compared with their appearance before staining.

……

……

……

……

Practical investigation 1.2:

Measuring cells, using an eyepiece graticule and stage micrometer

Introduction

In this activity, you will use an eyepiece graticule and stage micrometer to measure two types of plant cell. An eyepiece graticule is a little scale that fits inside the eyepiece of your microscope. When you look through the eyepiece, you can see the scale on the graticule at the same time as the object on the microscope stage. You can measure the size of the object in 'eyepiece graticule units'.

You then need to **calibrate** these graticule units. You do this using a stage micrometer. This is a slide with a scale with very small divisions on it, which you place on the microscope stage. The markings on this scale are very precisely drawn, and we know exactly how far they are apart.

Equipment

You will need:

- microscope, with a graticule in the eyepiece
- prepared slide of section through a leaf
- onion epidermis slide from Practical investigation 1.1

Access to:

- a stage micrometer

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- There are no significant safety issues for this practical investigation.

Method

Part 1 : Measuring cells using an eyepiece graticule

1 Place a prepared slide of a transverse section through a leaf onto the stage of your microscope.

2 Check that there is an eyepiece graticule inside the eyepiece of your microscope. Look down through the eyepiece and turn it around. You should see the scale on the eyepiece graticule turning around.

3 Using the smallest objective lens, focus on the leaf section. Move the slide until you can see palisade cells. If necessary, change to a different objective lens, until you can see a group of palisade cells clearly. Move the slide until the cells are placed vertically.

4 Turn the eyepiece graticule until the scale lies horizontally across the group of cells, as shown in Figure 1.5.

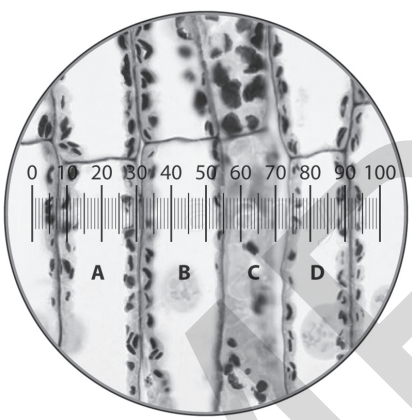


Figure 1.5

5 Move the slide until the 0 on the graticule scale lies exactly over the cell wall of one cell. Use the scale to measure the width of three or four cells in eyepiece graticule units.

..... palisade cells measure ..... graticule units.

Part 2: Calibrating the eyepiece graticule

1 Keeping the same objective lens over the slide, remove the slide from the stage and replace it with a stage micrometer.

2 Look down the eyepiece and focus on the stage micrometer scale. Move the eyepiece and/or the slide until the eyepiece graticule scale and the stage micrometer scale lie exactly next to each other, as shown in Figure 1.6.

HINT
If you get confused about which scale is the eyepiece graticule, and which is the stage micrometer, just turn the eyepiece. The scale that goes round is the eyepiece graticule scale.

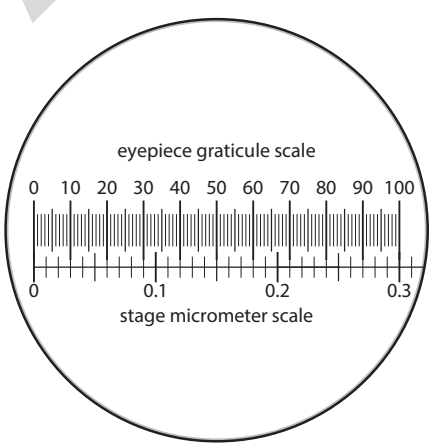


Figure 1.6

3 Look for a good alignment of marks on the two scales, as far apart as possible. In the example in Figure 1.6, there is alignment at 0, 0 and at 80 on the eyepiece graticule scale and 0.24 on the stage micrometer scale.

Write down the alignments on your scales:

4 The large divisions on the stage micrometer scale are 0.1 mm apart. The small divisions are 0.01 mm apart.

$$0.01\,\text{mm} = 0.01 \times 10^3\,\text{m} = 10\,\mu\text{m}$$

Use this information to calculate how many µm are represented by one small division on the eyepiece graticule scale.

1 small eyepiece graticule unit = ... µm

5 Go back to the measurement you made at the end of Part 1, where you measured the width of three or four cells in eyepiece graticule units.

Convert this measurement to µm.

..... palisade cells measure ..... µm.

6 Divide this value by the number of cells, to find the mean width of one palisade cell.

Mean width of one palisade cell = ... µm

7 Remove the stage micrometer from the microscope. Place a slide of onion epidermis cells onto the stage. **Using the same objective lens** as you did for the palisade cells, measure the width of a group of cells in graticule units.

..... onion epidermis cells measure ..... eyepiece graticule units.

8 Convert this measurement to µm, and then calculate the mean width of one onion epidermis cell.

Mean width of one onion epidermis cell = ... µm

Part 3: Calculating the magnification of a drawing

The **magnification** of an image is the number of times larger it is than the actual object.

magnification = size of image ÷ size of actual object

1 Measure the width of the group of onion cells in your drawing in Practical investigation 1.1. Record your answer in mm, and then multiply by 10^3 to convert it to µm.

Width of ..... cells in the drawing is ..... mm = ... µm.

2 Use your answer in Step 8 in Part 2 of this practical investigation to calculate the magnification of your drawing.

Practical investigation 1.3:
Comparing animal cells and plant cells

Introduction

In this activity, you will prepare a temporary slide of human cheek cells, and compare their size and structure with palisade cells and onion epidermis cells.

Equipment

You will need:

• microscope, with a graticule in the eyepiece • prepared slide of section through a leaf • onion epidermis slide from Practical investigation 1.1 (or you can make a new one) • clean microscope slides and coverslips • dropper pipette • iodine in potassium iodide solution • methylene blue stain • cotton bud or similar

Access to:

• a stage micrometer

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- There is a very small risk of pathogenic organisms in the saliva and cheek cell sample on the cotton bud. Place the bud in a container of disinfectant immediately after use.

Method

Part 1: Observing, recording and measuring cheek cells

1 Gently rub a cotton bud around the inside of your cheeks, as shown in Figure 1.7.

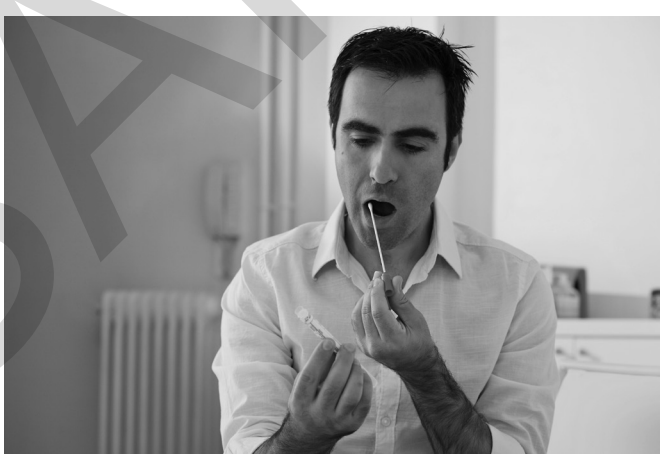


Figure 1.7

2 Rub the cotton bud onto the centre of a clean microscope slide. Note: you will not be able to see very much on the slide, but there should be a few cheek cells present. Place the bud in a container of disinfectant immediately after use.

3 Add a small drop or two of methylene blue stain to the part of the slide where you rubbed the cotton bud. This stain is absorbed by living cells. More is taken up by the nucleus than by the cytoplasm, so it makes the nucleus look dark blue and the cytoplasm pale blue.

4 Carefully lower the coverslip onto the slide (see Figure 1.2), trying to avoid trapping air bubbles. Clean the slide and coverslip using filter paper.

5 Look at the slide through the microscope. These cells are much smaller than the plant cells you have looked at earlier, so you may need to use a larger objective lens to view them.

In the space below, make a large labelled drawing of three or four cheek cells.

6 Use the eyepiece graticule to measure the diameter of three cheek cells in graticule units. (The cells will not be arranged in a neat row as in Practical investigation 1.2, so you will have to measure each one separately.) Calculate the mean diameter of one cheek cell, in eyepiece graticule units.

……

7 Now use the stage micrometer scale to convert the eyepiece graticule units to µm. (If you used the same objective lens as for the calculation in Practical investigation 1.2, you can use the same conversion factor. However, if you have used a different objective lens when measuring the cheek cells, you will need to follow Steps 1–4 in Practical investigation 1.2, Part 2 for this objective lens.)

……

8 Use your answers to Steps 7 and 8 to calculate the mean diameter of a cheek cell in µm.

……

9 Calculate the magnification of your drawing from Step 5. Show your working.

……

HINT
If you have time, you could try colouring cheek cells with iodine solution, or onion epidermis cells with methylene blue stain.

Part 2: Comparing cheek cells, palisade cells and onion epidermis cells

You are going to construct a table to compare the size and visible structures in the three types of cell you have been observing and measuring.

1 Using a ruler and pencil, draw a table with four columns in the space that follows. Label the first column 'Feature', and the other three columns with the type of cell.

2 On a piece of rough paper, make a note of possible features that you can compare. These could include the sizes of the cells, their shapes, the relative size of the nucleus compared with the size of the entire cell, the structures visible inside the cells, and the way the cells are grouped together. Remember that a comparison includes similarities as well as differences.

3 Complete your table to compare the three types of cell. Draw a ruled line beneath each set of features, so that it is easy for someone else to understand the information you are listing.

Chapter 2:
Biological molecules

Chapter outline

This chapter relates to Chapter 2: Biological molecules in the coursebook.

In this chapter, you will complete practical investigations on:

- 2.1 The biochemical tests used to identify different biological molecules
- 2.2 The semi-quantitative Benedict's test and serial dilutions
- 2.3 Using a semi-quantitative iodine test to compare the starch content of bananas

Practical investigation 2.1:
The biochemical tests used to identify different biological molecules

Introduction

It is important to be able to identify common biological molecules. You need to know the biochemical tests for starch, reducing sugars, non-reducing sugars, proteins and fats. All these tests are qualitative and only give an idea of the presence or absence of a molecule rather than the quantity.

Equipment

You will need:

• 10 test tubes • test-tube rack • Bunsen burner, tripod, gauze, heat-proof tile • test-tube holder • glass beakers, 500 and 50 cm^3 • pipette, 10 cm^3, and pipette filler • Benedict's solution, 25 cm^3 • biuret solution 25 cm^3 • iodine solution in a dropper bottle • ethanol, 200 cm^3 • distilled water, 50 cm^3 • dilute hydrochloric acid in a dropper bottle • sodium hydrogencarbonate (solid) • spatula • 20 cm^3 of 1% starch solution, 1% protein solution (albumin or casein), vegetable oil, 10% glucose, 10% fructose, 10% sucrose, 10% lactose, 10% maltose • 'unknown' solutions X, Y and Z, 20 cm^3

Access to:

• tap water, sink (to throw away solutions)

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- If you splash solution on skin, wash it off with water. Tie back long hair, and use Bunsen burners with care.
- Iodine solution should not be thrown away in water that could come into contact with aquatic life.

- Biuret solution is an irritant and should be handled carefully.
- Sodium hydroxide is corrosive and it is essential that eye protection is worn. If you splash sodium hydroxide into your eyes, wash eyes with lots of running water.
- Ethanol is highly flammable and so only carry out the lipid test when there are no naked flames (e.g. Bunsen burners).

Method

Part 1: The Iodine test for starch

1 Label three test tubes 1–3.

2 Using a pipette, place 5 cm^3 of 1% starch solution into the test tube labelled '1'.

3 Rinse out the pipette with water.

4 Place 5 cm^3 10% glucose solution into test tube 2, again rinsing out the pipette.

5 Place 5 cm^3 distilled water into test tube 3.

6 Place five drops of iodine solution into each tube. Mix each tube.

7 Record the colours of each tube in Table 2.1 of the Results section.

Part 2: The Biuret test for proteins

The biuret test for proteins can be carried out by adding a mixture of copper sulphate and sodium hydroxide (as biuret solution) or by adding the two solutions separately.

1 Label three test tubes 1–3.

2 Using a pipette, place 5 cm^3 of 1% protein solution into tube 1.

3 Wash the pipette with water.

4 Place 5 cm^3 10% glucose solution into tube 2, again rinsing out the pipette.

5 Place 5 cm^3 distilled water into test tube 3.

6 Place 5 cm^3 biuret solution[2] into each tube and mix.

7 Record the colours of each solution in Table 2.1.

Part 3: The reducing sugar test

Some sugars can donate electrons to other chemicals; this means that the sugars are reducing agents. In Benedict's solution, soluble Cu^{2+} ions are blue. If these Cu^{2+} ions gain an electron, they become Cu^{+} ions which are red and insoluble.

1 Label six test tubes 1–6.

2 Using a pipette, place 5 cm^3 of 10% glucose solution into tube 1. Wash the pipette with water.

3 Place 5 cm^3 sucrose, maltose, fructose, lactose and water into tubes 2–5, washing the pipette each time.

4 Place 5 cm^3 distilled water into test tube 6.

5 Add 5 cm^3 Benedict's solution to each tube.

6 Set up a boiling water bath by filling the 500 cm^3 beaker half full of water. Heat it using the Bunsen burner, tripod, gauze and heatproof mat as shown in Figure 2.1.

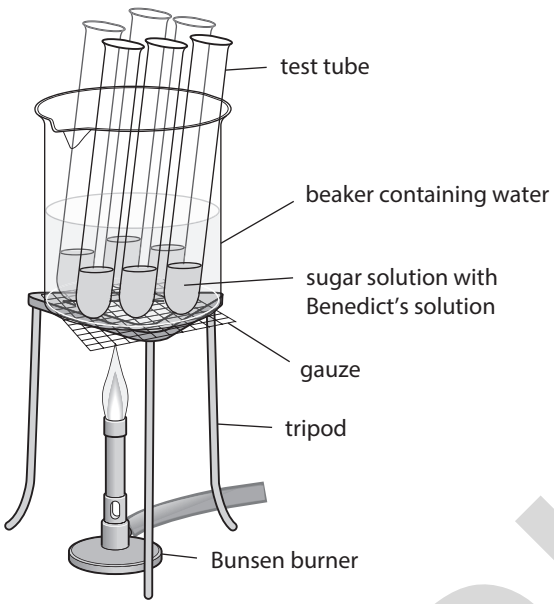


Figure 2.1

7 When the water bath is boiling, carefully place the test tubes into it.

8 After 5 min, turn off the Bunsen burner and carefully remove the test tubes using a test-tube holder. Place the test tubes into a rack.

9 Record the colour and consistency (cloudy or clear) of each solution in Table 2.1.

HINT
Remember that if you are testing for non-reducing sugars, you need to do a Benedict's test first to show that there are no reducing sugars present.

Part 4: The non-reducing sugar test

1 Label two test tubes 1 and 2.

2 Using a pipette, place 5 cm^3 distilled water into tube 1 and 5 cm^3 10% sucrose solution into test tube 2.

3 Place two drops of dilute hydrochloric acid into each tube.

4 Set up a boiling water bath.

5 Carefully place the tubes into the boiling water bath for 2 min.

6 Remove the tubes and place them into a rack.

7 Using a spatula, tap small amounts of solid sodium hydrogencarbonate into the solutions until they no longer fizz (this neutralises the acid).

8 Using a pipette, add 5 cm^3 Benedict's solution to each tube.

9 Place the tubes into the boiling water bath for 5 min.

10 Turn off the Bunsen burner and carefully remove the test tubes.

11 Record the colour and consistency of each solution in Table 2.1.

Part 5: The emulsion test for lipids (fats and oils)

1 Label two test tubes 1 and 2.

2 Using a pipette, place 5 cm^3 ethanol into each tube.

3 Add two drops of vegetable oil to tube 1 and mix thoroughly to dissolve the oil in the alcohol.

4 Add 5 cm^3 water to each tube and mix thoroughly.

5 Record the colour and consistency of each solution in Table 2.1

Part 6: Testing the 'Unknown' solutions

1 There are three solutions labelled X, Y and Z.

2 The solutions contain different combinations of starch, protein, sucrose and glucose.

3 Carry out biochemical tests to determine the contents of each solution.

4 Draw a results table for these three solutions in the space beneath Table 2.1.

Results

Biological molecule	Final colour of solution after biochemical test				
	iodine	biuret	reducing sugar	non-reducing sugar	emulsion test
1% starch					
1% protein					
10% glucose					
10% fructose					
10% maltose					
10% lactose					
10% sucrose					
vegetable oil					
water					
ethanol					

Table 2.1

Draw up a results table for your 'unknown' solutions in the following space. Give your table headings. The first column should be given the heading, 'Solution'.

Data analysis

a **i** Look at your table of results and use it to decide what substances were in each unknown solution.

Contents of unknown solution X ……

Contents of unknown solution Y ……

Contents of unknown solution Z ……

ii Why can you not be certain as to the presence or absence of sucrose in solutions X and Z?

……

……

b Use your completed Table 2.1 to complete the following:

- Monosaccharides that are reducing sugars include:

  ……

- Disaccharides that are reducing sugars include:

  ……

- Disaccharides that are non-reducing sugars include:

  ……

HINT
The reducing sugar test works because Benedict's solution contains dissolved Cu^{2+} ions which are blue in colour. Some sugar molecules are able to donate electrons to the Cu^{2+} ions making them red, insoluble Cu^{+} ions. This means that a red precipitate forms.

Evaluation

c Explain why each biochemical test (except the emulsion test) was also carried out with water.

……

……

d Explain whether these biochemical tests are qualitative or quantitative.

……

e The non-reducing sugar test should have given a positive result with both glucose and sucrose.

i Explain why glucose produced a positive result.

……

ii Explain why sucrose produces a positive result in the non-reducing sugar test but a negative result in the reducing sugar test.

……

……

iii Describe how you could use the biochemical tests to distinguish between a solution of glucose and a solution of sucrose.

……

……

f A student carried out a reducing sugar test on a sample of an unknown solution. This produced a positive result. They then carried out a non-reducing sugar test on another sample of the same solution. This produced a positive result but with a lot more precipitate. Explain this result.

……

……

Practical investigation 2.2:
The semi-quantitative Benedict's test and serial dilutions

Introduction

In this investigation, you will produce a range of dilutions of glucose by serial dilution and use the semi-quantitative Benedict's test to estimate the concentration of a solution of glucose.

Equipment

You will need:

• nine test tubes • test-tube rack • pipettes, 1 cm^3 and 10 cm^3, and pipette fillers • 50 cm^3 of glucose solution, 10% concentration • 20 cm^3 of glucose solution, unknown concentration • distilled water, 100 cm^3 • Benedict's solution, 100 cm^3 • beaker, 50 cm^3, 500 cm^3 • Bunsen burner, tripod, gauze, heat-proof tile • test-tube holder

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- If you splash your skin, wash with water.
- Use Bunsen burners with care and tie back long hair.

Method

Part 1: Preparing the different concentrations of glucose by serial dilution

It is important to be able to understand how to make different concentrations of solution by using the technique of serial dilution (Figure 2.2 shows the beginning of this process).

1 Label the test tubes 1–7.

2 Using a pipette, place 10 cm^3 10% glucose solution into tube 1.

3 Remove 1 cm^3 of this solution from tube 1 and place into tube 2.

4 Add 9 cm^3 distilled water to tube 2 and mix. This is now diluted to 1% glucose.

5 Remove 1 cm^3 of the 1% glucose solution from tube 2 and place into tube 3.

6 Add 9 cm^3 distilled water into tube 3 and mix. This is now diluted to 0.1% glucose.

7 Remove 1 cm^3 of 0.1% glucose from tube 3 and place into tube 4.

8 Add 9 cm^3 distilled water to tube 4 and mix. This is now diluted to 0.01% glucose.

9 Remove 1 cm^3 of 0.01% glucose from tube 4 and place into tube 5.

10 Add 9 cm^3 distilled water to tube 5 and mix. This is now diluted to 0.001% glucose.

11 Remove 1 cm^3 of 0.001% glucose from tube 5 and place into tube 6.

12 Place 9 cm^3 distilled water into tube 6.

13 Remove 1 cm^3 of 0.001% glucose from tube 6 and throw it away.

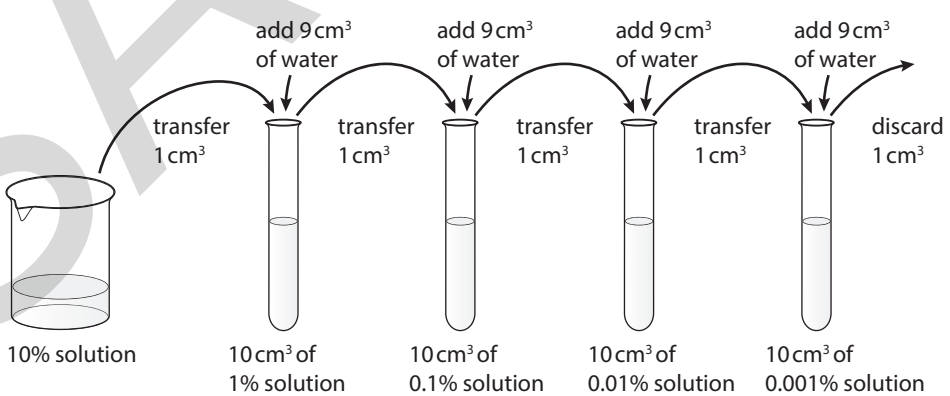


Figure 2.2

Part 2: Carrying out the Benedict's test

1 Set up a boiling water bath as shown in Practical investigation 2.1.

2 Place 9 cm^3 unknown glucose solution into tube 7.

3 Add 5 cm^3 Benedict's solution to each of tubes 1–6.

4 Carefully place tubes 1–6 into the boiling water bath for exactly 5 min.

5 Turn off the Bunsen burner and using a test tube holder, carefully remove the test tubes and place them in a rack in order 1–6.

6 Record the colours of the test tube by either taking a photograph of the rack and sticking it in this workbook, or colouring the test tubes in Figure 2.3 in the Results section below the appropriate colours.

7 Compare the colour of the 'unknown' solution with the colours of the known standards. Identify the concentration of the standards which have the most similar colour. If the colour is not exactly the same as the standard, look at the colours of the solutions with higher and lower concentrations. This can help you to suggest a range within which the concentration lies.

Results

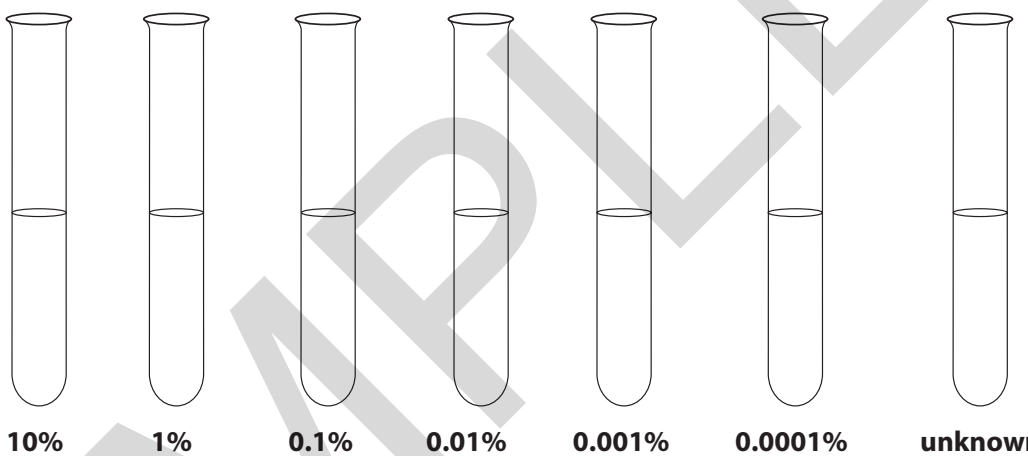


Figure 2.3

Data analysis

a The concentration of glucose in the 'unknown' solution is approximately ……..%. It may lie within a range of ……% to ……%.

Evaluation

b Explain why this method is considered a semi-quantitative method rather than a quantitative one.

……

……

……

c How certain can we be about the exact concentration of glucose in the unknown solution?

……

……

……

d Why was 1 cm^3 thrown away from the last dilution?

……

……

……

e Several variables were kept constant for all the tubes. List as many of these control variables as possible and explain why it is essential to keep them constant.

……

……

……

……

……

f It is important that the amount of Benedict's solution added is greater than the glucose. Explain how it could affect the result if there were too little Benedict's solution.

……

……

……

g Explain the purpose of tube 6.

……

……

……

h Suggest an alternative method that could be used to make the test fully quantitative.

……

……

……

i Serial dilution can be used to make many different concentration ranges. This experiment used a dilution factor of 10 each time. Calculate the concentrations of glucose solutions that would have been produced if 5 cm^3 of water and 5 cm^3 glucose solution were used each time instead of 9 cm^3 water and 1 cm^3 glucose solution.

……

……

……

Practical investigation 2.3:
Using a semi-quantitative iodine test to compare the starch content of bananas

Introduction

In this investigation, you will make a series of standard starch concentrations by diluting a stock, observe if different starch concentrations give a different colour with iodine solution and compare the starch content of bananas of different ripeness.

Equipment

You will need:

• nine test tubes • test-tube racks • iodine solution in a dropper bottle • 1% starch suspension, 50 cm^3 • distilled water, 100 cm^3 • pipettes, 10 cm^3, 1 cm^3, pipette filler • pieces of banana flesh from green, yellow and black bananas • knife or scalpel • Bunsen burner, tripod, gauze, heatproof tile • test-tube holder • glass beakers, 500 cm^3 and 50 cm^3 • spatula

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- If you splash your skin, wash with water.
- Use Bunsen burners with care and tie back long hair.
- Care should be taken when working with sharp blades.
- Iodine solution should not be thrown away in water that could come into contact with aquatic life.

Method

Part 1: Making the standard concentrations of starch

1 In Practical investigation 2.2, you made serial dilutions of glucose. Use the same method to make 9 cm^3 of each of the following concentrations of starch suspensions starting with a 1% stock:

- 1%
- 0.1%
- 0.01%
- 0.001%
- 0.0001%

Label each of the five test tubes appropriately.

2 Place 9 cm^3 distilled water into a sixth test tube.

3 Add three drops of iodine solution to each tube and mix.

4 Put all the test tubes into a rack in order of decreasing starch concentration.

5 Either colour each tube the appropriate colour in Figure 2.5 in the Results section or take a photograph of the tubes and stick it into this workbook.

Part 2: Making the banana extracts

1 Label three test tubes, A, B and C.

2 Use a knife and cut out a 1 cm^3 piece of green, unripe banana (without the peel) and place it into a beaker (see Figure 2.4).

3 Add 10 cm^3 distilled water to the beaker and mash the banana with a spatula to make a suspension of banana. Transfer the extract to tube A.

4 Repeat the procedure for the yellow (ripe) and black (over-ripe) bananas, transferring the extracts into tubes B and C respectively.

5 Place all three test tubes into a boiling water bath for 5 min.

6 Remove the tubes from the water bath and leave to cool for 10 min.

7 Add five drops of iodine to each solution, mix and compare each with the standard dilutions.

8 Record the approximate concentrations of starch in each type of banana in the Results section.

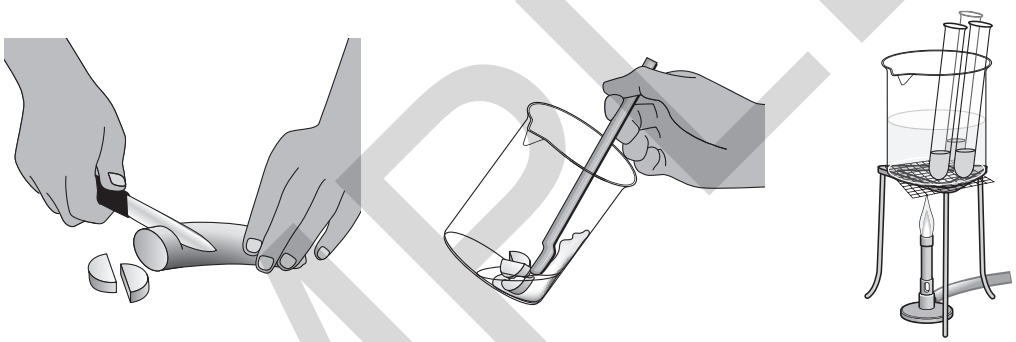


Figure 2.4

Results

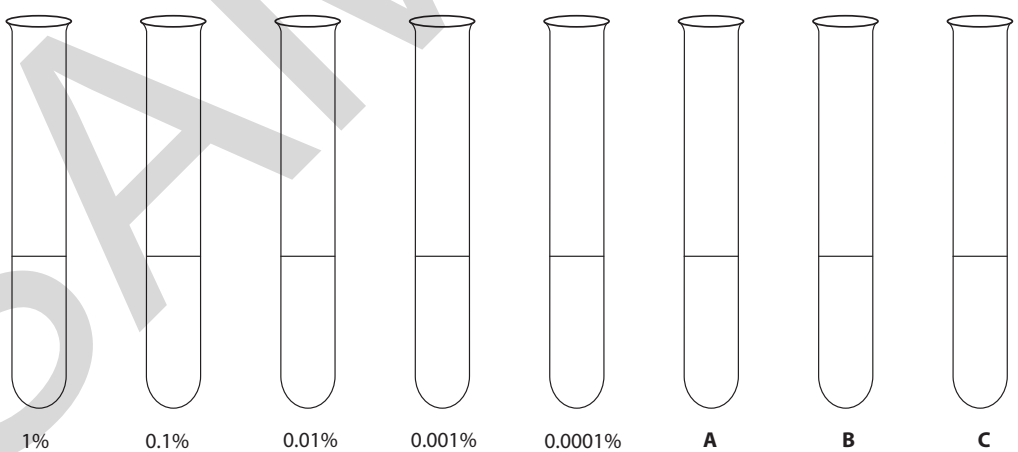


Figure 2.5

Data analysis

a The approximate starch concentrations and approximate ranges (the concentrations of the standards within which the colour lies) for each banana are:

- green (unripe): concentration…… range: …… to ……
- yellow (ripe): concentration…… range: …… to ……
- black (over-ripe): concentration…… range: …… to ……

b Explain what the investigation shows you about what happens to bananas as they ripen.

……

……

Evaluation

c Discuss the accuracy of this method and suggest how it could be improved to give more precise values of starch concentrations.

……

……

……

d Describe four limitations of this investigation.

1 ……

2 ……

3 ……

4 ……

Chapter 3:
Enzymes

Chapter outline

This chapter relates to Chapter 3: Enzymes, in the coursebook.

In this chapter, you will complete practical investigations on:

- 3.1 The time-course of an enzyme-catalysed reaction
- 3.2 The effect of substrate concentration on the rate of an enzyme-catalysed reaction
- 3.3 The effect of enzyme concentration on the rate of an enzyme-catalysed reaction
- 3.4 The effect of temperature on the rate of an enzyme-catalysed reaction
- 3.5 Immobilising urease
- 3.6 The effect of an inhibitor on the action of bromelain

Practical investigation 3.1:
The time-course of an enzyme-catalysed reaction

Introduction

Enzymes are proteins that act as catalysts. They increase the rate of a reaction, but are not changed themselves.

In this investigation, you will measure the rate of reaction by measuring the rate of formation of the product. You will investigate how this changes as the reaction takes place.

The enzyme you are using is catalase. This enzyme catalyses the breakdown of hydrogen peroxide to water and oxygen:

hydrogen peroxide → water + oxygen

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Hydrogen peroxide is the substrate in this reaction, and water and oxygen are the products.

Catalase is found in almost all living cells. In this investigation, you will macerate celery stalks in water. This will break up the cells, so the catalase inside them dissolves in the water.

HINT
The process of breaking something up in water is called macerating.

Equipment

You will need:

• two or three large stalks of celery • about 20 cm^3 of 10 volume hydrogen peroxide solution • an electric blender • a filter funnel and muslin • two 250 cm^3 beakers • a large test tube, preferably a side-arm test tube • a gas syringe • tubing to make an airtight connection between the test tube and the gas syringe • a timer (e.g. on a phone) • a retort stand, boss and clamp • apparatus for measuring small volumes, for example two 5 cm^3 or 10 cm^3 syringes or two graduated pipettes

Access to:

• distilled water

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- Hydrogen peroxide is a strong oxidising agent and bleach. Wear safety glasses throughout this practical. If you get hydrogen peroxide on your skin, wash with plenty of cold water.
- If oxygen is given off very quickly, the plunger of the syringe may move so fast that it shoots out of the end of the syringe and could hit someone. You can avoid this by tying the plunger loosely to the syringe with a piece of string.

Method

Part 1: Preliminary work

You are going to decide suitable concentrations of the enzyme and substrate to use when carrying out the main part of the investigation. This is difficult to predict, because you do not know how much catalase the celery contains, nor how fast the enzyme will work.

1 Break or cut one or two large stalks of celery into several pieces and place in an electric blender. Add approximately 400 cm^3 of distilled water. (Make a note of the quantity of celery and the volume of water that you use, as this may be helpful later.) Switch on the blender to make a suspension of celery extract in water.

2 Place some muslin in a filter funnel, and support the funnel over a beaker. Pour the celery extract into the funnel, and leave it so the liquid part of the extract passes through the muslin. You can squeeze it gently to speed up this process.

3 Connect a large, side-arm test tube to a gas syringe. Check that the plunger of the gas syringe moves freely. Support the test tube and gas syringe using a retort stand, boss and clamp.

Figure 3.1 also shows an alternative arrangement, if you do not have a side-arm test tube.

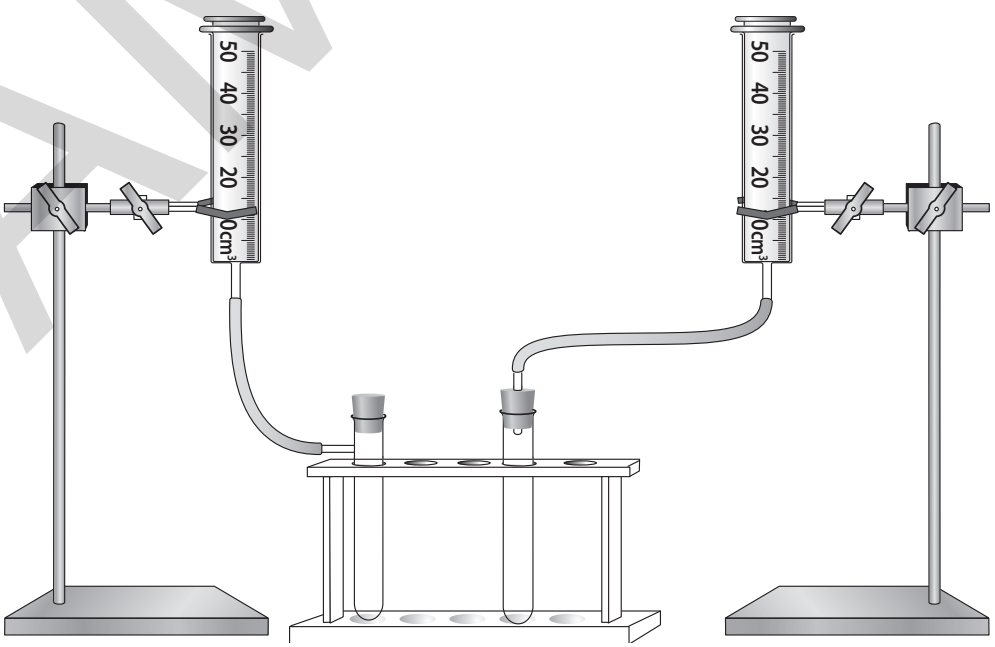


Figure 3.1

4 Measure 20 cm^3 of hydrogen peroxide solution and place in the test tube.

5 Measure 5 cm^3 of celery extract.

6 Add the celery extract to the hydrogen peroxide solution, and replace the bung as quickly as you can.

7 As oxygen is given off, the plunger of the gas syringe will move. Note how quickly this moves, and decide whether you will be able to measure the volume of oxygen in the syringe at 15 s or 30 s intervals. If the reaction is too fast for you to do this, consider how you can change the volume of either hydrogen peroxide or celery extract to slow it down. (It is unlikely that the reaction will be too slow, but if this happens try making a new celery extract using more celery and less water.)

8 Repeat Steps 4–7, trying different concentrations or volumes of enzyme or celery extract, until you are confident that you will be able to take readings of the volume of oxygen in the syringe at 15 s or 30 s intervals.

Part 2: Measuring the rate of oxygen formation over time

1 Ensure that your apparatus is completely clean and air-tight, with the gas syringe reading 0.

2 Add the required volume of hydrogen peroxide solution to the test tube.

3 Add the required volume of celery extract to the hydrogen peroxide in the tube. **Immediately** push the bung into the tube and start the timer.

4 Record the volume of oxygen in the gas syringe every 15 s or 30 s, in Table 3.1. Add more rows to the Results table as required. Continue until the rate of oxygen production starts to remain the same.

HINT
Think about the degree of precision with which you can read the volumes. This will depend on the scale on the gas syringe. For example, if it is marked off in cm^3, you can probably read the scale to the nearest 0.5 cm^3.

Results

Time / s	Volume of oxygen / cm^3

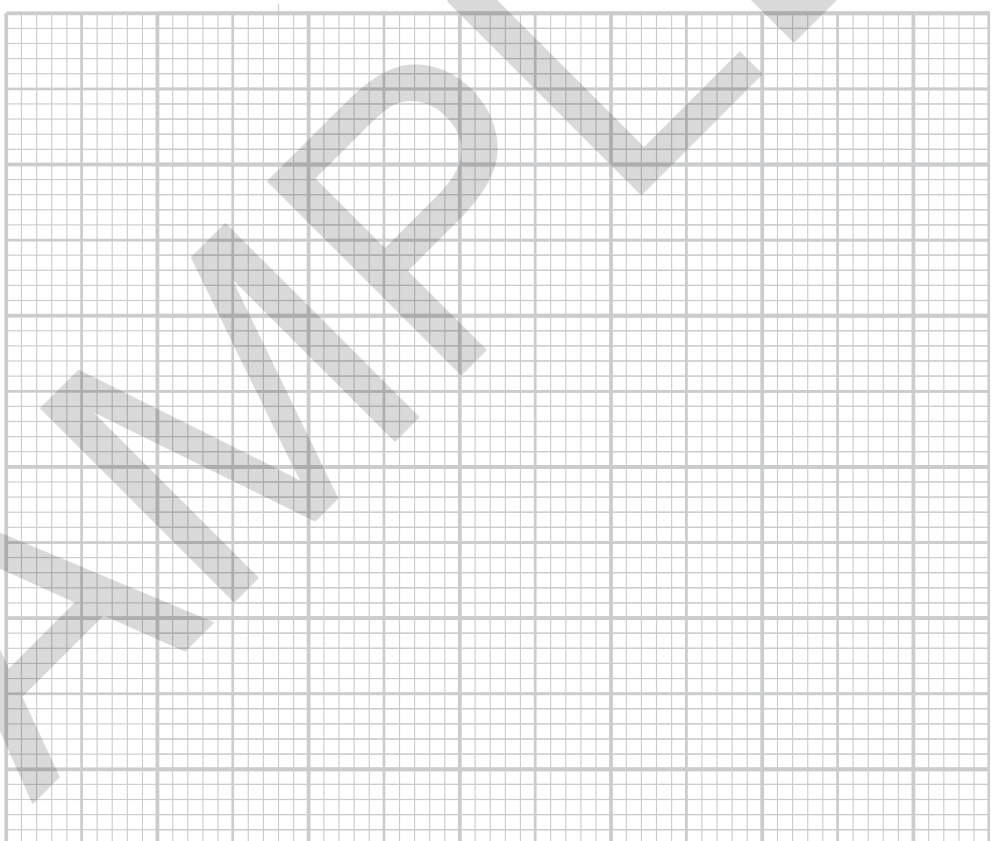
Table 3.1

Data analysis

a On the following grid, plot a line graph with Time / s on the *x*-axis and Volume of oxygen / cm^3 on the *y*-axis.

Take care to:

- use most of the graph paper
- use a scale on the *x*-axis that runs from 0 to your longest time measured
- use a scale on the *x*-axis that goes up in sensible, equal intervals, for example 15 or 30 s
- use a scale on the *y*-axis that runs from 0 to just above the greatest volume that you measured
- use a scale on the *y*-axis that goes up in sensible, equal intervals
- plot points with a very small, neat, carefully placed cross (x not +)
- draw a smooth best-fit line.



HINT
Remember that 'describe' means you simply write what you can see, without trying to say **why** the rate of reaction changes in this way.

b The steeper the slope (**gradient**) of the line, the greater the rate of the reaction.

Describe how the rate of the reaction changes with time.

……

……

……

c On your graph, at what time is the concentration of substrate greatest? Explain your answer.

……

……

d On your graph, at what time has all of the substrate been converted to product? Explain your answer.

……

……

HINT
Remember that 'explain' means you should give reasons, using your knowledge and understanding.

e Use your answers to **b**, **c** and **d** to explain the shape of the curve.

……

……

……

……

……

f You are now going to use your graph to find the **initial rate of reaction**. This is the rate of reaction as close to the start as you can measure.

- Draw a tangent to your curve as close to the origin as possible, perhaps at around 10, 20 or 15 s. Your tangent must be at least half as long as the line of the graph, so make it as long as possible (see Figure 3.2).

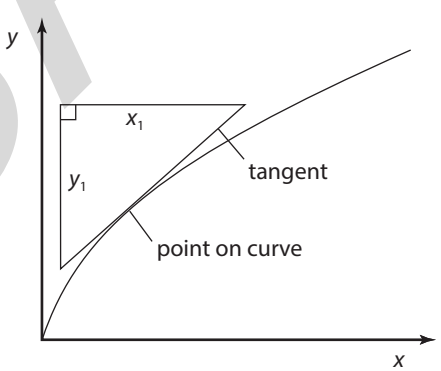


Figure 3.2

- Draw a right-angled triangle, with the tangent as the hypotenuse.
- Measure the lengths of x_1 and y_1. Use the scales on the *x*-axis and *y*-axis to record these as seconds and cm^3 of oxygen, respectively.

x_1 = …… s

y_1 = …… cm^3 of oxygen

- Calculate the gradient, by dividing y_1 by x_1. This gives you the initial rate of reaction in cm^3 of oxygen per second.

……

……

HINT
Sources of error can be **random errors**, where the size and direction of the error are not always the same. This can happen when a variable that should be kept constant changes unpredictably. For example, the temperature of the room might change, which could affect the result of the reaction.
Systematic errors are where the size and direction of the error is relatively constant. This can happen if a piece of apparatus is wrongly calibrated, or if there is a fault in the equipment that always makes your measurements larger or smaller than they should be.
You can read more about this in the Cambridge AS & A level Biology coursebook.

Evaluation

g In most enzyme investigations, you will want to find out the effect of a variable (e.g. substrate concentration, or temperature) on the rate of the reaction. You will need to keep all other variables constant.

Using your answers to **c** and **d**, explain why it is important to measure the rate of reaction as close to the start of the reaction as possible.

……

……

……

h This method involves several important sources of experimental error (uncertainty), which means we cannot be sure that we would get exactly the same results if we did the experiment again. In other words, the results may not be **repeatable**.

For each of the following sources of error listed, decide:

- whether it is a random error or a systematic error
- how it is likely to affect the results.

The first one has been done for you.

i The apparatus may not have been completely airtight.

systematic error: some gas may have escaped, so the volume of oxygen measured would be too small and the rate of the reaction would seem slower than it really was.

ii The bung may not have been placed into the test tube fast enough.

……

……

iii The readings may not have been taken at exactly correct time intervals.

……

……

iv The scale on the gas syringe may not have been totally accurate.

……

……

v It is difficult to decide exactly how to draw the tangent on the curve.

……

……

Practical investigation 3.2:

The effect of substrate concentration on the rate of an enzyme-catalysed reaction

Introduction

In Practical investigation 3.1, you measured the initial rate of reaction for the breakdown of hydrogen peroxide, catalysed by the enzyme catalase. In this practical, you use the same reaction and method to investigate how changing the concentration of the substrate affects the initial rate of reaction.

In this investigation:

- the **independent variable** (the one you change) is the concentration of the substrate, hydrogen peroxide
- the **dependent variable** is the rate at which oxygen is produced.

Equipment

You will need:

• two or three large stalks of celery • about 100 cm^3 of 10 volume hydrogen peroxide solution • an electric blender • a filter funnel and muslin • two 250 cm^3 beakers • five 100 cm^3 beakers or other small containers • method of labelling beakers, for example, glass marking pen • a large test tube • a gas syringe • tubing to make an airtight connection between the test tube and the gas syringe • a timer (e.g. on a phone) • a retort stand, boss and clamp • apparatus for measuring small volumes, for example two 5 cm^3 or 10 cm^3 syringes or two graduated pipettes

Access to:

• distilled water

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- Hydrogen peroxide is a strong oxidising agent and bleach. Wear safety glasses throughout this practical. If you get hydrogen peroxide on your skin, wash with plenty of cold water.

Method

1 Set up the apparatus as in Practical investigation 3.1.

2 Make up a celery extract as in Practical investigation 3.1. It will be helpful if you can use celery from a similar source, and in similar quantities, because you know that this produces a measurable rate of reaction.

HINT
See Practical investigation 2.3 to help you to design the table. You may also like to refer to the Cambridge AS & A Level Biology coursebook.

3 Use dilution to produce a range of concentrations of hydrogen peroxide solution. You could try 100, 80, 60, 40 and 20% of the concentration of the original solution.

Use the space here to construct a table showing how you prepared the different concentrations.

4 Read Steps 5–6, and then construct a results table in the space overleaf. You can use a similar design to the one in Practical investigation 3.1, but with extra columns for the different concentrations of substrate.

5 Using your highest concentration of hydrogen peroxide, measure and record the volume of oxygen produced over time, exactly as you did in Practical investigation 3.1.

6 Repeat for each concentration of hydrogen peroxide solution.

Results

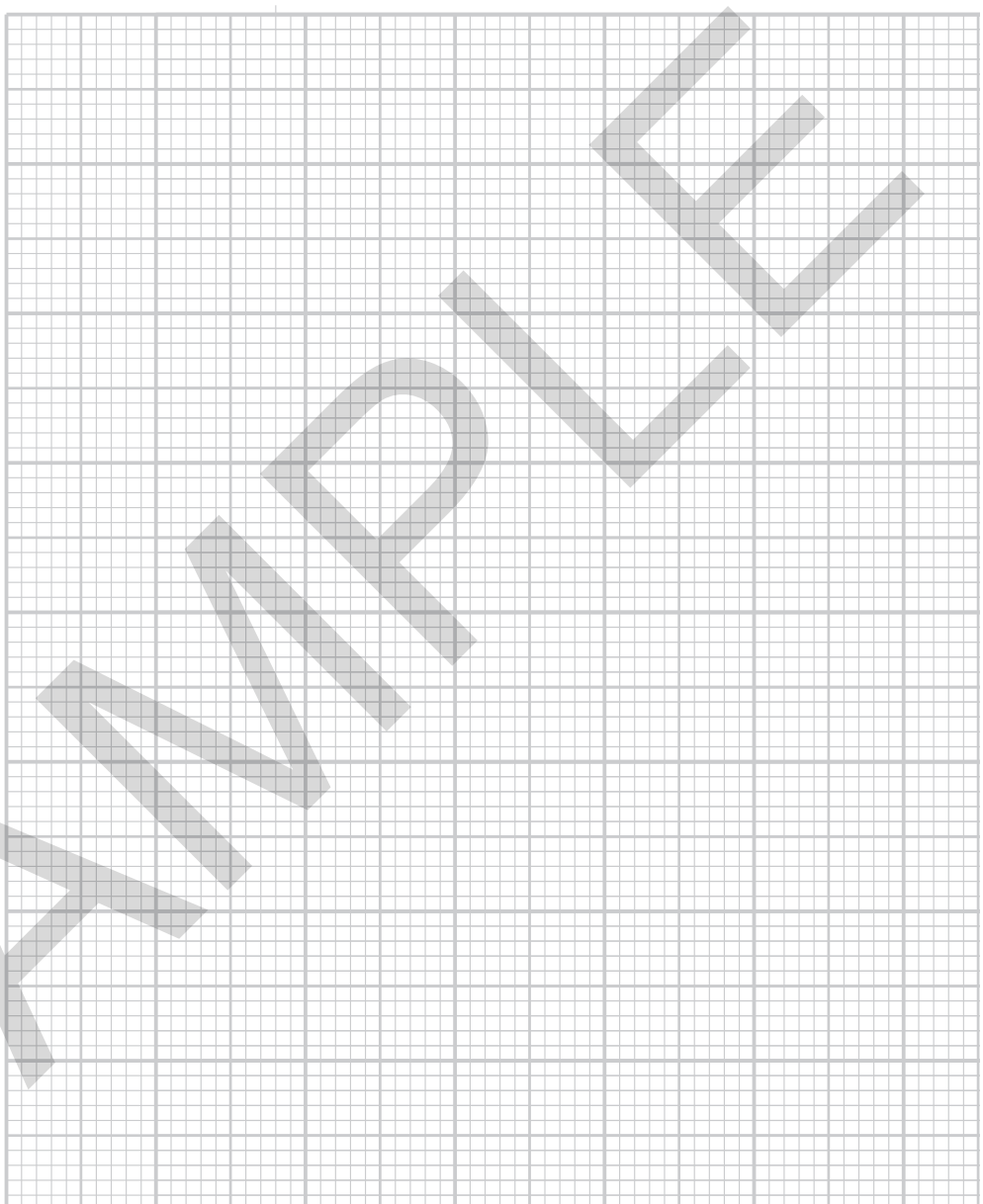
Take care to:

- draw the table using a ruler
- include full headings for each column, including units – do not include units with the individual readings
- write each reading to the same number of decimal places.

Data analysis

a On the grid, plot curves for each of the concentrations of hydrogen peroxide. Draw a best-fit line for each set of data.

Note: If you prefer, you can use separate sheets of graph paper to plot separate graphs for each concentration. If you do this, use the same scales for each graph.



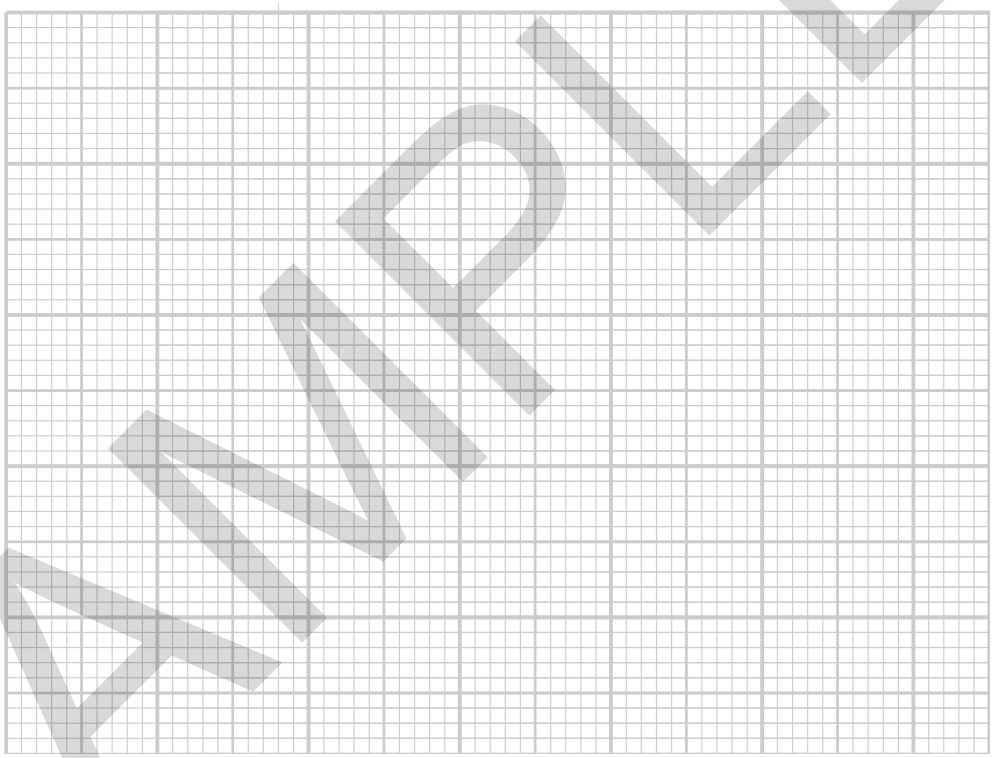
b Use the curves to calculate the initial rate of reaction for each concentration of hydrogen peroxide. Show your working in the following space.

Record your calculated results in Table 3.2.

Substrate concentration / percentage of original solution	Initial rate of reaction / cm^3 of oxygen s^{-1}

Table 3.2

c Use the results from Table 3.2 to plot a graph of initial rate of reaction against substrate concentration. Draw a smooth best-fit curve.



d Identify any values that do not fit the pattern shown by the other results. These are **anomalous** results.

e Use your results to write a brief conclusion about how the concentration of substrate affects the initial rate of reaction.

……

……

……

……

Evaluation

f Using your knowledge of how enzymes catalyse reactions, explain the reasons for the relationship that you have described in your answer to question part **e**.

……

……

……

……

……

g List **four** significant sources of error that reduce the reliability of your results.

At least **two** of these sources of error should be different from those listed in Practical investigation 3.1.

……

……

……

……

……

h Select **two** of the sources of error that you have listed.

For each one, suggest how you could adapt the method to reduce this error.

……

……

……

……

Practical investigation 3.3:
The effect of enzyme concentration on the rate of an enzyme-catalysed reaction

Introduction

In this investigation, you will use the enzyme amylase, which catalyses the hydrolysis of starch to maltose. Amylase is produced by many different organisms, including humans, some bacteria and some fungi.

In Practical investigations 3.1 and 3.2, you measured the rate of reaction by measuring the rate of formation of the product, oxygen. In this investigation, you will measure the rate of reaction by measuring the rate of disappearance of the substrate.

The reaction is:

starch → maltose + water

Equipment

You will need:

• about 50 cm^3 of a 1% solution of amylase (or as supplied by your teacher) • about 50 cm^3 of a 5% solution of starch (or as supplied by your teacher) • two 250 cm^3 beakers • five 100 cm^3 beakers or other small containers • method of labelling beakers, for example glass marking pen • at least 12 clean test tubes • six glass rods • a timer (e.g. on a phone) • apparatus for measuring small volumes, e.g. two 5 cm^3 or 10 cm^3 syringes or two graduated pipettes • iodine in potassium iodide solution, with a dropper • two white tiles, preferably with a series of hollows (a spotting tile or dimple tile) • starch-free paper for cleaning the glass rods

Access to:

• distilled water
• a thermostatically controlled water bath, or apparatus to make your own

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- If you get iodine in potassium iodide solution on your skin, wash it immediately with plenty of cold water.

Method

1 You are provided with a 1% solution of amylase. Use dilution to make up a range of four more concentrations of amylase: 0.8, 0.6, 0.4 and 0.2%.

Construct a table in the following space to show how you will do this.

2 Label six clean test tubes with the concentration of amylase that you will place in each one. One of them should contain no amylase.

3 Measure 5 cm^3 of each of your amylase solutions into each of five labelled test tubes. Measure 5 cm^3 of distilled water into the sixth tube. Wash the pipette or syringe between each solution, or use a different one for each. Stand all the tubes in a water bath set at 40°C.

4 Measure 5 cm^3 of starch suspension into six more test tubes. Stand all the tubes in the same water bath.

5 Leave all the tubes to stand for at least 10 min, to allow the temperature of their contents to come to the same temperature as the water bath. After 10 min, check these temperatures with a thermometer. Wash and dry the thermometer between each measurement, to avoid contaminating one tube with the contents of another. If necessary, leave the tubes for longer.

6 While you are waiting, prepare the spotting tiles.

- Label the tiles with the concentrations of enzyme you are testing.
- Place spots of iodine in potassium iodide solution onto the spotting tile or tiles. Arrange them in five rows, as shown in Figure 3.3.

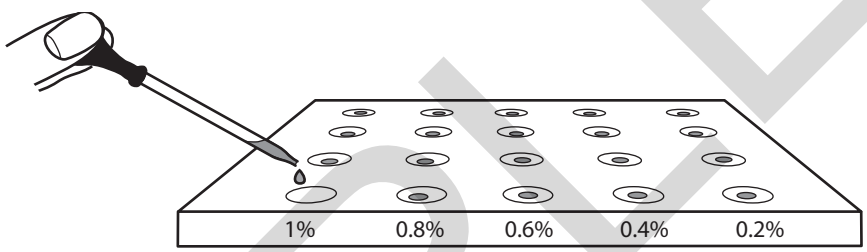


Figure 3.3

HINT
Glass rods that are not cleaned properly can completely spoil your experiment, by transferring amylase or starch from one tube or iodine spot to another. Note that paper towels often contain starch, so check this before you use them. If you do not have any starch-free paper, then wash the rod in absolutely clean water each time.

7 When you are satisfied that the contents of all of the tubes are at the correct temperature, dip a glass rod into one of the suspensions and then dip the rod into the first spot of iodine solution on the 1% row on the tile. Wipe or wash the glass rod clean.

8 Tip one of the starch suspensions into the tube containing 1% starch suspensions. Immediately stir with a glass rod and start the timer.

9 Take samples from the mixture every minute (or as advised by your teacher) and add them to the next spot of iodine solution on the tile. Do this by dipping a clean glass rod into the reacting mixture, and then immediately dipping the rod into the iodine solution.

10 Continue until the iodine solution remains orange–brown, or after 15 min (whichever is sooner).

11 Repeat Steps 7–9 for each of the other concentrations of enzyme.

12 Record your results in Table 3.3.

Results

Concentration of enzyme solution / %	Time taken for starch to disappear / s

Table 3.3

Data analysis

a Add a third column to Table 3.3.

Calculate the rate of reaction for each concentration of enzyme.

rate = 1 ÷ time

Multiply this number by 1000, and give your answer to one decimal place.

Add your calculated values to the third column in the Results table. Remember to add a suitable heading for this column.

b On the grid, plot a graph of rate of reaction against concentration of enzyme.

Join the points with ruled straight lines.

c Write a brief conclusion for your experiment.

……

……

……

……

Evaluation

d Using your knowledge and understanding of how enzymes work, explain your results. Use these terms in your explanation:

active site enzyme–substrate complex

……

……

……

……

……

e When investigating the effect of one variable (the independent variable) on another (the dependent variable), it is important to keep all other variables that might affect the independent variable the same.

These are called **control variables**.

List **two** important control variables in this investigation.

……

……

f Identify **three important** sources of experimental error in your investigation, which reduce the confidence that you have in your results and conclusion.

……

……

……

g Select **two** of the sources of error in your answer to **f** above. For each of these, suggest how you could adapt the method to reduce the source of error. Assume that you would have as much time as you need, and any other apparatus that you would require.

……

……

……

……

……

……

h Apart from reducing the sources of error, suggest **one** other way in which the investigation could be improved.

……

……

i Explain why you measured the time taken for disappearance of substrate to measure the rate of reaction in this experiment, whereas for the catalase reaction we measured the volume of oxygen produced over time.

……

……

……

……

Practical investigation 3.4:

The effect of temperature on the rate of an enzyme-catalysed reaction

Introduction

In this investigation, you will again investigate the reaction in which catalase catalyses the breakdown of hydrogen peroxide.

This time, you will find out how varying the temperature affects the rate of reaction.

You are going to plan part of this investigation yourself.

Equipment

You will need:

• one or two stalks of celery • about 100 cm^3 of 10 volume hydrogen peroxide solution • an electric blender or pestle and mortar • a filter funnel and muslin (fine cloth that can be used for filtering) • two 250 cm^3 beakers • five 100 cm^3 beakers or other small containers • method of labelling beakers, for example, glass marking pen • a large test tube • a gas syringe • tubing to make an airtight connection between the test tube and the gas syringe • a timer (e.g. on a phone) • a retort stand, boss and clamp • apparatus for measuring small volumes, for example two 5 cm^3 or 10 cm^3 syringes or two graduated pipettes

Access to:

• distilled water
• several thermostatically controlled water baths, and/or apparatus to make your own water baths using large beakers of water

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- Hydrogen peroxide is a strong oxidising agent and bleach. Wear safety glasses throughout this practical. If you get hydrogen peroxide on your skin, wash with plenty of cold water.

Method

For this investigation, you can use the same technique as the one described in Practical 3.1.

1 The independent variable in this investigation is temperature.

Decide on the **range** of the independent variable. The range is the spread between the lowest temperature and the highest temperature that you will investigate.

Range for the independent variable will be ……

2 You should have at least five values for your independent variable.

Write down what these values will be.

……

3 How you change the independent variable will depend on the apparatus that is available to you. Use thermostatically controlled water baths if you can. Otherwise, you can make water baths using large beakers, ice and a source of hot water.

Describe how you will change and measure the independent variable. Think about how you will make sure that the mixture of enzyme and substrate really is at the temperature you want it to be.

……

……

……

……

4 The dependent variable in this investigation is the rate of reaction.

If you had time, you could measure and calculate the initial rate of reaction for each value of your independent variable, as you did in Practical investigation 3.2. However, to save time in this investigation, you can simply measure the volume of oxygen produced in a chosen time period.

Describe how you will measure your dependent variable.

……

……

……

……

……

5 All other variables that could affect the rate of the reaction must be kept the same. These are called **control variables**.

List **two** control variables.

For each one, explain how you will keep it constant.

control variable 1 ……

control variable 2 ……

6 Construct a results table in the Results section on the next page.

7 Carry out your method.

Use the following space to describe your method. There is no need to describe the technique in detail where it is the same as in Practical investigation 3.1.

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

Results

Construct your results table in the following space.

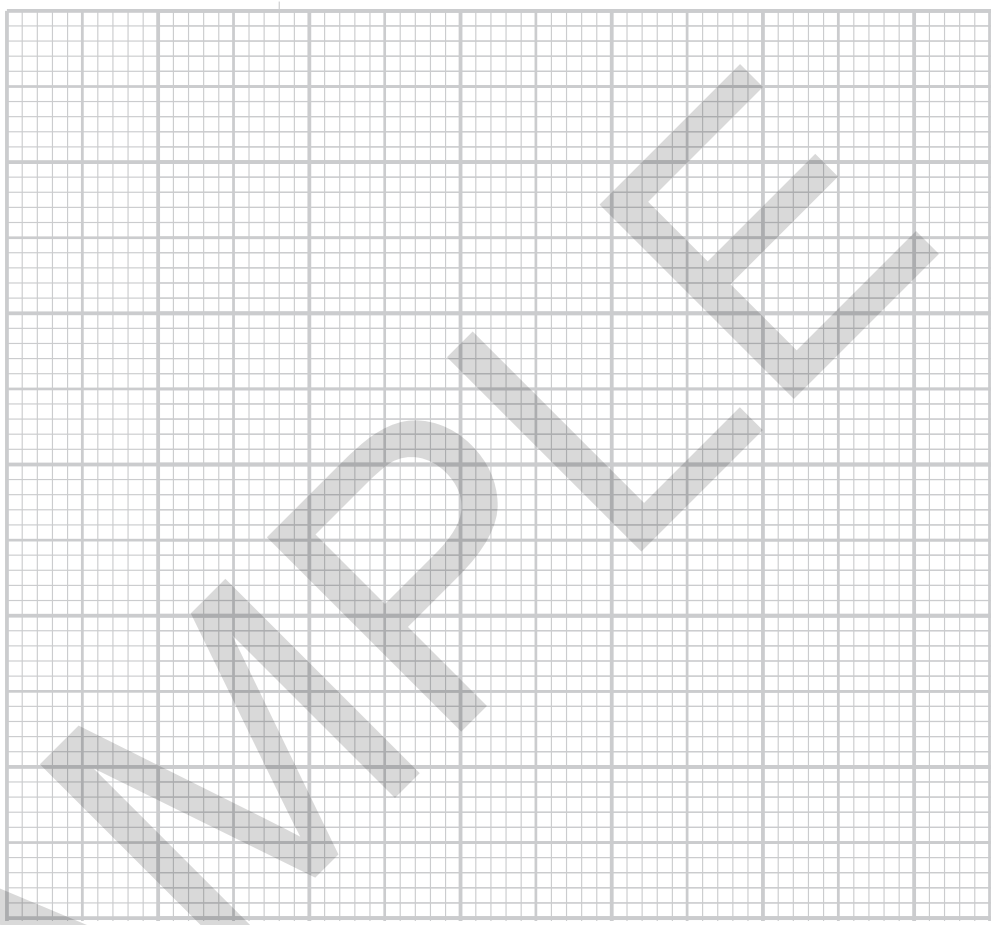
Put the independent variable in the first column, and the dependent variable in the second column.

Remember to make all your readings to the same number of decimal places.

Data analysis

a Draw a graph on the following grid.

Join the points with ruled, straight lines.



b Write a short, clear conclusion, describing how temperature affects the rate of this reaction.

……

……

……

Evaluation

c Using your knowledge and understanding of how enzymes function, explain your results.

……

……

……

……

d Describe how you could carry out a further investigation to determine the optimum temperature more precisely.

……

……

……

……

e Describe how you could use a similar method to investigate the effect of pH on the activity of catalase.

……

……

……

……

……

……

……

……

……

……

HINT
pH can be altered by using different buffer solutions. A buffer solution has a particular pH, which remains constant.

Practical investigation 3.5:
Immobilising urease

Introduction

In this investigation, you will use the enzyme urease. This enzyme catalyses the decomposition of urea into carbon dioxide and ammonia.

You are going to immobilise the enzyme. You will do this by trapping it inside beads of calcium alginate, which forms a jelly.

Ammonia forms an alkaline solution. You will measure the production of ammonia by monitoring the change in pH.

Equipment

You will need:

• about 50 cm^3 of 0.6% urease solution • about 100 cm^3 of 2% urea solution • a 20 cm^3 syringe barrel, with a short length of rubber tubing attached to its nozzle • a clamp to hold the tubing closed • a retort stand, boss and clamp to support the syringe barrel • a small piece of muslin • a tea strainer • several glass beakers (small or medium size) • about 80 cm^3 of 3% sodium alginate solution • about 100 cm^3 of 3% calcium chloride solution • a timer (e.g. on a phone) • apparatus for measuring small volumes, for example two 5 cm^3 or 10 cm^3 syringes or two graduated pipettes • a pH meter and probe • a dropper pipette with a fine nozzle

Access to:

• distilled water

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- Urease, like all enzymes, can irritate skin. Wash immediately with plenty of cold water if you get any on your skin.

Method

1 Cut a small piece of muslin, and push it into the syringe to cover the entrance to the nozzle. (This will prevent the beads of calcium alginate getting into the nozzle.)

2 Support the syringe barrel, nozzle downwards, in a clamp (Figure 3.4).

You are now going to immobilise the enzyme by trapping it in jelly beads.

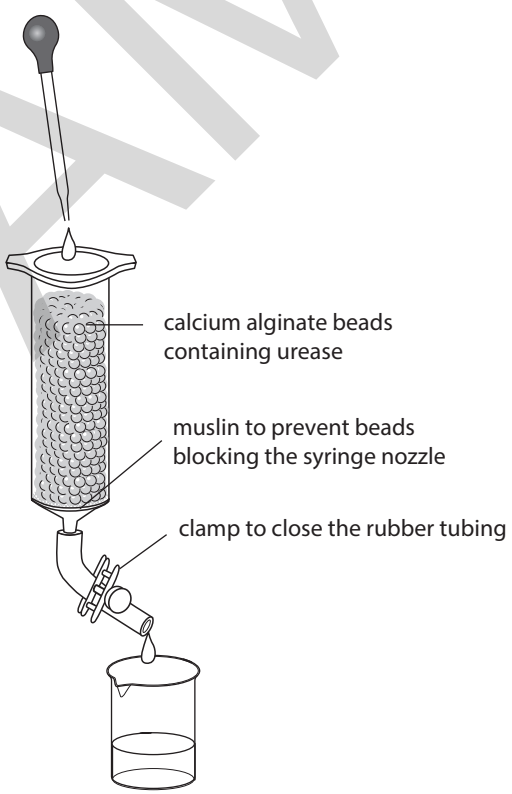


Figure 3.4

3 Measure 50 cm^3 of sodium alginate solution into a medium-sized beaker. Add 50 cm^3 of urease solution and mix thoroughly.

4 Using a dropper pipette with a fine nozzle, add a small drop of urea–sodium alginate solution to the calcium chloride. The calcium chloride and sodium alginate will react to produce calcium alginate, which forms a bead of jelly. Urease molecules will be trapped inside the jelly bead.

5 Repeat Step 4 many times, to produce as many jelly beads as you can. You need enough to fill the barrel of the syringe that you have supported in the clamp.

6 Carefully collect the beads (do not break them up) into the tea strainer, and wash gently using distilled water (Figure 3.5).

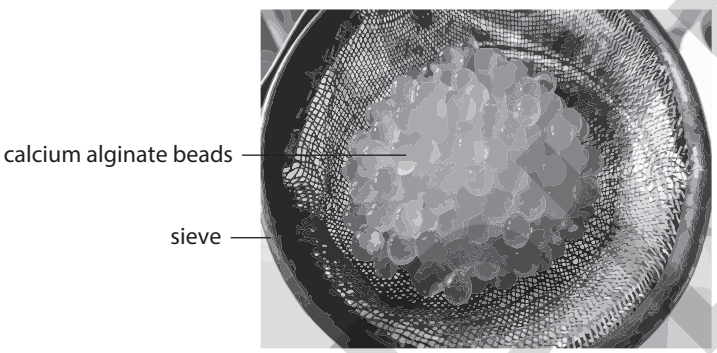


Figure 3.5

7 Place the beads inside the syringe barrel. Very gently push them together, or tap the syringe barrel, to reduce any large gaps between them.

8 Place a clean beaker underneath the syringe barrel.

9 Use the pH meter to measure the pH of the urea solution, and record it in a suitable table in the space in the Results section.

10 Partially open the clamp on the rubber tubing. Slowly pour the urea solution into the top of the syringe barrel, so that it flows slowly down over the beads and out through the nozzle of the syringe, into the beaker. You can adjust the speed at which it moves through the beads by loosening or tightening the clamp on the rubber tubing.

11 Measure the pH of the liquid in the beaker and record it.

Results

Data analysis

a Explain your results.

……

……

……

……

……

b You could check if the solution in the beaker still contains any urea using the following method:

- Put some of the solution into a small beaker.
- Place some beads containing immobilised urease into the solution in the beaker.
- If the solution contains urea, the urease in the beads will break it down and release carbon dioxide. (If there is enough carbon dioxide, the bubbles that stick to the beads may cause them to float to the surface.)

  Describe and explain your results.

  ……

  ……

  ……

Practical investigation 3.6

Investigating the effect of an inhibitor on the action of bromelain

Introduction

In this investigation, you will investigate the effect of copper ions on the activity of a protease enzyme, bromelain, found in fresh pineapple. This enzyme hydrolyses proteins – in this case, gelatin – to soluble amino acids.

Equipment

You will need:

• about 50 cm^3 of an extract made from fresh pineapple • a small quantity of boiled pineapple extract • five small Petri dishes containing coloured jelly, made using gelatin • about 10 cm^3 of 1 mol dm^{-3} copper sulfate solution • a cork borer • a ruler to measure in mm

Safety considerations

- Make sure you have read the Safety advice section at the beginning of this book and listen to any advice from your teacher before carrying out this investigation.
- If you get copper sulfate solution on your skin, wash immediately with cold water.

Method

1 You are provided with 1 mol dm^{-3} copper sulfate solution. Use this solution to make up 10 cm^3 each of solutions of 0.1, 0.01 and 0.001 mol dm^{-3}.

Describe how you will do this. You can do this in words, or present your results in a table.

2 Use a cork borer to cut one small, neat well in the agar jelly near the centre of each Petri dish (Figure 3.6). Try not to touch the rest of the jelly with your fingers as you do this.

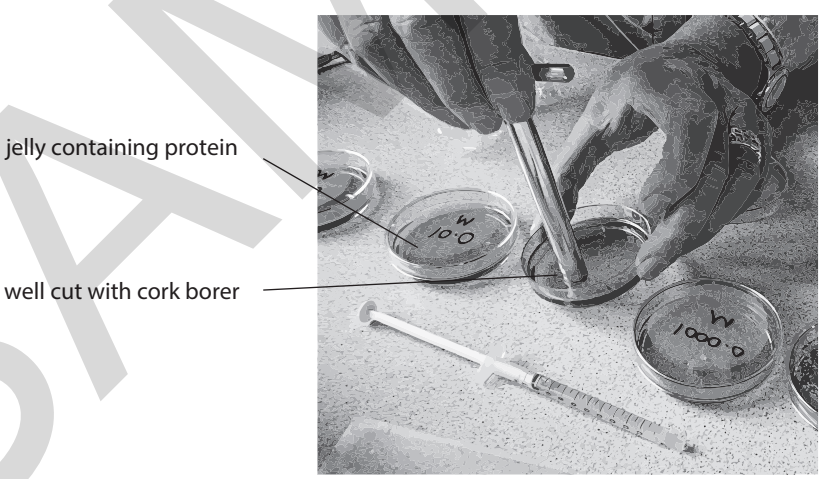


Figure 3.6

3 Using small syringes, add the same total volume of liquid (determine how much you think is suitable – it will depend on the depth and diameter of the holes you have made) to each well, as follows:

Petri dish 1: boiled pineapple extract and water

Petri dish 2: fresh pineapple extract and water

Petri dish 3: fresh pineapple extract and 1 mol dm^{-3} copper sulfate solution

Petri dish 4: fresh pineapple extract and 0.1 mol dm^{-3} copper sulfate solution

Petri dish 5: fresh pineapple extract and 0.01 mol dm^{-3} copper sulfate solution

Petri dish 6: fresh pineapple extract and 0.001 mol dm^{-3} copper sulfate solution

4 Place lids on each Petri dish, and label them. Leave the dishes for approximately 1 h, or as suggested by your teacher.

5 Use a small pipette to very carefully extract the liquid from the hole in Petri dish 1. Measure the diameter of the well. Repeat for all six dishes. Record your results in a suitable results table here.

Results

Data analysis

a Describe your results.

……

……

……

……

……

Evaluation

b Explain your results.

……

……

……

……

……

……

……

……

c Identify the most significant sources of experimental error in your investigation.

……

……

……

……

……

d Suggest how this investigation could be improved, to give more confidence in your results and conclusion.

……

……

……

……

……