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Biology

Practical Teacher's Guide

Mary Jones and Matthew Parkin

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Contents

Introduction

Practical work is an essential part of any advanced Biology course. For Cambridge International AS & A Level Biology, Paper 3 and Paper 5 focus on the assessment of practical skills. In addition, first hand knowledge of specific practical work is required by several learning objectives in the syllabus content, which is assessed on the theory papers.

The practical investigations in the workbook have been carefully chosen to:

- meet the requirements of all the learning objectives that require learners to undertake specific practical activities
- provide progressive guidance and practise in the Assessment Objective 3 (AO3) skills.

The practical skills grid on the following pages summarises the practical skills that are assessed in Paper 3 (AS) and Paper 5 (A level). You can use this grid to search for practical investigations that involve a particular skill. The learning objectives and skills that are supported are also listed at the beginning of each practical investigation.

Practical work is time-consuming but it is an essential part of scientific study. You should expect to spend around one quarter of your teaching time on practical work. For learners, doing a hands-on practical themselves is infinitely more useful than learning about a technique in theory only. Nevertheless, you will probably not be able to do every one of the practical investigations in this workbook, and will need to make a selection of those that you feel are most useful for your learners, and for which you are able to supply equipment and materials.

Biology experiments are notoriously unreliable, in comparison with Chemistry and Physics. This is because we are often dealing with complex systems where we are unable to control all variables adequately, and where measurement techniques may be difficult or imprecise.

Learners do, naturally, want to get the 'right' results. However, this will not always happen, and you and they should not expect it to. The important learning experiences, when carrying out practical work, are the range of skills that are being used and developed – the **processes** of planning, carrying out, observing, recording, analysing and so on. Unexpected results (or even no results at all) should not be dismissed as showing that the experiment 'did not work'. Instead, learners should look back at what they have done, and search for possible reasons for the unexpected results that they have obtained. This in itself is a highly valuable activity, and can encourage the development of higher order thinking skills such as evaluation and analysis. We have provided a set of sample results for each practical investigation, which you can give to learners who have not managed to obtain a complete set of results themselves, so that they can continue to answer all of the questions.

The Practical Investigations in this book have not been designed to reflect the structure of examination questions. This is because these investigations are designed to help learners to **develop** skills, rather than be assessed on them. Learners should also be aware that there is guidance on tackling Paper 3 and Paper 5 in Chapters P1 and P2 of the Coursebook in this series. There are also numerous exercises in the workbook that will help them to develop and practise these skills.

The practical investigations in this resource have been split into various sections, to help you when planning and carrying out these investigations. Where appropriate, tips to support students who may struggle with some aspects of the practical work have been given. These are indicated by this symbol:  . Ideas to challenge more advanced learners have also been included and are indicated by this symbol:  .

Safety

Safety of learners, teachers and technicians is paramount when planning and carrying out biology investigations. Most biology investigations have a relatively low level of risk, but even so, potential risks should never be ignored.

It is the responsibility of the biology teacher to carry out a thorough risk assessment before each investigation, and to ensure that learners and technicians are not exposed to any unnecessary risks. This should meet the standards set out by your local authority or educational provider.

The table in the Safety section in the Practical Workbook summarises the main types of risk associated with biology investigations.

It is strongly recommended that you refer to the CLEAPSS website, http://science.cleapss.org.uk/ for information about the risks associated with each chemical that is used in your laboratory, and that you obtain copies of the CLEAPSS Hazcards for each one.

These tell you the type of hazard involved with each chemical, and advice for handling the chemical, and for dealing with spillages or contamination. This information should be available to learners as they work in the laboratory, so that everyone is aware of the risks and how to handle them.

You may also like to download the free Student Safety Sheets from the CLEAPPS website. These can be printed and provided to learners. Word versions are available, so that you can modify these if you wish to suit the particular circumstances in your laboratory.

Cambridge Assessment International Education also provides excellent advice about all aspects of designing and using science laboratories, including safety, in the document *Guide to Planning Practical Science*. You can find this document as a downloadable pdf on the cambridgeinternational.org website.

AS Practical Skills

The following grids map the practical investigations from the workbook to the mark categories for Papers 3 and 5, as listed in the Cambridge International AS & A Level Biology syllabus.

The grids are designed to aid you when planning practical and theory lessons, to ensure learners develop the practical skills required as part of this course.

Manipulation of apparatus, measurement and observation: MMO

Chapter number:	1	2	3	4	5	6	7
Making decisions about measurements							
(a) identify independent and dependent variables			3.2	4.4		6.5	
(b) decide the range and interval of the independent variable and how to achieve this			3.2, 3.4	4.2, 4.4, 4.6		6.5	
(c) decide how to identify a biological molecule, and how to estimate its quantity		2.1, 2.2, 2.3					
(d) describe an appropriate control for an investigation		2.1				6.5	
(e) decide which variables to standardise, and how to do this			3.3, 3.4	4.2, 4.4		6.5	
(f) decide how to measure the dependent variable			3.4	4.2, 4.4		6.5	
(g) use a range of techniques to measure the dependent variable			3.2, 3.3, 3.4, 3.5, 3.6	4.7		6.3, 6.4	
(h) decide on the frequency of measurement of the dependent variable			3.1, 3.2, 3.4	4.2, 4.4		6.5	
(i) decide whether to replicate or repeat measurements			3.4	4.4, 4.7		6.5	
(j) decide on an appropriate number of significant figures for measurements			3.1, 3.3, 3.4, 3.6				
(k) decide how to measure an area using a grid						6.3	
(l) set up a microscope to view and observe specimens, in order to make plan diagrams or draw cells	1.1, 1.2, 1.3			4.6	5.1, 5.2	6.1, 6.2	
(m) draw and label diagrams to show distribution of tissues						6.1, 6.6	7.3, 7.4
(n) identify cells seen through the microscope and label their structures	1.1, 1.3				5.1	6.2, 6.6	7.1, 7.4
(o) stain and make a slide of cells	1.1, 1.3				5.1		
(p) calibrate and use an eyepiece graticule and stage micrometer	1.2, 1.3					6.3	
(q) estimate the number of cells or organelles in a whole slide or field of view using a sample or using a grid					5.2	6.2	

Chapter number:	1	2	3	4	5	6	7
Successfully collecting data and observations							
(a) follow instructions to collect results	1.1, 1.3	2.1, 2.2, 2.3	3.1, 3.2, 3.3, 3.5, 3.6	4.1, 4.2, 4.3, 4.5, 4.6, 4.7, 4.8	5.1, 5.2	6.3. 6.4	7.2, 7.4
(b) assess the risk of a procedure						6.5	
(c) take readings using a range of apparatus			3.1, 3.2, 3.4, 3.5	4.1		6.4, 6.5	
(d) measure using counting				4.6	5.2	6.3	
(e) use clear descriptions of qualitative results		2.1, 2.2, 2.3		4.1, 4.8		6.1, 6.2, 6.6	7.1, 7.2, 7.3, 7.4,
(f) make observations of the distribution of tissues in a specimen, and record as a plan diagram						6.1, 6.6	7.2, 7.3, 7.4
(g) draw cells in a specimen to show correct shapes, thicknesses of cell walls, relative sizes and observable cell contents	1.1, 1.3					6.2	7.1
(h) measure cells and tissue layers using a calibrated eyepiece graticule, scale bars and/or appropriate magnification	1.2, 1.3					6.1, 6.2	
(i) use sampling, a grid and/or tally counts to collect data about the number of cells or cell organelles in a specimen				4.6	5.2	6.3	
(j) observe similarities and differences between two specimens	1.3					6.6	

Presentation of data and observations: PDO

Chapter number:	1	2	3	4	5	6	7
Recording data and observations							
(a) record raw data in a fully ruled table		2.1	3.1, 3.2, 3.3, 3.4, 3.5, 3.6	4.2, 4.3, 4.7, 4.8	5.2	6.3	
(b) prepare a results table with headings for independent and dependent variable and units in headings		2.1	3.2, 3.4, 3.5, 3.6	4.2, 4.3, 4.4, 4.7, 4.8			
(c) record quantitative data to an appropriate number of significant figures			3.1, 3.2, 3.3, 3.4, 3.6	4.1, 4.2, 4.3, 4.4			
(d) record qualitative data using clear descriptions		2.1, 2.2, 2.3		4.1, 4.8		6.1, 6.2	7.2
(e) draw plan diagrams to record detailed shapes and positions of tissue layers seen on a microscope slide or micrograph						6.1, 6.6	7.3, 7.4
(f) draw diagrams to show detail of individual cells seen on a microscope slide or micrograph	1.1, 1.3	2.1			5.1	6.2	7.1

Chapter number:	1	2	3	4	5	6	7
Displaying calculations and reasoning							
(a) show all the steps in a calculation			3.1, 3.2	4.1		6.3, 6.4	
(b) show all steps in calculations involving the calibration of an eyepiece graticule, finding the actual size of a specimen and/or linear magnification	1.2, 1.3					6.3	
(c) show all steps in calculations involving finding a total number from a sample, finding a mean, and determining the simplest ratio	1.2, 1.3					6.3, 6.5	
Layout of data or observations							
(a) select whether to display data as a graph or chart							
(b) decide which variable to place on which axis			3.2, 3.3, 3.4	4.2, 4.4, 4.5, 4.6		6.5	
(c) select suitable scales for graph axes and label graph axes fully including units			3.1, 3.2, 3.3, 3.4	4.1, 4.2, 4.4, 4.5, 4.6		6.5	
(d) plot points accurately, either as a small cross or an encircled dot			3.1, 3.2, 3.3	4.1, 4.2, 4.4, 4.5, 4.6		6.5	
(e) connect points with a best-fit line, a smooth curve or a set of ruled straight lines to join the points			3.1, 3.2, 3.3, 3.4	4.1, 4.2, 4.4, 4.5, 4.6		6.5	
(f) plot bar charts accurately, using a thin ruled line for the bars				4.1	5.2		
(g) decide whether to separate the bars, or to join the bars on a bar chart					5.2		
(h) make unshaded drawings with finely drawn, unbroken lines that use most of the available space and show all relevant features	1.1, 1.3					6.1, 6.2, 6.6	
(i) organise comparative observations to show differences and/or similarities	1.3	2.2, 2.3				6.6	

Analysis, conclusions and evaluation: ACE

Chapter number:	1	2	3	4	5	6	7
Interpreting data or observations and identifying sources of error							
(a) use quantitative results or provided data to calculate an answer using the correct number of significant figures			3.1, 3.2	4.1, 4.2, 4.3, 4.5, 4.6	5.2		
(b) find an unknown value from a graph			3.1, 3.2	4.5, 4.6			
(c) estimate the concentration of an unknown solution from qualitative results		2.2, 2.3		4.7		2.2, 2.3	
(d) identify the contents of unknown solutions using biological molecule tests		2.1, 2.2, 2.3				2.1, 2.2, 2.3	
(e) identify and deal appropriately with anomalous results			3.1, 3.2, 3.3, 3.4	4.1, 4.4, 4.5, 4.6			

Chapter number:	1	2	3	4	5	6	7
(f) describe patterns and trends in results			3.1, 3.2, 3.3, 3.4, 3.6	4.1, 4.2, 4,3, 4.4, 4.5, 4.6, 4.7, 4.8			
(g) identify significant sources of error in an investigation, and determine whether these are systematic or random errors		2.2, 2.3	3.1, 3.2, 3.3, 3.6	4.1, 4.2, 4.4, 4.6		2.2, 2.3	
(h) calculate actual or percentage error				4.2			
(i) estimate and evaluate the effect of errors on the confidence with which conclusions may be made		2.2, 2.3	3.1	4.4, 4.5, 4.6, 4.7		2.2, 2.3	
(j) calculate correct answers, to the correct number of significant figures, relating to size and magnification of specimens	1.2						
(k) compare observable features of specimens, using micrographs and prepared microscope slides	1.1, 1.3						
Drawing conclusions							
(a) make conclusions		2.1, 2.2, 2.3	3.2, 3.3, 3.4	4.2, 4.4, 4.6, 4.7, 4.8		6.3, 6.5	7.2, 7.3
(b) make scientific explanations using knowledge and understanding		2.3	3.1, 3.2, 3.3, 3.4, 3.5, 3.6	4.1, 4.7, 4.8		6.3, 6.5	
(c) give scientific explanations of observations of specimens, calculated values, and adaptations of a specimen for a particular habitat		2.1, 2.2, 2.3				6.6	7.3, 7.4
Suggesting improvements or modifications to extend an investigation							
(a) suggest how to better standardise relevant variables to improve an investigation		2.3	3.2, 3.6	4.8		6.3	
(b) suggest how to use a different measurement method to improve an investigation		2.3	3.2, 3.3, 3.6	4.8			
(c) suggest how to use replicates to obtain more data and calculate a mean			3.2, 3.3, 3.6	4.8	5.2	6.5	
(d) suggest how to modify or extend an investigation to answer a new question		2.3	3.4	4.8			
(e) suggest improvements to a procedure that will increase the accuracy of observations, including using measurement methods with smaller intervals		2.3	3.2, 3.3, 3.4, 3.6	4.8		6.3	

Planning: P

Chapter number:	8	9	10	11	12
Defining the problem					
(a) express the aim of an experiment or investigation as a quantifiable, testable, falsifiable prediction or hypothesis	8.2		10.1, 10.5	11.4, 11.5, 11.7	12.4
(b) identify the independent variable(s)	8.2	9.1, 9.4, 9.5	10.1	11.4, 11.7	
(c) identify the dependent variable(s)	8.2	9.1, 9.4, 9.5	10.1	11.4, 11.7	
(d) where the dependent variable cannot be measured directly, identify a measurable feature or aspect in the investigation	8.2	9.3, 9.4		11.7	
(e) identify the **key** variables to be standardised	8.1, 8.2	9.1, 9.2, 9.4	10.1	11.4, 11.5, 11.7	
Methods					
(a) use information provided in a scenario to describe how to vary the independent variable with accuracy	8.2		10.1	11.4	
(b) use information provided in a scenario to describe how to measure the dependent variable, including measuring instruments to use and a suitable number of significant figures	8.1, 8.2	9.1, 9.2, 9.4	10.1	11.4, 11.7	
(c) describe appropriate methods for standardising each of the other key variables	8.2	9.1, 9.2	10.1	11.7	
(d) describe suitable volumes and concentrations of reagents, including how to prepare different concentrations in % (w / v) and / or mol dm^{-3}	8.3	9.5	10.2	11.7	
(e) describe how to make different concentrations of solution using serial dilution or proportional dilution	8.3	9.5	10.2		
(f) describe any appropriate control experiments	8.1, 8.2	9.3	10.1		
(g) describe, in a logical sequence, the steps involved in the procedure	8.2, 8.4	9.1, 9.2	10.1, 10.5	11.4, 11.5, 11.7	12.4
(h) describe how to ensure the quality of results by considering any anomalous results, and the spread of results by inspection	8.1, 8.2	9.2	10.3	11.7	
(i) describe how to use standard deviation, standard error or 95% confidence intervals to assess the quality of results			10.3	11.7	
(j) describe how to ensure the validity of results by considering success of measuring the dependent variable and the precision required	8.2	9.2	10.3	11.4, 11.7	
(k) assess the risks involved in a procedure, identify areas where accident or injury is most likely and most serious	8.2			11.4, 11.7	12.4
(l) describe suitable precautions to minimise risk	8.2			11.4, 11.7	12.4

Analysis, conclusions and evaluation: ACE

Chapter number:	8	9	10	11	12
Dealing with data					
(a) decide which calculations are necessary to draw conclusions, including error levels, confidence limits and statistical tests	8.2		10.3, 10.5	11.2, 11.4, 11.5, 11.7	12.4
(b) use tables and graphs to identify key points in quantitative data, including their variability	8.1, 8.2, 8.4	9.1, 9.2, 9.4	10.3, 10.4, 10.5	11.2, 11.3, 11.4, 11.7	
(c) sketch or draw suitable graphs, including confidence limit error bars, calculated using standard error	8.2	9.2, 9.4	10.4,	11.2, 11.3, 11.4, 11.7	12.3
(d) choose appropriate calculations to simplify or explain data	8.2	9.2	10.2, 10.3, 10.4, 10.5	11.4, 11.7	
(e) carry out calculations to compare data, including percentage, percentage gain or loss and rate of change	8.1, 8.2	9.2	10.2, 10.4, 10.5	11.2, 11.3, 11.7	
(f) calculate species diversity index					12.2
(g) carry out calculations to estimate population size					12.1
(h) use standard deviation or standard error, or graphs with standard error bars, to determine whether differences between mean values are likely to be statistically significant			10.3	11.3, 11.7	
(i) choose statistical tests appropriate to the type of data collected, and justify the choice				11.4, 11.7	12.4
(j) state a null hypothesis for a statistical test			10.5	11.3, 11.7	
(k) use the *t*-test appropriately			10.5	11.3, 11.7	
(l) use the chi-squared test appropriately					
(m) use Pearson's linear correlation appropriately					12.4
(n) use Spearman's rank correlation appropriately	8.2	9.2			12.4
(o) calculate the degrees of freedom for a data set				11.3, 11.7	
(p) use a probability table to determine the significance of a calculated value for the *t*-test and the chi-squared test			10.5	11.3, 11.7	
(q) recognise the different types of variable (qualitative: categoric or ordered and quantitative) and the different types of data presented					
Evaluation					
(a) identify anomalous values in given data and suggest how to deal with them	8.1, 8.2, 8.3	9.2	10.3, 10.5	11.2, 11.5	
(b) suggest possible explanations for anomalous readings	8.1, 8.2	9.2	10.3		
(c) assess whether provided data has been replicated sufficiently, and whether the range and interval of the independent variable were suitable	8.3, 8.4	9.2, 9.4			12.2
(d) explain why replicating data is important and the practical limits on replication	8.2	9.4	10.5	11.4, 11.7	12.1
(e) identify instances where more measurements need to be taken with an increased or decreased value of the independent variable to provide a wider range of values	8.2, 8.3	9.4			
(f) identify instances where more measurements need to be taken within the original range of the independent variable, to provide more detailed information	8.2	9.2, 9.4			

Chapter number:	8	9	10	11	12
(g) assess whether the method of measurement of the dependent variable is appropriate	8.1, 8.3	9.1, 9.5		11.4	
(h) assess the extent to which other variables have been effectively standardised	8.2	9.2		11.4	
(i) use evaluations to make informed judgements about how much confidence can be placed in conclusions	8.2	9.2, 9.3	10.3, 10.4, 10.5	11.4, 11.5, 11.7	12.1, 12.2
(j) use evaluations to make informed judgements about the validity of the investigation	8.3	9.2, 9.3	10.3, 10.5	11.4, 11.5	12.4
(k) use evaluations to make informed judgements about the extent to which the data can be trusted for testing the hypothesis		9.2	10.3, 10.5	11.5	12.4
Conclusions					
(a) make conclusions that include key points of the raw data, processed data, graphical representations and statistical test results	8.1, 8.2, 8.3, 8.4	9.2, 9.5	10.2, 10.4	11.2, 11.4, 11.5, 11.7	12.4
(b) quote relevant figures from raw or processed data	8.1, 8.2	9.2, 9.5	10.2, 10.3	11.5, 11.7	12.4
(c) decide whether a given hypothesis is fully supported or not supported by the experimental data	8.2			11.3, 11.5, 11.7	
(d) identify strengths and weaknesses of any support for or against the hypothesis	8.2	9.4		11.7	
(e) give detailed scientific explanations of the data and conclusions	8.1, 8.2, 8.3, 8.4	9.1, 9.2, 9.4	10.1, 10.2		12.3
(f) make further predictions and hypotheses, based on conclusions		9.1			12.4
(g) make relevant suggestions about how an experiment could be improved	8.2	9.3	10.4, 10.5	10.2, 11.4, 11.5, 11.7	12.2, 12.5

Chapter 1: Microscopy

Chapter outline

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

In this chapter, learners 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

Learning objective: 1.1(a)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (l), (m), (n), (o) Successfully collecting data and observations: (a), (g)
PDO	Recording data and observations: (f) Layout of data or observations: (h)

Duration

This practical is likely to take around 1 h. Learners who are not familiar with using a microscope may need a little longer.

Preparing for the investigation

- Most learners will have used a microscope before, but some may not. It is also likely that the microscopes available for them to use in the ASAL course are different from those used in previous courses. You may like to demonstrate the use of the microscopes before asking learners to explore their own.
- It is generally a good idea to allocate a particular microscope to each learner, or to a pair of learners, and for them to use the same microscope each time. This can help them to become very familiar with it, and also encourages them to be responsible for taking good care of it. However, if learners will eventually be taking their practical examination in an unfamiliar laboratory using unfamiliar microscopes, you may prefer to ensure that each learner has experience using a different kind of microscope from time to time.
- For assessment of the learners' drawings in Part 2, you could provide a list of criteria and ask learners to exchange drawings with each other and assess the drawings against the list. See Table 1.1 for a sample assessment sheet. This encourages them to think hard about these criteria, and involves them more closely in their own learning than if you simply mark their drawings and provide feedback. Later, you can add your own assessments to their work.
- Note that this diagram is a high-power detail, showing individual cells and the structures within them. It is suggested that you do not introduce this term until later, when learners learn how to draw low-power plans that show tissues only, with no individual cells.

Equipment

Each learner or group will need:

- a light microscope, preferably a high quality A level-type microscope equipped with a ×10 eyepiece and at least two objective lenses
- a source of illumination (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

Access to:

- iodine in potassium iodide solution

Feature of drawing	How well was this achieved?	Comment
takes up at least half of the space available		
drawn using a sharp pencil		
all lines are clear and single, with no overlaps and no gaps		
cell walls are shown as double lines		
proportions of different structures are correct		
there is no shading		
label lines are drawn with a ruler and touch what they are labelling		
labels are written clearly and do not overlap the drawing		

Table 1.1

Additional notes and advice

- You could either provide whole onion bulbs for learners to use, or you could cut sections from the layers in the bulbs and place these in beakers of water, ready for them to use.
- Microscopes should be the best quality that your school can afford. It is frustrating for learners to have to use microscopes that do not focus clearly. Ensure that microscopes are regularly serviced and checked.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Learners should be shown how to handle a sharp blade safely.
- Iodine in potassium iodide solution generally contains ethanol as a solvent and may therefore be flammable. Learners should wear eye protection and wash off any spills on their skin or clothes. (Unlike solid iodine, this solution does not produce iodine vapour.) Low hazard.

Carrying out the investigation

- It is unlikely that any major problems will occur. Learners may have difficulty in spreading out the epidermis in the drop of water without folding it, but with practice this is generally achieved by most.
- It is possible that the onion cells may contain starch grains; if so, these will stain dark blue when the iodine is added. However, starch grains are not always present, so learners should not be surprised if there are none.
- There is a strong tendency for many learners to be so focused on 'getting it right' that they may draw an idealised diagram of the epidermis, rather than drawing what they can see. It is important to emphasise that they are being asked to **observe carefully** and to **record their observations**. This exercise is all about developing these practical skills, not their recall of the structure of plant cells. If possible, move around and look at the learners' slides through their microscopes, and compare their drawings with what they can see.

Learners who finish within the allocated time, and have made diagrams that you consider to be good quality, could be provided with a prepared slide of TS root and asked to draw three or four cortex cells.

Sample results

Method

Part 2

3

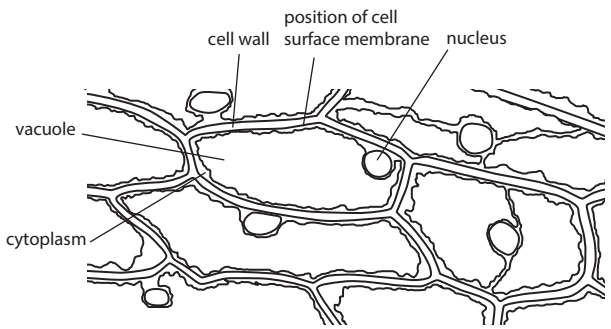


Figure 1.1

4 See Figure 1.1.

Part 3

3 It is possible that the onion cells may contain starch grains; if so, these will stain dark blue when the iodine is added. However, starch grains are not always present, so learners should not be surprised if there are none.

Practical investigation 1.2: Measuring cells, using an eyepiece graticule and stage micrometer

Learning objective: 1.1(b) and (c)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (l), (p) Successfully collecting data and observations: (a), (h)
PDO	Recording data and observations: (c)
	Displaying calculations and reasoning: (b)
ACE	Interpreting data or observations and identifying sources of error: (a), (j)

Duration

This practical is likely to take 1 h. You may like to extend this time to provide more practice in using a graticule and micrometer.

Preparing for the investigation

- Learners often become confused when using an eyepiece graticule and stage micrometer. It could be helpful to use Exercise 1.5, in the Cambridge AS & A level Biology workbook, *Using an eyepiece graticule and stage micrometer*, to practise this skill theoretically before doing this investigation. On the other hand, some learners may understand the procedures more easily when they are actually handling the graticule and micrometer themselves.
- Eyepiece graticules can be obtained relatively cheaply, but stage micrometers tend to be expensive. Cambridge Assessment International Education supply a low cost kit for you to make your own. You can download the publications list from the cambridgeinternational.org website. Click on the 'support for teachers' tab. This list also includes many different prepared slides.
- The instructions ask learners to make their first set of measurements using a prepared slide of a transverse section through a leaf. This ensures that they are looking at some clearly visible cells, and also may be more interesting for them than spending yet more time looking at onion epidermis cells. However, you (and they) may prefer to make the measurements using the onion epidermis cells first. Either sequence is fine.
- If learners always use the same microscope, then once they have calibrated a particular objective lens, they can use this calibration for all future measurements using that same objective lens and same eyepiece. Note, however, that a new calibration will be required for each of the objective lenses.

Equipment

Each learner or group will need:

- a microscope, with a graticule in the eyepiece
- prepared slide of section through a leaf
- onion epidermis slide from Practical investigation 1.1 (or a new one can be made)

Access to:

- a stage micrometer

Safety considerations

- Learners should have read the safety guidance section of the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- There are no additional significant safety issues associated with this investigation.

Carrying out the investigation

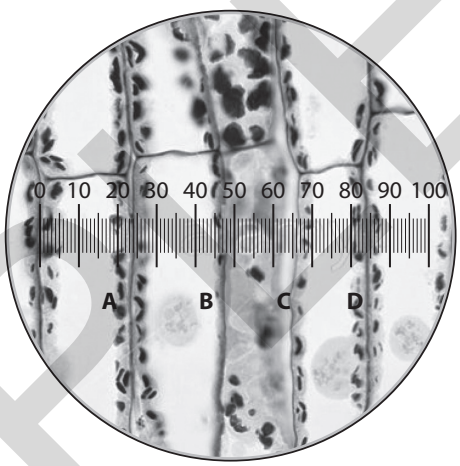
Some learners may become confused about which scale they are looking at. Swivelling the eyepiece causes the eyepiece graticule scale to move but not the stage micrometer scale.

Learners can often carry out each step in the measurement and calibration when following instructions, but find it difficult to remember what to do when they need to think about this for themselves. Step-by-step instructions are provided for measuring the palisade cells, but not the onion epidermis cells. You could provide a 'help sheet' to take learners through, step-by-step, when measuring the onion epidermis cells. For some learners, it could be valuable to repeat this several times, measuring different types of cell each time.

Some learners may have difficulty converting mm to µm, or may forget to do so when calculating magnification. See *Exercise 1.1, Units for measuring small objects*, and *Exercise 1.2, Magnification calculations*, in the Cambridge AS & A level Biology workbook, for guidance and practise in doing this.

Sample results

Part 1



Answers to the workbook questions (using the sample results)

Part 1

5 Four palisade cells measure 84 graticule units.

Part 2

3 Alignments at 0,0 and 80, 0.24.

4 24 small divisions on the stage micrometer scale = 24 × 10 µm = 240 µm

This equals 80 divisions on the eyepiece graticule scale.

So 1 division on the eyepiece graticule scale = 240 ÷ 80 = 3 µm.

5 Using the answer to Part 1 Question 5, 84 graticule units = 3 × 84 µm = 252 µm.

6 This was the width of 4 cells, so the mean width of one cell is 252 ÷ 4 = 63 µm.

7 Sample value for the width of 6 onion epidermis cells = 156 graticule units.

8 156 graticule units = 156 × 3 = 468 µm

So the mean width of one onion epidermis cell = 468 ÷ 6 = 78 µm.

Part 3

1 The answer will depend on the learner's drawing.

2 The answer will depend on the width of the drawing measured in 1. Their calculation here should be:

magnification = size of drawing ÷ size of actual cell(s)

The answer should be given as a number with a **x** sign in front of it, and no units, for example, **×40**.

Practical investigation 1.3:
Comparing animal cells and plant cells

Learning objective: 1.1(a)

Skills focus

The following skills are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (l), (m), (n), (o), (p) Successfully collecting data and observations: (a), (g), (h), (j)
PDO	Recording data and observations: (c), (f) Displaying calculations and reasoning: (b) Layout of data or observations: (h), (i)
ACE	Interpreting data or observations and identifying sources of error: (a), (j), (k)

Duration

This practical is likely to take about 1 h.

Preparing for the investigation

- Learners should be quite confident using a microscope by now, and should also be beginning to build confidence in using an eyepiece graticule and stage micrometer to make measurements. They can now use these skills to compare the appearance of three types of cell.
- If learners move straight into this activity from Practical investigation 1.1 or 1.2, they will already have temporary slides of onion epidermis. If not, they will need to make a new slide.

Equipment

Each learner or group will need:

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

Access to:

- a stage micrometer

Additional notes and advice

- Check local regulations about the use of human cheek cells. If this is not possible, then you can try scraping cells from the inner surface of a trachea from an animal such as a sheep (obtainable from a butcher).
- Cotton buds are compacted cotton wool attached to the end of a short plastic stick (Figure 1.3). They can generally be obtained from pharmacies or supermarkets, where they are sold for use with cosmetics or for baby bathing.

Figure 1.3

- Methylene blue stain can be bought from biological suppliers. It is very commonly used as a vital stain (i.e. one that is taken up by living cells) in temporary slides. If you have difficulty obtaining it from a biological supplier, you may be able to buy it from a shop selling fish for people to keep in ponds or tanks, where it is used as a treatment for fungal infections of fish. However, the concentration of the stain from this source may not be ideal for staining cells.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.

- Ensure that cotton buds are placed into a container of disinfectant (e.g. Lysol) immediately after use. This prevents the unlikely event of pathogenic bacteria being transferred from the mouth of one learner to another.
- Make sure that learners understand that they should simply run the cotton bud gently over the inner surface of their cheek. They should **not** dig the bud into the skin. There will be loose cells on the cheek surface which will readily adhere to the cotton wool.
- Methylene blue is classed as harmful (it is a reducing agent, and has various uses in medicine). Learners should avoid getting it on their skin or clothes, and should wear eye protection. If it does stain fingers, however, it is very unlikely to cause any harm.

Carrying out the investigation

- When making the cheek cell mount, learners may be puzzled that they cannot see anything on the microscope slide when they have rubbed the cotton bud onto it. They should continue with adding the stain; in most cases, cells will then become visible.
- Learners may have difficulty in locating the stained cells on the slide. They should be reminded to begin with the lowest power objective lens, and search the slide systematically until they see something. They can then move onto a higher power lens to look for individual cells.
- Some learners may construct their comparison table using what they **know** about cells, rather than using what they can **observe and measure**. This will become obvious if they mention structures such as cell membranes or endoplasmic reticulum, which they will not be able to see.

Some learners may need more support in constructing the comparison table. You could consider having a 'help sheet' available with an outline table for them to copy or complete, perhaps with one or two rows already filled in for them.

Learners who require a challenge could work in a group to research the functions of these three types of cell, and consider how each is adapted to its functions.

Sample results

Part 1

5

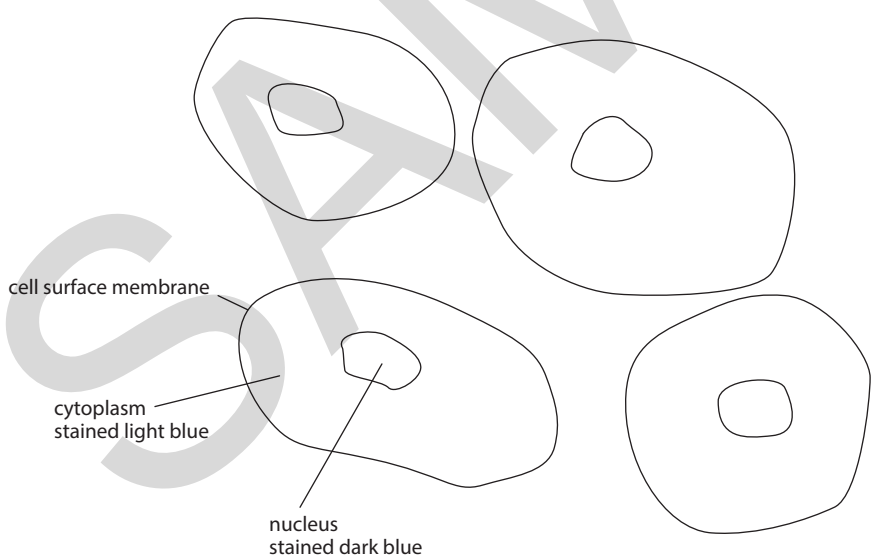


Figure 1.4

Answers to the workbook questions (using the sample results)

Part 1

6 Width of cheek cells in eyepiece graticule units: 14, 11, 13

Mean width of one cheek cell = 12.7 eyepiece graticule units

7 Using the same conversion factor as in Practical investigation 1.2, one division on the eyepiece graticule scale = 3 µm.

8 Therefore the mean width of one cheek cell = 38.1 µm.

9 Learners should measure each of the three cheek cells they have drawn along exactly the same positions as they made the measurements on the microscope slide. The measurement should be made in mm.

They should then calculate the mean width of the three cheek cells they have drawn, in mm.

The calculation is then:

$$\text{magnification of drawing} = \frac{\text{mean width of cheek cells in drawing in mm} \times 1000}{\text{mean width of cheek cells in answer to 9}}$$

The magnification should be given as a whole number, preceded by the **×** sign, and with no units.

Part 2

Answers will depend on the learner's observations and measurements. Some possible table entries are given in Table 1.2:

Feature	Onion cells	Palisade cells	Cheek cells
mean diameter / µm	78 µm	63 µm	38.1 µm
cell wall present	yes	yes	no
cytoplasm present	yes	yes	yes
chloroplasts present	no	yes	no
nucleus present	yes	yes	yes
position of nucleus	near the side of the cell	near the side of the cell	approximately central
shape of cell	approximately rectangular	approximately rectangular	approximately circular
large vacuole present	yes	yes	no
cell grouping	cells joined to one another	cells joined to one another	cells not joined

Table 1.2

Chapter 2:
Biological molecules

Chapter outline

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

In this chapter, learners 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

Learning objective: 2.1(a)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (c), (d) Successfully collecting data and observations: (a), (e)
PDO	Recording data and observations: (a), (b), (d)
ACE	Interpreting data or observations and identifying sources of error: (d) Drawing conclusions: (a)

Duration

The work should take approximately 1 h to complete but it could be split over two lessons if time is an issue.

Preparing for the investigation

- The practical work in this investigation is quite simple although learners will need to work through it methodically.
- Learners should know how to set up Bunsen burners safely. They will need to be shown how if they have not used them before.
- Most of the investigation is based upon observation and the recording of qualitative results.
- Learners should understand the main groups of biological molecules: carbohydrates (monosaccharides such as glucose, fructose, galactose; disaccharides such as sucrose, maltose and lactose and polysaccharides such as starch), proteins and lipids.
- Many test tubes are needed and learners should be encouraged to wash them out thoroughly between experiments. The emulsion test should be done last as it is difficult to remove all traces.
- The emulsion test for lipids should only be carried out at the end when all naked flames are extinguished.

Equipment

Each learner or group will need:

- minimum of 10 test tubes
- test-tube rack
- Bunsen burner, tripod, gauze and heat-proof tile (alternatively, whole class thermostatically controlled water baths may be used set to 85°C)
- test-tube holder
- glass beakers, 500 cm^3 and 50 cm^3
- pipette, 10 cm^3, and pipette filler (if pipettes are not available, small measuring cylinders or syringes may be used)
- Benedict's solution, 25 cm^3
- biuret solution, 25 cm^3
- iodine solution in a dropper bottle
- ethanol, 20 cm^3
- distilled water, 50 cm^3

- dilute hydrochloric acid (2M) in a dropper bottle
- sodium hydrogencarbonate (solid)
- spatula
- 20 cm^3 1% starch solution
- 20 cm^3 1% protein solution (albumin or casein)
- vegetable oil (any oil such as olive oil or sunflower oil. Avoid nut oils to reduce risk of allergic reactions)
- 20 cm^3 10% glucose
- 20 cm^3 10% fructose
- 20 cm^3 10% sucrose
- 20 cm^3 10% lactose
- 20 cm^3 10% maltose
- 20 cm^3 'unknown' solutions X, Y and Z

Additional notes and advice

Stock solutions

- Benedict's solution may be purchased ready made from suppliers. To make 1 dm^{-3}, dissolve 170 g sodium citrate crystals and 100 g sodium carbonate crystals in 800 cm^3 warm distilled water. In a separate beaker, dissolve 17.0 g copper(II) sulfate crystals in 200 cm^3 cold distilled water. Mix both solutions whilst constantly stirring.
- Biuret solution may be purchased ready made from suppliers. If it is not available, separate solutions of sodium hydroxide and copper sulfate may be used. Sodium hydroxide concentrations of between 1 and 2 dm^{-3} should work and a 1% solution of copper(II) sulfate made in distilled water is then added. Biuret solution should not be stored for long periods in glass bottles.
- Iodine solution may be purchased ready made from suppliers. To make 100 cm^3 iodine solution, grind 1 g iodine and 1 g potassium iodide in a mortar, gradually adding distilled water to dissolve the crystals. Pour the solution into a measuring cylinder and make up to 100 cm^3 with distilled water.
- 2 mol dm^{-3} hydrochloric acid. Dilute 200 cm^3 concentrated hydrochloric acid with 800 cm^3 distilled water.
- 1% starch solution. To make 500 cm^3, add 5 g soluble starch powder to approximately 300 cm^3 distilled water in a beaker. Heat to boiling point until all the starch dissolves then make up the solution to 500 cm^3 with cold distilled water. Store in refrigerator and use within two days.
- 1% protein solution. Either albumin or casein can be used. Slowly dissolve 1 g commercial albumin powder in 100 cm^3 cold water (it will take time). Alternatively, use a 1 in 10 dilution of raw egg white. For a 1% casein solution, dissolve 1 g casein powder in 100 cm^3 distilled water. Alternatively, powdered milk can be used but at slightly higher concentrations. Store in refrigerator.
- 1% glucose (and other sugars). To make 100 cm^3, weigh out 1 g of sugar and make up to 100 cm^3 with cold distilled water. Store in refrigerator for up to two weeks.
- Solution X: To make 100 cm^3, weigh out 1 g glucose, 1 g albumin. Dissolve in 100 cm^3 distilled water.
- Solution Y: To make 100 cm^3, weigh out 1 g sucrose. Dissolve in 100 cm^3 distilled water.
- Solution Z: To make 100 cm^3, weigh out 1 g glucose, 1 g starch. Dissolve in 100 cm^3 distilled water.

Safety considerations

- Learners should have read the safety section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Before carrying out the emulsion test all naked flames should be extinguished. This is extremely important as ethanol is highly flammable.
- When making up the solutions, special care should be taken with substances such as concentrated hydrochloric acid, sodium hydroxide and solid iodine.
- Eye protection should be worn always.
- Iodine solution should not be poured into natural water as it is harmful to aquatic organisms.
- If concentrated acids and alkalis are used when making up the solutions, extra care should be taken: high quality eye protection should be used and protective gloves.
- Some learners may be allergic to certain oils. Do not use oils such as groundnut / almond / peanut oil.

Carrying out the investigation

- It is important to trial the tests before the investigation to ensure that they give the correct results.
- If starch is kept for too long it can begin to break down, releasing glucose. It should be made up fresh and stored for only up to two days in the refrigerator.

- Biuret solution should not be kept for long periods in glass bottles as it will react with the glass and stop working.
- Test tubes should be rinsed out carefully but after reducing sugar tests there may be a residue that will not come off easily. It is advisable to have many other clean test tubes.
- Learners should be reminded that it is important to wash out pipettes thoroughly between different solutions.
- Sucrose can sometimes be contaminated with glucose and so give a positive reducing sugar test – it should be tested before the experiment.
- The reducing sugar test should have shown that sucrose did not cause Benedict's solution to change colour. To carry out a non-reducing sugar test, it is important that a negative result is obtained with the reducing sugar test before proceeding.

Help should be provided for any learners with physical disabilities in the laboratory when using boiling water baths. It is good practice to not sit down in the event of boiling water being spilled. Where this is not possible (for example, with learners who are in a wheelchair), alternative provisions such as thermostatically controlled water baths can be used.

Colour-blind learners may have difficulty discriminating between the colours of the Benedict's test. They may need another learner to read out colours.

Learners could be encouraged to trial food items that are available to identify the molecules – care should be taken to avoid risk of allergies.

As an extension activity, learners should try carrying out reducing sugar and non-reducing sugar tests on a mixture of glucose and sucrose solutions and comparing the results of the two tests.

Sample results

Biological molecule	Final colour of solution after biochemical test				
	Iodine	Biuret	Reducing sugar	Non-reducing sugar	Emulsion test
1% starch	blue / black				
1% protein		lilac / purple			
10% glucose	yellow / orange	blue	red / orange and cloudy		
10% fructose			red / orange and cloudy		
10% maltose			red / orange and cloudy		
10% lactose			red / orange and cloudy		
10% sucrose			blue and clear	red / orange and cloudy	
vegetable oil					white and cloudy
water	yellow / orange	blue	blue and clear	blue and clear	
ethanol					colourless

Table 2.1

Biological molecule	Final colour of solution after biochemical test			
	Iodine	Biuret	Reducing sugar	Non-reducing sugar
solution X	yellow / orange	lilac	red / orange and cloudy	red / orange and cloudy
solution Y	yellow / orange	blue	blue	red / orange and cloudy
solution Z	blue / black	blue	red / orange and cloudy	red / orange and cloudy

Table 2.2

Answers to the workbook questions (using the sample results)

a **i** Contents of unknown solution X: protein, reducing sugar (glucose), non-reducing sugar (sucrose) may or may not be present

Contents of unknown solution Y: non-reducing sugar (sucrose)

Contents of unknown solution Z: starch, non-reducing sugar (glucose), non-reducing sugar (sucrose) may or may not be present

ii X and Z both contain a reducing sugar which will give a positive result with both reducing sugar and non-reducing sugar tests. As the tests are qualitative, it is difficult to determine if there is extra precipitate or not.

b
- Monosaccharides that are reducing sugars: glucose, fructose, galactose
- Disaccharides that are reducing sugars: lactose, maltose
- Disaccharides that are non-reducing sugars: sucrose

c To act as a control and show what a negative test looks like for comparison.

d The tests are qualitative as they do not give an indication of the number of molecules present.

e **i** Both reducing and non-reducing sugar tests use a Benedict's test. This means that glucose would react with both tests.

ii The non-reducing sugar tests hydrolyses the sucrose into glucose and fructose. These are both reducing sugars and then react with the Benedict's solution. When bound together in sucrose, neither the glucose nor fructose is able to donate electrons.

iii Carry out reducing sugar tests on both solutions. This will show which one solution is glucose as it will give a positive test. Then carry out a non-reducing sugar test on the other solution to confirm it is sucrose.

f The solution contains a mixture of both reducing and non-reducing sugars. If it contains both glucose and sucrose, for example, the reducing sugar test will generate a precipitate from just the free glucose. If a non-reducing sugar test is carried out, the sucrose is hydrolysed into glucose and fructose so that the solution now contains additional glucose and fructose. These will now react with Benedict's solution to produce additional precipitate.

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

Learning objective: 2.1(b), 2.2(d)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (c) Successfully collecting data and observations: (a), (e)
PDO	Recording data and observations: (a), (b), (d) Layout of data or observations: (i)
ACE	Interpreting data or observations and identifying sources of error: (c), (d) Drawing conclusions: (a) Interpreting data or observations and identifying sources of error: (g), (i)

Duration

The work should take approximately 1 h to complete.

Preparing for the investigation

- Learners should have carried out Practical Investigation 2.1 and so understand the reducing sugar test.
- Solutions should be tested before the investigation to ensure that they work correctly.
- Individual boiling water baths can be set up by learners but the experiment can also be carried out using thermostatically controlled water baths set at 85°C.
- Comparing colours is very subjective and this should be discussed as a source of error. It is worthwhile different groups of learners comparing what they decided the colours matched up to.
- This experiment is a useful way of introducing the ideas of quantitative and qualitative data. It is also an appropriate

point to discuss the idea of uncertainty in the results as only an approximate concentration can be inferred.

Equipment

Each learner or group will need:

- nine test tubes
- test-rube rack
- Bunsen burner, tripod, gauze and heat-proof tile (alternatively, whole class thermostatically controlled water baths may be used set to 85°C)
- test-tube holder
- glass beakers, 500 cm^3, 50 cm^3
- pipette, 10 cm^3 and 1 cm^3 and pipette filler (if pipettes are not available, small measuring cylinders or syringes may be used)
- Benedict's solution, 100 cm^3 (see Practical investigation 2.1)
- distilled water, 100 cm^3
- 10% glucose solution, 50 cm^3
- 'unknown' concentration of glucose, 20 cm^3 (see Practical investigation 2.1). This should be 0.05% concentration.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- There are no additional significant safety issues associated with this investigation.

Carrying out the investigation

- This should be quite a straightforward practical.
- Learners will often find the idea of serial dilutions difficult and will often ask for help.
- It is important that the tubes are mixed carefully for each dilution before removing 1 cm^3.
- Learners may need to set up two water baths or share additional water baths.

Colour-blind learners may have difficulty discriminating between the colours of the Benedict's test. Another learner may need to help them identify the colours.

Learners could also be provided with a range of ready made fruit juice drinks and asked to estimate the sugar concentrations of each.

Sample results

Test-tube number	Glucose concentration / %	Colour of Benedict's solution
1	10	brick red
2	1	orange
3	0.1	yellow
4	0.01	green
5	0.001	pale green
6	0.0001	blue

Table 2.3

The colour of the unknown solution was green/yellow.

Answers to the workbook questions (using the sample results)

a The concentration of glucose in the 'unknown' solution is approximately 0.1 / 0.01%. It may lie within a range of 0.1–0.5%. Learners' answers may vary here depending on how they saw the colours – it is very subjective.

b A fully quantitative method would give an accurate, exact concentration of glucose. This method allows learners to approximate the amount of glucose without being certain of the exact concentration.

c The exact concentration can only be judged to lie between 0.1% and 0.01% as there is no colour standard for 0.05%. There would also be too little difference in colour between standard concentrations that were made between 0.1 and 0.01%. The human eye would not be able to detect the differences.

d All the standard solutions and the test solution had a volume of 9 cm^3 to which 5 cm^3 Benedict's solution were added. If 1 cm^3 were not discarded, it would have had 10 cm^3 glucose which could affect the reaction.

e Control variables included:

- temperature of water bath: higher or lower temperatures may affect the reaction speed
- length of time in water bath: leaving the solutions longer may have resulted in more reaction. (Ideally, the length of time should be sufficient to ensure the reactions are complete)
- volume of glucose solution: more solution would result in more intense colour due to increase of glucose
- volume of Benedict's solution: different amounts would affect whether the reaction had gone to completion
- concentration of Benedict's solution: different amounts would affect whether the reaction had gone to completion.

f If the amount of Cu^{2+} runs out before all the glucose molecules have oxidised / donated electrons, it is not an accurate measure of glucose concentration. Theoretically, if more Benedict's solution were added, more precipitate would form. If insufficient, the glucose concentration would be estimated to be lower than it is.

g Tube 6 is a control. This shows the colour that Benedict's solution turns when heated. It could be argued that simply heating Benedict's solution causes it to change colour even without glucose.

h Alternative methods could be:

- filtering the solution to collect the precipitate. This would be dried and weighed. A calibration graph could be plotted of mass of precipitate against glucose concentration and used to determine glucose concentrations of other solutions.
- using a colorimeter to measure the red colour or blue colour or turbidity. A calibration graph could be plotted of absorbance against glucose concentration and used to determine glucose concentrations of other solutions.

i 10%, 5%, 2.5%, 1.25%, 0.625%, 0.3125%

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

Learning objective: 2.1(a)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (c) Successfully collecting data and observations: (a), (e)
PDO	Recording data and observations: (a), (b), (d) Layout of data or observations: (i)
ACE	Interpreting data or observations and identifying sources of error: (c), (d), (g), (i) Drawing conclusions: (a), (b) Suggesting improvements or modifications to extend an investigation: (a), (b), (c), (d), (e)

Duration

The work should take approximately 1 h to complete. It is easier to complete within the time period if learners work in pairs.

Preparing for the investigation

- Learners should understand how to make solutions of different concentrations from stocks by using proportional and serial dilutions. This practical asks learners to plan their own dilutions – these should be checked before they start the practical.
- The practical requires the sourcing of bananas that are as green as possible. If very green bananas are not available, plantains may be used. Ripe, yellow bananas are needed. Black, over-ripe bananas should be prepared in advance.
- Learners should understand that unripe bananas contain large quantities of starch which is then gradually converted to sucrose, glucose and fructose as the bananas ripen.
- The practical could be modified to compare different fruit and vegetables such as new or old potatoes, sweet potatoes, apples, etc.

Equipment

Each learner or group will need:

- nine test tubes
- test-tube racks
- iodine solution in a dropper bottle
- 1% starch solution, 50 cm^3
- distilled water, 100 cm^3
- pipettes, 10 cm^3, 1 cm^3, pipette filler (if pipettes are not available, small measuring cylinders or syringes may be used)
- pieces of banana flesh from green, yellow and black bananas
- knife or scalpel
- Bunsen burner, tripod, gauze, heat-proof tile (alternatively, whole class thermostatically controlled water baths may be used set to 85°C)
- test-tube holder
- glass beakers, 500 cm^3 and 50 cm^3
- spatula.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Iodine solution should not be flushed into natural water where it can harm aquatic organisms.

Carrying out the investigation

- Learners will need support in working out the dilutions. They should show these to the teacher before they start to make them.
- Learners may need support in determining the differences in colour intensity.
- The starch concentrations in the different bananas may be variable and, depending on the banana, may not yield the predicted differences.
- The banana extract may need diluting with distilled water if it is too concentrated. This should be discussed when determining the actual starch concentration.

Help should be provided for any learners with physical disabilities in the laboratory when using boiling water baths. It is good practice to not sit down in the event of boiling water being spilled. Where this is not possible (for example, with learners who are in a wheelchair), alternative provision such as thermostatically controlled water baths can be used.

Learners with dyspraxia may need support when cutting up the bananas pieces.

Learners with visual impairments may need support when comparing the colours of different solutions.

As extension work, other fruits and vegetables could be tested. Learners could also predict how the water potential of the banana would change as starch is converted to sucrose. They could plan a further experiment to estimate how water potential changes with ripeness of bananas.

Sample results

Test-tube number	Concentration of starch / %	Colour of iodine solution
1	1	black
2	0.1	dark blue
3	0.01	pale blue
4	0.001	very pale blue
5	0.0001	orange

Table 2.4

The colour of the iodine solution with each of the bananas was:

green: dark blue

yellow: pale blue–blue

black: orange–very pale blue

Answers to the workbook questions (using the sample results)

a The approximate starch concentrations may be very variable and will depend very much on the bananas the learners use.

For example:

- green (unripe): concentration: 1.0%; range: >1.0%
- yellow (ripe): concentration: 0.25%; range: 0.25–0.5%.
- black (over-ripe): concentration: 0.1%; range: 0.1–0.25%

b As bananas ripen, the starch is hydrolysed into glucose. Some of this is converted into other sugars such as fructose and sucrose.

c The estimated ranges of starch concentrations are very high so no differences would be detected when, for example, starch concentrations of 0.1 and 0.5% are tested. This could be improved by using a colorimeter to produce a calibration curve with known standards (this could be demonstrated to learners).

d Limitations:

- lack of sensitivity at low concentrations of starch
- difficult to detect differences in intensity of colour at higher concentrations of starch
- it is highly subjective due to use of human eye (lack of precision)
- the estimated ranges are very high and so inaccurate
- the banana extracts may not have solubilised all the starch
- sections of a banana may have different starch concentrations
- the investigation is unreliable as only one banana of each type was used.

Chapter 3: Enzymes

Chapter outline

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

In this chapter, learners 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 Immobilised urease
- 3.6 Investigating the effect of an inhibitor on the action of bromelain

Introduction

Enzyme experiments are an important part of any AS Biology course. They provide a very wide range of opportunities to access many of the skills that will be tested in practical exams, as well as helping learners to understand the facts and concepts covered in the learning objectives on this topic, which will be tested in theory exams.

There is a very large number of possible enzyme experiments that learners could be asked to do. We have selected six investigations in this chapter, for these reasons:

- This set of practical investigations enables learners to become familiar with several different enzymes – catalase, amylase, urease and protease.
- These enzymes are generally possible for any school to obtain, as all of them can be found in biological materials – they do not need to be bought in.
- This set of investigations gives learners experience of measuring rate of reaction by either measuring the disappearance of substrate (amylase and protease) or appearance of product (catalase and urease).
- It provides experience of several different techniques for measurement.
- It covers the learning objectives relating to enzyme investigations in Section 3.2 of the syllabus.

There are many other enzymes that you can use, such as lipase and lactase. You can also, if you wish, change the enzymes that are used for the investigations in this chapter. For example, you could use catalase in Practical investigation 3.3, instead of amylase. You could use lactase instead of urease in Practical investigation 3.5, and test for its activity by allowing milk to flow over it and testing for glucose using glucose test strips.

Enzyme experiments, like all Biology experiments, have a well-deserved reputation for not always behaving in the way that you expect. As explained in the introduction to the Practical Workbook, it is very important to appreciate that getting the 'right' results is not the main point of the exercise – it is the **process** that is more important. Of course, learners will be more satisfied if their results are what they expect, but they should not feel that their experiment 'has not worked' if their results are unexpected. Such results provide good opportunities for looking critically at the experimental procedure, to identify possible reasons for the unexpected results obtained.

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

Learning objective: 3.1(d)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (h), (j) Successfully collecting data and observations: (a), (c)
PDO	Recording data and observations: (a), (c) Displaying calculations and reasoning: (a) Layout of data or observations: (c), (d), (e)

ACE Interpreting data or observations and identifying sources of error: (a), (b), (e), (f), (g), (i)
Drawing conclusions: (b)

Duration

The practical work will take about 1 h, depending on the familiarity of learners with the apparatus and techniques. The analysis and evaluation questions will take 45–60 min.

Preparing for the investigation

- Learners should know and understand the terms substrate, product and rate of reaction. They should understand how enzymes function, in terms of random collisions between substrate and the enzyme's active site.
- This experiment uses catalase extracted from celery stalks, and it is not possible to know the concentration or activity of the enzyme. Learners are therefore asked to do preliminary work themselves to determine appropriate volumes and concentrations of enzyme and substrate, and this is very valuable for them, helping them to think deeply about what they are doing and why, rather than simply following a set of instructions. However, if you are short of time, you could do this preliminary work yourself, and simply ask the learners to do the second part of the experiment, in which they measure the rate of oxygen production over time.

Equipment

Each learner or group will need:

- two or three stalks of celery
- about 20 cm^3 of 10 volume hydrogen peroxide solution (this can generally be obtained cheaply from a local pharmacy)
- an electric blender
- a filter funnel and muslin (fine cloth that can be used for coarse filtering; using filter paper will take too long)
- 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
- an apparatus for measuring small volumes, for example two 5 cm^3 or 10 cm^3 syringes or two graduated pipettes.

Additional notes and advice

- Any biological material will contain catalase. If celery is not available, try other plant material, such as potato, carrot, apple or other fruit or vegetable. Animal tissues, such as liver, can also be used, but the catalase in these is often so active that it is very difficult to measure the rate of reaction.
- If you have 20 volume hydrogen peroxide rather than 10 volume, you can dilute it by 50%. Alternatively, learners could be provided with 20 volume hydrogen peroxide.
- If no electric blender is available, learners can grind pieces of celery stalk with water in a pestle and mortar. However, this is time-consuming, and only small quantities can be dealt with at a time.
- This method uses gas syringes to collect the gas evolved. This is by far the simplest and most reliable method of measuring the rate of oxygen formation. If you do not have gas syringes, you may be able to borrow them from the Chemistry department. If you have good quality 'ordinary' plastic syringes, you can try using these instead, but do make sure that they are gas-tight, and that the syringe barrel moves smoothly.
- If no suitable syringes are available, you could try one of the methods shown in Figure 3.1.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Learners should take care when using sharp blades to cut the celery stalks.
- Hydrogen peroxide 20 or 10 volume irritates eyes and skin. Safety glasses should be worn throughout. Hydrogen peroxide decomposes slowly even with no catalyst present, so oxygen gas may build up in a bottle. Store in a cool, dark place and take care when opening.
- If the gas is given off very quickly, the plunger in the gas syringe can shoot out of the syringe barrel with some force, and travel quite a distance. To avoid this, tie the plunger loosely to the barrel, so that if it does shoot out, it cannot travel far.

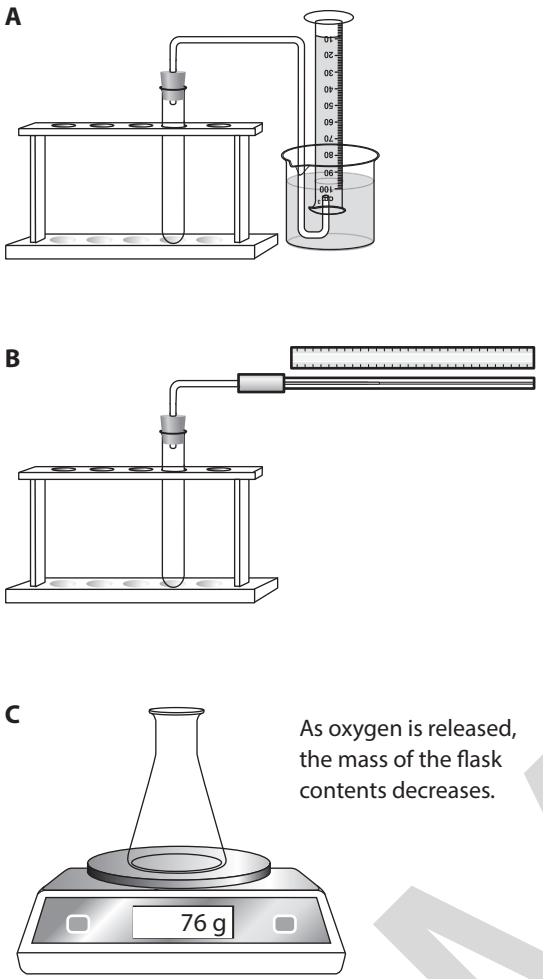


Figure 3.1

Carrying out the investigation

- It is strongly recommended that you encourage learners to make their own decisions when carrying out the preliminary work. This increases their engagement with the problems to be solved, and increases their understanding of the reaction and how they are measuring its rate. Only intervene when it is clear that learners cannot progress without your guidance.
- It is possible that their first attempts will result in a rate of production of oxygen that is too fast to measure. They can try diluting the enzyme extract, or diluting the hydrogen peroxide solution, or reducing the ratio of the volume of enzyme extract: volume of hydrogen peroxide solution. They may need several attempts before arriving at a concentration and volume of enzyme and substrate that produces a measurable rate. Encourage them to be systematic in their approach.
- If the rate of production of gas is too slow, they could try preparing a fresh enzyme extract using more celery and less water.
- The most common problem encountered while doing the experiment is that the apparatus is not airtight. Check all joints and ensure that suitable diameters of rubber tubing have been used to join the glassware. If problems persist, use petroleum jelly to seal suspect joins. Melted candle wax also works well, but can be difficult to clean off afterwards.
- After adding the enzyme extract to the hydrogen peroxide, it is impossible to completely place the bung back into the test tube immediately. This means that some gas will escape, and the measurement of volume will be too low. (This is a source of error that learners should identify and comment on as they answer question **h**.) The time delay is reduced if learners work in pairs or small groups.

Learners may find it difficult to work out what to do in the preliminary work, if the rate of reaction is too high or too low. Use questioning to help them to work this out for themselves, for example ask: *What do you think will happen to the rate of reaction if you decrease the concentration of the substrate*?

Some learners, especially those whose English language skills are not strong, may have difficulties in wording their answers to some of the questions, for example **b** and **e**. You could provide sentences with gaps for them to complete, and/or provide a list of words and phrases that they can use in their answers.

Question **g** is difficult. You could ask each group to discuss the question among themselves, and then ask each group to present their decisions to the rest of the class. Discuss their answers as a whole class, and then ask each learner to write down their individual answer in their own words.

A valuable additional exercise would be to provide learners with different methods of collecting the gas and measuring its volume. Some suggestions are shown in Figure 3.1. You could allocate different methods to different groups, and then discuss which apparatus provides the most reliable results and why. This will help learners to develop their understanding of making decisions about how to measure the dependent variable.

Ask learners to suggest how they could use this technique to compare the rates of activity of catalase taken from two different vegetables, or from celery stalks that have been kept in different conditions (e.g. in a fridge and in a warm kitchen). They can begin to think about the variables that they will try to keep the same. If time allows, they could carry out their investigation.

Common learner misconceptions

- The words 'catalase' and 'catalyse' are very similar, and it is worth spending a few moments pointing this out to learners, ensuring that they understand each term.
- When interpreting the graph, learners may not appreciate that the reaction has stopped when the oxygen volume no longer increases. They may read the graph as showing that oxygen continues to be given off, expecting the line to fall to 0 when no more is produced.

Sample results

Time / s	30	60	90	120	150	180	210	240	270	300	330	360	390	420	450	480	510
Volume of oxygen / cm^3	2.0	5.5	7.5	9.5	11.5	13.0	14.5	16.0	17.0	18.5	19.5	20.5	21.0	21.0	21.5	22.0	22.5

Table 3.1

Answers to the workbook questions (using the sample results)

It is highly preferable that learners should use their own results when answering the questions. In this case, you will need to check their answers to questions **a, b, c, d, e** and **f** against their results.

The following answers relate to the sample results.

a

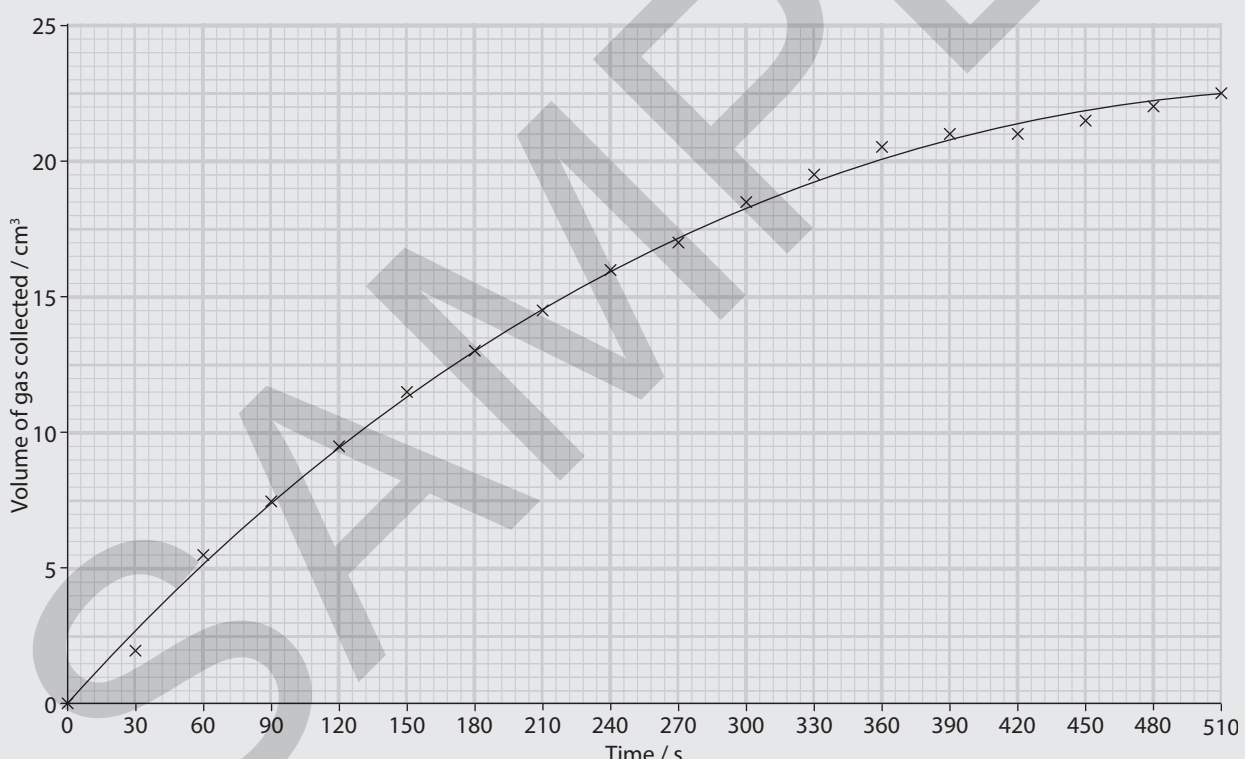


Figure 3.2

b The rate of reaction is rapid at first, and gradually becomes slower with time.

c At time 0, before the enzyme has begun to break down the hydrogen peroxide.

d The graph has not levelled off, so there is still some oxygen being released, meaning that there is still some substrate that has not been broken down even after 510 s.

e When the catalase is first added to the hydrogen peroxide, the concentration of substrate is highest. The frequency of collisions between the catalase molecules and the hydrogen peroxide molecules, and the rate of formation of enzyme–substrate complexes, is high. As time progresses, the concentration of substrate gradually decreases, as it is broken down by the enzyme. The frequency of collisions therefore also decreases. Eventually, there would be no substrate left, so the rate of reaction would become zero.

f

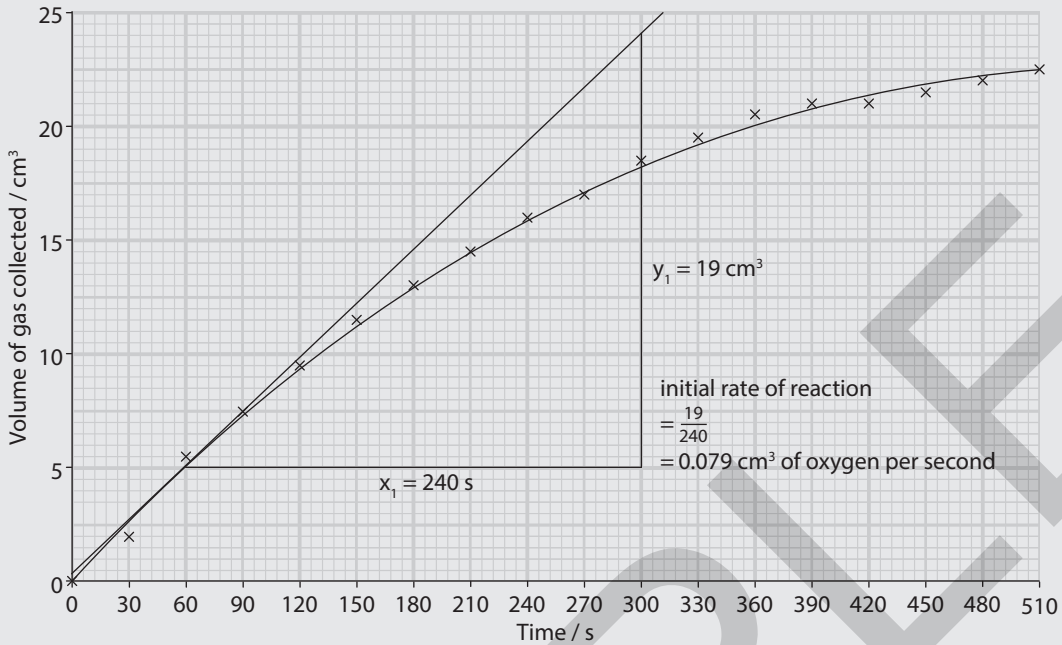


Figure 3.3

g As the reaction progresses, the concentration of substrate decreases. If we want to investigate the effect of substrate concentration on the rate of reaction, then substrate concentration is our independent variable, and we need to measure it and know what it is. We cannot do this if the substrate concentration is changing. We therefore need to measure the rate of reaction right at the start of the reaction, before the substrate concentration has decreased significantly. Only then can we be sure that the substrate concentration is the same for each experiment.

h **ii** A random error. Gas will escape from the test tube at the start of the reaction. After that, once the bung is in place, it will not escape. We therefore lose some gas at the start, so the total volume of gas collected is too low. However, once the bung is in place, all the gas is collected and the *change* in volume will be correct.

iii A random error. Readings may be taken a little bit before or a little bit after the required time. This is likely to produce points on the graph that do not lie neatly on a curve, because a point plotted at, say, 30 s actually belongs at 31 s.

iv A systematic error. If the scale is not quite correct, then each reading is likely to be 'out' by the same value each time. It could give readings that are either too high or too low, depending on what is wrong with the scale.

v A random error. It is a single error that takes place just once. The tangent should be drawn as close to the origin as possible, but this is difficult to do, and the curve is very steep (almost a straight line) here. Drawing the tangent in the wrong place or at the wrong angle will have a significant effect on the calculated value for the initial rate of reaction, which could be either too high (if the tangent is drawn at too vertical an angle) or too low (if the tangent is drawn sloping too much to the right).

Practical investigation 3.2:
The effect of substrate concentration on the rate of an enzyme-catalysed reaction

Learning objective: 3.2(a)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (a), (b), (g), (h) Successfully collecting data and observations: (a), (c)
PDO	Recording data and observations: (a), (b), (c) Displaying calculations and reasoning: (a) Layout of data or observations: (b), (c), (d), (e)
ACE	Interpreting data or observations and identifying sources of error: (a), (b), (e), (f), (g) Drawing conclusions: (a), (b) Suggesting improvements or modifications to extend an investigation: (a), (b), (c), (e)

Duration

The practical work will take between 60 and 90 min, partly depending on how quickly learners are able to determine how to make up the different concentrations of substrate, and to do this. The analysis and evaluation questions will take around 30 min.

Preparing for the investigation

- Learners should have carried out Practical investigation 3.1, so that they are familiar with the enzyme and the technique for measuring rate of reaction.

Equipment

Each learner or group will need:

- two or three large stalks of celery
- approximately 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, e.g. two 5 cm^3 or 10 cm^3 syringes or two graduated pipettes.

Additional notes and advice

- See Practical investigation 3.1 for suggestions for alternative equipment.
- You may prefer to use 20 volume hydrogen peroxide, as this will give you a greater possible range of substrate concentrations.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Learners should take care when using sharp blades to cut the celery stalks.
- Hydrogen peroxide 20 volume or 10 volume irritates eyes and skin. Safety glasses should be worn throughout. Hydrogen peroxide decomposes slowly even with no catalyst present, so oxygen gas may build up in a bottle. Store in a cool, dark place and take care when opening.
- If the gas is given off very quickly, the plunger in the gas syringe can shoot out of the syringe barrel with some force, and travel quite a distance. To avoid this, it is important to tie the plunger loosely to the barrel, so that if it does shoot out, it cannot travel far.

Carrying out the investigation

- Please see the points described for Practical investigation 3.1.
- If learners begin with the lowest concentration of substrate, they may find that the reaction scarcely takes place at all, and may waste a lot of time watching their apparatus while no measurable quantity of gas is given off. It is therefore suggested that you encourage them to begin with the highest concentration and work systematically downwards.

Some learners may have difficulty in deciding how to make up the different concentrations of substrate. They need to become confident with doing this, as it is often expected in examination questions, so it is important to encourage them to try to work it out for themselves. If this fails, refer them to Figure P1.2 in the coursebook. If this is not available, prepare a handout showing Figure 3.4.

Learners who require a further challenge, and who have a set of results that has produced a suitable graph, could use their graph to calculate the Michaelis–Menten constant, K_m. There is guidance on how to do this in the Biology workbook.

Example of how the learners could prepare the different concentrations

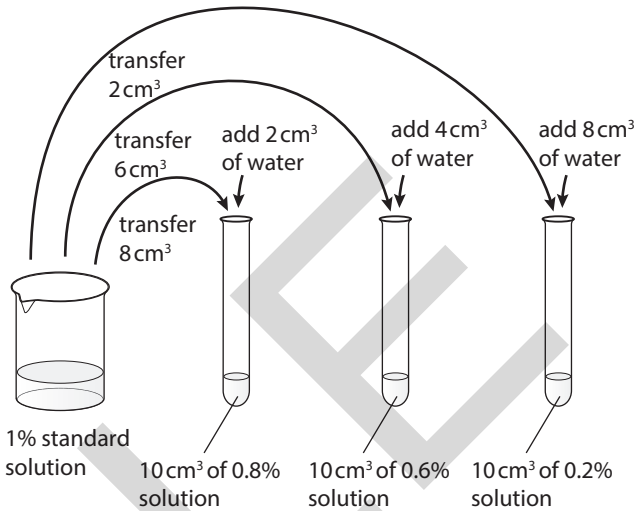


Figure 3.4

Sample results

Time / s	Volume of oxygen / cm³				
	100% substrate	80% substrate	60% substrate	40% substrate	20% substrate
0	0	0	0	0	0
30	2.0	3.0	2.0	1.0	0
60	5.5	4.5	4.0	2.0	0.5
90	7.5	6.5	6.0	3.5	0.5
120	9.5	8.0	8.0	5.0	1.0
150	11.5	10.0	9.5	6.0	1.0
180	13.0	11.0	11.0	7.0	1.5
210	14.5	12.5	12.0	8.0	2.0
240	16.0	13.5	13.0	8.5	2.0
270	17.0	15.0	13.5	9.0	2.5
300	18.5	16.0	14.0	10.0	3.0
330	19.5	16.5	14.5	10.5	3.0
360	20.5	17.0	15.0	10.5	3.5
390	21.0	17.5	15.0	11.0	3.5
420	21.0	18.0	15.5	11.0	4.0
450	21.5	18.5	15.5	11.5	4.0
480	22.0	18.5	16.0	11.5	4.5
510	22.5	19.0	16.0	12.0	4.5

Table 3.2

Answers to the workbook questions (using the sample results)

a

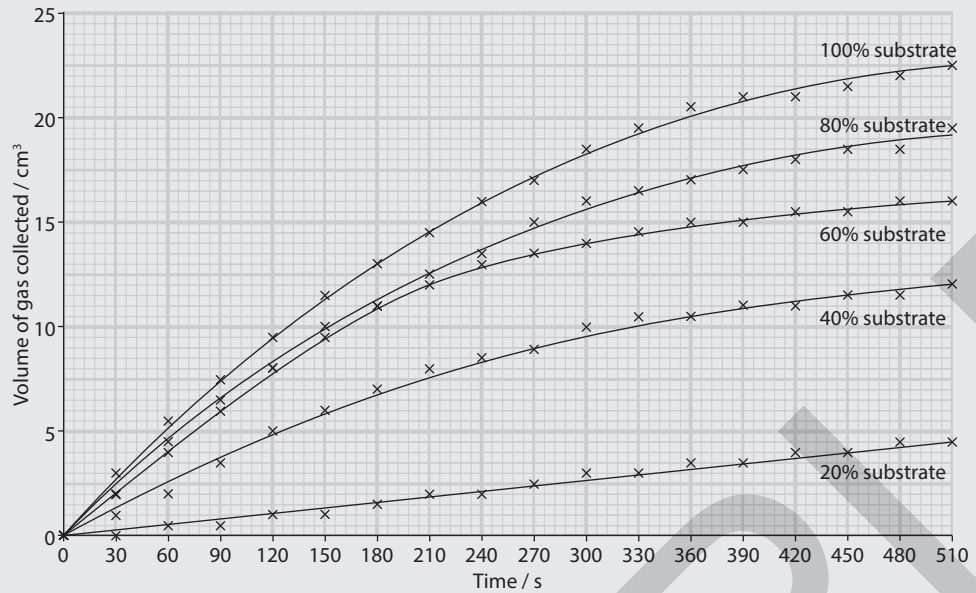


Figure 3.5

b The results given here are approximate; much depends on exactly where and how the tangent is drawn to the curves.

Substrate concentration as percentage of stock solution	Initial rate of reaction / cm^3 oxygen s^{-1}
100	0.42
80	0.41
60	0.34
40	0.15
20	0.02

Table 3.3

c

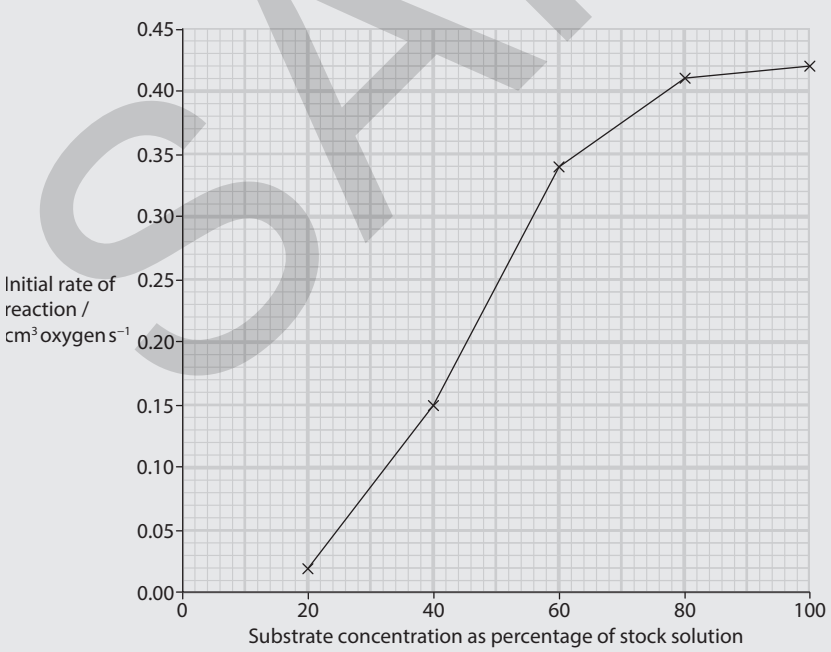


Figure 3.6

d In the raw results, the volume of oxygen produced after 30 s is greater for the 80% substrate concentration than for 100% substrate concentration.

e The greater the concentration of substrate, the greater the initial rate of reaction.

Some learners may find that their graph levels off at higher concentrations of substrate, in which case their conclusion should be:

The greater the concentration of substrate, the greater the initial rate of reaction. At very high substrate concentrations, however, a maximum rate of reaction is reached.

f The greater the concentration of substrate, the more hydrogen peroxide molecules are present in any given volume of the reacting mixture. This increases the chance that a hydrogen peroxide molecule will collide with an active site of a catalase molecule. The more frequent these collisions, the faster the rate of reaction.

(Note that it is the **frequency** of collisions that is important, not the **number** of collisions.)

At very high concentrations of substrate, all active sites may be occupied all of the time, so that increasing the substrate concentration even more does not increase the rate of reaction.

g, h Any of the errors listed in question **h** in Practical investigation 3.1 can be included.

New sources of error include those involved in making up the dilutions of the hydrogen peroxide solutions, and keeping the control variables constant.

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

Learning objective: 3.2(a)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (e), (g), (j) Successfully collecting data and observations: (a)
PDO	Recording data and observations: (a), (c) Layout of data or observations: (b), (c), (d), (e)
ACE	Interpreting data or observations and identifying sources of error: (e), (f), (g) Drawing conclusions: (a), (b) Suggesting improvements or modifications to extend an investigation: (a), (c), (e)

Duration

The practical work will take between 60 and 75 min. The analysis and evaluation questions will take around 30 min.

Preparing for the investigation

- Learners will find it helpful to have done Practical investigations 3.1 and 3.2 before this one. They should know how to make up a range of concentrations by dilution, and be able to identify random and systematic errors.
- Amylase bought from educational suppliers is usually obtained from fungi or bacteria. There may be information with the amylase stating information such as its optimum temperature (which may be as high as 80°C). However, you should test the activity of the amylase yourself. Make up a 1% solution of amylase (that is, dissolve 1 g of amylase in a little cold water, and then make up to 100 cm^3) and a 5% solution of soluble starch. Add 1 cm^3 of amylase solution to 1 cm^3 of starch solution, and mix thoroughly. Take samples every minute or so, checking for the continuing presence of starch. Ideally, you want all the starch to disappear within a time range between 5 and 15 min. If it disappears too quickly, try diluting the amylase solution. If too slowly, dilute the starch solution.
- The instructions do not suggest controlling pH. Ideally, this should be a control variable, and you could keep it constant by using a buffer solution that matches the optimum pH of the enzyme. However, in practice pH will not vary, so it may be better to bypass this step and simply use distilled water.

Equipment

Each learner or group will need:

- about 50 cm^3 of a 1% solution of amylase (or the concentration you find works well)
- about 50 cm^3 of a 5% solution of starch (or the concentration you find works well)
- 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, for example, 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.

Additional notes and advice

- If you do not have a thermostatically controlled water bath, learners can make their own by filling a large beaker with water at room temperature. They can measure the temperature at intervals throughout their experiment, to check that it stays constant. In practice, it is not possible to hold the temperature absolutely constant, so this will become a significant source of error to discuss.
- If you cannot buy amylase, then learners could use their own saliva as a source. Saliva can be collected in a boiling tube.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Although all enzymes can produce allergic reactions in a small minority of learners, the concentration of amylase used in this investigation is unlikely to pose any significant risk.

Carrying out the investigation

- This is a notoriously unreliable experiment. Amylase can sometimes fail to digest the starch at all, or it may do it so quickly that you cannot measure any differences in rate between the different concentrations. It is essential that you trial the experiment before asking learners to do it, to try to find suitable concentrations of enzyme and substrate.
- The starch solution should be made up with soluble starch, but some schools have problems in obtaining soluble starch that reacts appropriately with iodine in potassium iodide solution. Check that the starch solution gives a strong positive result (a strong blue–black colour) when tested with the iodine solution.
- A very common source of problems is that learners contaminate one solution with another. They may fail to clean a glass rod that has been in contact with one solution before inserting it into another. This can be so significant that it can completely prevent any useful results being obtained at all. Cleaning with paper towel may not be sufficient, particularly if your supply of paper towels contain starch (test one with iodine in potassium iodide solution to find out). It is recommended that a different glass rod is used for testing each solution, and this is washed thoroughly in clean water after each test. If paper is used for drying or cleaning the glass rods, this must be entirely starch-free.
- It is very difficult, if not impossible, to decide exactly when the iodine solution no longer gives a positive result for starch. It can be helpful to have two reference iodine solution spots – one to which a drop of starch solution has been added, and one to which a drop of water has been added.

Some candidates may have difficulty in deciding how to make up the different concentrations of amylase. See the suggestions in this section in Practical investigation 3.2.

Some learners may have difficulty in understanding how the colour of the iodine solution can be interpreted in terms of the reaction. Try using questioning to help them to work this out for themselves. For example, if they get an orange–brown colour when adding a sample to the spot of iodine, you could ask: What does this colour mean? If you had done this test before you added the enzyme, what colour would you have got? Why? So where has the starch gone? How can we tell how long the starch took to disappear? If it disappeared quickly, what does that tell us about the rate of the reaction?

Learners who complete the investigation quickly and successfully, could be asked to suggest why adding iodine solution to the reacting mixture and watching for a colour change, rather than taking samples and adding to drops of iodine solution, might not be a good

method to use. (The answer is that the presence of iodine molecules could affect the rate of the reaction.)

Common learner misconceptions

- The results of this practical investigation are less intuitive to understand than those involving catalase. For catalase, we can see the product and measure its volume. For amylase, there is no visible change when starch is hydrolysed to maltose. We begin with a colourless solution, and end with a colourless solution, with no gas being given off. The detection of disappearance of substrate (starch) involves an extra step that, for some learners, makes it difficult for them to understand exactly what is happening.

Sample results

Concentration of enzyme solution / percent	Time taken for starch to disappear / s
1.0	before the first test
0.8	60
0.6	120
0.4	120
0.2	180

Table 3.4

Answers to the workbook questions (using the sample results)

a

Concentration of enzyme solution / percent	Time taken for starch to disappear / s	Rate of reaction / 1000 ÷ time
1.0	before the first test	not measurable
0.8	60	16.7
0.6	120	8.3
0.4	120	8.3
0.2	180	5.6
0.0	did not disappear	0.0

Table 3.5

b

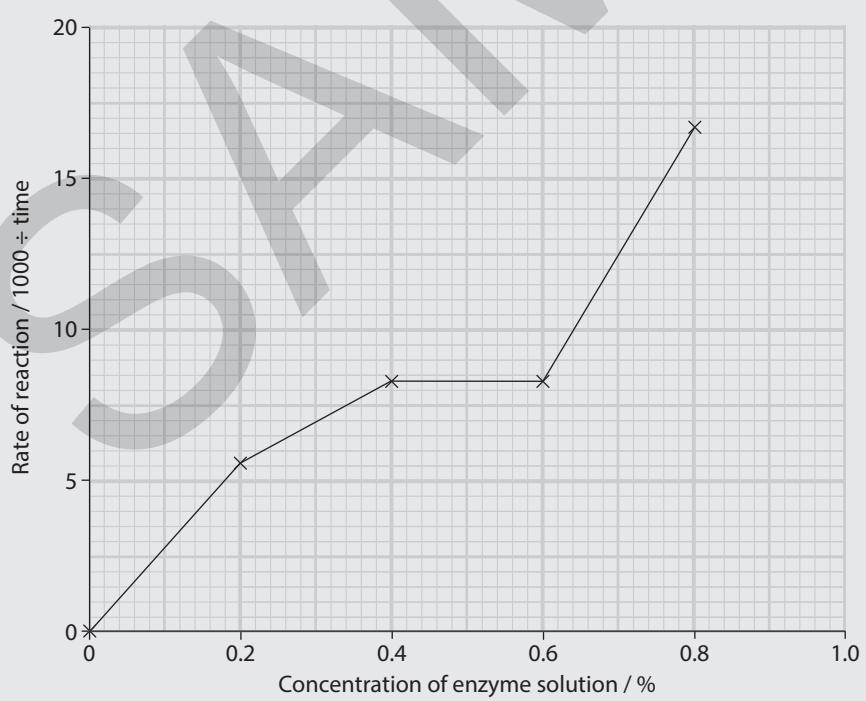


Figure 3.7

c The greater the enzyme concentration, the greater the rate of reaction. (Learners may find that at very high enzyme concentrations the rate of reaction does not increase any further as enzyme concentration increases.)

d The greater the concentration of enzyme, the greater the chance of a starch molecule colliding with an active site of an amylase molecule. The more frequent these collisions, the faster the rate of formation of enzyme–substrate complexes, and the faster the rate of reaction.

(Note that it is the **frequency** of collisions that is important, not the **number** of collisions.)

e Temperature, the concentration of substrate. Learners may also mention pH, even though this has not been controlled.

f, g Learners will probably 'reuse' sources of error from Practical investigations 3.1, 3.2 and 3.3. Take care that they select sources that are applicable to **this** investigation and that, where necessary, they reword them.

Possible answers include:

- Apparatus used for measuring volume may be inappropriate. For example, if a measuring cylinder or syringe was used, modify by using a graduated pipette.
- pH could fluctuate during the reaction; modify by add the same volume of the same buffer solution to each reacting mixture.
- Samples were taken at only 1-min intervals, so we cannot judge the time for the starch to disappear any more precisely than to the nearest minute (this can explain the same time being recorded for two different concentrations of amylase in the sample results); modify by taking samples more frequently.
- Judgement of the colour of the iodine solution; modify by comparing against a set of standards or a colour chart.

h Make sure that answers relate to *this* investigation, and do not suggest testing other enzymes or other variables.

- Do three repeats for each enzyme concentration, and calculate a mean value for the time taken for the starch to disappear. (Note: it is not enough just to do repeats; a mean must also be calculated.)
- Use a wider range of enzyme concentrations, and more intermediate values (that is, smaller intervals of the independent variable), to obtain more points on the graph and therefore a clearer picture of its shape.

i In this reaction, the product is maltose. There is no quick way of measuring its production – we would need to do a quantitative Benedict's test, which is time-consuming. It is much easier to measure the disappearance of starch, as this can be quickly and simply tested for using iodine in potassium iodide solution.

In the catalase reaction, the product is oxygen, which is a gas and is therefore given off from the reaction vessel and can be collected and measured easily. It would not be easy to measure the disappearance of the substrate, as there is no easy test for hydrogen peroxide.

Practical investigation 3.4:
The effect of temperature on the rate of an enzyme-catalysed reaction

Learning objective: 3.2(a)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (b), (e), (f), (g), (h), (i), (j) Successfully collecting data and observations: (c)
PDO	Recording data and observations: (a), (b), (c) Layout of data or observations: (b), (c), (d)
ACE	Interpreting data or observations and identifying sources of error: (e), (f) Drawing conclusions: (a), (b) Suggesting improvements or modifications to extend an investigation: (d), (e)

Duration

The practical work will take between 60 and 75 min. The analysis and evaluation questions will take around 30 min. Please see also the section *Carrying out the investigation*, for the possible effect of group size on the duration of the investigation.

Preparing for the investigation

- Learners will find it very helpful to have done Practical Investigations 3.1, 3.2 and 3.3 before this one. This will give them experience of the reaction and methods of measuring the rate of production of oxygen, and also how to use water baths to maintain a chosen temperature. They should know how to make up a range of concentrations by dilution, and be able to identify random and systematic errors.
- Learners are asked to suggest their own values for the independent variable, temperature. However, if you are using thermostatically-controlled water baths, they will not have a choice about this. You could involve the class in discussing the temperatures at which they would like the water baths to be set.

Equipment

Each learner or group 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
- an 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.

Additional notes and advice

- If you do not have thermostatically controlled water baths, learners can make their own. They should use a large beaker of water, and adjust the temperature by adding ice or hot water from a kettle. Temperatures above room temperature can be maintained by placing the beaker on a tripod and gauze above a heat source (Bunsen burner or spirit burner) and constantly adjusting the heat to try to maintain a constant temperature.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.

- Standard laboratory safety procedures should be followed always.
- Learners should take care when using sharp blades to cut the celery stalks.
- Hydrogen peroxide 20 volume or 10 volume irritates eyes and skin. Safety glasses should be worn throughout. Hydrogen peroxide decomposes slowly even with no catalyst present, so oxygen gas may build up in a bottle. Store in a cool, dark place and take care when opening.
- If the gas is given off very quickly, the plunger in the gas syringe can shoot out of the syringe barrel with some force, and travel quite a distance. To avoid this, it is important to tie the plunger loosely to the barrel, so that if it does shoot out, it cannot travel far.
- Some of the water baths will need to be at quite high temperatures, for example 80°C. If learners are using beakers on tripods as water baths, they should make sure that these are standing well back from the edge of the bench.

Carrying out the investigation

- Please see the issues described for Practical investigation 3.1.
- If you do not have thermostatically-controlled water baths, it can be difficult for learners to maintain the temperature they want in their water baths. There is no easy solution to this; they should discuss it as a significant source of experimental error.
- If many groups are sharing the same thermostatically-controlled water baths, a careless learner in one group may disrupt another group's apparatus. All groups should also label their apparatus using a waterproof marker, to avoid confusion.
- If learners are working in pairs, they may need to work with one temperature at a time rather than trying to run the experiments for each temperature simultaneously. This means that a longer time will be needed to carry out the investigation. If they are in larger groups, they may be able to organise themselves so that different members of the group work on different temperatures, but there is then a risk that different individuals will do something slightly different that may affect their results (in other words, they are introducing an uncontrolled variable).

This is the first task in this book in which learners are asked to plan part of their method. They are greatly helped by having used the technique before, but still may feel insecure in deciding on the temperatures to use (Steps 1 and 2). Use questioning to help them with this, for example ask: What is the highest temperature you can get using a water bath? What is the lowest temperature you can get? What do you think the optimum temperature of the enzyme might be? So which five/six temperatures will you investigate?

They may also need help in deciding their control variables (Step 5). Again, use questioning to support them. Ask: What is your independent variable in this investigation? What other variables do you know about, that affect the rate of an enzyme-catalysed reaction? So which other variables do you need to try to keep constant?

Learners who complete the investigation quickly and successfully could carry out their suggested method for investigating the effect of pH on the activity of catalase (see question **e**). This would be a useful activity, as it provides an opportunity to become familiar with the use of buffer solutions.

Common learner misconceptions

- Learners may expect the catalase to have an optimum temperature of 37°C. This is unlikely to be the optimum temperature for an enzyme extracted from a plant; their optimum temperatures are often significantly lower than this.

Sample results

The volume of oxygen was collected after 2 min.

Temperature / ˚C	Volume of oxygen collected at 120 s / cm^3
0	0.5
20	12.5
40	10.5
60	6.5
80	1.0

Table 3.6

Answers to the workbook questions (using the sample results)

a

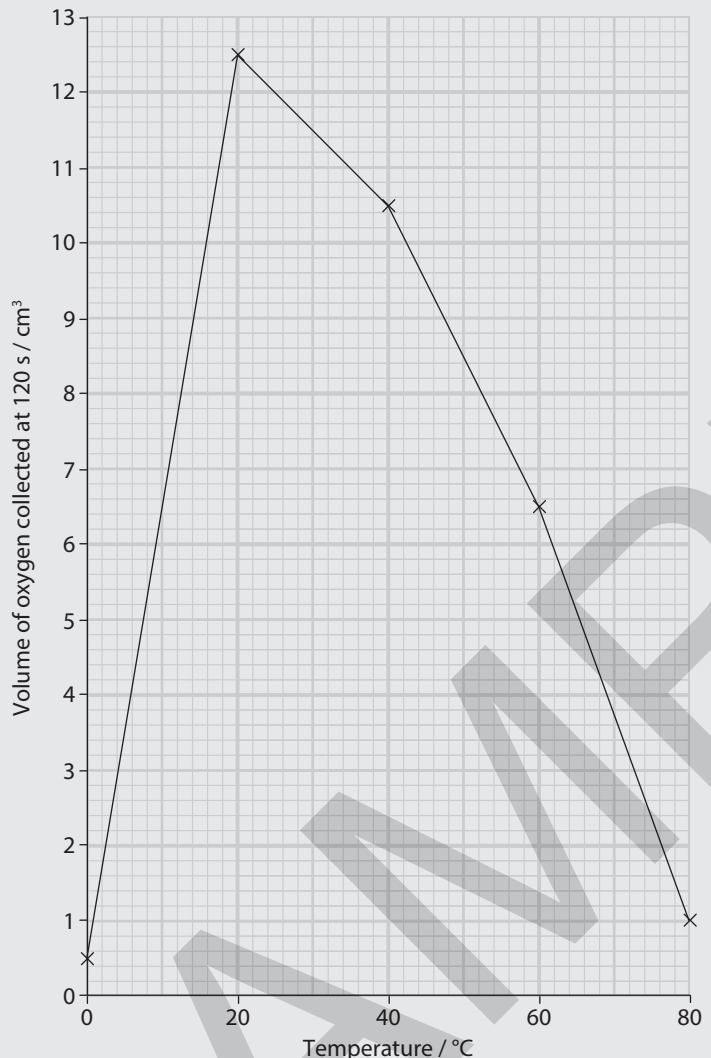


Figure 3.8

b The conclusion should be a concise statement relating temperature to rate of reaction. For example, for the sample results, the conclusion could be:

Increasing temperature from 0 to 20°C causes an increase in the rate of reaction. The optimum temperature for this enzyme lies somewhere between 0 and 40°C. Increasing the temperature above 40°C causes a decrease in the rate of reaction.

Notice that, although the maximum volume of oxygen was collected at 20°C, we do not know if more would have been collected at a temperature below this or above it. We cannot determine the optimum temperature of the enzymes, only say that it lies somewhere between 0 and 40°C.

c As temperature increases from 0 to 20°C, the kinetic energy of the catalase molecules and hydrogen peroxide molecules increases. This increases the frequency of collisions of hydrogen peroxide molecules with the active site of catalase, and therefore increases the rate of reaction.

Above 40°C, the high temperatures cause breakage of hydrogen bonds within the catalase molecule, disrupting the shape of the active site and making it less likely that hydrogen peroxide can bind with it. At the highest temperatures, all the catalase molecules are denatured, so no hydrogen peroxide can bind and no reaction takes place.

d The learners' results will have given them a range of temperatures between which the optimum temperature must lie. They could do further experiments using temperatures within this range. For the sample results, temperatures of 5, 10, 15, 25, 30 and 35°C could be trialled.

e A range of buffer solutions should be made up, using at least five values – for example, pH 2, 4, 6, 8 and 10. Temperature should be kept constant by standing the tube containing the hydrogen peroxide and catalase in a water bath at a temperature close to the optimum for the enzyme. Concentration and volume of enzyme and substrate should be kept constant, as the learners have just done in their investigation into the effect of temperature.

Practical investigation 3.5:
Immobilising urease

Learning objective: 3.2(d)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (g) Successfully collecting data and observations: (a), (c)
PDO	Recording data and observations: (a), (b)
ACE	Drawing conclusions: (b)

Duration

The practical work will take between 40 and 45 min. The analysis and evaluation questions will take around 30 min.

Preparing for the investigation

- Check the activity of the urease, to ensure that it does produce sufficient ammonia to give a measurable change in pH when added to urea. A 0.6% solution of urease is suggested below, but you may need to use a more or less concentrated solution.
- The sodium alginate solution should be made up beforehand. Follow any instructions with the sodium alginate powder to dissolve it. It may need warming, and will need stirring. It can take some time to dissolve; if you have a magnetic stirrer and a hot plate this will help considerably. The solution will be quite jelly-like, but must be sufficiently liquid to be able to be picked up in a dropper pipette; dilute it with water if necessary.
- Learners may like to know that urease breaks down urea to ammonia and carbon dioxide:

$$CO(NH_2)_2 + H_2O \longrightarrow 2NH_3 + CO_2$$

Ammonia dissolves in water to produce ammonium hydroxide, which is a strong alkali. Although carbon dioxide dissolves to produce a weak acid, the overall effect is an increase in alkalinity – that is, an increase in pH.

Equipment

Each learner or group 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 (this can often be obtained more cheaply from suppliers of cooking ingredients than from educational suppliers)
- about 100 cm^3 of 3% calcium chloride solution
- a timer (e.g. on a phone)
- an apparatus for measuring small volumes, e.g. 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.

Additional notes and advice

- If you cannot buy urease, you can extract it from dried soya beans. Soak the beans in water overnight and then liquidise them in an electric blender (or use a pestle and mortar). Filter; the enzyme will be present in the filtrate. Fresh soya beans can also be used. If you use beans, test the extract to determine an appropriate concentration to use in this experiment.
- A tea strainer is a small piece of kitchen equipment used to strain drinks such as tea (Figure 3.9). If you do not have one, you can use muslin or other cloth to separate the beads of jelly from the liquid.

Figure 3.9

- If you do not have pH probes, you can use strips of Universal Indicator paper to measure pH.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Although all enzymes can produce allergic reactions in a small minority of learners, the concentrations of urease used in this investigation are unlikely to pose any significant risk.

Carrying out the investigation

- It is important to ensure that the enzyme–alginate mixture is as uniform as possible, so that all beads contain the same concentration of urease. As previously described, make sure that it is sufficiently liquid to use in a dropper pipette.
- Learners may have difficulty in producing beads of similar sizes, although most are able to do this with a little practice.
- The jelly beads are quite fragile, and may be broken up if learners handle them too vigorously.
- The pH probes need to be calibrated shortly before the practical begins. Make sure that each probe is carefully cleaned between measuring the pH in one solution and the next. Even a tiny quantity of urease that has contaminated the probe will be able to break down urea that the probe later comes into contact with.
- If the urea solution runs through the column of beads too quickly, or if the spaces between the beads are too large, then not enough urea solution will come into contact with the urease in the beads, and only a little ammonia will be produced. This can be remedied by packing the beads more tightly (smaller beads pack better than large ones) and/or by reducing the rate of flow through the column by partly closing the clamp over the rubber tubing.

Learners who complete the investigation quickly and successfully could plan and carry out an investigation to try to answer one of the following questions:

- Does the activity of the urease in the beads remain relatively constant, or does it decrease as successive batches of urea solution are poured through the beads?
- When beads containing urease are added to urea solution, the gases produced form bubbles on the beads. These bubbles can cause the beads to rise to the surface. The faster the production of bubbles, the faster the beads rise. Use this information to devise a way in which the beads could be used to find the concentration of a urea solution.

Note that, for the second question above, it would be important to test the technique before doing the experiment. Carbon dioxide is a soluble gas and relatively dense, and if the beads themselves are also dense (as may happen if the sodium alginate solution is very thick) there may not be sufficient carbon dioxide adhering to them to cause a reduction in density below that of the urea solution – so the beads may not rise at all.

Sample results

Initial pH of urea solution	pH of urea solution after passing through the column of beads
7.7	9.0

Table 3.7

Answers to the workbook questions (using the sample results)

a The pH of the urea solution rose from 7.7 to 9.0 after passing through the beads. This happened because the urease broke down the urea, releasing ammonia which formed an alkaline solution and caused the pH to increase.

b It is likely that learners will find that some bubbles are formed, indicating that there is still urea present. Not all of the urea in the solution has been broken down by the urease in the beads, perhaps because not all of the urea molecules came into contact with the active site of a urease molecule.

Practical investigation 3.6: Investigating the effect of an inhibitor on the action of bromelain

Learning objective: 3.2(a)

Skills focus

The following skill areas are developed and practised (see the skills grids at the front of this guide):

MMO	Making decisions about measurements: (g), (j) Successfully collecting data and observations: (a)
PDO	Recording data and observations: (a), (b), (c) Layout of data or observations:
ACE	Interpreting data or observations and identifying sources of error: (f), (g) Drawing conclusions: (b) Suggesting improvements or modifications to extend an investigation: (a), (b), (c), (e)

Duration

Making the copper sulfate solutions and setting up the Petri dishes will take about 45 min. You could reduce this time by providing learners with ready-made copper sulfate solutions.

Learners will need to return later on the same day, or the next day, to measure the diameters of the wells and record their results.

Preparing for the investigation

- Learners should have knowledge of enzyme inhibitors. Copper sulfate is a non-competitive inhibitor.
- The jelly (gel) for the Petri dishes should be made up the day before, and kept in a refrigerator until used. The jelly should be of a similar consistency to agar jelly – sufficiently solid to be able to cut wells that hold their shape. If it is very solid, however, this will slow down the digestion of the protein and it will take longer to obtain results. It is important to use jelly that is based on gelatin, not on agar (which is not a protein). In most countries, cubes or powder can be bought to make edible jellies – the packet should indicate if this contains gelatin. Use a strongly-coloured jelly if possible, as this makes it easier to measure the diameters of the wells.

  (Note: in some countries, the word 'jelly' refers to a fruit preserve that can be spread on bread. This is ***not*** what is intended here.)
- The pineapple extracts should also be made up beforehand. Cut some fresh pineapple into chunks, liquidise, and filter through two layers of muslin. Take a small quantity of this fresh extract and boil, then cool.
- You should trial the investigation, to determine a suitable time for which to leave the dishes after learners have set up their experiment. Depending on the activity of the enzyme in the pineapple extract, and the ambient temperature, you might get results in as little as 1 h. It could, however, take considerably longer than this.

Equipment

Each learner or group will need:

- about 50 cm^3 of an extract made from fresh pineapple (see above)
- a small quantity of boiled pineapple extract
- five small Petri dishes containing coloured jelly, made using gelatin (see above)
- about 10 cm^3 of 1 mol dm^{-3} copper sulfate solution

- a cork borer
- a ruler to measure in mm.

Additional notes and advice

- If you do not have small Petri dishes, learners could use large ones and make several wells in each one. However, this does mean it is possible for wells to merge if the bromelain is very active and digests a lot of protein quickly.

Safety considerations

- Learners should have read the safety guidance section within the workbook before carrying out this investigation.
- Standard laboratory safety procedures should be followed always.
- Copper sulfate solution should be washed off with cold water if it gets onto hands or clothes.

Carrying out the investigation

- This is a relatively reliable investigation, as long as you are successful in making a suitable jelly.
- Learners may find it difficult to cut the wells neatly. You could provide a few spare dishes of jelly for them to practice. Twisting the cork borer slightly as you lift the core of jelly out can often work well.
- They may also have difficulty in deciding where the edge of the well is, when measuring its diameter. There is no easy fix for this, so it will need to be noted as a source of error.

Some learners may still not be confident in using serial dilution to make up solutions, although by now they will probably have done so on several occasions. You could provide a help sheet for them if needed.

Alternatively, ask learners to refer to the Practical Workbook, Figure 2.2. You may also like to refer to Figure 3.4 on page 23 in this Teacher Guide.

Learners who would benefit from a challenge could plan an investigation to compare the activity of bromelain from pineapple with proteases found in other fruits (e.g. pawpaw, kiwi fruit or figs). If time allows, they could carry out their planned investigation.

Sample results

Wells were cut with an 8 mm diameter cork borer.

0.8 cm^3 of pineapple extract and three drops of water or copper sulfate solution were added to each well.

Pineapple extract	Copper sulfate solution concentration / mol dm^{-3}	Diameter of well after 2 h / mm
boiled	0	8
fresh	0	37
fresh	0.001	16
fresh	0.01	9
fresh	0.1	8
fresh	1.0	8

Table 3.8

Answers to the workbook questions (using sample results)

a The wells containing boiled pineapple extract, and the fresh extracts to which 0.1 and 0.01 mol dm^{-3} copper sulfate solution had been added, were unchanged after 2 h.

The well containing fresh pineapple extract had increased in diameter to 37 mm, an increase of 25 mm. The well containing fresh pineapple extract and 0.001 mol dm^{-3} copper sulfate solution had also increased in diameter, but only by 8 mm, while the well containing fresh pineapple extract and 0.01 mol dm^{-3} copper sulfate solution had increased very slightly to 9 mm diameter.

b The wells increased in diameter because protease enzymes in the pineapple extract digested the protein in the jelly.

The enzyme in the boiled extract was denatured by boiling – the hydrogen bonds in the enzyme molecules were broken, so the active site lost its shape and could no longer form complexes with protein molecules. The protein around the well containing boiled extract was therefore not digested.

Copper sulfate has acted as an inhibitor. Even the lowest concentration of 0.001 mol dm^{-3} has reduced the activity of bromelain. Concentrations of 0.1 mol dm^{-3} appear to have completely prevented its activity, although it would be interesting to see if any activity did occur if the dishes were left for longer.

Copper ions act as non-competitive inhibitors. They bind permanently with the enzyme at a position other than the active site, distorting the 3D shape of the enzyme, so that its active site is no longer a complementary shape to the protein molecules that normally act as a substrate.

c The most significant source of error is likely to be the measurement of the diameter of the wells. The edges are often not clear-cut, and the wells and the digested areas may not be absolutely circular. Learners are likely to have had to make estimates of the diameters rather than precise measurements.

Another source of error is the volume of extract and/or inhibitor added to each well. These are very small volumes, and it is not easy to measure these accurately. Even small variations could affect results.

d The most obvious way to improve the investigation would be to use repeats. Three or five sets of dishes could be set up, and a mean diameter calculated for each well. This would allow any anomalous results to be detected more easily.