Cambridge Assessment International Education

Practical Booklet 1
Measuring cell size

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To prepare temporary slides of plant tissue to measure the size of the cells using an eyepiece graticule and stage micrometer.

Outcomes

Syllabus learning objective 1.1 (c)

use an eyepiece graticule and stage micrometer scale to measure cells and be familiar with units (millimetre, micrometre, nanometre) used in cell studies

Skills included in the practical

AS Level skills	How learners develop the skills
MMO collection	Using different methods to measure the size of cells
PDO recording	Recording quantitative data in a table
ACE analysis	Calculating a mean
PDO display	Showing all the steps in their calculations
ACE evaluation	Deciding which method provides the most accurate results and therefore develop an understanding of how modifying a procedure can increase accuracy

Method

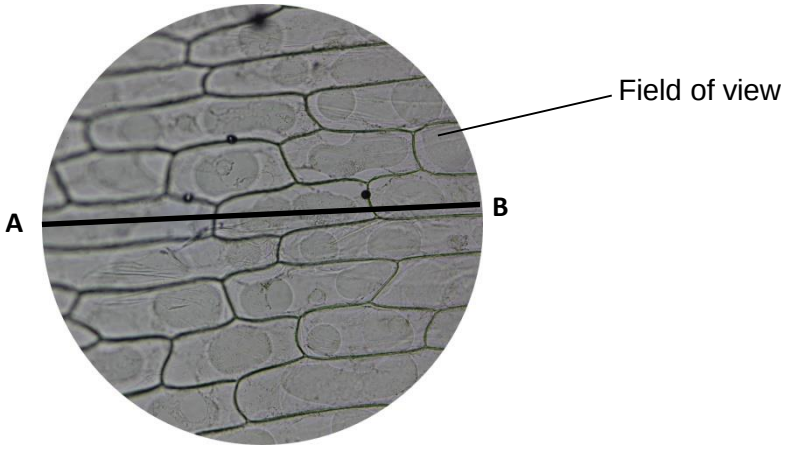
In preparation for this practical learners should have a basic understanding of how to use a light microscope. There are two experiments to measure the size of onion cells under a light microscope, the first using a ruler, the second using a stage micrometer and eye piece graticule.

Safety glasses must be worn when preparing the slide.

Experiment 1: Measuring the size of onion cells using a ruler on the stage.

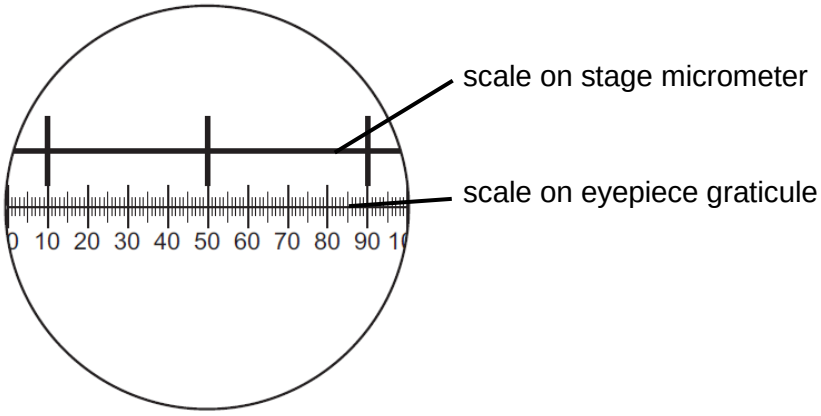
- Learners make slides of onion epidermis stained with iodine and view these under a light microscope using low power. They can identify the cell wall and other visible organelles such as the nucleus.
- They will count the number of cells across the field of view of their microscope from one side to the other. For example between points **A** and **B** on the photomicrograph below.
- This exercise should be repeated a number of times, viewing different areas of the slide to obtain an average number of cells.
- The slide is removed from the microscope and a transparent ruler is now placed onto the stage of the microscope. Learners will measure the diameter of the field of view in mm, using the same magnification that was used to view the cells.

Guidance for teachers, *continued*



Experiment 2: Measuring the size of onion cells using a stage micrometer and eye piece graticule.

- Learners will now use an eyepiece graticule to measure the length of the cells.
- A stage micrometer should be used to calibrate the eyepiece graticule. This can be done as explained in the following example.



- The diagram shows a stage micrometer, with divisions 0.1 mm apart, viewed through an eyepiece containing a graticule. There are 40 divisions of the eyepiece graticule in every division of the stage micrometer, so each division of the eyepiece graticule is 0.0025 mm or 2.5 μm.
- The eyepiece graticule can then be used to measure individual cells in the field of view. Learners need to sample the slide and take measurements from different areas of the slide. The need for a large sample size to should also be emphasised.

Guidance for teachers, *continued*

Results

Experiment 1: Measuring the size of onion cells using a ruler on the stage.

- The raw results from the experiment are recorded in a table.

Sample	Number of cells across the diameter of the field of view
1	
2	
3	
4	
5	

Experiment 2: Measuring the size of onion cells using a stage micrometer and eye piece graticule.

- The raw results from the experiment are recorded in a table. At this stage, the units are in epu (eyepiece graticule units).

Sample	Length of cell / epu
1	
2	
3	
4	
5	

Interpretation and evaluation

Experiment 1: Measuring the size of onion cells using a ruler on the stage.

- Learners can calculate the length of one onion cell by dividing the diameter of the field of view by the number of cells they have counted.
- The length of the onion cells can be converted from millimetres into micrometers which is a more suitable unit of measurement for cell studies.

Sample	Number of cells across the diameter of the field of view	Length of 1 cell / mm	Length of 1 cell / μm
1			
2			
3			
4			
5			

- This method is inaccurate as it is based on the assumption that each onion cell is the same length – this can be discussed. This error can be reduced by collecting a large number of results and calculating a mean length of the onion cells. This could be done as a class activity. Learners should be encouraged to show how they calculated the mean length by showing every step in the calculation.
- The idea of raw results (the number of cells and the diameter of the field of view) and processed results (the length of the cells and the mean length) can be introduced.

Guidance for teachers, *continued*

Experiment 2: Measuring the size of onion cells using a stage micrometer and eye piece graticule.

- Learners use the calibration of their eyepiece graticule to calculate the actual length of each cell. They should be asked to show all the steps in one of their calculations.

Sample	Length of cell / epu	Length of cell / μm
1		
2		
3		
4		
5		

- The two methods used to measure the length of the cells can be evaluated and a conclusion drawn about which method provides the most accurate results.
- The eyepiece graticule method is not reliant on the assumption that all the cells are equal size, and will almost certainly confirm that this assumption is incorrect, and therefore it is a more accurate method. The divisions on the eyepiece graticule are finer than those of the ruler allowing more accurate results to be collected.

Information for technicians

Each learner will require:

- Microscope with eyepiece graticule inserted
- 1 × piece of onion
- 1 × white tile
- 1 × knife
- 1 × forceps
- 1 × glass slide
- 1 × cover slip
- iodine in potassium iodide solution, labelled **iodine solution [H]**
- 1 × dropping pipette (teat)
- 1 × piece of filter paper or paper towel
- 1 × mounted needle
- 1 × transparent ruler
- 1 x stage micrometer
- Safety glasses

Hazard symbols

C = corrosive substance

F = highly flammable substance

H = harmful or irritating substance

O = oxidising substance

N = harmful to the environment

T = toxic substance

Worksheet

Aim

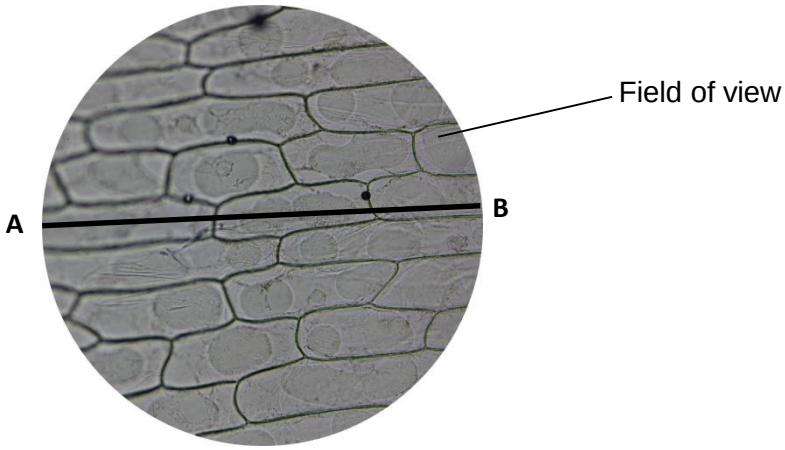
To prepare temporary slides of plant tissue in order to measure the size of the cells using an eyepiece graticule and stage micrometer.

Method

Safety glasses must be worn when preparing the slide.

Experiment 1: Measuring the size of onion cells using a ruler on the stage.

1. Prepare a slide of onion epidermis stained with iodine solution.
2. View the slide under a light microscope using low power.
3. Count the number of cells observed across the field of view from one side to the other. For example, on the photomicrograph below, this would be between points **A** and **B**. Record your results.

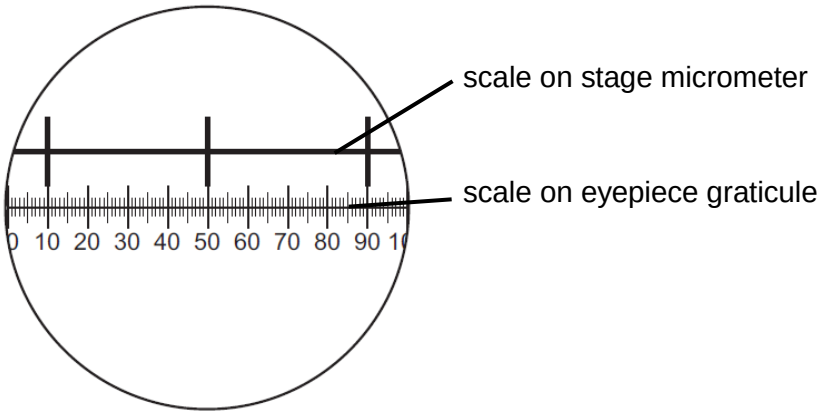


4. Repeat step **3** several times, viewing different areas of the slide to obtain a mean number of cells.
5. Remove the slide from the microscope and put to one side. You will need it during Experiment 2.
6. Place a transparent ruler onto the stage of the microscope.
7. Use the ruler to measure the diameter of the field of view in mm. Record your result. Ensure the same magnification is used as in Experiment 1.

Experiment 2: Measuring the size of onion cells using a stage micrometer and eye piece graticule.

8. Place the stage micrometer on the stage of the microscope. You must calibrate the stage micrometer using the eyepiece graticule. Use the example below to help you calibrate your micrometer.

Worksheet, *continued*



The diagram shows a stage micrometer, with divisions 0.1 mm apart, viewed through an eyepiece containing a graticule. There are 40 divisions of the eyepiece graticule in every division of the stage micrometer, so each division of the eyepiece graticule is 0.0025 mm or 2.5 μm.

9. Remove the stage micrometer from the microscope.
10. Place your prepared slide back onto the stage of the microscope. Use your eyepiece graticule to measure the length of an individual cell. Record your result.
11. Repeat step **10** several times, viewing different areas of the slide to obtain a mean length of cell.

Results

Record your results in the tables provided.

Experiment 1: Measuring the size of onion cells using a ruler on the stage.

Sample	Number of cells across the diameter of the field of view
1	
2	
3	
4	
5	

Diameter of field of view _____ mm

Worksheet, *continued*

Experiment 2: Measuring the size of onion cells using a stage micrometer and eye piece graticule.

Sample	Length of cell / epu
1	
2	
3	
4	
5	

Interpretation and evaluation

Experiment 1: Measuring the size of onion cells using a ruler on the stage.

1. Calculate the length of one onion cell by dividing the diameter of the field of view by the mean number of cells you observed. Convert your answer from mm to μm. Record your results in the table below.

Sample	Number of cells across the diameter of the field of view	Length of 1 cell / mm	Length of 1 cell / μm
1			
2			
3			
4			
5			

2. Is this an accurate method for measuring the length of onion cells?
3. Pool your results with the rest of your group and calculate a mean length of onion cell.

Experiment 2: Measuring the size of onion cells using a stage micrometer and eye piece graticule.

4. Use the calibration of your eyepiece graticule to calculate the actual length of each cell. Show all the steps in one of your calculations. Record your results in the table below.

Sample	Length of cell / epu	Length of cell / μm
1		
2		
3		
4		
5		

5. Which method provides the most accurate result? Evaluate both methods.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 2

Testing for biological molecules

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To use tests for biological molecules to determine which molecules are present in solutions of unknown composition.

Outcomes

Syllabus sections 2.1 (a) and (b)

Skills included in the practical

AS Level skills	How learners develop the skills
MMO decisions	Decide which tests should be used to identify the biological molecules present in unknown solutions
MMO collection	Complete a risk assessment Make qualitative observations of colour changes Collect quantitative results, time for a colour change
PDO recording	Record qualitative observations and quantitative results in appropriate tables
ACE conclusions	Conclude the composition of each unknown solution from their results
MMO decisions	Decide which tests should be used to identify the biological molecules present in unknown solutions

Method

Safety glasses must be worn when preparing the slide.

- In preparation for this practical, learners need to be familiar with the tests for biological molecules outlined in sections 2.1 (a) and (b) of the syllabus.
- The method for each of the tests should be reviewed before the practical begins.
- Learners are provided with five beakers labelled **A**, **B**, **C**, **D** and **E,** each containing a different solution.
- The solutions are as follows:

Solution label	Contents
A	1.0% sucrose solution
B	0.5% glucose solution
C	1.0% starch solution
D	4.0% glucose solution
E	5.0% sucrose solution

- Learners are told that the five solutions lost their content labels and were muddled up. Their task is to identify the composition of each solution.
- They are told that the solutions are as follows:
    - 5.0% sucrose solution
    - 1.0% sucrose solution
    - 1.0% starch solution
    - 4.0% glucose solution
    - 0.5% glucose solution

Guidance for teachers, *continued*

- A risk assessment identifying the hazards in the method must be carried out by each learner and they should assign an appropriate level of risk to the investigation. This will allow the teacher to ensure that the planned method is safe. The risk level is **medium** as learners will be using chemicals that are harmful. Safety glasses must be worn when carrying out this investigation.
- The learners will need to decide in which order to carry out the tests. They will also need to develop a method for each test. These activities can be done as a class activity prior to the practical.
- A suggested order would be to test all five solutions for starch using iodine in potassium iodide solution. This will allow the identification of the starch solution. The test for reducing sugars could then be carried out to identify the glucose solutions, and the time for the first appearance of a colour change recorded to enable identification of the more concentrated glucose solution. Finally the non-reducing sugar test can be carried out on the two sucrose solutions. If learners record the time taken for the first appearance of a colour change when performing the Benedict's test after hydrolysis, they should be able to distinguish between the two different concentrations of sucrose.

Results

- Learners should have prepared a table similar to the one below. They should be reminded that this is an appropriate results table because the:
  - table has been drawn with lines separating each of the columns and rows
  - independent variable is in the first column
  - table has descriptive column headings
  - units are included in the column headings, not next to each result recorded in the table.

Solution	Colour with iodine in potassium iodide solution	Colour with Benedict's solution	Time taken for colour change with Benedict's solution / s	Colour with Benedict's solution after hydrolysis	Time taken for colour change with Benedict's solution after hydrolysis / s
A					
B					
C					
D					
E					

Interpretation and evaluation

- Learners are asked to identify the contents of each solution by completing a table.

Solution label	Contents
A	
B	
C	
D	
E	

Extension

The need for reliable results and the importance of taking repeat results could be discussed. If time allows this could be carried out.

Information for technicians

Each learner will require:

- safety glasses
- at least 30 cm^3 5.0% sucrose solution, labelled **E**
- at least 30 cm^3 1.0% sucrose, labelled **A**
- at least 30 cm^3 4.0% glucose solution, labelled **D**
- at least 30 cm^3 0.5% glucose solution, labelled **B**
- at least 30 cm^3 1.0% starch solution, labelled **C**
- 50 cm^3 Benedict's solution **[H]**
- at least 10 cm^3 iodine in potassium iodide solution, labelled **iodine solution [H]**
- at least 20 cm^3 of 1 mol dm^{-3} hydrochloric acid [H]
- sodium hydrogen carbonate
- 1 × spotting tile
- 1 × glass rod or dropping pipette
- 2 × 5 cm^3 syringes and the means to wash them out
- 5 × large test-tubes (boiling tubes)
- 1 × test-tube rack to hold large test-tubes
- 1 × Bunsen burner
- 1 × tripod
- 1 × gauze
- 1 × bench mat
- at least 400 cm^3 beaker with water suitable for a water-bath (at approximately 40 – 45 ^oC)
- 1 × thermometer, 0 ^oC to 110 ^oC
- 1 × stop clock
- 1 × glass marker pen

Additional instructions

The sodium hydrogen carbonate should be provided with a spatula or an alternative that learners usually use in the test for non-reducing sugars.

Hazard symbols

C = corrosive substance

F = highly flammable substance

H = harmful or irritating substance

O = oxidising substance

N = harmful to the environment

T = toxic substance

Worksheet

Aim

To use tests for biological molecules to determine which molecules are present in solutions of unknown composition.

Method

Safety glasses must be worn when preparing the investigation.

1. You are provided with five beakers labelled **A**, **B**, **C**, **D** and **E**, each containing a different solution. The labels showing the contents of the beakers have fallen off and the beakers have been muddled up.
2. Your task is to plan an investigation using tests for biological molecules to decide what the content of each beaker is.
3. The solutions are as follows:
    - 5.0% sucrose solution
    - 1.0% sucrose solution
    - 4.0% glucose solution
    - 0.5% glucose solution
    - 1.0% starch solution
4. You must carry out a risk assessment identifying the hazards in your method. You should assign an appropriate level of risk to the investigation.

Results

Record your results in an appropriate table. When drawing a results table remember that you should:

- put the independent variable in the first column
- use descriptive column headings
- include units in the column headings only

Interpretation and evaluation

Complete the table below identifying the contents of each solution.

Solution label	Contents
A	
B	
C	
D	
E	

Extension

How could you amend your method to increase the reliability of your results?

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 3
Investigating osmosis

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To determine the concentration of potato cell content using sucrose solutions of known concentration.

Outcomes

Syllabus section 4.2 (e)

Skills included in the practical

AS Level skills	How learners develop the skills
MMO decisions	Carry out a simple dilution of the 1 mol dm^{-3} sucrose solution
MMO collection	Take quantitative readings of the mass of potato cylinders using a balance
PDO recording	Record quantitative results appropriately in a table
PDO display	Show all steps in the calculation of percentage change in mass
PDO layout	Draw a graph of concentration of sucrose solution against percentage change in mass using processed data
ACE analysis	Find the zero percentage change in mass from the graph drawn
ACE conclusions	Explain the trend shown by the graph and the reason why zero percentage change in mass shows the sucrose concentration with the same water potential as the potato cells

Method

- Learners will need an understanding of the process of osmosis and the concept of water potential before carrying out this practical. It is important that they understand how the net movement of water molecules into or out of a cell is affected by the water potential gradient between the cytoplasm of the cell and its environment. They should also have been taught that when the water potential of the cytoplasm in a cell is equal to that in its environment there will be no net movement of water molecules by osmosis.
- Learners are supplied with a 1 mol dm^{-3} sucrose solution. They are asked to carry out a simple dilution to produce 6 concentrations between 1 mol dm^{-3} and 0 mol dm^{-3}. They will need 25 cm^3 of each concentration. This task gives learners the opportunity to make decisions about which concentrations they should select and the volumes of sucrose solution and distilled water that they need in order to make these solutions. The solutions can be made directly into test-tubes which have been labelled by the learner.
- Learners are asked to cut 6 cylinders from a fresh potato; each should be 5 cm long. This could be done using a cork borer or chips could be made using a sharp knife. It is important that the surface area of the potato cylinders is constant and that the potato skin has been removed.
- The potato cylinders should be gently blotted dry with a paper towel. This will remove any liquid which has leaked out of cells that were damaged when the cylinders were cut.
- The learners will measure the mass of a potato cylinder. The mass should then be recorded in their results table. The potato cylinders are unlikely to start at the same mass so it is important that the starting mass of each potato cylinder is recorded next to the appropriate concentration of sucrose solution into which the potato will be placed. This will allow the starting mass of a cylinder to be compared to the final mass of the same cylinder after it has been soaked in a sucrose solution.

Guidance for teachers, *continued*

- Each potato cylinder should be put into the appropriate sucrose solution using forceps. The learner should place a bung into each test-tube. The potatoes should then be left to soak for one hour. The importance of the bung can be discussed with learners. It prevents evaporation which could cause a change in concentration of the solution. In this experiment this would be unlikely to be a significant error as the tubes are only left for an hour. However, if they were to be left overnight, the error due to evaporation would become more significant.
- After an hour the potatoes should be removed from the solutions. Learners should gently blot each potato dry. This is important as it removes the solution that is clinging to the outside of the potato cylinder. If blotting is not done then this would be an error as the volume of solution on the outside of the cylinder would vary between cylinders. The mass is being recorded to give an indication of the volume of water in the cytoplasm of the cells in the potato.
- Learners will then measure the final mass of the cylinders. Again it is important that the final mass of each cylinder is recorded in the table next to the concentration of solution that the potato was removed from.

Results

Learners should have prepared a table similar to the one below. They should be reminded that this is an appropriate results table because the:

- table has been drawn with lines separating each of the columns and rows
- independent variable is in the first column
- column headings are descriptive
- units are included in the column headings, not next to each result recorded in the table.

concentration of sucrose solution / mol dm^{-3}	start mass of potato cylinder / g	final mass of potato cylinder / g
0.0		
1.0		

The need to record the same number of significant figures for each measurement should be emphasised.

Interpretation and evaluation

- As each potato cylinder started with a different mass it is not possible to draw conclusions from the raw data, so it must be processed to allow the cylinders to be compared.
- Learners are asked to calculate the percentage change in mass of each cylinder and they should be encouraged to show each step in one worked example. They should draw a new table to record the processed data. The need to record the same number of significant figures for each calculation should be emphasised.

Guidance for teachers, *continued*

- The results of the experiment will be used to plot a graph to show the relationship between the concentration of sucrose solution and the percentage change in mass of the cylinder. This provides an opportunity to plot a graph which has both positive and negative values on the *y*-axis. When drawing the graph it would be helpful to re-enforce the following points:
  - the independent variable should be on the *x*-axis and the dependent variable on the *y*-axis
  - the axes should have descriptive labels, including units
  - the scale should enable more than half the graph paper to be used for plotting points
  - the points should be drawn with a sharp pencil, as a small cross or a small dot in a circle
  - the points should be connected with straight lines drawn with a ruler from the centre of each cross.
- Learners are then asked to read from their graph the concentration of sucrose solution at which the percentage change in mass of the potato cylinder is zero. This is the sucrose solution which has the same concentration as the contents of the cytoplasm within the cells in the potato cylinders.
- Learners will write a conclusion explaining the trend shown by the graph using their knowledge of osmosis and water potential. They should be able to explain why:
  - some potato cylinders gained mass
  - some potato cylinders lost mass.
- Learners should conclude that a potato with a zero percentage change in mass was in a sucrose solution with the same water potential as the potato cells.

Information for technicians

Each learner will require:

- at least 100 cm^3 1.0 mol dm^{-3} sucrose solution
- at least 100 cm^3 distilled water
- 2 × 10cm^3 syringes
- 6 × large test-tubes (boiling tubes) and bungs
- 1 × test-tube rack to hold large test-tubes
- 1 × marker pen
- 1 × potato
- 1 × cork borer
- 1 × white tile
- 1 × knife
- 1 × ruler
- 4 × paper towels
- access to a balance
- 1 × stopwatch
- 1 × glass rod
- 1 × forceps

There are no specific hazards for this investigation.

Worksheet

Aim

To determine the concentration of potato cell content using sucrose solutions of known concentration.

Method

You need to carry out a simple dilution of the 1.0 mol dm^{-3} sucrose solution to produce 6 different concentrations of sucrose solution ranging from 1.0 mol dm^{-3} to 0.0 mol dm^{-3}.

1. Complete the table below by writing in the concentration of sucrose solutions you will prepare.
2. Decide the volumes of 1.0 mol dm^{-3} sucrose solution and distilled water you will need to make 25 cm^3 of each of the concentrations of sucrose shown in the table below. Record these in the table.

concentration of sucrose solution / mol dm^{-3}	**volume of 1 mol dm^{-3} sucrose solution / cm^3**	**volume of distilled water / cm^3**
1.0	25	0
0.0	0	25

3. Label large test-tubes with these concentrations.
4. Prepare the 6 concentrations of sucrose solutions in the labelled test-tubes.
5. Cut 6 cylinders of potato using a cork borer, each should be 5 cm long.
6. Each cylinder should be gently blotted with a paper towel.
7. Measure the mass of one of the potato cylinders.

   Note: This potato cylinder is going to be soaked in 1.0 mol dm^{-3} sucrose solution.
8. Record the mass of this potato cylinder in a results table.
9. Put the potato into the test-tube containing the 1.0 mol dm^{-3} sucrose solution.
10. Repeat steps **7** to **9** for each of the other sucrose solutions you have prepared.
11. Leave the potato cylinders to soak for one hour.
12. After one hour, remove the potato cylinder from the 1.0 mol dm^{-3} sucrose solution.
13. Gently blot the cylinder with a paper towel.
14. Measure the mass of the potato cylinder and record its mass in a results table.
15. Repeat steps **12** to **14** with each of the other potato cylinders. You should remove them from their solutions in the same order that you put them in.

Worksheet, *continued*

Results

Record your results in an appropriate table. When drawing a results table remember that you should:

- put the independent variable in the first column
- use descriptive column headings
- include units in the column headings only.

Interpretation and evaluation

1. Calculate the percentage change in mass of each cylinder. You should show all the steps in your calculation for one cylinder. Record these processed results in a table.
2. Plot a graph to show the relationship between the concentration of sucrose solution and the percentage change in mass of the cylinders.
3. Use your graph to find the concentration of sucrose at which the percentage change in mass of the potato cylinder is zero.
4. Explain the trend shown by the graph using your knowledge of osmosis and water potential. You should be able to explain why:
    - some potato cylinders gain mass
    - some potato cylinders lose mass
    - there is a concentration of sucrose at which there is no percentage change in mass of the potato cylinder.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 4

Substrate concentration and enzyme activity

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To investigate the effect of substrate concentration on the activity of the enzyme catalase.

Outcomes

Syllabus section 3.1 (d) and 3.2 (a)

Skills included in the practical

AS Level skills	How learners develop the skills
MMO decisions	Carry out a simple dilution of hydrogen peroxide solution and suggest a suitable control experiment
MMO collection	Tally count the number of bubbles in a minute
PDO recording	Record quantitative results appropriately in a table
PDO layout	Draw a graph to show how rate of production of bubbles varies with substrate concentration
ACE analysis	Describe the trend shown in the graph
CE conclusions	Explain the trend shown in the graph using an understanding of how enzymes work
ACE evaluation	Identify the significant source of error in the experiment and suggest a modification to increase the accuracy of the results Suggest how to extend the investigation to answer a new question

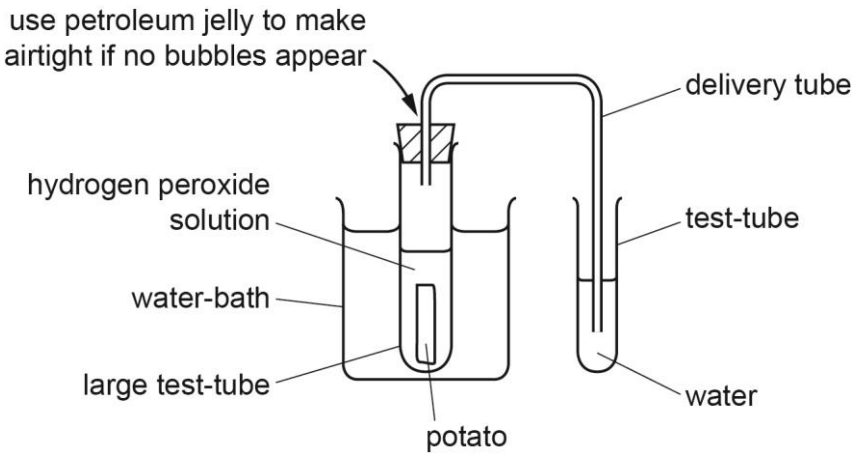
Method

Safety glasses must be worn when preparing the slide.

- Hydrogen peroxide is a harmful waste product of metabolic processes. It is broken down by the enzyme catalase into water and oxygen.
- During this investigation learners will study the effect of the concentration of hydrogen peroxide solution on the activity of catalase by counting the number of bubbles of oxygen produced in a set time.
- Learners should be provided with about 100 cm^3 of 20 vol hydrogen peroxide solution in a beaker, labelled 100% hydrogen peroxide solution, and the same volume of distilled water in a separate beaker, labelled distilled water.
- They will carry out a simple dilution of this hydrogen peroxide solution to provide 6 concentrations ranging between 100% and 50%. They will need 20 cm^3 of each concentration. This task gives learners the opportunity to make decisions about which concentrations they should select and the volumes of hydrogen peroxide solution and distilled water that they need in order to make these solutions.
- The solutions can be made directly into large test-tubes (boiling tubes) which have been labelled by the learner, or in separate beakers, which can then be used as stock solutions.
- Using beakers in this way provides the opportunity to repeat the experiment and assess the reliability of the results. Learners should be asked to process the results they collect and calculate means. If stock solutions are made in beakers, learners will have to transfer 20 cm^3 of each solution into a labelled test-tube and the method will need to be amended accordingly.

Guidance for teachers, *continued*

- The test-tubes should then be put into a water-bath at 37 ˚C to equilibrate for 5 minutes. The water-bath could be a thermostatically controlled water-bath or one made by learners using hot and cold water. The use of the water-bath can be discussed. It ensures that the enzymes are working at or close to the optimum temperature and so the number of bubbles produced is measurable. Leaving the test-tubes in the water-bath for 5 minutes ensures that they are all at the same temperature during the investigation.
- The potato provides the source of catalase. Learners will cut 6 cylinders from a fresh potato; each should be 5 cm long. This could be done using a cork borer or rectangular pieces could be cut using a sharp knife. It is important that the surface area of the potato cylinders is constant and that the potato skin has been removed.
- One potato cylinder or rectangle will be added to the test-tube with the highest concentration of hydrogen peroxide solution, a bung and delivery tube inserted and the number of bubbles produced in one minute should be counted and recorded. This procedure can then be repeated for each of the other concentrations of hydrogen peroxide solution. The diagram below shows how the apparatus should be set up for this experiment.



Results

Learners should record their results in a table and they should be reminded that they should:

- draw a table which includes lines separating each of the columns and rows
- put the independent variable in the first column
- use descriptive column headings include units in column headings, not next to each result recorded in the table.

Interpretation and evaluation

- Learners will plot a graph to show the relationship between substrate concentration and rate of reaction. They then use this graph to describe and explain the relationship. This provides an opportunity to emphasise the difference between a *description* and an *explanation*.
  - A description should state the relationships seen on the graph and use data taken from the graph to support each statement.
  - An explanation should include scientific reasons which explain **why** the relationship between the variables has occurred.

Guidance for teachers, *continued*

- The experiment also provides the opportunity to discuss the idea of a control experiment. Suitable control experiments should be discussed, such as replacing the hydrogen peroxide solution with an equal volume of distilled water to show the results are due to the presence of the hydrogen peroxide solution and not some other factor.
- There is an opportunity to identify possible sources of error and how they may affect the trend and accuracy of the results. The most significant error in this investigation is the fact that the bubbles produced may be of different size. Learners could then suggest how the experiment should be improved to reduce this error, for example by collecting the oxygen in a measuring cylinder or burette using the downward displacement of water.
- As an extension activity learners can design an experiment that uses this method but investigates the effect of temperature on the rate of catalase activity. They should identify that the substrate concentration must be standardised and the temperature of the thermostatically controlled water-bath changed. They should be able to suggest at least 5 suitable temperatures to investigate and these should be chosen from both sides of the optimum used in their investigation.

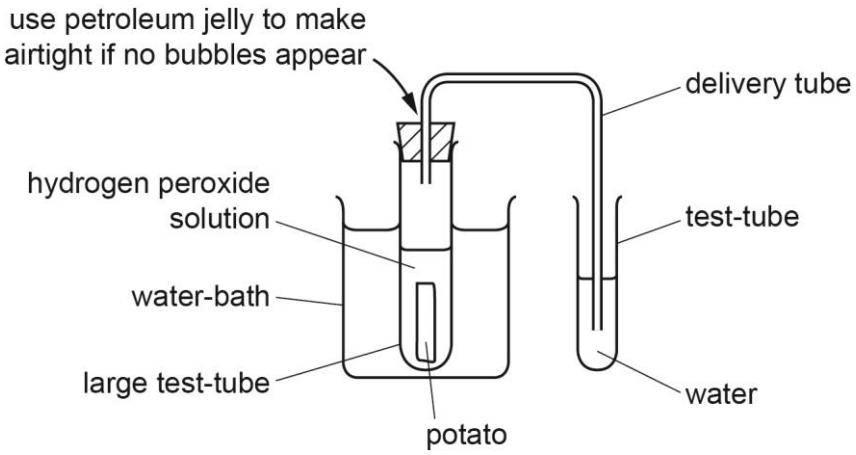
Information for technicians

Each learner will require:

- safety glasses
- at least 100 cm^3 hydrogen peroxide solution (20 vol) in a breaker, labelled 1**00% hydrogen peroxide solution [H]**
- at least 50 cm^3 distilled water
- six large test-tube (boiling tubes)
- one test-tube rack to hold large test-tubes
- one marker pen
- 1 × 5 cm^3 syringes
- 1 × potato
- 1 × cork border
- 1 × white tile
- 1 × knife
- 4 × test-tube
- one delivery tube with a bung that fits into the top of the test-tube
- 1 × stopwatch

Additional instructions

Learners are required to set the apparatus up as shown in the diagram. The bung must make an airtight seal both with the test-tube and the delivery tube to allow the collection of oxygen.



Hazard symbols

C = corrosive substance

F = highly flammable substance

H = harmful or irritating substance

O = oxidising substance

N = harmful to the environment

T = toxic substance

Worksheet

Aim

To investigate the effect of substrate concentration on the activity of the enzyme catalase.

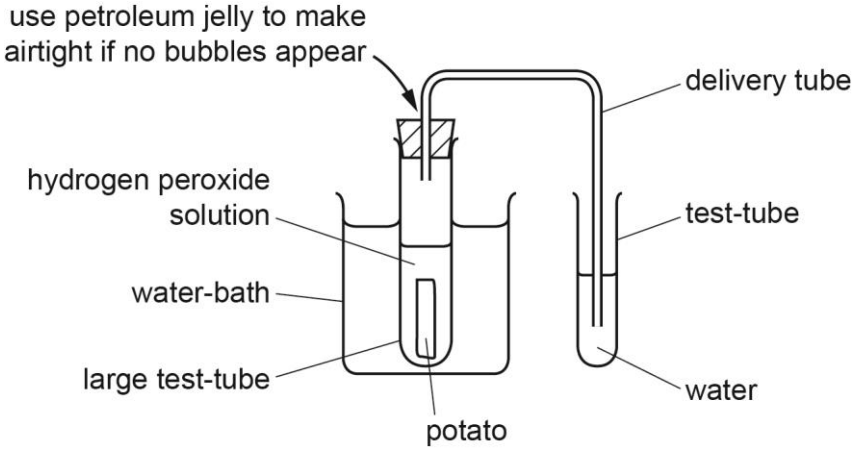
Method

Safety glasses must be worn when carrying out this investigation

1. Decide the volumes of distilled water and hydrogen peroxide solution you will need to make 20 cm^3 of each concentration of hydrogen peroxide solution shown in the table below.
2. Complete the table.

percentage concentration of hydrogen peroxide solution	volume of distilled water / cm^3	volume of 100% Hydrogen peroxide solution / cm^3
100		
90		
80		
70		
60		
50		

3. Label test-tubes with these concentrations.
4. Prepare these concentrations of hydrogen peroxide solution in the test-tubes.
5. Put the test-tube containing the 100% hydrogen peroxide solution into a water-bath at 37 °C and leave for 5 minutes to equilibrate.
6. Cut a cylinder or rectangle of potato 5 cm long using a cork borer or knife.
7. Put the potato cylinder or rectangle into the test-tube and quickly add the bung and delivery tube as shown on the diagram below.
8. Put the delivery tube into a test-tube containing water and count the number of bubbles given off in 1 minute.



9. Repeat steps **5** to **8** for each concentration of hydrogen peroxide solution you have prepared.
10. Record your results in a table.

Worksheet, *continued*

Results

Record your results in an appropriate table. When drawing a results table remember that you should:

- put the independent variable in the first column
- use descriptive column headings
- include units in column headings.

Interpretation and evaluation

1. Plot a graph to show the relationship between substrate concentration and rate of reaction.
2. Use the graph to describe the relationship between the concentration of hydrogen peroxide solution and the rate of reaction.
3. Use the graph to explain the relationship between the concentration of hydrogen peroxide solution and the rate of reaction.
4. Describe how you would set up a control for this experiment.
5. State the main source of error in this investigation.
6. Describe how you could improve the investigation and therefore the accuracy of your results.

Extension

Describe how you would modify this experiment to investigate the effect of temperature on the activity of the enzyme catalase.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 5

Investigating the progress of an enzyme-catalysed reaction by measuring the rate of disappearnce of a substrate

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To determine the rate of hydrolysis of starch using the enzyme amylase.

Outcomes

Syllabus section 3.1 (d)

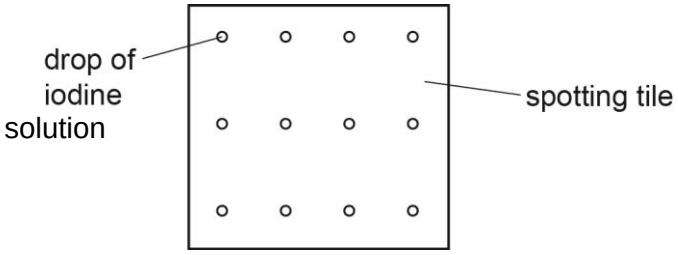
Skills included in the practical

AS Level skills	How learners develop the skills
MMO decisions	Describe an appropriate control experiment
MMO collection	Make qualitative observations about colour changes
PDO recording	Collect quantitative results, time for a colour change
ACE concluding	Record qualitative observations and quantitative results in appropriately in a table
PDO display	Explain the reason for the change in the colour of the iodine solution over the course of the experiment
ACE analysis	Calculate a mean and calculate the rate of the reaction showing every step in the calculation
ACE evaluation	Modify the procedure to investigate the new question, how temperature affects the rate of hydrolysis of starch by amylase

Method

Safety glasses must be worn when preparing the slide.

- Learners should be familiar with the structure of starch, and the breakage of glycosidic bonds in polysaccharides. They should also have an understanding of the mode of action of enzymes.
- Starch is a polysaccharide made up of many α-glucose molecules joined by glycosidic bonds. The enzyme amylase hydrolyses starch to produce the disaccharide maltose.
- During this investigation learners will follow the course of this reaction using iodine solution. The practical follows the disappearance of starch as it is hydrolysed by amylase. The intensity of the blue colour produced when samples of starch and amylase solutions are added to iodine solution is recorded regularly over a set period of time. As starch is broken down the blue-black colour will become less intense and eventually disappear.
    - A spotting tile should be prepared with a drop of **iodine solution** placed in every cavity.



- Learners are provided with a 1% amylase solution and a 1% starch solution. They should put 10 cm^3 of the 1% starch solution into a large test-tube and 2 cm^3 of 1% amylase solution into a second large test-tube.

Guidance for teachers, *continued*

- A beaker with warm water (about 37 °C) should be prepared to act as a water-bath. This should be used to equilibrate the starch and amylase solutions. The two test-tubes should be left in the warm water-bath for 5 minutes. The purpose of this is to equilibrate both the substrate and enzyme to the same temperature. The use of a thermometer in each tube as well as one in the water-bath can be discussed as a more accurate way of determining that the equilibration is complete.
- The starch solution should be poured into the amylase solution and mixed with a glass rod and a stop clock started immediately. After 15 seconds a drop of liquid can be removed from the mixture using a glass rod and placed into the iodine solution in one of the cavities in the spotting tile. This will be repeated every 15 seconds, and a drop of the starch amylase mixture added to the next cavity in the spotting tile.
- At the start of the experiment the drop of iodine solution should turn blue-black because starch is present. As the amylase hydrolyses the starch to maltose the samples should turn the iodine solution a less intense blue-black colour until eventually the drop of iodine solution remains orange-brown. This is the end-point of the experiment and the time taken for the complete hydrolysis of starch will be recorded. Learners may need more than one spotting tile to follow the reaction to its endpoint.
- To act as a control, the experiment should be repeated with 2 cm^3 of water in place of the amylase solution. The experiment should be stopped after a maximum of 5 minutes as without the amylase the starch will not break down and an end point will not be reached. This is a simple experiment to reinforce the purpose of a control experiment.

The experiment is repeated twice more, to allow a mean to be calculated. This allows the reliability of the experiment to be discussed.

Results

- Learners record their results in a table. The colour of the drop of iodine solution will be recorded every 15 seconds. This can be done using adjectives such as ‘dark’ or ‘pale’. Alternatively they can use the symbol, + with a key to represent the degrees of colour e.g. +++++ very dark blue to + very pale blue.

time after the enzyme and substrate are mixed / s	**colour of iodine solution**
0	
15	
30	
etc.	

Guidance for teachers, *continued*

Interpretation and evaluation

- Learners calculate the mean time taken to reach the end-point of the experiment. This allows the opportunity to discuss the accuracy of observing colour change.
- Learners calculate the rate of hydrolysis of starch using the formula: rate = 1 / time. The need to consider the number of significant figures the answer is given to can be emphasised.
- This calculation is based on the assumption that the rate of hydrolysis will be constant. This assumption is incorrect and can be discussed.
- Learners are asked to consider how they would modify this experiment to investigate the effect of temperature on the rate of hydrolysis of starch. They should identify the need to change the temperature of the water-bath and be able to suggest 5 temperatures to investigate, including temperatures both above and below the one used in this investigation. The concentration of the starch and amylase solutions should be kept constant.

Information for technicians

Each learner will require:

- safety glasses
- at least 10 cm^3 iodine in potassium iodide solution, labelled **iodine solution [H]**
- 6 cm^3 of 1% amylase solution **[H]**
- 30 cm^3 1% starch solution
- 1 × dropping pipette
- 2 × spotting tiles
- 1 × 400 cm^3 beaker to be used as a water-bath (at approximately 15-40 ^oC)
- 1 × glass rod
- paper towels
- 1 × stop clock
- 2 x large test-tube rack
- 2 × 5 cm^3 syringes
- at least one thermometer, three if available

Hazard symbols

C = corrosive substance

F = highly flammable substance

H = harmful or irritating substance

O = oxidising substance

N = harmful to the environment

T = toxic substance

Worksheet

Aim

To determine the rate of hydrolysis of starch using the enzyme amylase.

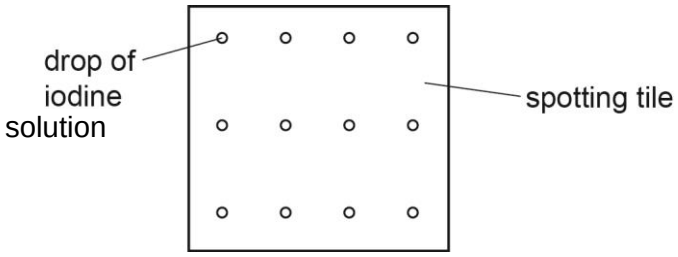
Method

Starch is a polysaccharide made up of many α-glucose molecules joined by glycosidic bonds. The enzyme amylase hydrolyses starch to produce the disaccharide maltose.

You will follow the course of this reaction using iodine solution. The practical follows the disappearance of starch as it is hydrolysed by amylase. Over a set period of time you will regularly record the intensity of the blue colour produced when samples of starch and amylase solution are added to iodine solution. As starch is broken down the blue-black colour will become less intense and eventually disappear.

Safety glasses must be worn when preparing the slide.

1. Add a drop of iodine solution to every cavity on the spotting tile as shown below.



2. Add 10 cm^3 of 1% starch solution into a large test-tube and label it accordingly.
3. Add 2 cm^3 of 1% amylase solution into a second large test-tube and label it accordingly.
4. Prepare a beaker with warm water (about 37 °C). This will act as a water-bath and will be used to equilibrate the starch and amylase solutions to the same temperature. Leave the two test-tubes in the warm water-bath for approximately 5 minutes. Use a thermometer to determine when the contents of the tubes have reached the required temperature.
5. Pour the 1% starch solution into the test-tube containing the 1% amylase solution and mix with the glass rod. Start the stop clock immediately.
6. After 15 seconds a drop of liquid can be removed from the mixture using a glass rod and placed into the iodine solution in the first cavity of the spotting tile. Record the colour of the mixture and its intensity in a results table.
7. Repeat step **6** every 15 seconds, placing a drop of the starch-amylase mixture into the iodine solution in the next cavity on the spotting tile.
8. You will need to determine when the end-point of the experiment has been reached.
9. Repeat steps **1 – 8** twice more.
10. Decide what would be an appropriate control experiment to perform. Your teacher may allow you to carry this out if time allows.

Worksheet, *continued*

Results

Record your results in an appropriate table. When drawing a results table remember that you should:

- put the independent variable in the first column
- use descriptive column headings
- include units in the column headings only.

Interpretation and evaluation

1. Calculate the mean time to reach the end-point of the experiment.
2. Calculate the rate of hydrolysis of starch using the formula below. Consider the number of significant figures carefully.

   Rate of hydrolysis = 1 / time
3. How could you modify this experiment to investigate the effect of temperature on the rate of hydrolysis of starch?

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 6
Using a potometer

Cambridge International AS & A Level
Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To determine the uptake of water by a leafy shoot and investigate how leaf area affects this.

Outcomes

Syllabus learning objective 1.1 (c)

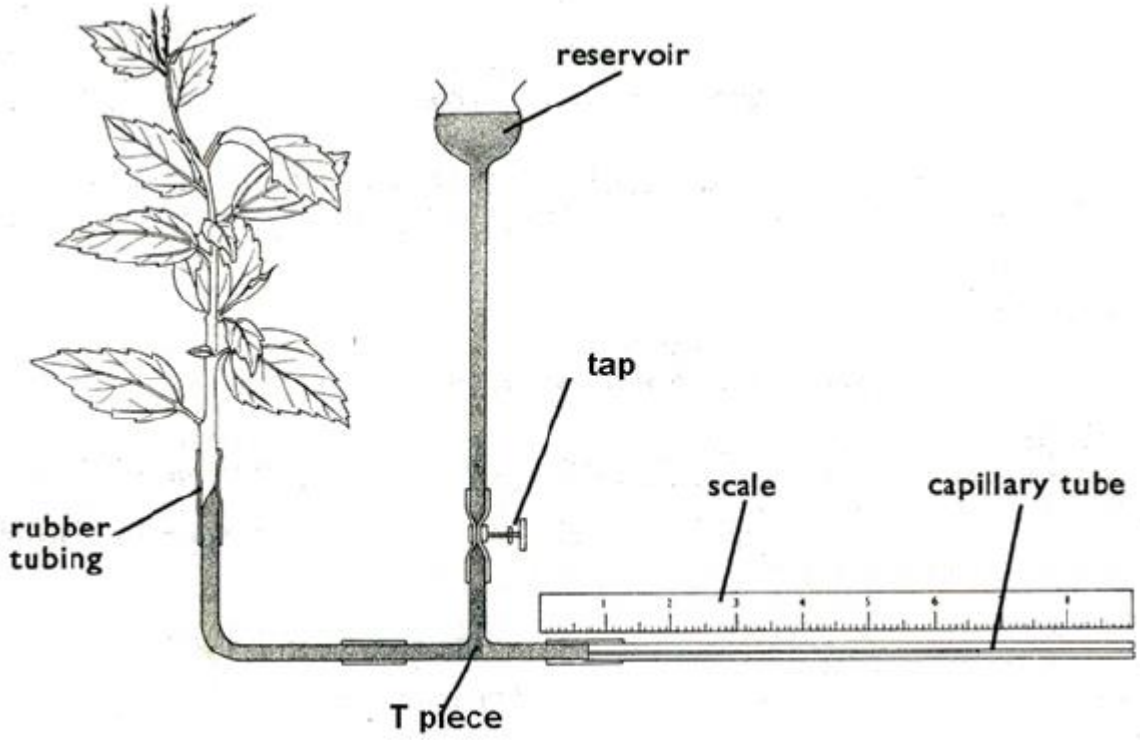
Skills included in the practical

AS Level skills	How learners develop the skills
MMO decisions	Measure the area of a leaf using a grid
MMO collection	Collect quantitative results - distance moved by bubble in the potometer
PDO recording	Record quantitative results in an appropriate table
PDO layout	Draw a graph to show the relationship between surface area of leaves removed and rate of transpiration
ACE analysis	Identify anomalous results Describe the trend shown in the graph drawn
ACE conclusions	Explain the trend shown in the graph using scientific understanding of the factors that affect the rate of transpiration

Method

- Learners should be familiar with the movement of water through a plant and the loss of water from the leaves by transpiration.
- A potometer will need to be provided for each group of students as shown in the diagram below. To set up the potometer the rubber tubing must be attached to the capillary tube and the water reservoir to the T piece. The reservoir, capillary tube and rubber tubing should be filled with water. This can be done by placing them under water and gently squeezing the rubber tubing until all the air has been removed.
- A leafy shoot can be cut at an angle underwater and the cut end inserted into the rubber tubing of the potometer while it is also still under water. This ensures that there are no air bubbles trapped in the xylem, which will prevent the transport of water. The tap on the water reservoir can then be closed and the apparatus removed from the water and attached to a clamp stand or support. The leaves should be allowed to dry before the apparatus is used to measure the rate of transpiration.

Guidance for teachers, *continued*



- A simple potometer could be used if the above apparatus is not available. This would consist of a piece of glass capillary tube and the rubber tubing. This can be put under water to fill with water and the rubber tubing can be squeezed to remove air bubbles from the apparatus. The leafy shoot can then be inserted into the simple potometer as described above. A single air bubble is introduced into the potometer and the rate of movement of this bubble is taken as an indication of the rate of transpiration.
- A ruler is placed along the capillary tubing. Learners will measure the distance a bubble in the capillary tube moves in one minute. Repeat readings are taken.
- To investigate the effect of leaf area on the rate of transpiration, the experiment is repeated and a leaf removed from the shoot. The distance the bubble travels in one minute is recorded. Several repeats can be taken. This experiment repeated, removing a second and third leaf etc. until sufficient data has been collected to draw a graph (at least 5 values of the independent variable should be investigated). Alternatively individual leaves could be covered in petroleum jelly so that the stomata are blocked to prevent transpiration.

As each leaf is removed it needs to be labelled and its surface area determined. This can be done by tracing round each leaf onto squared paper and then counting the number of squares within the leaf outline to determine the area. Squares that are half or more covered by the leaf should be counted as whole squares and those squares that are less than half covered should not be counted.

Guidance for teachers, *continued*

Results

- Learners record their raw data in tables.
- For the first experiment they will record the distance the bubble moves after each minute.

trial	distance bubble moves in one minute / mm

- For the second experiment the table of results should show the number of leaves removed from the shoot and the distance the bubble moved in one minute.

number of leaves removed	distance bubble moves in one minute / mm

- Learners can be reminded of the need to put the independent variable in the first column and the dependent variable in the second column. They can also be reminded of the need for descriptive column heading with units in the column headings rather than next to each result in the table.
- The surface area of each leaf removed should be recorded in a table. Then the total surface area of the leaves removed for each experiment can be calculated by adding the surface area of all the leaves removed before the experiment begins.
- A table showing these processed results and the results for the dependent variable should be produced.

total surface area of leaves removed / mm^2	distance bubble moves in one minute / mm

Interpretation and evaluation

- The processed data will be used to plot a graph. The graph can be used to identify any anomalous results. Learners are asked to describe the relationship shown on the graph. They can be reminded of the need to use data from the graph to support each statement made in their description.
- The experiment provides the opportunity to discuss different types of errors which may affect the accuracy of the results.
- Learners can use their understanding of transpiration and the factors which affect it to explain the shape of the graph.

Information for technicians

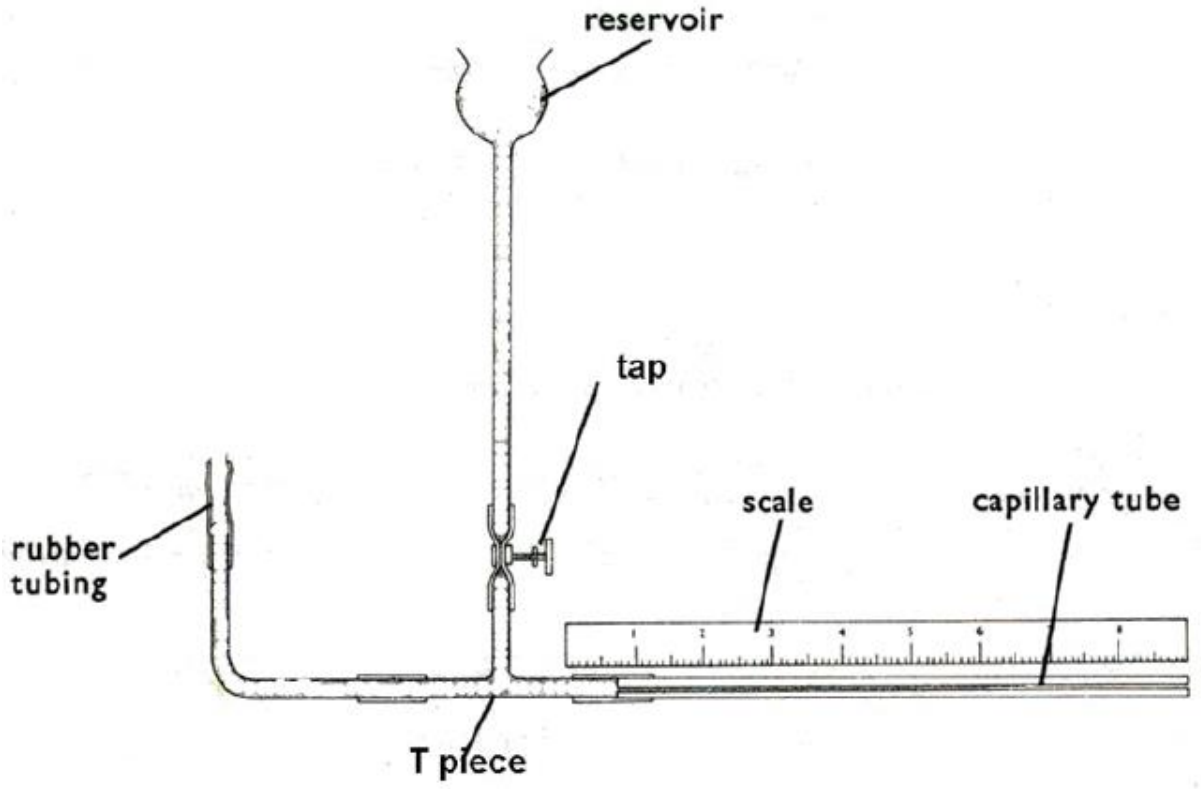
Each learner will require:

- 1 × freshly cut leafy shoot that has been put immediately into fresh water
- 1 × photometer set up as shown in the diagram below
- ruler with mm scale to place alongside the capillary tube
- stop watch or clock
- petroleum jelly (optional)
- 1 × marker pen

Additional instructions

There are no specific hazards for this investigation

Set the equipment up as shown below



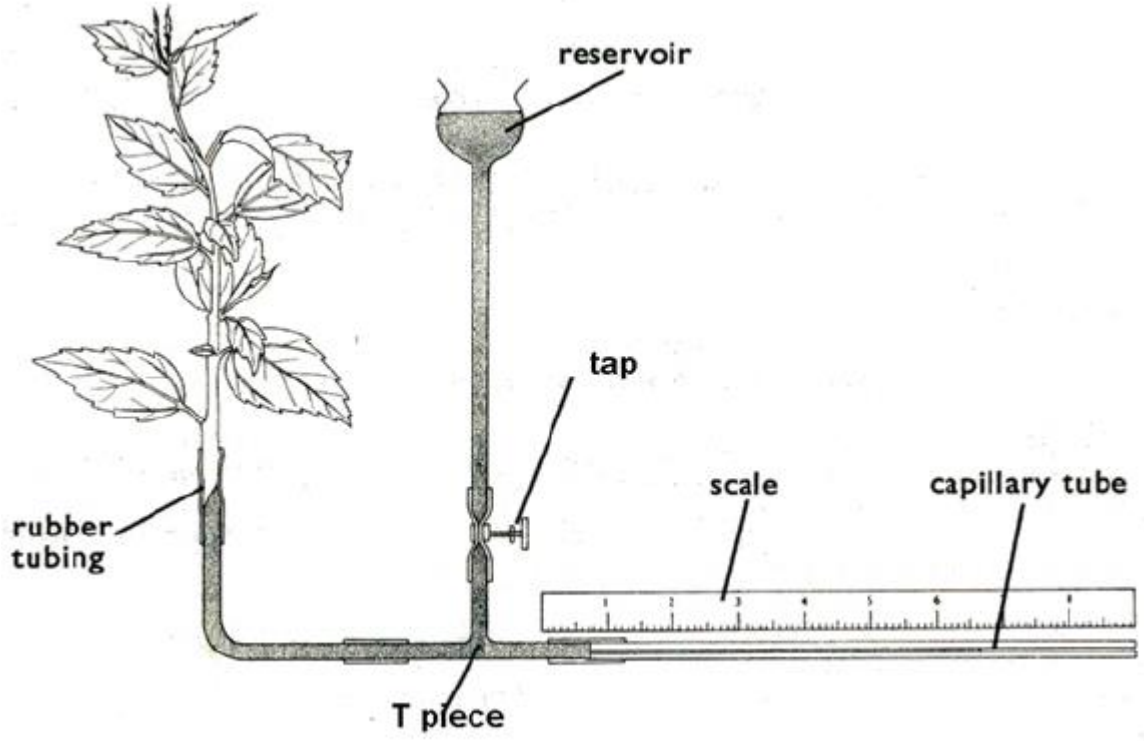
Worksheet

Aim

To determine the uptake of water by a leafy shoot and investigate how leaf area affects this.

Method

You will be provided with a potometer which has been set up for you as shown below.



Experiment 1

1. Place a ruler along the capillary tubing and note the position of the air bubble.
2. Measure the distance the bubble in the capillary tube moves in 1 minute. Record your results.
3. Repeat steps **1** and **2** several times. Record your results.

Worksheet, *continued*

Experiment 2

1. Remove one leaf from the shoot and label it **leaf 1**. You must not remove the shoot from the potometer when you do this.
2. Place a ruler along the capillary tubing and note the position of the air bubble.
3. Measure the distance the bubble in the capillary tube moves in 1 minute.
4. Repeat steps **2** and **3** several times.
5. Record your results in a results table.
6. Remove a second leaf from the shoot and label it **leaf 2**.
7. Repeat steps **2** – **5**.
8. Continue the process above until you have removed a total of 5 leaves from the shoot.
9. Determine the surface area of each leaf removed from the shoot by tracing round each leaf onto squared paper.
10. Count the number of squares within the leaf outline to determine the area. Squares that are half or more covered by the leaf should be counted as whole squares and those squares that are less than half covered should not be counted.
11. Record your results in a table.

Results

1. Record your results in appropriate tables. When drawing a results table remember that you should:
   - put the independent variable in the first column
   - use descriptive column headings
   - include units in the column headings only.

   You should have:
   - one table recording the number of leaves removed from the shoot and the distance the bubble moved in one minute
   - one table recording the surface area of each removed leaf.
2. Calculate the total surface area of the leaves removed for each experiment.
3. Record these processed results and the results for the distance the bubble moved in 1 minute in a new table.

Worksheet, *continued*

Interpretation and evaluation

1. Plot a graph to show the relationship between the total surface area of leaves removed and the distance the bubble moved in one minute.
2. Describe the relationship shown on the graph.
3. Explain the trend shown in the graph by using your understanding of transpiration and the factors which may affect it.
4. Identify any results you think are anomalous.
5. List the possible errors which may have occurred when collecting your data. How might they have affected the trend and accuracy of your results?

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 7

Measurement of gas exchange using a respirometer

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To determine the volume of oxygen consumed and the volume of carbon dioxide released by respiration. To use these volumes to calculate RQ for the organism tested. The procedure for measuring oxygen uptake is used as a model to develop a plan to find the optimum temperature for respiration.

Outcomes

Syllabus sections 12.1 (h) and 12.2 (m)

Skills included in the practical

A Level skills	How learners develop the skills
Planning	Identify hazards and produce a simple risk assessment Modify a method to investigate a different independent variable
Analysis	Calculate volume using the appropriate formula Calculate RQ value Calculate rate of oxygen consumption
Evaluation	Evaluate the methods used and their effect on the accuracy and reliability of the results
Conclusions	Use scientific theory and their results to determine which substrate was used

This practical provides an opportunity to build on essential skills introduced at AS Level.

AS Level skills	How students develop the skills
MMO collection	Measure distance moved by dye
PDO recording	Record quantitative results appropriately in a table

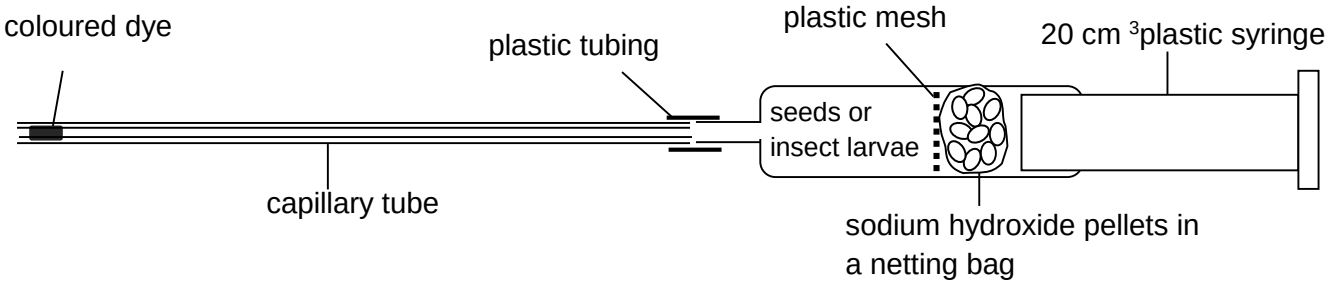
Guidance for teachers, *continued*

Method

Safety glasses must be worn when preparing the slide.

- Respiration is a process that consumes oxygen and releases carbon dioxide. The volume of oxygen consumed and carbon dioxide released can be measured using a respirometer.
- Learners need to understand how a respirometer works so a preliminary discussion is needed to encourage learners to think of a respirometer as a closed air-tight system from which the test organisms take oxygen and release carbon dioxide. The carbon dioxide is absorbed by the sodium hydroxide, causing the dye to move. The potential hazards of this apparatus should also form part of the discussion so learners can choose suitable safety precautions. This could take the form of a question and answer session or demonstration of the apparatus.
- Learners should be provided with a respirometer and asked to set up and use it by following the learner worksheet. This task gives learners the opportunity of re-visiting AS skills in a more complex procedure. The learners should work in pairs and each measure the gas exchange of a different organism, either germinating seeds or insect larvae. This gives an opportunity for learners to compare gas exchange in different organisms. The procedure for learners takes approximately one hour to complete. Learners may need help in setting up and making sure the respirometer is air-tight.

Diagram of a simple respirometer set up ready to use:



Results

1. Learners record their results in a table, making use of the skills developed during the AS Level course to produce a suitable table. The table will be more complex than those used for AS and will help to develop A Level skills in interpreting complex tables.

    - They can be reminded of the expected form of tables from AS.
    - They can be prompted to think about how to present two sets of measurements taken over time.

2. A further table of total volume of oxygen and total volume of carbon dioxide should be generated by pooling the results of all the learners.

3. Learners will then process their results by calculating the total volume of oxygen consumed and the total volume of carbon dioxide produced. This provides an opportunity for learners to practice mathematical skills that are an integral part of A Level. Learners can work out the volume of a 10 mm length of the capillary tubing and use this to work out the total volume, or they can use the total distance measured to work out the volume. This activity provides an opportunity to revisit the idea of significant figures and sensible rounding of numbers generated by calculators.

Guidance for teachers, *continued*

Interpretation and evaluation

1. Learners will then carry out a further calculation to find the RQ value for the organisms tested. At this point the idea of RQ and how it relates to respiratory substrate can be introduced. Each pair of learners can compare their results from germinating seeds or from insect larvae and draw conclusions about the substrate being used for respiration.
2. Learners will then describe the pattern shown by their results, noting if there is any change in the distance moved as time increases.
3. The idea of using a respirometer as a means of estimating the rate respiration can be introduced, as learners then carry out a further calculation to work out the rate of oxygen consumption in $mm^3 g^{-1} s^{-1}$. This provides learners with an opportunity to manipulate a sequence of calculations.
4. The experiment provides an opportunity for discussion about the reliability of the results in relation to the method used. The main considerations are the lack of replication of the measurements and the absence of a control. Learners could then suggest that at least three complete sets of measurements should be taken for both oxygen consumption and carbon dioxide release from which a mean can be calculated. They should also suggest a suitable control, such as replacing the test organism with an inert material such as beads or stones and be able to explain how the results of the control should be used.
5. Learners should then be asked to modify the method they have used in order to produce an experimental method to find the optimum temperature for respiration. This method should be suitable for another learner to use and ideally should be trialled. This gives another opportunity for learners to use a respirometer again and to follow this up by deciding how to use the results to find the optimum temperature.

Information for technicians

Each learner will require:

- 1 × respirometer
- 10 cm^3 coloured solution (water and blue or red food dye)
- 20 Mung bean seeds (*Vigna radiata*) previously soaked for 24 hours in tap water or 15 below fly larvae, approximately 2cm in length
- 1 × sheet of 2 mm graph paper
- 10-15 sodium hydroxide pellets in a mesh bag made from fabric (nylon, cotton or polyester) **[H] [C]**
- 1 × 10 cm x 10 cm square of cotton, polyester or nylon mesh fabric. Muslin or window curtain netting can be used.
- 30 cm length of cotton suitable for trying seeds inside a fabric bag
- 1 × clamp stand, boss and clamp or other means of support for capillary tubing
- 1 × pair of forceps for handling sodium hydroxide
- 1 × 15 cm or 30 cm ruler, marked in mm
- access to top pan balance or scale weighing to 0.00 g
- 1 × pair of safety glasses
- 1 × pair of gloves – plastic or vinyl

For follow-on investigation into effect of temperature

- 5 x containers suitable to use as water-baths
- 1 x thermometer
- Access to a supply of hot and cold water

Information for technicians, *continued*

Additional instructions

To make a simple respirometer:

- 20 cm^3 syringe with tight fitting plunger that does not leak when immersed in water
- 20 cm length of capillary tubing of 1 mm bore
- 3 cm length of plastic tubing

The capillary tubing is attached to the syringe by plastic or rubber tubing. The tubing must be tight enough to prevent air leakage. This can be tested by immersing the joint in water.

Centres that have commercially produced simple respirometers of similar design could substitute these. U-tube manometers are not suitable substitutes.

Housefly larvae can be used instead of blow fly larvae, but approximately 25 will be needed to give results in the time. Fishing suppliers often have blowfly and housefly larvae (maggots) which can be kept in a refrigerator for several days before use and allowed to warm up 2 – 3 hours before the practical.

Hazard symbols

C = corrosive substance

F = highly flammable substance

H = harmful or irritating substance

O = oxidising substance

N = harmful to the environment

T = toxic substance

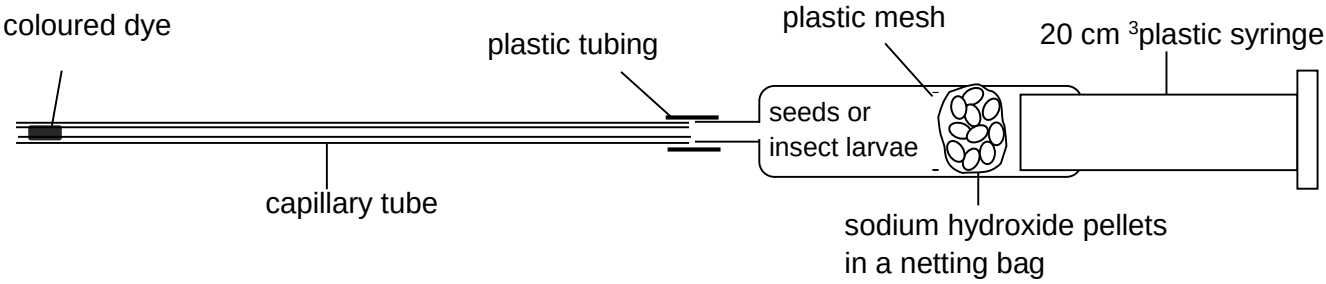
Worksheet

Aim

To determine the volume of oxygen consumed and the volume of carbon dioxide released by respiration. To use these volumes to calculate RQ for the organism tested. The procedure for measuring oxygen uptake is used as a model to develop a plan to find the optimum temperature for respiration.

Apparatus

Simple respirometer set up ready to use.



Method

Safety glasses must be worn when preparing the slide.

1. Identify hazards and appropriate safety precautions for this investigation.
2. Remove the plunger from a 20 cm^3 syringe that is attached to a capillary tube.
3. Place 20 soaked mung bean seeds **or** 15 blowfly larvae into a piece of netting.
4. Fold the netting around the seeds and tie the open end with cotton to make a bag.
5. Find and record the mass of the seeds or insect larvae in their netting bag.
6. Place the bag of seeds or insect larvae into the syringe.
7. Insert a piece of plastic mesh to keep the bag of seeds or insect larvae at the bottom of the syringe, making sure it does not touch the bag containing the organisms.
8. Place a netting bag containing sodium hydroxide pellets into the open syringe so that it lies next to the plastic mesh.
9. Replace the plunger of the syringe and leave the assembled respirometer for 5 minutes.
10. Prepare a scale using the graph paper provided so it can be attached to the capillary tube.
11. Place the end of the capillary tube into the dye solution and use the syringe plunger to pull in a 1 cm length of the dye.
12. Attach the graph paper scale and use a support to keep the capillary tube horizontal.
13. Note the time.
14. Measure the distance moved by the dye at 1 minute intervals for 10 minutes.
15. If the dye reaches the end of the capillary scale before 10 minutes, reset the respirometer by pushing the plunger until the dye reaches the beginning of the scale.
16. Remove the syringe plunger and allow fresh air to enter the respirometer.
17. Remove the bag of sodium hydroxide pellets and replace them with a bag containing beads or small stones of the same mass.
18. Replace the syringe plunger and repeat steps **10** to **15**.

Worksheet, *continued*

Results

1. Record the raw results in a table.
2. Use the formula for volume of a cylinder to:
   - Calculate the oxygen consumption after 10 minutes in mm^3
   - Calculate the carbon dioxide production after 10 minutes in mm^3.
3. To calculate the RQ value use the formula

$$RQ = \frac{\text{carbon dioxide released}}{\text{oxygen consumed}}$$

4. Calculate the rate of respiration from the oxygen consumption in $mm^3\,g^{-1}\,s^{-1}$.

Interpretation and evaluation

1. Summarise how a respirometer works, emphasising which steps in the procedure are essential to obtain reliable results.
2. Analyse the raw data and comment on the pattern including:
   i. whether the rate of oxygen uptake is constant or changes during the 10 minute period
   ii. the volume of carbon dioxide produced in comparison to the oxygen consumed
   iii. what the RQ value indicates about the substrate being used for respiration.
3. Comment on the reliability of the results including:
   i. use of apparatus
   ii. the control
   iii. the number of replicates.
4. Plan an investigation using a respirometer to find the optimum temperature for respiration germinating seeds of insect larvae. This plan should be detailed enough for another person follow.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 8

Separation of leaf pigments by chromatography

Cambridge International AS & A Level

Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To separate and identify the pigments present in leaves using chromatography.

Outcomes

Syllabus sections 13.1 (e)

Skills included in the practical

A Level skills	How learners develop the skills
Analysis	Calculate *R*f values
Evaluation	Evaluate the methods used and their effect on the accuracy and reliability of the results
Conclusions	Draw conclusions about the pigments shown in leaves of different species and different colours Use scientific knowledge to explain why leaves have these pigments

This practical provides an opportunity to build on essential skills introduced at AS Level.

AS Level skills	How learners develop the skills
MMO collection	Measure distance moved by pigment
PDO recording	Record quantitative results appropriately in a table

Method

Safety glasses must be worn when preparing the slide.

The room should be well ventilated and an extraction cabinet used if available.

- Leaves appear green due to the presence of chlorophyll but contain other pigments that are hidden by the colour of chlorophyll. Chromatography is a technique used to separate mixtures of chemicals by their solubility. There is a support layer, the paper, on which the mixture to be separated is placed. Chemicals in the mixture dissolve in the solvent, propanone, and are carried through the support layer. The support layer interacts with the solvent and the dissolved chemicals, attracting the chemicals in the solvent onto the support layer. The least soluble chemicals are left on the support first, the most soluble last.
- During this investigation learners will use chromatography to separate and identify the pigments in leaves.
- Learners should be introduced to the principle of chromatography by:
  - asking questions about their experience of what happens when water is spilt against the edge of dry paper or cloth
  - demonstrating what happens when a spot of black ink (not biro) is placed at one end of a piece of filter paper and then dipped into water.

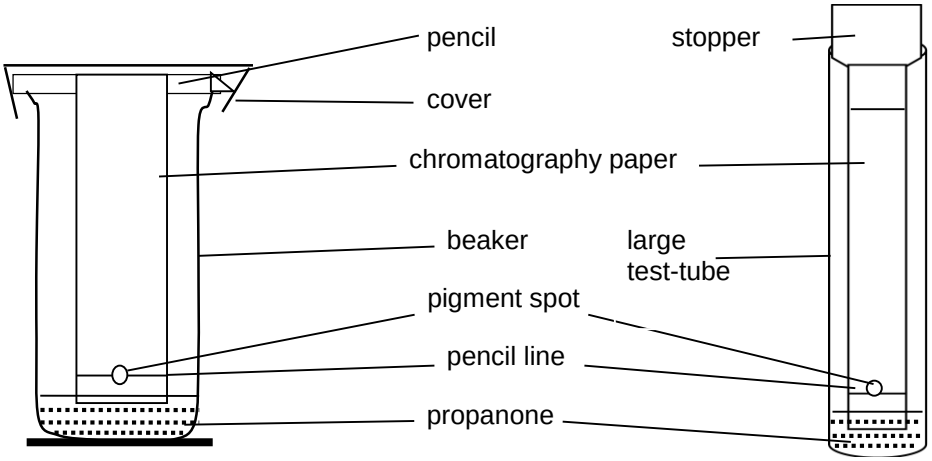
Guidance for teachers, *continued*

- Learners are asked to make a leaf extract by cutting leaves into small pieces and then grinding in a mortar and pestle with a small quantity of propanone (acetone). A variety of leaves can be used. Soft leaves are easier to grind and dark green leaves usually give better extracts. If there are local species that have red or orange coloured leaves these can be used for comparison. This activity provides an opportunity for learners to experience the difficulty of breaking open plant cells and to understand why it is necessary. If the leaves are very thick, a small quantity of sand can be added to the mortar.
- Learners will then filter their extract. If too much propanone has been used the extract will be too dilute and will need to be concentrated. This can be done in hot water, but as propanone is flammable and has irritant vapour this should only be done in an extractor cabinet and with no naked flames in the room.
- Learners will then place one or more samples of their leaf extract onto paper. Chromatography paper (Watman no.1) gives a good separation, but standard filter papers used in filter funnels or coffee filters can be used. For small scale chromatography, strips of filter paper that fit inside a large test-tube can be used. These are suitable for one pigment sample. Wider pieces of filter paper that fit inside a 250 cm^3 beaker can be used for two or three samples. To place samples onto filter paper learners should:
  - draw a line in pencil about 15 mm from one end of the filter paper
  - make a pencil mark on this line where the samples are going to be placed. This is the origin. For single samples this should be in the centre. For two or more samples these should be at least 20 mm from the edge and evenly spaced, at least 20 mm apart
  - use a capillary tube to place a spot of extract on the marked places. Spots should be no more 2 mm in diameter
  - leave the spot to dry and then place another spot on top of the first and leave to dry. At least 5 spots are needed, more if the extract is dilute.

  Thin layer chromatography can also be used for comparison if facilities are available for making thin layers of cellulose powder onto one surface of glass microscope slides.
- After spotting the chromatography paper learners will place it into the solvent to run. To do this, learners should place propanone into a container to a depth of 10 mm. The paper is then placed into the container so that the end with the leaf sample is just touching the propanone. Learners must be instructed to make sure that the propanone does not go above the level of the pencil line. If a large test-tube is used, the top edge of the paper can be folded over the edge of the tube and a tightly fitting stopper inserted. If a beaker is used, the free edge of the paper can be folded over a pencil laid across the beaker and then covered by metal foil or parafilm.

Guidance for teachers, *continued*

Diagram of apparatus ready to use



- The apparatus should then be left to run so that the propanone rises up the paper and carries the different pigments with it. Learners should be instructed to watch the separation and to observe the order in which each of the different colours appear. They should not move the container during this time. Learners should also be instructed to make sure that the propanone does not run off the end of the paper. When they see the rising edge of the propanone (the solvent front) about 10 mm from the end of the paper, they should lift the paper from the solvent and draw a pencil line at the solvent front. The waiting time provides an opportunity to discuss why pencil is used for marking chromatography paper, why the spots must be concentrated and how the propanone (solvent) is able to separate out the pigments.
- The separation will take between 20 – 30 minutes depending on the type of paper used for separation. Coffee filters will be the fastest, but give the least separation.
- Removing the chromatograms must be done in a well-ventilated area and the paper left to dry. This is best carried out in an extractor cabinet, but if this is not available then the papers can be hung to dry in an open, well-ventilated space. The containers with propanone should be recovered and left in a safe place for safe disposal.
- Propanone can be flushed down a sink using a lot of water. Crushed leaves can be wrapped in paper and left outside or in an extractor cabinet until all the propanone has evaporated and then disposed of with normal waste.

Guidance for teachers, *continued*

Results

1. Once the paper is dry, learners are instructed to use pencil to draw around the shapes of the coloured spots and to record the colours. This needs to be done immediately as the colours often fade within 24 hours. At least three spots should be seen, but a good separation should give up to six. Dark yellow or orange carotenes should be close to the solvent front, blue-green chlorophyll a and green chlorophyll b should be next in sequence, yellow xanthophyll is closest to the starting line. A good separation will give two xanthophyll spots and a grey-green phaeophytin spot between carotene and the chlorophylls.
2. Learners are then instructed to calculate the R_f (retention values). This provides an opportunity for discussion about R_f values and how they can be used to identify specific compounds because every compound has a specific R_f value in every specific solvent.

   Learners are instructed to measure from the origin to the solvent front and then to the centre of each pigment spot and use these to calculate the R_f value.

   $$R_f = \frac{\text{distance moved by pigment spot}}{\text{distance moved by solvent (propanone)}}$$

   Measurements of R_f are likely to be inaccurate as spots tend to spread and 'trail' at the edges. Learners are then instructed to measure to the front of a spot and to the back of the same spot and take the average of these as the position of the spot.
3. If different leaves are used then R_f values can be compared. There should be the same pigments, whatever the type of leaf. Leaves with a colour other than green may have additional pigments, but carotene and chlorophylls should be present.

   This could also be an opportunity to introduce the idea of 2-dimensional chromatography to separate compounds that have the same solubility in one solvent but a different solubility in a different solvent.

Interpretation and evaluation

There is an opportunity to discuss results and the reason why learner's results may not match the expected results. For propanone these are:

Carotene	*0.95*
Chlorophyll a	*0.60*
Chlorophyll b	*0.50*
Xanthophyll	*0.35*

Learners should discuss possible improvements, such as better application of spots, longer chromatography paper to give more time and distance for separation and different supporting material. This provides an opportunity to introduce other types of chromatography, for example, silica gel (thin layer chromatography), column chromatography using cellulose powder, solvents that are mixtures and two-way chromatography.

Other uses of chromatography could also be discussed, for example using two-way chromatography to work out the sequence of reactions in the light independent stage of chromatography.

Information for technicians

Each learner will require:

- propanone in closed containers, labelled **Highly Flammable Irritant**.**[H]** Nail varnish remover can be used as an alternative to propanone. The volume required will depend on the container used for chromatography. **[F]**
- 5 – 10 fresh leaves.
- 1 × pair of scissors
- 1 × mortar and pestle
- 2 or 3 x 10 cm length capillary tubing or ignition tubes
- large test-tube or small beaker. Transparent plastic drinking glasses can be used Expanded polystyrene should not be used.
- 1 x Pasteur pipette
- Watman® no.1 chromatography paper, or filter paper or coffee filter
- metal foil or parafilm for covering chromatography container
- support for large test-tube if required, e.g. a rack or clamp stand, boss and clamp.
- 1 × pair of safety glasses
- 1 × 15 or 30 cm ruler, marked in mm
- 1 x pencil
- 1 x container for disposal of leaves and used propanone
- Access to hot water if required

Additional instructions

An extractor cabinet or fume cupboard should be used if possible to run the chromatograms. Otherwise a well-ventilated room with windows that open is necessary. Air conditioning should not be used. There should not be any naked flames in the area.

Capillary tubes can be made from glass tubing by heating the centre of a length of glass tubing until it softens and then pulling out. The thin tubing and then cut into sections. These should be made before the practical. Heat proof gloves and eye protection should be worn.

Hazard symbols

C = corrosive substance

F = highly flammable substance

H = harmful or irritating substance

O = oxidising substance

N = harmful to the environment

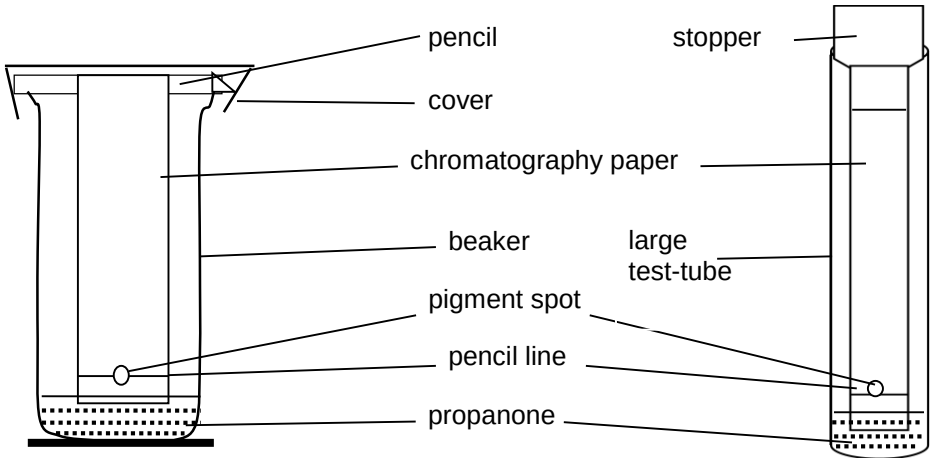
T = toxic substance

Worksheet

Aim

To separate and identify the pigments present in leaves using chromatography.

Apparatus



Using a beaker

Using a large test-tube

Method

You are using propanone, which is a highly flammable and irritant chemical. You must make sure there are no naked flames in the room at any time. You should try not to breathe too many fumes and keep containers with propanone covered.

Safety glasses must be worn when preparing the slide.

1. Cut up some leaves into small pieces.
2. Put the leaf pieces into a mortar to about 2 cm depth.
3. Add about six drops of propanone using a Pasteur pipette.
4. Grind the mixture with a pestle for at least 3 minutes. If the leaves are very thick, add a small quantity of sand to help the cells to break.
5. Cut a strip of chromatography paper to fit into a large test-tube or small beaker.
6. Draw a pencil line 15 mm from one end of the chromatography paper.
7. Using a pencil, mark places on this line where the leaf extract will be placed. This is the origin.
8. Use a capillary tube to draw up liquid from around the crushed leaves.
9. **Touch** the capillary on to the origin mark on the chromatography paper. The leaf extract will flow onto the paper. You must keep the spot as small as possible (no more than 2 mm).
10. Allow the spot to dry and then add another spot on top.
11. Add 5 more spots of solution, letting each one dry before putting on the next. This should give a very concentrated small spot on the paper.
12. Put propanone into a large test-tube or small beaker until there is a 10 mm depth.

Worksheet, *continued*

13. If you are using a large test-tube:
    - place it into a rack or other support. Lower the chromatography paper into the test-tube with the origin towards the propanone. The end of the paper should just touch the propanone. Do not allow it to rise above the origin. Fold the other end of the paper over the edge of the test-tube and insert a stopper. Try not to move the test-tube.

    If you are using a beaker:
    - fold the top end of the paper over a pencil and lower the paper into the beaker with the origin towards the propanone. The end of the paper should just touch the propanone. Do not allow it to rise above the origin. Cover the beaker with metal foil or parafilm. Try not to move the beaker.
14. Leave the apparatus to allow the propanone to rise up the paper and separate the pigments in the leaf extract. This is called 'running a chromatogram'.
15. Observe the order in which the different colours of pigment appear.
16. When the propanone is about 10 mm from the top of the paper, remove the paper and mark the position of the solvent. This is the solvent front.
17. Leave the chromatogram to dry.
18. The crushed leaves and the used propanone must be disposed of safely.

Results

1. Outline the shape of each spot you can see in pencil and label the colour. The colours fade very quickly so you should do this as soon as the chromatogram is dry.
2. Identify each pigment by its colour.
3. Find the R_f value of each pigment on your chromatogram.
    a) Measure from the origin to the solvent front
    b) Measure from the origin to the centre of each pigment spot
    c) Calculate the R_f value.

$$R_f = \frac{\text{distance moved by pigment spot}}{\text{distance moved by solvent (propanone)}}$$

If your spots have spread, to find the centre measure to the front of a spot and to the back of the same spot. The average of these is the centre of the spot.

Compare your results to the results from other species of leaf and describe the similarities.

Interpretation and evaluation

1. Draw conclusions about the different pigments observed in your leaf and those shown in the leaves of different species. Explain why leaves have these pigments.
2. Review the procedure used and identify sources of error. Make suggestions about improvements. This could include using other solvents and other types of chromatography.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 9

Measuring the effect of wavelength of light on photosynthesis

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To use a redox dye, DCPIP, to measure the effect of light on photosynthesis by varying the wavelength of light. This practical is intended to focus on planning, in particular, defining the problem and identification of variables.

Outcomes

Syllabus section 13.2 (d)

Skills included in the practical

A Level skills	How learners develop the skills
Planning	Identify the independent and dependent variables Make a hypothesis and express this in words Identify the variables that should be controlled
Analysis	Calculate rates, standard deviation (s) and standard error (S_M) Draw a graph and add standard error bars
Evaluation	Calculate rates, standard deviation (s) and standard error (S_M) Draw a graph and add standard error bars
Conclusions	Describe and explain the relationship between light wavelength and photosynthesis Explain the relationship between this experiment and the light dependent reactions of photosynthesis

This practical provides an opportunity to build on essential skills introduced at AS Level.

AS Level skills	How learners develop the skills
MMO collection	Make qualitative observations about colour changes Record quantitative results, time for colour to change
PDO recording	Record qualitative observations and quantitative results in appropriate tables

Method

Safety glasses must be worn when preparing the slide.

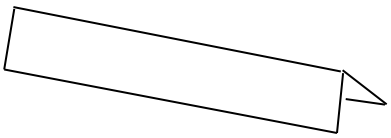
- In the light dependent reaction of photosynthesis, electrons are excited using energy from light. These high-energy electrons are passed through chains of electron carriers into NADP, which becomes reduced NADP by accepting the electrons and hydrogen ions from the photolysis of water. This provides an opportunity to remind learners that the movement of electrons through provides the driving force for ATP synthesis.
- Learners need to understand that chloroplasts contain a variety of pigments that can absorb light of different wavelengths, but that chlorophyll uses some wavelengths of light more effectively than others. A preliminary discussion about the visible spectrum of light and wavelengths of light can be used to encourage learners to think about questions such as, ‘Why do leaves look green?’ to develop an understanding that some wavelengths are reflected and some are absorbed. The idea of the absorption spectrum could also be introduced.

Guidance for teachers, *continued*

- Learners need to understand that chloroplasts contain all the necessary pigment, electron carriers and enzymes to reduce NADP and synthesise ATP. If chloroplasts are extracted from leaves, the same reactions occur and other oxidised materials can accept the electrons and hydrogen ions, releasing oxygen. This is called the Hill reaction after its discoverer, Robert Hill.
- Some coloured chemicals can act as electron and hydrogen ion acceptors and change colour as they are reduced. Oxidised 2, 6-dichlorophenolindophenol (DCPIP) is bright blue, and when reduced, for example by high-energy electrons and hydrogen ions from the light dependent reaction of photosynthesis, it becomes colourless. Methylene blue can be used but DCPIP works best as it is very sensitive.
- DCPIP provides a way of measuring how fast the light dependent reaction is happening as the time taken for the colour to change from blue to colourless can be timed.
- Learners should be given a summary of the information about the light dependent process of photosynthesis, the visible light spectrum, the absorption spectrum and the information about how DCPIP can be used to demonstrate the release of electrons and hydrogen ions. Using this information, learners should then be asked to plan an investigation into the effect of light wavelength on the rate of photosynthesis.
- Learners should be asked to use the information given to:
  - Identify and write down the independent and dependent variables. Help may be needed as learners often confuse the independent and dependent variables. They should work out that the variable being **changed** is wavelength of light (independent variable) and the variable being **measured** is the time for the blue DCPIP to change to colourless (dependent variable). Learners should be encourage to think in terms of what is actually **measured**, so 'rate of reaction' is not the actual dependent variable.
  - Write down the hypothesis that can be tested. The precise hypothesis will vary from student to student, depending what information they use to help guide them. Accept any valid hypothesis, e.g. the time for the DCPIP to become colourless will vary with the wavelength of light; DCPIP will decolourise faster in blue light.
  - Sketch a graph to represent the hypothesis. This should be consistent with their hypothesis.
  - Decide which are important variables that should be standardised (controlled). Learners need to be encouraged to think about which variables are likely to have an effect on photosynthesis e.g. temperature; volumes and concentrations of extraction medium and DCPIP; leaf area / volume of extract used and species / type of leaf; light intensity; pH.
  - Describe how each of these variables could be controlled. Learners need to be encouraged to think about realistic ways of achieving this related to the apparatus being used, e.g. it would not be practical to use a water-bath to control the temperature during the timing, so it might be appropriate to work at room temperature and assume that the temperature will not vary greatly so the error will be random and affect all the samples in the same way.
- Learners are instructed to make a chloroplast extract. Leaves should be cut into small pieces and placed into the plastic specimen tube until it is half full and 2 cm^3 of extraction medium is added. The glass rod should then be used to grind the leaves with the extraction medium for approximately 1 minute. The liquid should then be decanted or filtered through muslin into the petri dish and covered with metal foil. If the room is hot, then the dish may need to be placed onto ice. This provides an opportunity to discuss the experimental procedure so that learners consider why some of the processes are necessary for the investigation to work, for example, why the extract must be kept cold, why the extraction medium contains sucrose at the same water potential a cell and why the extract must be decanted or filtered.

Guidance for teachers, *continued*

- Learners are then instructed to use the equipment to test the effect of different wavelengths of light on photosynthesis. Coloured filters are used that allow specific wavelengths of light to pass through; purple, blue, green, orange and red. Coloured plastic sheets can be obtained from photographic or theatrical suppliers that only allow a specific wavelength to pass. Transparent coloured plastic folders from stationary suppliers will work but the results will not be as clear. These should be cut into pieces about 10 cm x 5 cm, so they can be folded length ways to form a tent as shown in the diagram.



- Learners should place one of the capillary tubes vertically into the leaf extract in the Petri dish and draw up some leaf extract. This should be laid on its side on the white tile. This is the control and is used as a colour comparison. The same one is used throughout the investigation. DCPIP should then be added to the extract in the petri dish until it becomes blue. Learners need to be instructed to only add about 5 drops at a time and to stop as soon as the colour changes. If too much DCPIP is added the colour is very intense and takes more time to reduce. The dish should then be covered with metal foil immediately.
- Learners should then prepare the coloured filters before filling another capillary tube with the chloroplast extract and DCPIP mixture. The capillary tube containing DCPIP is a test capillary and should be laid next to the control capillary on the white tile and the purple filter placed over both tubes. A bench lamp should be placed facing one side of the filter and switched on. A stop watch should be started at the same time as the light is switched on. The stop watch should be stopped as soon as the tube containing DCPIP becomes the same colour as the control. To check the colour, learners should be directed to lift the side of the filter furthest from the light.
- Learners should then fill a clean capillary with chloroplast extract and DCPIP mixture and repeat timing for the blue filter. Each of the coloured filters should be tested in turn using the same procedure. If time is limited then different learners can test different colour filters and share results. Times will vary depending on the intensity of the DCPIP colour, the quality of the extract and the colour of the filter. The shortest times are expected for purple and blue (commonly 1-2 minutes), the next shortest times for red (commonly 2-3 minutes) and the longest times for green (commonly 8-10 minutes). Learners should be instructed to stop timing if an extract takes longer than 10 minutes and record ‘no colour change’.

Guidance for teachers, *continued*

Results

1. Learners should construct a table in which to record the time at one minute intervals until the test capillary tubes become the same colour as the control. The total time for each wavelength should then be recorded.

   Class results should be pooled, and another table constructed to record all of the results. Learners should evaluate these results and identify those which may be anomalous.

2. Learners calculate the mean value for each colour of filter, taking into account anomalies, and calculate the rate of reaction using the formula:

$$\frac{1}{\text{time / s}}$$

3. Learners use these values to calculate the standard deviation (s) and standard error (S_M) using the formulae:

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}} \quad \text{and} \quad S_M = \frac{s}{\sqrt{n}}$$

   where n = sample size (number of observations), $\bar{x}$ = mean, Σ= 'sum of'

4. Learners then plot a graph of the mean results and add error bars using the values of S_M they have calculated.

These activities provide an opportunity to build on AS Level skills of graph plotting and will develop A Level skills in using statistical formulae. Learners should be reminded about the expected orientation of graphs, with the independent variable on the *x*-axis with correct units and the independent variable on the *y*-axis with correct units. Graphs can be drawn using the colours of light, but it is better to use the actual wavelengths as a more representative graph will be obtained. If the filters used do not have a specific wavelength then approximate ones, according to the colour, can be given to learners to use.

colour	wavelength / nm
purple	425
blue	450
green	525
orange	625
red	675

Interpretation and evaluation

1. The length of the error bars will then be used to assess the reliability of the data collected. Learners need to understand that long error bars indicate less reliability than short error bars.
2. Learners should describe the effect of the wavelengths of light on the rate of photosynthesis.
3. The shape of the graph should then be compared to the shape of the graph of the absorption spectrum and conclusions made about the relationship between the wavelengths absorbed and the rate of photosynthesis. This provides an opportunity to introduce the idea of an action spectrum and also to relate the presence of different pigments to the absorption of different wavelengths of light. It also provides an opportunity to discuss the energy available from different wavelengths of light, which could be extended to discuss the effect on plants that live in different depths of water.

Guidance for teachers, *continued*

Extension

Learners could then be asked to use this procedure to write out a method to test the effect of different light intensity on the rate of photosynthesis. They could be provided with information that neutral density grey filters that reduce the intensity of light passing through are available.

description	% of light transmitted
pale grey	70
mid-grey	50
dark grey	25

This provides an opportunity for learners to follow the model used in paper 5, of giving information from which a method has to be devised. It should be stressed that this method should have practical details that would allow another person to use it without any further information about the procedure.

Information for technicians

Each learner will require:

- 2 cm^3 of very cold extraction medium, labelled **extraction medium.** In a hot room this should be placed inside a water and ice mixture
- 2 cm3 solution DCPIP (2, 6-dichlorophenolindophenol) solution, labelled DCPIP solution
- 1 x fresh green cabbage or spinach leaf. Any soft, green, non-toxic dicotyledonous leaf would be suitable
- 1 × shatterproof plastic specimen tube (minimum 3 cm x 1 cm) that will withstand being squeezed
- 1 x thick short glass rod that will fit into the specimen tubes (15 cm)
- 1 x white tile
- 1 x Petri dish base or top
- 1 x desk lamp
- 6 ignition tubes (thin wall capillary tubes 10 cm long) or six pieces of capillary tube cut to a length of 4-10 cm each, with any sharp edges removed
- metal foil sufficient to cover the Petri dish
- coloured filters, each allowing a specific wavelength of light to pass through:

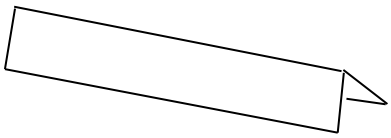
Colour	**Wavelength / mm**
purple	425
blue	450
green	525
orange	625
Red	675

- 2 × 2 cm^3 syringes
- 1 x dropping pipette
- 1 x pair of safety glasses

Information for technicians, *continued*

Additional instructions

1. The extraction medium must be made before the investigation. It does not keep more than 48 hours in a refrigerator and must be very cold when used. The extraction medium consists of phosphate buffer, sucrose and potassium chloride (KC*l)*. The best concentration to use is 34.23 g sucrose and 0.19 g KC*l* in 250 cm^3 of phosphate buffer solution.
2. DCPIP can be bought as a ready-made solution or made from the powder and phosphate buffer or made from the powder, KC*l* and phosphate buffer. A concentration of DCPIP that work well is made by dissolving 0.4 g DCPIP and 0.93 g KC*l* in 250 cm^3 phosphate buffer solution **at room temperature.** It does not keep more than 48 hours in a refrigerator.
3. Phosphate buffers consist of disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$) and potassium dihydrogen phosphate (KH_2PO_4) in varying proportions, depending on the precise use. For this investigation a buffer that works well is made by dissolving 4.48 g $Na_2HPO_4.12H_2O$ and 1.7 g KH_2PO_4 in 500 cm^3 of distilled water. This keeps for several weeks if stored in a refrigerator. Learners do **not** need to be given any of the phosphate buffer solution.
4. Coloured plastic sheets can be obtained from photographic or theatrical suppliers that only allow a specific wavelength to pass. Transparent coloured plastic folders from stationary suppliers will work but the results will not be as clear. These should be cut into pieces about 10 cm x 5 cm, so they can be folded length ways to form a tent as shown in the diagram.



Note: These recipes are needed as the solutions are very specific and the experiment will not work if they are incorrect.

Worksheet

Aim

To use a redox dye, DCPIP, to measure the effect of light on photosynthesis by varying the wavelength of light. This practical is intended to focus on planning, in particular, defining the problem and identification of variables.

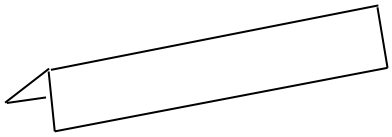
Method

Safety glasses must be worn when preparing the slide.

Preparation of leaf extract.

1. Put the leaf onto a tile.
2. Cut out and discard any large veins. Then chop the leaf into small pieces.
3. Put the pieces into a plastic tube and add 2 cm^3 of very cold extraction medium.
4. Grind with a glass rod for one minute to give a green liquid (the leaf extract).
5. Decant (pour) the leaf extract slowly into a Petri dish with one edge.

   If there is a lot of leaf debris, filter through muslin or fine netting.
6. Place a metal foil cover over the Petri dish to keep light out.
7. Fold the different coloured filters along their length to make little tents, and put them on the white tile like the one in the diagram.



Preparation of capillary tubes and making observations. Steps 4 and 5 need to be done fast.

1. Dip the end of one of the capillary tubes into the leaf extract in the Petri dish so that some extract rises up the tube. This is the control. Lay this tube on the white tile.
2. Add 5 drops of DCPIP solution to the leaf extract in the Petri dish and mix. If no blue colour is visible add another 5 drops and mix. Repeat until the green leaf extract is a blue-green colour. Cover with the metal foil immediately.
3. Lift the edge of the metal cover and dip the end of another capillary tube in the blue-green leaf extract / DCPIP mixture. Recover the dish. This is a test extract. Lay this on the tile next to the control. Cover the two capillary tubes with a tent of a purple filter.
4. Switch on the lamp so that the light falls evenly onto the filter and start timing. At one minute intervals lift the filter on the side opposite to the lamp and record the colour of the test extract. If the extract is still blue after 10 minutes, record as '>10 minutes'.
5. Discard the test capillary as soon as it has changed back to green. Leave the control on the white tile.
6. Repeat step **3** but place a blue filter over the two capillary tubes. Then repeat steps **4** and **5**.
7. Repeat steps **3**, **4** and **5** for each of the coloured filters in turn.

Worksheet, *continued*

Results

1. Prepare a table to record the colour of each tube at 1 minute intervals.
2. Record the total time taken for the blue colour to disappear from each tube in the same table.
3. Prepare a second table to record the pooled class results of the time taken for the blue colour to disappear from each tube.
4. Identify any anomalies in the pooled data.
5. Calculate the mean time taken for the blue colour to disappear from each tube.
6. Calculate the mean rate of reaction using 1 / time taken for blue colour to disappear. If the time is recorded as >10 minutes, then record 1 / time taken for blue colour to disappear as 0.
7. Use the formulae below to calculate the standard deviation (s) and standard error (S_M) for each wavelength of light.

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}} \quad \text{and} \quad S_M = \frac{s}{\sqrt{n}}$$

where n = sample size (number of observations), $\bar{x}$ = mean, Σ= 'sum of'

8. Plot a graph showing the effect of light intensity on the rate of photosynthesis. Add error bars using your calculations of standard error.

Interpretation and evaluation

1. Use the length of the error bars to assess the reliability of the data collected.
2. Describe the effect of the wavelengths of light on the rate of photosynthesis.
3. Compare your graph to the shape of the graph of the absorption spectrum and draw conclusions about the relationship between the wavelengths of light absorbed and the rate of photosynthesis.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 10

Measuring the effect of gibbererllin on the amylase activity of germinating maize

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To expose germinating maize grains to different concentrations of applied gibberellin (gibberellic acid) to stimulate amylase activity. To then assay the activity of the enzyme using cut halves of the maize grains and starch agar and measuring the area of starch digestion.

Outcomes

Syllabus sections 15.2 (c), 16.3 (d) and 17.1 (c)

Skills included in the practical

A Level skills	How learners develop the skills
Planning	Decide how to dilute a stock solution
Analysis	Calculate area of a circle Calculate rate of reaction Draw a graph and add standard error bars Calculate standard deviation (s) and carry out a t-test, including: • stating a null hypothesis • calculating t • calculating degrees of freedom • use a probability table • decide if results are significant Produce a calibration curve to find the actual concentration of amylase – extension work, optional
Evaluation	Evaluate the method used and suggest sources of error and how these might be improved
Conclusions	Describe the effect of the concentration of gibberellin on the activity of starch amylase in the maize halves with the embryo and those without the embryo Explain their results using appropriate theory

This practical provides an opportunity to build on essential skills introduced at AS Level.

AS Level skills	How learners develop the skills
MMO collection	Record quantitative results, measuring diameter and using a grid
PDO recording	Record quantitative results in appropriate tables

Guidance for teachers, *continued*

Method

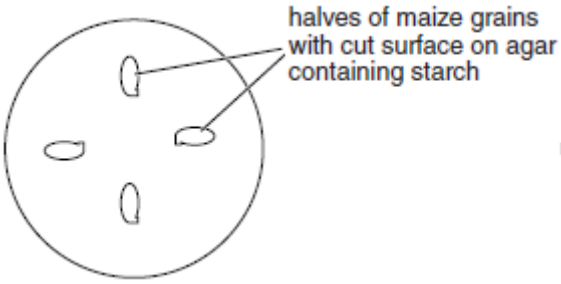
- Cereal grains, such as barley and maize, contain an embryo and endosperm, which is a storage tissue. During germination, amylase enzymes are produced in the aleurone layer around the endosperm. These enzymes diffuse into the endosperm and catalyse the breakdown of starch reserves to maltose.
- The production of amylases in the aleurone layer is triggered by the release of gibberellin from the embryo in response to water. Gibberellin is also known as gibberellic acid and as GA3.
- Amylase activity is measured by the breakdown of starch to give the reducing sugar maltose. It is possible to measure amylase activity by placing grains that are cut in half onto starch-agar in Petri dishes and measuring the area of starch digested. Learners should be reminded about AS knowledge of enzymes and how to test for the presence of starch and reducing sugars.
- The natural concentration of gibberellin in plant tissue is very low, approximately 346×10^{-6} g dm^{-3} which is equivalent to 1.0 μmol dm^{-3}. Gibberellins can be supplied to seeds to promote germination and in the brewing industry are sprayed onto germinating barley to increase maltose production from starch.
- This investigation has three main stages and two periods of time where the investigation has to be left for at least 24 hours. This means planning lesson time to take this into account.

Stage	Activity	Approximate time to complete / minutes
1	Making solutions and soaking grains. These need to be left for **24 hours**. Optional: learners make their own starch agar plates.	40 40–60
2	Cutting maize grains and placing then onto starch-agar plates. These need to be left for **24 hours**.	30
3	Measuring areas on agar plates.	40

- Learners should be provided with dry maize grains and a 3 mmol dm^{-3} stock solution of gibberellin and sodium hypochlorite solution. The learners are asked to decide how to dilute the stock solution to give a suitable range of concentrations of gibberellin to test on the maize. This provides an opportunity to review AS knowledge of serial dilution and simple dilution and to discuss which would be most appropriate for this investigation. Learners should also discuss how many different dilutions should be used in terms of the range, intervals between concentration as well as the time and equipment available to carry out the investigation.
- Learners should then prepare the dilutions they have decided. They will surface sterilise maize grains and place 6 grains in each dilution. Barley can be used for this investigation, but the grains are much smaller and difficult to handle. These will need to be left for 24 hours to soak. If learners are to make their own starch-agar plates this should also be prepared and left for 24 hours. If resources are limited then this can be carried out by pairs of learners.

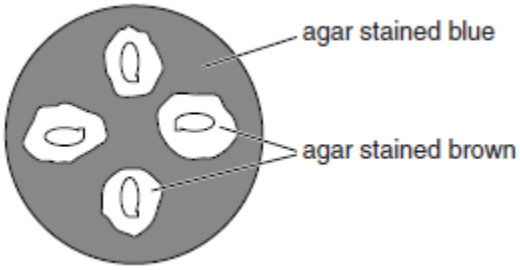
Guidance for teachers, *continued*

- Learners should use two starch-agar plates for each dilution of gibberellin. They are instructed to label the Petri dishes on the underside with their name and the dilution of gibberellin that grains have been soaked in. Learners should then select four maize grains from the highest concentration of gibberellin and cut them vertically into two halves. The halves from two of the grains should then be placed cut surface downwards on one of the labelled starch-agar plate, as shown below. The embryo should be removed from the remaining two grains and then placed cut surface downwards on the second starch agar plate. This should then be repeated for each concentration of gibberellin. Learners should be instructed to take care to keep the lids on the Petri dishes containing starch-agar and to only lift them when placing the maize grins onto the starch-agar.



- Once all the starch-agar plates have been completed the learners should stack the Petri dishes on top of each other and leave them for 24 hours in a dark place. If the results cannot be obtained after 24 hours, the plates can be left for 48 hours at room temperatures of 20 °C – 25 °C. At higher temperatures they should be placed in a refrigerator after 36 hours to prevent the digested areas from overlapping.
- To obtain results, learners are instructed to add 10 drops of iodine solution and swirl the plate to spread the iodine over the plate.

  The expected appearance is shown in the diagram.



Results

1. Learners are instructed to measure the area of the starch-agar plate that has been digested. This provides an opportunity to discuss how this might be achieved. Learners should be able to suggest measuring the diameter of the brown zone and using the formula πr^2. Some may also suggest tracing onto graph paper and counting squares.

   Learners should be directed to observe the shape of the areas stained brown and led towards the idea of measuring several diameters to obtain a mean to use in the calculation of area. Learners should also be introduced to transparent grids as an alternative to graph paper.

Guidance for teachers, *continued*

2. Learners should construct a table to record their results for each dilution of gibberellin for both the halves with the embryo and those without the embryo. They may need to be reminded that since there are 4 halves on each starch–agar plate, a mean can be calculated. A graph showing the concentration of gibberellin and the area of starch digestion should be plotted. If the exact time is known then a rate can be worked out by dividing the area by the time. Learners should be reminded about the rules for orientating both tables and the graphs and that units are need.
3. Learners should then describe the effect of concentration of gibberellin on the activity of amylase and also comment on any differences between the maize halves with the embryo and those without the embryo.
4. Learners should then be directed to think about the reasons for their observed results and draw conclusions about the effect of gibberellin.
5. To carry out a *t*-test, class results need to be pooled to give sufficient data. 10 sets of data should be sufficient. Individual values should be used, so 3 students will have a total of 12 values for each of the maize halves with the embryo and those without the embryo.

   Learners should:

   - explain why the *t*-test can be used for the results of this investigation
   - state a null hypothesis
   - use the formula to find the standard deviation (*s*) for maize halves with the embryo and for those without the embryo and the *t*-test to obtain a value of ***t***.

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}} \quad \text{and} \quad t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

   - work out the number of degrees of freedom (*v)*
   - use a probability table to find the critical value at 0.05 probability
   - decide whether the difference in amylase activity of the maize halves with the embryo and those without the embryo is significant.

   Learners often find statistical analysis confusing. It is important that learners understand that a null hypothesis always makes an assumption that there is no significant difference between the two populations being tested, in this case, amylase activity of the maize halves with the embryo and those without the embryo. A null hypothesis needs to state clearly what is being compared.

   Learners also need to understand that degrees of freedom are worked out from the number of samples. Assuming that 12 measurements are recorded for maize halves with the embryo and those without the embryo, then n = 12 and:

   - degrees of freedom for maize halves with the embryo is n – 1, i.e. 12 – 1 = 11
   - degrees of freedom for maize halves without the embryo is also 12 – 1 = 11
   - the total degrees of freedom is 22, which is the row of the probability table that the learners need to use to find the critical value with which to compare the calculated value of ***t.***

Guidance for teachers, *continued*

Interpretation and evaluation

Learners should evaluate the reliability of the results. This could be done by discussing the methods used for obtaining the results, listing all the possible sources of error and deciding if there is any way the reliability could be improved.

- Some problems identified may include measuring the area of an irregular shape, observing the 'edge' where the colour changes and being unable to calculate the rate of starch breakdown accurately. They should also suggest alternative ways of estimating the amylase activity, such as making an extract from the grain and testing it with a starch solution of known concentration.
- Learners should also be directed to think about a control and whether this could be used to improve the reliability of the results.
- The way in which variables that have been standardised should also be considered to decide if all the variables that might influence the results have been taken into account and whether the method of standardising could be improved.
- Other issues such as uncontrolled or non-standardised variables could also be considered, such as rate of diffusion of enzyme from the grain and the pH of the starch-agar. This provides an opportunity to discuss the idea that there may be variables that cannot be standardised.

Extension

1. A calibration curve could be made by using a known concentration of starch and measuring the rate of disappearance of starch with different known concentrations of amylase.
    - Learners should be provided with 2% amylase solution to make a simple dilution series of: 1.5%, 1%, 0.5%, and 0.25% amylase solution.
    - A 1 cm^3 sample of each amylase solution is added to 1 cm^3 of a 1% starch solution and tested at 30 second intervals using a spotting tile.
    - Learners place drops of iodine solution in rows on a tile and remove a sample from each dilution of amylase at 30 second intervals until the iodine remains brown. The rate of starch hydrolysis can be calculated in mg s^{-1}.
    - Learners can calculate the number of mg of starch in 1 cm^3 of a 1% starch solution and divide by time in seconds.
    - The calibration curve is plotted with concentration of amylase as the *x*-axis and rate of starch hydrolysis as the *y*-axis.
2. To find the amylase concentration in a maize grain, the two halves of a grain should be crushed in water and filtered. 1 cm^3 of the filtrate is added to 1 cm^3 of a 1% starch solution and tested in the same way as the known amylase concentrations. The rate of reaction is then found on the *y*-axis of the calibration curve and the amylase concentration found on the *x*-axis.

Information for technicians

Each learner will require:

Stage 1

- 1% sodium hypochlorite (sodium chlorate) solution for surface sterilising the grains **[H]**
- 1 g dm-3 solution of gibberellin (gibberellic acid)
  1 g dm-3 solution is approximately 3 × 10-3 mol dm-3 (3 mmol dm-3) solution of gibberellin
- 5 × containers for making dilutions, e.g. 100 cm^3 beakers
- supply of distilled or deionised water
- 36 maize grains
- 4 × 2 cm^3 syringes or pipettes

Stage 2

- 20 starch-agar plates
- 1 × sharp scalpel or single edge razor blade
- 1 × forceps
- 1 × tile or cutting board

Stage 3

- Iodine in potassium iodide solution
- 1 × transparent 2 mm grid
- 1 × 15 cm or 30 cm ruler with mm divisions

Additional instructions

- Barley can be used for this investigation, but the grains are much smaller and difficult to handle.
- To make gibberellin solution, 2 cm^3 of 95% ethanol per dm^3 should be added to dissolve the gibberellin before adding distilled water. Learners will use this to make their own dilutions so sufficient quantity should be prepared for the number of learners in a class.
- Starch-agar plates may be prepared as follows using sterile apparatus:
  1. Take 100 cm^3 of water.
  2. Use a small volume of this water to make a paste with 1 g soluble starch.
  3. Boil the rest of the water and add to the starch paste. Stir until dissolved.
  4. Add 2 g of agar powder until the agar is dissolved.
  5. Boil the starch-agar solution for several minutes.
  6. Allow the starch-agar to cool to about 60 °C and pour into sterile Petri dishes to a depth of approximately 3 mm.
  7. Leave the agar to set.
  8. Store the starch-agar plates in a refrigerator until required.

Information for technicians, *continued*

Transparent grids can be made by photocopying 2 mm graph paper onto heat resistant transparencies or printing using a laser printer from an internet source onto heat resistant transparencies. Ink jet printers are not suitable.

Hazard symbols

C = corrosive substance

F = highly flammable substance

H = harmful or irritating substance

O = oxidising substance

N = harmful to the environment

T = toxic substance

Worksheet

Aim

To expose germinating maize grains to different concentrations of applied gibberellin (gibberellic acid) to stimulate amylase activity. To then assay the activity of the enzyme using cut halves of the maize grains and starch agar and measuring the area of starch digestion.

Method

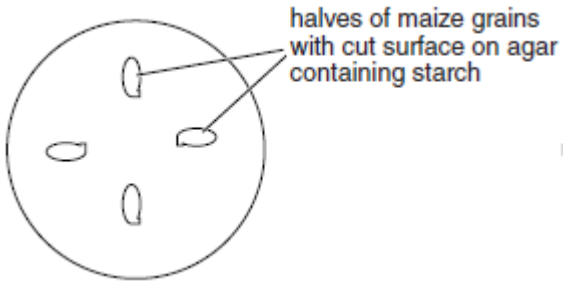
You are provided with a 1 g dm^{-3} solution of gibberellin. You must decide how to dilute this stock solution to give a suitable range of concentrations of gibberellin to test on the maize.

Stage 1

1. Prepare the dilutions of gibberellin solution that you have decided to use.
2. Surface sterilise the maize grains using the sodium hypochlorite solution.
3. Put 6 maize grains in each dilution and leave to soak for 24 hours.

Stage 2

4. Label 2 starch- agar plates with your name and the highest concentration of gibberellin solution you have made.
5. Remove 2 maize grains from the solution containing the highest concentration of gibberellin and cut them vertically into two halves.
6. Lift the lid of one Petri dish and place these halves face downward onto the starch-agar as shown in the diagram. Replace the lid as quickly as possible.



7. Remove another 2 maize grains from the solution containing the highest concentration of gibberellin and cut them vertically into two halves.
8. Remove the embryos from the bottom of the grains and then place the grain halves face down onto the starch-agar in the second Petri dish.
9. Repeat steps **3** to **6** for each of the concentrations of gibberellin solution you have made.
10. Leave the Petri dishes containing starch agar to incubate for 24 hours.

Stage 3

11. Add 6 drops of iodine solution to the surface of the starch agar and rotate the petri dish to spread the iodine solution evenly.

Worksheet, *continued*

Results

1. Prepare a table to record the results for each concentration of gibberellin solution. This should have sufficient cells to record, separately, each of the 4 maize halves with the embryo and each of the four halves without the embryo and to calculate the mean for each concentration.
2. Measure the area of the starch-agar stained brown. Use two different methods:
   - use a ruler to measure the diameter of the brown area and use the formula for the area of a circle to calculate the area
   - turn the starch-agar plate upside down and place the transparent 2 mm grid over the brown area. Trace the shape of the area onto the grid and then use systematic counting to find the area.
3. Plot a graph of the mean area of starch-agar stained brown against the concentration of gibberellin solution. If you know the exact time for which the starch-agar plates were left, then calculate the rate as $mm^2\ h^{-1}$.
4. Describe the effect of the concentration of gibberellin on the activity of starch amylase in the maize halves with the embryo and those without the embryo.
5. Use your knowledge of the role of gibberellin to give reasons for your results and explain any difference between the maize halves with the embryo and those without the embryo.
6. Choose a concentration of gibberellin that appears to have a clear difference between the maize halves with the embryo and those without the embryo. Use the pooled class results to collect at least 6 other results for the maize halves with the embryo and those without the embryo from the same concentration of gibberellin solution.
7. Use the data you have collected in step **6** to find out if the difference between the maize halves with the embryo and those without the embryo is significant, using the formulae for standard deviation (*s*) and the *t*-test.

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}} \quad \text{and} \quad t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

Interpretation and evaluation

Evaluate the reliability of the results by discussing the methods used for obtaining the results, listing all the possible sources of error and describing any ways the reliability could be improved. You should consider:

- problems such as measuring the area of an irregular shape
- the role of a control
- whether all the variables that might affect the results have been taken into account
- whether there are any variables that cannot be controlled.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 11

Invesitgating biodiversity of an ecosystem

Cambridge International AS & A Level Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To use a variety of techniques to study the distribution of species in an ecosystem, to estimate population size and consider any relationships between the distribution of species and specific environmental factors.

Outcomes

Syllabus sections 18.1 (d) (e) (f)

Skills included in the practical

A Level skills	How learners develop the skills
Analysis	To use correlation analysis of the distribution of a species in relation to an environmental factor To use chi-squared test to determine any significant differences in populations of the same species in different environments Calculate Spearman's rank correlation coefficient
Evaluation	Evaluate the methods used and the accuracy and reliability of their results.
Conclusions	Decide whether a hypothesis is supported by their data

- This practical provides an opportunity to build on essential skills introduced at AS Level.

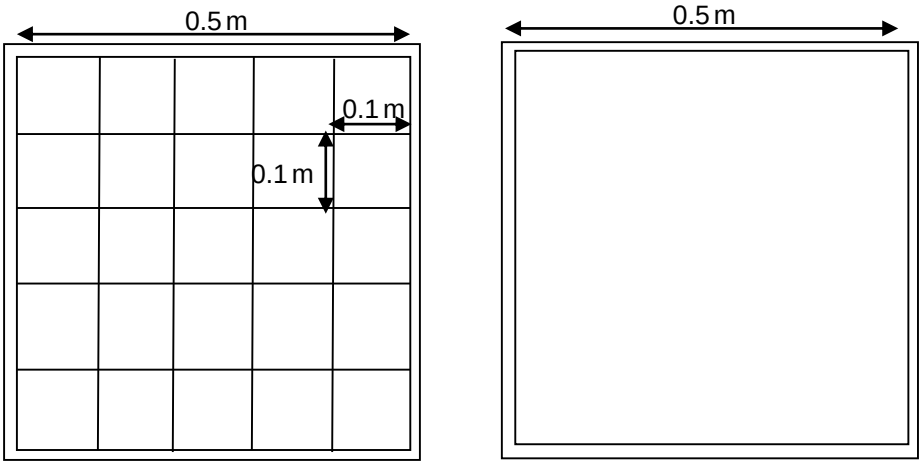
AS Level skills	How learners develop the skills
MMO collection	Record quantitative results
PDO recording	Record quantitative results in appropriate tables

Method

- Biodiversity is concerned with the variety and numbers of different species that exist in particular ecosystems and the genetic variation within species. It can be studied at many different levels from the entire world to the underside of a stone on the bottom of a stream.
- The biodiversity of specific ecosystems such as an area of woodland, sand dunes of a coastal area, a desert, the surface of the sea could be studied. This could provide an opportunity for learners to discuss their understanding of an ecosystem and identify those that are local. It is also an opportunity to remind learners that humans are part of ecosystems and can have far reaching effects on biodiversity.
- To study an ecosystem can be very time consuming so if possible at least a complete day should be allowed for learners to use ecological sampling techniques and collect data. For population estimations at least 24 hours between sampling times is needed. Depending on the quantity of data collected it may take several hours to process the results and draw conclusions. An ideal situation would be to take learners to a specific ecosystem and stay in residential accommodation so that evenings can be used to follow up on data collected during the day. If an extended time cannot be arranged, the sampling techniques may have to be carried out at different times. There may be organised courses available where the field study instructors are familiar with the species in that ecosystem. Otherwise, learners will need field guides to help identify species and teachers need to be familiar with the area and its species.

Guidance for teachers, *continued*

- As the ecosystems studied will be very different, general principles only are considered here:
  - Learners should be familiar with the use of a transect line and how to decide where to lay a line. The location of the line will usually depend on the purpose of the study. In some ecosystems this may be horizontal, for example across grass land with a footpath or along a beach, parallel to the coast line. In other cases it may be vertical, for example down the side of a slope to study changes with the slope or at right angles to an estuary to study zonation (stratified sampling).
  - Transect lines can laid using a long tape, e.g. 20 m. A transect line can be made from thin rope or cord that is knotted at 1 m intervals.
- The use of transects can be practiced in a class room by giving learners a tape or transect line and asking them to lay it across the class room. It is likely that these will be crossed over each other, so learners should be able to work out that this means some organisms will appear in two places so their numbers may be overestimated. Learners should then be able to suggest that lines should be parallel to each other and equidistant. Learners can then try point sampling by recording every object that touches the transect line at each 1 m interval. Learners need to understand that only objects that touch at that point should be recorded. Using different numbers and coloured shapes to represent species and scattering them on the floor is one way of showing how individual species can be missed.
- Learners should also be familiar with the use of quadrats and how they can be used either as part of a belt transect or for random sampling within an area.
  - Quadrats can be metal or wooden frames and have sides of 0.5 m or 1 m.
  - Some quadrats can also have internal division forming 10 cm squares with the frame. These are useful when estimating abundance.



Guidance for teachers, *continued*

- The use of quadrats can also be practiced in the classroom, either with or without a transect line.
  - For random sampling learners can use random number tables, or calculators to generate random numbers. If these are not available then telephone numbers can be used. Learners stand in the middle of the room and use the first random number, e.g. 5, to walk 5 paces in one direction, then the second random number, e.g. 3, to walk 3 paces at right angles to the first direction. For most purposes this is sufficient, but up to 4 numbers can be used, walking in a different direction each time. The quadrat is then placed on the floor with one corner on the random point. The number of each object within the quadrat is counted and recorded. The use of random numbers can also be used to determine where a transect line is to be laid.
  - For systematic sampling the quadrat is placed at 1 m intervals along the transect line. If 1.0 m x 1.0 m quadrats are used, this will give a complete 'belt' along one side of the transect line. If 0.5 m x 0.5 m quadrats are used, there will be gaps. This provides an opportunity to discuss the different ways of using a belt transect and the advantages and disadvantages of each. For example a continuous transect is very time consuming so for a quick survey quadrats may be placed at 2 m intervals. For an extended survey, quadrats may be placed both sides of the transect line.
  - The use of subdivided quadrats for estimating percentage cover can also be demonstrated by learners counting the number of squares occupied by specific objects. This could include a large number of small shapes, e.g. cut out from a hole-punch, to represent abundant species like grasses or paper clips to represent lesser numbers of larger objects. This provides an opportunity to discuss what to do when 'species' overlap, or lie on top of each other.
- If the class room activities are used then learners can be asked to discuss how well the results represent the actual classroom. This should help learners to understand difficulties in getting reliable data about an ecosystem and the need for monitoring at intervals to get a truer picture.
- During a field activity learners should work in groups to make best use of the time and at the end share results. This enables several transects to be studied. Groups of three are the most efficient, one to position quadrats and identify species, one to the count/estimate species cover, one to record the data. Tasks can be rotated. Sampling of the abiotic factors can be taken at the same time, e.g. pH of soil / water, air / soil / water temperature, wind speed, light intensity. Soil and water samples can also be taken for laboratory testing, for example humus content of soil, mineral content of soil or water, biological oxygen demand of water. Kits for environmental sampling are available that have probes and chemical tests that can be carried out in the field.
- Learners should be instructed to collect data that will enable them to look for a correlation between the distribution of a species and an environmental factor. Unless there is plenty of time available it is best to decide before hand which correlations the learners are going to look for. With a large group of learners they could be split into groups and look at more than one correlation. The correlations will depend on the ecosystem being studied. On a slope a possible correlation is water content of soil and the numbers of a specific species of grass. In woodland it could be the percentage cover of leaf litter and the numbers of a detritivores in that ecosystem.
- Learners should also to carry out a chi-squared test. This can be done using data from random quadrats. Two species are chosen and the total numbers of each species in all the quadrats put into a 2 x 2 contingency table. These tables can be used to look for associations between species and for using the formula for the chi-squared test.

Guidance for teachers, *continued*

species **A**	species **B**		
	present	absent	totals
present			
absent			
totals			

- Estimations of animal populations need a least 24 hours between sampling times. It is easier to use a relatively small ground living invertebrate species that are easy to identify and have easy to find habitats. e.g. molluscs, crustacean, ground beetles, millipedes. Winged insects, birds and mammals require special trapping and marking methods. Learners should choose an inconspicuous place to mark the chosen species and a non-toxic marker. This provides an opportunity to discuss what parts of the chosen species is inconspicuous and how it might affect the results if the mark is easily visible. Learners should collect a sample from one area. This can be a randomly determined area of known dimensions e.g. 5 m x 5 m or a particular habitat e.g. under low growing bushes or a hedge. Learners could do this as a separate exercise in a garden or park. A sample of between 50 and 100 organisms are collected and marked. After 24 hours another 50 -100 organisms are collected and the number of marked organisms counted.
- **Safety**. When preparing for a field study, learners need to be aware of hazards which will vary according to the ecosystem studied, e.g. times of tides in coastal regions, steep slopes, marshy or muddy ground, dangerous plants or animals. General safety precautions should be discussed, e.g. not going away from the group, not running, not throwing quadrats. If the ecosystem is in an isolated area then a mobile telephone or radio telephone should be available in case of accidents.

Results

1. During the field study learners need to complete a data collection sheet that shows either distance along a transect line or quadrat number and the number or percentage cover of each species found. Bare soil / ground / rock within the quadrat should also be recorded. The most useful design is:

species found	distance along transect / m **or** quadrat number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
number	2						1													
% cover																	30	70		

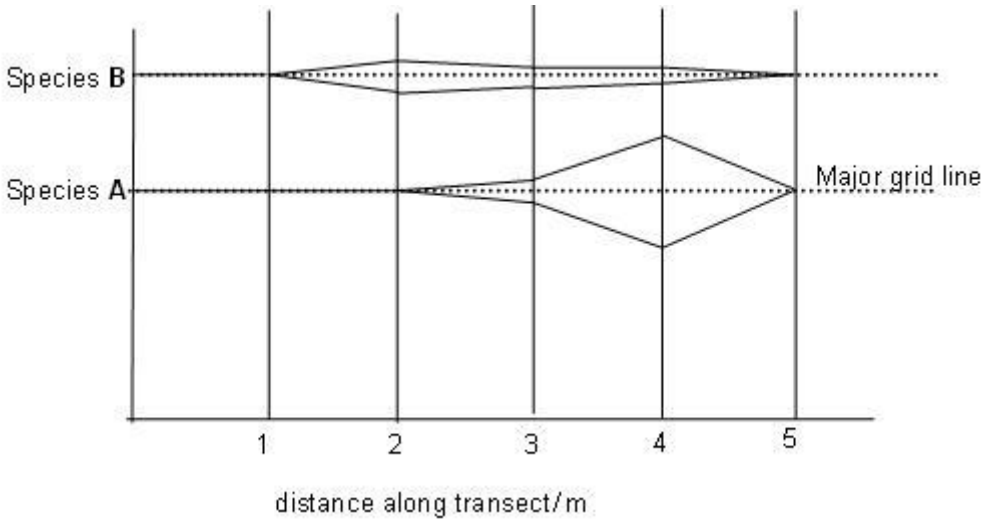
Species can be added as they are found.

Guidance for teachers, *continued*

2. Graphs showing distribution along a transect line can be drawn. The most useful type is a kite graph, particularly for species with a high percentage cover. An arbitrary scale can be used to convert % cover to a number or the 1 mm squares of a grid scaled. e.g.

percentage cover	value / arbitrary units
1-20	1
21-40	2
41-60	3
61-80	4
81-100	5

The independent variable is the distance along the transect line. The dependent variables are the percentage cover of each species, plotted as arbitrary values above and below a major grid line. Kite graphs allow changes in the distribution of species along a transect line to be easily seen.



3. Simpsons Index of Diversity can be calculated using the data collected using the formula

$$D = 1 - \left(\Sigma \left(\frac{n}{N}\right)^2\right)$$

n = number of individuals of each type present in the sample (types may be species and/or higher taxons such as genera, families, etc.)

N = the total number of all individuals of all types

4. Spearman's rank correlation coefficient should be calculated from the data collected for this purpose using the formula:

$$r_s = 1 - \left(\frac{6 \times \Sigma D^2}{n^3 - n}\right)$$

Where n is the number of pairs of items in the sample and D is the difference between each pair of ranked measurements.

Guidance for teachers, *continued*

5. Chi-squared should be carried out using the data collected into contingency tables using the formula:

$$x^2 = \Sigma \frac{(O-E)^2}{E} \qquad v = c - 1$$

6. Associations can also be considered from these tables. e.g.

species **A**	species **B**		
	present	absent	totals
present	80	95	170
absent	105	60	170
totals	185	155	340

- No association is present if the numbers are almost equal in both columns
- Positive association is when there are greater numbers in the present / present column and in the absent / absent column
- Negative association is when there are greater numbers in the present / absent column

7. Population size should be estimated using the Petersen or Lincoln index using mark-release-recapture data using the formula:

$$N = \frac{n_1 \times n_2}{m_2}$$

N = population estimate
*n*1 = number of marked individuals released
*n*2 = number of individuals (both marked and unmarked) captured
*m*2 = number of marked individuals recaptured

If population data is collected from two habitats then chi-squared test can be used to find out if the difference in population size is significant.

Guidance for teachers, *continued*

Interpretation and evaluation

Once all the data has been processed learners should describe and draw conclusions about the ecosystem studied. This should include:

- species diversity
- species distribution in relation to abiotic factors, e.g. light, temperature, pH, and biotic factors, e.g. predators, competition. Any human influences should also be considered, e.g. litter, trampling, fishing, bait collecting
- any correlations or associations between species
- population size.

Learners should also evaluate the methods used for obtaining results and the reliability of their results. Suggestions for improvements should also be made. They should be encouraged to consider the 'snap shot' idea of the results being only on that day at that time, so they may not be valid on another day. To make results more reliable, data has to be collected at different times over a long period of time.

Learners should also think about the accuracy as well as the reliability of the techniques, e.g. misidentification, not noticing rare species, miscounting the number of different species if males and females have different phenotypes, miscounting individual animals that move quickly.

Information for technicians

Each learner will require:

For a land-based field study you may require (depending on ecosystem sampled)

- transect line or tape
- frame quadrat
- clip board with a plastic bag cover if rain is likely
- 3 × recording sheets
- species identification guide
- pooter
- pitfall trap / jam jar and suitable cover to prevent water entry
- tray for hand sorting

For abiotic measurements:

- thermometer or temperature probe
- universal indicator paper or pH probe
- light meter
- 20 small plastic specimen tubes with lids, for soil or water samples
- marker pen

For laboratory measurements of soil water and humus:

- crucibles
- oven or incubator at a temperature of 80 ^{o}C. A Bunsen burner, tripod and gauze or heater can be used but care must be taken not to burn the soil.
- Bunsen burner, tripod and gauze or heater table to reach temperatures hot enough to burn soil
- balance

Additional instructions

Optional chemical tests for nitrate, chloride and other mineral ions can be carried out using standard laboratory chemicals. Methods for these tests can be obtained from the internet. Environmental testing kits can be purchased that contain all the equipment need for abiotic testing.

Worksheet

Due to the open-ended nature of this task, no worksheet is provided.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org

Cambridge Assessment International Education

Practical Booklet 12
Separating DNA using electrophoresis

Cambridge International AS & A Level
Biology 9700

Version 2.0

Cambridge Pathway

In order to help us develop the highest quality resources, we are undertaking a continuous programme of review; not only to measure the success of our resources but also to highlight areas for improvement and to identify new development needs.

We invite you to complete our survey by visiting the website below. Your comments on the quality and relevance of our resources are very important to us.

www.surveymonkey.co.uk/r/GL6ZNJB

Would you like to become a Cambridge International consultant and help us develop support materials?

Please follow the link below to register your interest.

www.cambridgeinternational.org/cambridge-for/teachers/teacherconsultants/

Introduction

Practical work is an essential part of science. Scientists use evidence gained from prior observations and experiments to build models and theories. Their predictions are tested with practical work to check that they are consistent with the behaviour of the real world. Learners who are well trained and experienced in practical skills will be more confident in their own abilities. The skills developed through practical work provide a good foundation for those wishing to pursue science further, as well as for those entering employment or a non-science career.

The science syllabuses address practical skills that contribute to the overall understanding of scientific methodology. Learners should be able to:

1. plan experiments and investigations
2. collect, record and present observations, measurements and estimates
3. analyse and interpret data to reach conclusions
4. evaluate methods and quality of data, and suggest improvements.

The practical skills established at AS Level are extended further in the full A Level. Learners will need to have practised basic skills from the AS Level experiments before using these skills to tackle the more demanding A Level exercises. Although A Level practical skills are assessed by a timetabled written paper, the best preparation for this paper is through extensive hands-on experience in the laboratory.

The example experiments suggested here can form the basis of a well-structured scheme of practical work for the teaching of AS and A Level science. The experiments have been carefully selected to reinforce theory and to develop learners' practical skills. The syllabus, scheme of work and past papers also provide a useful guide to the type of practical skills that learners might be expected to develop further. About 20% of teaching time should be allocated to practical work (not including the time spent observing teacher demonstrations), so this set of experiments provides only the starting point for a much more extensive scheme of practical work.

Guidance for teachers

Aim

To separate dyes by electrophoresis and to use this to develop an understanding of the principles by which DNA can be separated. Dyes which have different charges are used to simulate DNA so that they move different distances along an agarose gel, imitating the actual events in DNA separation.

Outcomes

Syllabus section 19.1 (d)

Skills included in the practical

A Level skills	How learners develop the skills
Evaluation	Evaluate which variables would need to be standardised when carrying out the procedure using DNA fragments Discuss how they may affect the accuracy and reliability of the results
Conclusions	Explain how gel electrophoresis is used to separate DNA fragments, using their scientific knowledge and linking it to their results

This practical provides an opportunity to build on essential skills introduced at AS Level.

AS Level skills	How learners develop the skills
MMO collection	Collect qualitative results
PDO recording	Record qualitative results appropriately

Method

- Learners need to be introduced to the idea of electrophoresis as a technique that is used to separate molecules by a combination of their charge and molecular size. It is often confused with chromatography, so it is helpful to demonstrate how to set up the electrophoresis apparatus while discussing how it works. As the electrophoresis gel needs to run for at least 90 minutes the practical will need to be conducted in two halves. Once a gel has ‘run’ it can be removed from the tank and stored in a container, covered by buffer, in a refrigerator.
- There is a supporting surface, usually agarose gel, on which the mixture of molecules to be separated is placed. The gel is placed in a tank and covered by a conducting buffer, which makes sure that an electrical current passes through the gel. Electrodes are immersed in the buffer at each end of the gel and a current passed through.
- DNA is a negatively charged molecule so it moves towards the anode (positive electrode). The agarose gel is a very complex molecular network with many narrow twisted passages. It can be thought of as a 3-dimentional sieve. Small molecules move quickly through these passages, but large ones move more slowly. This provides an opportunity to revisit AS knowledge about the structure of DNA and understand why it is negatively charged.
- DNA can be extracted from cells and cut into fragments using enzymes called restriction enzymes or restriction endonucleases. These enzymes cut DNA at specific points called restriction sites that occur throughout DNA molecules, so DNA fragments of different sizes are formed. This can be demonstrated using a ribbon or string with marks at irregular intervals to represent the restriction sites.

Guidance for teachers, *continued*

- The mixture of DNA fragments is placed at the cathode (negative electrode) end of an agarose gel. This end of the gel has wells into which DNA samples can be placed. When the current is passed through, the DNA fragments move towards the anode and separate according to their size. The DNA fragments are often added to glycerol so they sink into the wells more easily and a tracking dye is used to see how far the DNA fragments have moved and prevent them running off the end of the gel.
- DNA is colourless, so the gel has to be stained with a dye that binds to DNA, to see where each of the DNA fragments has reached. The DNA shows up as a band and is sometimes called 'visualisation'.
- Learners should then use electrophoresis to separate a mixture of dyes. These dyes represent DNA molecules. To do this separation the learners must:
  - Make an agarose gel by dissolving agarose powder in TBE buffer. This is done by adding the agarose to the buffer and heating using a Bunsen burner or heater until the powder dissolves. If a microwave is available then the mixture can be heated for 30 seconds and then for 10 seconds at a time until the agarose dissolves. When the agarose dissolves it becomes transparent.
  - Leave the agarose to cool to between 55–60 °C. This takes 6–8 minutes.
  - Prepare the electrophoresis tank by putting in casting gates and a comb for the wells.
  - When the agarose is cool, pour it carefully into the tank, between the two casting gates and leave to set. This takes about 15 minutes and provides an opportunity to explain how the agarose acts as a sieve through which molecules have to move.
  - When the gel has set, remove the casting gates and comb very carefully so the gel does not tear.
  - Pour TBA buffer into the tank until the surface of the gel is covered.
  - Use a micropipette and tips or capillary tubes to load dyes into the wells. If equipment is limited 2 or 3 learners can share a gel. The dyes can be provided separately and as mixtures, so that the idea of markers can be discussed when the results are interpreted.
  - Place the electrodes at the correct ends of the tank so that the positive electrode is at the same end as the wells loaded with dye.
  - Connect the battery pack and leave to run for a minimum of 90 minutes and a maximum of 180 minutes.
- It is possible to buy kits that use actual DNA samples, but these can be very expensive.

Guidance for teachers, *continued*

- Learners need to understand that for a complete DNA fingerprint there are six stages. They also need to understand that the dyes represent what happens to DNA during electrophoresis.
  1. Isolation of DNA (DNA extraction).
  2. Cutting DNA into fragments using restriction enzymes.
  3. Gel electrophoresis to sort DNA fragments by size.
  4. DNA denaturation (to make DNA single stranded).
  5. Southern blot onto nitrocellulose paper – this transfers the single stranded DNA onto a permanent medium (the gel is not permanent).
  6. Fluorescent probes are added to the Southern blot and base pair with the single stranded DNA on it. This is called hybridisation. The places where these specific probes bond can then be seen.

Results

1. Learners should draw a diagram of the completed gel and label the position of the dyes.
2. Explain why the dyes have separated differently.
3. Discuss what would happen if actual DNA fragments had been separated. This provides an opportunity to introduce the idea of DNA fingerprinting as a way of visualising the sequence of base pairs in a DNA sample and identifying to whom it belongs. To 'map' the entire sequence of an individual's DNA would be too difficult, so specific sections of DNA are used, which can be obtained using restriction enzymes. These specific sections are known as variable number tandem repeats (VNTRs) and are inherited genetically. The VNTR of every individual is different, so when two samples are compared it is be possible to tell the difference between the DNA of two different people. It would also be possible to identify individuals who were related to one another.

Interpretation and evaluation

1. Learners should discuss the method they have carried out and decide which variables are likely to affect the reliability of results when used for an actual DNA sample. These variables should be listed and suggestions made about how they should be standardised.
2. For DNA fingerprinting the learner should also consider what errors could occur in the procedure and how this could affect the results.

Information for technicians

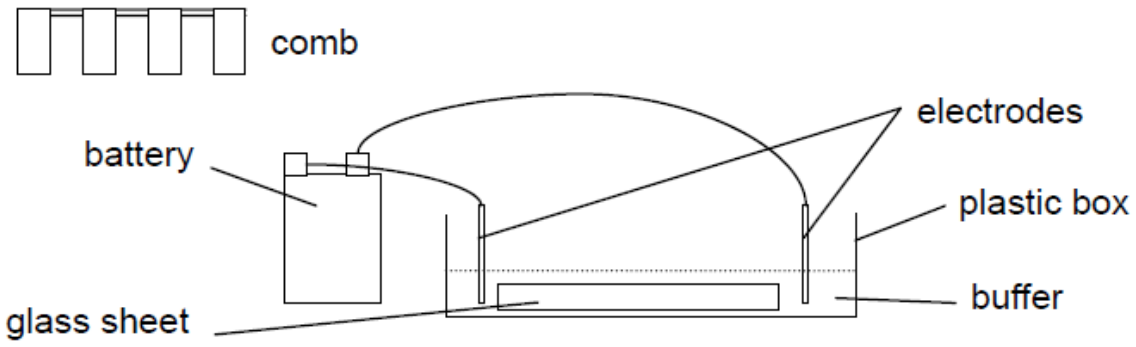
Each learner will require:

- 75 cm^3 TBE buffer **[H]**
- fluorescein (see note on dyes below) **[H]**
- methylene blue (see note on dyes below) **[H]**
- crystal violet (see note on dyes below) **[H][N]**
- orange G (see note on dyes below) **[H]**
- 0.35 g agarose powder
- one micropipette
- micropipette tips – one for each dye used. Capillary tubes can be used if micropipettes are not available.
- one Bunsen burner, tripod and gauze or access to a microwave
- once conical flask
- either one gel electrophoresis tank with battery pack, electrodes, wires, and clips or a commercial electrophoresis kit.

Additional instructions

- Once prepared, agarose gel has a short shelf life and should be discarded after removal from the electrophoresis tank.
- TBE buffer is effective to a minimum of a 10% working concentration and has a shelf life of approximately 12 months.
- Possible variables to control include: temperature, pH, concentration of TBE buffer, volume of TBE buffer, electrical charge through the gel.
- It is convenient to make more of the reagents than is required in order to give sufficient quantities for accurate measurements.
- Very small quantities of dye solutions are needed. 1 cm^3 is sufficient for 3 or 4 learners. These can be provided in a number of ways, either separately or in various mixtures. Other dyes that might be used are bromophenol blue and bromocresol green. Alternatively, red food colouring, blue food colouring, green food colouring, yellow food colouring can be used. The dye samples should be provided in small specimen tubes, labelled **A**, **B**, **C**, **D**, etc.
- It is expected that an electrophoresis kit will be available. Each kit usually has its own individual way of connecting a battery pack but will include instructions. For a practical that incorporates more aspects of DNA fingerprinting, including cutting DNA with restriction enzymes for example, the purchase of a kit that includes all the necessary materials is advised. A number of companies produce kits with instructions for practical activities.
- If electrophoresis kits are not available, they can be made using plastic boxes as buffer tanks, glass sheets for supporting a gel, a pair of electrodes (platinum is preferred although carbon will work) and a 9 V battery. A thin plastic sheet can be cut to form a comb.

Information for technicians, *continued*



Hazard symbols

C = corrosive substance

F = highly flammable substance

H = harmful or irritating substance

O = oxidising substance

N = harmful to the environment

T = toxic substance

Worksheet

Aim

To separate dyes by electrophoresis and to use this to develop an understanding of the principles by which DNA can be separated. Dyes which have different charges are used to simulate DNA so that they move different distances along an agarose gel, imitating the actual events in DNA separation.

Method

Preparing and pouring the agarose

1. Add 0.35 g of agarose powder to 35 cm^3 of TBE buffer in a conical flask.
2. Heat the mixture over a Bunsen burner using a tripod and gauze, swirling the flask occasionally to prevent any lumps forming. If a microwave is available, heat for 30 seconds, then in units of 10 seconds until the agarose dissolves.
3. Agarose becomes transparent when it boils. When it reaches this stage, remove from the heat and leave to cool for 6-8 minutes or until the temperature of the agarose is between 55 °C and 60 °C.
4. Whilst the gel is cooling, prepare the electrophoresis tank by inserting the casting gates and comb.
5. When the gel has cooled sufficiently to the desired temperature it can be carefully poured into the tank, between the two casting gates.
6. Leave the gel to set for at least 15 minutes.

Loading the gel

7. Once the gel is set, carefully remove the casting gates.
8. Very gently remove the comb, taking care not to rip the gel. The comb should have introduced ‘wells’ into the gel for loading the dyes.
9. Pour 40 cm^3 TBE buffer into the tank. This should completely cover the surface of the gel.
10. Fill a micropipette or capillary tube with dye **A**.
11. Position the pipette or capillary inside the mouth of the first well in the gel and dispense the dye into it.
12. Load dye **B** into the next well in the same way.
13. Repeat the loading procedure for each of the dyes available.
14. Connect the battery pack. The negative electrode should be at the same end as the wells loaded with dye.
15. After a minimum of 1.5 hours and a maximum of 3 hours, disconnect the battery pack and record the results.

Worksheet, *continued*

Results

1. Draw a diagram to represent the final positions of the dyes on your gel.
2. Explain why the dyes a have separated differently.
3. Discuss what would happen if actual DNA fragments had been separated.

Interpretation and evaluation

Evaluate the method, considering the following:

1. Which variables are likely to affect the reliability of results when using a sample of DNA? Suggest how these variables should be standardised.
2. When carrying out DNA fingerprinting, list the errors which could occur in the procedure and how they could affect the reliability of the results.

Cambridge Assessment International Education
The Triangle Building, Shaftesbury Road, Cambridge, CB2 8EA
t: +44 1223 553554 f: +44 1223 553558
e: info@cambridgeinternational.org www.cambridgeinternational.org