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

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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.

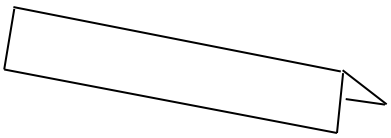
- In the light dependent reaction of photosynthesis, electrons are excited using energy from light. These high-energy electrons are passed through chains of electron carriers into NADP, which becomes reduced NADP by accepting the electrons and hydrogen ions from the photolysis of water. This provides 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:

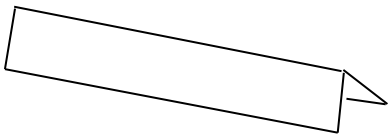
Colour	**Wavelength / mm**
purple	425
blue	450
green	525
orange	625
Red	675

- 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.

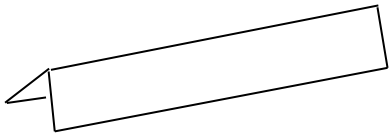
Method

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.

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