

Appendix 2 – practicals for which full details are provided

Practical 1 - M(b)(c) The effect of light intensity on rate of the Hill reaction

Defining the Problem

This practical focuses on Planning - Defining the problem. You will be developing other assessed skills throughout the practical.

Intended learning outcomes

By the end of this practical and its write-up you should be able to:

- Identify the independent and dependent variables
- Make a hypothesis and express this in words and graphically
- Identify the variables that should be controlled
- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the relationship between light intensity and photosynthesis
- Explain the relationship between this experiment and the light dependent reactions of photosynthesis

Safety information



You should wear eye protection throughout this practical.

There are no particular hazards in this practical, however you must follow your laboratory rules.

Background information

- 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, also using hydrogen ions from photolysis of water.
- If chloroplasts are broken, the enzymes that are involved in this process can reduce other oxidised materials in the same way. This is termed the Hill reaction after its discoverer.
- Oxidised DCPIP is bright blue, and when reduced, for example by high-energy electrons and hydrogen ions from the light dependent reaction of photosynthesis, becomes colourless.
- DCPIP provides a way of measuring how fast the light dependent reaction is happening, as well as giving an interesting insight into the light dependent reaction itself.

You will investigate the effect of **light intensity** on the **rate of DCPIP reduction** by the Hill reaction.

- Read the information above.
- Identify and write down the independent and dependent variables.
- Write down the hypothesis.

- Represent the hypothesis as a sketch graph.
- List the variables that should be controlled.
- Outline how each such variable might be controlled.

Light intensity in this case is varied by using filters to absorb some of the available light.

filter	light intensity as percentage of available light
none	100
pale	70
medium	50
dark	25
under foil	0

Method

Preparations

- 1 Put the leaf onto a tile.
- 2 Discard large veins and chop the leaf finely.
- 3 Put it into a plastic tube and add 2 cm³ of very cold extraction medium.
- 4 Grind with a glass rod for 1 minute to give green juice (the leaf extract).
- 5 Decant (pour) the leaf extract slowly into a Petri dish with one edge resting on the clean white tile.
- 6 Place a loose aluminium foil cover over the Petri dish to keep light out but be easy to remove to take samples.
- 7 Fold the three grey filters along their length to make little tents, and put them on the tile.
- 8 Make up a table to include the colours of the contents of tubes A, B, C, D, E and F every minute from 0 to 10 minutes.

Preparation of capillary tubes and making observations - steps 4 and 5 need to be done fast

- 1 Stand one of the capillary tubes in the leaf extract in the Petri dish so that some extract rises up the tube. This is tube A. Lay tube A on the tile.
- 2 Add 5 drops of DCPIP solution to the leaf extract in the Petri dish. Mix, and if not blue colour is visible add another 5 drops, repeating until the green leaf extract is a blue-green colour. Cover.
- 3 Stand another capillary tube in the leaf extract/DCPIP mixture. Lay on the tile under a foil cover as tube B. Cover the Petri dish.
- 4 Stand four more capillary tubes in the dark blue-green leaf extract/DCPIP mixture. On the tile, put tube C under the dark filter, tube D under the mid-grey filter, tube E under the pale filter and tube F without a cover.
- 5 Switch on the lamp so that the light falls evenly all over the tile and start timing. Every minute lift the filters or covers and record the colour of each tube in the table.

Calculations

- 1 Record the time taken for the blue colour to disappear from each tube. If it is still blue, record '>10 minutes'
- 2 Calculate the rate of reaction using $1/\text{time}$ taken for blue colour to disappear. If >10 minutes, then record $1/\text{time}$ taken for blue colour to disappear as 0.
- 3 Record your rate of reaction for each light intensity in the class results table on the board or flipchart.
- 4 When all the results have been recorded in the class results table, calculate the mean rate of reaction for each light intensity.
- 5 (Optional - calculate the standard error for each light intensity.)

Write-up

- Plot a graph to show the mean rate of reaction for each light intensity
- (Optional - add error bars to your graph.)
- Make an evaluation considering:
 - the limitations of the methods used,
 - anomalous values if any,
 - replication and range of values of independent variable,
 - effectiveness of control of selected variables,
 - the confidence with which conclusions should be drawn
- Draw conclusions considering:
 - detailed description of the features of the results,
 - the meaning of the results in relation to the hypothesis,
 - scientific explanation of the results and conclusions,
 - potential Improvements and further predictions

Lesson Plan

The effect of light intensity on rate of the Hill reaction - Defining the Problem

Context

A practical investigation set in the context of 9700 Syllabus - aspects of the light dependent reaction and light as a limiting factor in photosynthesis.

It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that they will have been given learning opportunities before this so that they know how to identify the variables involved and make hypotheses in writing and graphically.

Key aims of lesson

This practical is designed to develop the skill of Planning - Defining the problem. Students will be developing other assessed skills throughout the practical.

Intended learning outcomes

By the end of this practical and its write-up the student should be able to

- Identify the independent and dependent variables
- Make a hypothesis and express this in words and graphically
- Identify the variables that should be controlled
- Experience relevant methods, analysis, conclusions and evaluation.
- Describe and explain the relationship between light intensity and photosynthesis.
- Explain the relationship between this experiment and the light dependent reactions of photosynthesis

Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

Planned activities (timings can be altered to suit shorter or longer lessons)

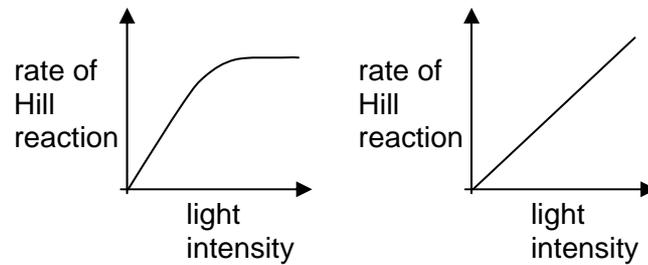
Timings/ minutes	Teacher / Student Activities
end of previous lesson	Preparation - 2 page student worksheet given out for students to read in preparation for the practical lesson, and to consider the identification of variables, hypothesis formulation and listing of control variables, reinforcing previous learning and preparing for this lesson
0-4	Introduction to the aims, intended outcomes and shape of the lesson - teacher led oral presentation
4-8	Context - review of Hill reaction, light independent reactions of photosynthesis, and on light as a limiting factor in photosynthesis. Teacher-led questioning, student responses / discussion, students building a multicoloured learning outline on the board.

8-12	Introduction to method - teacher demonstration of cutting up leaf, adding extraction medium, grinding, putting extract in Petri dish, loading capillary tube, adding DCPIP to an appropriate green-blue colour, handed round for students to see (this will go green in a minute or two in normal daylight - add some more DCPIP if needed).
12-20	Identification of variables, writing and graphing hypotheses and listing control variables - pupils work through second box on page 1 of the student worksheet - teacher circulates, answering specific queries, praising students who are making a good effort and discussing responses that are not detailed enough or incorrect to help guide students in the right direction. Student activity will be completed for homework
20-50	Carrying out the practical - students carry out the practical work, entering their results into a table on the board and tidying away apparatus as soon as they have finished.
50-60	Drawing together the threads - teacher-led class discussion on the skills that have been developed, as well as the results and their meaning - teacher led introduction to write-up, which should include class-work, finished off if necessary, (identification of variables, writing and graphing hypotheses and listing control variables), the method sheet to show what was done, annotated to include any modifications that were made, and a full, detailed write-up as described on page 2 of the student worksheet.

Useful Information

- 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, also using hydrogen ions from photolysis of water.
- If chloroplasts are broken, the enzymes that are involved in this process can reduce other oxidised materials in the same way. This is termed the Hill reaction after its discoverer.
- Oxidised DCPIP is bright blue, and when reduced, for example by high-energy electrons and hydrogen ions from the light dependent reaction of photosynthesis, becomes colourless.
- DCPIP provides a way of measuring how fast the light dependent reaction is happening, as well as giving an interesting insight into the light dependent reaction itself.
- The Independent variable is light intensity; the dependent variable is rate of the Hill reaction, measured as how fast the blue DCPIP is reduced to colourless.
- The precise hypothesis will vary from student to student, depending what information they use to help guide them. Accept any valid hypothesis, e.g.
 - Students who make use of the curve of light intensity against photosynthesis will come up with something equivalent to: at low light intensities; as light intensity increases, the rate of the Hill reaction also increases (as light is the limiting factor), but at high light intensities; as the light intensity increases, the rate of photosynthesis remains constant (as some other factor is limiting).

Appendix 2



- Other students may view the process energetically as all the raw materials are present in excess, and will anticipate a straight line in which: as light intensity increases, the rate of the Hill reaction will increase (as the light supplies the energy, and the more energy is supplied, the faster the reaction will go).
- Possible variables to control include:
 - temperature;
 - volumes and concentrations of extraction medium and DCPIP;
 - leaf area / volume of extract used and species / type of leaf;
 - light intensity falling on each filter;
 - spectrum of light falling on each tube ('light temperature');
 - pH;
 - atmospheric pressure;
 - time of exposure of each treatment to bright light during the making of observations;

Technical Information

The effect of light intensity on rate of the Hill reaction - Defining the Problem

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

For some of the chemicals, it is convenient to make up more than is required in order to give sufficient quantities for accurate measurement.

- 1 1 fresh green cabbage or spinach leaf per student / group. Any soft, green, non-toxic dicotyledonous leaf would be suitable.
- 2 1 white tile
- 3 1 scalpel or sharp knife
- 4 1 shatterproof plastic specimen tube (minimum 3cm x 1 cm) that will withstand being squeezed and being used as a mortar for grinding the soft green leaves.
- 5 1 glass rod that will fit into the specimen tubes. Very thin rods will break easily, so use reasonably thick glass rod and round off the ends with heat
- 6 1 paper towel
- 7 1 Petri dish base or top (either is suitable)
- 8 3 different plastic neutral density grey filters 1.5 cm by 10 cm cut from a large sheet. There are several manufacturers of such filters, details of which are given below:

description	% of light transmitted	Lee	Cotech	Roscolux
pale grey	70	298	298	397
mid-grey	50	209	209	97
dark grey	25	210	210	98

- 9 1 desk lamp, which could be a proper articulated lamp, or a bulb-holder safely screwed to a small wooden base.
- 10 6 melting point tubes (thin wall capillary tubes 10 cm long) or 6 pieces of capillary tube cut to a length of 4-10 cm each, with any sharp edges removed.
- 11 aluminium foil
- 12 2 syringes, 5 cm³ or 2 cm³
- 13 Make up 500 cm³ of phosphate buffer solution to use as below, using 4.48 g Na₂HPO₄.12H₂O and 1.7g KH₂PO₄ made up to 500 cm³ with distilled water. Store in a fridge. Students do **not** need to be given any of this solution.
- 14 2 cm³ of very cold extraction medium, labelled **extraction medium**. To make up 250 cm³ of extraction medium, dissolve 34.23 g sucrose and 0.19 g KCl in phosphate buffer solution (see above) and then make up to 250 cm³ with the phosphate buffer solution. Store in a fridge for no more than 48 hours and supply very cold for use.
- 15 2 cm³ DCPIP solution labelled **DCPIP solution**. To make up 250 cm³ of DCPIP solution, dissolve 0.4 g DCPIP and 0.93 g KCl in phosphate buffer solution **at room temperature** and make up to 250 cm³ with phosphate buffer solution.

Store in a fridge for no more than 48 hours but **supply for use at room temperature.**

Safety Precautions/Risks.

No specific hazards identified.

A risk assessment should be carried out as a matter of course.