Bio Factsheet

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Practical Investigations For Photosynthesis

Photosynthesis is a favourite topic for:
1. Coursework and in-class practicals
2. Exam questions – in the exam, photosynthesis investigations can be used to test your understanding of the theory and your ability to design investigations and fair tests.

This Factsheet will look at photosynthesis-related investigations.

1. Coursework and in-class practicals

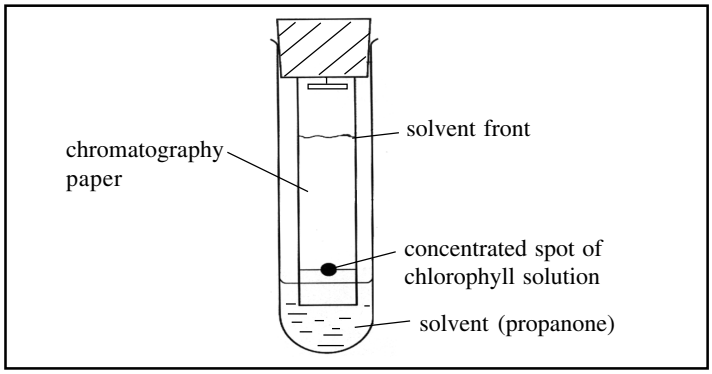
The most common and successful investigations involve:
A. Chromatography of chlorophyll pigments;
B Comparison of chlorophyll concentration in sun and shade leaves;
C Absorption spectrum;
D Action spectrum;
E Effect of limiting factors and the rate of photosynthesis;
F Identifying factors that affect the compensation point of a plant ;
G Comparing C3 and C4 plants when they are competing for CO_2.

A) Chromatography of chlorophyll pigments

Exam Hint: *Whenever you are doing chromatography try to handle the chromatography papers as little as possible – your sweaty fingers will contaminate the paper with amino acids.*

Outline method
1. Remove any large veins from the leaves. Cut up green leaves and macerate with a solvent eg propanone. Add a spatual of sand. Maceration breaks cell walls, releasing chlorophyll, which dissolves.
2. Use a micro-pipette to build up a dark green, concentrated spot of chlorophyll solution on the bottom of a piece of chromatography paper. Allow the spot to dry before each new application.
3. Suspend the bottom edge of paper but not the chlorophyll spot in solvent. Seal with a bung (Fig 1). The solvent diffuses up the paper and the individual chlorophyll pigments dissolve and are carried up the paper. Since they are differentially soluble, they move different distances. This will take a couple of hours.
4. Cut out the individual pigments and redissolve to obtain individual pigment solutions.

Fig 1 Separation of chlorophyll pigments

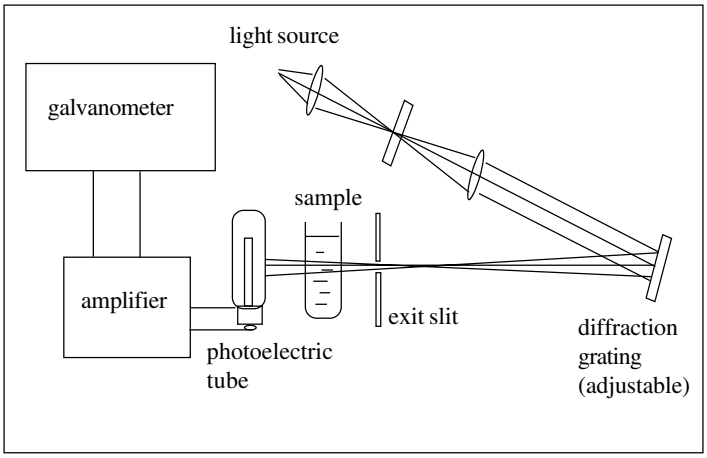


B) Chlorophyll in sun and shade leaves

The sun leaves growing at the top of a rainforest tree are different in structure than the shaded leaves at the bottom of the tree. The sun leaves at the top of the tree:
- Are smaller
- Are thicker
- Are more effective at photosynthesis in strong light
- Have smaller but more numerous chloroplasts

It is quite easy to carry out investigations about whether native and non-native trees in Britain also have sun and shade leaves and how the different morphology of these leaves might affect rates of photosynthesis.
- Identify trees where the light intensity reaching high leaves is much greater than the light reaching lower leaves
- Collect samples of different heights
- Measure length and width of leaves to determine length to width ratio
- Measure the thickness of the leaves by taking transverse sections
- Measure the number of stomata on upper and lower leaf surfaces by using nail varnish / sellotape to take sections and then count the number per area and multiply up.
- Measure the relative concentration of chlorophyll in leaves by extracting from a known area and then using a spectrophotometer to measure light absorption.

Fig 2. Spectrophotometer

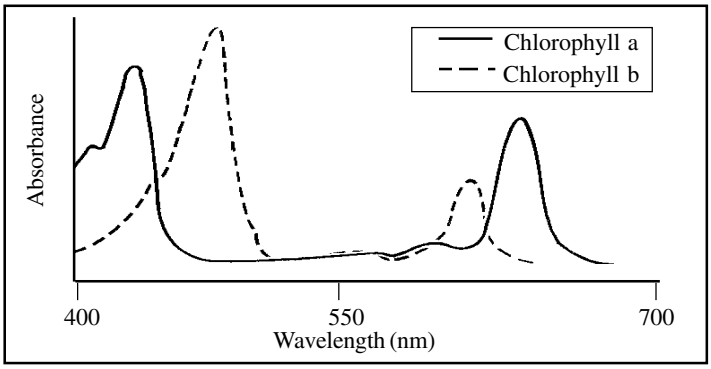


C) Absorption spectrum

Chlorophyll absorbs light from the visible part of the electromagnetic spectrum. Chlorophyll is made up of a number of different pigments: chlorophyll a, chlorophyll b, chlorophyll c along with other pigments such as carotenoids. Each of these absorb different wavelengths of light so that the total amount of light absorbed is greater than if a single pigment were involved.

Not all wavelengths of light are absorbed equally. An **absorption spectrum** is a graph showing the percentage absorption plotted against wavelength of light (Fig 3).

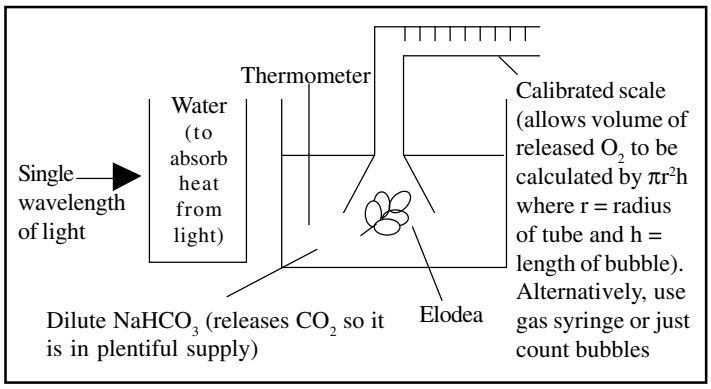
Fig 3. The absorption spectra



To measure the absorption spectrum you need a Spectrophotometer.

D) Action Spectrum
This is usually carried out using Elodea

Fig 4. Using Elodea



Constructing an action spectrum
Individual wavelengths of light are projected onto Elodea and the amount of oxygen (No. of bubbles/minute or volume/minute) given off from each wavelength is measured.

Typical exam question
What precautions should be taken when using this apparatus?
1. Each wavelength must be of similar intensity and be shone for the same length of time.
2. Temperature of solution surrounding Elodea must not change.
3. All light, other than wavelength being tested, must be excluded.

Identify the possible sources of errors in the above method
1. Bubbles may become trapped and therefore not be measured.
2. If No. of bubbles is used, there is an assumption that all bubbles are of the same volume - this is unlikely.
3. Temperature/rate of release of CO_2 from $NaHCO_3$ may vary.

E) Limiting factors on rate of photosynthesis
These investigations are conducted using the basic set up of Fig 4. The index of rate of photosynthesis is the evolution of oxygen.

Table 1. Limiting Factors

Factor	How vary?	Precautions
Light intensity	Move light different distances from Elodea. $LI \propto \frac{1}{d^2}$ where d = distance from lamp	1. Exclude background light 2. Keep T^o, $[NaHCO_3]$ and wavelength constant and time period for each LI equal.
Temperature	Add cold or warm water and measure with thermometer	Keep LI, $[NaHCO_3]$ and wavelength constant and time period for each T^o equal.
$[CO_2]$	Use different concentration of $NaHCO_3$	Keep LI, T^o and wavelength constant and time period for each $[CO_2]$ equal.

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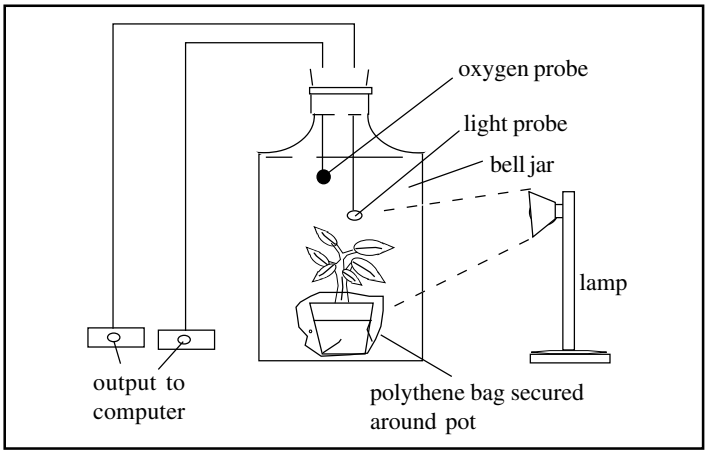
F) The light compensation point
The **light compensation point** of a plant is the light intensity at which CO_2 intake by photosynthesis equals CO_2 output from respiration. i.e. the net uptake is zero. Since one molecule of oxygen is released for every molecule of CO_2 taken in, when the CO_2 uptake/ output is balanced, so too is the oxygen uptake / output. It is easier to measure oxygen evolution than carbon dioxide uptake so often the light compensation point is established by measuring the light intensity at which there is no net oxygen uptake / output (Fig 5).

Fig 5. Light compensation point equipment



This investigation can be used to compare the light compensation points of plants of diffferent ages, of sun and shade plants and of healthy and diseased plants

Outline method
1. Completely seal a *Pelargonium* plant in a pot using polythene
2. Set up a 100W light close to the plant, wait for 10 minutes and then record the pattern of oxygen concentration in the closed jar for 10 minutes.
3. Use the oxygen probe to construct a graph of the oxygen output over time and the light probe to determine the average light intensity which reached the plant.
4. Repeat these measurements with the lamp at different distances.
5. Switch off the light, wait for 10 minutes, and determine the net oxygen exchange of the plant in the dark over 10 minutes.
6. Plot light intensity against net oxygen input or output. The light compensation point is the light intensity at which there is no net oxygen exchange.

Comparing C3 and C4 plants
Outline method
1. Plant 3 oat and 3 corn seeds in each of 5 small pots.
2. Place the pots under a light bank or in a bright light. Record percentage germination daily and the height of all seedlings daily once they have germinated.
3. When the seedlings are 5cm tall, identify the single strongest oat and corn seedling in each pot and pinch off all the rest of the seedlings in the pots.
4. Seal one pot completely in using transparent film. Leave the other pots open.
5. Place the pots in bright light.
6. Water the plants in the open pots if required. The plants in the sealed pot should not need water.
7. After two weeks, compare the growth of both types of plants in the open and sealed pots

Corn is a C4 plant (so –called because the first product of the light independent stage has 4 carbons, whereas in normal photosynthesis, a 3 carbon compound is made first). C4 plants are more efficient than C3 plants (such as the oat seedling) at absorbing very low concentrations of carbon dioxide. It is interesting to note the result of competition for carbon dioxide between the corn and oat seedlings and useful comparisons can be made between the growth of the corn and oat in the sealed pot and in open pots.