Chloroplast absorption spectra using the CLEAPSS (Open-frame) colorimeter (DRAFT)

Why do this?

To help students understand how the different pigments present in chloroplasts absorb different wavelengths of light (the absorption spectrum)

To allow investigations of the variability of absorption spectra of plants harvested from the environment

To help students understand the use of a colorimeter to analyse the spectrum of incident light

Possible curriculum links:

Biochemistry of photosynthesis, Light Dependent reactions

Adaptation of plants to lighting conditions in different parts of the environment

This *Practical Procedure* draws on information from the following guidance

- GL192 A technical guide to setting up and using the CLEAPSS colorimeter
- GL174 Make it guide – DIY Colorimeter
- CLEAPSS video "Setting up a DIY colorimeter"
- Hazcard 40A (ethanol)

Suitability

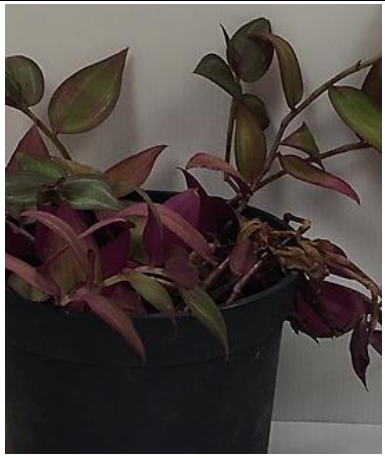
Y12/13

Outline method with control measures

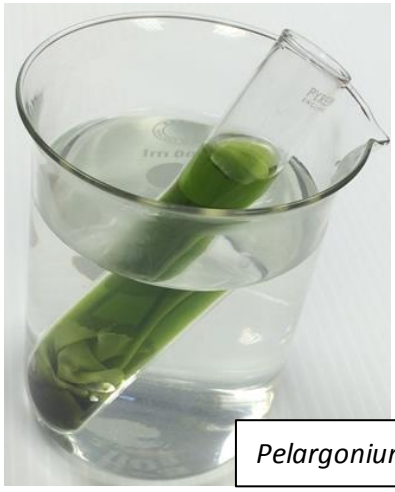
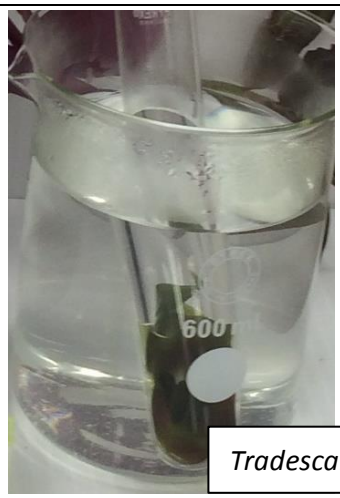
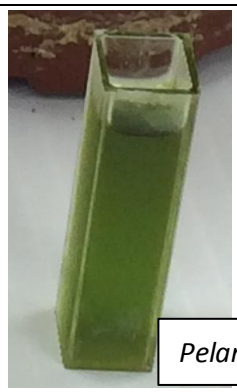
Use fresh leaves, here *Pelargonium sp.* (geranium) and *Tradescantia sp.* leaves were used. The practical involves heating plant leaves in ethanol, wear eye protection and heat water in a kettle, to avoid use of naked flames.

This practical procedure is made up of 3 elements

1. Extracting chloroplast pigments into ethanol
2. Using the CLEAPSS Open-frame colorimeter to find the relative transmission of light from a range of single wavelength LEDs
3. Using a Spreadsheet to calculate the absorption spectrum of the chloroplast extract

Pelargonium (commonly known as Geranium) and *Tradescantia* were selected in this investigation, as they have contrasting leaf colours	 ***Pelargonium***	 ***Tradescantia***

Procedure 1 - Preparing chloroplast extracts

<table>
<tr><td>Place a leaf from the plant into a 250ml beaker.
Pour on hot water taken from a freshly boiled electric kettle, to the 150ml mark.
Leave in the boiling water for 30 seconds
The boiling kills the cells and softens the cell walls.</td><td colspan="2">  </td></tr>
<tr><td>Use forceps to remove the leaf from the water, and to place the leaf in a boiling tube.
Add ethanol to the boiling tube so that the leaf is completely covered.
Place the boiling tube in the beaker of hot water.
The beaker should be left for 5 minutes, to allow the ethanol to dissolve the pigments from the leaf.</td><td> 
Pelargonium</td><td> 
Tradescantia</td></tr>
<tr><td>Use a pipette to fill a colorimeter cuvette with a sample of the chloroplast extract</td><td> 
Pelargonium</td><td> 
Tradescantia</td></tr>
</table>

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Tel: 01895 251496; Fax: 01895 814372; E-mail: science@cleapss.org.uk; Web site: www.cleapss.org.uk

Procedure 2: Using the CLEAPSS Open-frame colorimeter to find the absorption of different light wavelengths by chloroplast pigments

This activity can be carried out using the CLEAPSS DIY colorimeter, see the following CLEAPSS resources:

- the CLEAPSS video: Setting up a DIY Colorimeter
- the Open-frame colorimeter in GL192 Setting up and using the CLEAPSS colorimeter
- GL174 Make it guide: the DIY colorimeter

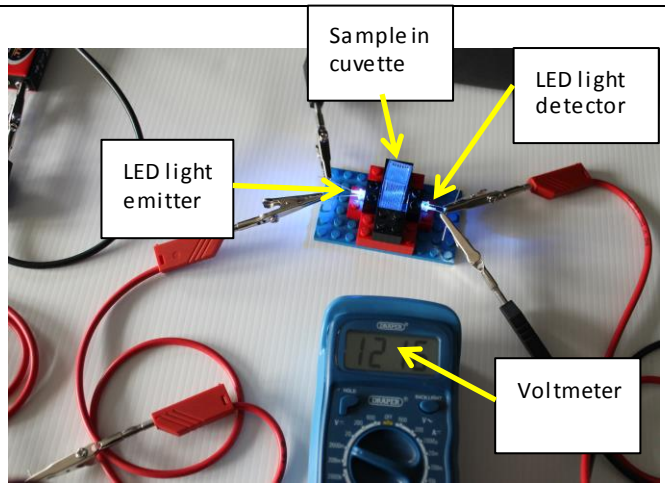
The CLEAPSS colorimeter uses a pair of LEDs. One acts as a light source and the other as a light detector.

The light produced by the emitter LED is transmitted through the cuvette, and this light produces a voltage that is read on the voltmeter.

For absorption spectra, use an IR LED (850nm, 5°) as a light detector.

A full range of LEDs will be used as light emitters.

(see GL174 Make-it Guide, for details of components).



Place a single spectrum LED emitter opposite the detector LED, in the LEGO holder.

Place an absorbent non-reflective cover over the detector LED, to exclude ambient light.

Place the cuvette containing chloroplast extract into the slot in the colorimeter, and obtain a voltmeter reading.

Use the shortest peak wavelength LED as the first emitter, and then replace this emitter with others of longer wavelength.

Obtain readings for all the available LEDs, ensuring that you cover the range 450-470nm (blue) to 625-650 nm(red)) (V_s). If possible use some LEDs outside this range.

For each reading with a chloroplast cuvette, obtain another for a cuvette containing just the solvent (ethanol) (V_0).

Cuvette containing chloroplast extract

Cuvette containing equivalent ethanol & water mixture.

Calculate values of Relative Absorbance (RA) for each sample.

For full details of this calculation see the technical guide GL192

You could set up a spreadsheet to do the calculations for you.

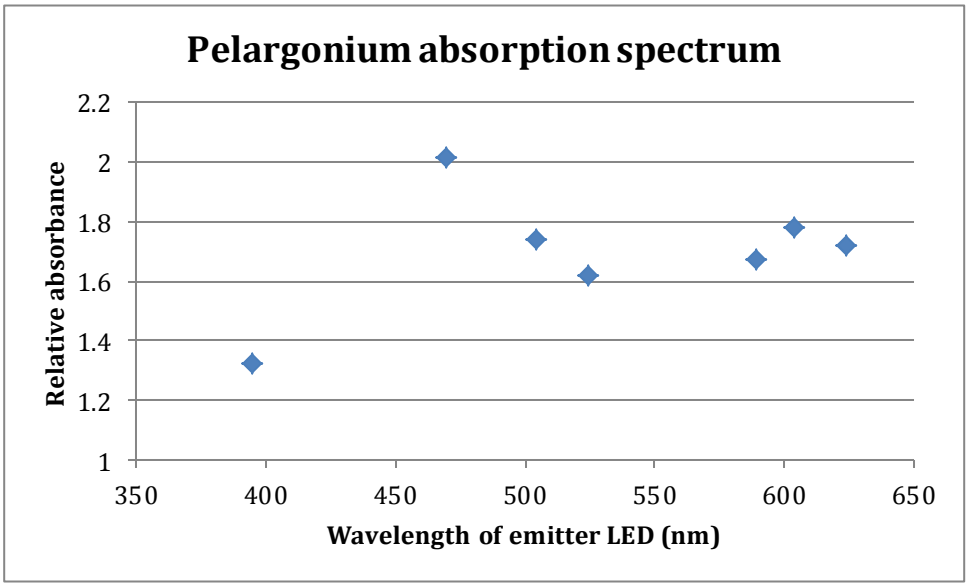
$$\text{Relative absorbance} = \text{Log}_{10}\ \frac{V_o}{V_s}$$

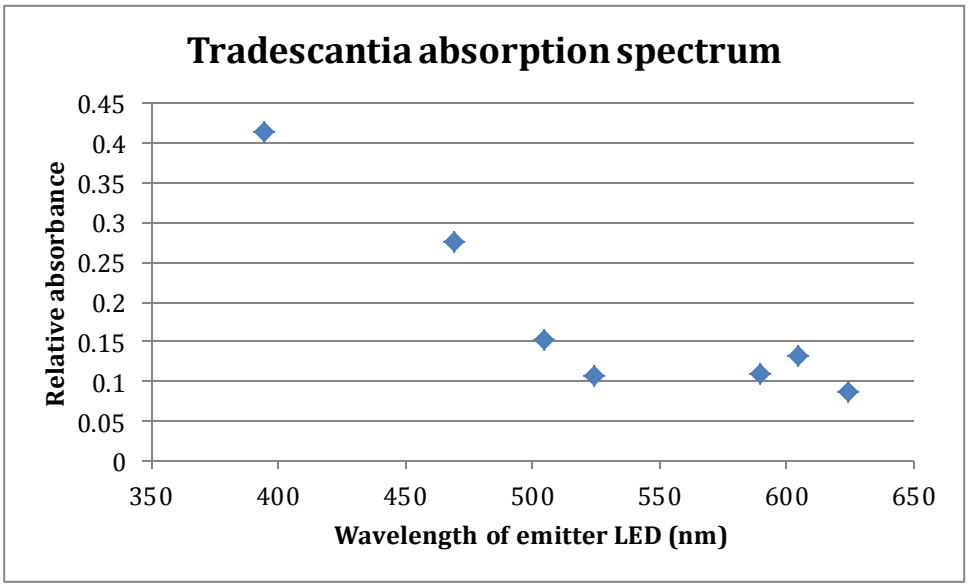
V_o = voltage for reference cuvette (ethanol only)

V_s = voltage for sample cuvette

Plot a graph of wavelength of LED emitter (x axis) vs Relative Absorbance (y axis)

Results from our trials





Conclusions

The two spectra both show strong absorbance in the 450nm (blue) end of the spectrum, with very little absorbance in the 500-600nm mid spectrum.

The Pelargonium spectrum shows greater absorbance in the 600-650nm part of the spectrum (red), and the Tradescantia shows more absorbance in the UV (400nm) part of the spectrum.

The results explain the yellow/green colour of the Tradescantia extract, compared with the blue/green colour of the Pelargonium extract.

The differences in absorbance patterns may reflect the more shady conditions in which Tradescantia is found relative to Pelargonium.

Biology notes

The pigments found in plant chloroplasts act as an antenna unit, with associated ATP generating enzymes. Collectively these form Photosystems 1 and 2.

The absorption spectra of the extracts shown here indicate that 450 nm absorbance is found widely, but UV absorbance is less common.

Students can research the implications of their findings to efficiency of photosynthesis.

Suggested apparatus and materials

- Pipettes
- Forceps
- 250ml glass beakers
- Hot water
- Boiling tubes and racks
- Ethanol
- Cuvettes
- CLEAPSS Open-frame Colorimeter

Apparatus and materials notes

- CLEAPSS video *Setting up a DIY colorimeter*
- GL 192 *The CLEAPSS DIY colorimeter*
- GL174 *Setting up a CLEAPSS DIY colorimeter*

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