

**Note to markers:** My e-textbook came squished (I don't know how to unsquish it) and is nearly 8000 pages long, meaning my references are technically all wrong. To remedy this, I put *my* page numbers and the chapters/sub-headings/figures I reference from so you know the general area I got my information from – like (Urry A L et. al.; 2022:914) (Concept 10.3; Figure 10.14). I made them in distinct parentheses to qualify for in-text citation criteria and to have more accurate, though less formal information. I apologise in advance; it was confusing for me too.

## Concept map

Q1. Electrons

Q2. ATP

Q3. Water ( $2\text{H}^+$  and  $\frac{1}{2}\text{O}_2$ )

Q4. Light energy

Q5. NADPH

Q6. (i) Photosystem II and (ii) Photosystem I

(Urry A L et. al.; 2022:914) (Concept 10.3:Figure 10.14)

Q7.  $\text{NADP}^+$  (SLE132 Unit Coordinators; c.2023:6)

## Introduction

2,6-dichlorophenolindophenol (DCPIP) is known to lose colour as it gain electrons. This phenomenon can be exploited to determine the rate of reaction of processes like the light reactions in photosynthesis (SLE132 Unit Coordinators; c.2023:6). The goal was to gather data on this and to the consensus on this property of DCPIP. It was aimed to investigate the different effects light has on the colour of DCPIP when mixed with chlorophyll extract, measured using a light spectrophotometer. It was hypothesised that the light would have a measurable impact on the absorbance of 590nm (blue) light in the experimental assay and no change in the controls.

Photosynthesis is a sugar-producing process consisting of two distinct light and dark reactions that occurs in all plants for the purposes of energy, storage, and growth (Geiger; 2020). The light reactions, in Photosystem II (PSII), feed electrons to Photosystem I (PSI). These electrons come from external photons exciting chlorophylls pigment electrons. Water is oxidised ( $\text{H}_2\text{O} \rightarrow 2\text{H}^+ + \frac{1}{2}\text{O}_2 + 2$  electrons) to replace pigment electrons. The chlorophylls may fluoresce and release heat as byproducts; the  $\frac{1}{2}\text{O}_2$  and  $2\text{H}^+$  are also byproducts. To reach PSI, the excited electrons are accepted into P680+, which is then oxidised, permitting the electrons into the primary electron acceptor. The electrons are used to produce a proton gradient and subsequently ATP via an electron transport chain (ETC). Light excites the PSI electron acceptor, P700, so PSII electrons can be accepted. On each light excitation, these reinvigorated electrons pass through another ETC and allow an NADP to be reduced to the high-energy NADPH. NADPH needs two of these electrons. The NADPH is then used in the dark reactions (Urry A L et. al; 2022:912-914) (Chapter 10: "Linear Electron Flow"). In this experiment, NADP is substituted for DCPIP as the electron acceptor (SLE132 Unit Coordinators; c.2023:6).

The dark reactions are a cycle of reactions with carbon dioxide to form sugars and CO<sub>2</sub> acceptor rejuvenation to allow the cycle to repeat. For every two glyceraldehyde 3-phosphate (G3P) the sugar-forming section produces, one glucose, isomer, or derivative of results. During these processes, NADPH is oxidised to form NADP<sup>+</sup> to allow the light reactions to continue (Urry A L et. al; 2022:925-928) (Concept 10.4). The DCPIP may prevent this light-dark-light cycle from continuing as it is not specialised for the processes involved like NADPH is, though this is out of scope for the experiment.

## Method

A table was cordoned off where a ruler and a lamp were placed. The lamp was turned off and placed at the 0m of the ruler, facing towards the 1m end. At the 26cm point three blue tack blobs were placed to hold the control and treatment cuvettes. Three cuvettes were picked and labelled as C (control), DC (Dark Control), and E (experimental) accordingly. Buffer, DCPIP, and 50 microlitres of chloroplast extract was added to each then covered with parafilm in accordance with the experimental guidelines found in the SLE132 Practical 4 manual supplied by the SLE132 Unit Coordinators (c.2023:9). All were lightly shaken to homogenise the solution. The assays then had their initial absorbance at 590nm read in the light spectrophotometer. Each was placed on separate blue tack blobs and the DC covered with a tin can. The lamp and a timer for two minutes were turned on. After the two minutes expired, the assays had their absorbance at 590nm measured and written down before being lightly shaken then set back to their conditions pre-measurement. This was repeated 5 more times for a total of 7 readings and 12 minutes elapsed (SLE132 Unit Coordinators; c.2023:8-9).

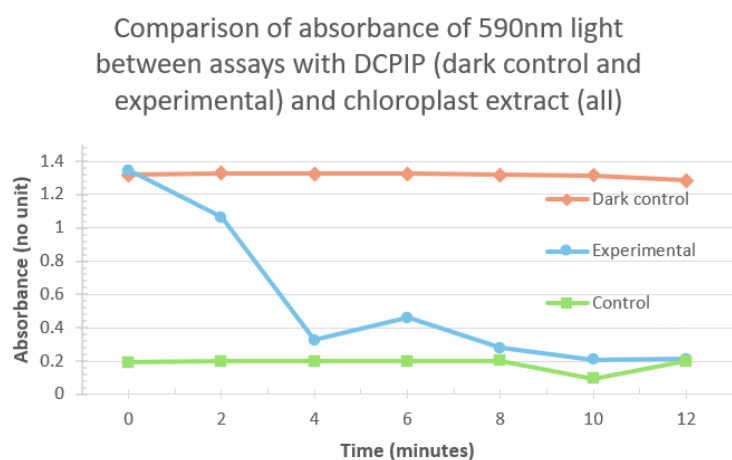
E was expected to show a decreasing absorbance as the DCPIP gained electrons from ferredoxin (Urry A L et. al; 2022:913) (Chapter 10: "Linear Electron Flow"). E was used to determine the level of absorbance change from being exposed to light. C was expected to not show any absorbance changes due to it lacking the electron-gaining DCPIP. C was used to establish that there would be no change in absorbance levels if no electron acceptor was present. DC was expected to show no absorbance changes due to the lack the light preventing the light reactions from occurring. DC was used to compare against E to determine if light was the cause for lowering absorbance.

## Results

Fig. 1. Line graph comparing the absorbance of 590nm light using 2 controls and 1 experimental unit, all with chloroplast extract (n=3).

There were 7 measurements taken for each from 0-12 minutes, taken initially and every two minutes after using a light spectrophotometer. The red line shows the dark control with DCPIP, buffer, chloroplast extract, and a dark environment at a largely stable rate with an average of 1.32

absorbance. The blue line shows the experimental with DCPIP, buffer, chloroplast extract, and a light environment with an initial reading of 1.344 absorbance, rapidly declining to 0.326, then stabilising



at 0.211. The green line shows the control with buffer, chloroplast extract, and a light environment at a median of 0.199 absorbance.

The dark control (DC) and control (C) were primarily level throughout measurements. DC had an average of 1.316 absorbance and a standard deviation of 0.014. DC fluctuated most from the minute 10 to 12 with a difference of 0.028, which was 0.018 higher than the next biggest fluctuation at minute 0 to 2 of 0.010.

C had a median of 0.199 absorbance and standard deviation of 0.039. Median was chosen rather than average to ignore a measurement mistake on the 10-minute measurement which erroneously affected the average. This error caused the largest change to occur between minutes 8 to 10, dropping in absorbance by -0.107. Ignoring this measurement error, the largest change was between minutes 0 to 2, with a difference of 0.006 absorbance and the lowest standard deviation at 0.003.

The experimental (E) quickly deviated from the initial reading it closely shared with DC. It had a downwards trend, having less absorbance at every measurement except at the 6<sup>th</sup> minute. All measurements appeared invisible to the naked eye. The average was 0.556 absorbance, and the standard deviation was the largest of all assays at 0.457. Minute 2 to 4 had the largest change of absorbance at -0.737 and minute 10 to 12 with the smallest at +0.002. Absorbance was raised by +0.134 from minutes 4 to 6 after the aforementioned large change.

## Discussion

There were minor changes in absorbance in both controls and significant in the experimental.

C absorbance stayed highly stable until a cuvette was turned the wrong way on minute 10, causing a strong dip in absorbance. It then rose back to previous absorbance levels on minute 12 when the mistake was found and rectified. The fluctuations in both C and DC were thought to be caused by three possible reasons. One idea was that there were positioning errors of the cuvettes due to a poor understanding of how they slotted into the spectrophotometer. Another theory was that fingerprints or other blemishes occurred during handling (SLE132 Unit Coordinators; c.2023:8) while undertaking the measurements which affected the spectrophotometer light beam. The last pertains only to DC, where it may have been allowed to undergo light reactions during measurement due to exposure to natural light, allowing DCPIP to be reduced and alter absorbance like in E. This last option is believed to not have been able to occur in C as DCPIP was not present and therefore unlikely to have affected the measurements from light exposure. The fluctuations mean there was indeed change, not supporting the hypothesis. Better care in handling, more training in spectrophotometer usage, and an improved setup for the measurement of dark controls is needed.

E had lowered absorbance over the minutes, supporting the hypothesis. However, the sudden gain in absorbance at 6 minutes did not support the hypothesis. The gain was unexpected as the DCPIP was expected to continue in a downwards trend. A theory on this is that an error occurred similar to C and DC, where fingerprints are thought to have interfered with measurements occurred. It is believed that this could not have occurred as it did in C and DC, as marks for them are suspected to have lowered absorbance, not raised it.

Another theory on why this occurred is that the DCPIP was oxidised in an unknown way, shifting the colour to blue and therefore altering the absorbance. This is thought at DCPIP oxidises slowly in

aerobic and faster in anaerobic environments (Jahn B et al.; 2020). It is therefore proposed that the DCPIP oxidised between minutes 4 and 6, raising the absorbance reading. This theory potentially has a major weakness however, as the chloroplasts should theoretically be producing oxygen and accordingly the oxidation would be slower. The levels of CO<sub>2</sub> and O<sub>2</sub> within each cuvette was unknown too. This theory is only partially in harmony with DC, as DC's fluctuations may be explained by DCPIP oxidation, but the absorbance gains were far less drastic than E. More refined setups with aerobic and anaerobic environments are needed to determine if this could have occurred.

The primary factors in the rate of light reactions are the intensity of light and CO<sub>2</sub> concentration, both being a potential limiting factor to the other (Smith L E; 1938:1). Light intensity proportionally affects photosynthesis rates until limited by CO<sub>2</sub> concentration. The rates are affected due to more photons being absorbed by chlorophyll pigments (RSC; n.d.:para.2) and therefore allowing a greater number of excited electrons to be transferred to the primary electron acceptor (Urry A L et. al; 2022:912) (Chapter 10: "Linear Electron Flow").

### Extension question

A cuvette with a water to oxygen blocking compound and DCPIP would likely demonstrate a marginal negative change of absorbance as electrons from chlorophyll pigments continue to PSI and then reduce DCPIP. This would not last as the pigments would eventually exhaust their electrons and as water is blocked from oxidation, there would be no provisions of electrons.

### Reference list

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