**Final todos:**

* Reference methodology
  + Ask Jess about referencing the prac manual
* Rewrite graph title
  + Decide whether we should add “(no units)” to absorbance

**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 (2H+ and ½ O2)

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. NADP+ (SLE132 Unit Coordinators; c.2023:6)

The light reactions use pigments like chlorophyll which readily absorbs photons, exciting the pigment electrons. After a cascade of excitations from pigment to pigment, the excited electron reaches P680.

**Introduction**

1. Intro (400 words)
   1. Aim/purpose/hypothesis
   2. Photosynthesis overview
   3. Light and dark reactions purpose in photosynthesis
   4. All (by)products of light reactions – concept of them too
   5. DCPIP purpose/substitution

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. 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 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.

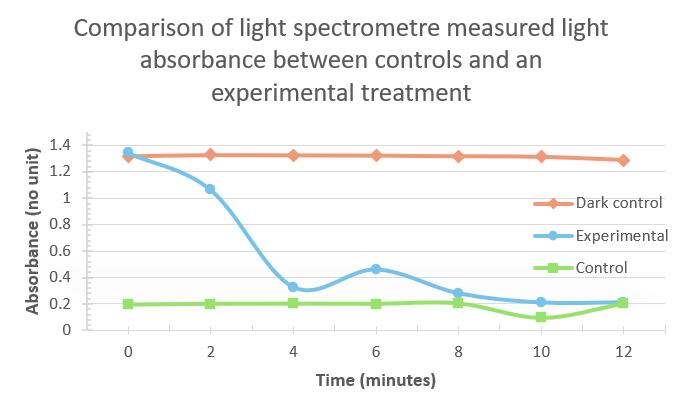
Photosynthesis is a sugar-producing process consisting of two distinct light and dark reactions that occurs in all plants for the purposes of energy, storage, and growth (Geiger; 2020). The light reactions, in Photosystem II (PSII), feed electrons to Photosystem I (PSI). These electrons come from external photons exciting chlorophylls pigment electrons. Water is hydrolysed (H2O -> 2H+ + ½O2 + 2 electrons) to replace pigment electrons. The chloropylls may fluoresce and release heat as byproducts; the ½O2 and 2H+ are also byproducts. To reach PSI, the excited electrons are accepted into P680+ then 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 revigorated 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.

The dark reactions are a cycle of reactions with carbon dioxide to form sugars and CO2 acceptor rejuvenation to allow the cycle to repeat. For every two glyceraldehyde 3-phosphate (G3P) the sugar-forming section produces, one glucose (or isomer/derivative of) results. During these processes, NADPH is oxidised to form NADP+ 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. 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. These were the assays for the experiment. 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 **(Page 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.

The experimental treatment, *E*, was expected to show a decreasing absorbance as the DCPIP gained electrons from ferredoxin (**Talk about Photosystem 1 instead or have a leadup to this**) and became colourless (Prac manual reference). *E* was used to determine the level of absorbance change from being exposed to light. The control, *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. The dark control, *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**

Figure legend/caption:

* Techniques, equipment, absorbance freq.
* Controls and experimental(s)
* Sample size
* If added to the graph, mention error bars, SD, range, etc.
* Leave noteworthy (interesting, unusual, or errors) results out of this
  + Don’t mention why things are like something

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.

May need more description of the experiment

https://www.scribbr.com/dissertation/results/

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) had a downwards trend, having less absorbance every measurement except at the 6th 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 large drop in absorbance.

1. Reference list

Work on methods > results > aim/purpose/hypothesis > discussion

**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 controls 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 unable 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 in C and DC, marks 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 oxidisation would be slower. The levels of CO2 and O2 within each cuvette was unknown too. This theory is only partially in harmony with DC, as DC’s fluctuations may be explained by DCPIP oxidisation, 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 CO2 concentration, both being a potential limiting factor to the other (Smith L E; 1938:1). Light intensity proportionally affects photosynthesis rates until limited by CO2 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 oxidisation, there would be no provisions of electrons.

C/DC/E fluctuation reason 4: wrongly set spectrophotometer settings

Reference list:

Jahn B, Jonasson S W N, Hu H, Singer H, Pol A, Good M N, Op den Camp M J H, Martinez-Gomez C N, and Dauman J L; (2020); ‘Understanding the chemistry of the artificial electron acceptors PES, PMS, DCPIP and Wurster’s Blue in methanol dehydrogenase assays’; *Journal of Biological Inorganic Chemistry*; 25(2):199-212; [10.1007/s00775-020-01752-9](https://doi.org/10.1007%2Fs00775-020-01752-9)

Geiger D; (2020); ‘Plant glucose transporter structure and function’; *Pflügers Archiv - European Journal of Physiology*; 472(9):111-1128; [10.1007/s00424-020-02449-3](https://doi.org/10.1007/s00424-020-02449-3)

SLE132 Unit Coordinators; (c.2023); Practical 4 manual, Form and Function SLE133, Deakin University

Urry A L, Meyers N, Cain L M, Wasserman A S, Minorsky V P, Orr B R, Burke da Silva K, Parkinson A, Lluka L, Chunduri P; (2022); *Campbell Biology: Australian and New Zealand Version*, 12th ed.; Ganner J, Taylor M-L; Pearson Australia, Melbourne

Royal Society of Chemistry (RSC); (n.d.); ‘Rate of photosynthesis: limiting factors’; accessed 6 January 2024; <https://edu.rsc.org/download?ac=12620>

Smith L E; (1938); ‘LIMITING FACTORS IN PHOTOSYNTHESIS: LIGHT AND CARBON DIOXIDE’; *Journal of General Physiology*; 22(1):21-35; [10.1085/jgp.22.1.21](https://doi.org/10.1085%2Fjgp.22.1.21)