Spectrophotometry and the Absorption Spectrum of Chlorophyll

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Lab #2
Spectrophotometry and the Absorption Spectrum of Chlorophyll

Introduction

This laboratory experiment will allow observation of the chlorophyll absorption spectrum measured at different wavelengths on two spectrophotometer machines. The results from a Spectronic 20 Spectrophotometer will be compared with graphed values of the Varian Cary WinUV Spectrophotometer. The absorbance spectrum will specifically be measuring for acetone-soluble plant pigments.

Materials

Spectronic 20 Spectrophotometer
Varian Cary WinUV Spectrophotometer
Waring blender and tissue homogenizer
Balance
2 weighing trays
Funnel, beakers, and cuvettes
500 mL Erlenmeyer flask
100 mL graduated cylinder
Filter Paper
250 mL acetone
5.0 g leaf tissue
3 scalpels
2 pairs tweezers
Cheesecloth

Procedure

Sliced, fresh spinach leaves were weighed on a balance to 4.950 g. The spinach and 250 mL of acetone were blended in a Waring blender. Acetone dissolved the lipid bonds of chlorophyll, allowing the acetone-soluble plant pigments to dissolve with the solvent. This dissolving enabled the pigments to move with the solvent through two filtration mechanisms. The double filtration was completed by pouring the blended spinach solution through a quadruple layer of cheesecloth (Photo 1) followed by pouring the cheesecloth-strained liquid through filter paper. 10 mL of the filtered chlorophyll pigment was diluted with 90 mL of acetone for insertion into the Spectronic 20 Spectrophotometer. The Spectrophotometer was set to 380 nm wavelength to do readings in increments of 20 nm up to 700 nm. This procedure was performed once each by two student teams. The pigment solution was then inserted into the Varian Cary WinUV Spectrophotometer to compare the data with the Spectronic 20 results.

Photo 1. First filtration of the spinach solution through cheesecloth.

Results and Discussion

The results of Team 1 differed from the results of Team 2 (Table 1, Figure 1). Team 1 obtained a peak for blue at a higher intensity and higher wavelength than Team 2 results. Team 2 obtained a peak for red light absorbance at a lower intensity, but higher wavelength then Team 1.

Table 1: Wavelength and Absorbance

	Team 2	Team 1
Wavelength	Absorbance	Absorbance
380	0.17	0.23
400	0.212	0.28
420	0.29	0.41
440	0.251	0.56
460	0.22	0.31
480	0.137	0.19
500	0.049	0.06
520	0.027	0.04
540	0.035	0.03
560	0.029	0.05
580	0.035	0.06
600	0.039	0.07
620	0.05	0.08
640	0.07	0.18
660	0.115	0.11
680	0.052	0.03
700	0.03	0.02

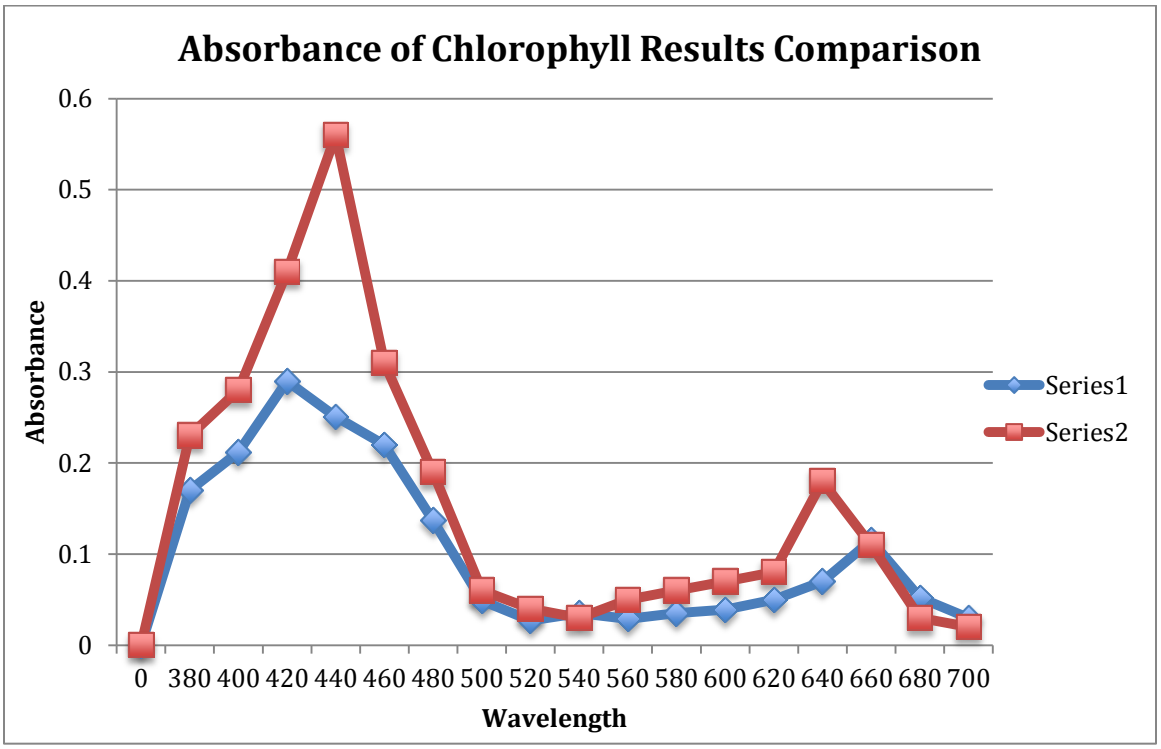

Figure 1. This graph compares the absorption results of Teams 1 and 2. Series 1 =Team 2. Series 2 = Team 1.

The chlorophyll absorption for Team 1(Table 1, Figure 2) had a blue absorbance peak at 440 nm and a red absorbance peak at 640 nm.

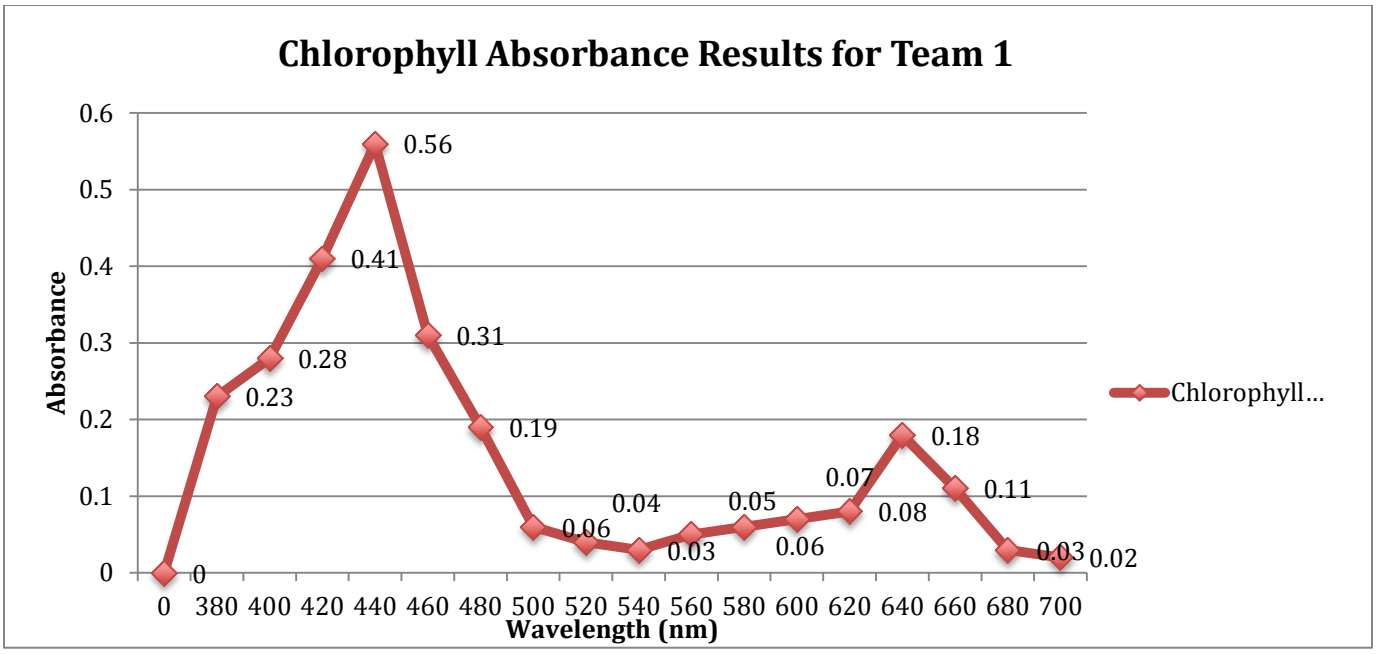

Figure 2. Chlorophyll Absorbance for Team 1 shows a peak right at 440 and a second peak at 640.

Team 2 obtained different results with a blue peak at 420 and a red peak at 660 (Table 1, Figure 3). The red peak is closer to accuracy, but the blue peak was too early for the expected outcome.

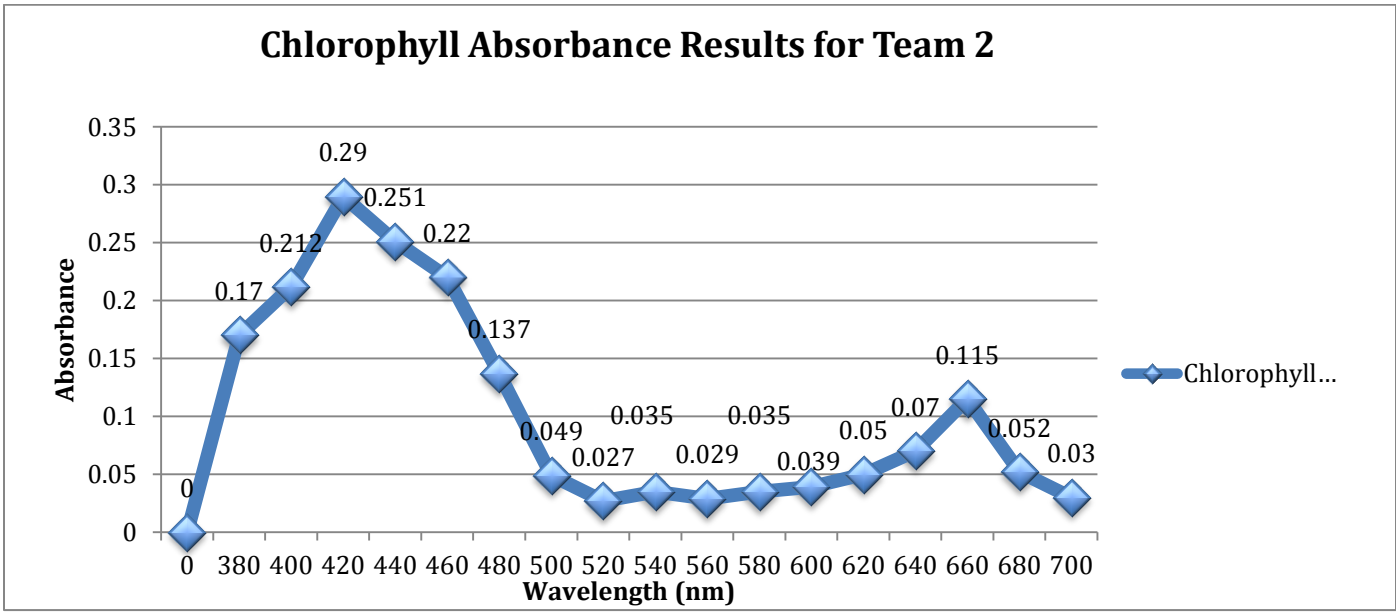

Figure 3. Chlorophyll Absorbance Results for Team 2 show more accuracy in red absorbance, than blue.

Chlorophyll will absorb a short wavelength of blue and long wavelength of red. It cannot absorb green light, so green light is reflected. As the chlorophyll molecule absorbs photons, energy is transferred between electrons; This is called inductive resonance, and causes the porphyrin to be in an excited state! The Spectronic 20 Spectrophotometer used a single beam to pass through the chlorophyll solution, and the light intensity was measured as it passed through the chlorophyll. The Varian Cary WinUV Spectrophotometer produces a double beam; One beam passes through the reference standard and the other beam passes through, or illuminates, the sample to be measures; This can have a higher ability of detection. The results of the Spectronic 20 were compared to the Varian Cary to define our sample results even further (Figures 4 and 5). Team 1 Varian Cary results show an absorbance of blue light at 0.453 at 435 nm, and an absorbance of red light at 0.219 and 660 nm (Figure 4). Team 2 Varian Cary results show an absorbance of blue light at 0.318 at 435 nm and an absorbance of red light at 0.153 at 660nm. See Table 2 for ease of comparison between the two types of Spectrophotometers for Teams 1 and 2. From Table 2, it is visible that the absorbance of light from the Team 1 sample was stronger than the sample of Team 2. This goes back to the probability that there is human error in the filtering and/or diluting of the chlorophyll pigment of the Team 2 sample. What also can be noticed is the wavelength differences read by the two machines. The Varian Cary WinUV gave readings at identical wavelengths for the two peaks of light in each samples, where the Spectronic 20 gave varying wavelengths in for the two peaks in each sample.

Table 2. Comparison of Spectrum 20 results to those of Varian Cary WinUV results

	Team 1 Spec 20	**Team 1 Varian**	**Team 2 Spec 20**	**Team 2 Varian**
Blue light	0.56 at 440 nm	0.453 at 435 nm	0.251 at 440 nm	0.318 at 435 nm
Red light	0.18 at 640 nm	0.219 at 660 nm	0.115 at 660 nm	0.153 at 660 nm

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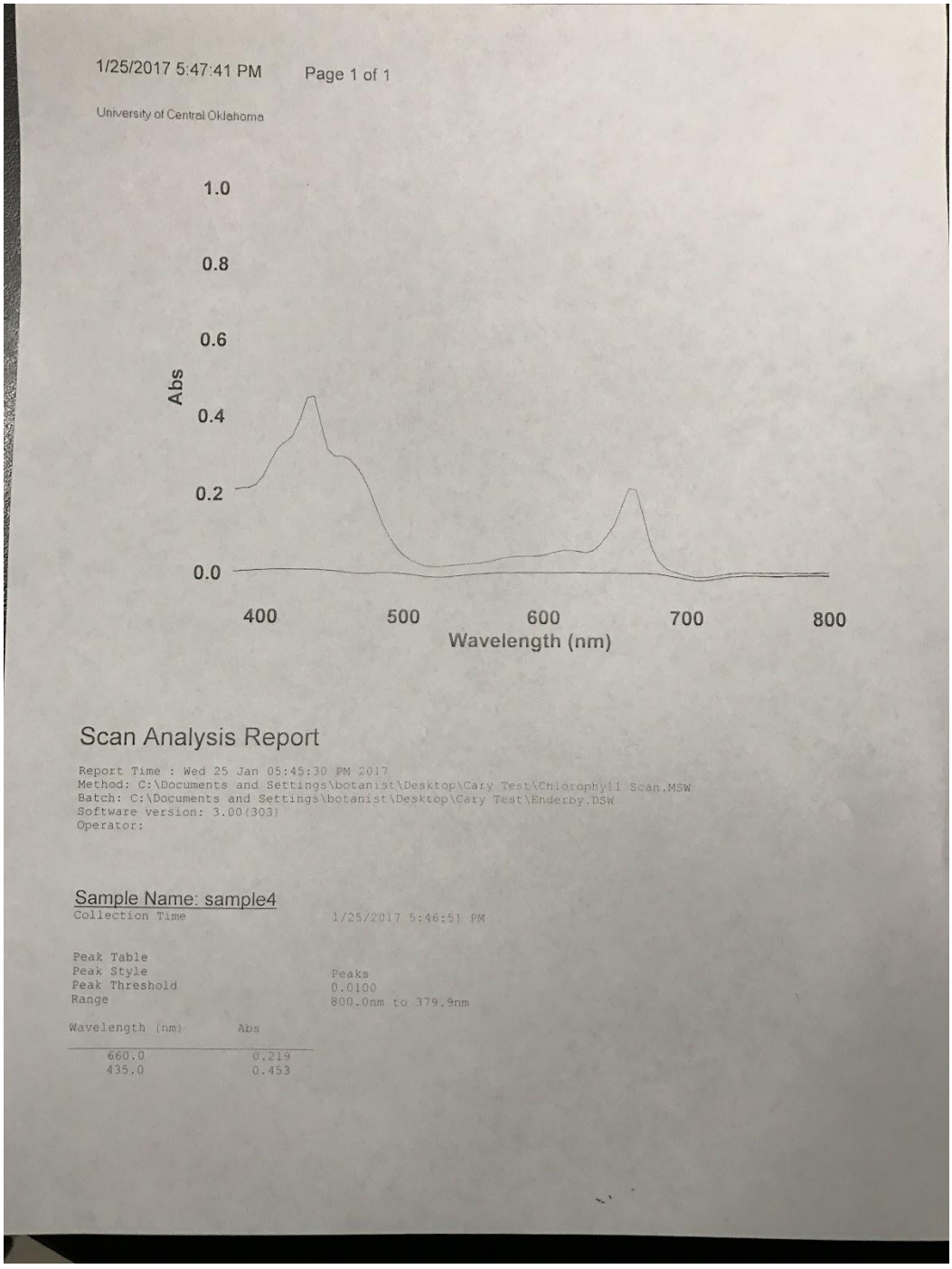


Figure 4. Team 1 graph from the Varian Cary WinUV Spectrophotometer.

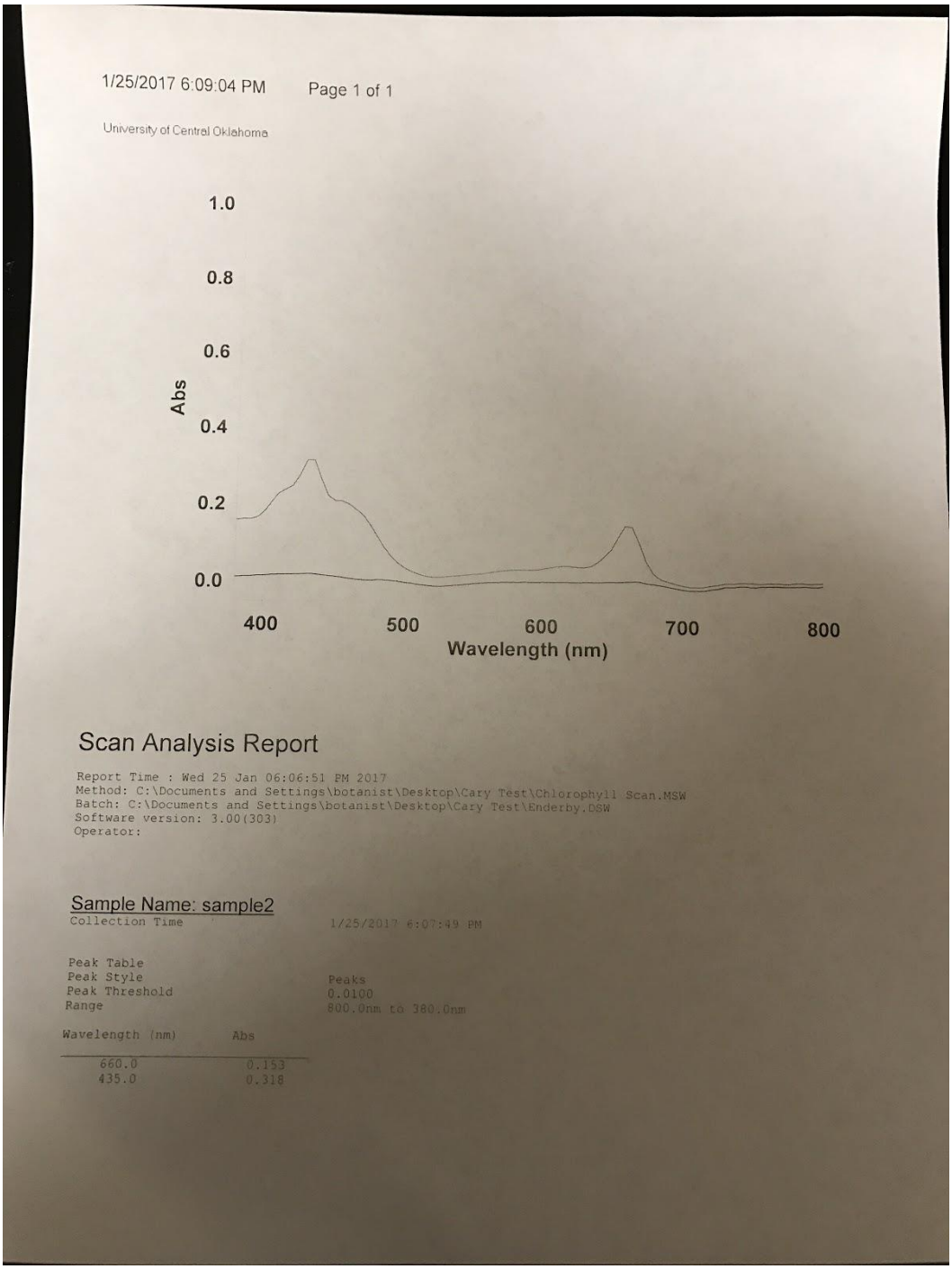
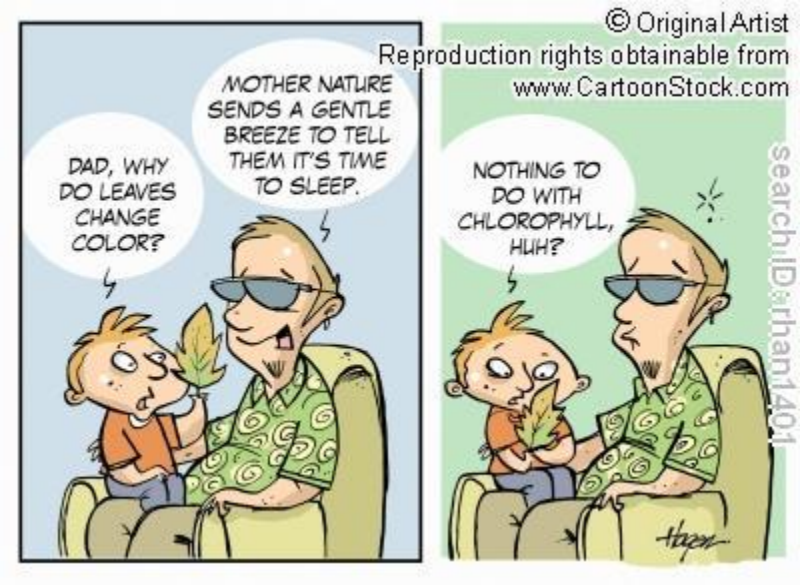


Figure 5. Team 2 graph from the Varian Cary WinUV Spectrophotometer

The result of Team 2 (Table 1, Figure 3) with blue absorbance at a lower wavelength could have been a result of human error in diluting the pigment after double filtration. Team 1 results (Table 1, Figure 2) show a lower wavelength for red absorbance than at 640 nm. Other than human error, the differing wavelength values between Teams 1 and 2 could be related to the slightly different wavelength

absorbance of chlorophyll a an b. Chlorophyll a absorbs blue light at approximately 420 nm and red light at 670 nm, while chlorophyll b absorbs blue light at approximately 470 nm and red light at 650 nm (Taiz, 175). The value of wavelength for red absorbance peak for Team 1 is closer to the value of chlorophyll b than that of chlorophyll a. For Team 2, the value of blue absorbance obtained at 440 nm is more closely related to that of chlorophyll a than b.



Conclusion

The differences in chlorophyll pigment absorbance peaks at varying wavelengths was probably due to human error, Spectrophotometer capabilities, and/or Chlorophyll a and b absorbance differences. The readings obtained in this experiment had an expected wavelength range of approximately 435 nm to 460 nm for maximum blue light absorbance and 660 nm to 675 nm for red light absorbance (Taiz, 175). Taking all the above into consideration, Team 2 results are more accurate to the expected outcome for the experiment than Team 1 results, as Team 2 findings more closely resemble the expected outcome.

References

Taiz, Lincoln, et al. *Plant Physiology and Development*. Sunderland, MA., 2015.