Spectroscopy of Fast Green Solution and Chlorophyll A and B

Introduction

The purpose of this lab is to be introduced to measure the concentration of an unknown solution using spectroscopy and to isolate the individual pigments of a chloroplast extract to measure the light absorption of chlorophyll A and B. Fast green is Light is a photon, a particle without mass however is a wave. (Petrucci, 2010)

In these experiments the visible light spectrum having a wavelength between 400 nm to 700 nm was used. A spectrophotometer separates light into distinct bands of energy, allowing on to focus a particular band of energy to measure its absorption from 0 to 100%. (Jones et al., 2007) The spectrophotometer tells the observer the absorbance based on whatever wavelength one wants to find. (Jones et al., 2007) An essential part of observing a substance under a spectrophotometer is that of using a blank. The role of a blank and the spectrophotometer is to set the absorbance of the spectrophotometer to zero allowing the absorbance of one substance to be shown. (Jones et al., 2007)

In experiment one; water was used as the blank. Based on the results from the spectrophotometer, on is able to make a concentration curve to find the concentration of the unknown solution. (Jones et al., 2007) A spectrophotometer is an instrument that measures the intensity of the light entering a sample and the light exiting a sample and compares the two intensities. Information about the two intensities can be expressed as transmittance. (Vogelman and Evans, 2002) The transmittance is the ratio of the intensity of the exiting light to the entering light. (Jones et al., 2007) This value is expressed as a percentage. Different materials absorb different wavelengths of light. (Karp, 2010) Therefore, the wavelength of maximum absorption by a material is one of the characteristic properties of that material. (Karp, 2010) The

transmittance can be related to the absorbance (A) by the formula A is equal to 2 – (log(%T)). For example if the transmittance is 60 percent because 40 percent of the intensity of the light is absorbed passing through the sample.(Jones et al., 2007) The absorption would be 2 – log 60 which is 0.22184 A. Beer's Law states that the absorbance is directly proportional to the concentration of a solution. (Vogelman and Evans, 2002)

If you plot absorbance versus concentration, the resulting graph yields a straight line. This is called an absorptions spectrum which illustrates the intensity of light absorbed to its relative wavelength. (Karp, 2010) The equation for the straight line can be used to determine the concentration of an unknown solution. (Karp, 2010) This is how the unknown concentration fast green is found in experiment number one. (Karp, 2010)

Materials and Methods

The protocol and materials used for the experiment were completed as written in the Department of Biology of the University of Waterloo Fall term 2010 Biology 130L Lab Manual, pages 34 to 37. No changes were made to the procedure.

Results

Dilution Sample Calculations

$C_1V_1 = C_2V_2$

$C_2 = (C_1V_1) / V_2$

$C_2 = (0.015mg/mL)(10mL)/(20mL)$

$C_2 = 7.5 \times 10^{-3}$

Table 1) Absorption Values of Stock Fast Green Solution (0.015 mg/mL) for Wavelengths 460 – 700 nm

Wavelength (nm)	Absorption (A)
460	0.038
480	0.014
500	0.018
520	0.028
540	0.069
560	0.159
580	0.310
600	0.525
615	0.854
620	0.946
625	0.970
630	0.963
640	0.643
660	0.146
680	0.020
700	0.008

Table 1 shows the results of the testing of absorption of the 0.015 mg/mL stock fast green solution using the spectrophotometer. The maximum absorbance or peak is observed at a wavelength of 625 nm.

Table 2) The Absorption Values of each Concentration of Fast Green at Wavelength 625nm

Fast Green Solution Concentration (mg/mL)	Absorption(A)
1.5×10^{-2}	0.970
7.5×10^{-3}	0.440
3.75×10^{-3}	0.257
1.875×10^{-3}	0.169
9.375×10^{-4}	0.119
Unknown # 207	0.666

Table 2 shows the results of the testing absorptions of the different known concentrations of fast green solution and of the unknown # 207. The absorbances are all measured at the wavelength of 625nm.

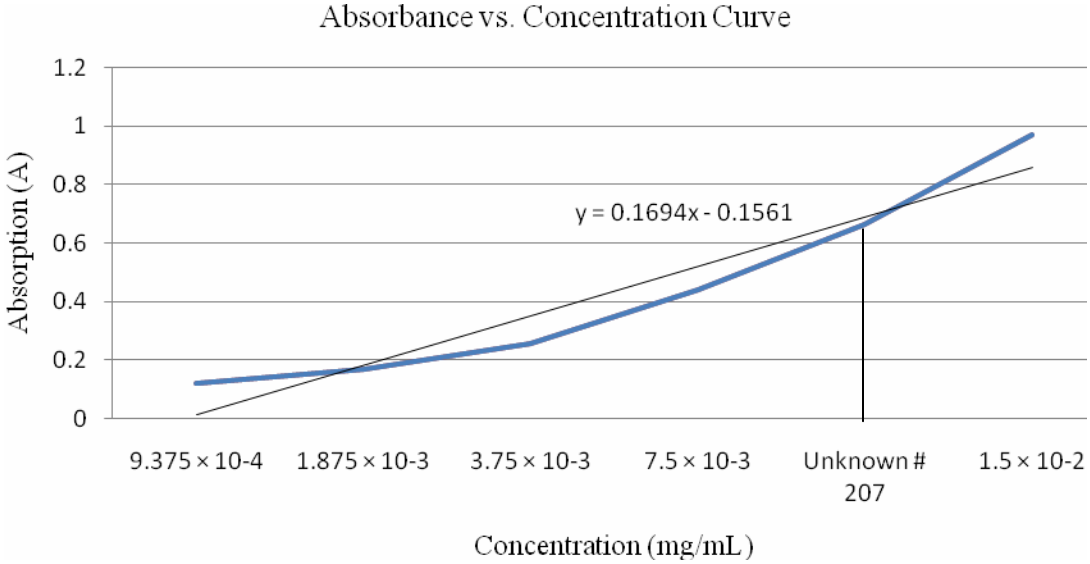

Figure 1) Concentration Curve illustrating the Absorbance at Wavelength 625 nm

Figure 1 illustrates the results for the concentration curve of test tubes one through five of this experiment at a wavelength of 625 nm. This is done so the concentration of the unknown # 207 can be calculated. The equation of the line of best fit is y = 0.1694x – 0.1561. By analysing the graph the concentration of the unknown may be about 1.125×10^{-2}mg/mL.

Figure 2) Drawing of Chromatogram of Chloroplast Pigments from Homogenized Spinach

The chromatogram illustrates the four chloroplast pigments which where separated from a chloroplast extract. The solvent, 90 petroleum ether: 10 acetone, dissolved the extract and allowed the pigments to migrate along the matrix. Carotene moved up the highest and it is the

colour orange. Next is the yellow Xanthophylls, then the green chlorophyll A and finally the olive green chlorophyll B.

Table 3) Absorption Spectrum of Chlorophyll A and B

Wavelenght (nm)	Chlorophyll A Absorption(A)	Chlorophyll B Absorption(A)
400	0.375	0.140
415	0.456	
420	0.454	0.200
425	0.541	
430	0.588	
435	0.497	
440	0.351	0.270
445		0.305
455		0.400
460	0.143	0.396
465		0.319
480	0.061	0.090
500	0.029	0.027
520	0.028	0.025
540	0.032	0.029
560	0.050	0.034
580	0.056	0.039
600	0.056	0.050
620	0.083	0.046
635		0.095
640	0.087	0.118
645		0.109
655	0.270	
660	0.371	0.105
665	0.396	
670	0.289	
680	0.069	0.020
700	0.007	0.005

Table 3 shows the absorptions for chlorophyll A and B at different wavelengths. It is used to find the two peeks for each pigment. For chlorophyll A, the peaks are at wavelengths 430 nm and 665nm. For chlorophyll B, the peaks are at the wavelengths 455 nm and 640 nm.

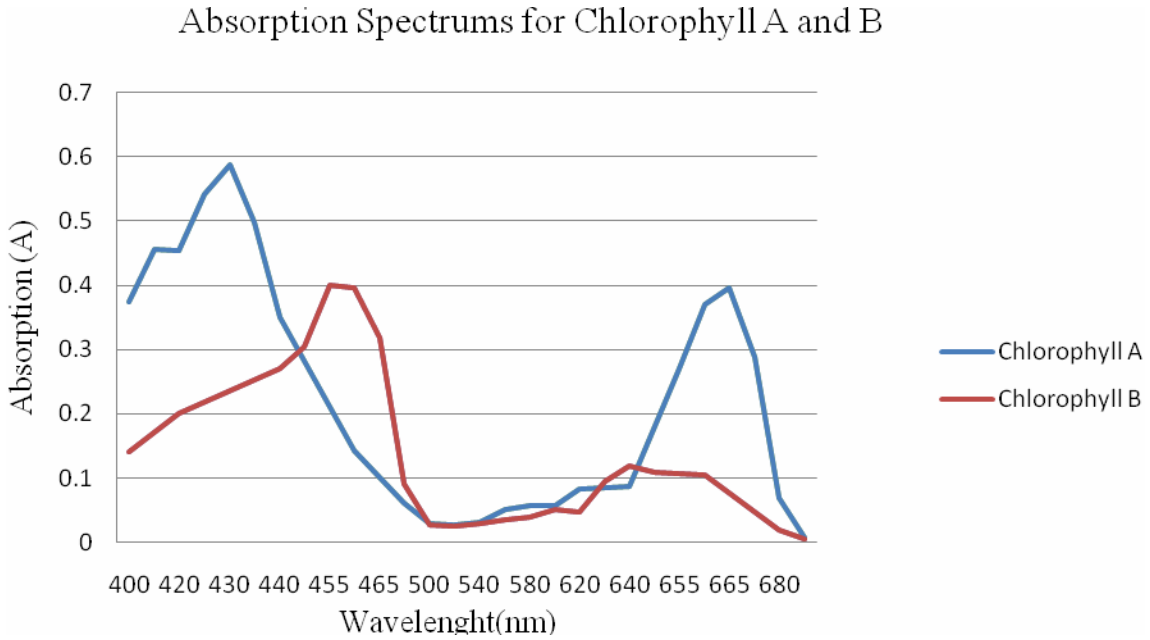

Figure 3) Absorption Spectrums for Chlorophyll A and B

Figure 3 shows the peaks of both chlorophyll A and B. For chlorophyll A, the peaks are at wavelengths 430 nm and 665nm. For chlorophyll B, the peaks are at the wavelengths 455 nm and 640 nm.

Discussion

In experiment 1, a spectrophotometer took absorbance at specific wavelengths to make a graph illustrating the absorption of fast green solution. From this information, the maximum absorption was found and each of the remaining test tubes were measured at this wavelength to find their absorbencies. From these absorbencies, concentrations were calculated these were used to make a concentration curve. This concentration curve illustrated Beer's Law which states that as concentration increases so does the absorption. (Vogelman and Evans, 2002)

However an error such as not properly handling the cuvettes or a reading mistake causes the graph to not be straight and linear. The curvature of Figure 1 does not disprove Beer's Law but instead it shows that there is a source of error in the experiment. Because of this error the unknown concentration cannot be properly calculated. In other words, concentration and absorption are proportional. The absorbance of the unknown concentration can be used to find the concentration by drawing a line from the unknown absorbance and y axis to the point on the graph.

In experiment 2, chromatography paper was used to isolate the various pigments of chlorophyll of which include chlorophyll A, chlorophyll B, carotene and xanthophylls. (Karp, 2010) This resulted in the paper having many different colours and layers. These layers were very specific carotene (orange) at the top, xanthophylls below (yellow), and following this was chlorophyll A and B. (Karp, 2010) The solvent, 90 petroleum ether : 10 acetone, allowed the pigments to migrate up the matrix, chromatography paper. These pigments are compounds that appear coloured because they absorb only certain wavelength of light in the visible spectrum. Leaves on most plants are green because they contain chlorophyll. (Karp, 2010) Chlorophyll absorbs the red and blue light of the spectrum which leaves the colour green to be reflected to our eyes. (Karp, 2010) As seen in Figure 3, the chlorophyll A peaks at wavelengths 430 nm and 665nm and chlorophyll B peaks at the wavelengths 455 nm and 640 nm. Visible light ranges from 390nm to 760nm. (Petrucci, 2010) The colours which correspond with the peak wavelengths are blue which is between 450nm to 500nm and red which is between 650 nm to 700nm. (Petrucci, 2010) The chlorophyll absorbs the red and blue visible light photons which is used in photosynthesis. (Karp, 2010) Because the chlorophyll does not absorb the green light it is reflected. Since the human eye is able to detect wavelengths of light between 400 nm and

750nm, it is able to pick up the green wavelengths which have a wavelength of 500nm to 550nm. (Karp, 2010)

In conclusion, this lab experiment helped be become familiarized with spectroscopy and learn measure the concentration of an unknown solution and isolate the individual pigments of a chloroplast extract to measure the light absorption of chlorophyll A and B.

References

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