Section 1

Section 1 Purpose: The purpose of this lab was to separate plant pigments using chromatography, calculate Rf values using the collected data, and study photosynthesis with isolated chloroplasts. Light energy Light energy Background Information (Activity A): In photosynthesis, plant cells convert light energy into chemical energy that is stored in sugars and other organic compounds. It is an endergonic and anaerobic reaction. Critical to the process is chlorophyll, the primary photosynthetic pigment in chloroplasts. The chemical equation for photosynthesis is: 36 ATP + 6CO2 + 6H2O C6H12O6 + O2 (From: " LabBench for Lab 4." LabBench. PHS School, n. d. Web. 22 Dec. 2012. .) Pigments are chemical compounds which reflect only certain wavelengths of visible light. This makes them appear " colorful". Flowers, corals, and even animal skin contain pigments which give them their colors. More important than their reflection of light is the ability of pigments to absorb certain wavelengths. Because they interact with light to absorb only certain wavelengths, pigments are useful to plants and other autotrophs --organisms which make their own food using photosynthesis. In plants, algae, and cyanobacteria, pigments are the means by which the energy of sunlight is captured for photosynthesis. However, since each pigment reacts with only a narrow range of the spectrum, there is usually a need to produce several kinds of pigments, each of a different color, to capture more of the sun's energy. Four pigments are usually found in many leaves: carotene, xanthophyll, chlorophyll a and chlorophyll b. Carotene is very soluble in the solvent used in the lab. Its molecules don’t form hydrogen bonds with cellulose, an important polysaccharide in cell walls used for support. Carotene makes a faint yellow to yellow-orange band. Xanthophyll is less soluble than carotene in the solvent. It forms some hydrogen bonds

with cellulose. Xanthophyll produces a yellow band. Both chlorophyll a and chlorophyll b easily make hydrogen bonds with cellulose. Chlorophyll a makes a bright green to blue-green band, while chlorophyll b produces a yellow-green to dark olive green band. (From: " Photosynthetic Pigments." Photosynthetic Pigments. N. p., n. d. Web. 22 Dec. 2012. . & Carolina Student Guide for AP Biology Laboratory 4: Plant Pigments and Photosynthesis) Background Information (Activity B): In the light reactions of photosynthesis, light energy is taken in by chlorophyll, the pigment that makes plants green, and is used to excite electrons, the negatively charged subatomic particle. The excited electrons then enter one of two electron transport chains. One chain turns ADP + P to ATP. The other chain changes NADP + H to NADPH. In this part of the lab, we will add a solution of DPIP, which is a blue dye to a suspension of chloroplasts, the plant cell organelle that conducts photosynthesis. The DPIP will replace NADP in the light reactions: DPIP + H DPIPH. DPIPH is colorless, so as the light reactions occur, the blue color of the solution will decrease. We will use this color change as an indication that the light reactions are occurring and we will use the rate at which the color change is happening as a measure of the rate of the light reactions. Independent Variable: The amount of light and the boiling/unboiling/no chloroplasts in the suspension Dependent Variable: % of light transmittance Hypothesis: If a cuvette contains boiled chloroplasts or has unboiled chloroplast in the dark, then they will have a lower percentage of light transmittance than the cuvette containing unboiled chloroplasts exposed to light. Section 2 Materials (Activity A) * Chromatography jar tightly capped with solvent * Chromatography paper * Green Leaf * Coin * Small staples or paper clips * Ruler Procedure (Activity A) 1. Obtain an 8-cm square piece of

chromatography paper and one fresh spinach (or other) leaf. 2. Make two pencil marks 1. 5 cm from one edge of the chromatography paper. 3. Lay the leaf on the chromatography paper, near one edge. Using the marks as a guide, lay a ruler on top of the leaf so that the edge of the ruler is on the paper exactly 1. 5 cm from and parallel to the edge of the paper. 4. Using the ruler as a guide, roll a coin over the leaf, driving the leaf pigments into the paper in a straight line 1. 5 cm from the edge of the paper. You should see a dark green stripe of pigment. If not, repeat this step suing the same 1. 5 cm line, but reposition the leaf so that you roll the coin over fresh leaf tissue. Use a pencil to mark the location of the bottom of the pigment line on the paper. Use this line as the origin. 5. Form a cylinder with the chromatography paper by stapling or paper-clipping each end, so that the two edges do not overlap. Place the chromatography paper in the jar so that the pigment-streaked end of the paper is barley immersed in the solvent. The pigment stipe itself should not be in the solvent. CAUTION: Avoid breathing fumes from the solvent. 6. Tightly cap the jar. Do not disturb the jar for several minutes, but continue to observe the chromatography paper within. 7. When the solvent is about 1 cm from the top margin of the paper, remove the paper from the jar and immediately mark the location of the solvent front before it evaporates. 8. Mark the bottom of each pigment band. 9. Beginning at the origin line, measure the distance traveled by the solvent front and each of the pigment bands. Record the results in Table 1. Number the bands so that Band 1 is the pigment band nearest the origin line at the bottom of the paper. 10. For a given solvent and substrate system (in this case, cellulose), each pigment will move a distance that is proportional to the distance moved by the solvent. This is expressed as the Rf value and it is

a constant for the solvent/substrate/pigment. Calculate Rf values for each of the pigment bands you have identified. Record this data in Table 1. 11. Using the data you have collected, make a t least tentative identifications of the chlorophyll band(s) and other major bands on your chromatography paper. Record these in the “ Band Color/Identification" column of Table 1. Materials (Activity B) * Spectrophotometer/colorimeter * 5 cuvettes * Aluminum foil * Heat sink (aquarium filled with water) * Lamp * 4 dropping pipets * Vial of unboiled chloroplasts * Vial of boiled chloroplast suspension * Vial of 0. 1 M phosphate buffer * Vial of DPIP solution * Distilled water * Lens tissue * Bucket of ice * 4 squares of Parafilm * Labels * Ruler * Calculator * Clock/timer * Test tube rack Procedure (Activity B) Important: Do not add the chloroplast until you are completely ready to put it into the test chamber. Handle the cuvettes by their tops only. If you touch the sides, you will leave a fingerprint that may interfere with light transmission. Wipe the sides of a cuvette with lens tissue before inserting it into the test chamber. 1. Turn on the spectrophotometer. 2. Once the spectrophotometer has warmed up, set it to read light transmission at 605 nm. 3. Set up a work area with the lamp, cuvettes, and heat sink. (The water in the aquarium will absorb infrared radiation (heat) that could damage the chloroplasts.) 4. Label your cuvettes 1, 2, 3, 4 and 5. If your cuvettes have caps, label the caps also. If not, place the labels near the tops of the cuvettes. The labels must not block the light beam used by your instrument. 5. Use a new, clean dropping pipet to add 4 mL of distilled water (H_2O) to Cuvette 1. 6. Use the same pipet to add 3 mL of distilled water to cuvettes 2-5. 7. Use the same pipet to add 3 additional drops of distilled H_2O to Cuvette 5. 8. Still using the same pipet, add 1 mL of phosphate buffer to each cuvette. 9. Use a new, clean 9second0 pipet to add

1 mL of DPIP to cuvettes 2-5. 10. Fashion an aluminum foil cover for Cuvette 2. The cover must prevent light from entering the cuvette. 11. Obtain a vial of unboiled chloroplast suspension. Keep these vials on ice throughout this activity. 12. Mix the unboiled chloroplast suspension by inverting the vial (make sure cap is secure). Use a new, clean (third) pipet to add 3 drops of the unboiled chloroplast suspension to Cuvette 1. 13. Cap or cover Cuvette 1 with Parafilm and gently mix the contents. Insert Cuvette 1 into the test chamber and adjust the light-control knob to get a 100% transmittance reading. 14. Mix the unboiled chloroplast suspension and use the 3rd pipet to add 3 drops of the suspension to Cuvette 2. Immediately mix the contents of Cuvette 2. Remove Cuvette 2 from its foil cover, insert it into the test chamber, and read its % transmittance. Record the results in Table 3 under 0 min. Return Cuvette 2 to its foil cover, and place it in the test tube rack. Turn on the lamp. Repeat readings at 5, 10 and 15 minutes. Mix the contents of the cuvette each time before taking the reading. 15. Mix the unboiled chloroplasts suspension and use the third pipet to add 3 drops of the suspension to Cuvette 3. Immediately mix the contents of Cuvette 3. Insert it into the test chamber and read its % transmittance. Record the results in Table 3 under 0 min. Place Cuvette 3 in the test tube rack. Repeat readings at 5, 10, and 15 minutes. Mix the contents of the cuvette each time before taking the reading. 16. Mix the boiled chloroplast suspension and use the last (third) pipet to add 3 drops of the suspension to Cuvette 4. Immediately mix the contents of Cuvette 4. Insert it into the test chamber and read its % transmittance. Record the results in Table 3 under 0 min. Place Cuvette 4 in the test tube rack. Take readings at 5, 10, and 15 minutes. Mix the contents of the cuvette each time before taking the reading. 17. Mix the contents of

Cuvette 5. Insert it into the test chamber and read its % transmittance. Record the results in Table 3 under 0 min. Place Cuvette 5 in the test tube rack. Repeat readings at 5, 10, and 15 minutes. Mix the contents of the cuvette each time before taking the reading. Chromatography of Plant Pigments Chromatography of Plant Pigments DATA (Activity A) Solvent Front Solvent Front Distance from origin (mm) Distance from origin (mm) 0. 887096774(0. 88) 0. 887096774(0. 88) 0. 193548387(0. 19) 0. 193548387(0. 19) 0. 129032258(0. 12) 0. 129032258(0. 12) 0. 080645161(0. 08) 0. 080645161(0. 08) Rf Value Rf Value Yellow Orange Yellow Orange Yellow Yellow Blue Marine Blue Marine Olive Green Olive Green Band Color/Identification Band Color/Identification 55 mm 55 mm 12 mm 12 mm 8 mm 8 mm 5 mm 5 mm 62 mm 62 mm Band # Band # 1 1 2 2 3 3 4 4 Plant Pigmentation/Photosynthesis-Lab #4 - Class Data collected- 12-11-12(Activity B) OUR GROUP= Group 7 Unboiled dark | | Time (minutes) | | | | 0 | 5 | 10 | 15 | Group 1 | 37. 6 | 74. 8 | | | Group 2 | 61. 6 | 72 | 72. 2 | 70 | Group 3 | 47. 2 | 51. 8 | 51. 2 | 51. 4 | Group 4 | 41. 6 | 61. 3 | 62 | 49. 2 | Group 5 | 64 | 66 | 70 | 69 | Group 6 | 63. 1 | 75. 1 | 70. 1 | 67. 8 | Group 7 | 72. 2 | 95 | 96. 8 | | | | | | Average | 48. 4125 | 62 | 52. 7875 | 38. 425 | Legend Legend Unboiled light | | Time (minutes) | | | | 0 | 5 | 10 | 15 | Group 1 | 55. 8 | 31. 4 | | | Group 2 | 58. 4 | 97 | 97. 4 | 92. 2 | Group 3 | 32 | 51. 4 | 49. 2 | 48. 6 | Group 4 | 39. 3 | 56. 8 | 57. 4 | 46. 7 | Group 5 | 58. 6 | 73. 8 | 84. 6 | 84. 4 | Group 6 | 67. 1 | 114. 7 | 107. 7 | 106. 8 | Group 7 | 62. 2 | 77. 6 | 76. 6 | | | | | | Average | 46. 675 | 62. 8375 | 59. 1125 | 47. 3375 | Legend Legend Boiled light | | Time (minutes) | | | | 0 | 5 | 10 | 15 | Group 1 | 27. 6 | 63 | | | Group 2 | 38. 2 | 57. 4 | 58. 8 | 55. 2 | Group 3 | 21. 4 | 32. 6 | 30 | 27 | Group 4 | 48. 9 | 62. 6 | 72. 6 | 59. 1 | Group 5 | 58. 2 | 60. 1 | 62. 2 | 59 |

Group 6 | 61. 9 | 77. 6 | 67. 9 | 61. 8 | Group 7 | 108. 2 | 137. 6 | 128. 6 | | | | | | | Average | 45. 55 | 61. 3625 | 52. 5125 | 32. 7625 | Legend Legend No Chloroplasts | | Time (minutes) | | | | 0 | 5 | 10 | 15 | Group 1 | 128 | 30. 8 | | | Group 2 | 86. 2 | 86 | 85. 8 | 85. 2 | Group 3 | 62. 2 | 62. 8 | 62. 6 | 62. 2 | Group 4 | 83 | 88 | 90. 8 | 72. 6 | Group 5 | 95 | 94. 2 | 93. 8 | 92. 6 | Group 6 | 78 | 88. 3 | 84. 9 | 83. 8 | Group 7 | 118. 8 | 99. 4 | 99. 6 | | | | | | | Average | 81. 4 | 68. 6875 | 64. 6875 | 49. 55 | Legend Legend Calculations distance of solvent front from origin distance of solvent front from origin Activity A: Rf values= distance of pigment from origin Band Color Rf VALUE Olive Green: 5 mm/62 mm= 0. 080645161 mm Blue Marine: 8 mm/62 mm= 0. 129032258 mm Yellow: 12 mm/62 mm= 0. 193548387 mm Yellow Orange: 55 mm/62 mm= 0. 887096774 mm Activity B: Measurements of distilled H2O, phosphate buffer, and DPIP in ml & obtaining the reading on the spectrophotometer for the percent of light transmittance. Additionally, calculating time for each reading. Section 3 Analysis The graphs above show a number of significant things. For instance, the % of light transmittance - which was the dependent variable in this experiment-was highest for our group at the boiled light chloroplasts suspension; however for the class data, it was highest at no chloroplasts. This may be because the no chloroplasts could absorb much of the light because it was filled with only distilled H2O. However, in our group’s data, the chloroplast suspension of boiled light had the most light transmittance. The class data, however, shows that the majority of the class had low percentages of light transmittance. This is probably because the chloroplasts/s enzymes have become denatured by boiling it and therefore are less effective in absorbing light. This was a very common trend shown in the graphs above. Another pattern seen in the data

would be that most of the no chloroplast suspensions stayed the same. There was almost no change in the amount of light transmitted for most of the class data. In fact, between 5 and 10 minutes for our group's data, the change was only moved up by . 2. This shows that because this suspension contained no chloroplasts, there could be no photosynthesis; therefore no light was being transmitted. Basically, the class data showed that the lowest amount of light transmittance was seen in the boiled/light chloroplast suspension because of the denatured enzymes in the chloroplasts. Next, no chloroplast had a low percentage of light transmittance. Then, unboiled/dark was also had a low light transmittance percentage mainly because the aluminum foil around the suspension prevented almost all light from entering the suspension. Lastly, unboiled/light had the highest light transmittance percentage, because its chloroplasts enzymes were not denatured; therefore they could function at optimum levels. If temperature became a variable in the experiment, the data would change. Temperature would change the independent variable and the dependent variable. The amount of light transmittance in any given chloroplast suspension would increase to a point and then denature from extreme temperature. However, our results would have been slightly different had the data been accurate, but still a bit similar. Conclusion In summation, my hypothesis was correct. The class data (though it had numerous errors such as a group that didn't exist and some groups data was taken incorrectly) showed that the suspensions that contained boiled chloroplasts and the suspension that was in the dark had a lower percentage of light transmittance than the unboiled chloroplast suspension in the light. The no chloroplast suspension had very little change as seen in the data. The boiled chloroplast had denatured

chloroplast because they were boiled, which caused them to have a lower % of light transmittance than the chloroplast suspension with unboiled chloroplasts and kept in the light. The chloroplast suspension that had unboiled chloroplasts but was kept in the dark also had a lower % of light transmittance. Although the suspension had unboiled chloroplasts and the enzymes were working correctly, it was kept in the dark, which made it harder for light to enter the suspension, thus reducing the amount of light transmittance. Lastly, the suspension with unboiled chloroplasts and kept in light was in optimal conditions for photosynthesis; therefore it had the highest amount of light transmittance. Error Analysis There were many possible sources of error. For instance, the oil on our fingers could have gotten on the cuvettes when putting them in the spectrometer and mess up the reading. When measuring the liquids such as distilled H2O, DPIP, and the phosphate buffer, one could measure too much or too little. The time may have also been inaccurate when taking the reading. One may have put the suspension in too soon or too late. When the chloroplast suspensions were put in front of the fish tank and heat lamp, some may have received too much heat, thus changing the results and giving inaccurate data. I learned that photosynthesis can be affected by a number of divergent conditions such as boiling/unboiling, and light/dark. Photosynthesis is decreased when enzymes in the chloroplasts are denatured or there is little light to be absorbed. Plants also have many different pigments of different colors in them and can be seen when doing chromatography. Optimal conditions for plant photosynthesis are high (to an extent) light intensity, unboiled chloroplasts, high CO2 amount, and high (to an extent) temperature. All these things combined along with habitable environment well suited to the

plant will result in a high % of light transmittance, thus high level of photosynthesis. Our class data was slightly incorrect. There was a group that was counted that didn’t exist and got 0 for everything, which brought the average down. Additionally, some groups (including our own) had outlier data-data that was either too high or too low that affected the average. Therefore, this lab was not completely accurate and conclusions that were drawn from the data may not necessarily be correct. Real Life Application This lab can be applied to real life for farmers. Farmers could see from this lab that extreme conditions can affect the amount of photosynthesis in plants. If a plant does not go through photosynthesis, then the plant will struggle to survive, as it has no other way to feed itself. The autotroph would then eventually die and the crop harvest would be horrible. Farmers