PLANT PIGMENTS AND PHOTOSYNTHESIS

Driving Question

How do we know what the different photosynthetic pigments are and how can photosynthesis be measured?

Materials and Equipment

- Chromatography solvent, 5 mL
- Colorimeter sensor with interface
- Cuvettes (5)
- Glass jar, 10 to 12 cm tall
- Disposable graduated pipet (2), 1-mL
- Chloroplast suspension, 2 mL
- 0.1 M phosphate buffer, 4 mL
- DPIP in small amber bottle, 3 mL
- #1 Whatman® chromatography paper
- Spinach
- Quarter or other coin
- Kimwipes® or other lint-free tissue
- Distilled water, 13 mL
- Floodlight, 100 watt
- Heat sink (large beaker or flask filled with water)
- Aluminum foil
- Cheesecloth
- Ice

Background

Plants are photosynthetic organisms, able to harness light energy from the sun to convert carbon dioxide gas from the atmosphere into sugar through photosynthesis as follows:

$$6CO_2 + 6H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2.$$

The photosynthetic pigments absorb light energy from the sun. As light hits the chloroplasts, electrons are excited and passed along an elaborate electron transport chain within the thylakoid membrane of the chloroplasts. The electrons eventually reduce the molecule nicotinamide adenine dinucleotide phosphate ($NADP^+$) to form NADPH.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety glasses and lab coats or aprons.
- Due to the volatility of the chromatography solvent, ensure all containers remain tightly sealed.

Procedure

1. Connect to the colorimeter sensor.

2. Open the AGR 06 Plant Pigment and Photosynthesis.spklab file.

3. Prepare the incubation area. You will need a flood light and a heat sink (a large beaker or flask filled with water). Place the flood light directly in front of the heat sink. The heat sink will absorb the heat from the flood light while still allowing light to pass through to the cuvettes that are placed a few inches behind the flask.

4. Obtain five cuvettes and label the tops from "1"to "5". Obtain five cuvettes and label the tops from "1"to "5". Fill each of the cuvettes according to Table 4.1 below, <u>but do not add either the unboiled or boiled chloroplasts</u> yet.

Table 4.1: Setup for photosynthesis experiment

Contents	Cuvette 1 Blank (no DPIP)	Cuvette 2 Unboiled chloroplasts (Dark)	Cuvette 3 Unboiled chloroplasts (Light)	Cuvette 4 Boiled chloroplasts (Light)	Cuvette 5 No chloroplasts (Light)
Phosphate buffer	1 mL	1 mL	1 mL	1 mL	1 mL
Distilled water	4 mL	3 mL	3 mL	3 mL	3 mL + 3 drops
DPIP	None	1 mL	1 mL	1 mL	1 mL
Unboiled chloroplasts	3 drops	3 drops	3 drops	None	None
Boiled chloroplasts	None	None	None	3 drops	None

5. Add 3 drops of the unboiled chloroplast suspension to cuvette 1 (blank no DPIP). Screw the lid onto the cuvette and mix by inverting the cuvette several times. Wipe the sides of the cuvette gently with a Kimwipe® or other lint-free tissue.

6. Insert the cuvette into the cuvette holder on the colorimeter and close the colorimeter lid tightly. Calibrate the colorimeter. This step is added to help calibrate the sensor and establish a baseline.

7. Remove the cuvette. Do not replace the aluminum foil.

8. For cuvette 2 add 3 drops of the unboiled chloroplast suspension.

9. Remove the aluminum foil from the cuvette, and insert into the cuvette holder on the colorimeter. Close the colorimeter lid tightly.

10. Display % Transmittance in a digit display. Start recording data. Record the % Transmittance value in Table 4.2.

11. Remove cuvette form the colorimeter.

12. Recover the cuvette with foil.

13. For cuvettes 3-5 add the prescribed drops of unboiled or boiled chloroplasts listed in table 4.1. **Note**: for cuvette 5 add 3 drops of distilled water instead of chloroplasts.

14. Repeat steps 9-11. Note: for cuvettes 3-5 don't recover with foil.

15. Place all cuvettes in the incubation area. Note the time that the cuvettes are put in the incubation area.

16. Measure the % Transmittance of cuvettes 2-5 again at 5 minutes, 10 minutes and 15 minutes then record the data in Table 4.2.

Table 4.2: % Transmittance

Cuvette	Conditions	% Transmittance (Initial)	% Transmittance (5 min)	% Transmittance (10 min)	% Transmittance (15 min)
#2	Unboiled, Dark				
#3	Unboiled, Light				
#4	Boiled, Light				
#5	No Chloroplasts, Light				

17. Obtain a piece of chromatography paper about as long as the height of the glass jar. Cut one end of the paper into a point and draw a line in pencil across the width of the paper 1.5 cm above the point.

19. Lay a spinach leaf on the paper above the pencil line. Deposit the plant pigments onto the paper by firmly rolling the edge of a quarter over the leaf about 15 times until a heavy green line appears on the paper.

20. Lower the paper into the jar ensuring that only the tip of the paper touches the solvent. The green line must be above the solvent. Tightly close the lid of the jar.

21. When the solvent has traveled to about 1 cm below the top of the paper, remove the paper from the jar.

22. Using a pencil, quickly mark the location of the solvent's furthest point of travel before the solvent evaporates. Measure the distance the solvent traveled (the distance between the two lines) and record this value in Table 4.1

23. On the paper, mark the location of the top of each of the pigments. Measure the distance each pigment traveled from the origin to the top of each band. Record these measurements in Table 4.3.

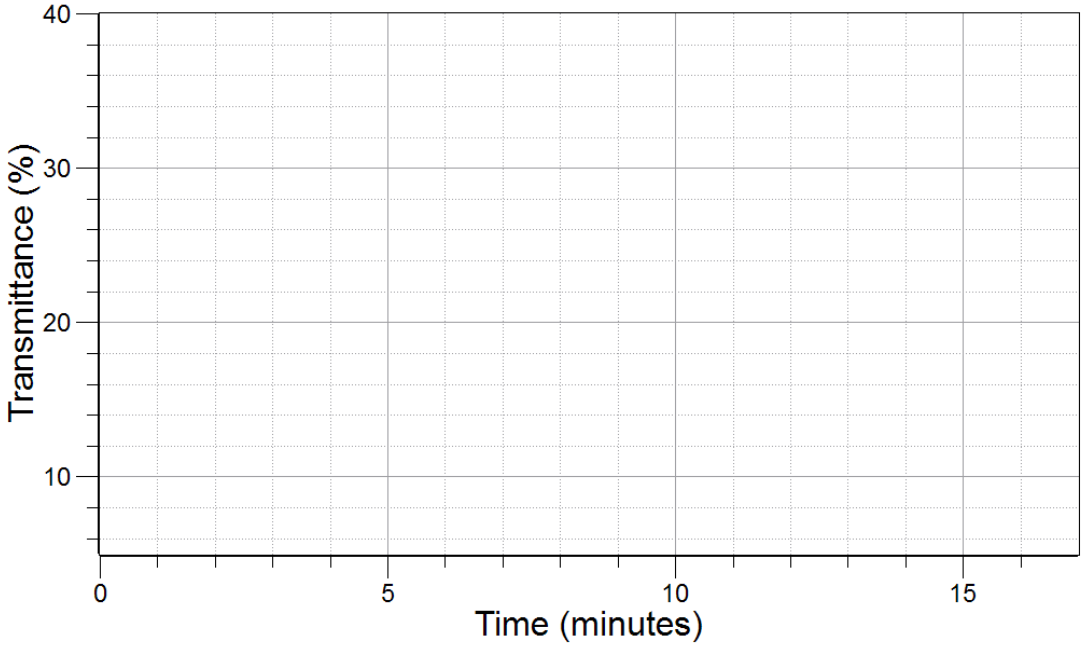
24. Measure the distance each pigment traveled from the origin to the top of each band. Record these measurements in Table 4.3.

Table 4.3: Distance moved by pigment bands (millimeters)

Band Number	Distance (mm)	Band color	R*f*
1			
2			
3			
4			
5			

Analysis & Questions

graph of % Transmittance versus Time



1. Which of the pigments that you observed in the chromatography experiment is most easily dissolved by the solvent? How do you know?

2. How would the *Rf* value change if a different solvent were used?

3. What is the role of DPIP in this experiment? What would happen to your results if you forgot to add the DPIP to cuvette #3?

4. Compare the % Transmittance in cuvettes #3 and #4.

5. Explain in your own words why the colorimeter was used in this lab. What data did it help you collect?

6. Relate leaves changing color in the fall to the accessory pigments.