Plant Pigments and Photosynthesis

EXERCISE A: Plant Pigment Chromotography

Paper chromatography is a useful technique for separating and identifying pigments and other molecules from cell extracts that contain a complex mixture of molecules. The solvent moves up the paper by capillary action, which occurs as a result of the attraction of solvent molecules to the paper and the attraction of solvent molecules to one another. As the solvent moves up the paper, it carries along any substances dissolved in it. The pigments are carried along at different rates because they are not equally soluble in the solvent and because they are attracted, to different degrees, to the fibers in the paper through the formation of intermolecular bonds, such as hydrogen bonds.

The relationship of the distance moved by a pigment to the distance moved by the solvent is a constant (for a given solvent) called R_f. It can be calculated for each pigment in a mixture using the formula:

$$R_f = \frac{\text{distance pigment migrated}}{\text{distance solvent migrated}}$$

Procedure

Paper chromatography is used separate the pigments extracted from a spinach leaf using the apparatus in shown in Figure 1. After 20 minutes, the bottom of each pigment band is marked and the distance each pigment migrated from the bottom of the pigment origin to the bottom of the separated pigment band is recorded. The results are recorded in Table 1. Calculate the R_f value for each pigment

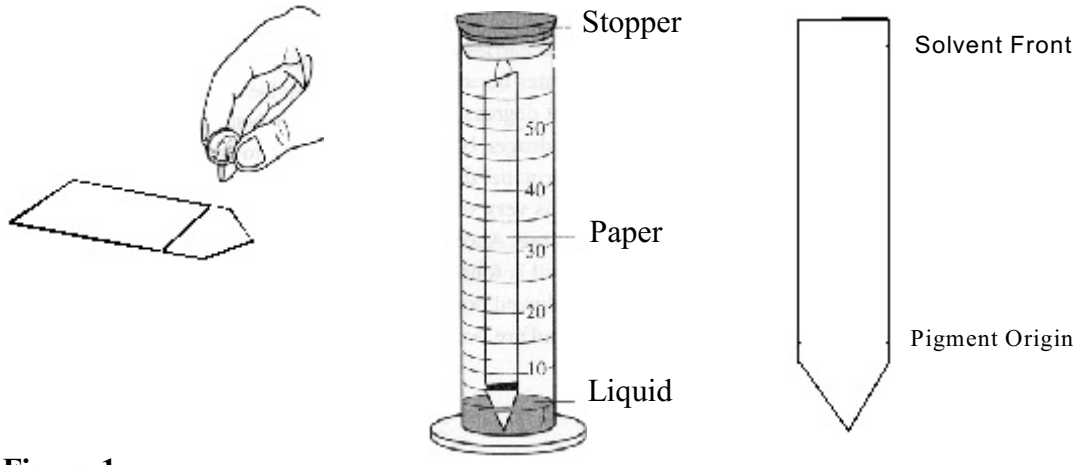

Figure 1

Table 1: Distance moved by Pigment bands (mm)

Band Number	Distance	Band Color
1.	17	yellow green
2.	38	blue green
3.	61	yellow
4.	178	yellow-orange

Solvent Distance: 190 mm

Table 2: R_f Values for all pigments

R_f	Pigment
	carotene (yellow to yellow-orange)
	xanthopyll (yellow)
	chlorophyll a (bright green to blue green)
	chlorophyll b (yellow green to olive green)

EXERCISE B: Photosynthesis: The Light Reactions

Photosynthesis may be studied in a number of ways. For this experiment, a dye-reduction technique will be used. The dye-reduction experiment tests the hypothesis that light and chloroplasts are required for the light reactions to occur. In place of the electron acceptor, NADP the compound DPIP (2,6-dichlorophenol-indophenol), will be substituted. When light strikes the chloroplasts, electrons boosted to high energy levels will reduce DPIP. It will change from blue to colorless.

In this experiment, chloroplasts are extracted from leaves and incubated with DPIP in the presence of light. As the DPIP is reduced and becomes colorless, the resultant increase in light transmittance is measured over a period of time using a spectrophotometer.

Data are collected by shining light through each cuvette and measuring how much of that light is absorbed (or transmitted) by the solution in the cuvette (Figure 2).

Table 4 Time (min)

Cuvette	0	5	10	15
2 Unboiled/Dark	31.3	32.5	35.5	34.8
3 Unboiled/Light	32.7	54.5	63.7	65.1
4 Boiled/Light	32.7	32.9	33.1	32.5
5 No Chloroplasts	31.3	31.3	31.3	31.3

Plot the percent transmittance versus time for the four cuvettes.

Questions

1. What factors are involved in the separation of the pigments?
2. Would you expect the R_f value of a pigment to be the same if a different solvent were used? Explain.
3. How is paper chromatography useful?
4. What is the purpose of DPIP in this experiment?
5. What molecule found in chloroplasts does DPIP "replace" in the experiment?
6. What is the source of the electrons that will reduce DPIP?
7. What is measured with the spectrophotometer in this experiment?
8. What is the effect of darkness on the reduction of DPIP? Explain.
9. What is the effect of boiling the chloroplasts on the subsequent reduction of DPIP? Explain.
10. What reasons can you give for the difference between percent transmittance between the unboiled chloroplasts that were incubated in the light and those that were kept in the dark?
11. Identify the purpose of each of the five cuvettes.