Activity 3. Exploring plant pigment

Activity 3.1. Independent reading exercise

In this part you are expected to develop your understanding of the structure of chloroplasts. Read the text given by your teacher and answer to the questions given below:

1. The photosynthetic pigment that is essential for the process to occur is:

a) chlorophyll *a;* b) chlorophyll *b;* c) beta carotene; d) xanthocyanin; e) fucoxanthin.

2. When a pigment reflects red light:

a) all colours of light are absorbed; b) all colours of light are reflected; c) green light is reflected, all others are absorbed; d) red light is reflected, all others are absorbed; e) red light is absorbed after it is reflected into the internal pigment molecules.

3. Chlorophyll *a* absorbs light energy in the colour range:

a) yellow-green; b) red-orange; c) blue violet; d) a and b; e) b and c.

4. The individual flattened stacks of membrane material inside the chloroplast are known as: a) grana; b) stroma; c) thylakoids; d) cristae; e) matrix.

5. The fluid-filled area of the chloroplast is the: a) grana; b) stroma; c) thylakoids; d) cristae; e) matrix

6. The chloroplasts of plants are closest in size to: a) unfertilised human eggs; b) human cheek cells; c) human nerve cells; d) bacteria in the human mouth; e) viruses.

Activity 3.2. Experiment: Plant pigment chromatography[1]

Paper chromatography is a technique used to separate substances in a mixture based on the movement of the different substances up a piece of paper by capillary action. Pigments extracted from plant cells contain a variety of molecules, such as chlorophylls, beta carotene, and xanthophyll, that can be separated using paper chromatography.

A small sample of plant pigment placed on chromatography paper travels up the paper due to capillary action.

The ratio of the distance moved by a pigment to the distance moved by the solvent is

[1] Adapted from http://www.phschool.com/science/biology_place/labbench/lab4/design1.html

a constant, R_f. Each type of molecule has its own R_f value.

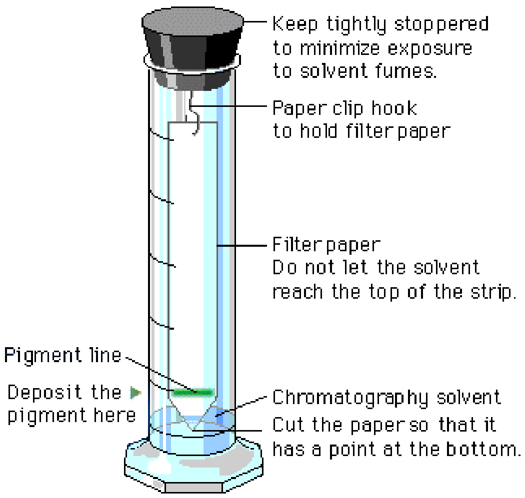
$$R_f = \frac{\text{distance travelled by pigment}}{\text{distance travelled by solvent}}$$

Materials

50 mL graduated cylinder

chromatography paper
(spinach) leaves
coin

cork stopper
pencil
scissors
Solvent
(1 part acetone with 1 part ethanol)
Ruler



Be sure the tip of the filter paper touches the solvent, but keep the pigment line above it.

Procedure

Obtain and wear goggles! ***Caution:*** *The solvent in this experiment is flammable and poisonous. Be sure there are no open flames in the lab during this experiment. Avoid inhaling fumes. Wear goggles at all times. Notify your teacher immediately if an accident occurs.*

1. Obtain a 50 mL graduated cylinder with 5 mL of solvent in the bottom.

2. Cut the chromatography paper so that it is long enough to reach the solvent. Cut one end of the paper into a point.

2. Draw a pencil line 2.0 cm above the pointed end of the paper.

3. Use the coin to extract the pigments from the spinach leaf. Place a small section of the leaf on top of the pencil line. Use the ribbed edge of the coin to push the plant cells into the chromatography paper. Repeat the procedure 10 times making sure to use a different part of the leaf each time.

4. Place the chromatography paper in the cylinder so the pointed end just touches the solvent. Make sure the pigment is not in the solvent.

5. Stopper the cylinder and wait until the solvent is approximately 1 cm from the top of the paper. Remove the chromatography paper and mark the solvent front before it evaporates.

6. Allow the paper to dry. Mark the bottom of each pigment band. Measure the distance each pigment moved from the starting line to the bottom of the pigment band. Record the distance that each of the pigments and the solvent moved, in millimetres.

7. Identify each of the bands and label them on the chromatography paper.

- beta carotene: yellow to yellow orange
- xanthophyll: yellow
- chlorophyll *a*: bright green to blue green
- chlorophyll *b*: yellow green to olive green

9. Staple the chromatogram to the front of your lab sheet.
10. Discard the solvent as directed by your teacher.

Band number	Distance travelled (mm)	Colour	Pigment
1			
2			
3			
4			
5*			
Distance solvent front moved =......................mm			

* The fifth band may not appear.

Processing the data
Calculate the R_f values and fill the Table 2.

Table 2	
Molecule	R_f
beta carotene	
xanthophyll	
chlorophyll *a*	
chlorophyll *b*	

1. What factors are involved in the separation of the pigments?

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2. Why do the pigments become separated during the development of the chromatogram?

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3. Look again at the chromatogram you completed in the previous exercise. Which of the following is true for your chromatogram?
   a. The R_f for carotene can be determined by dividing the distance the yellow-orange pigment (carotene) migrated by the distance the solvent front migrated.
   b. The R_f value of chlorophyll *b* will be higher than the R_f value for chlorophyll a.
   c. The molecules of xanthophyll are not easily dissolved in this solvent, and thus are probably larger in mass than the chlorophyll *b* molecules.
   d. If this same chromatogram were set up and run for twice as long, the R_f values would be twice as great for each pigment.
4. If a different solvent were used for the chlorophyll chromatography described earlier, what results would you expect?
   a. The distances travelled by each pigment will be different, but the R_f values will stay the same.
   b. The relative position of the bands will be different.
   c. The results will be the same if the time is held constant.
   d. The R_f values of some pigments might exceed 1.0