Separating pigments present in plants using Thin Layer Chromatography (TLC) – Student instructions

Introduction

Pigments commonly found in the leaves of green plants include chlorophylls, xanthophyll and carotenes. Their structures are shown in Figures 1,2 and 3.

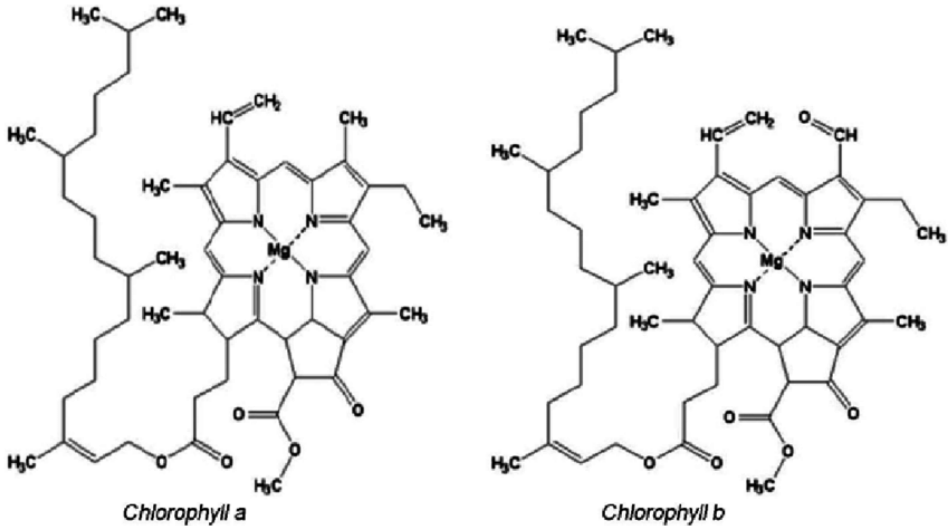


Figure 1. Chlorophylls a and b.

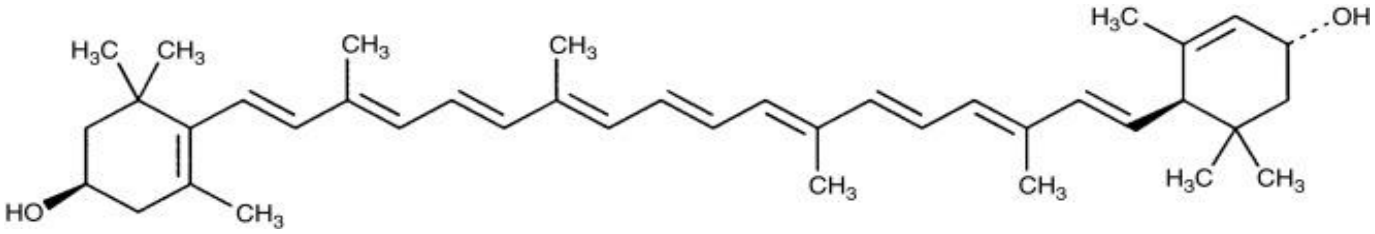


Figure 2. Xanthophyll.

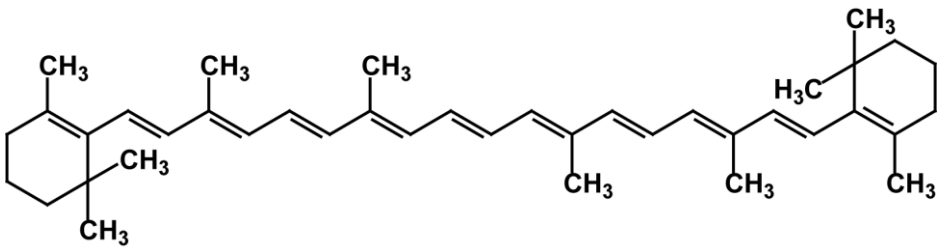


Figure 3. Beta-carotene.

Chlorophyll a, $C_{55}H_{72}O_5N_4Mg$, is a yellow-green coloured pigment that is involved in oxygenic photosynthesis. Chlorophyll b, $C_{55}H_{70}MgN_4O_6$, is a green coloured pigment that gives plants the ability to absorb higher frequency blue light for use in photosynthesis. The structure of chlorophyll a is very similar to that of chlorophyll b. Xanthophyll, $C_{40}H_{56}O_2$, is a yellow pigment. Beta-carotene, $C_{40}H_{56}$, is a red-orange pigment. Xanthophyll and beta-carotene are classified as carotenoids.

One way of extracting, separating and identifying these pigments is to use thin layer chromatography (TLC). This works on the same principle as paper chromatography: different compounds will be attracted to stationary phase and the mobile phase to different extent, so will be carried different distances up the stationary phase by the mobile phase in a given time.

The difference is that instead of being made of paper, the stationary phase is a glass or metal plate at coated with a thin layer of a solid substance such as aluminium oxide or silica gel. In this experiment the surface of the plate will consist of silica gel. The mobile phase will consist of a mixture of acetone and cyclohexane, which is significantly less polar than the silica gel.

Figure 4 illustrates how this works.

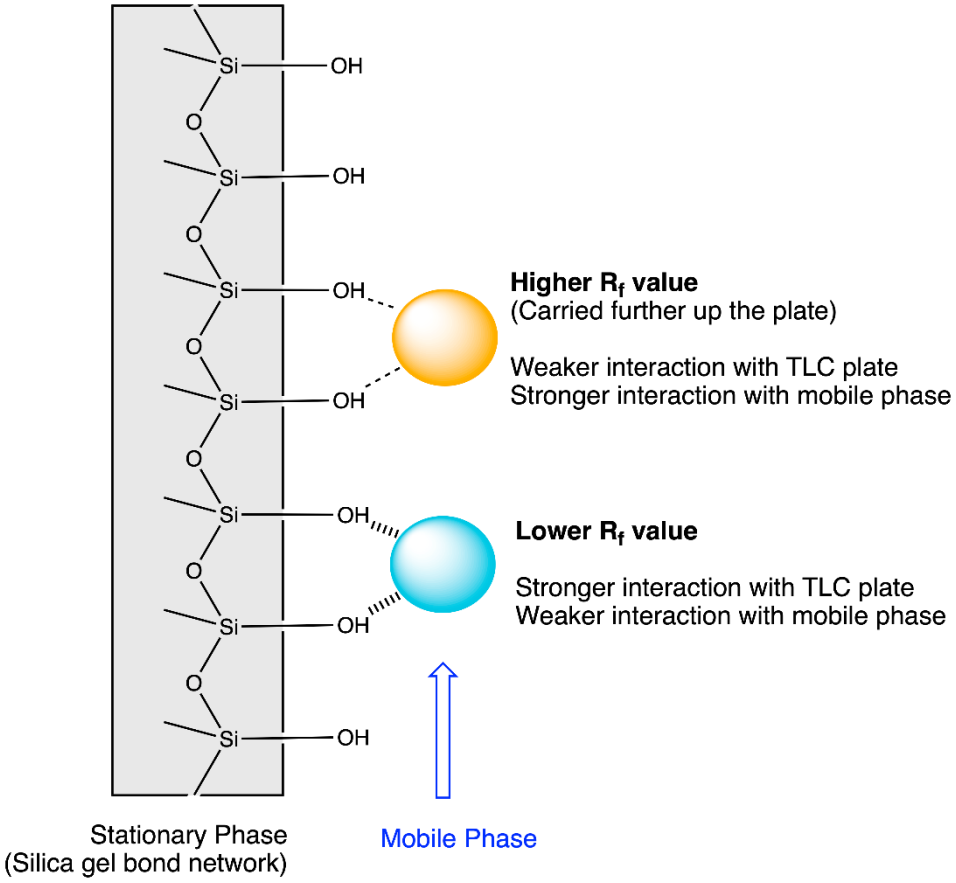


Figure 4. How TLC works. It can be seen that the silica gel has many highly polar OH groups at its surface.

The retention factor, R_f, of a compound is given by

$$R_f = \frac{\text{distance travelled from the origin by the compound}}{\text{distance travelled from the origin by the mobile phase}}$$

Introduction

This class investigation will involve extracting pigments from different plant leaves by chopping them into fine pieces, mixing them with a small amount of acetone and grinding them to extract the pigments. Some of this concentrated solution will be drawn up in a very fine capillary melting point tube and spotted onto the origin line on the TLC plate until a small concentrated dot is obtained. The plate will then be placed into the acetone / cyclohexane mixture. The separation and appearance of the pigments will be observed. The process will then be stopped and the R_f values of the different pigments calculated. The plate will also be observed under ultraviolet (UV) light. The results for different plant materials will then be compared

Aims

1. To separate and identify pigments present in green leaves from three different plant sources using thin layer chromatography.
2. To measure the R_f values of the different pigments.
3. To observe the plate when under UV light, to see if further pigments are present.

Safety precautions

1. **Acetone and cyclohexane are toxic, gloves must be worn and the activity performed in a well-ventilated room.**
2. **Acetone and cyclohexane are flammable. There must be no sources of ignition in the laboratory.**
3. **Eyes can be damaged if exposed to UV light. Proper eye protection must be worn when using UV lamps.**
4. **At the end of the experiment all wastes must be placed in the residue jars provided, and not down the sink.**

Materials required per group

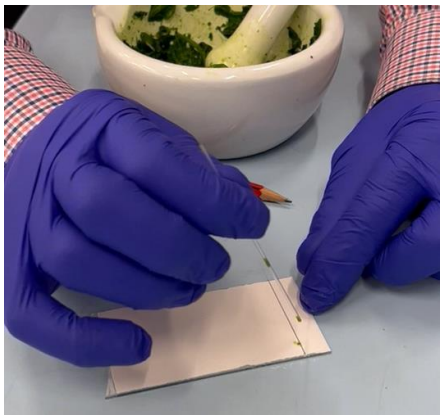
- Finely chopped spinach leaves
- Finely chopped lilly pilly leaves
- Sealed bottle of acetone (in fume cupboard)
- Sealed bottle of hexane (in fume cupboard)
- Small phial of sand
- 2 x mortars and pestles
- Small spatula
- 2 x 10 mL measuring cylinders
- 2 x 100 mL beakers
- Glass stirring rod
- 2 x glass capillary melting point tubes
- 600 mL beaker
- 4 cm x 6.5 cm TLC plate
- Graphite pencil
- 30 cm ruler
- Paper clip

Materials required per class

- UV light sources and orange glasses (to be worn when using this light source)
- Waste containers
- Paper towel

Procedure

1. Using a graphite pencil, draw a line 1.0 cm from the bottom on the TLC plate to form the origin. Do not press too heavily. Also use the pencil to lightly initial your group name in the top right corner. Draw three pencil dots evenly spaced along the origin. Stay in from the sides.
2. Add 20 grams of chopped plant material to the mortar. Use a measuring cylinder to add 10 mL of acetone for spinach (or 30 mL acetone for native plant samples). Do this in the fume hood. Add a spatula of fine sand.



3. Use the pestle to grind up the plant material into a smooth paste. This can be done at the bench.
4. Dip a capillary tube into the plant extract and use the solution that rises into the capillary to draw a dot of the spinach extract on the left most dot along the origin on the TLC plate. Add a dot of your chosen plant extract on the middle dot and draw a dot of the lilly pilly extract on the right most dot.
5. Allow the solvent to dry naturally.
6. Add 2 mL acetone and 2 mL cyclohexane to a fresh 100 mL beaker. Use a glass stirring rod to mix the solutions.
7. Place the chromatography plate in the beaker so that the bottom of it is in the acetone / cyclohexane mix. The solvent should be lower than the origin, as shown in diagram 1. Invert the 600 mL beaker over the chromatography run. The run will take about 10 minutes. The actual time is temperature dependent. Take pictures as the run occurs to record the separation.
8. Allow the solvent to rise up the plate until it is 1 cm from the top.
9. Remove the plate and immediately use a pencil to draw in the level the solvent reached and the position of each component. The colours of the components will fade in sunlight. Record these at the time of the run.

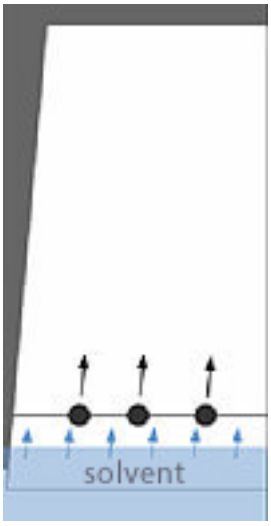
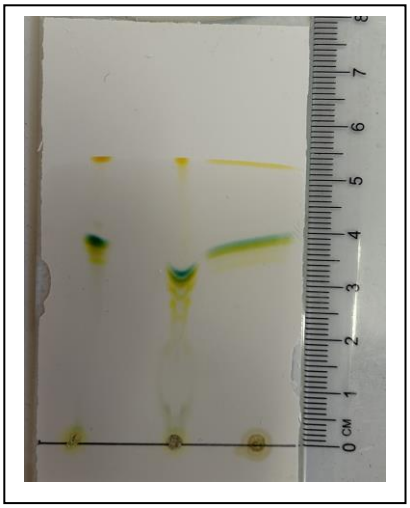


Diagram 1

10. Examine the chromatograph under UV light. More spots may be evident.
11. Record a digital image of your chromatogram as shown on the right.



Results for spinach (5 marks)

Components and solvent front		TLC distance from origin (cm)	Rf
Solvent front			
βcarotene (orange-yellow),			
chlorophyll a (blue-green)			
chlorophyll b (yellow green)			
Xanthophyll (pale yellow)			

Results for chosen plant: (5 marks)

Plant name:

Components and solvent front	TLC distance from origin (cm)	Rf
Solvent front		
βcarotene (orange-yellow),		
chlorophyll a (blue-green)		
chlorophyll b (yellow green)		
Xanthophyll (pale yellow)		

Discussion: *All calculations must be shown.*

1. Use the positions of the components to determine the retardation factor for βcarotene (yellow), chlorophyll a (blue-green), chlorophyll b (yellow green) and Xanthophyll in each plant sample.

2. Explain how temperature can affect the run time for TLC. (2 marks)

3. Explain why the line where the extract is placed on the plate must be above the level of the solvent at the beginning of the experiment. (1 mark)

4. Why was the line representing the origin drawn in pencil? (1 mark)

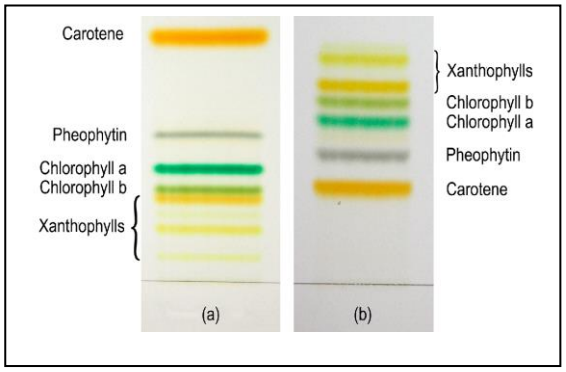
5. Compare your result to the other groups.
   a. Are your results reproducible? Explain

   b. Are your results repeatable? Explain (2 marks)

6. Name a random error which could have occurred with this experiment and explain how this would have affected the results. Suggest an improvement to the method to eliminate this error. (3 marks)

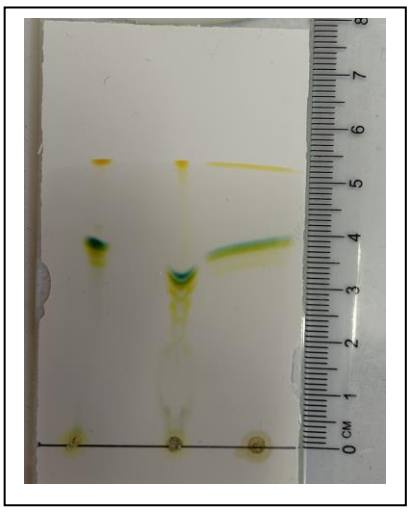
7. Compare the components of each mixture, the spinach, your chosen plant and lilly pillly leaves. Give a hypothesis as to why they differ. ` (4 marks)

8. Consider the two chromatograms a and b shown on the right.

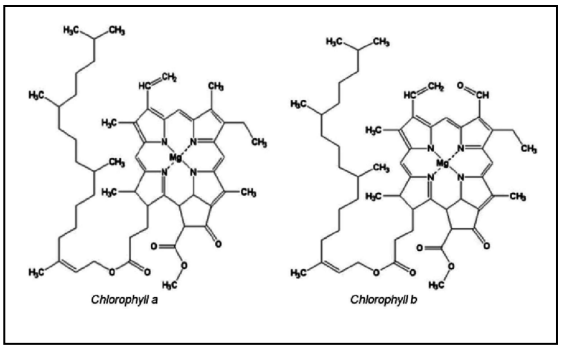


   a. Which chromatogram, a or b, was formed using normal-phase TLC. Explain. (2 marks)

   b. Consider the chromatogram shown on the right. The βcarotene has an R_f value close to 1. Given the stationary phase (silica gel) cannot be changed, what modifications can be made to allow for the βcarotene to separate further from the solvent front? Explain. (2 marks)



9. Consider the chemical structure of both chlorophylls a and b, shown on the right.



   a. Excluding carbon-to-carbon double bonds and cyclic structures, name the functional group common to both molecules and circle this functional group on each molecule. (2 marks)

   b. Circle and name one functional group that is unique to chlorophyll a. (2 marks)

   c. Given an explanation as to why chlorophyll b has a higher R_f value than chlorophyll a as measured from your chromatogram. (2 marks)

<u>Conclusion:</u>