Thin layer chromatography of plant pigments

TLC plates

The plates used here have a thin silica layer on a polyester backing. Aluminium oxide plates are also available.

Although expensive (~ £80 for a box of 50 plates, 20 cm x 5 cm), each plate can easily be cut (with sharp scissors) into smaller strips.

For example, 1 large plate → 15 smaller ones (1.3 cm x 5 cm). The cost of each small strip is then < 15 p.

Some plates are doped with a UV_{254} fluorescent material to allow detection of colourless spots using a UV light in the 254 nm range.

Procedure for chromatography

Wear eye protection. Do not inhale the solvents. Work in a well-ventilated room.

1. Add a small spatula measure of anhydrous sodium sulfate(VI) to the bottom of a plastic Eppendorf tube (Figure 1). This is to remove any moisture that may be present.
2. Place small pieces of a soft green leaf in the bottom of the Eppendorf tube. Add 5 drops of **Solvent A** (the 'extracting' solvent).
    - *An Eppendorf tube is used here but any small container or test tube would work.*
    - *Solvent A is a 2 : 3 mixture (by volume) of ethyl ethanoate and propanone.*

Figure 1

3. Press/stir the leaf with a metal probe (eg, end of forceps) to extract as much colour as possible!
4. Now use a fine paint brush (Figure 2) to place a **small** dot of the coloured solution about 5-8 mm from the bottom edge of the TLC plate. Gently blow on the dot to dry it before putting another dot of solution on top of it.
    - *Check that the dot is not too faint/strong before going on to the next step.*
    - *Mark the start level of the coloured spot with a small mark on the side edge of the plate (eg, use the corner of the spatula to scrape off a little of the silica layer).*
5. Using a dropper pipette, add ~ 0.5 cm^3 of **Solvent B** (the developing or 'eluting' solvent) into a clean vial to a depth of about 5 mm.
    - *The solvent level must start below the level of the coloured dot on the plate.*
    - *Solvent B is a 5:3:2 mixture (by volume) of cyclohexane, ethyl ethanoate & propanone.*

Figure 2

6. Put the TLC plate into the vial (Figure 3) and replace the screw-top lid.
    - *Ensure that the vial is kept still while the chromatogram is running.*
7. After a few minutes, when the 'solvent front' has moved 7/8ths of the way up the plate, remove the plate from the vial (use forceps/tweezers). Mark the position of the solvent front and allow the plate to dry.
8. Take a photograph of the result as soon as the solvent has evaporated away. The coloured compounds detected are light sensitive and will fade.

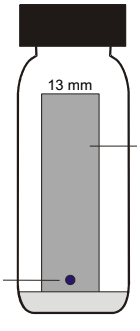
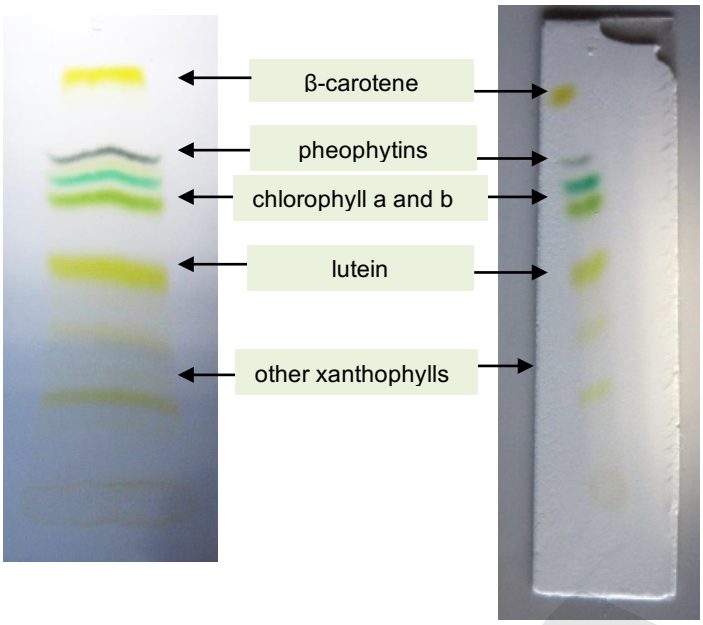
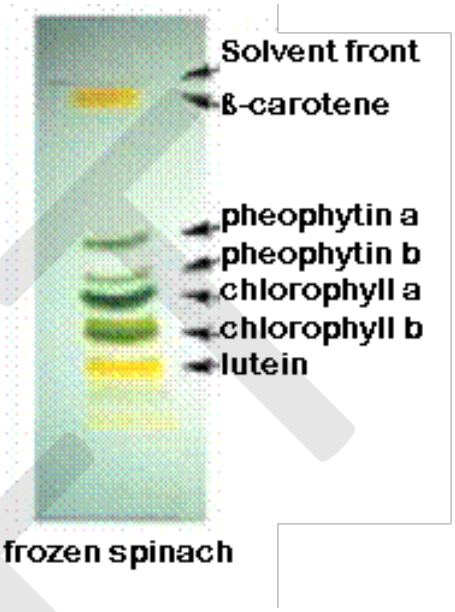


Figure 3

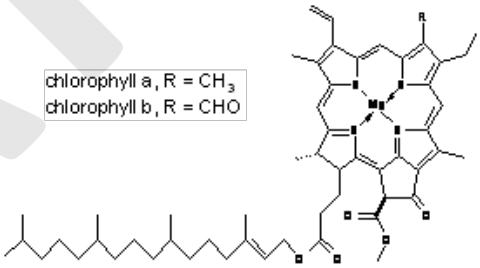
Expected observations/results

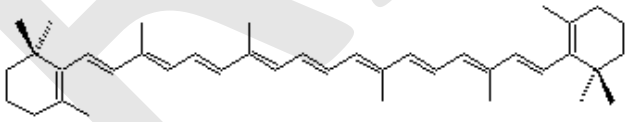
Our results from geranium leaves are compared below to some published values that used a slightly different solvent but the arrangement of spots is very similar. *Separation of Plant Pigments by Thin Layer Chromatography* from Quach, H. T.; Steeper, R L.; Griffin, G. W. *J. Chem. Educ.* **2004**, *81*, 385-7.



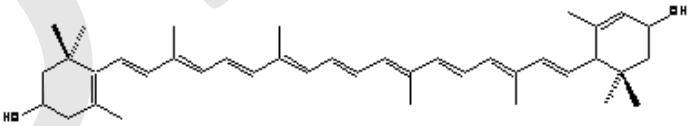


- The green in leaves is due mainly to chlorophyll a and chlorophyll b.
- Pheophytins are decomposition products in which chlorophylls a and b have lost their magnesium ions.
- The yellow dyes include beta-carotene and xanthophylls.
- Xanthophylls are derivatives of beta-carotene that contain oxygen. The xanthophyll in spinach is about 96% lutein and 4% zeaxanthin.

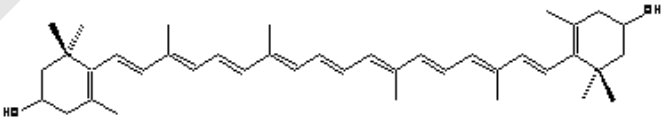




Beta-carotene



Lutein



Zeaxanthin

Zeaxanthin differs from lutein in the position of a double bond in the ring on the right.