

## Leaf Pigment Chromatography

**Summary:** My group and I attempted to separate plant pigments from a sample of spinach extract solution. We did this by using thin layer chromatography in an acetone solvent. Our results showed that four distinct pigments appeared: orange for carotenes, yellow for xanthophylls, blue-green for chlorophyll a, and olive-green for chlorophyll b. Our findings were confirmed by the general knowledge that chlorophyll a and b, along with carotenoids are found in most chloroplasts (Raven et al., 2008). Xanthophylls are also known to be found in the chloroplasts, offering special pigments for the purpose of light absorption (Gilmore, 1997).

**Methods & Results:** My group and I obtained a long piece of filter paper to use as a strip for thin layer chromatography. We spotted a point in the middle about 2cm up from the bottom with plant pigment obtained from spinach extract solution. We then affixed the top of the filter paper to a stir rod with a paper clip so that it could be suspended over a solvent. To begin the experiment the filter paper was placed inside of a tall beaker containing a small amount of acetone solvent, with the bottom of the filter paper submerged about 1cm in the solvent. We left the filter paper arranged this way until the solvent front came within 1in from the top.

Upon removal, four distinct pigments were found: orange for carotenes (3cm), yellow for xanthophylls (5.5cm), blue-green for chlorophyll a (2.2cm), and olive-green for chlorophyll b (0.9cm). The solvent front traveled a distance of 11.7cm. This produced the following table of data:

Pigment	Color	Distance Traveled	R <sub>f</sub> Value
Carotene	Orange	3cm	0.25
Xanthophyll	Yellow	5.5cm	0.47
Chlorophyll a	Blue-Green	2.2cm	0.19
Chlorophyll b	Olive-Green	0.9cm	0.077

R<sub>f</sub> values were calculated for each the pigments by dividing the distance traveled of the pigment by the distance traveled by the solvent front:  $R_f = (\text{Pigment Distance})/(\text{Solvent Front Distance})$ . A visual representation of our results is shown below in diagram 1.

### Diagram 1

#### References:

Gilmore, A.M., 1997. Mechanistic Aspects of Xanthophyll Cycle-dependent Photoprotection in Higher Plant Chloroplasts and Leaves, *Physiologia Plantarum*, 99(1), pp. 197-209.  
Raven, P. & Johnson, G. & Losos, J. & Mason, K. & Singer, S., 2008. *Biology*, 8<sup>th</sup> ed., McGraw-Hill.