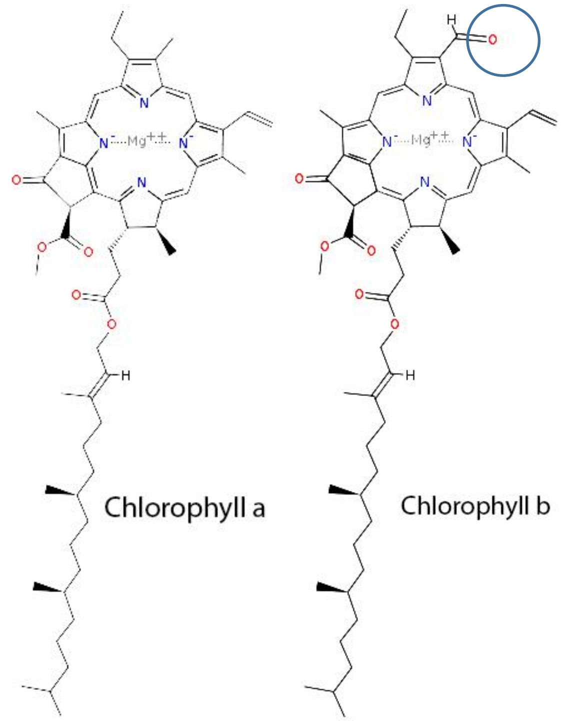
Photosynthesis: Pigments and Chromatography

Without pigments photosynthesis would not occur. Pigment structure determines the wavelengths of light the pigment can absorb and also the solubility of the pigment. Select pigments in plants intercept light energy and use that energy funneled through specialized reaction center chlorophylls to generate the materials needed to fix carbon dioxide. Chlorophyll is probably the best known of the photosynthetic pigments. There are two forms of chlorophyll found in higher plants, chlorophyll a and chlorophyll b. Examine the two chlorophyll structures shown to the right, how are they different? Notice the blue circle around the double bonded oxygen in the upper right hand corner of chlorophyll b. That is the only atomic difference in these two molecules, yet their light absorption and solubility characteristics are slightly different.



The ring structure of chlorophyll is responsible for intercepting photons of light and performing photochemistry. The long hydrocarbon tail of chlorophyll is non-polar or hydrophobic and inserts into the lipid layer of the thylakoid membrane of the chloroplast.

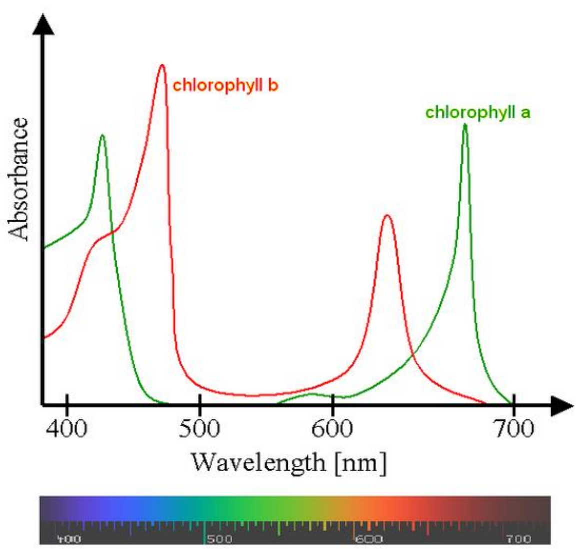
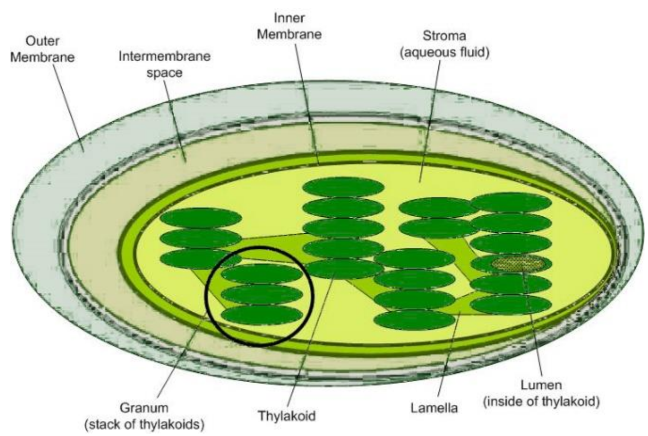


Image by Daniele Puliesi



Scientists use a graphic called an absorption spectrum to demonstrate how pigments respond at various wavelengths of light. Remember, visible light is sometimes called white light and is actually composed of a continuum of colors that we identify by wavelength. Violet light has a shorter high energy wavelength and red light has a longer lower energy wavelength. The spectra are made by exposing plants to specific wavelengths of light and then measuring the pigments absorption of light. Chlorophyll a and chlorophyll b have overlapping absorption spectra in the red and blue color range. Chlorophyll a and chlorophyll b absorb light in the red, blue and violet range and reflect yellow and green light. That is why plant leaves appear green; the green color is reflected light from chlorophyll.

A second class of pigments called the carotenoids are often found in plants and also play an important role in photosynthesis. Xanthophyll and beta-carotene are two common carotenoids. The carotenoids absorb in the blue, green, and violet light. Because carotenoids absorb in the green light range, they can absorb and funnel green light energy to chlorophyll, thus expanding the range of photosynthetically usable wavelengths of light. The structure of beta carotene is shown below. Beta carotene is hydrophobic.

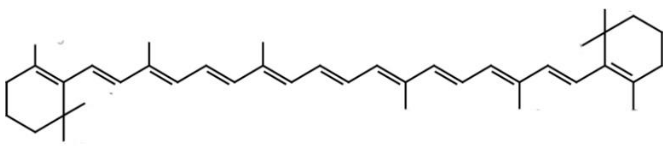
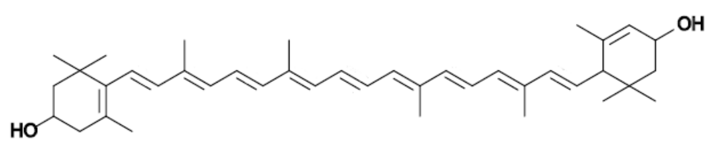


Image by Polimerek edited by S. Finazzo

One of the xanthophylls found in spinach is lutein. Compare lutein below, to carotene above, how are they structural different? Although lutein's structure is similar to carotene, lutein is slightly more water soluble because of the hydroxyl groups found in the molecule.



Chromatography is a technique used to separate components of mixture based on solubility and molecular size. In this activity pigments will be applied to a paper substrate. The tip of the paper substrate, chromatography paper, will then be immersed in a chromatography solvent. The solvent will diffuse up the paper. As the solvent meets the applied pigments, if the pigments are soluble in the solvent they will dissolve in the solvent and be carried up the paper. Because each pigment has a different solubility in the solvent it will travel a different distance from the origin.

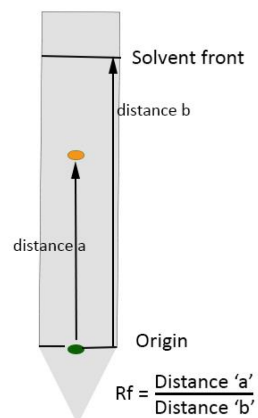
Materials

Spinach
Coin
Chromatography solvent
Test tube (18 mm x 150 mm)
Cork
Paperclip
Scissors
Chromatography paper
Pencil
Ruler
Test tube rack

Procedure

1. Pick up a spinach leaf and section of chromatography paper. Handle the paper by the edges, do not get your fingerprints on the paper. The chromatography paper should be trimmed to a point.

2. Use a pencil to draw a line 1.75 cm from the bottom pointed tip.
3. Lay the spinach leaf across the pencil line.
4. Carefully roll the coin across the leaf along the line, pressing down on the leaf. Pressing on the leaf is crushing the leaf cells and adding the pigments to the chromatography paper.
5. Lift the leaf and move a ‘fresh’ piece of leaf over the line. Roll the coin across the leaf again.
6. Repeat step 5, two more times. Allow the paper to dry for 5 minutes.
7. Add 5 mL of chromatography solvent to the test tube. Insert the cork in the test tube. Place the test tube in the test tube rack.
8. Open the paper clip and insert the paper clip into the cork.
9. Hang the chromatography paper from the paper clip.
10. Suspend the chromatography paper in the test tube. The tip of the paper should be in the solvent, the line with the pigments should not.
11. Allow the chromatogram to develop for 30 minutes or until the solvent front has moved 5-6 cm.
12. Remove the chromatogram from the test tube and mark the location of the solvent front.
13. Identify each pigment. You should see chlorophyll a (blue green), chlorophyll b (yellow green), xanthophyll (light yellow) and carotene (yellow orange).
14. Your instructor may ask you to determine the Rf value for the pigments. Rf is the ratio of the distance the pigment has moved in relation to the distance the solvent front has moved.
    a. How far did the solvent travel? (Measure the distance in mm from the origin (line where pigments were applied) to the second line you drew on the paper when you withdrew it from the test tube) ________
    b. How far did lutein travel? Measure from the origin to the center of the lutein spot. ___________
    c. How far did chlorophyll a travel? Measure from the origin to the center of the chlorophyll a spot. ___________
    d. How far did chlorophyll b travel? Measure from the origin to the center of the chlorophyll b spot. ___________
    e. How far did carotene travel? Measure from the origin to the center of the carotene spot. ___________



Calculating Rf

1. Rf_{Lutein} = b/a = ________________
2. $Rf_{Chlorophyll\ a}$ = c/a = __________________
3. $Rf_{Chlorophyll\ b}$ = d/a =_______________
4. $Rf_{Carotene}$ = e/a = __________________

Draw the results of your chromatogram below or insert a picture.

Which pigment was the most hydrophobic? How do you know?

Which pigment was the least hydrophobic? How do you know?