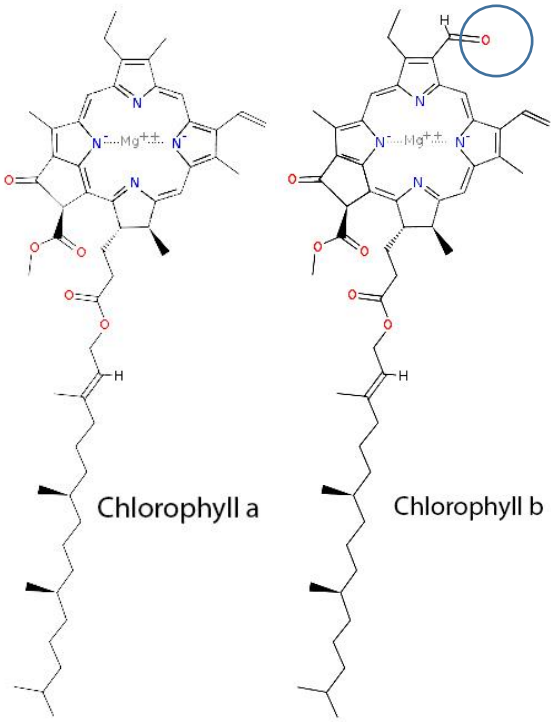
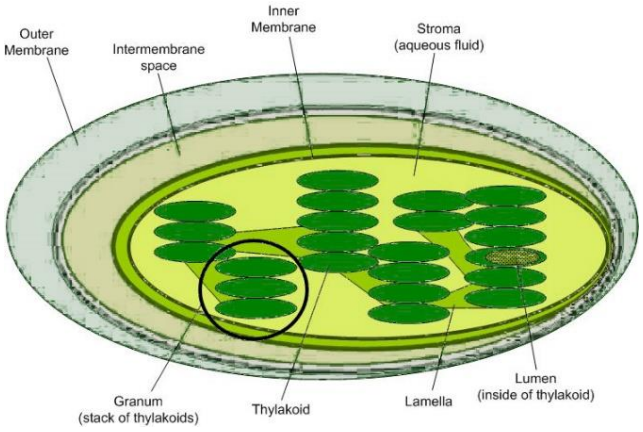
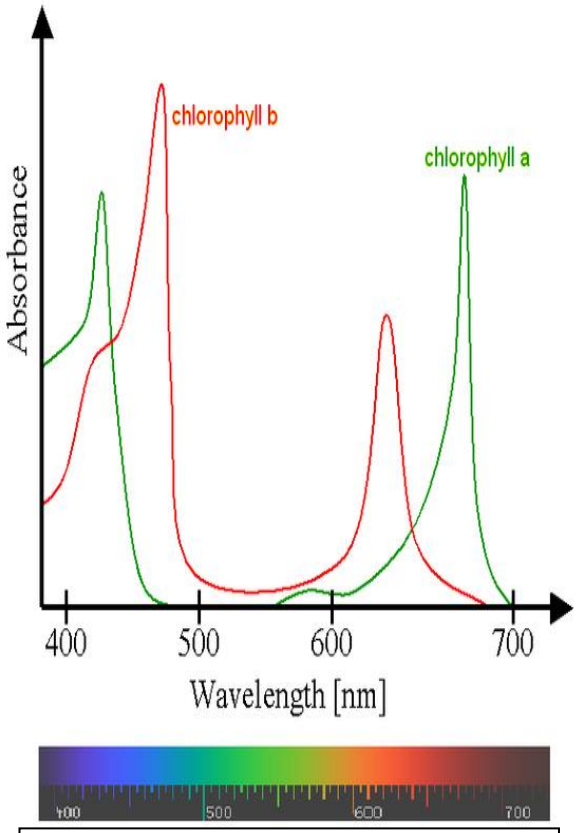
Photosynthesis: Pigments and Chromatography

Without pigments photosynthesis would not occur. Pigments are compounds that absorb and reflect light. The pigment's structure determines the wavelengths of light that can be absorbed and also the solubility of the pigment. Specific plant pigments 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 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 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 the molecule into the lipid layer of the thylakoid membranes of the chloroplast.





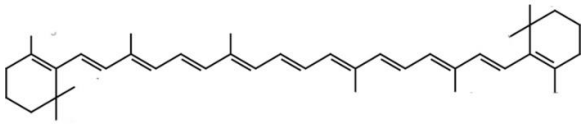
Absorption Spectrum
Image by Daniele Puliesi

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 the shortest wavelength and red the longest. The spectra are made by exposing plants to specific wavelengths of light and then measuring the

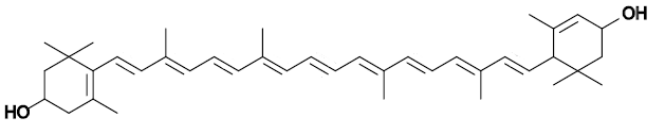
pigments absorption of light. Chlorophyll a and chlorophyll b have overlapping absorption spectra in the red and blue color range. Chlorophyll a and chlorophyll b absorb light in the red, blue and violet range and reflect yellow and green light. That is why plant leaves appear green; the green color is reflected light from chlorophyll.

A second class of pigments called the carotenoids are often found in plants and play an important role in photosynthesis. Xanthophyll and beta-carotene are two common carotenoids. Carotenoids absorb 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

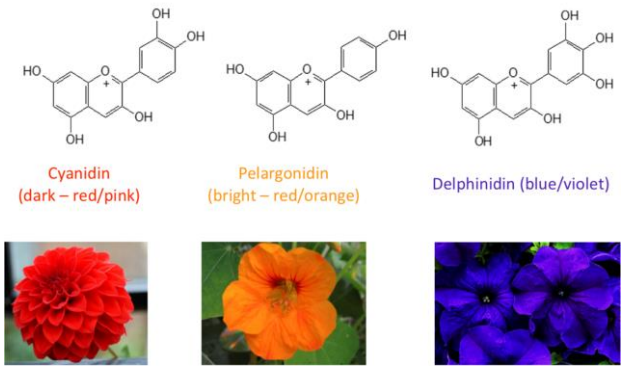


One of the xanthophylls found in beets is lutein. Compare lutein below, to carotene above, how are they structural different? Although lutein's structure is similar to carotene, lutein is slightly more water soluble because of the hydroxyl groups found in the molecule.



Flavonoids are yet another class of pigments found in plants. Flavonoids are found in the cytoplasm and central vacuole of cells throughout the plant body including tissues in the root, stem, leaves, flowers and fruits. Flavonoid pigments can be red, blue or violet colors. Anthocyanins are common flavonoids found in plants. Anthocyanins and the flavonoids in general do not directly participate in the light reactions of photosynthesis but are thought to protect the photosynthetic apparatus from UV light and environmental stressors. Look at the structure of the flavonoid examples below, notice all of the hydroxyl groups found on each molecule.
Flavonoids are water soluble unlike the pigment molecules directly involved in photosynthesis.

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



Examples of Anthocyanins. Image by FlowerPowerH2020 downloaded with attribution from https://commons.wikimedia.org/wiki/File:Chemical_structures_of_the_three_main_types_of_anthocyanins.png

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

Beet leaves
Sand
Chromatography solvent
Test tube (18 mm x 150 mm)
Cork stopper
Paperclip or thumb tack
Scissors
Chromatography paper
Pencil
Ruler
Gloves - optional
Graduated cylinder – 15 mL
Cheesecloth – 4 layers
Beaker – 25 mL
Test tube rack
Mortar and pestle or beaker (250 mL) and spoon
70% Ethanol
Capillary pipette, toothpick, plastic coffee stirrer

Procedure

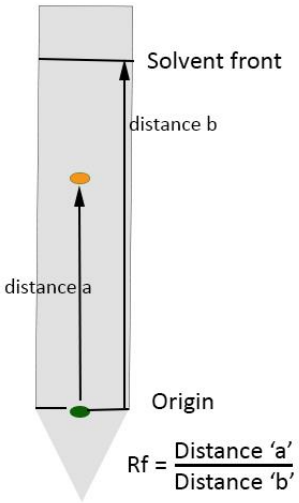
1. Pick up a beet leaf and section of chromatography paper from the supply table. Handle the paper by the edges, do not get your fingerprints on the paper. The chromatography paper should be trimmed to a point.
2. Use a pencil to draw a line 1.75 cm from the bottom pointed tip.
3. Get a beaker and spoon or mortar and pestle from the supply table. Add a small scoop (1/2 tsp) of sand to the mortar.
4. Use the scissors to cut the beet leaf into small pieces (1 cm x 1 cm). Do not include the large central vein. Place the pieces in the mortar.
5. Use the graduated cylinder to add 5 mL of 70% ethanol to the mortar.
6. Grind the beet leaf into the sand and ethanol with the pestle or spoon to produce a colored slurry. If necessary add another 1 to 2 mL of ethanol.
7. Cover the 25 mL beaker with cheesecloth and pour the contents of the mortar onto the cheesecloth to filter the pigments. Once the solution has drained through, dispose of the cheesecloth.
8. Concentrate the sample by allowing the ethanol to evaporate for 15 minutes. Continue on to step 9 while you are waiting.
9. Add 5 mL of chromatography solvent to the test tube. Insert the cork stopper. Place the test tube in the test tube rack.
10. After the pigment extract has concentrated for 15 minutes use a toothpick, capillary tube or plastic coffee stirrer to apply the extract to the pencil line drawn on the chromatography paper. Insert the capillary tube into the beaker, place your finger over the open end of the tube. Touch the capillary tube to the center of the pencil line drawn on the chromatography paper for just a second to make a small spot (3 mm).
11. Wait 2 or 3 minutes for the spot to dry and then reapply another spot of extract to the same spot. Repeat step 10, 4 more times. Let the paper dry for 5 minutes.
12. Open the paper clip and insert the paper clip into the cork.
13. Hang the chromatography paper from the paper clip.
14. 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.

15. Allow the chromatogram to develop for 30 minutes or until the solvent front has moved 5-6 cm.
16. Remove the chromatogram from the test tube and mark the location of the solvent front.
17. Identify each pigment. You should see chlorophyll a (blue green), chlorophyll b (yellow green), xanthophyll/lutein (light yellow), anthocyanin (red) and carotene (yellow orange). 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 pigment were applied) to the second line you drew on the paper when you withdrew it from the test tube) _____
    b. How far did carotene travel? Measure from the origin to the center of the carotene spot. _____
    c. How far did lutein travel? Measure from the origin to the center of the lutein spot. _____
    d. How far did chlorophyll a travel? Measure from the origin to the center of the chlorophyll a spot. _____
    e. How far did chlorophyll b travel? Measure from the origin to the center of the chlorophyll b spot. _____
    f. How far did anthocyanin travel? Measure from the origin to the center of the anthocyanin spot. _____

    Calculating Rf

    1. $Rf_{Carotene}$ = b/a = _____
    2. Rf_{Lutein} = c/a = _____
    3. $Rf_{Chlorophyll\ a}$ = d/a =_____
    4. $Rf_{Chlorophyll\ b}$ = e/a = _____
    5. $Rf_{Anthocyanin}$ = 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?