

During this lab you will extract and analyze the pigments of various plants. You will analyze the pigments of each plant using two methods, and then compare pigment composition between plants.

#### Determining light absorption

1. Remove the stems and veins from the leaves before beginning the pigment extraction. Work with one species of plant at a time.
2. Using about 5 ml acetone, grind several grams of leaf tissue in a mortar. It is necessary only to split a few leaves (you do not need to make a mash) and collect enough pigment to tint the acetone green. (If you're not sure you have enough pigment collected, let the ground leaves sit several minutes in the acetone. Why would this help? Be cautioned: the absorbance of a sample too dark will not be accurately recorded with our instruments. This is no problem, however, because you may simply dilute your sample later.)
3. Filter the pigment into a spectrophotometer tube. Repeat procedure for each species.
4. Record the absorbance of the pigment at 20 nm intervals from 400 nm to 700 nm. You will need a blank and must zero the spectrophotometer at each new wavelength.

#### Separation of leaf pigments

1. Remove the stems and veins from the leaves before beginning the pigment extraction. Work with one species of plant at a time.
2. Using the chromatography solvent (9:1 petroleum ether:acetone), grind several grams of leaf tissue in a mortar. You may grind well, and let the tissue sit with the solvent for several minutes, or until the solvent is dark with pigment.
3. While you are waiting, prepare chromatography chambers. Carefully drop chromatography solvent in the bottom of a test tube until it covers the bottom 0.5 cm of the tube. Cut a long piece of chromatography paper with a 1 cm pointed tip which will sit nicely in the waiting solvent.
4. When the chromatography solvent is saturated with pigment, use a toothpick or fine tipped pipette to transfer a thin line of pigment to the chromatography paper. THIN. This requires patience. Wait for the solvent to dry and reapply lines for best results.
5. Place chromatography paper in the chromatography chamber and cork the test tube. Be sure the tip of the paper (but not the band of pigment) is resting in the solvent at the bottom of the tube.
6. Watch the paper carefully and stop the chromatography when the solvent front has reached about 1 cm from the top of the paper. Remove the paper carefully, and lay to dry. Dispose of all solvents in the hazardous waste jar.

#### Results (6):

- Include data table indicating absorbance for each species at various wavelength.
- Graph of wavelength vs. absorbance for each species on its own graph. Connect the dots in a smooth-flowing fashion, as modeled in Fig. 8.7 of your text.
- Identify wavelengths show peak absorption for each species
- Staple chromatograph in notebook. Identify the number of pigments present.

## Discussion (9):

- Based on your graphs, identify the number and types of pigments are present in each species.
- Do the data from your graphs and chromatographs support each other? Explain.
- Is measuring the light absorption a useful tool in identifying the pigments present in a plant? Explain.
- Given the species you tested, do your data make sense?
- What further analysis could be done to strengthen your conclusions regarding the pigment composition of these plants? Propose a method to go about this analysis.

