

Procedure for leaf pigment extraction from student notebook. This same procedure was used by other classes for: spinach leaves, fall foliage such as oak and maple, and for chloroplasts isolated from spinach leaves using a sucrose step gradient.

Absorption spectrum of pigment extracted from Chokecherry.(Prunus Virginianis)

Purpose: To compare our best wavelength for photosynthesis (leaf flotation assay) with the absorption spectra of the leaf extract.

Materials:

95% ethanol	Filter paper
Hot plate	Scale
Funnel	Cuvettes
Test tube	Large chokecherry leaf
PPE	spectrophotometer

Procedure:

1. Set up boiling water bath with 200mls water in 600 ml glass beaker, set to 120 deg C
2. Turn spec on
3. Break up a large chokecherry leaf into fragments, remove stem and determine mass
4. Place the leaf in a KIMAX glass bottle and cover with 95% ethanol
5. Place the bottle in a boiling water bath on a hot plate and heat to boiling.
6. Remove from heat
7. Fold a piece of filter paper in quarters and place in a funnel and moisten it with 95% Ethanol
8. Turn on spectrophotometer to warm up
9. Place the end of the funnel into a test tube and pour the pigment extract through the funnel
10. Take 0.5 mls of the extract and put it into a cuvette containing 0.5 mls of ETOH
11. After zeroing spec with cuvette containing 1 ml of 95% ethanol, take absorption readings at 20nm intervals between the wavelengths of 400nm and 720 nm.
12. Graph absorbance units vs wavelength (nm).

Results:

See graph in lesson sequencing document.

Note: This activity can also be used to look at the pigment profile of fall leaves, after chlorophyll has been resorbed into the autumn leaves. In this case, the leaves should sit for at least one hour in the boiled ethanol after step 6. This will maximize the yield of the non-chlorophyll pigments.