ANALYZING THE ABSORPTION SPECTRUM OF LEAVES

Plants use light energy to synthesize energy rich chemical compounds like sugars and starches from carbon dioxide (CO_2) and water (H_2O).

What colors of light or wavelengths do chloroplasts actually need for this process?

The leaves of many plants are green, which indicates that most of the green light falling on leaves is being reflected or transmitted, since you can see it! Other colors are absorbed by the leaf. This is similar to the energy absorption that occurs when wearing a dark T-shirt on a sunny day, it is much warmer than wearing a light colored shirt. As the seasons change some leaves change from green to red, orange, yellow, and brown. This happens when leaves stop producing chlorophyll and other pigments become visible such as carotenoids or anthocyanins. Analyzing the absorption spectrum (what wavelengths are absorbed vs transmitted) can tell us what pigments are present in a leaf and what wavelengths of light will support the photosynthetic process.

Materials

- Spectrometer
- Cuvettes and Lids (4)
- 3 disposable pipets
- Plastic or glass funnel
- Lint free cloth/wipes
- 3 leaf samples
- 3 test tubes
- 80 mL of 95% ethanol
- Filter paper
- Test tube rack
- 20-mL graduated cylinder
- Scissors
- Distilled water

Procedure

Calibration

1. Plug the spectrometer into a computer using the USB cable or wirelessly pair the spectrometer to a computer or tablet running PASCO's Spectrometry Application (http://pasco.com/spectrometer/)
2. Open the spectrometry application.
3. Select ANALYZE SOLUTION from the menu at the top.
4. Select CALIBRATE DARK from the menu at the bottom of the screen. The spectrometer will turn off light sources to perform a calibration. Cover the sample well with your finger to block any ambient light from entering the detector. A check mark will appear when the calibration is complete.
5. Fill a cuvette 3/4 full with 20% ethanol to use for the reference calibration. Handle the cuvette on the ribbed sides and wipe the smooth sides clean with a lint free cloth. Place the cuvette into the spectrometer so that a clear side is facing the white light source icon.
6. Select CALIBRATE REFERENCE from the menu at the bottom. A check mark will appear when the calibration is complete.

Sample Prep

7. Using a balance, measure 0.50g of each leaf sample. Avoid veins and stems if possible by cutting out small leaf tissue sections with a scissors.
8. Place the sample in the clean mortar and add 20mL of 95% ethanol.
9. Grind the mixture with the pestle for 2-3minutes until the mixture is homogenized as much as possible.
10. Filter the solution using the filter paper and funnel into a labeled test tube.
11. Fill a cuvette 3/4 full with the filtered leaf extract.
12. Repeat steps 1-4 with additional samples and store them on ice until ready for testing.

Data Collection

13. Using a lint free cloth clean the smooth sides of the cuvette with the first leaf extract. Place the cuvette into the sample well and select START RECORDING in the lower left of the screen. The absorbance spectrum for the extract will appear.

    Note: if the data plateaus at any point in the scan remove the sample and dilute with 95% ethanol. Dilute additional samples with the same amount of 95% ethanol.
14. In the tools slide-out adjust the smoothing and number of scans to average. Select STOP RECORDING when you are satisfied with the result.
15. Rename the run or make a note indicating which sample or species was analyzed.
16. Repeat steps 13-15 with the remaining leaf extract samples.

Data Analysis

17. Using the ADD COORDINATE tool locate the peak(s) for each sample. SNAPSHOT each run with the coordinates active or record the values for each sample in a data table.
18. Table 1 (below) shows the absorption peaks for the most common plant pigments. Based on your data, which pigments are visible in your leaf extract samples?

Table 1

PIGMENT	PEAK ABSORPTION Wavelengths (nm)
Chlorophyll A	430 and 662
Chlorophyll B	453 and 642
Carotenoids	460-550
Anthocyanins	520*

**The absorption wavelength of anthocyanins is pH dependent. The value shown is at a pH of 4.5. At lower pH values the pigments undergo a structural change and do not absorb visible light wavelengths.*

19. Will the visible pigments in a leaf from the same plant species change throughout the year? Explain your reasoning.


CALIBRATE DARK


CALIBRATE REFERENCE


START RECORDING


STOP RECORDING


SCALE TO FIT

ANALYZE SOLUTION

CONCENTRATION


SHOW LINEAR FIT


ADD COORDINATE


EXPERIMENT OPTIONS