

# SPECTRAL ANALYSIS OF AUTUMN LEAVES

## LAB VIS 10

### INTRODUCTION

Sunlight is composed of all colors. When sunlight falls on green leaves, chlorophyll, the green pigment in leaves, absorbs red and blue light. The chlorophyll does not absorb green light; it is transmitted or reflected. Forests are green because leaves reflect green light. In the autumn, as daylight hours decrease and temperatures start to drop, production of chlorophyll in leaves declines and their green color fades. Now other pigments present in the leaves give them color. For example, leaves containing *carotenoids* appear yellow, orange, and brown. We see these colors each fall in deciduous trees such as hickory, ash, maple, aspen, birch, black cherry, and cottonwood. Carotenoids are present in leaves all of the time but remain unseen until the fall. *Anthocyanins* are another group of pigments. Unlike the carotenoids, however, they are not present in the leaves all of the time. Rather, they develop in the leaves in the late summer. Anthocyanins often combine with carotenoids to give the deeper oranges, fiery reds and bronzes typical of many deciduous trees.

### PURPOSE

The purpose of this experiment is to use spectrophotometry to analyze leaf samples by their absorption of light. Data from different samples will provide an opportunity to compare the pigments present in leaves of various types of trees and to predict what causes the leaves to change color.

### MATERIALS

Spectronic 20 Genesys	fresh leaf samples
1 test tube/ leaf extract sample	1 mortar and pestle
1 funnel	1 test tube rack
2 disposable pipets/ leaf extract sample	Kimwipes
20 mL 95% ethanol/ leaf extract sample	1 graduated cylinder
filter paper	2 cuvettes

### SAFETY

Always wear goggles and an apron in the lab.

## **PROCEDURE**

### **Preparing Leaf Extracts**

1. Using a balance, measure a 0.50 gram sample of leaves that have been cut or torn into small pieces.
2. Place the sample in the mortar and add 20 mL of 95% ethanol.
3. Grind the mixture with the pestle for several minutes.
4. Filter the resulting solution using a funnel and filter paper. Collect the extract in a labeled test tube. **Note:** If you do not use the leaf extract immediately, store it in an ice bath.

### **Analyzing Leaf Extracts**

1. Ensure that the spectrophotometer has warmed up for at least 20 minutes. Use the same instrument for the entire experiment.
2. Adjust the wavelength to 350 nm.
3. Fill a cuvette about 3/4 full with 95% ethanol. This is the “blank”.
4. Using a Kimwipe, wipe off the cuvette containing the blank. Make sure the solution is free of bubbles. Do not touch the clear sides of the cuvette. Place the cuvette in the sample compartment with the triangle on the cuvette facing the front of the instrument. Close the lid.
5. Press
6. Remove the blank cuvette from the instrument.
7. Fill another cuvette about 3/4 full with leaf extract and make sure it is clean and dry and that the solution is free of bubbles. Record the source of the leaf extract on the top of one of the columns in the Data Table.
8. Place the cuvette containing the leaf extract into the spectrophotometer. Make sure that the triangle on the cuvette is facing the front of the instrument. **Do not press** .
9. Record the absorbance of the leaf extract in the Data Table.
10. Reset the wavelength to 375 nm and repeat steps 4 – 9.
11. Continue to repeat steps 4 – 9, adjusting the wavelength up 25nm each time until reaching 750nm.

**DATA SHEET**

Name \_\_\_\_\_  
Name \_\_\_\_\_  
Period \_\_\_\_\_ Class \_\_\_\_\_  
Date \_\_\_\_\_

**SPECTRAL ANALYSIS OF LEAVES**

**DATA TABLE**

**Absorbance**

Wavelength (nm)	<u>Leaf Extract:</u>	<u>Leaf Extract:</u>	<u>Leaf Extract:</u>	<u>Leaf Extract:</u>
350				
375				
400				
425				
450				
475				
500				
525				
550				
575				
600				
625				
650				
675				
700				
725				
750				

## **PROCESSING THE DATA**

1. Use a spreadsheet or graph paper to make a graph of absorbance vs. wavelength for each of the leaf extracts. Absorbance should be plotted on the y-axis, and wavelength on the x-axis.
2. If possible, overlay the graphs of absorbance vs. wavelength for the different leaf extracts.

## **QUESTIONS**

1. Compare the graphs of absorbance vs. wavelength for the different extracts. What do you observe?
2. For the graph of each leaf extract, how many peaks are there? At what wavelength do the peaks occur? Make a table summarizing your answers to this question.
3. What peak(s) are due to chlorophyll?
4. What do the graphs suggest happens when the leaves change color? Explain.