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.

Adapted from Jack W. Sipe, Jr., "Spectral Curve of Autumn Leaves", Advancing Science Program, Gettysburg College, Gettysburg, PA 17325.

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

5. Fill one of the cuvettes about 3/4 full with leaf extract. Record the source of the leaf extract on the top of one of the columns in the Data Table.
6. Fill another cuvette about 3/4 full with 95% ethanol. This is the "blank cuvette".
7. Set the spectrophotometer to 350 nm.
8. Place the blank cuvette into the sample compartment of the spectrophotometer with the triangle on the cuvette facing the front of the instrument.

   Note: Before inserting a cuvette into the spectrophotometer, wipe it clean and dry with a kimwipe, and make sure that the solution is free of bubbles. Do not touch the clear sides of the cuvette.
9. Press 0 ABS 100%T.
10. Remove the blank cuvette from the instrument.
11. 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 0 ABS 100%T.**
12. Record the absorbance of the leaf extract in the Data Table.
13. Reset the wavelength to 375 nm and repeat steps 8 – 12.
14. Repeat steps 8 – 12, recording absorbances every 25 nm until you reach 750nm.

DATA SHEET

Name _____
Name _____
Period _____ Class _____
Date _____

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DATA TABLE

	Absorbance			
Wavelength (nm)	**Leaf Extract: _____**	**Leaf Extract: _____**	**Leaf Extract: _____**	**Leaf Extract: _____**
350				
375				
400				
425				
450				
475				
500				
525				
550				
575				
600				
625				
650				
675				
700				
725				
750				

PROCESSING THE DATA

1. Using a spreadsheet or by hand, make a graph of absorbance vs. wavelength for the leaf extract. 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.