

CHLOROPHYLL AND VISIBLE LIGHT

LAB VIS 7

INTRODUCTION

The range of the wavelengths or frequencies of electromagnetic radiation is called the *electromagnetic spectrum*. Visible radiation is just a small part of the entire electromagnetic spectrum. It is, however, the only region that can be seen by the human eye. Visible radiation has wavelengths from approximately 400 to 750 nanometers (nm, $1 \text{ nm} = 10^{-9} \text{ m}$) and ranges in color from violet to red. White light, or sunlight, is composed of all of these colors. The table below shows the various colors of visible light and their corresponding wavelengths.

Wavelength (nm)	Color
400 - 435	Violet
435 - 480	Indigo
480 - 500	Blue
500 - 560	Green
560 - 580	Yellow-green
580 - 595	Yellow
595 - 610	Orange
610 - 750	Red

Consider what happens when white light shines on a transparent but colored solution. Some of the light's wavelengths are *absorbed* or removed by the solution. We see the wavelengths that are not absorbed. These wavelengths are *transmitted* by the solution. For example, a solution that appears green absorbs blue and red light, and transmits green light. Chlorophyll looks green to us because it absorbs blue light and red light, and reflects green light. Forests and meadows are green because plants absorb green light the least.

PURPOSE

The purpose of this experiment is to use a spectrophotometer to find the wavelength of maximum absorbance for a solution of chlorophyll extracted from spinach, and then graph the data.

SAFETY

Always wear goggles and an apron in the lab.

EQUIPMENT/MATERIALS

test tube	filter paper
2 cuvettes	disposable pipet
mortar and pestle	fresh spinach leaves
25 mL 95% ethanol	Kimwipes
Spectronic 20 Genesys	wash bottle with deionized water
balance	glass funnel
ring stand and small ring	stirring rod

PROCEDURE

1. Ensure that the spectrophotometer has warmed up for at least 20 minutes. Use the same instrument for the entire experiment.
2. Set the spectrophotometer to 350 nm.
3. Using a balance, measure a 1 gram sample of fresh spinach leaves.
4. Place the sample in the mortar and add 20 mL of 95% ethanol.
5. Grind the mixture with the pestle until it becomes semi-liquid.
6. Filter the resulting solution using a funnel and filter paper. Collect the extract in a test tube.
7. Fill one of the cuvettes about 3/4 full with the chlorophyll solution.
8. Fill the other cuvette about 3/4 full with 95% ethanol. This is the "blank cuvette".
9. 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.

10. Press **0 ABS 100%T**.
11. Remove the blank cuvette from the instrument.
12. Place the cuvette containing the chlorophyll solution 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.**
13. Record the absorbance of the chlorophyll solution in the Data Table.
14. Reset the wavelength to 375 nm and repeat steps 9– 13.
15. Repeat steps 9 – 13, recording absorbencies every 25 nm until you reach 750nm.

PROCESSING THE DATA

1. Using a spreadsheet or graph paper, make a graph of absorbance vs. wavelength for the chlorophyll solution. Absorbance should be plotted on the y-axis, and wavelength on the x-axis.

DATA SHEET

Name _____

Name _____

Period _____ Class _____

Date _____

CHLOROPHYLL AND VISIBL E LIGHT

DATA TABLE

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

QUESTIONS

1. What is the wavelength of maximum absorbance for the chlorophyll solution?
2. What color of light corresponds to the wavelength of maximum absorbance?
3. What is the wavelength of minimum absorbance for the chlorophyll solution?
4. What color of light corresponds to the wavelength of minimum absorbance?
5. Using your results, what color of light does your chlorophyll solution absorb the most?
6. Using your results, what color of light does your chlorophyll solution transmit the most?
7. What color of light starts and sustains photosynthesis in spinach? Explain your reasoning.
8. Use the results from this experiment to explain why plants containing a large amount of chlorophyll are green.