



# **Why Do the Colors of Leaves Change in the Fall?**

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## *Suggestions for Teachers*

### **Purpose:**

The color change of the leaves on deciduous trees is one of the most striking signals that summer is ending and fall is beginning. What is it that causes this change in color? We all know that leaves contain chlorophyll – giving them their green-color but why do they change from green to the palette of colors we see in the fall? In this investigation you will determine what pigment molecules that green plants contain and what happens to those molecules as the season progresses.

### **Objectives:**

At the conclusion of this investigation, students will be able to...

Perform an extraction of the pigmented material contained in green plants.

Determine the frequency of light that the pigments contained in the extract respond to.

Determine if there are changes in the relative amounts to these pigments as the season progresses.

### **Background Information:**

In this investigation you will be using a visible light spectrophotometer in order to determine the wavelengths that pigments contained in green leaves respond to. Once that absorbance spectrum has been determined you will use the absorbance maximum to establish a standard curve (Beer's Law Plot) of the extracted pigments so that you will be able to determine a relative concentration of pigments as the seasons progress. For information on the use of the spectrophotometer see the following web site:

[http://www.101science.com/spectroscopy\\_links\\_www.htm](http://www.101science.com/spectroscopy_links_www.htm)

### **Materials and Methods:**

#### **Materials – Part 1 – Chromatography Separation of Plant Pigments:**

40 grams of green leaves	Acetone	400 mL beaker
Mortar and pestle	Petroleum ether	10 x 20 cm chromatography paper
Fine sand	Distilled water	Parafilm
Fine paint brush	100 mL beakers	

**Procedure – Part 1:**

1. Prepare 100 mL of extraction solvent by mixing acetone, petroleum ether, and distilled water in a 9:3:1 ratio.
2. Grind 40 grams of green leaves in a mortar containing a small quantity of fine sand and 20 mL of the extraction solvent.
3. Filter and save the solvent.
4. Carefully draw a pencil line one-centimeter from the bottom of the filter paper rectangle (20 cm side is the bottom) and with a small paintbrush, brush the extract along the pencil line. Allow this to dry and repeat this procedure around ten times.
5. Pour ½ cm of solvent into the bottom of the 400 mL beaker, seal with parafilm and allow to equilibrate for five minutes.
6. Bring the edges of the chromatography paper together to form a cylinder, but be sure to not let the edges touch each other. Staple the edges together. Carefully place the cylinder into the beaker and reseal. Make sure that you handle the chromatography paper by the edges only.
7. Allow the system to run until the solvent front gets to within about a centimeter of the top edge of the cylinder (about 20 minutes).
8. Take the cylinder out of the beaker, remove the staples and allow the filter paper to dry.
9. Look at the pigment banding on the filter paper. You should be able to see four color bands. Cut the bands apart and place each strip in a separate 100 mL beaker containing 20 mL of solvent in order to leach the pigment from the paper.

**Materials – Part 2 – Establishment of a Standard Curve for the Plant Pigments:**

Spectrophotometer  
Pigments extracted from filter paper bands  
2 cuvettes  
Extract solvent

**Procedure – Part 2:**

1. Turn on the spectrophotometer and allow it to warm up about 5 minutes.
2. Set the zero and 100% transmittance using the extract solvent.

3. Run an absorbance spectrum on each of the extract samples. Record both the transmittance and the absorbance.
4. Graph wavelength vs. absorbance and determine the wavelength of light that is maximally absorbed.
5. Make a serial dilution of each of the pigments and using the absorbance maximum wavelength determined for the pigments – run a Beer's Law Plot to be used as a standard for determining the relative concentration of the pigments in the next part of the investigation.

### **Procedure – Part 3 – Degradation of Plant Pigments With Time:**

You are now ready to begin the experimental part of the investigation. We are interested in seeing if there is a difference in the relative amounts of pigments extracted from green leaves as the season changes toward the fall of the year. We are also interested in determining a reason for the shift if there is one.

Work in groups of three or four students.

With the other groups, determine the experimental procedure that the entire class is going to use. The analysis techniques that you used at the beginning of this investigation should allow you to design an experiment that might solve this problem. The teacher must first approve any procedure that is used. Your group does not have to run the procedures every day, but there must be a procedure run each day (take turns). At the end of the investigative period you should pool your data with that collected by the other groups.

Your group should also access the Internet to gather any relevant information regarding this project. You should consider things like the chemical composition of the extracted pigment, the purpose of these pigments, any physics that might be involved in this investigation, and any astronomical phenomenon relevant to the investigation.

### **Assessment:**

The following items will be used to assess your degree of success in this project:

Successful pigment extraction.

Successful pigment separation.

Graphed absorbance spectrum for each of the separated pigments.

Correctly done data table for recording the collected data.

Correctly done graph of the data collected.

Reasonable conclusion drawn from data collected and from references and any searches that you happened to make.

Write up of the investigation (following the approved format) with any references cited according to standard MLA format.

A presentation of the results of the investigation will be made to the teacher. During the creation of this presentation, each group must appoint one member to a committee

responsible for coming up with the presentation. However, each member of the class will be held responsible for the work done by this committee.

**References and Resources:**

The following references and resources might prove useful to this investigation:

<http://www.gac.edu/cgi-bin/user/~cellab/phpl?appds/appd-g.html>

[http://members.aol.com/ledodd/spectroscopy\\_links\\_www.htm](http://members.aol.com/ledodd/spectroscopy_links_www.htm)

<http://biology.wsc.mass.edu/biology/courses/concepts/labs/spec/operatespec20.html>

<http://www.spaceweather.com/>

<http://library.thinkquest.org/16468/hist-d1.htm>

[http://imagine.gsfc.nasa.gov/docs/science/know\\_11/emspectrum.html](http://imagine.gsfc.nasa.gov/docs/science/know_11/emspectrum.html)

<http://webster.commnet.edu/mla.htm>

<http://www.geocities.com/Athens/Oracle/4184/>

<http://www.esf.edu/pubprog/brochure/leaves/leaves.htm>

<http://photoscience.la.asu.edu/photosyn/education/colorchange.html>

<http://virtual.clemson.edu/groups/FieldOps/Cgs/leaves.htm>