Measure the absorbance of chlorophyll at three different wavelengths

Purpose
To measure the absorbance of light by chlorophyll at three different wavelengths within the visible spectrum.

Overview
This exercise is designed to familiarize the student with the concept of spectroscopy or the study of the interaction of matter with electromagnetic radiation. Students will prepare solutions and measure absorbance at different wavelengths.

Time
One class period.

Key Concepts
Matter and energy interact.
White light is made up of all wavelengths (colors).
Objects appear colored when they absorb or reflect different wavelengths of light to different degrees.
The color that we see is the transmitted or reflected color.
Chlorophyll helps to convert light energy into chemical energy by absorbing electromagnetic radiation.
This is the basis of photosynthesis!

Skills
Collecting data
Making observations
Graphing data
Forming hypotheses

Materials
Mortar and pestle or glass blender (do not use plastic if you will be using nail polish remover as a solvent)
Spinach or dried algae preparations (can be obtained from health food store as dietary supplement. About five capsules will do)
Nail polish remover (acetone or ethyl acetate) or rubbing alcohol or ethanol (30 - 50 mL)
Erlenmeyer flask (or a jelly jar)
Funnel
Filter paper (or coffee filters)
Vernier colorimeter interfaced to a CBL system.
Cuvettes (3)
Pasteur pipettes
Facilitator Preparation

This experiment works best if students work in groups. Some students will be responsible for operation of the spectrophotometer; others will collect and record data. Discuss the electromagnetic spectrum. Point out the relationship between wavelength and color in the visible region of the electromagnetic spectrum. (Remember the pneumonic Roy G. Biv)

<table>
<thead>
<tr>
<th>Red</th>
<th>Orange</th>
<th>Yellow</th>
<th>Green</th>
<th>Blue</th>
<th>Indigo</th>
<th>Violet</th>
</tr>
</thead>
<tbody>
<tr>
<td>700 nM</td>
<td>600 nM</td>
<td>580 nM</td>
<td>550 nM</td>
<td>475 nM</td>
<td>430 nM</td>
<td>400 nM</td>
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Before the exercise, show students a solution of chlorophyll. Ask students what color it is. Ask students what wavelength corresponds to that color. Ask students what wavelength of light chlorophyll absorbs. Most will say 500 nM. Hopefully they will change their answer at the end of the exercise.

Students should generate a curve with absorbance a maxima around 635 and 470 nM and minimum at 565 nM.

Background

We see color when light is reflected by objects around us. White light is made up of a spectrum or combination of all colors. When light hits the surface of an object, or passes through an object, these different colors can be reflected or absorbed in differing intensities. The color we see depends on which colors are reflected and which are absorbed. An object that appears red absorbs more of the green and reflects the red parts of the white light.

When ocean color is observed from space, highly sensitive instruments monitor various wavelengths (depending on what one wants to look for). Different colors (wavelengths) may reveal the presence and concentration of phytoplankton, sediments, and dissolved organic chemicals. Because different types of phytoplankton have different concentrations and types of chlorophyll, they appear as different colors to sensitive satellite instruments such as the Sea-viewing Wide Field-of-View Sensor (SeaWiFS). Thus, looking at the color of an area of the ocean allows us to estimate the amount and general type of phytoplankton in that area, and tells us about the health and chemistry of the ocean. Comparing images taken at different periods tells us about changes that occur over time.

Procedure

Instructions are written for a Vernier colorimeter to be interfaced with a CBL system.

1. Grind spinach (a handful) or dried algae in a blender or mortar and pestle with some of your solvent. Filter the suspension into the Erlenmeyer flask through filter paper or coffee filter. Adjust the concentration so that you can see the color, but it is not so concentrated that you can’t see through the solution.
2. Set the wavelength at 470 nM
3. With no sample in the colorimeter; set the % Transmittance at 0%.
4. Put the blank in the colorimeter and set the %T at 100%.
5. Place the cuvette with the chlorophyll solution in the colorimeter. Read the %Transmittance = ________________________
6. Set the wavelength at 565 nM
7. Repeat steps 3-5. %T = ________________
8. Set the wavelength at 635 nM
9. Repeat steps 3-5. %T = ________________

Student Assessment
Students should be able to answer the following questions. Students should be able to explain why chlorophyll is green.

1. What is the wavelength of maximum absorbance?
2. What color does this correspond to?
3. What is the wavelength of minimum absorbance?
4. What color does this correspond to?
5. If you wanted to monitor chlorophyll concentration what wavelength of light would you monitor?
6. What color does this wavelength correspond to?