  Spectral Analysis 13

The Visible Spectra of   
Plant Pigments

Plants contain many different molecules directly or indirectly involved with photosynthesis, which may also impart color to the plant. The mixture of chlorophyll molecules found in spinach, for example, absorbs several wavelengths of visible light, with distinct absorbance peaks in the blue range (400–500 nm) and in the yellow-red range (600–700 nm). The combination of visible light that is not absorbed appears green to the human eye. Chlorophyll contains a porphyrin ring in its structure with a magnesium ion in the center. The porphyrin ring accounts for much of the molecule’s light absorbance. Chlorophyll is found in the thylakoid plate of a plant chloroplast.

Carotenoids, accessory pigments produced in chromoplasts, are associated with many colors observed in vegetation. There are hundreds of different types of carotenoids. Carrots get their color, which is often orange but is not restricted to orange, from carotene. And carotene is not so much a specific compound as a family name for several compounds that also go by the name terpene.

Another type of carotenoid phyto-pigment is called anthocyanin. The purplish color of a red cabbage and the rusty red of the flesh of a blood orange are a result of the presence of anthocyanins, which also have the curious property of changing color with changes in pH. Anthocyanins absorb UV light, which is used by plants to perform two important functions: to attract insects, which augment pollination, and as a “sunscreen” to protect the other parts of the plant cells such as DNA from harmful UV radiation.

In this experiment you will extract pigments from spinach and carrots and measure their visible absorbance spectra with a spectrometer. While you wait for the extracts to develop, you will measure the absorbance of blue and yellow food-colored water samples, which will provide an analogy to the absorbance of the plant pigment extracts.

OBJECTIVES

* Measure and analyze the visible light absorbance spectra of pigments from spinach and carrots.
* Measure and analyze blue and yellow food coloring to compare with the plant pigments.

MATERIALS

Chromebook, computer, or mobile device

Vernier Spectral Analysis app

spectrometer

cuvettes

two 10 mL graduated cylinders

funnel

filter paper

three small beakers

13 × 100 mm glass test tube or glass cuvette

fresh spinach

carrot slices or shavings

70% isopropanol (IPA)

acetone or petroleum ether

yellow and blue food colored solutions

distilled water

plastic Beral pipets

ring stand and ring

balance, ± 0.1 g accuracy

125 mL Erlenmeyer flask and stopper

mortar and pestle

PROCEDURE

1. Obtain and wear goggles.
2. Launch Spectral Analysis. Connect the spectrometer to your Chromebook, computer, or mobile device. Select Absorbance vs. Wavelength.
3. Prepare a blank by filling an empty cuvette 3/4 full with distilled water.
4. To calibrate the spectrometer, place the blank cuvette in the spectrometer and select Finish Calibration. Note: If necessary, wait for the spectrometer to warm up before selecting Finish Calibration.

Part I  Prepare the Plant Pigment Extracts

1. Measure out 0.5 g of fresh spinach. Tear the spinach into tiny pieces and grind them with a mortar and pestle. Add 20 mL of 70% isopropanol (IPA) and transfer the mixture to a small beaker. Allow the mixture to sit.
2. Measure out 0.5 g of carrot slices (or shavings) and place them in a 125 mL Erlenmeyer flask. Add 20 mL of either acetone or petroleum ether to the flask and stopper it. DANGER: Handle the acetone or petroleum ether, with care. The fumes may irritate your nasal passages. It is best to use these liquids in a hood.

Part II The Absorbance of Food Coloring

1. Conduct a full spectrum analysis of the blue food coloring.
   1. Empty the blank cuvette and rinse it twice with small amounts of the blue food coloring mixture. Fill the cuvette 3/4 full with the blue liquid.
   2. Place the cuvette containing the blue food coloring into the spectrometer. Note: Make sure to align the cuvette so that the clear sides are facing the light source of the spectrometer.
   3. Start data collection. A full spectrum graph of the blue food coloring sample will be displayed.
   4. Stop data collection. If any of the peak absorbance values are greater than 1.5, dilute your sample to bring the peaks down to a more reasonable level and repeat data collection.
2. Repeat Step 7 with the yellow food coloring sample. Note: The previous data set is automatically saved.
3. Mix equal amounts of the blue and yellow solutions into a small beaker. Repeat Step 7 with the mixture.
4. Tap y-axis and select the runs you want to display on the graph.
5. Examine the graph, noting the absorbance peak ranges for each sample.
6. Record your observations of the graph. Save your experiment file as directed.

Part III Measure the Absorbance of the Plant Pigment Samples

1. First, prepare a purified sample of your chlorophyll extract. Use a funnel and filter to slowly pour the IPA/chlorophyll extract into a clean beaker.
2. Calibrate the spectrometer. This time you will calibrate the spectrometer isopropanol because your solvent in the chlorophyll extract is isopropanol, not water.
   1. Click or tap File, , and choose New Experiment.



* 1. Select Absorbance vs. Wavelength.
  2. Prepare a blank by filling an empty cuvette 3/4 full with isopropanol.
  3. Click or tap Settings, , and choose Calibrate.



* 1. Place the blank cuvette in the spectrophotometer and select Finish Calibration. Note: If necessary, wait for the spectrophotometer to warm up before selecting Finish Calibration.

1. Measure the absorbance spectrum of the chlorophyll extract.
   1. Pour out the isopropanol from the cuvette, rinse, and fill it 3/4 full with the chlorophyll extract.
   2. Place the cuvette in the spectrometer.
   3. Start data collection. A full spectrum graph of the chlorophyll extract will be displayed.
   4. Stop data collection.
2. Examine the graph, noting the absorbance peak ranges for chlorophyll described in the introductory remarks. If any of the peak absorbance values are greater than 1.5, dilute your sample to bring the peaks down to a more reasonable level and repeat data collection.
3. Record your observations of the graph.
4. Save your experiment file as instructed.
5. Calibrate the spectrometer with a different solvent for testing the carrot extract.
   1. Click or tap File, , and choose New Experiment.



* 1. Select Absorbance vs. Wavelength.
  2. Obtain a 13 × 100 mm glass test tube. Mark the test tube so that you can align it in the spectrometer the same way each time you use it.
  3. Fill the test tube ~1/2 full with the solvent, acetone or petroleum ether that you used with the carrot slices. This test tube of solvent will serve as your blank.
  4. Click or tap Settings, , and choose Calibrate.



* 1. Place the blank test tube in the spectrometer, being careful to line it up with the mark, and select Finish Calibration. Note: If necessary, wait for the spectrometer to warm up before selecting Finish Calibration.

1. Measure the absorbance spectrum of the carrot extract.
   1. Pour out the solvent from the test tube, rinse, and fill it ~1/2 full with the carrot extract.
   2. Place the test tube in the spectrometer, being careful to line it up with the mark.
   3. Start data collection. A full spectrum graph of the carrot extract will be displayed.
   4. Stop data collection.
2. Examine the graph. If any of the peak absorbance values are greater than 1.5, dilute your sample to bring the peaks down to a more reasonable level and repeat data collection.
3. Record your observations of the graph. Save your experiment file as directed.

DATA TABLE

|  |  |  |
| --- | --- | --- |
| Trial | Sample | Peaks or unique features of the spectrum |
| 1 | Blue |  |
| 2 | Yellow |  |
| 3 | Mixture |  |
| 4 | Spinach extract |  |
| 5 | Carrot extract |  |

DATA ANALYSIS

1. Describe, in detail, the spectrum of each food coloring sample. With the mixture of blue and yellow food coloring, can you clearly distinguish the characteristics of each coloring? Explain.
2. Consult a reliable resource to identify the major absorbance peaks of chlorophyll a and chlorophyll b. Examine the absorbance vs. wavelength graph for your spinach extract. Does your graph clearly show these absorbance peaks? Are there other peaks on your graph that are not characteristic of chlorophyll? If so, speculate about what caused these peaks.
3. Consult a reliable resource to identify the major absorbance peaks of -carotene and β-carotene. Examine the absorbance vs. wavelength graph for your carrot extract. Does your graph clearly show these absorbance peaks? Are there other peaks on your graph that are not characteristic of carotene? If so, speculate about what caused these peaks.
4. How did your tests of the absorbance of the blue and yellow food colored solutions compare with the tests of the spinach and carrot extracts?

ExtensionS

1. Your spinach extract contains chlorophyll, which is a fluorescent molecule. Fluorescent molecules can absorb light of one wavelength and then reemit light at a new and longer wavelength of light. As you have seen in this exercise, chlorophyll absorbs light in the violet and blue regions of the spectra. If you were to shine a violet or blue light through a sample of spinach extract, you would see the solution turn red in color. The “Long-Wave UV Pen Light” from Bio-Rad Laboratories, Inc. (Catalog # 166-0530EDU) can be used for this purpose. Fill a new cuvette with 1 mL of 70% IPA. Add 1 mL of the spinach extract to the cuvette. Shine the light from the Long-Wave UV Pen Light through the cuvette. Does the solution in the light path turn red in color?
2. Fluorescence spectroscopy is a method that is used to quantify fluorescent compounds in solution. In fluorescence spectroscopy, a sample can be “excited” with a chosen wavelength of light and the resulting light that is emitted from the sample can be measured and quantified. Follow the directions below to measure the fluorescence of the spinach extract after it has been diluted 50% with 70% IPA.
   1. Launch Spectral Analysis. Connect the spectrometer to your Chromebook, computer, or mobile device. Select Fluorescence vs. Wavelength.
   2. Place the cuvette containing the diluted spinach extract into the cuvette slot of the spectrometer.
   3. Change the excitation wavelength to 405 nm in the Collection Settings menu.
   4. Change Integration Time to 150 ms in the Collection Settings menu.
   5. Start data collection. A full spectrum graph of the solution will be displayed. Note that one area of the graph contains a peak at approximately 675 nm. This peak is from chlorophyll. Stop data collection.
   6. The height of the peak should be between 0.6 and 1.0. If necessary, adjust the integration time to increase or decrease the size of the fluorescent peak and repeat the data collection.
   7. Print or save your experiment file as directed.