OBJECTIVES:

1. Extract chlorophyll from spinach
2. Acquire a visible absorbance spectrum of chlorophyll

BACKGROUND:

Visible spectroscopy is an instrumental method of observing the absorption or transmission of visible wavelengths of light by a substance.

Light consists of a very broad spectrum of different wavelengths. Most wavelengths of light are not visible to humans. The human eye is sensitive to a relatively narrow range of wavelengths, of about 400-700nm. Along that range, the eye detects and the brain perceives different spectral colors as given in Figure 1, below.

Table 1 – Wavelengths of the different spectral colors of visible light.

Wavelength (nanometers)	Color
400-450	Violet
450-495	Blue
495-570	Green
570-590	Yellow
590-620	Orange
620-700	Red

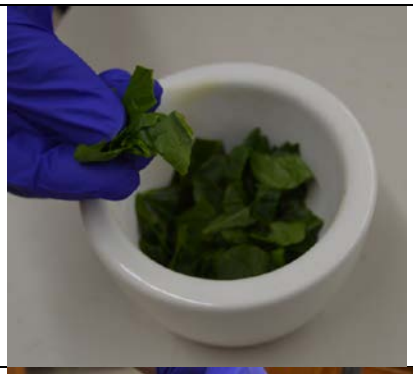
When light interacts with a substance, wavelengths can either be absorbed, reflected or transmitted by the substance. Grass appears to be green because of the pigment known as chlorophyll which is in the cells of the grass. Chlorophyll absorbs wavelengths from both ends of the visible spectrum and reflects or transmits the colors from the middle of the visible spectrum, including green, yellow and some orange wavelengths. Since the human eye is most sensitive to green light, grass appears green to most humans.

A **visible absorption spectrum** is simply a plot of how strongly each wavelength of visible light is absorbed by a given substance. Wavelength and absorbance data are plotted along the x and y axis respectively. In this experiment, A Spectronic 200 spectrophotometer will be used to produce a visible absorption spectrum of each sample.

Figure 1 – Top view of a Spectronic 200 spectrophotometer.

Figure 2 – Visible Absorption Spectrum of Chlorophyll. The vertical peak finder line is dialed over to the peak at 670nm.

PROCEDURE 1 – Extract chlorophyll from spinach leaves

Tear up five spinach leaves (without the stems), add about 20 mL of distilled water. Pulverize and mix the leaves into the water.	 
Vacuum-filter the liquid away from the mashed leaves.	
Disconnect the filter flask and pour the green liquid from the filter flask to a small beaker. Use a plastic pipette to transfer 10 mL of the green liquid into a 100mL graduated cylinder. Then, add distilled water to the cylinder to bring the total volume to 100mL. This effectively dilutes the green liquid to 1/10 of its initial concentration. It is done so that the absorption of light will not be out of range for the Spectronic 200.	 

PROCEDURE 2 – Visible spectrum of chlorophyll:

1. Use the Spec 200 to scan your diluted sample of chlorophyll from 400 to 700 nm.
2. Dilute the sample further if necessary to bring the peaks on scale.
3. Once you have acquired a good scan, show your instructor the Spec200 screen display. You may be asked by your instructor to adjust the dilution, re-zero the Spec200, etc.
4. Take a photo of the spectrum to include in your report.
5. Collect enough data from the display to generate an Excel plot for your report.

WASTE:

- All plant solids can go into the regular garbage.
- All liquids can go down the sink with plenty of water.
- DO NOT attempt to wash leaf solids down the sink. It will clog the drains.

NOTE TO INSTRUCTORS

- Return the mortar and pestle to the cupboard in the front desk
- Replace the ice in the spinach cooler and be sure to return the leftover bag of spinach to the cooler.