Spectrophotometry

# Introduction

Analytical chemistry is concerned with the accurate measurement of chemical substances. Two primary reasons for analysis are identification and quantification. For example, in both drug testing and environmental testing, the chemist must determine, first, *what* chemical species are in a sample, and second, *how much* of each compound is present.

One method used for measuring concentrations accurately is spectrophotometry, which is the measurement of light absorbed or released by matter. The full spectrum of light ranges from the infrared to the ultraviolet and beyond, but in this experiment only the absorption of visible light is measured.

An uncolored species does not have significant levels of absorption for any frequencies of the visible spectrum, while a black substance absorbs all frequencies of light. A colored substance does not significantly absorb the color seen but it does absorb others, especially its complimentary color. For example, an orange-colored liquid does not absorb orange light, but it does absorb all other colors of visible light, especially blue.

Percent transmittance is defined as

(1) %T = I/I0 X 100,

where I is the amount of light of a given frequency passing through a sample and Io is the amount of light passing through a similar solution with no absorbing species present. Absorbance is related to transmittance through the relation:

(2) A = -log (I0/I)

For many species at low concentrations, the relationship between absorbance and concentration follows an equation known as the Beer-Lambert law:

(3) A = ε b c

where ε is the absorptivity (a property of the chemical species that is also a function of wavelength), b is the path length that the light is traveling through, and c is the concentration. The path length is almost always one centimeter. If all measurements are made at the same wavelength, the absorptivity will be constant. Therefore, a graph of absorbance versus concentration should be a straight line.

Once a Beer-Lambert plot has been constructed, the concentration of an unknown solution can be found directly by measuring its absorbance and locating the correct concentration on the plot. Alternately, a linear fit of the data can be made and the value of ε can be determined directly.

Spectrophotometry may be used to identify compounds as well as determine concentrations. If a single solution is tested at different wavelengths, then a graph of absorbance versus wavelength shows the characteristic absorptivity of the compound. This spectral plot is, in effect, a fingerprint for a colored compound, since no two compounds will have the exact same color. Such a plot is also necessary to determine which wavelength should be used for a Beer-Lambert plot.

# Materials and Methods

Spectrophotometer, with cuvettes

Blue, red, and/or yellow food dyes

250 mL beaker, plus four other beakers

10 mL and 100 mL graduated cylinders

# Procedure

Part 1: A Transmittance plot

1. Obtain approximately 200 mL of tap water in a 250 mL beaker and add one or two drops of food coloring to the water. Stir the solution to make a uniform color.
2. Turn on the spectrophotometer. After the machine has completed its internal calibration, use the buttons on the right-hand side of the control panel to collect transmittance data from 400 nm to 750 nm.
3. Add tap water to a cuvette to serve as a blank. Place the cuvette in the sample holder, close the lid, and press the ‘zero’ button [0.0] to zero the machine.
4. Pour some of the colored solution into a second cuvette, about 2/3 full. Remove the blank and place the sample in the sample holder and close the lid.
5. Press the ‘go’ button. In a few minutes, the transmittance of the solution will appear in graph from on the control panel screen.
6. Use the knob on the right side of the machine to scan through the wavelengths (the green line). Record the percent transmittances at the wavelengths shown in the data table on the Report Sheet.

Part 2: A Beer-Lambert Plot

1. Use the data from Part 1 to identify a wavelength for the Beer-Lambert plot. A good wavelength has low transmittance, which corresponds to high absorbance. Record the wavelength used on the Report Sheet.
2. Use the stock solution to make 10%, 25%, 50%, and 75% dilutions. If you have trouble figuring out how to do that, the instructor will provide suggestions.
3. Change the settings for the spectrometer to Absorbance and Live Display. Set the wavelength to the value chosen in (1).
4. Record the absorbances of all five solutions, including the stock solution. Record the values in the data table on the Report Sheet.

# Data and Results

1. Construct a plot of percent transmittance (%T) on the y-axis versus wavelength, in nm, on the x-axis from the first data table.
2. Construct a Beer-lambert plot with absorbance on the y-axis and concentration on the x-axis from the second data table. The concentration will be expressed as a value between 0% and 100% of the stock solution.