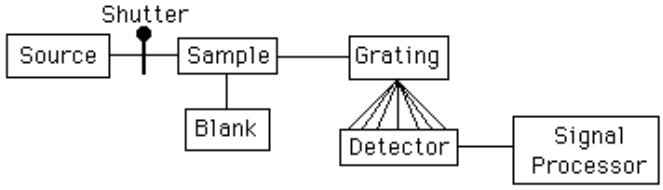
Visible Absorbance Spectra and Beer's Law

Why is orange juice orange? The simple answer is that one or more components of orange juice absorb selected wavelengths of visible light such that light passing through or reflecting off the orange juice appears orange; that is, the sample absorbs light whose color is the complement of orange. We can take advantage of this phenomenon to study molecules, atoms, and ions by measuring their ability to absorb electromagnetic radiation. Sometimes this information qualitative (What compound is this?) and other times it is quantitative (How much of this compound is present?); in the context of Chem 260, our interest is in the quantitative application of visible spectroscopy.

Spectrometers

A sample's ability to absorb light is measured using a spectrometer. The simplest visible spectrometer has five parts: (a) a place to put the sample; (b) a source of visible light, typically a tungsten lamp similar to those you might find at home; (c) a detector that measures the amount of radiation passing through the sample; (d) a means of dispersing the light, typically a prism or a diffraction grating, so that we can analyze for each wavelength, the light's interaction with the sample; and (e) a signal processor, such as a meter or computer, for manipulating and displaying the resulting measurements.

A simple cartoon of the spectrometer used in Chem 260 is shown here.



This instrument uses a diffraction grating to disperse the light over a series of individual detectors, each of which monitors absorbance simultaneously over a narrow band of wavelengths.

Transmittance vs. Absorbance

At any wavelength, the fraction of light not absorbed by a sample is defined as its transmittance, T

$$T = \frac{P_T}{P_0}$$

where P_T is the intensity of light transmitted through the sample and P_0 is the intensity of light from the source. Frequently the transmittance is expressed as a percentage, %T, where

$$\%T = T \times 100$$

A little thought will convince you that transmittance must fall within the range of 0 to 1 and that percent transmittance must fall within the range of 0 to 100%.

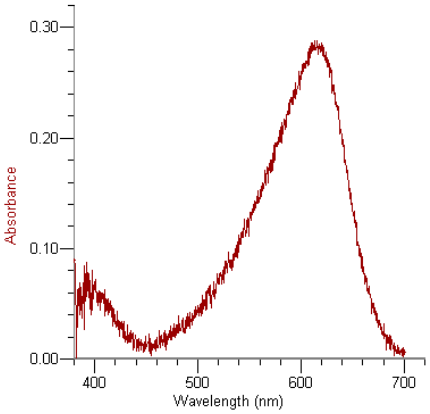
Because the relationship between transmittance and concentration is logarithmic (for reasons we will not consider here), it is more common to report absorbance, A, instead of transmittance, where

$$A = -\log(T) = 2 - \log(\%T)$$

Visible Absorbance Spectra

A spectrometer monitors the absorbance (or transmittance) over a range of wavelengths. A plot of absorbance as a function of wavelength is called an absorbance spectrum, a typical example of which

is shown to the right. Note that in this example the sample absorbs strongly at a wavelength of 618 nm.



Beer's Law

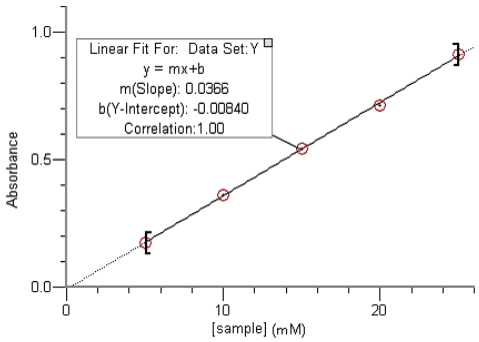
One of the most important applications of visible spectrometry is determining the concentration of the species absorbing light. As you might expect, a solution with a higher concentration of the absorbing species transmits less light and has a smaller percent transmittance and a greater absorbance. Unfortunately, the relationship between %*T* and concentration is logarithmic, as noted earlier, which complicates the conversion of a sample's %*T* to the concentration of the absorbing species in the sample. The relationship between concentration and absorbance, however, is linear. The exact relationship between absorbance and concentration is known as Beer's law

$$A = \varepsilon b C$$

where A is the sample's absorbance, ε is the molar absorptivity, a constant whose value depends on the absorbing species and the selected wavelength, b is the distance the light travels through the sample, and C is the molar concentration of the absorbing species. Values for ε are rarely known with sufficient accuracy, so they are determined by measuring the absorbance of a solution of known concentration. Usually we just combine the value for b with the value of ε and drop the stipulation that concentration is expressed as molarity. Beer's law reduces, therefore, to

$$A = kC$$

where k is a calibration constant and C is any concentration unit. To determine the calibration constant we prepare several solutions containing known concentrations of the absorbing species, measure the absorbance of each, plot A vs. C, and use linear regression to find the best-straight line through the data. The equation of this line is used to calculate the concentration of the absorbing species given its absorbance. An example of a Beer's law calibration curve is shown on the left.



In this case the slope of 0.0366 is equivalent to k and the y-intercept, as expected, is essentially zero. Knowing the calibration equation allows us to calculate the concentration of analyte in a sample. For example, if a sample has an absorbance of 0.372, then the analyte's concentration is

$$A = 0.372 = -0.00840 + 0.0366C$$

$$C = 10.4 \text{ mM}$$

Limitations to Beer's Law

Our treatment of Beer's law assumes the relationship between absorbance and concentration is linear. This strictly is not true for a variety of reasons that we will not explore here; however, Beer's law closely approximates a linear relationship if the absorbing species' concentration is sufficiently small and if the absorbance is not too large. For this reason, it generally is a good idea to limited absorbances to values that are smaller than one.