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 visible light such that the 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 is 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; (c) a detector that measures the amount of radiation that passes through the sample; (d) a means of dispersing the light, typically a prism or a diffraction grating, so that we can analyze at each wavelength the light's interaction with the sample; and (e) a signal processor, such as a meter or a computer, to manipulate and display the resulting measurements. A simple schematic diagram of the spectrometer used in Chem 260 is shown in Figure 1. 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.

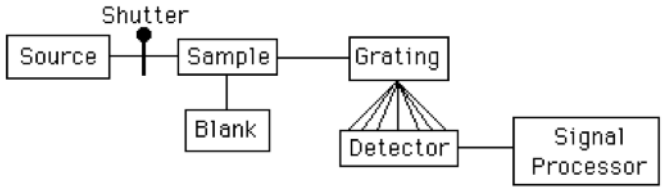

Figure 1: Schematic diagram of a simple diode array spectrometer.

Transmittance vs. Absorbance

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

$$T_\lambda = \left(\frac{P_T}{P_0}\right)_\lambda \tag{1}$$

where P_T is the intensity of light transmitted by the sample and P_0 is the intensity of light from the source. Frequently the transmittance is expressed as a percentage, $\%T_\lambda$, where

$$\%T_\lambda = T_\lambda \times 100 \tag{2}$$

A little thought should convince you that values for the transmittance must fall within the range 0 to 1 and that values for the percent transmittance must fall within the range 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_λ, instead of transmittance, where

$$A_\lambda = -\log(T_\lambda) = 2 - \log(\%T_\lambda) \tag{3}$$

Visible Absorbance Spectra

A spectrometer monitors 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 in Figure 2. Note that in this example the sample absorbs strongly at a wavelength of 618 nm.

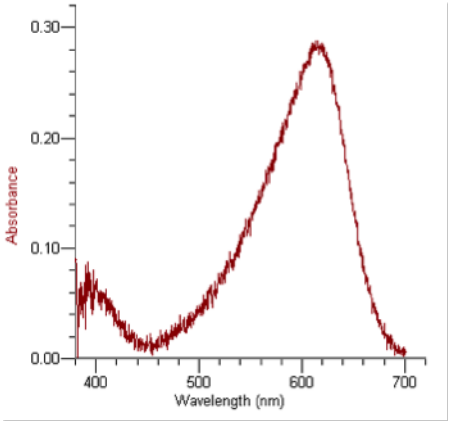

Figure 2: Typical visible absorbance spectrum.

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, as we see in Equation 3, the relationship between $\%T_\lambda$ and concentration is logarithmic, which complicates the conversion of a sample's $\%T_\lambda$ 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

$$A_\lambda = \epsilon_\lambda b C \tag{4}$$

where A_λ is the sample's absorbance at the wavelength λ, ϵ_λ is the molar absorptivity at that wavelength, 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_λ 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_λ and drop the stipulation that concentration is expressed as molarity. Under these conditions, Beer's law reduces to

$$A_\lambda = k_\lambda C \tag{5}$$

where k_λ is a calibration constant and C is any concentration unit.

To determine the calibration constant we prepare several solutions that contain known concentrations of the absorbing species, measure the absorbance of each, plot A_λ 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

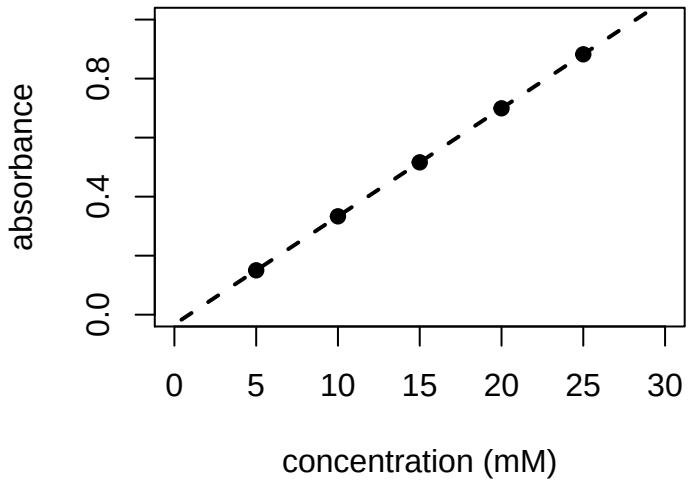

Figure 3: Example of a Beer's law calibration curve.

absorbing species given its absorbance. Figure 3 shows an example of a Beer's law calibration curve where a regression analysis gives a calibration curve of

$$A_\lambda = -0.00840 + 0.0366C \tag{6}$$

The equation for the calibration curve 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

$$A_\lambda = 0.372 = -0.00840 + 0.0366C$$

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