

## Appendix I

FV 10/26/05

### SPECTROPHOTOMETRY

*Spectrophotometry* is an analytical technique used to measure the amount of light of a particular wavelength absorbed by a sample in solution. This measurement is then related to relevant properties of the solution, e.g., concentration. The wavelengths of light used in ultraviolet-visible spectrophotometry range from about 250 nm to 750 nm, the visible range itself ranging from ~ 400 nm (violet) to ~ 700 nm (red). The instrument used to measure the amount of light absorbed by the sample is a *spectrophotometer*.

#### Solution Color and Light Absorption

Solutions which absorb visible light are generally colored. Colored solutions are therefore considered good candidates for analysis via spectrophotometry. It is important to recognize that the color of the solution is related to the wavelength of light absorbed by the solution in a *complementary* way. For example, a solution which appears blue does so because the blue wavelength region of the spectrum of the white light of the room, which is composed of all wavelengths, is *not* being absorbed by the solution, whereas significant absorption is taking place for the non-blue wavelength regions of the visible spectrum. A crude but useful tool for understanding this concept is the color wheel, shown in Figure 1. Solutions which appear to have a particular color, e.g., blue, absorb wavelengths of light associated with colors primarily on the *opposite* side of the wheel, e.g., orange, yellow, and red.

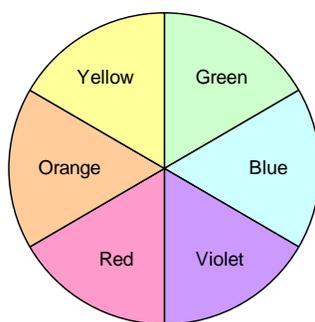


Figure 1. The color-wheel. Solutions which appear to a certain color absorb wavelengths associated with the colors on the opposite side of the wheel.

#### Basic Design Elements

A spectrophotometer has a source of light with a continuous spectrum in either the visible or ultraviolet region of the electromagnetic spectrum. A monochromator then selects light within a narrow band of wavelengths. For example, a prism in conjunction with a moveable, narrow slit will allow only light within a small range of wavelengths to pass. This selected light then passes through a pathlength  $\ell$  of the sample which is contained in a sample holder, usually a glass cuvette. The intensity of the selected light just before entering the sample,  $I_o$ , and the intensity of the light after passing through the sample,  $I$ , are measured with electronic detector systems. See Figure 2.

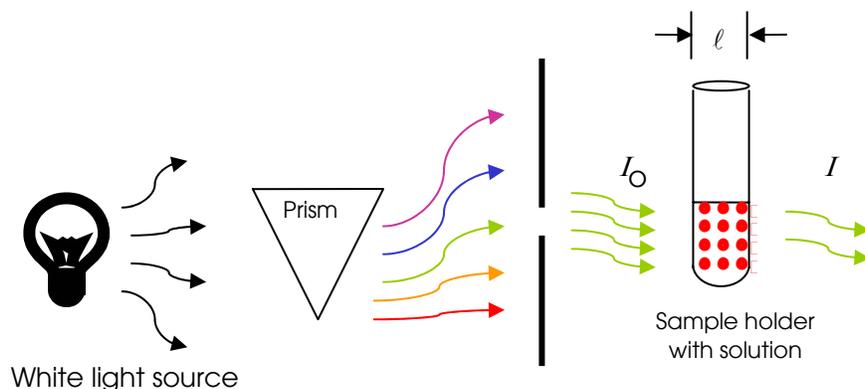


Figure 2. Schematic diagram of a spectrophotometer

### Percent Transmittance and Absorbance

The amount of light transmitted through a sample is expressed as the *percent transmittance* and is designated as %*T*. It is simply defined as the ratio of the transmitted light intensity to the intensity of the incident light, multiplied by 100%:

$$\%T = \left( \frac{I}{I_o} \right) \times 100\% \quad (1)$$

Percent transmittance is therefore a quantity directly measured by a spectrophotometer, since *I* and *I<sub>o</sub>* are directly measured by the electronic detector system.

The *absorbance* of a sample, designated by *A*, is related to the measured %*T* in the following way. A sample which allows 100% of the selected light to be transmitted clearly has zero absorbance. On the other hand, a sample which allows no light to be transmitted (zero percent transmittance) exhibits *infinite* absorbing power, i.e.,  $A = \infty$ . These limits are properly accounted for through the following logarithmic relationship between absorbance and percent transmittance:

$$A = -\log\left(\frac{\%T}{100}\right) \quad (2)$$

As will be shown below, the absorbance *A* is significantly more relevant to a sample's physical properties than %*T*.

*Practical Limitations.* Though this definition allows any measured %*T* to be converted to an absorbance value *A*, errors in %*T* measurements yield corresponding absorbance value errors in a non-uniform manner. Errors in %*T* readings which are too low (e.g., below ~12%) or too high (e.g., above ~70%) are significantly magnified in their corresponding *A* values. Thus, a practical guideline for avoiding this mathematical pitfall, if possible, is to manipulate the experimental situation so that only samples with %*T* values above 12% and below 70% are prepared and measured. This corresponds to solutions with absorbance values *A* between ~0.16 and 0.92.

### The Beer-Lambert Law

For a given sample, absorbance depends on six factors: (1) the identity of the absorbing substance, (2) its concentration, (3) the pathlength *ℓ*, (4) and wavelength of light, (5) the identity of the solvent, and (6) the temperature. Obviously, not all solution species absorb light in the same way. For a given species, a more concentrated solution has more absorbing substance present, resulting in more light

being absorbed. The longer the pathlength, the more absorbing substance the light will interact with, resulting in greater absorbance. Solutions with higher absorbance appear darker or more intensely colored than solutions with lower absorbance. The existence of colored solutions is indicative that not all wavelengths of light are absorbed equally strongly. Most solvents are colorless, including water, and do not absorb visible light to any appreciable degree. The effect of temperature is minimized since most solutions are studied at room temperature.

For solutions which are dilute enough, the above factors combine together to yield a linear relationship between absorbance, concentration, and pathlength, known as the Beer-Lambert Law:

$$A = \varepsilon \cdot \ell \cdot c \quad (3)$$

where  $A$  is the absorbance (unitless),  $\ell$  is the pathlength of the sample (cm),  $c$  is the concentration of the absorbing species (usually in molarity), and  $\varepsilon$  is an experimentally determined constant known as the molar absorptivity (if the concentration is in molarity) or the extinction coefficient. This proportionality constant accounts for the diversity of absorbing strengths of different chemical species as well as the variable absorbing strength for different wavelengths of light. In other words, the molar absorptivity  $\varepsilon$  is unique for a given solution species and a particular wavelength of light.

*Practical Limitations.* If a solution is too concentrated, the simple linear Beer-Lambert relationship no longer applies, and more complex equations would need to be used. For practical purposes, ionic species with concentrations below about 0.02 M are generally dilute enough and the Beer-Lambert Law of equation 3 adequately models these solutions. Less concentrated solutions obey the Beer-Lambert Law even more closely.

If the solution species does not absorb a particular wavelength of light very strongly (if  $\varepsilon$  is too small), it may be necessary to either (1) find a different wavelength, if possible, where absorption is greater, or (2) increase its concentration above 0.02 M in order to obtain measurable and reliable absorbance values. The linear Beer-Lambert Law could not be used appropriately in this latter case.

The challenge to the experimentalist is to manipulate the experimental parameters so that species being studied with spectrophotometry are both dilute enough (so that the Beer-Lambert Law is valid) and strongly absorbing enough (so that the solutions absorb in the most reliable portion of the scale, i.e., with  $A$  between  $\sim 0.16$  and  $0.92$ ). See Figure 3.

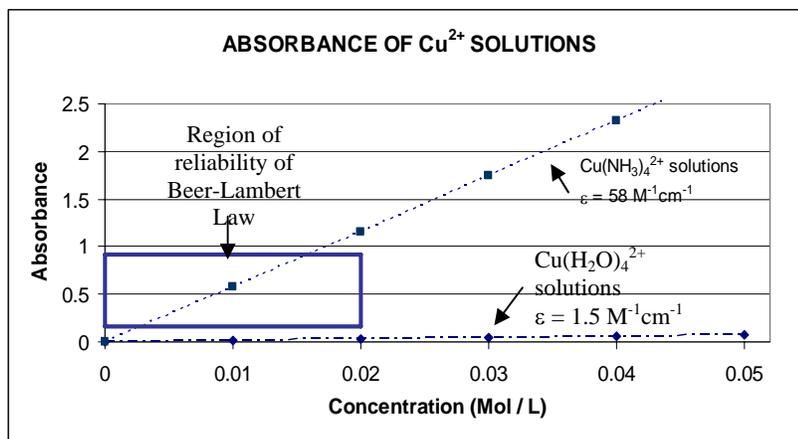


Figure 3. The most reliable concentrations and absorbances for spectrophotometric determinations. The  $\text{Cu}^{2+}$  solutions, with  $\varepsilon = 1.5 \text{ M}^{-1}\text{cm}^{-1}$  for a wavelength of 610 nm, have a pathlength,  $\ell$  of 1.0 cm. Evidently, simple aqueous solutions of  $\text{Cu}^{2+}$ , though blue in color, are not good candidates for spectrophotometric analysis. On the other hand, solutions of  $\text{Cu}(\text{NH}_3)_4^{2+}$ , with  $\varepsilon = 58 \text{ M}^{-1}\text{cm}^{-1}$ , are good candidates for such analysis, especially for the range of concentrations between about 0.002 M and 0.016 M.

Finally, it should be noted that solutions which are cloudy or inhomogeneous will generally not produce acceptable results. The techniques of spectrophotometry are thus limited to typical homogeneous solution samples.

### Calibration Curves

For dilute solutions, there is a linear relationship between absorbance and concentration. Normally, the same or similar sample holders (e.g., cuvettes) are used for each measurement, so that the pathlength  $\ell$  is constant. Therefore, a plot of absorbance vs. concentration gives a straight line at a particular wavelength and temperature (see Figure 4).

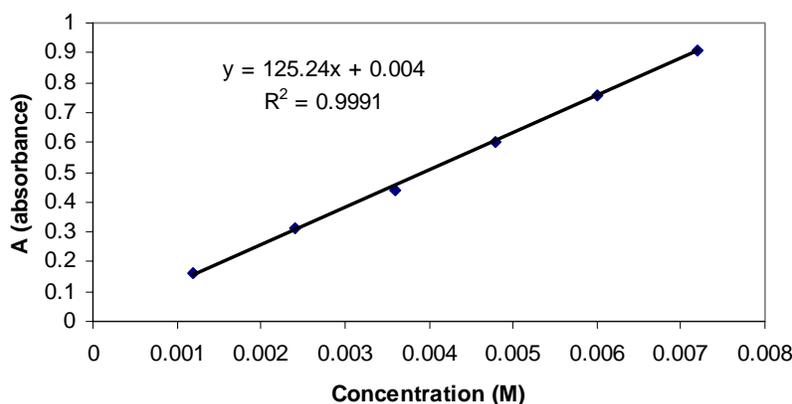


Figure 4. A calibration curve, including equation and  $R^2$  for best-fit line. The slope,  $125.24 \text{ M}^{-1}\text{cm}^{-1}$  for this example, is equivalent to  $\epsilon\ell$ .

Solutions of various known concentrations are prepared, followed by measurement of their respective % $T$ 's, which are then converted to absorbances. If the Beer-Lambert Law holds, these experimental data points should fall along a nearly perfect straight line. Using linear regression, the straight line which best fits these experimental points is obtained. This line is called the *calibration curve or line*. Samples of the same absorbing species, but with unknown concentration, can have their concentrations determined by performing the spectrophotometric measurement of absorbance, and then using the equation of the best-fit straight line to calculate the corresponding concentration.

Theoretically, one might predict that the best-fit straight line should pass through the graph's origin, i.e., a zero concentration solution should exhibit zero absorbance. In practice, however, this does not usually occur in this method of obtaining a calibration curve, as seen in Figure 3. There are several reasons why this may not occur, including glass of the sample holder or the solvent having a small effect on the absorption of the light, or the possibility that the linear Beer-Lambert Law is not perfectly obeyed for the data points at higher concentration. Whatever the reasons may be, one normally uses the best-fit equation, including both the slope and intercept, to convert the unknown solution's measured absorbance to concentration. The graph's origin is *not* included as a data point nor is it included in the regression analysis.

Of course, the slope of the best-fit line is a very good approximation to the quantity  $\epsilon\ell$ , which is the proportionality constant in the Beer-Lambert Law. Since the pathlength  $\ell$  is easily measured (typically, 1.0 cm), the value for  $\epsilon$ , the molar absorptivity, is easily determined.

## Absorption Spectra

Most of the discussion to this point has assumed a particular selection for the wavelength of the light being absorbed. Most solution species which absorb light do not absorb just a single wavelength, but instead absorb to varying degrees all of the wavelengths across a broad range of the electromagnetic spectrum. The *absorption spectrum* of an absorbing species is simply a plot of the absorbance vs. wavelength. Figure 5 shows the absorption spectrum for a solution containing the  $[\text{Ti}(\text{H}_2\text{O})_6]^{3+}$  ion (this solution appears orange-red in color), spanning the visible portion of the electromagnetic spectrum. Obtaining this spectrum is helpful for choosing the wavelength at which to study a particular solution species. Notice that the absorbance is at a maximum at a wavelength of about 510 nm. The wavelength of maximum absorbance,  $\lambda_{\text{max}}$ , is usually the wavelength chosen. This is done for three reasons. First, most monochrometers

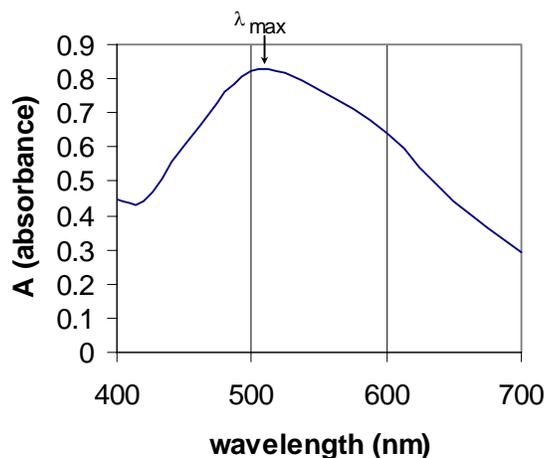


Figure 5. The visible absorption spectrum of  $[\text{Ti}(\text{H}_2\text{O})_6]^{3+}$

transmit a narrow band of wavelengths surrounding the selected wavelength. If the various wavelengths within this band absorbed with different strength, as would be the case for a selected wavelength along a sloping portion of the absorption spectrum, there would arise unwanted complications in the spectrophotometric analysis, yielding less reliable measurements. Second, if one needed to reset the selected wavelength, e.g., from day to day, any small error in that process would have a minimal impact on the reproducibility of the data since the absorbing strengths of those wavelengths near the maximum of the absorption spectrum are nearly the same. Third, the absorbance is at a maximum for  $\lambda_{\text{max}}$ , and less percent error is associated with larger signals.

### Solutions with Two Absorbing Species

If a sample is a mixture of two absorbing substances, the resulting absorption spectrum is the sum of the two individual absorption spectra. Figure 6 shows the absorption spectra of two absorbing species, A and B, and the spectrum of a mixture of A and B.

Species A has  $\lambda_{\text{max}}$  at 500 nm and species B has  $\lambda_{\text{max}}$  at 600 nm. The total absorbance of the mixture at 500 nm and at 600 nm is:

$$A_{500}(\text{total}) = A_{A,500} + A_{B,500} \quad (4)$$

$$A_{600}(\text{total}) = A_{A,600} + A_{B,600} \quad (5)$$

Using the Beer-Lambert Law (we are assuming dilute solutions of A and B), and substituting for the absorbance of the individual substances, one obtains:

$$A_{500}(\text{total}) = \epsilon_{A,500} \ell c_A + \epsilon_{B,500} \ell c_B \quad (6)$$

where  $\epsilon_{A,500}$  is the molar absorptivity for substance A at 500 nm,  $\ell$  is the pathlength, and  $c_A$  is the molar concentration of A, etc. Analogously for 600 nm,

$$A_{600}(\text{total}) = \epsilon_{A,600} \ell c_A + \epsilon_{B,600} \ell c_B \quad (7)$$

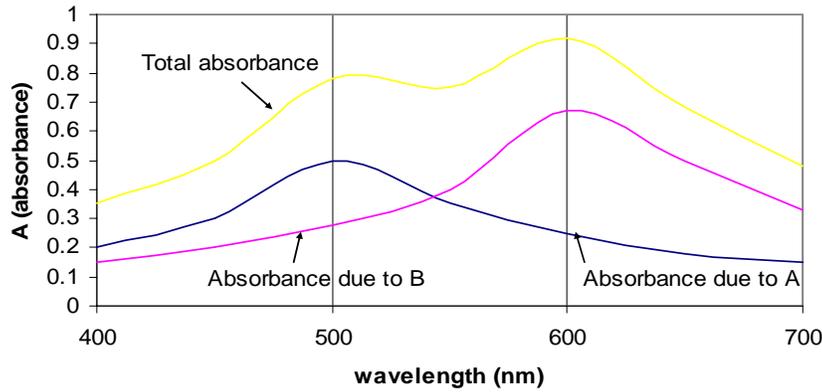


Figure 6. Absorption spectrum of A, B, and mixture of A and B

The molar absorptivities of A and B can be calculated from calibration curves of the individual substances at each wavelength, so when the absorbance of the mixture has been measured, equations (6) and (7) can be solved for the concentrations of substances A and B in the mixture.