Review of Concepts: Standard Curve and Beer's Law

Use of a Standard Curve In this technique a series of solutions with known concentrations is prepared and then a parameter such as absorbance is measured. This parameter is then plotted versus concentration to yield a standard curve (for Beer's Law how absorbance varies with concentration), which is often a straight line with some degree of experimental error. Analysis of the data allows determination of the best-fit line (e.g., with Excel). A subsequent measurement of the absorbance for an unknown sample allows determination of its concentration using the equation for the standard curve found for the line. The unknown concentration should lie in the concentration range of the standard solutions used to construct the standard curve for an accurate concentration determination.

Beer's Law Beer's Law relates the experimental absorbance value for a chromophore (a substance that absorbs light) to the concentration of that chromophore in solution. Beer's law has many forms, the most common is: $\boldsymbol{A_\lambda = \varepsilon\, l\, C}$. In this equation $\boldsymbol{A_\lambda}$ is the measured absorbance of the chromophore at the wavelength λ (usually at a peak maximum, or λ_{max}, determined from a spectrum spanning ultraviolet and/or visible wavelengths of light). The Greek letter epsilon, $\boldsymbol{\varepsilon}$, stands for the molar extinction coefficient ($M^{-1}cm^{-1}$), an experimentally determined constant for the specific chromophore at the same wavelength (this wavelength is always specified). The molar extinction coefficient is a quantitative measure of the light absorbance by the chromophore at that wavelength for a one molar solution and a one-centimeter path length. The value $\boldsymbol{l}$ is the path length, or the distance the light travels through the solution in the cuvette (container) used for the absorbance measurement. Lastly, $\boldsymbol{C}$ is the molar concentration of the chromophore (mol/L) used for the measurement. Beer's law says that the relationship between the absorbance of the chromophore and its concentration is linear, allowing construction of a standard curve by plotting absorbance versus concentration, such as shown in Figure 1. Notice that all of the units cancel upon multiplying $\boldsymbol{\varepsilon\, l\, C}$, consistent with $\boldsymbol{A}$ being a unitless quantity.

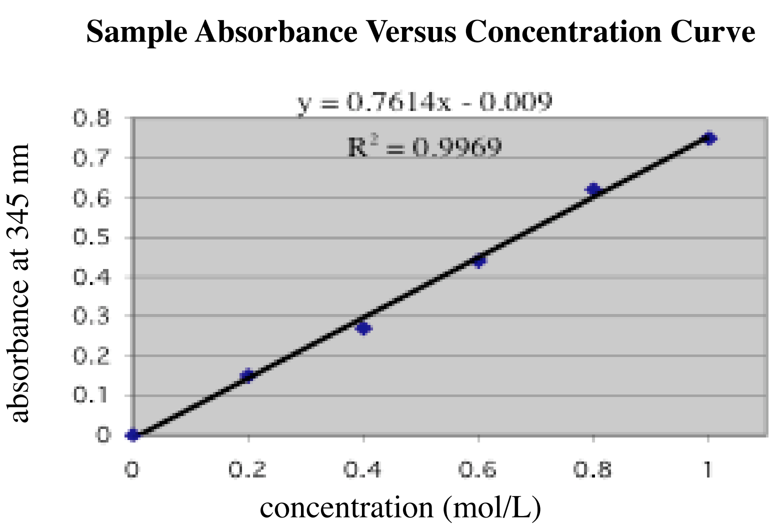

Figure 1. A typical absorbance vs. concentration standard curve based on Beer's Law. This particular curve is most reliable for absorbance values between 0 and 0.8. The concentration for a solution can be determined by measuring its absorbance and then substituting the absorbance value as y in the equation for the standard curve and solving for x.