How Is Protein Concentration Measured?

The standard procedure for measuring protein concentration is UV analysis, wherein in the protein concentration is calculated from the measured absorbance at a given wavelength, using the Beer-Lambert expression shown below, where A is the absorbance, ε is the molar absorptivity, b is the path length, and C is the molar concentration.

$$A = \varepsilon bC$$

A common mistake that is made in the use of Beer's Law to determine protein concentration, is failure to recognize that the expression is only valid within a narrow range of protein concentrations. Figure 1 shows the concentration dependence of the UV spectra for lysozyme in PBS (pH 7.6). The dashed line indicates the wavelength (281.5 nm) for the lysozyme absorptivity of 2.64 L cm^{-1} g^{-1} reported in the Worthington Enzyme Manual.

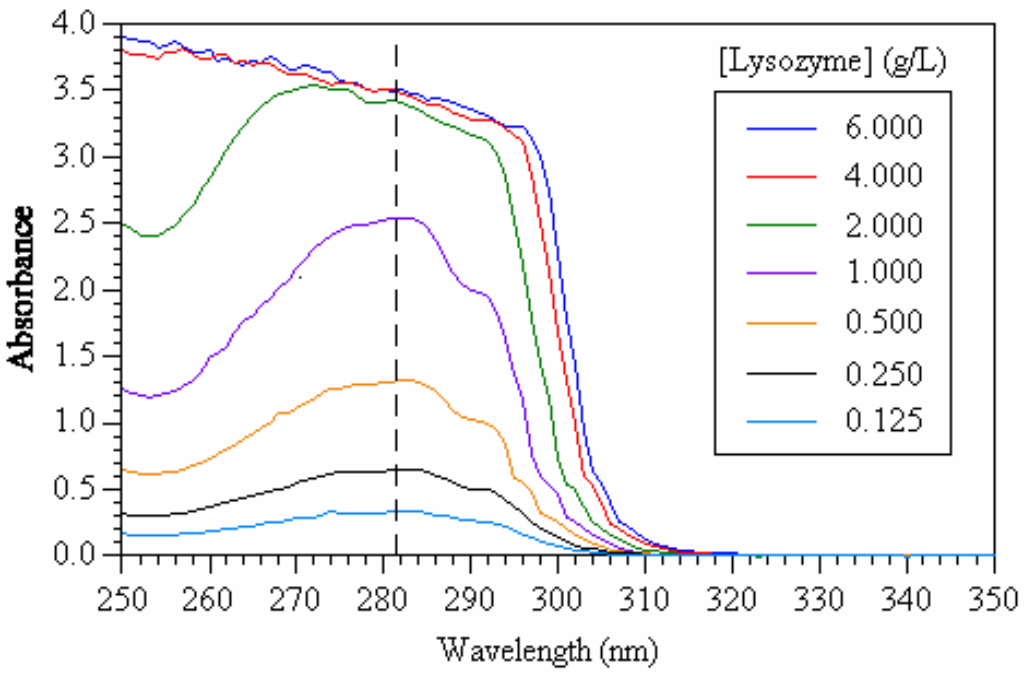

Figure 1: Concentration dependence of the UV spectra for lysozyme in PBS, measured in a 1 cm path length cell.

The concentration dependence of the absorbance at 281.5 nm for the lysozyme series is shown in Figure 2. As seen in this figure, a negative deviation from Beer's law is observed for concentrations > 1.0 g/L. When determining the concentration of an unknown lysozyme sample then, one would need to insure that the measurement was conducted under conditions wherein the concentration was < 1 g/L.

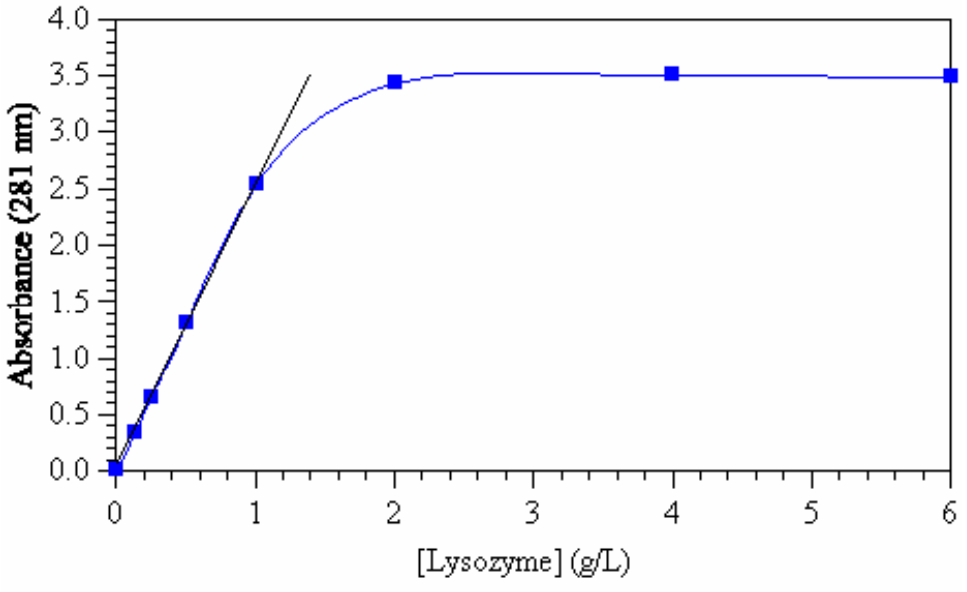

Figure 2: Beer's Law plot for lysozyme in PBS, measured at a wavelength of 281.5 nm.

The upper concentration limit for Beer's Law determination of protein concentration is unique to each protein. Consider for example, the Beer's Law plot shown in Figure 3 for BSA in 0.l M NaCl at pH 4.0. In contrast to lysozyme with an upper concentration limit of 1 mg/mL, BSA absorbance is linear with concentration up to circa 4 mg/mL.

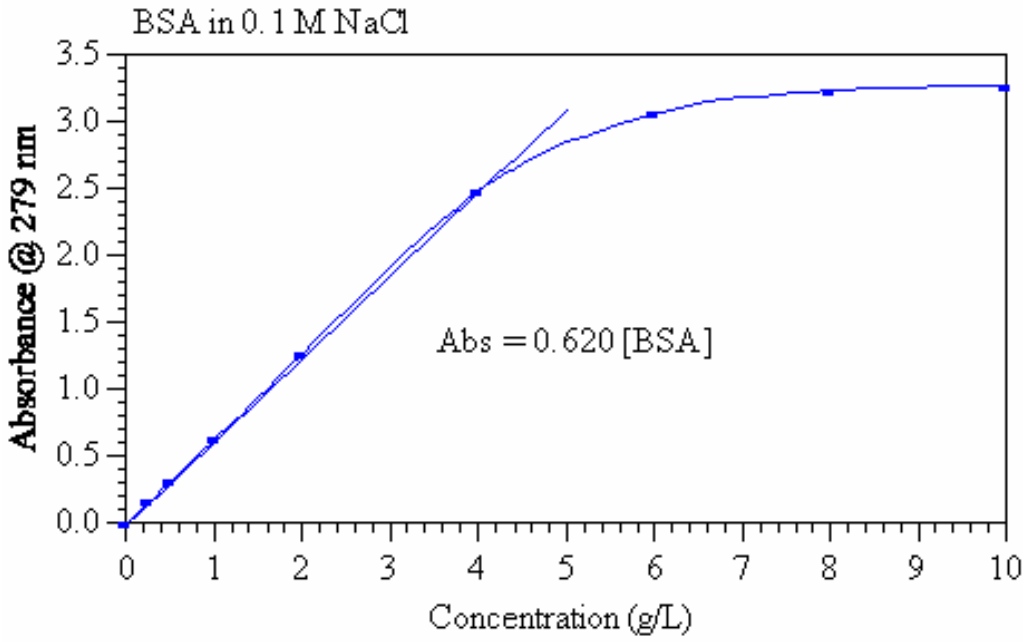

Figure 3: Beer's Law plot for BSA in 0.1 M NaCl & pH 4.0, measured at 279 nm.

The ideal conditions for protein concentration determinations using the Beer-Lambert expression can be identified by conducting an analysis of error propagation. In the absence of scattering, the wavelength dependent absorbance (A) and transmittance (T) of a solution of particles are related as shown below, where I is the sample intensity, I_o is the incident intensity, ε is the absorptivity, b is the cell path length, and C is the analyte concentration.

$$-\log\frac{I}{I_o} = -\log T = A = \varepsilon bC$$

After rearrangement, the differential of C with respect to T can be written as follows.

$$dC = \frac{dT}{T(2.303)\varepsilon b} = \frac{dT}{T(2.303)} \text{ x } \frac{C}{-\log T} = \frac{dT}{T \ln T} C$$

Assuming $dT \approx \Delta T$, the relative error in the concentration arising from instrumental error in the transmittance reading can then be approximated by the following expression.

$$\frac{\Delta C}{C} \text{ x } 100 \approx \frac{\Delta T}{T \ln T} \text{ x } 100$$

The relative error in the concentration as a function of %T and absorbance for arbitrarily selected ΔT values of 0.2 and 1.0 % is shown in Figures 4a & b. The relative error in concentration displays a minimum at 38.5 %Transmittance, independent of the magnitude of instrumental error. The corresponding optimum absorbance value is 0.415. Hence, when determining unknown sample concentrations via UV measurements, the effects of instrumental error can be minimized by incrementally diluting the samples to a UV absorbance value of circa 0.4.

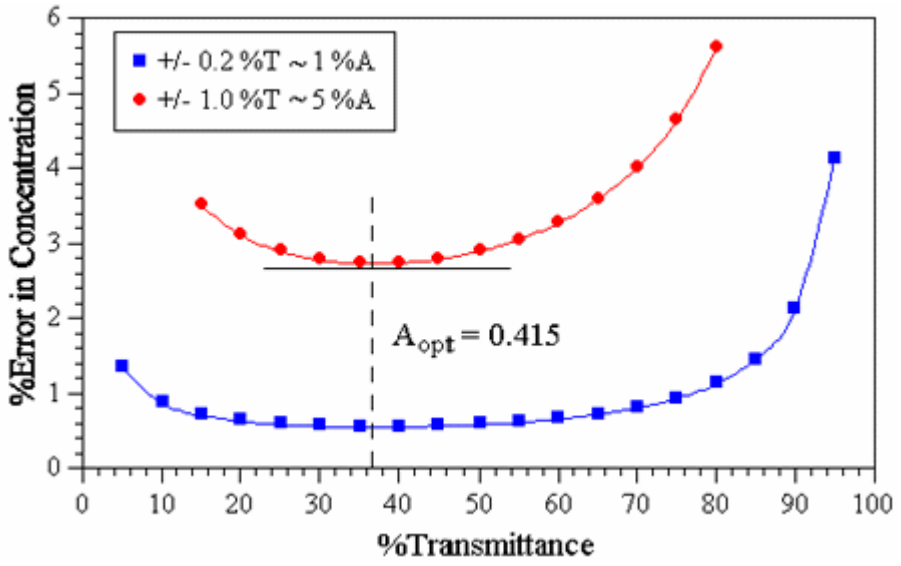

Figure 4a: The relative error in concentration as a function of %T for instrumental error (ΔT) values of 0.2 & 1.0 %T.

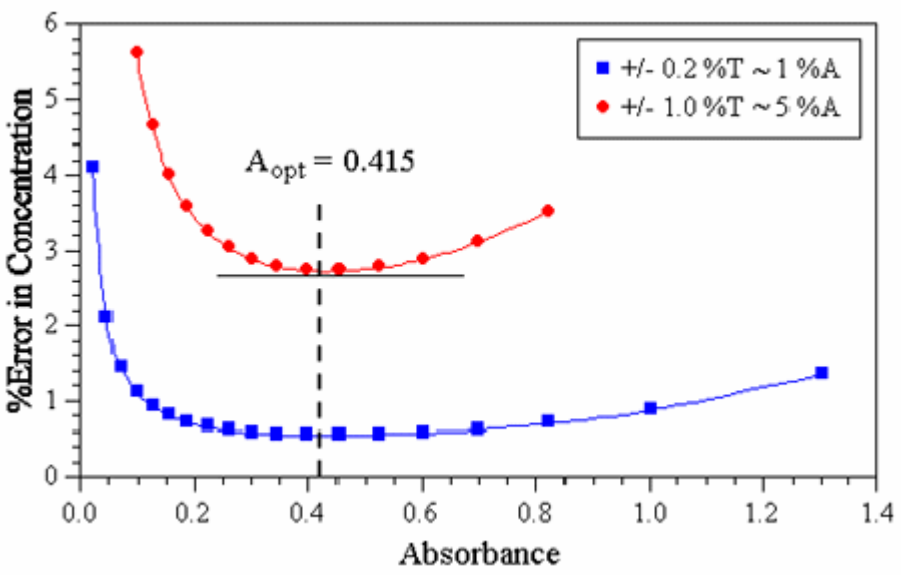

Figure 4b: The relative error in concentration as a function of the absorbance for instrumental error (ΔT) values of 0.2 & 1.0 %T.

For lysozyme (ε = 2.64 L cm^{-1} g^{-1}) and BSA (ε = 0.62 L cm^{-1} g^{-1}), this "ideal" absorbance of 0.415 corresponds to concentrations of 0.16 and 0.67 mg/mL respectively, if the measurements are collected in a 1 cm path length cell. If a shorter path length cell, such as a 2 mm cell is used, the optimal concentrations for UV measurements of lysozyme and BSA would be increased to 0.79 and 3.35 mg/mL respectively.

Malvern Instruments Ltd
Enigma Business Park • Grovewood Road • Malvern • Worcestershire • UK • WR14 1XZ
Tel: +44 (0)1684 892456 • Fax: +44 (0)1684 892789

Malvern Instruments Worldwide
Sales and service centers in over 50 countries for details visit www.malvern.com/contact