



The Power of Slope Spectroscopy[®]

E. Shih and M. Salerno
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C TECHNOLOGIES, INC.—The SoloVPE[®] System is an innovative instrument for UV-Vis-NIR spectroscopy. VPE (Variable Pathlength Extension) provides analysts easy access to another dimension of measurement using the Beer-Lambert law, specifically fine pathlength control. The ability of the SoloVPE System to rapidly generate absorbance vs. pathlength linear graphs is one of the unique measurement results

made possible by its pathlength-scanning capability. By using the slopes from the absorbance vs. pathlength relationships, quick, easy, and precise determinations of concentrations, extinction coefficients, and dilution accuracy are possible. We refer to this powerful analysis technique as Slope Spectroscopy[®].

What is Slope Spectroscopy?

The SoloVPE System enables users to quickly and easily measure the absorbance of a sample at various pathlengths. During operation, the pathlength is defined by the distance between the tip of the light-delivering Fibrette[®] and the bottom interior of the sample vessel. The pathlength is dynamically controlled by integrated hardware and software, precisely moving the Fibrette up and down through the sample. The SoloVPE software also includes useful data-analysis tools for both traditional and Slope Spectroscopy methods.

Figures 1 and 2 show typical SoloVPE data representations. Figure 1 shows a collection of spectrographs of the same sample (in this example, Myoglobin). Each of the spectra has been collected at a different pathlength and, as predicted by the Beer-Lambert law, the absorbance varies proportionally with respect to the changes in pathlength. Frequently, we are interested in the absorbance value at specific wavelengths, a peak or valley in the spectrograph. By using the wavelength cross section feature in the SoloVPE software, absorbance vs. pathlength plots can be generated at the desired wavelength(s) as

shown in Figure 2.

The SoloVPE Analysis tool enables the user to perform data regression on the wavelength cross-section plot. The linear-regression analysis provides two key parameters: the slope and the coefficient of determination (R^2). The coefficient of determination indicates the goodness of fit or how well the regression line matches the measured data. The slope term of the linear-regression equation drives the potential and power of Slope Spectroscopy.

Slope and Beer's Law

Slope Spectroscopy uses the slope term of the linear-regression equation to

make analytical determinations quickly and easily for solutions varying in concentration. Through simple manipulation of the Beer-Lambert law, the utility of the slope term is revealed.

The Beer-Lambert law is expressed as:

$$A = \alpha \times l \times c \quad (1)$$

where A is the measured absorbance, α is the wavelength dependent molar absorption coefficient, l is the pathlength, and c is the sample concentration. For a Slope Spectroscopy study, we move

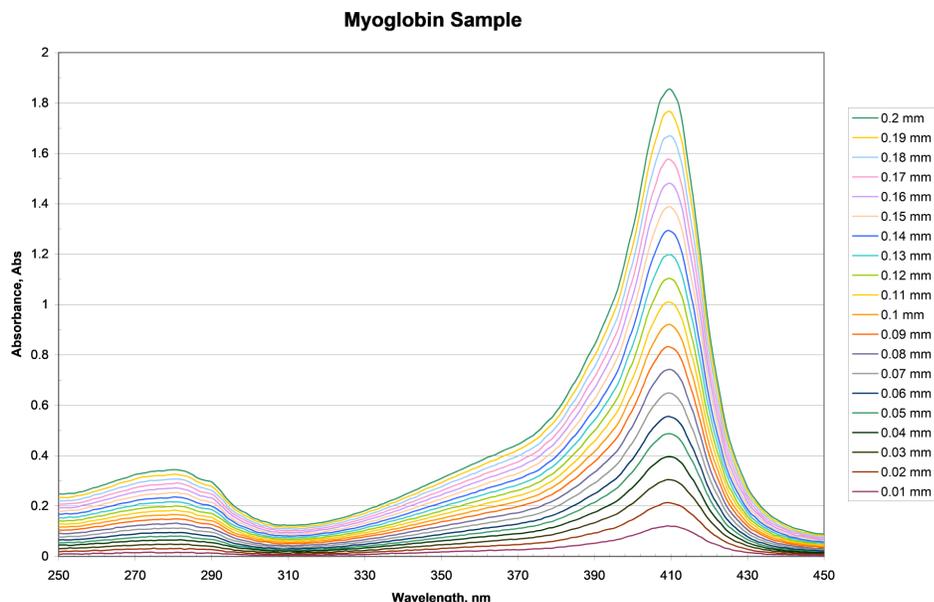


Figure 1. Pathlength Spectra Data Set

pathlength term l to the left-hand side of the equation, so the above equation becomes:

$$A / l = \alpha \times c \quad (2)$$

The linear equation from the regression of absorbance vs. pathlength data can be written as:

$$A = m \times l + b \quad (3)$$

where m is the slope of the regression line, and b is the y -intercept of the linear equation. It is known that the units of the slope term in the regression equation are absorbance/pathlength, in this case, Abs/mm. This dimensional equality allows direct replacement of the left-hand side of Eq. 2 (A / l) with the slope term (m) from Eq. 3. This substitution results in a new equation which we call the **Slope Spectroscopy Equation**:

$$m = \alpha \times c \quad (4)$$

Using this equation, if the molar absorption coefficient of a sample is known, the concentration of the sample can then be simply calculated by dividing it into the slope:

$$c = m / \alpha$$

Or if we know the concentration of the sample, we will be able to calculate the molar absorption coefficient by dividing the slope by the concentration:

$$\alpha = m / c$$

Interpreting the Regression Terms

If we closely examine the regression equation (Eq. 3) and the Beer-Lambert law (Eq. 1), we will find that there is some discrepancy when the pathlength is zero. According to Beer's law, zero pathlength should result in zero absorbance. However, the regression equation predicts a small but non-zero absorbance value when the pathlength is equal to zero. This absorbance offset comes from two primary sources: the effect of light scattering, and small pathlength deviations near zero. Applying commonly used scatter correction algorithms to the data reduces

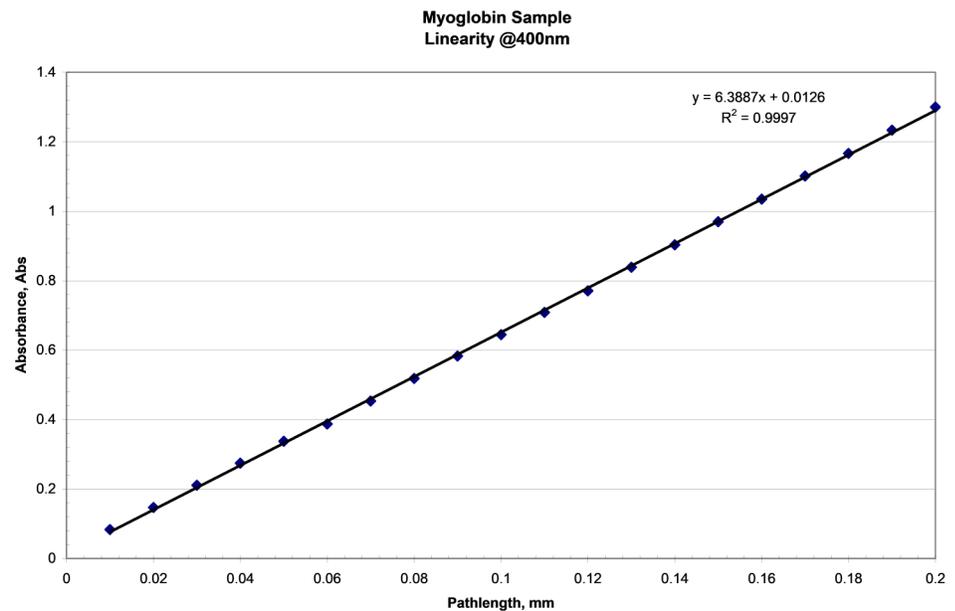


Figure 2. Linear Regression.

the contribution of scatter on the offset for most samples. Understanding the small pathlength deviations requires more explanation.

The SoloVPE System has an innovative method for defining the zero pathlength position. However, every mechanical system has a tolerance—no matter how small—and the SoloVPE System is no different. There is no absolute zero position, so there will be small variations in the measurement pathlength. The SoloVPE System does precisely control the pathlength changes with a resolution of five microns (0.005 mm). This is readily verified by examining the strength of correlation on regressed wavelength cross section plots. R^2 values close to one confirm that the absorbance values are changing proportionally with pathlength in accordance with Beer's law. Experimental results show that in addition to having very strong correlation results, the y -intercept term closely approaches zero as expected. How do we reconcile the remaining absorbance offset with the Beer's law?

There are two different methods for obtaining a regression line that passes through the origin. Option one is to employ a regression algorithm that forces the regression through the origin. However, that option has the effect of

changing the slope of the best-fit line. This could result in larger absorbance errors when using the regression equation to predict absorbance values well outside the range of measured pathlengths. Option two is based upon the theory that the x -intercept value is the pathlength offset of the system. This suggests that the regressed data set can be normalized for the pathlength based upon the x -intercept value. After shifting the predictive equation, it could yield more accurate results. However, in this case it is important to note that shifting the curve to make the x -intercept zero *does not change the slope of the regressed line*.

The Slope Spectroscopy method is so robust that despite the presence of pathlength deviations, even at the smallest pathlengths, fast and accurate determinations can be made when the absorbance vs. pathlength regression has a region of strong correlation across multiple data points (>5). The SoloVPE System, and its ability to quickly generate slope data, empowers users to take advantage of this highly useful Slope Spectroscopy technique.

For more information, visit: www.ctechnologiesinc.com