

The Power of

Slope
spectroscopy®E. Shih and M. Salerno
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The SoloVPE (pr. sōlō vē-pē-ē), Variable Pathlength Extension, from C Technologies, Inc. is an innovative instrument for UV-Vis-NIR spectroscopy. It provides analysts easy access to another dimension of measurement using the Beer-Lambert Law, specifically fine pathlength control. The ability of the SoloVPE 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*®.

Introducing Slope Spectroscopy:

The SoloVPE enables users to quickly and easily measure the absorbance of a sample at various pathlengths. In SoloVPE operation, the pathlength is defined by the distance between the tip of the light delivering Fibrette and the inside bottom of the sample vessel. The pathlength is dynamically controlled, through precise movement of the Fibrette up and down in the sample, by integrated hardware and software. 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 spectra has been collected at a different pathlength and, as predicted by the Beer-Lambert Law, the absorbance varies proportionally with respect to the pathlength

changes. Frequently, we are interested in the absorbance value at specific wavelength, 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 three key parameters: the slope and y-intercept of the best fit equation and the coefficient of determination. The coefficient of determination indicates the goodness of fit or how well the regression line matches the measured data. The y-intercept projects where

the regression line intercepts the Absorbance Axis. Manipulation of the regression equation will also reveal the x-intercept or where the regression line intercepts the Pathlength Axis. It is the slope term of the linear regression equation, with dimensions of Abs/mm, that drives the potential and power of Slope Spectroscopy®.

Slope and Beer's Law

While the regression equation itself is useful for predicting the absorbance value at pathlengths that are not directly measurable and for comparing solutions of different concentrations, Slope Spectroscopy is particularly interested in using the slope term of the linear regression equation to make analytical determinations quickly and easily. Through simple manipulation of the Beer-Lambert Law, the utility of the slope term is revealed

The Beer-Lambert Law is expressed as

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

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 pathlength term "l" to the left hand side of the equation so the above equation becomes

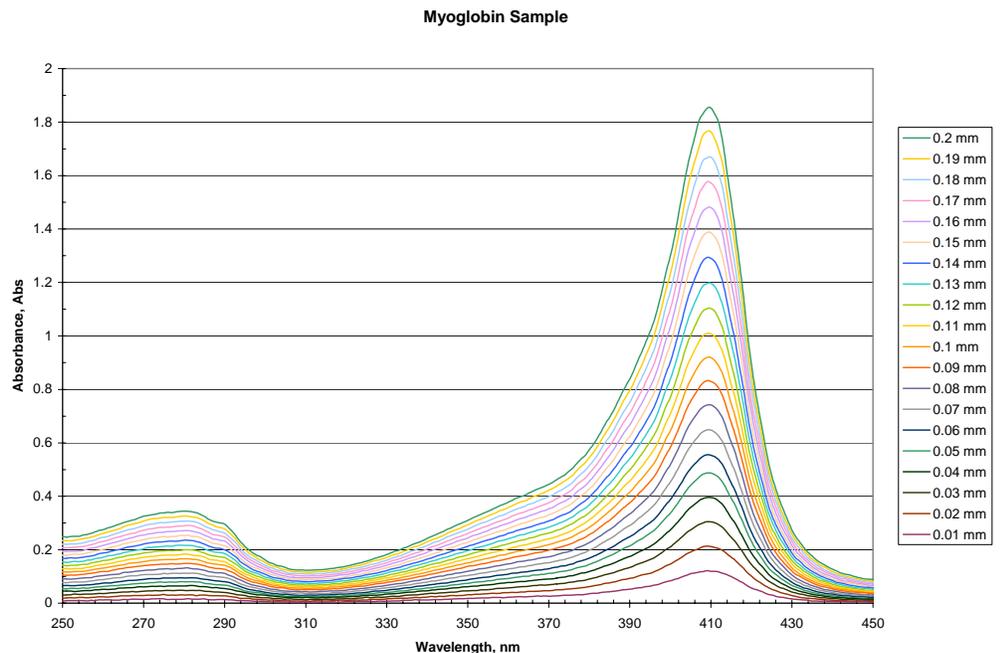


Figure 1: Pathlength Spectra Data Set

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

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

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

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 Abs/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 new equation which we call the **Slope Spectroscopy Equation**

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

Using the Slope Spectroscopy 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.

$$c = m / \alpha \quad (5)$$

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

$$\alpha = m / c \quad (6)$$

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

The SoloVPE has an innovative method for defining the “zero” pathlength position. However, every mechanical system has a

Myoglobin Sample
Linearity @400nm

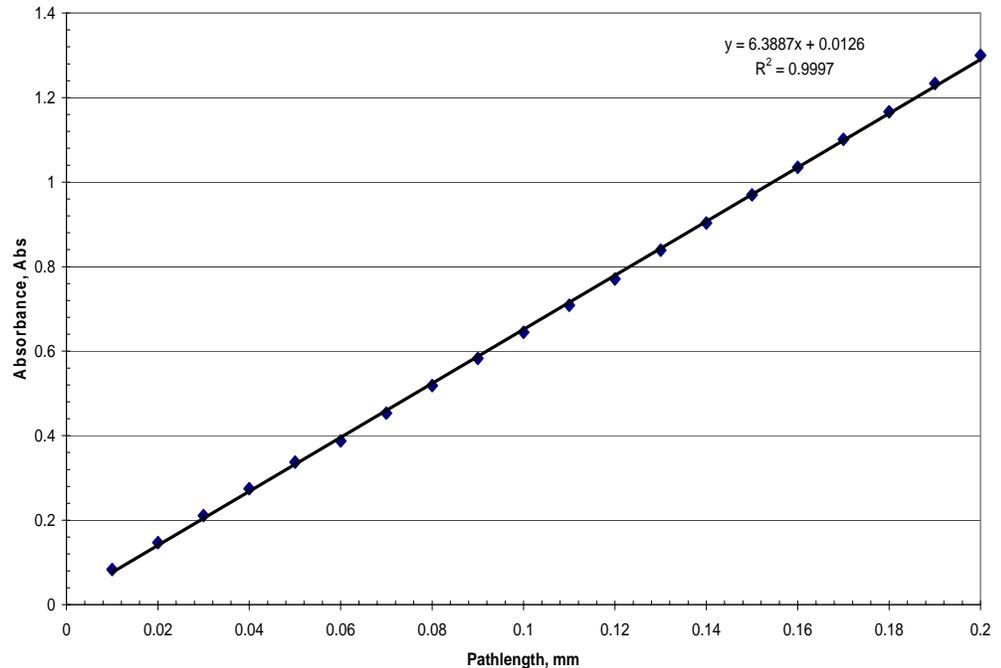


Figure 2: Linear Regression

tolerance, no matter how small, and the range of measured pathlengths. Option two is SoloVPE is no different. There is no absolute 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*. This means that regardless of pathlength accuracy, utilizing the slope of the regressed data set will yield an accurate prediction of absorbance and concentration.

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changing proportionally with pathlength in accordance with the Beer-Lambert Law. Experimental results show that in addition to having very strong correlation results, the y-intercept term closely approaches zero as expected. But how do we reconcile the remaining absorbance offset with the Beer-Lambert 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

the slope of the regressed line. This means that regardless of pathlength accuracy, utilizing the slope of the regressed data set will yield an accurate prediction of absorbance and concentration.

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 and its ability to quickly generate slope data, empowers users to take advantage of this highly useful Slope Spectroscopy technique. □

For more information go to: www.solovpe.com