VPE Software - Quick Slope Corrections: Baseline, Single λ Scatter, Dual λ Scatter, V3 Scatter Methods

Abstract: This document provides details on how Baseline Correction and Scatter Corrections work inside the Quick Slope application.

Applicability: This article applies to the Quick Slope application which is a part of the C Technologies, Inc. SoloVPE Software.

Introduction: Quick Slope is a tool that utilizes Slope Spectroscopy to analyze the concentration of a sample. Slope Spectroscopy works by taking absorbance readings at multiple pathlengths to form a slope of the sample. The slope is then used to calculate the concentration using the Beer's law equation. Baseline Correction is used to subtract any background contribution from unwanted peaks in found in the sample. Scatter Correction is used to eliminate any light scatter that may be present due to particles in the sample or optical density.

Detailed Info:

Baseline Correction:

There is an option inside the Quick Slope application allowing users to run a Baseline Correction with their sample. While running a Baseline Correction will not cause any issues with data obtained, it may be unnecessary to perform the action. Generally the SoloVPE does not require Baseline Corrections because the user is no longer looking at absolute absorbance. The SoloVPE instead uses Slope to determine the concentration. If the buffer being used does not have a slope (or little to none), then it should not contribute to the actual slope of the sample. Comparing the corrected slope with a non-corrected slope will most likely show the same results. This is very common in highly concentrated samples

With certain buffer types, such as ones containing methanol, Baseline Correction may be needed, most specifically when there is a non-zero slope that contributes to the sample or has pathlength dependency. Another reason a user might need Baseline Correction is if the sample is very dilute, less than 1(mg/ml). When measuring a sample that is very dilute it is important to remember that even a small non-zero buffer slope could be significant when measuring a sample that should already have a small slope.

To find out if Baseline Correction is needed, a user may run the buffer sample as if they were running a Quick Slope on the actual sample. The slope value obtained from running the Quick Slope will not be exactly zero. This is due to noise and optical loss from the Cary instrument to the SoloVPE. If the slope value is less than 0.01 Baseline Correction is typically not required to run the sample. Lastly, for testing your buffer, you will disregard the R^2 value. This is due to the lack of Slope presence within the sample. The Slope value will provide the information required to understand if the correction is required or not. See Figure 1 below.

Figure 1: Poor R-Squared value and low slope value.

If the Slope value is greater than 0.01 it is possible that the pathlength dependence of the buffer is real and Baseline Correction will be required.
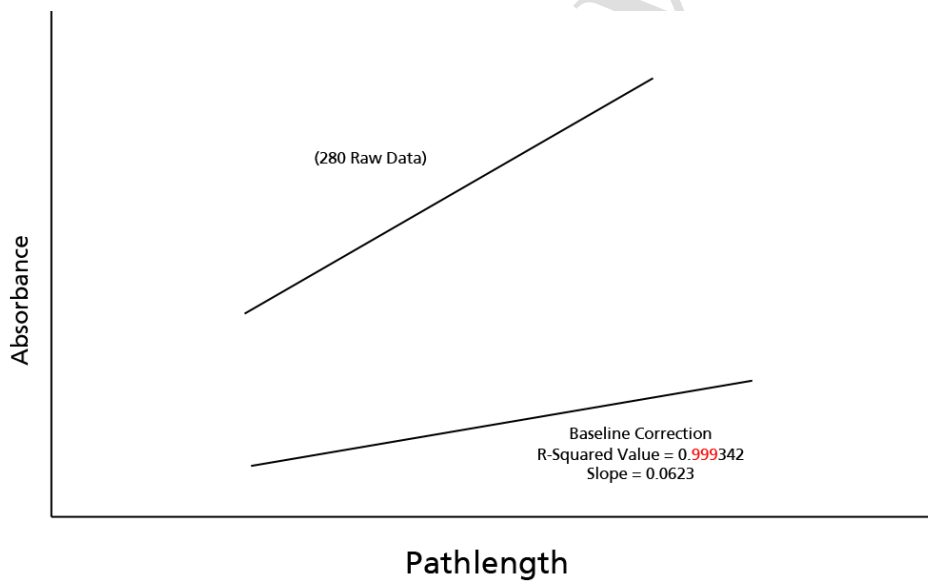

Figure 2: Baseline Correction is needed; good R-Squared value and high slope value.

A user may also run the sample with Baseline correction enabled. After the baseline has been run and Quick Slope has determined that a Baseline was not needed, an alert will appear resembling a blue cross-out circle with BL in the center. The SoloVPE software determines this when the Slope of the Baseline is so small that it may not be required.

When performing either test to find out if Baseline Correction is needed it is important to completely fill the selected Sample Vessel. Quick Slope works by using predefined pathlengths to create a Baseline Section that will cover the entire height of the Sample Vessel. Quick Slope interpolates the correction value from the baseline section data, since visiting every single pathlength would be impractical. Doing so allows the software to adapt to the threshold pathlength and step size that the Quick Slope application finds during the sample data acquisition.

Scatter Correction

UV-Spectroscopy is a commonly used practice for determining the concentration of a protein solution. However, in protein solutions where particles comparable to the size of the wavelength at which the absorbance is being determined appear, light scattering may occur. Light scattering can decrease the accuracy of the determined concentration of the protein and as a result any scattering must be corrected out. There are multiple methods of determining and correcting light scatter of which are described below.

Single Wavelength (SW) Scatter Correction

SW Scatter Correction works by taking data points at two different wavelengths at the same pathlengths. It then subtracts the absorbance at the Scatter Wavelength from the Absorbance at the Method Wavelength to create a Corrected Absorbance at the specified method wavelength. This will isolate the absorbance contribution of the key species being measured at the method wavelength.
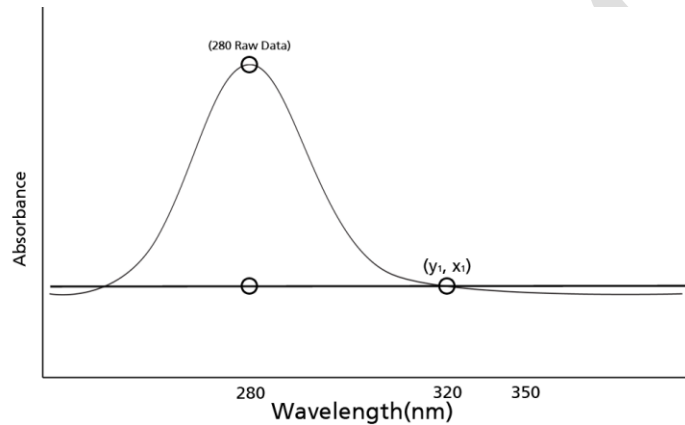

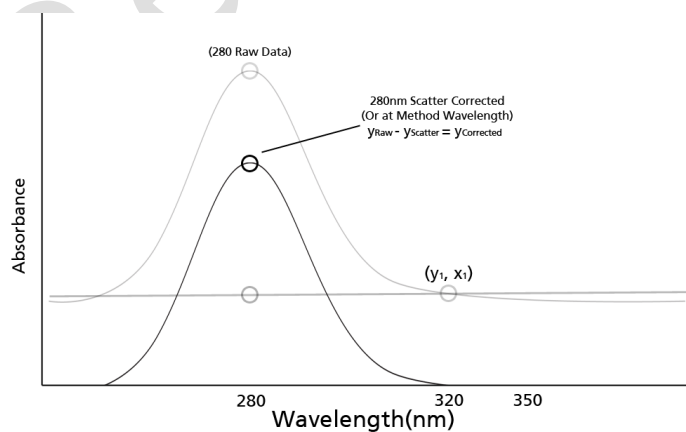**Figure 3: Data collected before Scatter Correction with SW Scatter.**

Figure 4: The Corrected Absorbance at the method wavelength.

Dual Wavelength (DW) Scatter Correction

Dual Wavelength Scatter Correction works differently than Single Wavelength Scatter Correction. Instead of subtracting one slope line from the other and shifting the results down, DW will use the two scatter wavelengths to project an absorbance value to be subtracted from your expected wavelength. The dual wavelength feature is similar to a 3-Point drop correct method, which quantifies a sloping scatter contribution line and makes a correction based off of the absorbance values obtained. Each individual pathlength will be calculated for separately, point for point as the section data is collected.
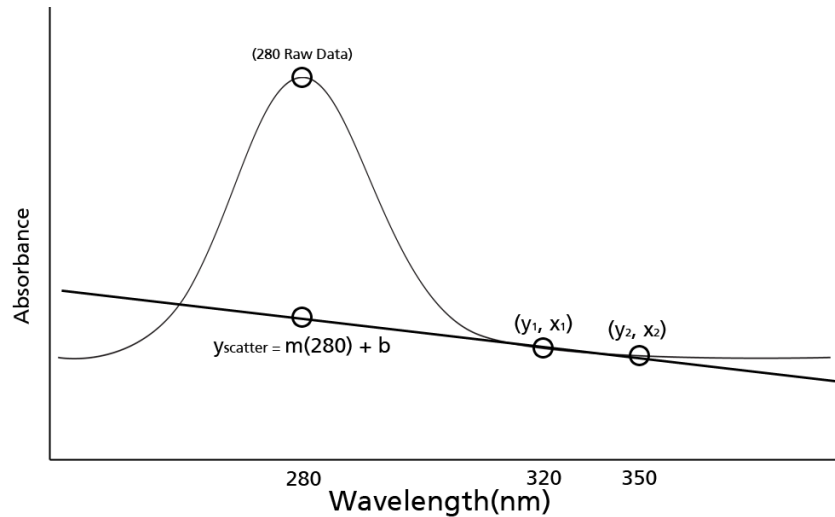

Figure 5: Data collected before Scatter Correction occurs.

Figure 5 shows the raw data taken before any scatter correction is calculated. In the above example the projected points will come from the 320nm and 350nm data points all taken at the same pathlength. The scatter contribution is determined by defining the line that passes through those two points using the equations below.

$$m = \frac{y_2 - y_1}{x_2 - x_1}$$

Where X_i and Y_i are points taken from the 320nm and 350nm wavelengths.

$$y_1 = mx_1 + b$$

After solving for the slope of the projected line, it is plugged in to solve for the y-intercept.

$$b = y_1 - mx_2$$

Once *b* has been solved for, we are able to calculate the corrected *y*-abs value by subtracting the absorbance from the scatter value from the absorbance at the method wavelength.

$$y_{Raw} - y_{Scatter} = y_{Corrected}$$
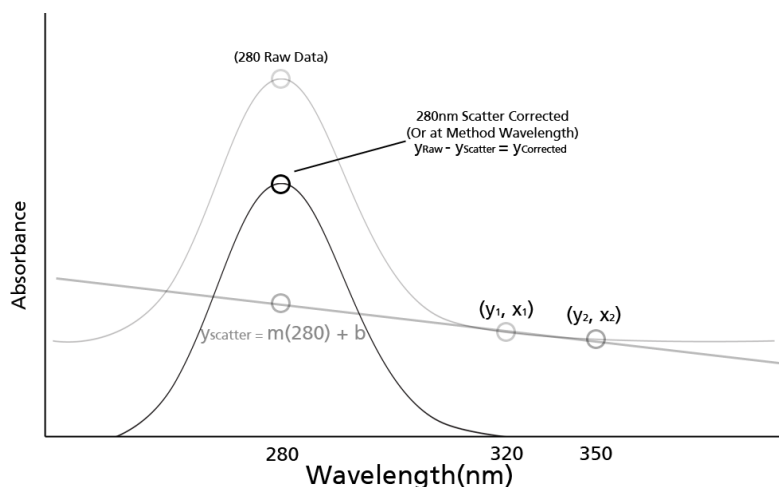$$(At\ the\ method\ wavelength)$$

Figure 6: Corrected absorbance at the Method Wavelength.

After performing the above calculation for each pathlength, the slope of the section data will remove the contribution of scatter. Figure 6 shows the Corrected Absorbance at the method wavelength after subtraction.

Scatter Correction in SoloVPE V3+

Dual Ln, Multi Ln and Scan WL Scatter Corrections

All three Scan, Dual Ln and Multi Ln are considered to be Rayleigh Scatter Correction methods. Each method will take a scan at specified pathlength(s) over a specified wavelength range to create a scatter absorbance value to correct the collected raw data. Scan WL will take an aggressive scan over the specified wavelength range. Dual Ln Scan will only take the two end data points of the specified wavelength range and Multi Ln will collect data at every 5 nm. The method for Multi Ln scatter correction is also described in the USP <1057>.

In each case the algorithm to project the scatter and then correct the raw data is the same aside for the amount of data used in the algorithm. The calculation begins by taking the Log of the raw WL data and the Log of the raw Absorbance Data and then creating a Log vs Log plot.

$$Ln(raw\ WL), Ln(raw\ Abs)$$

RAW DATA SCAN

**LN(ABSORBANCE) VS LN(WAVELENGTH)**
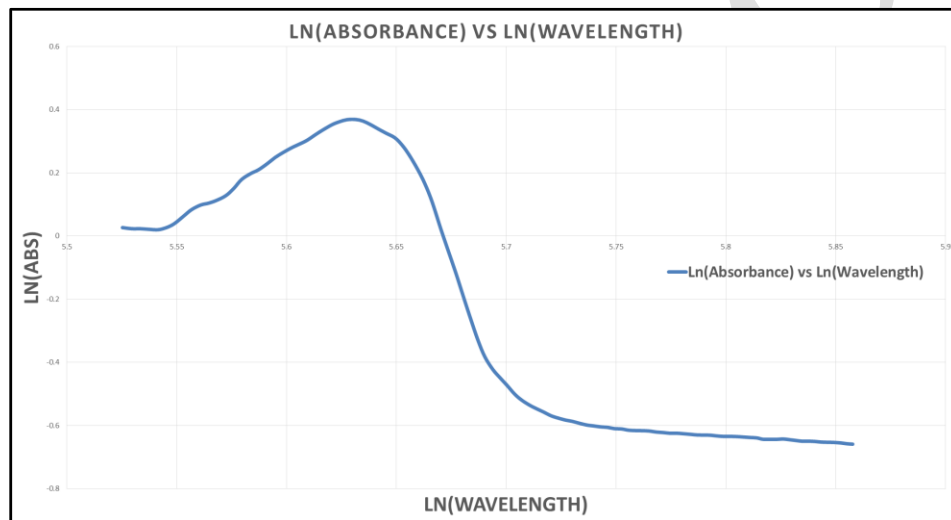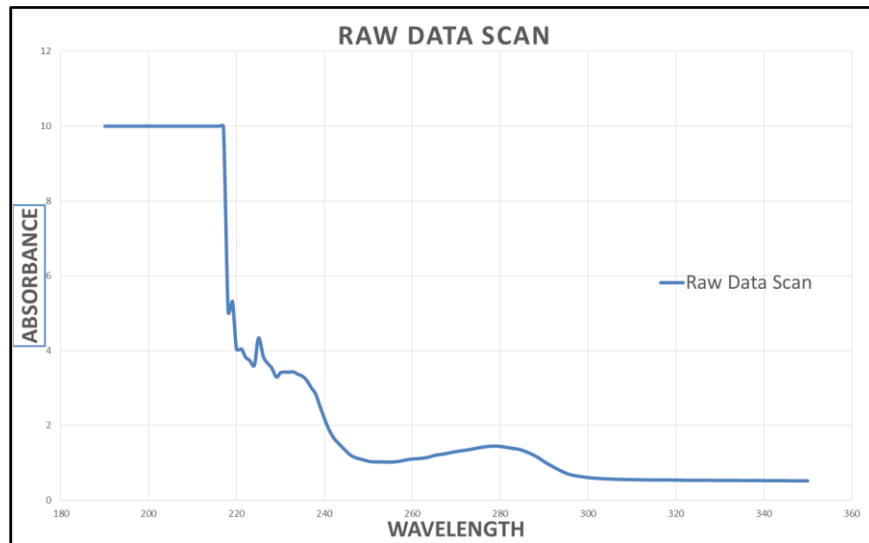
Calculate the slope of the Log vs Log scan by plotting the trend line over the scatter wavelength range. After which the Slope Equation may be used to calculate the Scatter Contribution or Optical Density (Ln(Scatter OD)). Where x is the Ln(Wavelength).

$$y = mx + b$$
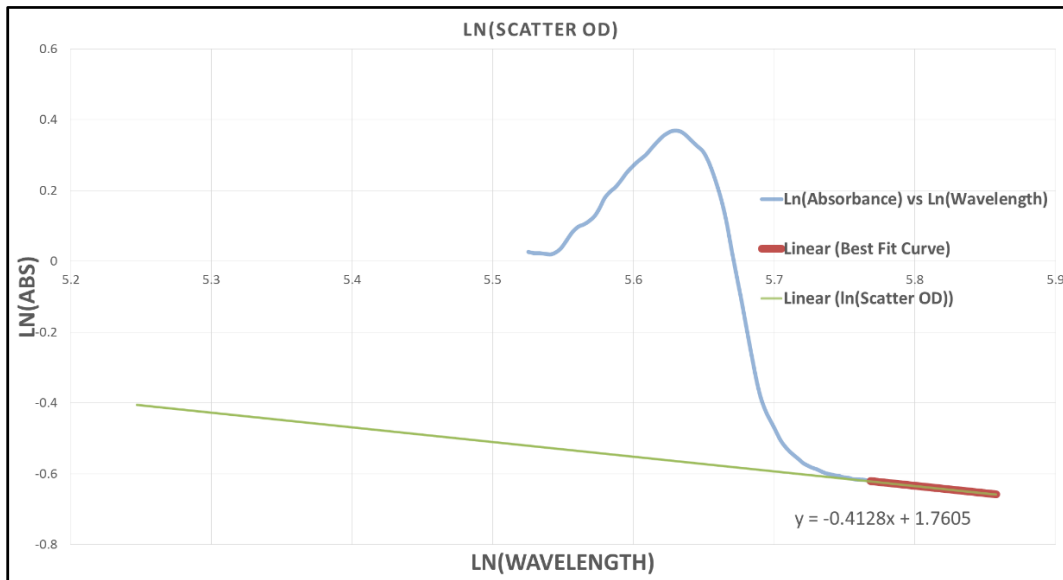
The Scatter Contribution is then converted back to Absorbance by taking the inverse log. The projected Absorbance is then subtracted from the raw data to plot the corrected Absorbance value(s). For Dual and Multi Ln corrections, the Scan would use less data to perform the above calculations.

$$e^{\ln(Scatter\ OD)}$$
$$(Raw\ Data\ Scan) - (Corrected\ _{Absorbance})$$
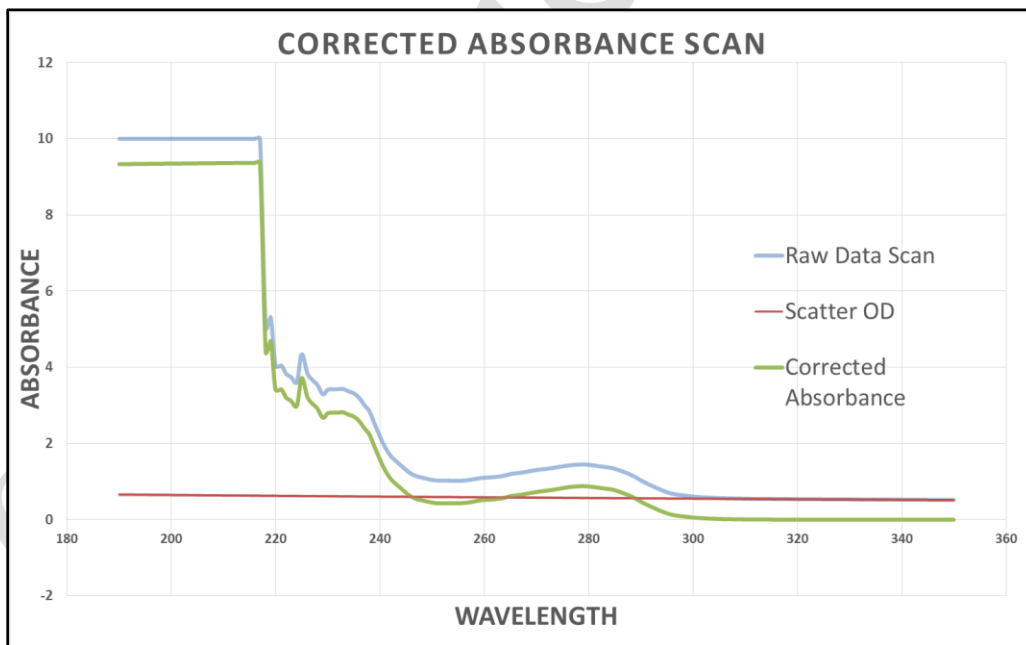
Dual Discrete Scatter Correction

Dual Discrete scatter correction is also known as the Mach and Middaugh approximation and is best described in the journal *Measuring Protein Spectra in the Presence of Light Scatter*[1]. The equation for the estimation is

$$As, 280 = 10^{2.5 log A_{320} - 1.5 log A_{350}}$$

Document Info:

Revision History				
Rev	**Date**	**Changes**		**Initials**
00	2014-06-16	Initial Release		GM
01	2016-10-12	Minor Grammar Edits Addition of V3 Scatter Methods		GM
02	2018-02-27	Included additional Slope references		JF

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[1] Mach, H., and Middaugh, C. R., 1993, Measuring Protein Spectra in the Presence of Light Scattering, *BioTechniques*, v. 15 No. 2, p. 240-242