APPLICATION NOTE

C TECHNOLOGIES, INC.

Solo VPE

Variable.Pathlength.Extension

Characterizing Gold Nanoparticle Detection Using Slope Spectroscopy

Application Note

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The SoloVPE System is the technological innovation behind the Slope Spectroscopy® technique. Unlike traditional UV-Vis methods that rely on a single Absolute Absorbance value, Slope Spectroscopy methods use Section Data (Absorbance vs. Pathlength Data) to determine a slope value for quantitation of sample concentration using the Slope Spectroscopy Equation (m = εc) which is derived from the Beer-Lambert Law. The variable pathlength technology in the SoloVPE allows even highly concentrated samples to be measured usually without dilution and baseline correction. The internationally patented SoloVPE is being deployed throughout global organizations allowing them to realize increased accuracy while saving time and money.

Summary/Abstract:

Virus-removal filters are a key component in many bio-pharmaceutical manufacturing processes. The gold particle test is a common and effective way to confirm that the filter pore size has remained unchanged throughout the process. Spectroscopic analysis of concentrated gold particle solutions pre and post filtration is the preferred method to validate filter and process integrity. To avoid the risks of passing ineffective filters and the costs of false positive results, the measurement technique used must be robust, accurate and highly sensitive. Since the post-filtration absorbance levels are in the range of 0.01 Abs on a 1 cm pathlength measurement, this verification technique can benefit from the dynamic range made possible by the variable pathlength technology in the SoloVPE System. A new Slope Spectroscopy® based method for filter verification offers time & cost savings while delivery the increased confidence associated with a slope based measurement.

Apparatus / Equipment

Section Data Plot
(Absorbance vs. Pathlength)
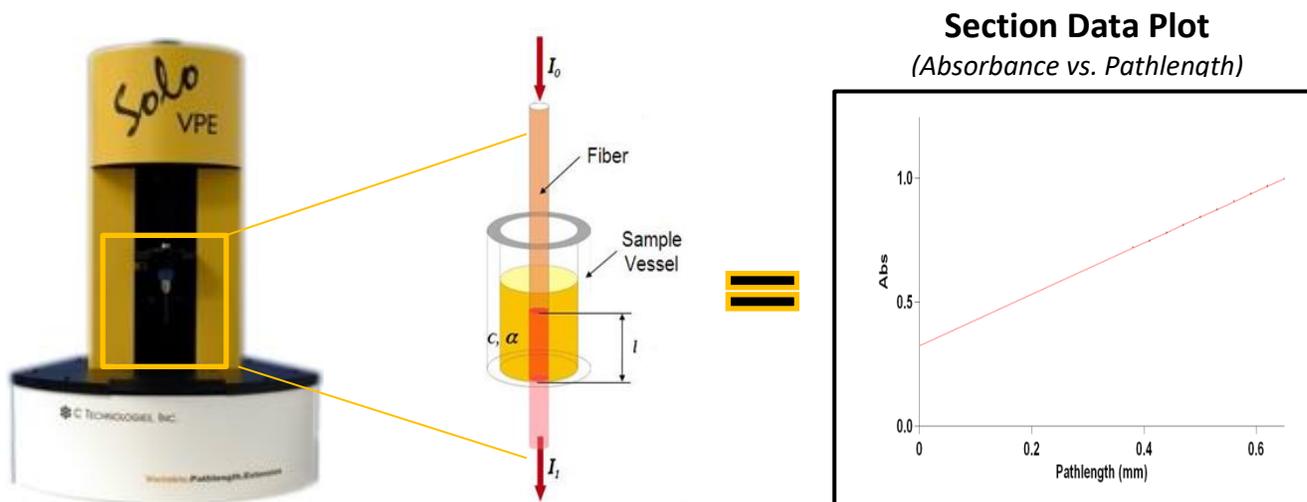
Method & Results

The UV-Vis absorption spectrum of the red solution shows the spectral band derived from the gold nanoparticles at 526 nm. Gold Particle Testing (GPT) is the most precise user-conducted integrity test available. It serves to confirm filter quality (pore size distribution) after filtration. The test utilizes a solution of colloidal gold particles similar in size to the viruses targeted by the filter. The gold particles are then filtered out of solution by the membrane during the course of the test. The filtered material is then tested for absorbance to ensure the pore size of the filter has not changed and the filter has performed as expected.

An UV-Vis method for testing the integrity of the filter membrane is comprised of filtering a solution through a membrane and analyzing the filtered solution to ensure that the filter has effectively removed the unwanted material. This application note discusses a *Slope Spectroscopy®* based test method in which the undiluted sample will be measured using the SoloVPE to generate Section (Absorbance vs. Pathlength) Data. Linear regression analysis of the Section Data will yield a slope value which can be used for quantitation. There are benefits to using a slope value based upon absorbance readings at various pathlengths over a single Absolute Absorbance value. The variable pathlength technology of the SoloVPE dynamically adjusts the measurement pathlengths to suit the sample being measured, this ensure that optimal readings are made. Additionally, the use of a multiple data points provides verification that the readings are compliant with the Beer-Lambert Law. The context of the slope measurement inherently provides a greater level of confidence that a single absorbance data point.

For this application note, we have used the *SoloVPE Variable Pathlength* system, which incorporates an Agilent Cary 60 UV-Vis spectrophotometer, to quantitate the concentration of the gold particle solution in a series of samples. As is the case for most SoloVPE methods, the required sample volume is related to the sample. Higher concentration samples require less volume than those that are more dilute. This constraint exists because the light travels vertically through the sample when using the SoloVPE and the height of the liquid in the sample vessel must exceed the maximum pathlength to be measured. The SoloVPE System accommodates a variety of

vessel styles, including disposable UV plastic vessels, to allow users to minimize the sample volume required. The measurements on the neat GPT standard solution required a volume less than 20 µl. For the diluted sample measurements the volume requirements increased to 100 µl for the 1 in 10 Dilution and 2 ml for the 1 in 100 and the 1 in 1000 dilution.

Analysis

How is Baseline/Buffer Correction eliminated? One of the most unique advantages of Slope Spectroscopy methods is the fact that baseline correction can frequently be eliminated. The condition for which this is true is simple to explain and easy to verify using the SoloVPE System. Baseline Correction is not required when the absorbance response of the buffer media does not display significant pathlength dependence. Stated another way, if absorbance measurements are made on the buffer media at different pathlengths, whether they be 0.010 mm, 1 mm, 8.4 mm of 15 mm etc., the absorbance value reported will not appear to be a function of pathlength, essentially the absorbance result variation will be attributable only to instrument variation. This can be easily verified using the SoloVPE and its variable pathlength technology because it is designed to make measurements at a different pathlengths. The buffer media can be run using the Quick Slope function in the SoloVPE software which is designed to rapidly and automatically measure Section Data to generate a slope value, where the slope value is the numeric measure of change in absorbance over the change in pathlength. When this value is close to zero, the Section plot will be a horizontal line and baseline correction is probably not required.

This condition was verified for the method proposed in this application note by measuring the standard buffer material used with the Gold Particle solutions. The resultant SoloVPE Quick Slope data showed that the absorbance response of the buffer was virtually unchanged when measured between 0.005 mm and 5.000 mm, corresponding to a slope a slope value very close to zero. Based upon this result, it can be concluded that baseline correction was not required for the slope measurements of the gold particle solutions.
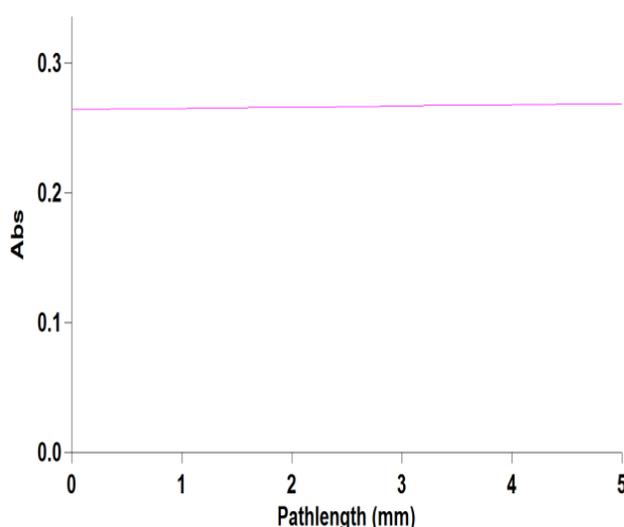
Regression Curve Name: SDS Blank @ 526.00nm
Start Pathlength (mm):   1.400
Stop Pathlength (mm):   5.000
Correlation Coefficient: 0.981323
Regression Equation: 0.00096 x PL & 0.26416
Regression Intercept (Abs):   0.26416

**SoloVPE Regression Slope (Abs/mm):   0.00096**

Since there is no Slope contribution (measurable pathlength dependence) at the wavelength of interest (526nm) there is no contribution from the buffer/blank. Therefore,

No Baseline Correction Required

Sample Measurement: Traditional abs measurements using a single abs value in a fixed 10mm cell have been diluted to fit within the linear range of the spectrophotometer. The issues with this measurement are the introduction error and additional measurement/prep time into the experiment. The SoloVPE is capable of measuring the neat sample of the gold particle standard in order to accurately verify concentration. The

Sample : Asahi Kasei Standard (neat)
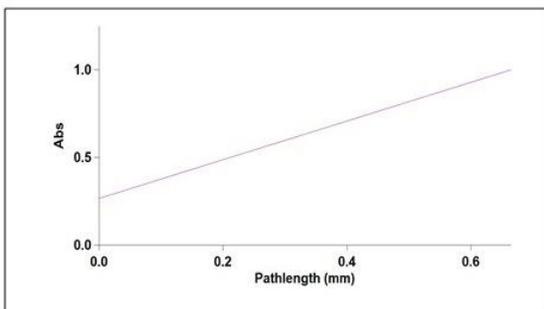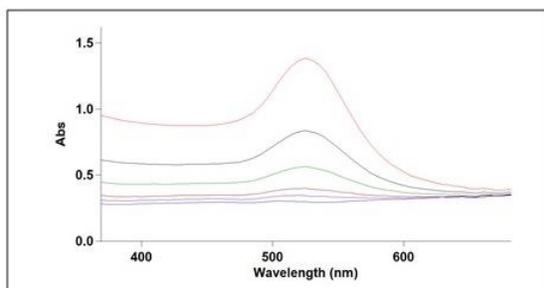
Pathlength Range
.005mm to .500mm

Linear range for sample (Beer's Law)
no dilution required

Regression Curve Name:	Asahi Kasei Std 1 @ 526.00nm
Start Pathlength (mm):	0.350
Stop Pathlength (mm):	0.665
Correlation Coefficient:	0.999991
Regression Equation:	1.09097 x PL & 0.26668
Regression Intercept (Abs):	0.26668

SoloVPE Regression Slope: 1.09097

Asahi Kasei COA Reported Abs Value: 10.820
= 1.082 (abs/mm)

accuracy in the measurement will be represented by the number of data points in the measurement and the R^2 value of the Slope regression line.

Measurements of Dilution Samples: Detection limitations for this test are crucial to be able to quantify what comes before and after the filter. The neat standard was diluted three times to simulate the types of expected measurements one can expect at this stage of particle detection. As you will see the SoloVPE is able to accurately measure down to a 1 in 1000 dilution while still maintaining an R^2 value of at least .999.

Discussion

The SoloVPE can be used to determine linearity within Beer's Law for the sample. This has an implication on stability at all phases of sample analysis. By measuring the slope value of the sample at varying concentrations, a

SoloVPE Method Linearity Summary

$y = 1.00797x + 0.00032$
$R^2 = 1.00000$

◆ Series1
— Linear (Series1)
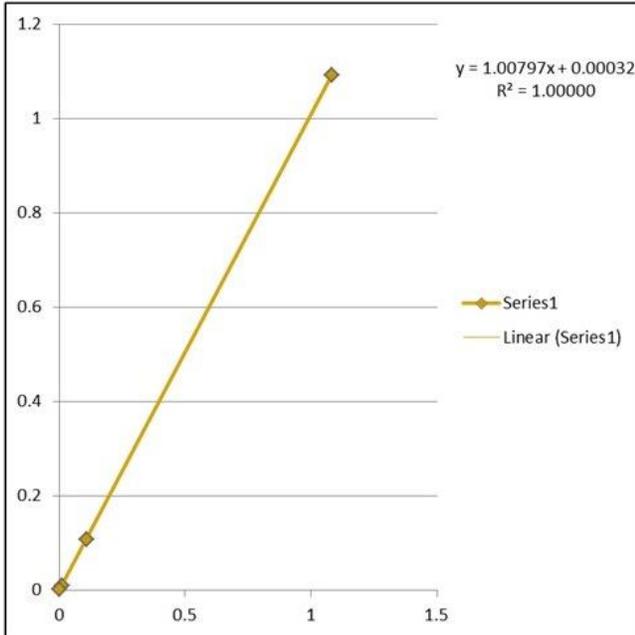
Current method based on serial assay shows nearly identical linearity

Since neat sample can be measured without dilution and samples prepared down to a 1 in 1000 dilution can be measured. It can be assured that sample traces measured after passing through the filter will be able to be quantified and confirmed if present or not.
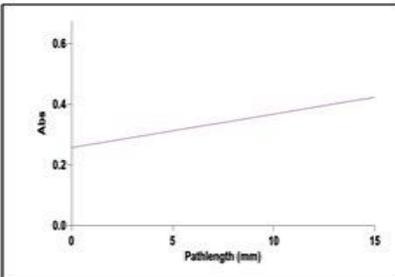
1 in 10 Dilution
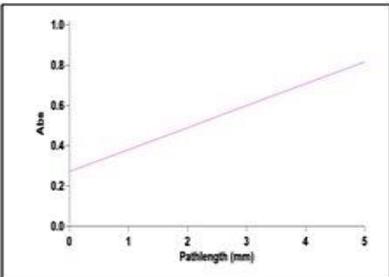
Regression Curve Name: std 1 to 10 dilution
Start Pathlength (mm):   1.400
Stop Pathlength (mm):   5.000
Correlation Coefficient:
0.999998
Regression Equation:
0.10910 x PL & 0.27164
Regression Intercept (Abs):
0.27164

SoloVPE Regression Slope (Abs/mm): 0.10910

Asahi Kasei 1 in 10 Dilution
Calculated Value: 0.109

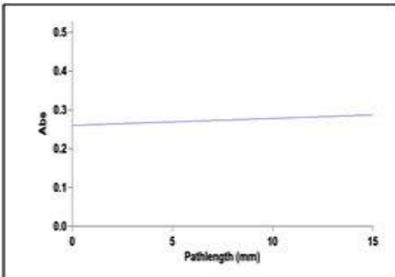### 1 in 100 Dilution

Regression Curve Name: std 1 to 100 dilution
Start Pathlength (mm):   4.200
Stop Pathlength (mm):   12.600
Correlation Coefficient:
0.999965
Regression Equation:
0.01109 x PL & 0.25694
Regression Intercept (Abs):
0.25694

SoloVPE Regression Slope (Abs/mm): 0.01109

Asahi Kasei 1 in 100 Dilution
Calculated Value: 0.0109

1 in 1000 Dilution

Regression Curve Name: std 1 to 1000 dilution
Start Pathlength (mm):   4.200
Stop Pathlength (mm):   13.800
Correlation Coefficient:
0.999695
Regression Equation:
0.00179 x PL & 0.26003
Regression Intercept (Abs):
0.26003

SoloVPE Regression Slope (Abs/mm): 0.00179

Asahi Kasei 1 in 1000 Dilution
Calculated Value: 0.00109

direct comparison of the sample to the ideal can be created.

Conclusions

Studies are ongoing to determine how this new parameter can be utilized to understand the stability of gold particle testing using the SoloVPE.

The SoloVPE is an effective tool for rapid acquisition of accurate Slope or Concentration values. This is a valuable asset for the formulation scientist. In addition the SoloVPE can be used to clearly understand the product at a basic level using a simple and quick slope based protocol to determine "delta absorbance". Therefore, the SoloVPE is an essential tool for studies from early stage to late stage. It allows for concentration and absorbance (Slope) measurement at the widest range.

References

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2. Ramsey Shanbaky Engineering/Sales C Technologies Inc. rshanbaky@ctechnologiesinc.com
3. Joe Lepore Technology Manager Glaxo Smith Kline King of Prussia, PA