APPLICATION NOTE

C TECHNOLOGIES, INC.

Solo VPE

Variable.Pathlength.Extension

Characterization of Gold Nanoparticle Detection Using Slope Spectroscopy *(SoloVPE Variable Pathlength UV)*

Author(s)

Joe Ferraiolo
Senior Product Specialist
C Technologies Inc.
jferraiolo@ctechnologiesinc.com
www.solovpe.com

Ramsey Shanbaky
Engineer/Sales
C Technologies Inc.
rshanbaky@ctechnologiesinc.com
www.solovpe.com

Application Note

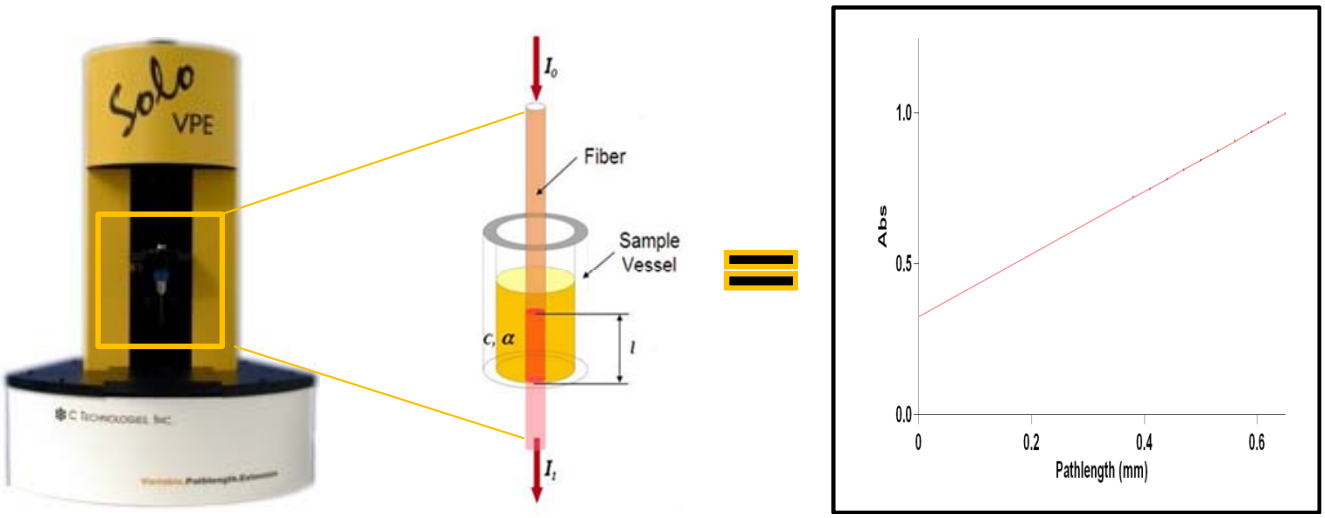
The SoloVPE and Slope Spectroscopy offer a new method of *Slope* measurement that ensures rapid, accurate and reproducible results. No longer are scientists bound to dilution factors and fixed path lengths. The SoloVPE uses a highly repeatable linear stage in the measurement of sample concentration and can quickly (<1min) and accurately find the linear range of Beer's Law for this sample. It will read between 5 and 10 data points through the sample and plot the absorbance vs. path length.

Summary/Abstract:

Virus-Removal filters are a key component of the bio-pharmaceutical manufacturing process. The gold particle test is an effective way to ensure the pore size of the filter has remained unchanged throughout the course of processing. Spectroscopic measurement of gold particle solution prior to and post filtration is a means to validate filter integrity post-use. An accurate and highly sensitive measurement of the spectroscopic solution, are important factors to guarantee no false positives or passing ineffective filters.

Concentrated gold particle solution is measured prior to filtration. After filtration the absorbance value at 10mm must be accurately read to values below .01 Abs to ensure filter integrity. The SoloVPE's slope sensitivity of .0005 Abs/mm and linear regression values provide the scientist with the confidence and accuracy of a slope based measurement for filter validation.

Apparatus / Equipment



Method & Results

The UV-Vis absorption spectrum of the red solution showed the spectral band derived from the gold nanoparticles at 526nm. Gold Particle Testing (GPT) is the most precise user-conducted integrity test on the market. It serves to reconfirm filter quality (pore size distribution) after filtration.

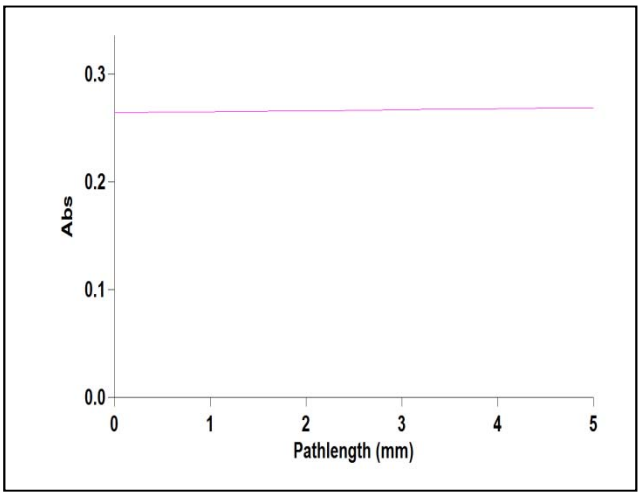
In each test, a solution of colloidal gold particles, similar in size to the target viruses, is introduced into the filter. The gold particles are then filtered out of solution by the membrane during the course of the test. The filtered material is then tested for absorbance to ensure the pore size of the filter has not changed and the filter has performed as expected.

An UV method of testing the integrity of the filter membrane is comprised of filtering a solution through a membrane and analyzed to ensure that the filter has effectively removed the unwanted material. The testing of an undiluted sample will yield a slope measurement within the linear regression of Beer's Law instead of a single absorbance value in a standard spectrophotometer that cannot be distinguished from the noise of the instrument.

For this test, the *SoloVPE Variable Pathlength UV* system used a Cary 60 Spectrophotometer to determine a quantitative level of gold particle solution using serial dilutions. Sample volume is determined by the concentration of the sample. For higher concentrations, the sample volume required for analysis is less due to smaller pathlengths used to plot the linear region of Beer's Law. In this analysis the neat GPT standard only required between 10/20ul. In the case of lower concentration samples, more volume is required in order to achieve linearity as longer pathlengths must be utilized. Samples 1 in 100 and 1000 required 2ml of sample as opposed to sample 1 in 10 which required 100ul.

Analysis

Buffer/Baseline Correction: Baseline Correction may not be required when the absorbance of the buffer does not display significant pathlength dependence. The way to determine whether baseline correction is required is to perform a Quick Slope measurement on your buffer media with no active in it. A Quick Slope result close to zero suggests that Baseline Correction may not be required.



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

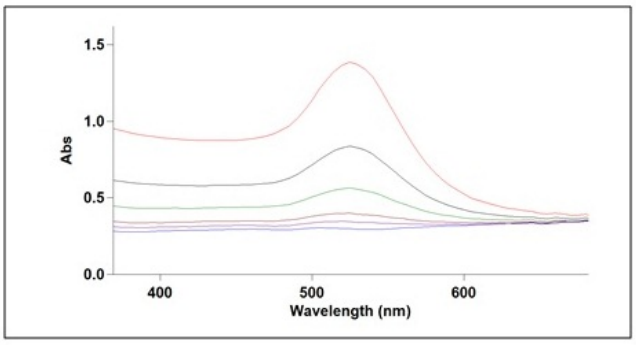
SoloVPE Regression Slope (Abs/mm): 0.00096

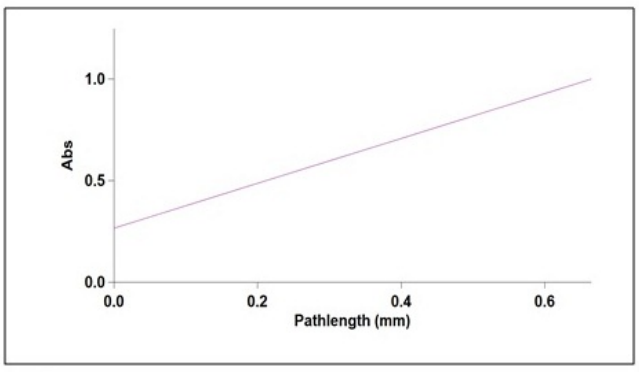
Proof that since there is no Slope contribution at wavelength of interest (526nm) there can be no contribution from buffer/blank

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 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

Sample : Asahi Kasei Standard (neat)





Pathlength Range .005mm to .500mm

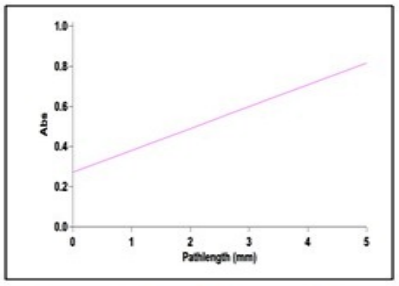
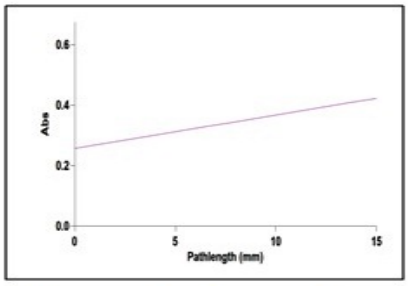
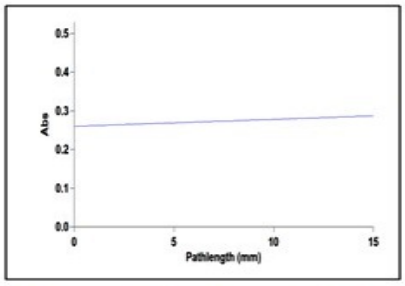
Linear range for sample (Beer's Law)
no dilution required

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

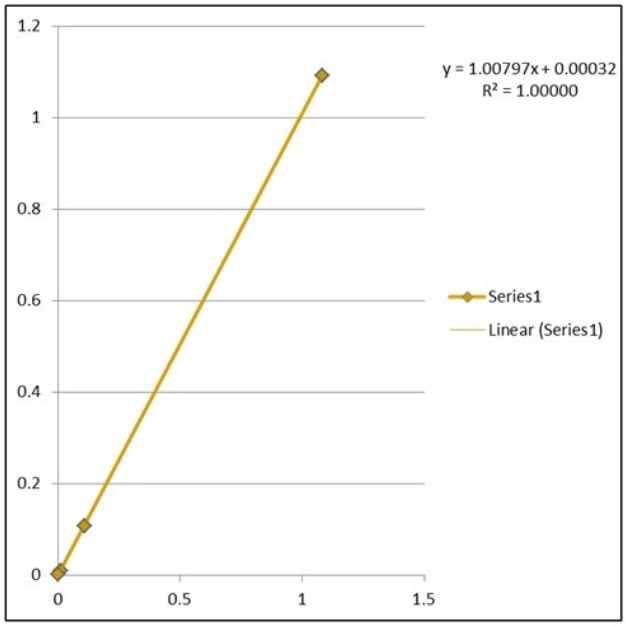
SoloVPE Regression Slope: 1.09097

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

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.

1 in 10 Dilution	1 in 100 Dilution	1 in 1000 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**	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**	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**

SoloVPE Method Linearity Summary



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.

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 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

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