

Characterizing Gold Nanoparticle Detection Using the Slope Spectroscopy® Method

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Abstract

The CTech™ SoloVPE® System is the technological innovation behind the Slope Spectroscopy® method. Unlike traditional ultraviolet-visible (UV-Vis) methods that rely on a single absolute absorbance value, the SoloVPE System uses section data (absorbance vs. pathlength) to determine a slope value for quantitation of sample concentration. The slope value derives from the Slope Spectroscopy equation ($m = \epsilon c$), which is a manipulation of the Beer-Lambert's law. The variable pathlength technology in the system allows even highly concentrated samples to be measured, usually without dilution and baseline correction. The internationally patented SoloVPE System is deployed throughout multiple global organizations, allowing them to realize increased accuracy while saving time and money.

Summary

Virus-removal filters are a key component in many biopharmaceutical manufacturing processes. The gold particle test (GPT) effectively confirms that the filter pore size has remained unchanged throughout the process. Spectroscopic analysis of concentrated pre- and post-filtration gold particle solutions is the preferred method to validate filter and process integrity. To avoid the risks of ineffective filters passing and the costs associated with 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 benefits from the dynamic range made possible by the variable pathlength technology in the SoloVPE System.

Apparatus/Equipment

Figure 1. Variable Pathlength Technology (VPT)

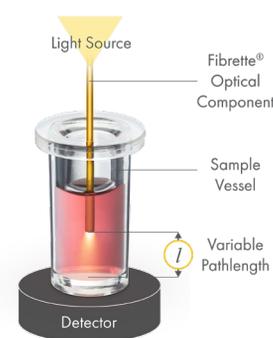
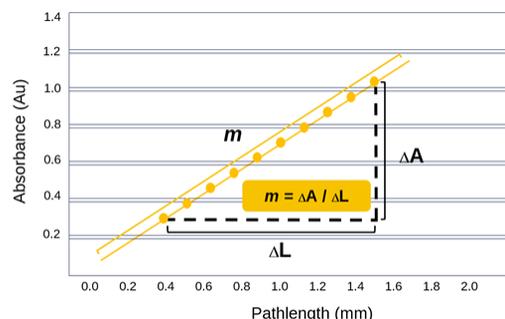


Figure 2. Variable pathlength UV-Vis absorbance reading.



Method and Results

The UV-Vis absorption spectrum of the red solution shows the spectral band derived from the gold nanoparticles at 526 nm, 530 nm, or 520 nm. The lambda max is dependent on the filter type that is being utilized. For this application note, the AGP-HA20 gold particle solution was used, which has a lambda max of 526 nm. The gold particle test 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.

This application note discusses a Slope Spectroscopy-based test method where the undiluted sample yield a slope value based on the SoloVPE System's linear regression analysis. The variable pathlength technology of the system dynamically adjusts the measurement pathlengths to suit the sample being measured, which ensures that optimal readings are made. Additionally, the multiple data points provide verification that the readings are compliant with the Beer-Lambert's law. The context of the slope measurement inherently provides a greater level of confidence than a single absorbance data point.

The system hardware and software offer several benefits that make it the optimal UV method for GPT. The system ensures that all measurements are accurate by requiring that samples have a R^2 value of 0.999 or higher. Also, the repeat modes within the software allow the system to measure multiple collections of the same sample. A %RSD of $\pm 2\%$ is required to ensure that the measured samples are repeatable. The software automatically reports the %RSD and average when the repeat modes are activated. The software is also capable of automatically calculating and reporting the absorbance ratio criteria. Users are no longer bound to manually calculating values after the measurement has been taken.

As is the case for most SoloVPE System methods, the required sample volume is related to the concentration of 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 system and the height of the liquid in the sample vessel must exceed the maximum pathlength to be measured. The system accommodates a variety of vessel types to allow users to minimize the

sample volume required. Both neat and diluted GPT samples were measured by the system. The measurements by the neat GPT standard solution required a volume less than 20 μl while 1 in 10 dilutions required 100 μl of volume. A volume of 2.5 ml was required for 1 in 100 dilutions and 1 in 1000 dilutions.

Analysis

How is baseline/buffer correction eliminated?

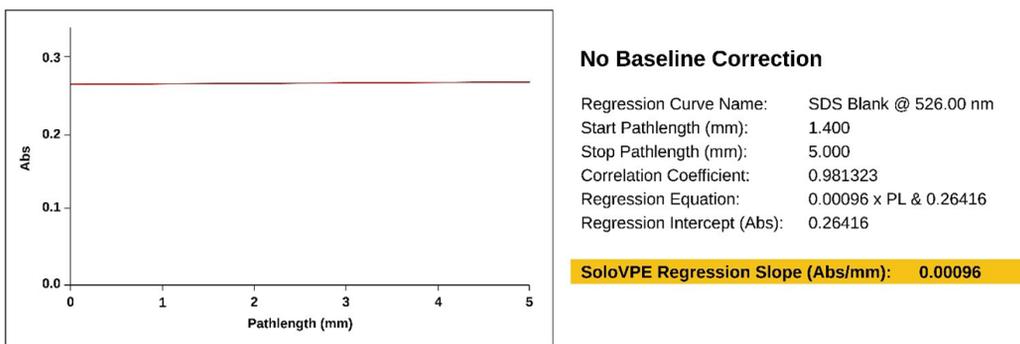
One of the unique advantages of the Slope Spectroscopy method is the fact that baseline correction can frequently be eliminated. Baseline correction is not required when the absorbance response of the buffer media does not display significant pathlength dependence. Stated another way, if absorbance measurements are made on the buffer media at different pathlengths (e.g., 0.010 mm, 1 mm, 8.4 mm, or 15 mm), 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 system software. The buffer media can be run using the Quick Slope function in the system software which is designed to rapidly and automatically measure section data to generate a slope value. When this value is close to zero (<0.01), 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 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 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 (see Figure 3).

How is baseline/buffer correction eliminated?

Traditional UV measurements using a single absorbance value in a fixed 10 mm cell have been diluted to fit within the linear range of the spectrophotometer. The issues with this measurement are the dilution errors and the

Figure 3. Baseline correction data



additional measurement/prep time into the experiment. The SoloVPE System is capable of measuring the neat gold particle standard in order to accurately verify absorbance (see Figure 4). The accuracy in the measurement will be represented by the number of data points and the R^2 value of the slope regression line.

Measurements of Diluted Samples

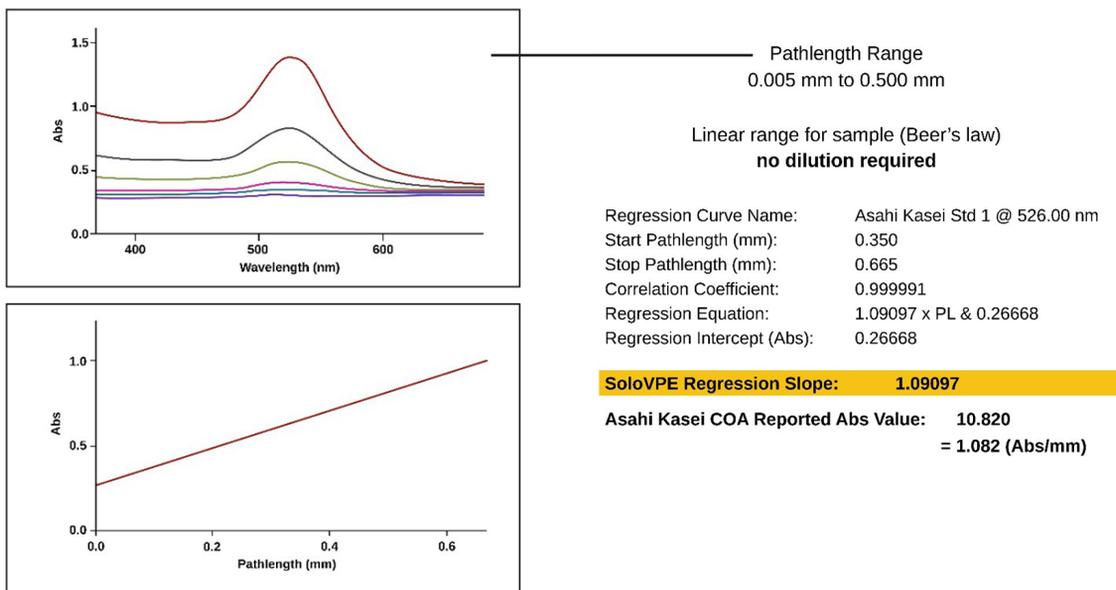
Detection limitations for this test are crucial to be able to quantify what comes before and after the filter. The neat standard was diluted three times to simulate the types of expected measurements one can expect at this stage

of particle detection. By measuring the slope value of the sample at varying concentrations, a direct comparison of the sample to the ideal can be created.

Discussion

The SoloVPE System can be used to determine linearity within Beer-Lambert’s law for the sample. As shown in Figure 5, the system is able to accurately measure down to a 1 in 1000 dilutions while still maintaining an R^2 value of at least 0.999. This has an implication on stability at all phases of sample analysis.

Figure 4. Sample - Asahi Kasei standard data (neat)

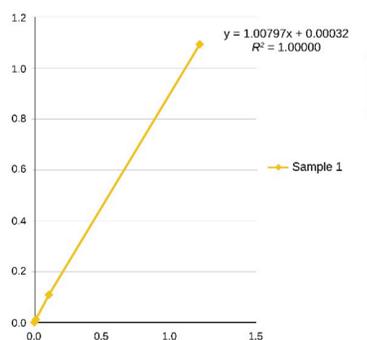


Conclusion

The CTech SoloVPE System is an effective instrument for rapid acquisition of accurate slope and concentration values. The system’s ability to accurately read neat and dilute gold particle solutions demonstrates its wide range of measurements. The system’s software makes it easy for users to automatically calculate their acceptance criteria associated with

the gold particle test. The system’s ability to acquire near-perfect linear data without the need of baseline correction, rigorous sample preparation and dilution, demonstrate its superiority over traditional UV methods. Its variable pathlength technology allows it to be the most optimal UV method for viral filtration detection.

Figure 5. Asahi Kasei serial assay data



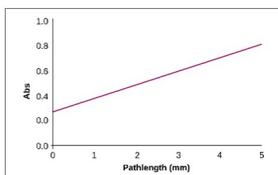
SoloVPE Method Linearity Summary

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.

1 in 10 Dilution

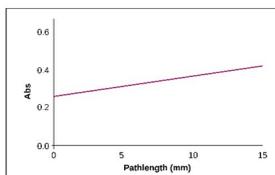


Regression
 Curve Name: Std 1 to 10 dilution
 Start Pathlength (mm): 1.400
 Stop Pathlength (mm): 5.000
 Correlation Coefficient: 0.999998
 Regression
 Equation: 0.10910 x PL & 0.27164
 Regression Intercept (Abs): 0.27164

SoloVPE Regression Slope (Abs/mm): 0.10910

Asahi Kasei 1 in 10 Dilution Calculated Value: 0.109

1 in 100 Dilution

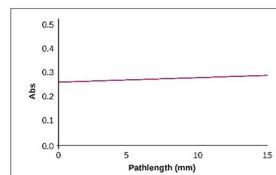


Regression
 Curve Name: Std 1 to 100 dilution
 Start Pathlength (mm): 4.200
 Stop Pathlength (mm): 12.600
 Correlation Coefficient: 0.999965
 Regression
 Equation: 0.01109 x PL & 0.25694
 Regression Intercept (Abs): 0.25694

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

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