Solo VPE

Variable.Pathlength.Extension

Polysorbate 80 UV/Vis Spectral and Slope Analysis

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Application Note

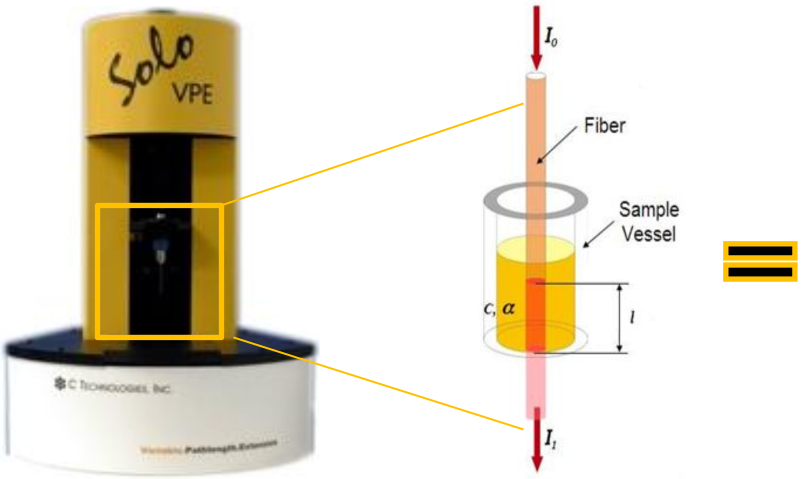
The SoloVPE and Slope Spectroscopy offer a new method of *Slope* measurement that ensures rapid, accurate and reproducible results. No longer are scientists bound to dilution factors and fixed path lengths. The SoloVPE uses a highly repeatable linear stage in the measurement of sample concentration and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

Summary/Abstract

Polysorbate 80 is used in the pharmaceutical industry as an additive to enhance solubility of non-polar compounds in formulation design during analysis. The wide UV/Vis absorbance range of Polysorbate 80 often makes it difficult to accurately quantitate pharmaceutically active compounds in solutions where the surfactant is present.

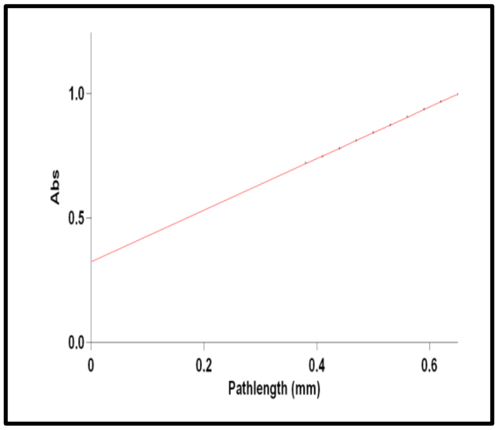
This application note will discuss compare and contrast the most commonly used methods for measuring Polysorbate 80 with a new slope based technique made possible by the variable pathlength technology of the SoloVPE System.

Apparatus / Equipment



Section Data Plot

(Absorbance vs. Pathlength)



Method & Results

Most companies are currently running several methods to determine polysorbate concentrations. Two of the most popular methods utilize solid phase extraction of polysorbate from samples and one utilizes a liquid extraction. The polysorbate is quantified from a generated standard curve.

This current method uses a standard spectrometer in a low volume covered cuvette of a path length of 1cm. Background correction is used at 690nm

Multiple assays are measured at varying concentrations

Method development is based off the compendia method (USP) for the identification of Polysorbate. The Analytical Sciences group has quantified the test using a standard curve of known concentrations.

The rules for regression ensure that all of the methods are compliant and meet ICH guidelines

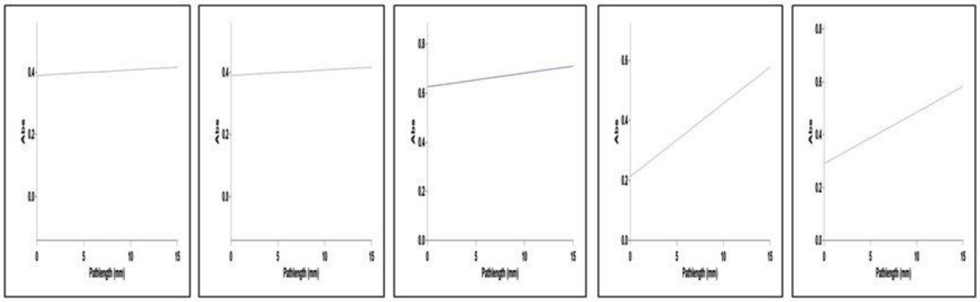
For this test, we have used the *SoloVPE Variable Pathlength UV* system with a Cary 60 Spectrophotometer to determine a quantitative level of Polysorbate 80. Sample volume is determined by the concentration of the sample. For higher concentrations, the sample volume required for analysis is less due to smaller pathlengths used to plot the linear region of Beer's Law. In this analysis the neat polysorbate stock only required between 50/100ul. In the case of lower more dilute samples, more volume is required in order to achieve linearity as longer pathlengths must be utilized.

Analysis – Traditional Method vs. Slope Method at 620 nm

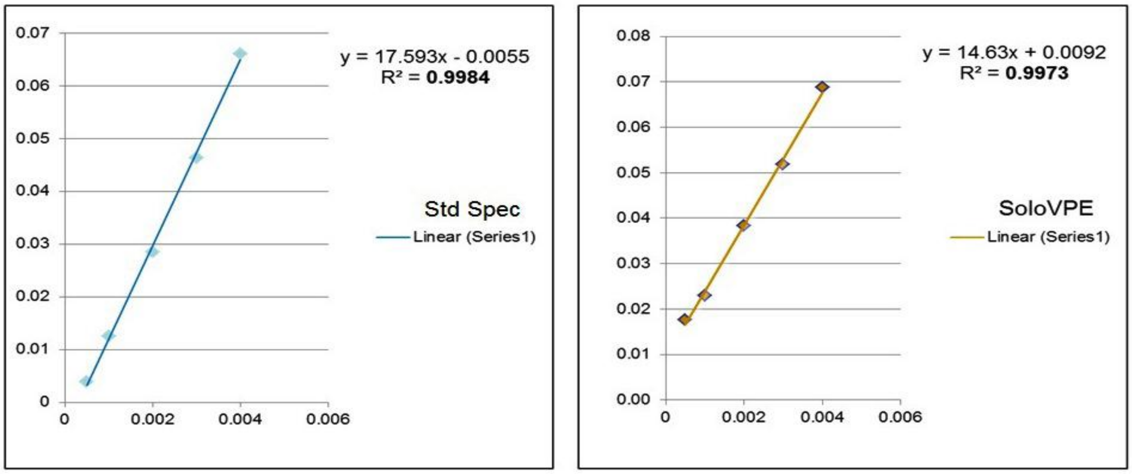
For this application note a comparison was made of the traditional executed serial assay method and a Slope Spectroscopy based method designed to mirror the current method but use slope values rather than absorbance values. The resultant standard curves, one absorbance based and one slope based show an essentially identical strength of correlation between the two methods, though the Slope Spectroscopy based method was completed more rapidly and without sample preparation.

Sample : PS 80 620nm Serial Assay Method

Expected	Std 1 : 0.0005	Std 2 : 0.001	Std 3 : 0.002	Std 4 : 0.003	Std 5 : 0.004
Std Spec	0.00389	0.01256	0.02851	0.04633	0.06603
SoloVPE	0.0177	0.0230	0.0384	0.0519	0.0688



Current Method Linearity Summary



Current method based on serial assay shows nearly identical linearity

Analysis – A Slope Spectroscopy Method for Polysorbate 80

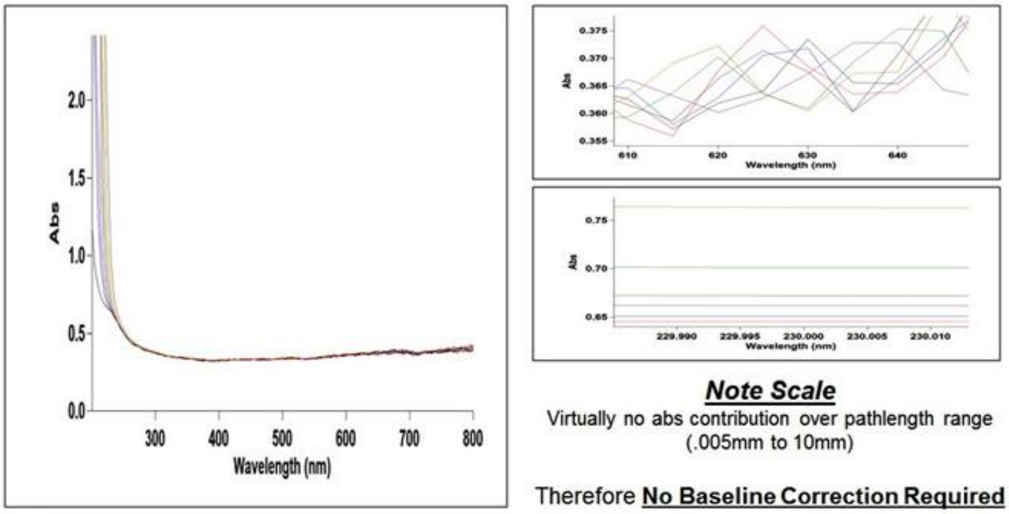
Traditional methods are developed using the tools available at the time and they frequently persist despite innovations that occur in the science due to the cost of change and inertia in the industry. The emergence of new technology and analytical techniques create opportunities for improvements but the cost of making the change must be weighed against the potential increase in accuracy, and reduction in cost. The shift to slope based concentration measurements has accelerated due in large part to the improved accuracy, the reduced time to result and the virtual elimination of sample prep. These advantages are certainly present for the proposed Slope Spectroscopy based method for the measuring of Polysorbate 80. The following method will show that neat Polysorbate 80 samples can be measured:

- Without Dilution
- Without Baseline Correction
- Without Serial Dilutions and Repeated Measurements
- Without the use of the hazardous substances Methylene Chloride or Cobalt

Leveraging the variable pathlength power of the SoloVPE system which can make move between pathlength as small as 0.005 mm and as large as 15 mm, the measurements will be made at the natural peak of 235nm rather than a the traditional used 620nm.

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.

Sample : MilliQ Water (Blank)



Note Scale

Virtually no abs contribution over pathlength range (.005mm to 10mm)

Therefore **No Baseline Correction Required**

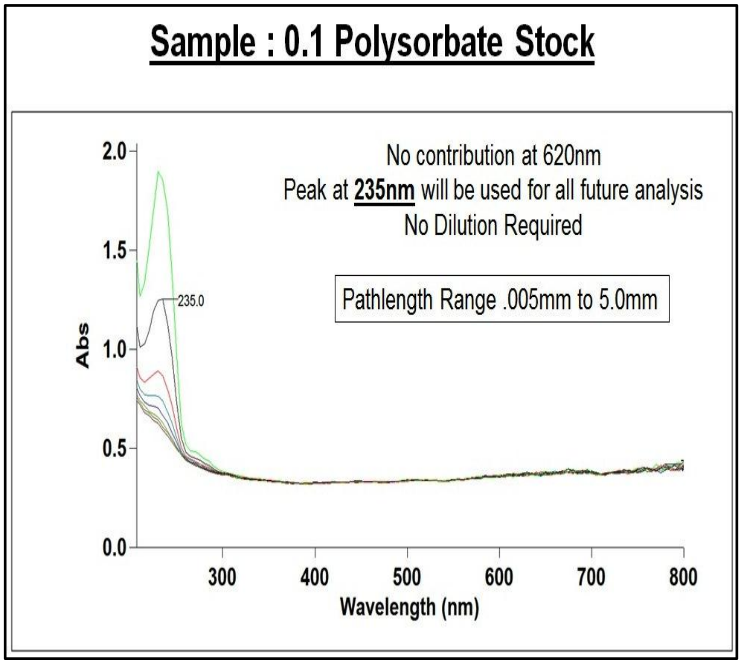
The Proposed Slope Spectroscopy Method

We will be using the SoloVPE Slope value at wavelength of interest. (All values based on Slope R2 values of at least .999)

No Baseline Correction/Blank, no Dilutions, no Serial Assays, and no Methylene Chloride or Cobalt

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

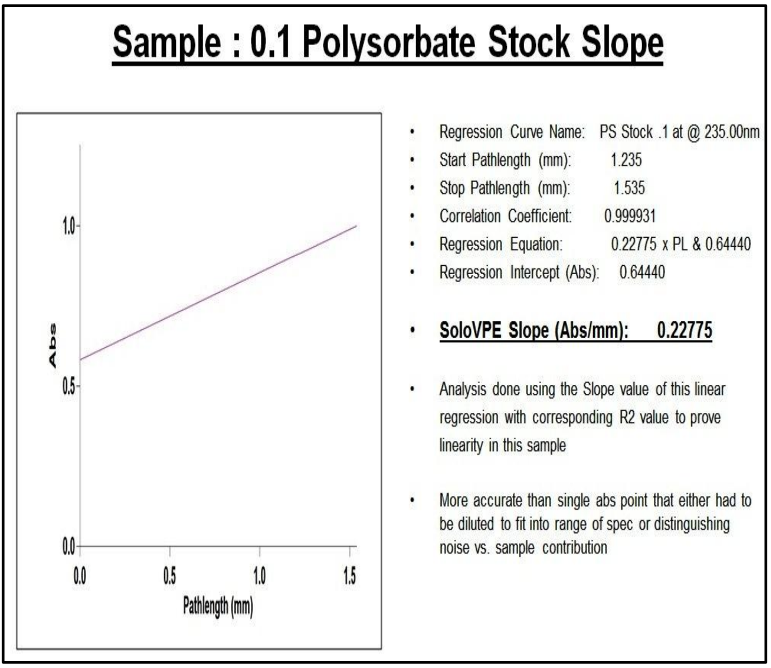
In the case of analyzing Polysorbate stock neat we will no longer be using the former wavelength of 620nm for our analysis. ***235nm*** provides a very prominent peak which will be used in the following testing using the SoloVPE.



Sample : 0.1 Polysorbate Stock

No contribution at 620nm
Peak at **235nm** will be used for all future analysis
No Dilution Required

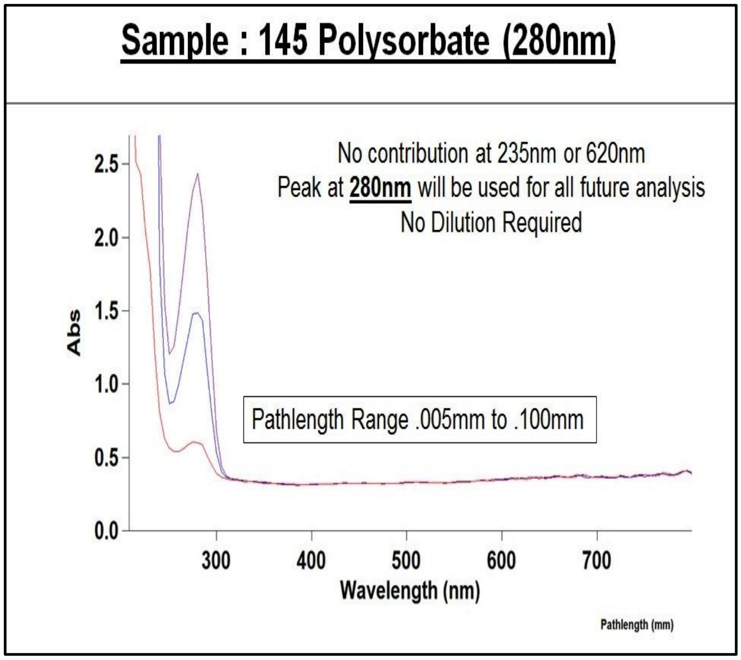
Pathlength Range .005mm to 5.0mm



Sample : 0.1 Polysorbate Stock Slope

- Regression Curve Name: PS Stock .1 at @ 235.00nm
- Start Pathlength (mm): 1.235
- Stop Pathlength (mm): 1.535
- Correlation Coefficient: 0.999931
- Regression Equation: 0.22775 x PL & 0.64440
- Regression Intercept (Abs): 0.64440
- **SoloVPE Slope (Abs/mm): 0.22775**
- Analysis done using the Slope value of this linear regression with corresponding R2 value to prove linearity in this sample
- More accurate than single abs point that either had to be diluted to fit into range of spec or distinguishing noise vs. sample contribution

Sample Measurement (combined with antibody)



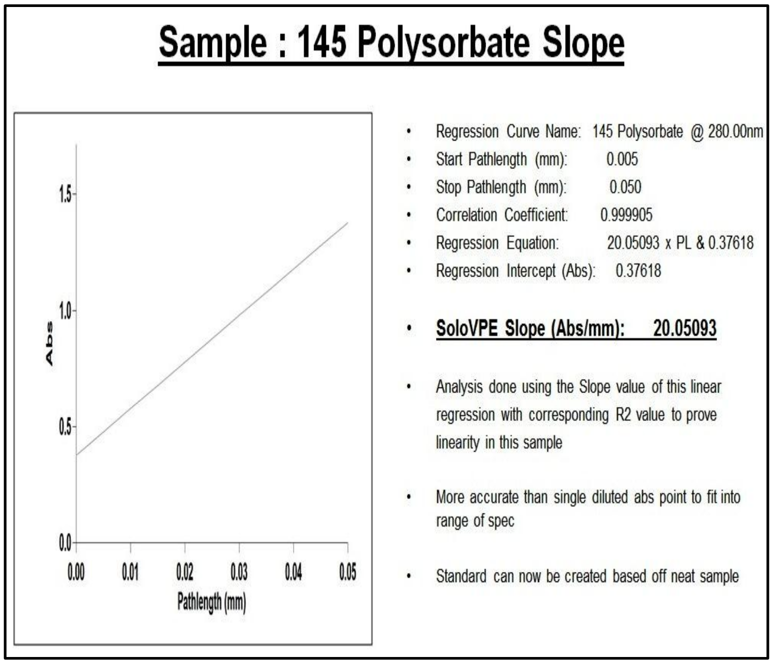
Sample : 145 Polysorbate (280nm)

No contribution at 235nm or 620nm

Peak at **280nm** will be used for all future analysis

No Dilution Required

Pathlength Range .005mm to .100mm



Sample : 145 Polysorbate Slope

- Regression Curve Name: 145 Polysorbate @ 280.00nm
- Start Pathlength (mm): 0.005
- Stop Pathlength (mm): 0.050
- Correlation Coefficient: 0.999905
- Regression Equation: 20.05093 x PL & 0.37618
- Regression Intercept (Abs): 0.37618
- **SoloVPE Slope (Abs/mm): 20.05093**
- Analysis done using the Slope value of this linear regression with corresponding R2 value to prove linearity in this sample
- More accurate than single diluted abs point to fit into range of spec
- Standard can now be created based off neat sample

Discussion/Summary

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.

Neat samples can now be measured accurately without:

- Dilution (associated time and error)
- Baseline Correction / Buffer Preparation
- Serial Dilution Assay
- Using Methylene Chloride or Cobalt (associated hazardous risks and disposal costs)

Additionally:

- Samples will require between 10ul and 50ul of volume for each experiment.
- All data acquired used between 5 to 10 data points with R^2 values of at least .999 rather than one point of extrapolated data.
- There were <u>no dilutions or baseline correction</u> required for any of the Solo VPE experiments
- Each Solo VPE experiment using Quick Slope took less than one minute per run.
- All samples had option of being recoverable after the testing.

Current Method Time:
(Extraction, Evaporation, Sample Prep, and Reading): 4.5 hours

Proposed SoloVPE Method:
(Extraction, Evaporation, and Reading): 1 Hour

Conclusions

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