Polysorbate UV-Vis Spectral and Slope Analysis: CTech™ SoloVPE® System Application Note for Polysorbate 80

Joe Ferraiolo (Repligen), Nigel Herbert (Repligen)

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 (Figure 1). The slope value derives from the Slope Spectroscopy equation ($m = \varepsilon c$), which is a manipulation of the Beer-Lambert's law. The Variable Pathlength Technology (VPT) in the system allows even highly concentrated samples to be measured, usually without dilution and baseline correction (Figure 2). The internationally patented SoloVPE System is deployed throughout multiple global organizations, allowing them to realize increased accuracy while saving time and money.

Polysorbate is a nonionic surfactant and emulsifier that is commonly used as a stabilizer in the formulation of protein drugs. It is generally prepared in water as a stock solution to meet a targeted polysorbate concentration. Formulated polysorbate concentration is crucial to support protein stability where excess amounts could result in oxidation or toxicity. The wide UV-Vis absorbance range of polysorbate often makes it difficult to accurately quantitate pharmaceutically active compounds in solutions where the surfactant is present. This application note will discuss and compare polysorbate analysis with two analytical methods; the traditional gravimetric UV-Vis method and the Slope Spectroscopy method. The limits of detection for this application are thoroughly evaluated by analyzing vendor-specific polysorbate 80 and polysorbate 20. The benefits of the Slope Spectroscopy method allow the SoloVPE System to be the most optimal method for polysorbate analysis.

Figure 1. Variable pathlength UV-Vis absorbance reading.

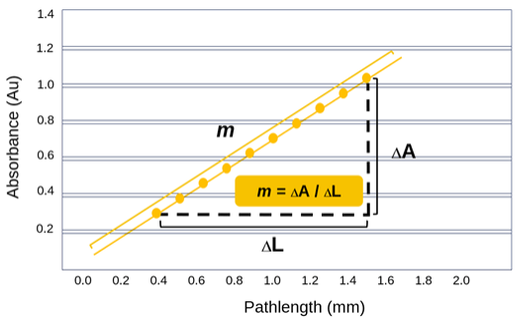
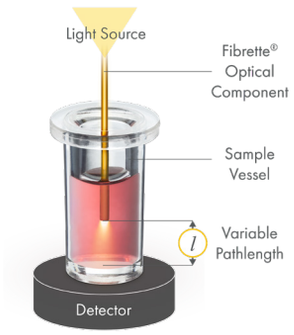


Figure 2. Variable Pathlength Technology (VPT)



Method and Results

There are two popular methods for determining polysorbate concentration; one utilizes solid phase extraction while the other utilizes liquid extraction. The polysorbate is then quantified by generating a standard curve. For this application note a serial assay of polysorbate 80 was made and evaluated using a standard

spectrophotometer. The same evaluation was mirrored with the SoloVPE System to compare the results of the traditional method with the results of the Slope Spectroscopy method.

Multiple assays of polysorbate 80 were first measured with the standard spectrophotometer. The polysorbate was formulated with water to create serial dilutions at varying concentrations. The samples were measured at 620 nm in a 1 cm cuvette and background correction was applied. The polysorbate stock solutions were then measured by the SoloVPE System for comparison. In this analysis, the neat polysorbate was measured in the small silica vessel and only required ~120 ul of volume. The more dilute samples required more volume with the large silica vessels to achieve linearity.

The limits of detection for polysorbate 80 and polysorbate 20 were analyzed by the SoloVPE System. Polysorbate 80 was provided by Sigma Aldrich, Thermo Fisher, JT Baker, and NOF Corporation while polysorbate 20 was provided by Sigma Aldrich, Thermo Fisher, and JT Baker. Serial dilutions of each brand were made and measured, starting with the brand's neat solution. The limits were determined by using the SoloVPE System's linearity requirement (R^2 ≥0.999) as the acceptance criteria. The samples were measured in triplicate at the natural peak of 235 nm. Higher concentration samples were measured with the small silica vessel while lower concentration samples were measured with the large silica vessel.

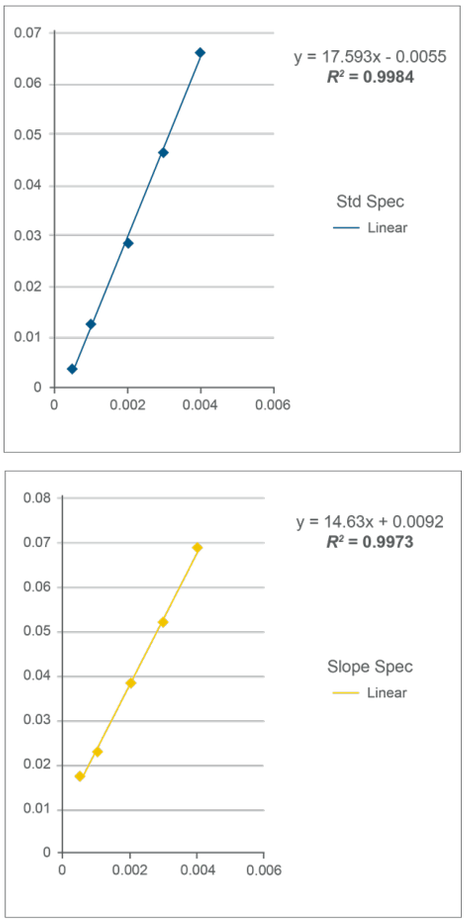
Analysis

Traditional Method vs. Slope Method

The slope-based results demonstrate near identical correlation with the traditional absorbance results shown in Table 1. The graphs in Figure 3 show identical linearity between the two analytical methods.

Traditional UV-Vis measurements using a single absorbance value in a fixed 10 mm cell have been diluted to fit within the linear range of the spectrophotometer. This introduces error into the measurement and additional measurement/prep time is required to effectively run the experiment.

Figure 3. Method linearity summary. Current method based on serial assay shows nearly identical linearity.



Polysorbate Limits of Detection

The SoloVPE System can measure a wide concentration range of polysorbate. The accuracy in the measurement will be

Table 1. PS80 serial assay

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 System	**0.0177**	**0.0230**	**0.0384**	**0.0519**	**0.0688**

represented by the number of data points and the R^2 value of the slope regression line. Various concentrations of PS20 and PS80 were measured to determine limits of detection.

Figure 4 demonstrates the upper and lower limits of each polysorbate 80 brand. Low concentration method development was required for most samples that generated a slope less than 0.01 Abs/mm. Overall, the SoloVPE System can accurately measure between ~0.0002% and 25% PS80.

Figure 4. Method linearity summary.

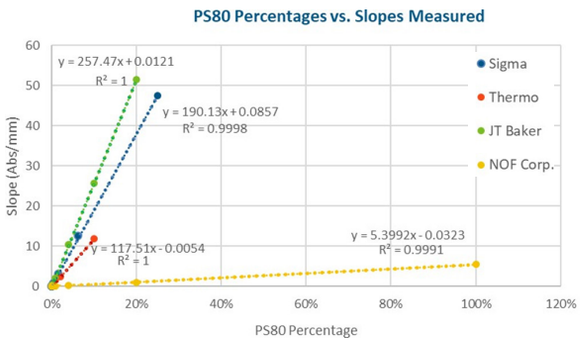
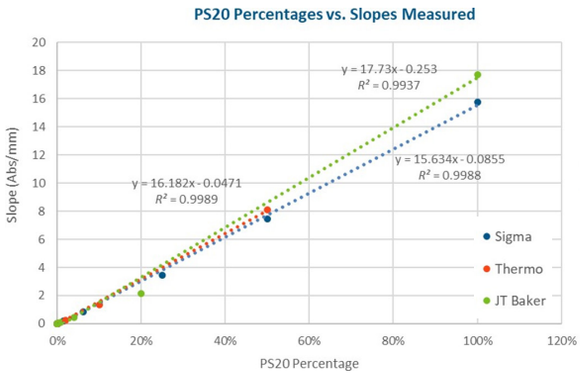


Figure 5 demonstrates the upper and lower limits of each polysorbate 20 brand. Low concentration method development was required for most samples that generated a slope less than 0.01 Abs/mm. Overall, the SoloVPE System can accurately measure between ~0.01% and 100% PS20. The PS20 demonstrated better correlation between the brands when compared to the PS80.

Figure 5. PS20 stock solution limit of detection.



Polysorbate was also formulated with BSA to determine the protein limit of detection. BSA demonstrated to have high absorbance contribution at 235 nm, which is interfering with the polysorbate. Even at low protein concentrations, the slopes were well above 0.01 Abs/mm. Further analysis must be done with specific proteins to determine which will work effectively with polysorbate.

Discussion

The Slope Spectroscopy method is the proposed technique for polysorbate analysis. In the case of analyzing polysorbate neat, we will no longer be using the former wavelength of 620 nm. The 235 nm wavelength provides a very prominent peak which is the recommended wavelength of interest for the SoloVPE System.

The Slope Spectroscopy method allows the system to measure a vast range of polysorbate 80 and polysorbate 20. As seen in Figure 4 and Figure 5, the SoloVPE System is able to detect higher concentrations of PS20 and lower concentrations of PS80. This is because PS20 is composed of lauric acid while PS80 is composed of oleic acid. Lauric acid is a 12-carbon atom fatty acid chain while oleic acid is an 18-carbon atom fatty acid chain. The increased number of carbon atoms in the oleic acid results in a higher absorbance profile for PS80. Therefore, the SoloVPE System can detect lower concentrations of the PS80 samples relative to the PS20 samples.

It was determined that the BSA expressed high absorbance contribution when formulated with polysorbate. We believe the amino acid chain of the protein is interfering with the polysorbate at the 235 nm wavelength. We cannot dictate which proteins will work effectively with polysorbate; however, we will continue to work with our customers to assist with developing a robust method.

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 measuring polysorbate. The

Slope Spectroscopy method will show that neat polysorbate 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.

Traditional UV method time (extraction, evaporation, sample prep, and reading): 4.5 hours

Proposed SoloVPE method time (extraction, evaporation, and reading): 1 hour

Discussion

The CTech SoloVPE System is an effective instrument for rapid acquisition of accurate slope and concentration measurements. The system's ability to accurately read neat and dilute polysorbate solutions demonstrates the effectiveness of the Slope Spectroscopy method. The system's software makes it easy for users to automatically calculate their acceptance criteria. 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 analyzing polysorbate.

Customer Support
analytics-support@repligen.com
+1 908-707-1009

Repligen Corporation
685 Route 202/206
Bridgewater, NJ 08807, USA
www.ctech.repligen.com