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RESEARCH ARTICLE

Determination of Polysorbate 80 Concentration of Inprocess Stock Solution by SoloVPE for Biopharmaceutical Formulation Development and Manufacture

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ABSTRACT

Polysorbate 80 as a non-ionic surfactant has been widely used as a stabilizer for protein products to minimize interface induced degradation during manufacture and long-term storage. During manufacture of therapeutic proteins, polysorbate 80 is usually prepared in water as a stock solution that is added to Bulk Drug Substance (BDS) to meet a targeted polysorbate 80 concentration. The viscous, lipophilic, and oily nature of polysorbate 80 make it extremely challenging for weighing and dissolving in water, especially in a manufacturing setting. Ensuring accurate weight and complete dissolution of polysorbate 80 in stock solutions prior to addition to BDS is crucial for Drug Product (DP) manufacture. Currently, available polysorbate 80 assays take a couple of days to obtain results, so the concentration of the polysorbate 80 stock solutions can't be confirmed before adding it to BDS. Therefore, significant risk is associated with the unverified polysorbate 80 concentration. Here, a new technology, Variable Path length Extension (Solo VPE), is employed to study the dissolution of polysorbate 80 stock solution. Thus, a fast and real time assay is developed to assess the dissolution of polysorbate 80 in water and ultimately quantify the polysorbate 80 concentration in the stock solution. Briefly, the polysorbate 80 extinction coefficient (ε) at UV195nm is determined from a fully dissolved polysorbate 80 standard in analytical scale. Then, the experimental ε is applied to measure the concentration of polysorbate 80 in a stock solution. The assay takes less than 1.5 hours, serving as a real time, in-process assay to ensure the accurate addition of polysorbate 80 to the BDS. Qualification of the method suggests that the variability of the assay is minimal with an intermediate precision of Relative Standard Deviation (RSD) ≤ 3% and a Limit Of Quantification (LOQ) of 0.03%, which is sufficient for polysorbate 80 stock solutions with concentrations ranging from 0.1 to 5%.

Introduction

Polysorbate 80 has been widely used as a stabilizer in the formulation of protein drugs including monoclonal antibodies (mAb) in biopharmaceutical industries [1]. Polysorbate 80 has been previously shown to have a typical structure (Figure 1) that contains approximately 20 groups of polyoxyethylene per molecule [1]. The polysorbate 80 concentration in the formulation is crucial

to support protein stability by preventing interface induced protein aggregation and particle formation [2-4]. Excess amounts of polysorbate 80 could result in oxidation or even be toxic at a high surfactant concentration [5]. Therefore, quantification of polysorbate 80 stock solution prior to addition to Bulk Drug Substance (BDS) is needed to ensure correct polysorbate 80 contents of the formulated Drug Product (DP).

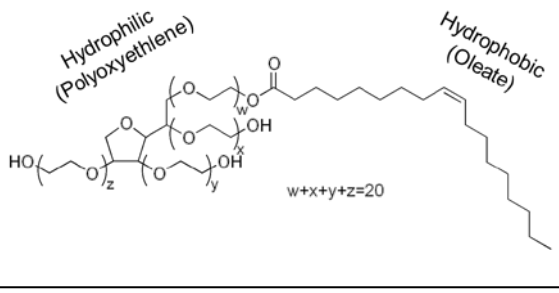

Figure 1: Schematic Chemical Structure of Polysorbate 80.

Several methods for quantification of polysorbate 80 in proteins have been described in the literature. A colorimetric method based on the formation of a blue complex between ammonium cobalt-thiocyanate and polyoxyethylene group was one of the classic polysorbate 80 assays utilizing extraction of polysorbate 80 complexes with methylene chloride [6-8].

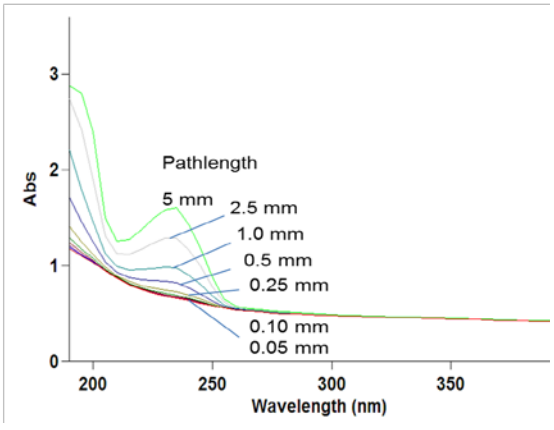

Figure 2: UV Spectra Collected from SoloVPE Spectrometer with 0.05% Polysorbate 80 at Various Path-lengths.

A more recent published technique was to quantify the fatty acid content generated from hydrolysis of polysorbate 80 [9-10]. In addition, several direct methods for the analysis of polysorbate 80 have been reported using High-Performance Liquid Chromatography (HPLC) coupled with Evaporative Light Scattering Detection (ELSD) [11] and Charged Aerosol Detection (CAD) [12]. All of these reported methods focus on measuring the polysorbate 80 content in protein solutions. Extensive publications describe absorption and extraction procedures whereas reports are lacking to ensure that the concentration of polysorbate 80 stock solution is accurate before addition to the BDS other than the calculation from the weight.

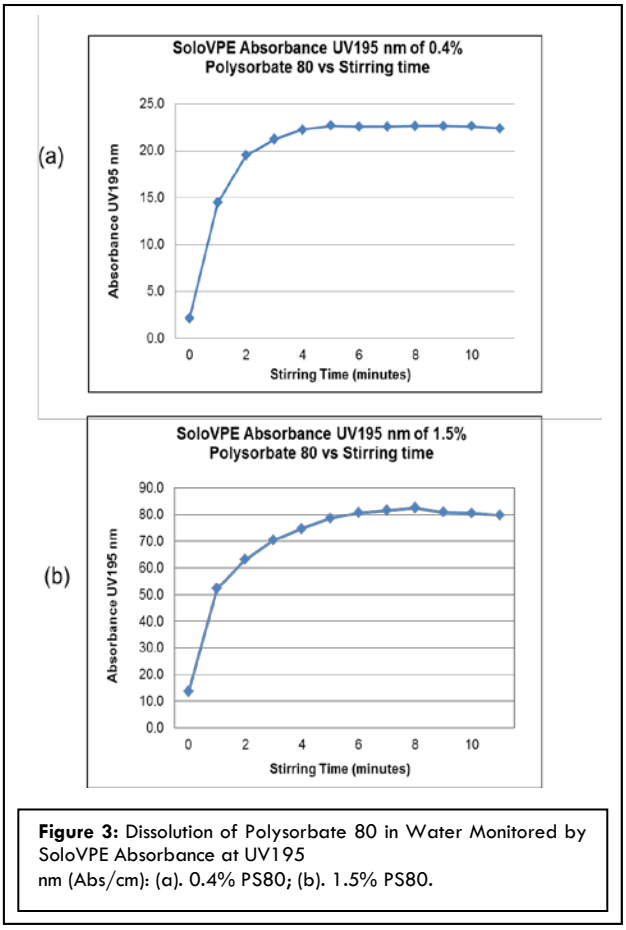

Figure 3: Dissolution of Polysorbate 80 in Water Monitored by SoloVPE Absorbance at UV195
nm (Abs/cm): (a). 0.4% PS80; (b). 1.5% PS80.

Since neat polysorbate 80 is a viscous oily liquid, it's not possible to add the neat polysorbate 80 directly to formulate DP. Usually a polysorbate 80 stock solution is made by dilution of the neat Polysorbate 80. Then it's added to the BDS based on the polysorbate 80 concentration and the volume of BDS. It is difficult to pipette the neat polysorbate 80 into a container and weigh them accurately because drops of the oily liquid are often on the wall or bottom of a container and not fully dissolved resulting in an incorrect polysorbate 80 concentration in DP. It is critical to control the

polysorbate 80 stock solution and confirm it's concentration for the intended use. The test method needs to be fast to provide analytical results in a couple of hours which allows manufacturing to re-make a polysorbate 80 stock solution if the polysorbate 80 concentration does not meet the inprocess control (IPC) limit.

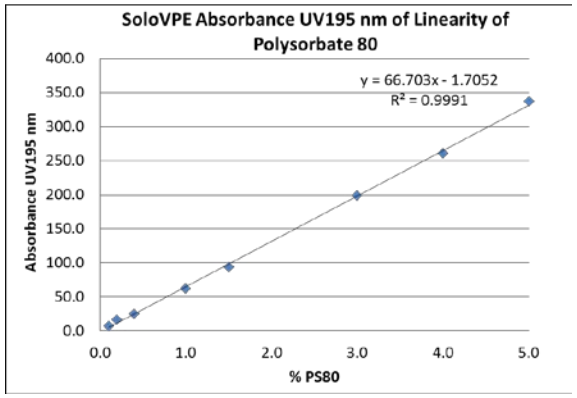

Figure 4: Linearity of UV Absorbance (Abs/cm) at 195 nm of Various Polysorbate 80 Concentrations Ranged from 0.1 to 5.0%.

Available polysorbate 80 assays such as Cobalt blue and HPLC assays are time consuming [6-8], which usually takes 1-2 days. In addition, these assays need a 30-50-fold dilution to make polysorbate 80 calibration standards resulting in large errors in the methods. In this manuscript, a simple efficient In Process Control (IPC) method is presented to determine polysorbate 80 concentrations in water stock solutions. The accuracy of polysorbate 80 stock solution concentration is the focus. The polysorbate 80 method utilizes the characteristics of UV spectra of polysorbate 80 [13] and a new technology SoloVPE instrument (variable path length extension) [14]. The SoloVPE has been developed for protein content determination and proven to be very efficient to measure absorbance from 0.01 to 450 Au for proteins without any dilution [15]. In this manuscript, the SoloVPE is employed to determine extinction coefficient (ε) from a known concentration of polysorbate 80 analytical standard, and then apply the experimental ε to test polysorbate 80 stock solution. The method takes less than 1.5 hours and it is truly a real-time test on the manufacture floor and provides accurate results in time to formulate BDS with confidence.

Experimental

1. Materials

Polysorbate 80, NF (Cat. # 4117-04) was purchased from Avantor (Pennsylvania, USA). 0.22 µm filter (Cat.# MPGL06CB1) was purchased from EMD Millipore (Massachusetts, USA). Ethyl alcohol (Cat. DSP-MD.43) was purchased from Decon Laboratories Inc (Pennsylvania, USA). HPLC grade acetone (Cat. # A949SK-4) was purchased from Thermo Fisher Scientific (Massachusetts, USA). The 250 mL Erlenmeyer flasks, non-pyrogenic polycarbonate (Cat. # 430183), were purchased from Corning (New York, USA).

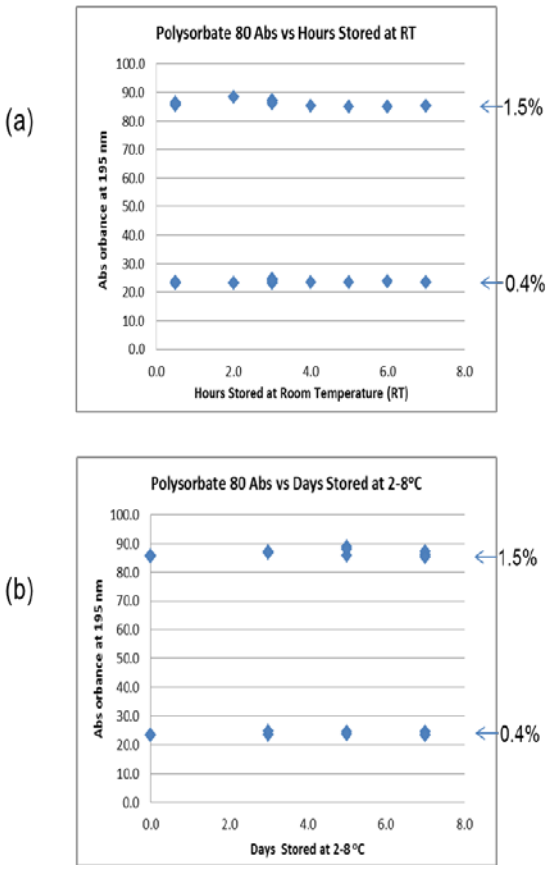

Figure 5: A195 nm Absorbance of PS80 Stock Solution Stability: (a). RT; (b). 2-8 oC.

2. Instrumental

All UV measurements were performed using SoloVPE instrument, C Technology Inc. (New Jersey, USA) installed onto an Agilent Cary 60 UV-Vis spectrometer (California, USA). The accessories including fibrettes (Cat. # OF 0002-P50), sample vessels (Cat.# OC0005-1, Cat.# OC0005-2, and Cat.# OC0009-1-P50), and

vessel cleaner (Cat. #: ACC-CLEANER-OC) were purchased from C Technology Inc. (New Jersey, USA). Analytical balance was purchased from Mettler-Toledo, LLC (Ohio, USA).A stir plate (Cat. # 11-500-49S) was purchased from Thermo Fisher Scientific (Massachusetts, USA).

about 2 Au (absorbance/cm, Abs/cm). Unlike traditional UV spectrometry, SoloVPE employs an advanced technique based on Slope Spectroscopy to rapidly collect linear absorbance data at variable path length, from which a slope in unit of absorbance/mm and

Table 1: The Impact of Sample Vessel Material and Sizes on DI Water Absorbance at 195 nm.

Sample Vessel ID #	Sample Vessel Material	Sample Vessel Size	R^2	Measured Slope (Abs/mm)	Measured Abs (Abs/cm)
1	Silica	2000 uL	0.99876	0.00287	0.0287
			0.99832	0.00263	0.0263
			0.99982	0.00353	0.0353
2	Silica	2000 uL	0.99897	0.00234	0.0234
			0.99940	0.00260	0.0260
			0.99908	0.00273	0.0273
3	Silica	200 uL	0.99937	0.00408	0.0408
			0.99960	0.00433	0.0433
			0.99939	0.00550	0.0550
			0.99911	0.00498	0.0498
4	Plastic	200 uL	0.46399	-0.00669	-0.0669
			0.87604	0.00294	0.0294
			0.60795	0.00712	0.0712

3. Preparation of Polysorbate 80 Solution

An aliquot of neat polysorbate 80 was weighed on an Analytical Balance (with an accuracy of 0.01g) in amount of 1.0 g, 3.75 g, or 12.5 g and q.s. with DI water up to 250 g in 250 mL Erlenmeyer flasks to achieve 0.4%, 1.5% or 5.0%(w/w) polysorbate 80 stock solution, respectively. The percentage unit in w/w will be applied for this entire document. The polysorbate 80 and DI water mixtures were stirred on a Stir Plate at a medium speed for 15 minutes or as specified in the text. During the mixing step, a clear vortex should be observed to ensure complete dissolution of the polysorbate 80 in DI water. The final concentration was calculated by weight of neat polysorbate 80 divided by total weight of polysorbate 80 and DI water, then it was expressed in unit of percentage (%) in weight by weight (w/w). The diluted polysorbate 80 has a density close to 1.0 g/mL. Thus, the mg/mL is equivalent to mg/g for the polysorbate 80 water stock solutions discussed in this manuscript.

4. Principle of SoloVPE for Polysorbate 80 Stock Solution

A conventional UV spectrometer uses a 1 cm cuvette as a fixed light path length and the detector is saturated at

regression coefficient constant (R^2) are determined [14,15]. Manually diluted calibration standards are not needed for a linearity curve. SoloVPE takes readings from path-lengths as low as 0.05 mm and allows high concentration samples to be measured without further dilution or background correction of a matrix blank. In fact, test sample concentrations can be as high as 200 times those measured using a 1 cm cuvette and the absorbance can be recorded linearly up to 450 Au (abs/cm) in SoloVPE.

To setup the SoloVPE experimentally, a Quick Check is performed to determine if the % transmission meets the system suitability. For all experiments reported here in this manuscript, the detection wavelength was set at 195 nm with Baseline Correction OFF and Scatter Correction ON for a single wavelength at 320 nm. SoloVPE instrument can be used to determine extinction coefficient for an analyte with a known concentration. It can also be employed to measure or confirm the concentration for the analyte with a known extinction coefficient (ε). These calculations are based on Beer's Law as stated in following equation:

$$\text{Abs (Au)} = \varepsilon\ [\text{mL/(mg.cm)}] * \text{B (cm)} * \text{C (mg/mL)}$$

When both extinction coefficient and concentration are not input in the method, the SoloVPE instrument would assume the extinction coefficient is 1.0 mL/(mg.cm).

Table 2: Absorptivity of Sample Vessel Cleaning Solvents at 195 nm Measured by SoloVPE.

Solvent Name	R^2	Measured Slope at 195 nm (Abs/mm)	Measured Absorbance at 195 nm (Abs/cm)
DI-WATER	0.99906	0.00308	0.0308
Ethanol 200 proof	0.99987	0.73654	7.3654
Acetone	0.82553	3.62213	36.2213

In any case, the slopes in unit of Absorbance/mm are recorded as raw data for all modes of SoloVPE measurements instead of Absorbance/cm (Au) in conventional UV spectrometer. In order to prevent confusion between the slope (abs/mm) of SoloVPE and the slope of calibration standard curve of conventional UV instrument, the slopes in Abs/mm of SoloVPE are converted to Au (Abs/cm) by multiplying by 10 as 1 cm equals to 10 mm which is expressed in the following equation.

$$\text{Abs (Au)} = \text{Slope (Abs/mm)} * 10 \text{ (mm)}$$

For specificity experiments, the slope in Abs/mm of the blank (i.e. DI Water) is recorded for 195 nm and then the Au (abs/cm) of the blank is calculated based above equation. To obtain the extinction coefficient for an analytical standard of 0.4% polysorbate 80, input the concentration of polysorbate 80 in mg/mL which is 4.0 mg/mL for 0.4% or 50.0 mg/mL for 5.0%. Finally, to measure an unknown concentration of polysorbate 80 stock solutions, the experimental extinction coefficient generated from 0.4% polysorbate 80 is input into the SoloVPE method and then the concentration in mg/mL of polysorbate 80 and % polysorbate 80 is calculated.

Results and Discussion

1. UV-Spectra of Polysorbate 80

Polysorbate 80 is a mixture of polymer with polyoxyethylene (in hydrophilic sites) and oleic acid (oleate as hydrophobic moiety) as major components. A schematic chemical structure is widely known as in Figure 1. The neat polysorbate 80 is an oily viscous yellow liquid with a density ranged from 1.06 to 1.09 g/mL [2] and the UV spectra from regular spectrophotometers were reported previously with absorbance maxima around 195 and 234 nm [13]. It was observed that the peak around 195 nm contains noisy spikes possibly due to interferences from solvents, cuvettes and UV detector of instrument. It was also previously stated that absorbance at 234 nm was attributed to by-products of polysorbate 80 manufacture process whereas absorbance at 195 nm originates from the double-bond of the fatty acid chain(oleic acid) (Figure 1).

Here, UV spectra were collected by the SoloVPE with quick survey mode for 0.05% polysorbate 80 at various path-lengths shown in Figure 2. The spectra suggest that there are two distinct peaks from 190-250 nm with Apexes around 195 nm and 234 nm. The Apex at 195 nm is not as clear as described in literature [13] possibly due to the quality of the UV lamps equipped with Cary 60. As there are no conjugated double bonds present in polysorbate 80 molecules, the absorbance of the Apex at 234 nm is likely due to a by-product of manufacture. This wavelength may only be used as surrogate to quantify polysorbate 80 when the same lot of polysorbate 80 is used for manufacture of DP and for the assay standard. It is obvious that the wavelength observed at 195 nm originated from the polysorbate 80 molecule and is a better choice if it works. Here, an extensive study has been conducted to prove that the use of A195 nm as a detection for polysorbate 80 stock solution is accurate and reproducible which are described in the following sections.

2. Impact of Sample Vessel Material and Sizes on Polysorbate 80 at A195 nm

Sample vessels are known to be important for UV measurement [16] in conventional spectrometer thus the UV cuvettes are made of high quality quartz with low absorptivity from 180 to 900 nm. SoloVPE instrument is equipped with four different sample vessels made of 2 different types of materials i.e. silica and plastic. The silica vessels come with volumes of 100 µL (micro), 200

Table 3: Extinction Coefficient [mL/(mg.cm)] at 195 nm of Polysorbate 80: (a). Obtained from Four PS80 Lots; (b). Determined from PS80 with Various Concentrations from Lot 0000005773.

(a)

Polysorbate 80 Lots	% PS80 (w/w) by Weight	Au (Abs/cm) by SoloVPE	# of Measurements	Ave Measured Ext. Coeff. [mL/(mg.cm)]	RSD
Lot 0000005773	0.403	23.192	3	5.748	2.8
Lot 0000090430	0.400	23.267	2	5.636	4.6
Lot K15584	0.400	23.581	1	5.896	N/A
Lot 0000068892	1.052	61.132	1	5.811	N/A
		Ave Ext. Coeff. of 4 PS80 Lots		5.773	
			Std Dev	0.1	
			RSD	1.9	

(b)

% PS80 (w/w) by Weight	Au (Abs/cm) by SoloVPE	Measured Ext. Coeff. [mL/(mg.cm)]	Ave Measured Ext. Coeff. [mL/(mg.cm)]	Std Dev	RSD
0.4	23.285	5.833	5.748	0.08	1.4
0.4	22.970	5.675			
0.4	23.321	5.736			
1.5	84.364	5.628	5.716	0.11	1.9
1.5	87.162	5.834			
1.5	85.521	5.687			
5.0	268.208	5.350	5.463	0.12	2.2
5.0	272.767	5.451			
5.0	279.362	5.587			
	Ave Ext. Coeff. of 9 Measurements		5.642		
		Std Dev	0.16		
		RSD	2.9		

µL (small) and 2000 µL (large) respectively, whereas the plastic vessel holds 200 µL of sample. For protein assays, it was proved that comparable data were produced using plastic and silica vessels detected at 280 nm [15]. Plastic vessels are inexpensive and therefore disposable, which eliminates contamination from cleaning and saves time.

Here we employ the SoloVPE to measure polysorbate 80 absorbance in far UV region at a wave length as low as 195 nm where many organic compounds including polymers with a double bond absorb significantly. Thus, the absorbance at 195 nm of sample vessels including both silica and plastic vessels is evaluated using DI water as blank sample in Table 1.

Clearly, the R^2 of silica vessels including both 200 µL and 2000 µL is ≥0.998 whereas the R^2 of plastic vessels is< 0.9, ranging from 0.5 to 0.9, and far below the required limit of 0.99. It is also noted that the average Au (Abs/cm) for the 2000 µL silica vessel is ≤ 0.03, slightly lower than 0.05 Au observed for the smaller 200 µL silica vessel. Although the differences are small, it is recommended to use 2000 µL vessels as long as the amount of polysorbate 80 stock solution sample is not limited. Also the 2000 µL vessel is easier to handle.

3. Absorptivity of Solvents at A195 nm

During UV measurements, the sample cuvettes or vessels are cleaned between different tests using DI water

followed by drying with organic solvents such as acetone or ethanol.

SoloVPE. Two polysorbate 80 solutions at concentrations of 0.4% and 1.5% were made (weight by weight).

Table 4: %Recovery Results.

PS80 Sample	%PS80 by Weight	%PS80 by Solo VPE	% Recovery	Ave % Recovery	RSD
0.4% -1	0.403	0.420	104	104.5	0.5
0.4% -2		0.420	104		
0.4% -3		0.420	104		
0.4% -4		0.425	105		
0.4% -5		0.423	105		
0.4% -6		0.424	105		
1.5% -1	1.504	1.544	103	103.2	0.4
1.5% -2		1.550	103		
1.5% -3		1.544	103		
1.5% -4		1.558	104		
1.5% -5		1.551	103		
1.5% -6		1.552	103		

DI water is also the solvent for polysorbate 80 stock solution. Thus, these solvents are tested for their absorptivity at 195 nm. Table 2 documents the absorbance of DI water, ethanol, and acetone. The absorbance of DI water is very low (<0.04 Au) which indicates there shouldn't be much interference with the polysorbate 80 measurements at 195 nm. However, the absorption of both acetone and ethanol are not neglectable, with acetone exhibiting an even higher absorbance at 195 nm than ethanol. This also emphasizes that the sample vessels need to be completely dried after washing with ethanolor acetone. To make sure that the residual organic solvents do not interfere with the SoloVPE measurement, it is recommended to rinse the sample vessels with the actual polysorbate 80 analytical standards and test solutions at least once prior to taking measurements when a large quantity of polysorbate 80 solutions is readily available for analysis. In addition, to minimize introduction of contamination from organic solvents, the sample vessel should n't be washed when consecutive duplicate measurements are conducted.

4. Dissolution of Polysorbate 80 in Water

Prior to finalizing the method, dissolution of polysorbate 80 in water at various stirring times is monitored by

An aliquot of sample was collected at one minute intervals up to 11 minutes, and the absorbance of these samples was measured by SoloVPE. The absorbance (Abs/cm) at 195 nm of polysorbate 80 is plotted against the stirring time (minutes) in Figure 3. In the first 3 minutes the absorbance rises rapidly. For 0.4% polysorbate 80 analytical standard solution, it takes about 4 minutes for polysorbate 80 to fully dissolve in water, while it takes about 6 minutes for the 1.5% stock solution. As it's easier to get 0.4% polysorbate 80 to dissolve than 1.5% polysorbate 80, it is beneficial to measure the extinction coefficient of 0.4% polysorbate 80 and then apply it to higher concentration solutions, possibly up to 5% polysorbate 80.

5. Measurement of Extinction Coefficient of Polysorbate 80 at A195 nm

It is known that polysorbate 80 compositions have some variability among the lots due to the manufacturing process in terms of fatty acid content [2] and absorptivity [13]. Thus, four polysorbate 80 stock solutions with concentrations ranging from 0.4% to 1% were made from 4 different lots of polysorbate 80 and the experimental extinction coefficient at UV195 nm of each stock solution was determined and shown in Table 3a. The average extinction coefficient of 4 different lots

Table 5: Repeatability and Intermediate Precision.

	Targeted 1.5% PS80 Stock Solution	# of Experiments	Ave %PS80 by SoloVPE	Absolute % Differences	% RSD
Repeatability #1	1.5%-Day 1	6	1.503	0.2	0.5
Repeatability #2	1.5%-Day 2	6	1.488	0.8	0.3
Repeatability #3	1.5%-Day 3	6	1.533	2.2	1.0
		Ave	1.508		
		RSD of 18 measurements	1.4		

is calculated as 5.77 mL/(mg.cm) with a relative standard deviation (RSD) of 1.9%. As mentioned earlier, polysorbate 80 is a mixture of polyoxyethylene (w+x+y+z=20) sorbitan mono-fatty acid with varying lengths. The double-bonds of carbonyl in ester group () and double bond of oleate acid (-C=C-) contribute to the absorption properties at 195 nm [13].

To evaluate the robustness of the extinction coefficient measurement, polysorbate 80 at various concentrations were tested. The individual measurement, average at each polysorbate 80 concentration, standard deviation (Std. Dev.) and RSD are described in Table 3b. The overall RSD of 9 measurements from three concentrations was 2.9% with an average extinction coefficient of 5.64 mL/(mg.cm). Therefore, an experimental extinction coefficient at 195 nm of a known polysorbate 80 concentration, i.e. 0.4% polysorbate 80, can be used to quantify unknown concentrations of polysorbate 80 stock solutions up to 5%. The assay qualification for its use as an IPC testis conducted and described in following sections including linearity, robustness, precision and accuracy.

Qualification of SoloVPE Polysorbate 80 as an IPC Test

1. Specificity

Specificity is to determine if the sample matrix significantly affects the measurement of the analytes. In order to demonstrate specificity, the absorptivity of A195nm of the sample blank, i.e. DI water in this assay, was measured. The R^2, slope and Au of DI water at 195nm are described in Table 1. The R^2 for all silica vessels including both sizes of 200 µL and 2000 µL are ≥ 0.998. The Au is ≤ 0.04 for 2000 µL vessel and 0.06 for 200 µL vessel, which is less than 0.3% of the absorbance of analytical standard at about 24 Au (Table 3), suggesting no significant interference from the water blank. The absorbance of the plastic vesselseems low as well, but sometimes with a negative value, does not have acceptable R^2 values and is not suitable for polysorbate 80 measurements.

2. Recovery

Samples at concentrations of 0.4% and 1.5% polysorbate 80 are measured 6 times at UV195 nm. The % recovery of polysorbate 80 is calculated by comparing the measured to the weight concentration and shown in Table 4. The extinction coefficient was pre-determined using 0.4% analytical standard (Table 3). The % recovery of each measurement ranged from 103% to 105% and the RSD of triplicate and all 6 measurements are ≤ 1%.

3. Linearity

A serial dilution from a 5% (w/w) polysorbate 80 stock solution to 0.1% was conducted and 2 mL of each was measured by SoloVPE. The absorbance (Abs/cm) vs % polysorbate 80 is plotted in Figure 4. A linear regression equation with R^2 of 0.9991 is displayed in Figure 4. The calculated absorbance based on linear regression and the Absolute % Difference between the

measured and calculated absorbance are≤ 5% for all concentration levels from 0.1% to 5%.

4. Estimation of LOQ Statistically

Linearity of UV absorbance at 195 nm of polysorbate 80 concentrations from 0.1 to 5% is plotted Figure 4 (R^2 of 0.9991) and was used to estimate LOQ. The LOQ is estimated by using the following equation recommended by FDA guidance [17].

$$LOQ = \frac{10 * \sigma}{S}$$

Where σ refers to residual standard deviation of a calibration curve and S refers to slope of calibration curve. One thing to mention is that the S (slope) from the calibration curve is completely different from the Slope obtained from the SoloVPE instrument. The calibration standard linear equation is as follow:

$$Y\ (\text{Abs/cm at 195 nm}) = 66.703 * X\ (\text{Conc. of PS80}) - 1.7052$$

Where σ (residual standard deviation) is 3.815 and S (slope) is 66.703. The LOQ is 0.572 Au and the LOQ in percentage of polysorbate 80 isestimated statistically as 0.034%.

5. Repeatability and Intermediate Precision

A polysorbate 80 stock solution with a concentration of 1.5% was analyzed at 195 nm 6 times with Fibrette Lot #1, 2000 µL Vessel #1, Analyst #1. The exact concentration of polysorbate 80 by weight, measured by SoloVPE, Ave, Absolute %Difference between Measured and by Weight, and RSD of the 6 measurements are documented in Table 5. A second and third polysorbate 80 stock solutions with concentration of 1.5% from different preparations were measured at 195 nm 6 times with different combinations of Fibrette Lot, Vessels, and Analysts, respectively. To determine intermediate precision, the concentrations of 1.5% polysorbate 80 measured by SoloVPE from 3 different assays are documented in Table 5. RSD of each repeatability assay or intermediate precision from the total of 18 measurements is ≤1.4%. The % absolute differences for each repeatability assay are ≤2%.

6. Polysorbate 80 Solution Stability at RT and Refrigerated at 2-8 oC

The absorbance of polysorbate 80 solutions with concentrations of 0.4% and 1.5% were measured at various time points after being kept at room temperature (RT) for up to 7 hours, or 2-8 °C for up to 7 days (Figure 5). One measurement was conducted at each time point. The Absolute % Difference of each timepoint compared to T0, Ave and RSD are calculated. As a result, the Absolute % difference is ≤7% and the RSD is ≤3% which suggests that the polysorbate 80 samples exhibit stable absorbance at 195 nm for at least 7 hours at RT and 7 days at 2-8°C. The stability of polysorbate 80 water solution at RT and 2-8 °C allows sufficient time to perform a robust assay for a real-time polysorbate 80 test sample from manufacture.

Accuracy

The accuracy of the measurements is demonstrated by the specificity, linearity, and recovery of 104% as the highest. Polysorbate 80 is weighed first and the measured % polysorbate 80 by SoloVPE is compared to the % calculated by weight values. The accuracy can be directly assessed by % recovery or % absolute differences in the following equation.

%Accuracy = 100% - Highest Absolute %Difference of Qualification Experiments

The largest absolute % difference of all qualification experiments observed is ≤7% (data extracted from Figure 5). Thus, the accuracy of this assay is >93%.

Robustness

For robustness experiments, the conditions are altered from those recommended to examine if the assay is performing for its intended use. Since there is little to be changed for sample preparation for polysorbate 80 or SoloVPE instrument settings, robustness tests were focused on the sample vessels which have been reported to be important [14-16]. As described earlier, 200 µL plastic vesselswere excluded due to interferences with the UV measurement at 195 nm. Here the silica vessels with 2 different sizes (200 µL vs 2000µL) were chosen for the robustness tests.

Polysorbate 80with a concentration of 1.5% was tested 6 times in 2000 µL and 200 µL vessels, respectively. The

Absolute % difference for both large and small vessels is ≤3% and RSD is ≤2% (Data not shown). During the robustness experiments, it was observed that it's easier to operate the large vessel than the small one. However, 200 µL of silica vessel can be used as a backup sample vessel when limited amounts of samples are available for analysis.

Conclusion

Quantification of polysorbate 80 stock solutions by SoloVPE has been developed and qualified. The method is simple, reproducible, accurate and efficient for its intended usage in the pharmaceutical industry during manufacturing of DP. In addition, the A195nm absorbance of polysorbate 80 is stable at room temperature for at least 7 hours and 2-8°C for at least 7 days, which allows for completion of the assay in time for assessment of the polysorbate 80 dissolution in a real-time manufacture setting. With the publication of this manuscript, the method may be widely used as an in-process control to ensure preparation of the required polysorbate 80 concentration in the drug product.

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