Comparison of protein concentration determination of a mAb drug substance using Variable Path Length Spectroscopy and UV/VIS Spectroscopy – The regulatory dilemma: Choosing the scientifically sound analytical technique or consistency with clinical trials?

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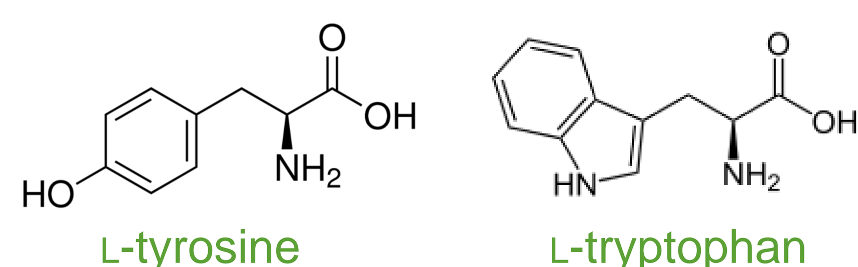
Abstract

In recent years a new technology known as "Variable Path Length Spectroscopy" (e.g. SoloVPE from C Technologies Inc.) has been developed for the determination of protein concentration in biologics such as monoclonal antibody drug substances (mAb DS). The advantage of this new technique is its increased dynamic range and resulting minimized sample handling, which leads to increased throughput and also mitigates the risk of dilution errors compared to conventional UV. In this case study for a mAb in late-stage clinical development, it was desirable to change from a traditional UV method to SoloVPE for DS release, and it was shown that the protein concentration was significantly impacted by the choice of method (SoloVPE or SoloVPE LS (Corrected for Light Scattering)) compared to the historical conventional UV method. The decision to implement SoloVPE for DS release was considered using a risk-based approach.

Introduction

Proteins, such as monoclonal antibodies (mAb), absorb UV light at 280 nm due to the presence of aromatic amino acids, mainly tyrosine and tryptophan:



L-tyrosine L-tryptophan

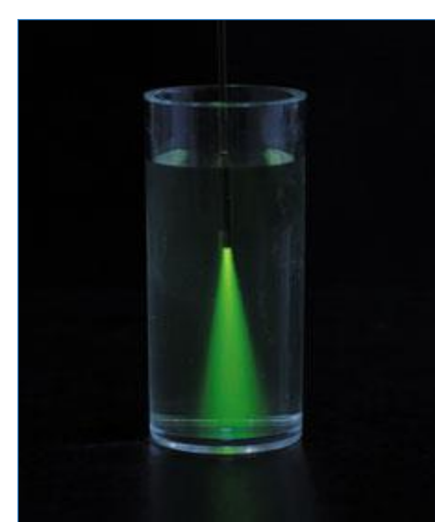
In conventional UV spectroscopy, the absorbance measured is converted to protein concentration as determined by the rearranged Beer-Lamberts law:

$$c = \frac{A}{\varepsilon \times l}$$

c = concentration ; A = absorbance ; l = path length ; ε = extinction coefficient

In Variable Path Length Spectroscopy, multiple measurements at varying path lengths produce a straight line with slope m = A / l. Substituting and rearranging, the concentration can be determined as follows:

$$c = \frac{m}{\varepsilon}$$



Photograph of a light path from the SoloVPE fibrette dipping into flourescein solution[1]. The optimal path length is automatically determined by the SoloVPE by searching for a straight line (Abs. vs path length) around 1.0 AU. The vertical position of the fibrette is adjusted accordingly.

SoloVPE results in a large dynamic range (0.05 – 300 mg/ml) leading to a minimized sample handling. Reduced sample handling mitigates the risk of dilution errors and increases testing throughput.

However, the increased dynamic range of the SoloVPE also brings challenges. As the protein concentration of many mAb DS is relatively high (50 mg/ml - 150 mg/ml) the contribution from light scattering could lead to an erroneous overestimation[2]. Hence, this study compares three analytical methods all capable of determining the protein concentration: SoloVPE, SoloVPE LS, and conventional UV.

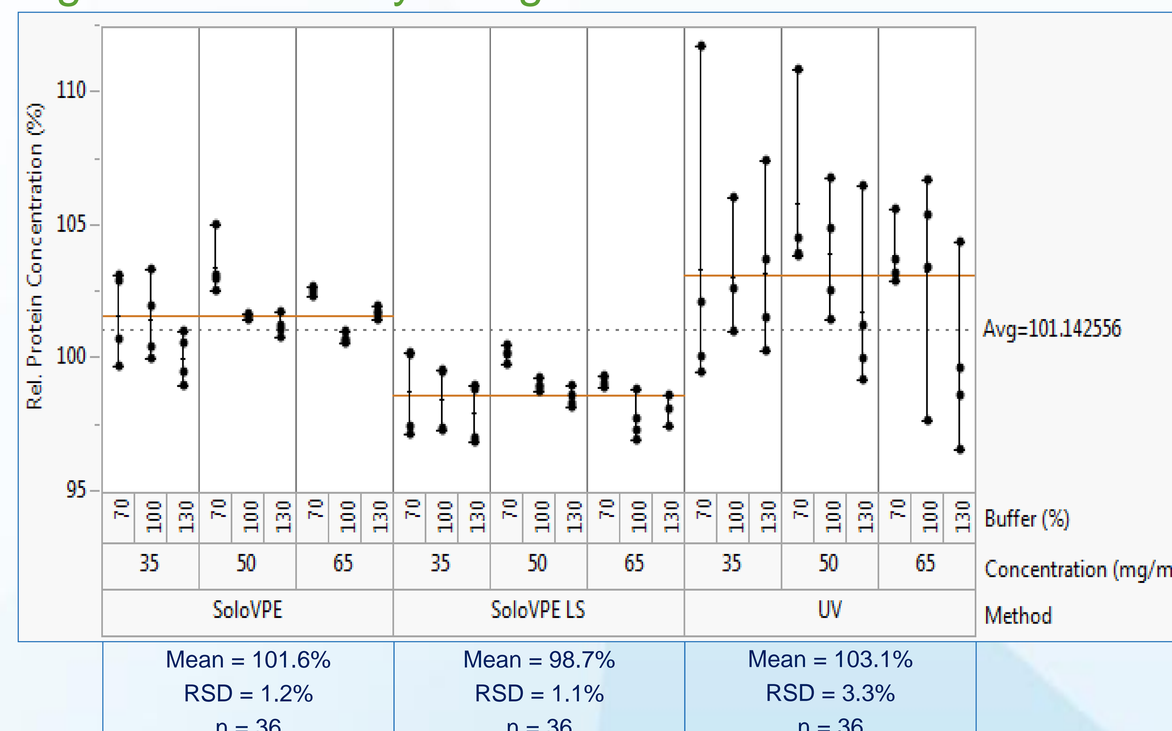
Experimental

Samples of mAb DS with a target protein concentration of 50 mg/ml were concentrated in Amicon Ultra-4 10 K centrifugal filters in a centrifuge (Sorvall RC4, 5000*g*) for 50 min at 21 °C. The concentrated samples were pooled and the buffer matrix shifted using PD-10 columns (GE Healthcare, spin protocol (1000*g*, 2 min.) to 70%, 100%, and 130% respectively, of the nominal mAb DS buffer component concentration (10 mM sodium phosphate, 140 mM sodium chloride, and 1% (w/v) sucrose). The protein concentration was determined using SoloVPE to be 66 mg/ml (70% buffer (A)), 65 mg/ml (100% buffer (B)), and 71 mg/ml (130% buffer (C)), respectively. Each of the solutions A, B, and C were diluted in duplicate to a protein concentration of 35 mg/ml, 50 mg/ml, and 65 mg/ml with the appropriate buffer (70%, 100%, and 130%). The protein concentration of the diluted samples were determined twice at 280 nm using SoloVPE, SoloVPE LS (dual wavelength scatter correction (320 nm and 360 nm)), and historical conventional UV (samples diluted to 0.5 mg/ml in 0.9% NaCl, quartz cuvette (1 cm path length), UV apparatus from Perkin Elmer (Model: Lambda 25)).

Results

Results from the analytical level of the diluted samples were back-calculated to protein concentrations on the stock solution level (A, B, and C) by multiplying with the appropiate dilution factor. The pre-determined protein concentrations of A, B, and C were then set to index 100 and relative protein concentration results of each of the diluted samples were derived thus allowing evaluation of the possible impact of the difference in buffer matrices (70%, 100%, and 130%) and protein concentration results (35 mg/ml, 50 mg/ml, and 65 mg/ml); see Figure 1.

Figure 1: Variability Gauge Chart



In Table 1, the difference in % between the mean protein concentrations as well as the p-values from the statistical comparison of means are presented.

Table 1: Overview of Results

Comparison/ Parameter	SoloVPE vs. UV	SoloVPE LS vs. UV	SoloVPE vs. SoloVPE LS
Difference of Means (%)	1.5%	4.4%	2.9%
Comparison of means (p-value)	0.0135	< 0.0001	< 0.0001

Discussion

As seen in Table 1, a downward shift in protein concentration of 1.5% (p=0.0135) or 4.4% (p<0.0001) would be expected if the historical conventional UV method was to be replaced by the SoloVPE or SoloVPE LS method respectively. The difference in % seen between the means of the SoloVPE and SoloVPE LS methods show that a 2.9% (p<0.0001) overestimation of the protein concentration occurs when the result obtained at 280 nm is not corrected for light scattering. From a method performance point of view either method would be superior compared to conventional UV as the SoloVPE and SoloVPE LS methods would reduce variation to almost the same degree (% RSD: 1.2 and 1.1 respectively; UV = 3.3) (Figure 1). Hence, the scientifically sound decision would be to replace the conventional UV method with the SoloVPE LS method. However, specification limits for the protein concentration of commercial mAbs are normally in the range of target concentration ±10% or tighter. Hence, the latter option would in theory mean a critical downward shift which would significantly increase the risk of OOS results. An option to mitigate this risk would be to increase the target concentration of the mAb DS correspondingly; however this would impact all other test results accordingly (e.g. potency). Also, the adjusted protein concentration would be inconsistent with the protein concentration of the batches originally used in the clinical trials. The % difference between the means of the SoloVPE and UV method is 1.5% and thus within the RSD% of the UV method. A downward shift of this magnitude would not be critical and consistency with clinical trials would not be compromised. Originally the historical conventional UV method was maintained without correction for light scattering while pursuing a more accurate and precise methodology which now has been found. However, before SoloVPE LS is allowed to replace the historical conventional UV method, the clinical aspects and the impact on specifications/other test results should be thoroughly investigated using a risk-based approach.

Conclusion

- **This case study concludes that the method performance of SoloVPE is superior compared to conventional UV when used to determine protein concentration in a mAb drug substance solution (50 mg/ml)**
- **If light scattering is evident, the regulatory aspects of replacing the UV method with SoloVPE, such as continuity and consistency, should be considered using a risk-based approach**

1. Photograph presented with permission from C Technology Inc.
2. Santosh V. T. et al. "An application of Ultraviolet Spectroscopy to Study Interactions in Protein Solutions at High Concentrations", Journal of Pharm. Sciences, (2012)

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