

In-Line UV-Vis Variable Pathlength Spectroscopy to Measure Concentration During Manufacturing Using Novel CTech™ Beams™ Light Source

Brandon Goldberg, Repligen • Oleksandr Karpenko, Repligen • Matt Muller, Repligen



Introduction

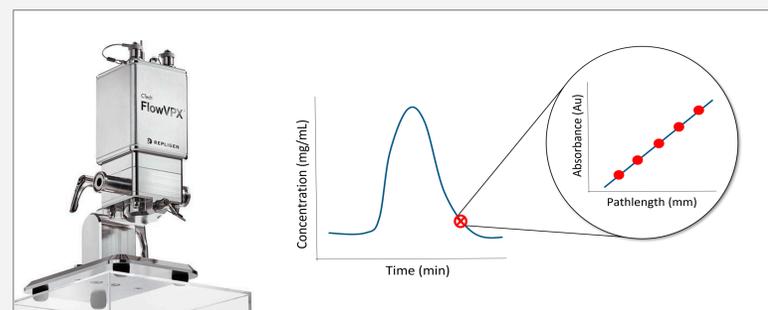
With the Biopharmaceutical industry requiring such time consuming and stringent testing to release drug products, inline PAT tools are the best solution. According to the process analytical technology (PAT) initiative of the US Food and Drug Administration, in recent years the preference in process analytics is given to inline methods, which provide the real time continuous data allowing constant monitoring and control [1]. The FlowVPX using variable pathlength technology is able to do just that, produce results in a timely matter while being able to cover a wide range of downstream processes. The FlowVPX is an in-line analytics tool that has the ability to go from 1 micron up to 5 millimeters in pathlength range to measure very dilute samples up to over 300 mg/ml samples every 5 seconds while being inline in the process.

Traditional UV-Vis vs. Variable Pathlength Technology

Ultraviolet-visible (UV-Vis) spectroscopy is a popular analytical technique in the world of biopharmaceuticals to assess the quality of drugs for a number of reasons, such as it is minimally affected by water and rarely has any deviations from Beer-Lambert law due to adsorption to the measurement cell walls, which can impair the quantification limits [2]. However, a challenge of UV spectroscopy is the limited linear range of traditional UV-Vis instruments [3], forcing scientists to take samples and dilute to the limits of the spectrometer, which sharply raises the risk of human error.

Traditional UV-Vis Spectroscopy Single absorbance measurement	Variable Pathlength Spectroscopy Multiple absorbance measurements for slope-based concentration results
$A = \epsilon l c$ <ul style="list-style-type: none"> Pathlength (l) is a fixed value Concentration (c) is the variable, requires sample dilution to ensure concentration falls within the linear range of the spectrophotometer 	$A = \epsilon l c$ <ul style="list-style-type: none"> Pathlength (l) is a variable value, capturing multiple absorbance measurements in less than 1 minute Concentration (c) is a fixed value, and does not require sample dilution

Variable pathlength technology (VPT) uses the Slope Spectroscopy® equation, which is derived from Beer-Lambert law ($A = \epsilon l c$), to read a wide range of concentration without involving dilution and human error ($m = \epsilon \times c$). Variable pathlength technology works by using a specific search algorithm to accurately find 1.0 Absorbance and measure 5 to 10 datapoints going down to generate a slope. That slope is then used to calculate a concentration point in real time. By widening the range that the FlowVPX System is able to read, while giving it the ability to read in-line, it becomes the perfect UV-Vis PAT tool for concentration measurements.



VPT In-Line Applications: Ultrafiltration/Diafiltration (UF/DF)

Ultrafiltration/Diafiltration (UF/DF) finds applications across the entire spectrum of biopharmaceutical processes, ranging from research studies to final formulation, owing to its ability to facilitate product concentration, buffer exchange, and identification of product limitations. UF/DF however has its own limitations due to mass calculation. By having the FlowVPX instrument in-line, instead of basing the concentration off of the change in mass, the concentration is being read in real time, eliminating the need to wait for samples and avoiding any errors related to holdup volume and density changes.

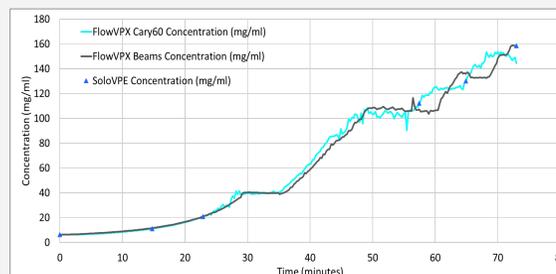
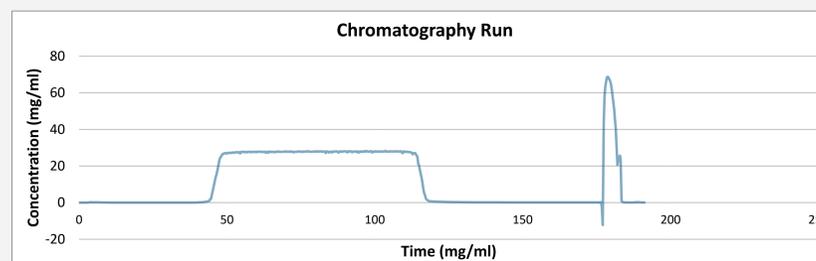


Figure 1. Concentration measurements comparing the CTech Beams light source versus the Agilent Cary 60 in a UF run.

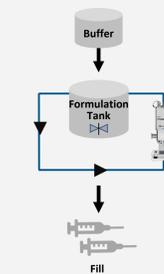
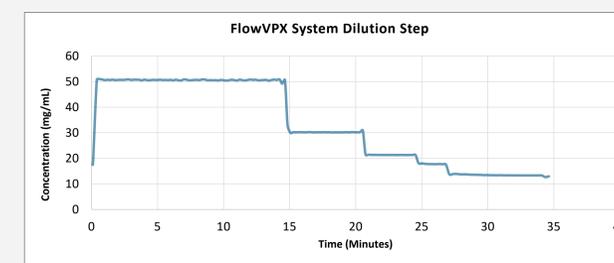
VPT In-Line Applications: Chromatography

Chromatography is an essential first step in the downstream process of biopharmaceuticals, as it enables the achievement of high purification rates. By adding the FlowVPX instrument into the process, scientists can now monitor titers in real time, pinpoint the breakthrough percentage, fully see the elution peak, and calculate the mass of the load and elution.



VPT In-Line Applications: Formulation

One primary use of the FlowVPX System in the context of GMP is for formulation purposes. It involves incorporating an in-line UV-Vis system that can constantly measure concentration in recirculation with the formulation tank, just before the fill finish. This approach provides scientists with the assurance that the concentration of the formulation is precisely what they require before collection. Moreover, the use of the FlowVPX System eliminates the need for the manufacturing team to obtain samples and wait for results from Quality Control or third parties.



GMP Compatibility

The FlowVPX instrument is a versatile system that can be used for process development and manufacturing due to its ability to accommodate flow cell sizes ranging from 3 mm to 2 inches. This flexibility allows the system to be utilized in various laboratory settings. Furthermore, the system includes CTech™ ViPER® ANLYTX Software, a platform that can enable 21 CFR Part 11 compliance and can connect to other manufacturing-suite control software. Through the use of OPC UA, or analog output, the ViPER Software can communicate with other software to automate the process based on concentration. Additionally, in terms of hardware, the FlowVPX System employs Repligen's CTech™ Beams™ System, a monochromatic LED light source and data acquisition module for integration with the FlowVPX System. For single wavelength applications, it is IP65- and C1D2-rated, making it ideal for use in the manufacturing suite. The system is made entirely of 316 L-grade stainless steel and has closed compartments that make it waterproof, allowing it to be hosed down before entering a clean space.

CTech™ Beams™ Advantages

The new Beams light source has a smaller footprint, simplifying integration onto a skid. Its single-wavelength, modular design ensures ease of use and compliance with USP, EP, and JP requirements for spectral bandwidth.

- Facilitated installation and integration in a process skid due to the compact design
- Much smaller footprint by a factor of 16
- Greater measurement speed—at least x2
- Higher measurement resolution as a result of lower full width at half maximum (FWHM) value
- Full compliance with USP, EP and JP requirements
- Uniform single wavelength modular design (available in 260 nm, 272 nm, 280 nm, 310 nm canisters)
- IP65 and C1D2



Conclusion

The utilization of the FlowVPX System has the potential to revolutionize the way in which biopharmaceutical companies assess the quality of their drugs using UV-Vis. This can be achieved through the elimination of human error, the need for sample preparation, and wait times for concentration readings. The application of variable pathlength technology in-line ensures that downstream processes are streamlined by increased accuracy, automation, and a simplified understanding of the process. The CTech Beams Light Source is a valuable upgrade for FlowVPX System users, as it enables them to integrate a more space-efficient solution into their workflows. Moreover, for intricate measurements involving multiple wavelengths, the FlowVPX platform allows effortless switching between the Beams and Cary 60 light sources. This has enabled Repligen to expand its offerings of VPT light sources, catering to the varied requirements of the biopharmaceutical industry.

References

- Rathore, A.S.; Bhambure, R.; Ghare, V. Process analytical technology (PAT) for biopharmaceutical products. *Anal. Bioanal. Chem.* **2010**, *398*, 137–154. DOI: 10.1007/s00216-010-3781-x.
- Skoog D A, West D M, Holler F J, Crouch SR. 2013. Fundamentals of analytical chemistry. Nelson Education.
- Rüdt M, Briskot T, Hubbuch J. Advances in downstream processing of biologics-spectroscopy: An emerging process analytical technology. *J Chromatogr A* **2017**;1490:2–9.



Learn more at repligen.com/flowvpX