High-concentration protein measurement with Repligen FlowVPX® in-line analytical system equipped with the new compact Beams™ light source

Oleksandr Karpenko, Repligen • Brandon Goldberg, Repligen • Gicell Schaenzler, Repligen • Joe Ferraiolo, Repligen

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Introduction

Effective and reliable analytics is critical for the biopharmaceutical industry, as it plays a crucial role in the discovery, development, and production of drugs. It becomes especially challenging when keeping in mind the constant increase in product concentrations across the industry [1]. Another important challenge of biopharmaceutical product development is finding methods which can be efficiently scaled up and effectively transferred to new platforms or stages of production [2].

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 methods that provide real-time, continuous data and allow constant monitoring and control; these criteria are best met by in-line methods, which analyze the sample without removing it from the process stream [3]. This means that scalable, in-line analytical methods should be considered and introduced at the drug development stage in order to achieve maximum productivity. UV spectrometers are among the most frequently used tools in the modern biopharmaceutical industry. However, due to the trend of increasing concentrations in modern bioprocessing, the effectiveness of traditional UV spectrometers in in-line applications is limited.

Variable Pathlength Technology (VPT)

Variable Pathlength Technology (VPT) is an alternative approach to UV-based analytics, based on the Beer-Lambert law, where absorbance is proportional to both concentration and pathlength. In contrast to conventional UV-Vis spectroscopy, where the pathlength remains constant, VPT adapts the pathlength to the concentration of the solution, enabling measurements of highly concentrated solutions that would otherwise only be possible with dilution [4]. This enables effective in-line measurements throughout the whole development and production process, making it possible to successfully implement a VPT instrument as a PAT tool for in-process monitoring and control of protein concentration.

The first in-line analytical UV-Vis spectrometer using VPT was the revolutionary CTech™ FlowVPE® System, introduced in 2017. This system was followed by the CTech FlowVPX® System in 2021, which provided increased GMP compatibility, improved connectivity, and optimized software integration.

Beams™ System: New Compact Light Source For FlowVPX

The FlowVPE and FlowVPX Systems both rely on the Agilent Cary 60 UV-Vis spectrophotometer as their light source. Being a robust instrument, the Cary 60 reliably fulfills its role as the systems' light source and provides a wavelength range from 190 to 1100 nm. Unfortunately, the size of the Cary 60 housing – 477 mm x 567 mm x 196 mm – may cause spatial challenges for some users. Although in most Research & Development (R&D) settings, the size of the light source in not a critical point, Manufacturing Science & Technology (MSAT) and Manufacturing (MFG) groups usually see the Cary 60's size as a complication and possibly a fundamental obstacle to the implementation of the FlowVPX in some processes.

Some Process Development (PD) groups also decline to adopt it due to footprint issues when transferring the process to downstream stages. Yet another obstacle is the inconsistent compliance of the Cary 60 instrument with the Japanese Pharmacopoeia (JP), which is an important factor for certain companies.

Due to the reasons above, and in line with the goal of continuous product improvement, Repligen has developed the CTech™ Beams™ light source for the FlowVPX in-line analytical system. The Beams module is a monochromatic LED light source and data acquisition system for single-wavelength applications, integrating source and detection capabilities as a Cary 60 alternative. The standout feature of this product is its compact form factor, which not only makes it easy to install and integrate into existing processes, but also requires minimal space to do so. Additionally, its simplified single-wavelength modular design ensures ease of use, while compliance with the USP, EP, and JP acceptance criteria for wavelength accuracy and resolution supports its suitability for use in spectroscopy applications.

When introducing improvements to existing analytical instruments, it is essential to demonstrate that the measurement quality and efficiency is comparable to or better than the previous iteration. For this purpose, a series of comparative field studies in various conditions and applications, including UF/DF, chromatography, formulation and others, was performed to showcase the comparability between the two light sources of FlowVPX System.

Materials and Methods

Two FlowVPX Systems were used to continuously monitor concentration during a tangential flow filtration (TFF) process. One unit used the Cary 60 as a light source, while the other used the 280 nm Beams light source.

3 mm Flow Cells were used for both FlowVPX devices. The CTech SoloVPE® System (using the Cary 60 light source) was used for at-line testing as a positive control; the SoloVPE has been adopted in numerous companies all over the world, mostly in quality control labs, and is considered by many to be a gold standard for QC measurement.

The TFF system was the KrosFlo® KR2i TFF System equipped with TangenX® SIUS® PD flat sheet cassettes (0.02 m^2, 5 kDa). The TFF was run in concentration mode with transmembrane pressure kept at 0.68 Bar. The TFF runs were performed using Trastuzumab (brand name Herceptin®), starting at 6 mg/ml and concentrated to 160 mg/ml. The CTech™ ViPER® (VPT software) measurement method applied in both cases was Quick Mode at 280 nm wavelength with continuous read and an extinction coefficient of 1.44 $(mg/ml)^{-1}cm^{-1}$.

Results and Discussion

The results indicate high comparability between the FlowVPX System using the novel Beams light source and the system using the Cary 60 as its light source. Both systems also compared well to the measurements by at-line SoloVPE System.

As seen from Figure 1 and Table 1, the real-time concentration readings obtained using FlowVPX with Beams are comparable to those obtained using FlowVPX with Cary 60 and SoloVPE across a wide range of concentrations, from 6 mg/ml to almost 160 mg/ml. The Beams system shows a similar concentration trend to the Cary 60 system.

Figure 1. Trastuzumab concentration dynamics during the TFF run measured by FlowVPX with Cary 60 and FlowVPX with Beams light source compared with SoloVPE at-line measurement

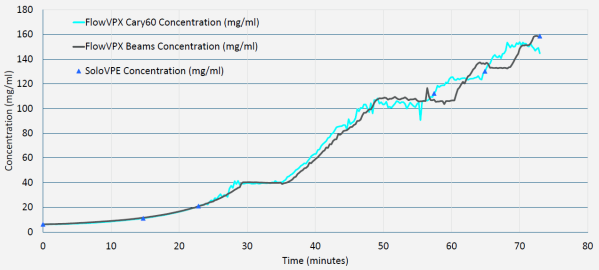

Table 1. Trastuzumab concentration comparison between FlowVPX equipped with Cary 60, FlowVPX equipped with Beams and SoloVPE at-line measurement

FlowVPX Beams concentration (mg/ml)	FlowVPX Cary 60 concentration (mg/ml)	SoloVPE concentration (mg/ml)
6.23	6.27	6.24
11.56	11.18	11.05
20.08	21.03	20.98
107.19	111.93	112.18
135.97	129.79	130.14
158.32	152.44	158.58

Conclusions

The newly introduced CTech Beams System is a reliable, compact light source for the FlowVPX in-line analytical system. The Beams module has several advantages when compared to the Agilent Cary 60:

- Easy installation and integration in a process skid due to the compact design
- Much smaller footprint, by a factor of 16
- Greater measurement speed, by a factor of at least 2
- Higher measurement resolution as a result of lower full width at half maximum (FWHM)
- Full compliance with USP, EP, and JP acceptance criteria
- Uniform single-wavelength modular design (available in 260 nm, 272 nm, 280 nm, and 310 nm canisters)
- IP65 certificate

The CTech Beams light source is an excellent option for both existing and new FlowVPX users looking to implement a more compact system into their processes. In addition, for applications requiring multi-wavelength measurements, the FlowVPX system allows users to easily switch between the Beams and Cary 60 light sources. As a result, Repligen now offers a range of light sources that can fully meet the diverse needs of FlowVPX system users.

References

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