Application of an Effective In-Line Analytical Instrument for Biopharmaceutical Development and Manufacture

BioProcess International
SPECIAL REPORT

Application of an Effective In-Line Analytical Instrument for Biopharmaceutical Development and Manufacture

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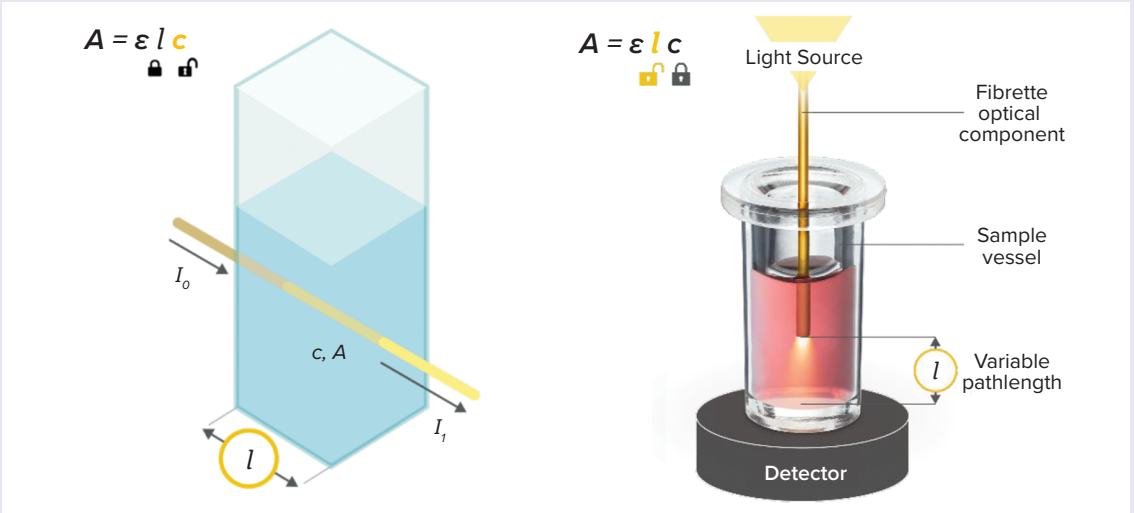
The rapid advancement and competitive environment of the modern biopharmaceutical industry, accompanied by the need for continuous quality improvement, demand robust analytical instruments (**1**). Analytical technology is one key factor contributing to the quality and safety of finished products. Ongoing improvements in analytical instruments are needed to address new challenges, including specificity of target substances, high complexity of matrices, and multiple production stages with a number of input and output parameters and peculiarities (**2**). Those factors point to the demand for a versatile solution, one that can be used accurately and reliably for analyses of intermediate and end products in a number of bioprocesses.

Ultraviolet-visible (UV-vis) instruments constitute a large portion of the analytical tool set currently used in the biopharmaceutical industry (**3**), although most traditional UV-vis technologies have a narrow detection range that can limit functionality in direct applications. Because the light absorbance value in UV-vis is directly proportionate to pathlength of the light and sample concentration (according to the Beer–Lambert law), the fixed-pathlength principle of conventional UV-vis analytical techniques is a key obstacle to expanding the concentration range of an instrument. That calls into question the feasibility of in-line applications of UV-vis instruments. Even off-line measurements with such devices can be problematic because of manual and human error. In this regard, significant benefits can come from an instrument that is capable of high-speed continuous data collection, broad concentration-range measurement, and in-line application. The CTech FlowVPX instrument is an in-line system that fulfills all of the above demands as an analytical tool in the biopharmaceutical industry.

Figure 1: Comparison of traditional UV spectroscopy with variable pathlength technology



Variable Pathlength Technology Principle

The FlowVPX system, as well as its off-line analog, the SoloVPE system, uses patented variable pathlength technology (VPT) based on the Beer–Lambert law. According to this law,

$$A = E \cdot l \cdot C$$

where A is light absorbance, E is the extinction coefficient (characteristic to each individual substance to be detected), l is the pathlength (the distance that light travels through a sample), and C is the concentration of the substance in the sample. It can be posited that absorbance is proportional to both concentration and pathlength. Unlike conventional UV-vis spectroscopy, in which the pathlength value remains constant, VPT enables alteration of the pathlength to obtain a slope: the characteristic of linear regression between light absorbance and pathlength (Figure 1). VPT also allows users to keep the light absorbance value in the linear range by varying the pathlength rather than the analyzed product concentration in the sample, which often requires sample dilution (**4**).

When combining the Beer–Lambert law equation with the linear regression equation, a Slope Spectroscopy equation may be derived:

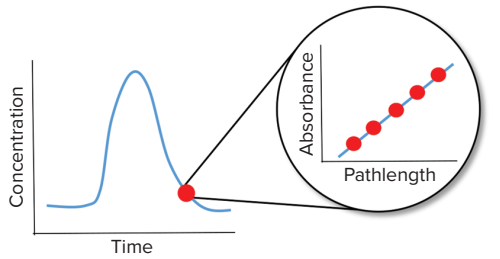
$$m = E \cdot C$$

where m is the slope of the linear regression between light absorbance and pathlength. This approach dramatically broadens the concentration range that can be analyzed, as well as the accuracy and precision of measurement (Figure 2).

Main Advantages of VPT: The broad dynamic pathlength range (0.001–5.000 mm) of VPT removes the need for dilution during measurement by using the system's search algorithm, which optimizes pathlengths to stay within the linear range of the detector. This unique feature of the FlowVPX system enables in-line application — and thus provides users with means for adapting to the rising demands of modern bioprocessing (such as for analyzing samples with increasingly high expression titers) (**5**).

Measurement speed is of great importance for decision-making during a manufacturing process, as is maintaining optimal operation while increasing effectiveness. The FlowVPX instrument can take one concentration measurement in as little as five seconds, which allows for process automation, enables responses to critical process conditions, accelerates process development, and saves substantial time. In most cases, measurement doesn't require baseline correction (although the instrument has this capability, which further simplifies and speeds up the analytics. All these factors have become significant amid the trends toward increased automation and continuous manufacturing in recent decades.

Figure 2: Each point on the concentration curve is a slope value of the linear regression between absorbance and pathlength, based on five or more absorbance readings.



Measurement speed is of great importance for decision-making during a manufacturing process, as is maintaining OPTIMAL operation while increasing effectiveness.

Because different flow cells are available (3 mm, 10 mm, and 22 mm), the FlowVPX device also can operate in a broad range of flow rates: up to 160 L/min. This versatility allows for seamless scaling of a process from laboratory to pilot and ultimately manufacturing scales. Last but not least is the advantage of little to no product loss, which is critical for the manufacture of high-value biopharmaceutical products.

The following sections expand on well-established applications of the FlowVPX system and its suitability for current good manufacturing practice (CGMP) implementation, as well as prospective uses currently under development.

Chromatography

In biopharmaceutical manufacturing, chromatography usually is the initial step of downstream processing (DSP) after removal of cells. Using a number of separation mechanisms, chromatography serves as the primary step for isolation of an end product (**6**). As with upstream processing (USP) stages, the emphasis for DSP is placed on increased efficiency and automation implementation. The trend toward increasing antibody titers in USP has put a strain on the

Figure 3: Application of the FlowVPX instrument in a chromatography skid

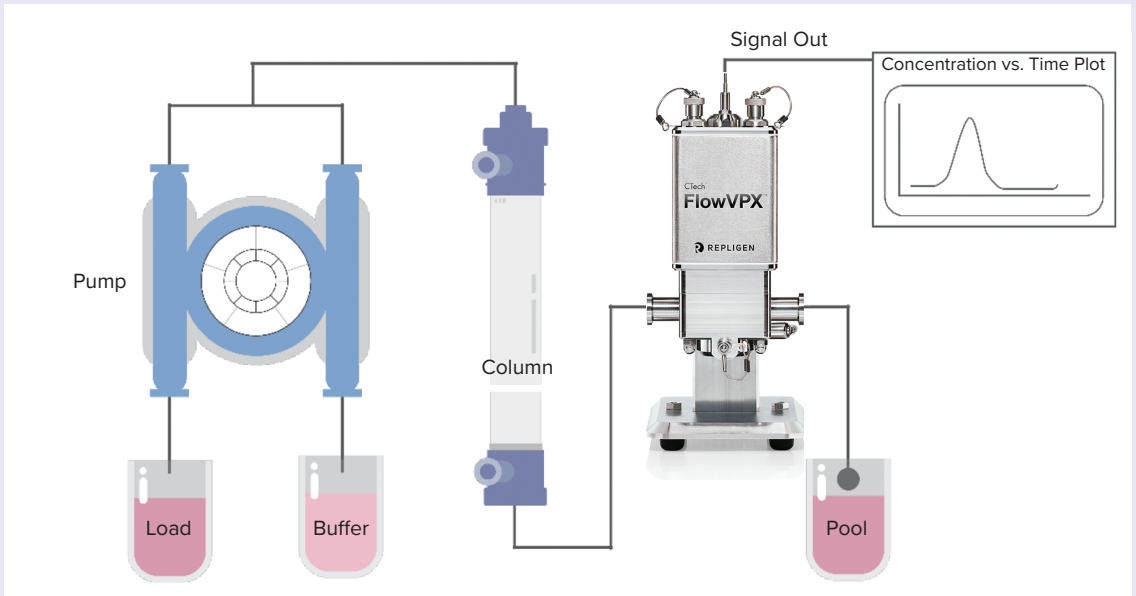
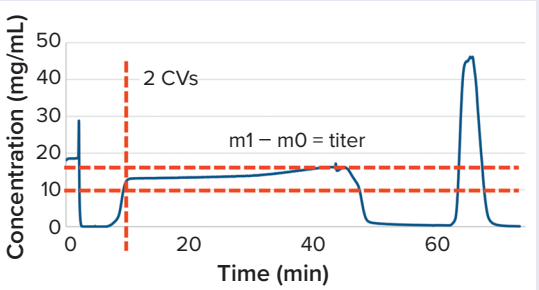


Figure 4: Protein A chromatography overloading and elution



chromatography stages, requiring new means for improving efficiency. Development of new, more effective chromatography resins and efforts to maximize column loading have led to new continuous chromatography techniques for which in-line process analytics are crucial. It should be noted that chromatography also is one of the most expensive steps in an entire DSP sequence, which contributes to the importance of optimizing analytics for this step. Doing so also can lead to considerable cost savings (**7**). Because the FlowVPX system has a broad dynamic range and in-line application, it can serve effectively as the main analytical tool during a chromatography step. Figure 3 demonstrates typical positioning of a FlowVPX instrument in a chromatography setup.

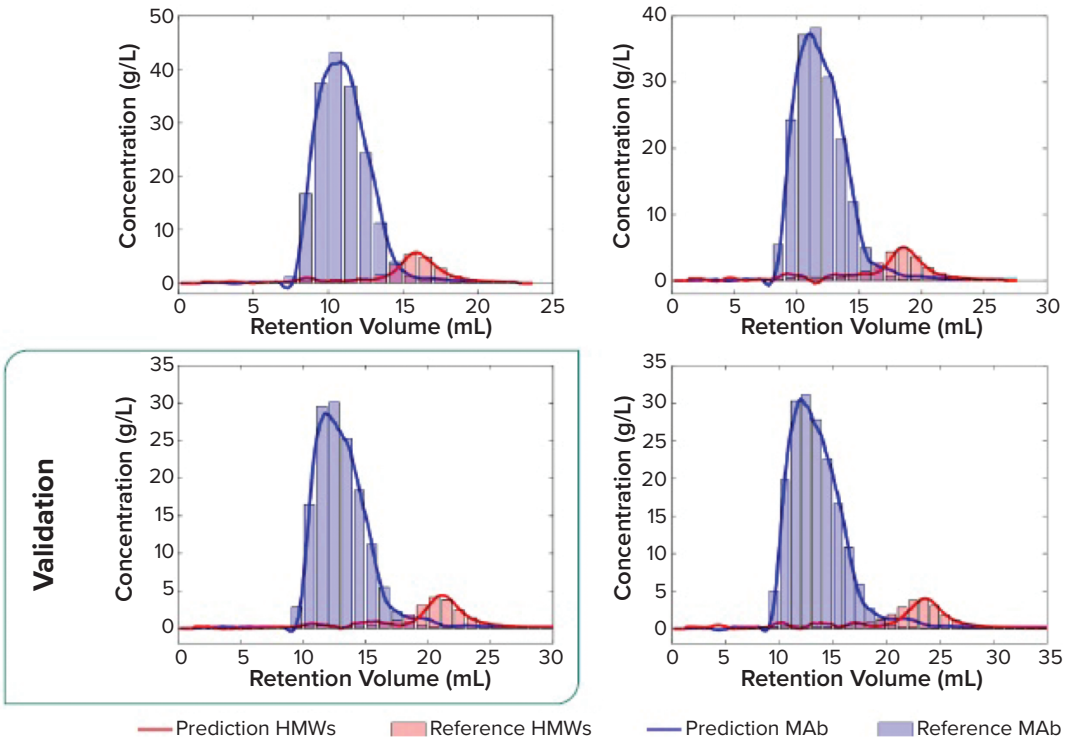
VPT allows for a deepened understanding of a process as well as acquisition of real-time product concentration. For example, during the protein-A chromatography stage, the device can register high product concentrations in real time. It also enables determination of product titer by taking the concentration value in the column effluent after two column volumes (CV) and subtracting it from the concentration value of the clarified cell-culture fluid (Figure 4).

Other case studies of FlowVPX system application in chromatography purification steps are presented below, although the range of applications is by no means limited to these examples.

Dynamic Binding Capacity (DBC): DBC of a chromatography column indicates the maximum amount of a protein that can be isolated under predetermined flow conditions before the beginning of a breakthrough of unbound protein. In other words, it indicates the highest effective loading of a column (most often, 10% of a column's breakthrough). High amounts of nontarget proteins, which are not bound to a column but should be taken into account in calculations, often lead to the saturation of a fixed-pathlength UV sensor, precluding in-line concentration measurement using that analytical technique. Traditional off-line measurements have costly drawbacks, including long wait times and high error rates.

The FlowVPX system and other VPT instruments, however, avoid the above drawbacks in DBC determination while offering high accuracy and efficiency during chromatography. For instance, Bhangale et al. (**8**) have shown that the FlowVPE system, the FlowVPX system's predecessor, was used successfully for precise DBC determination of a chromatography column. The authors

Figure 5: Comparison of partial least squares (PLS) model prediction for monoclonal antibody (MAb) monomers and high molecular weight variants (HMWs) with the results of the off-line reference analytics



demonstrated the viability of analytical VPT for accurate and rapid DBC determination for different classes of monoclonal antibodies (MAbs) and fusion proteins with concentrations >60 mg/mL.

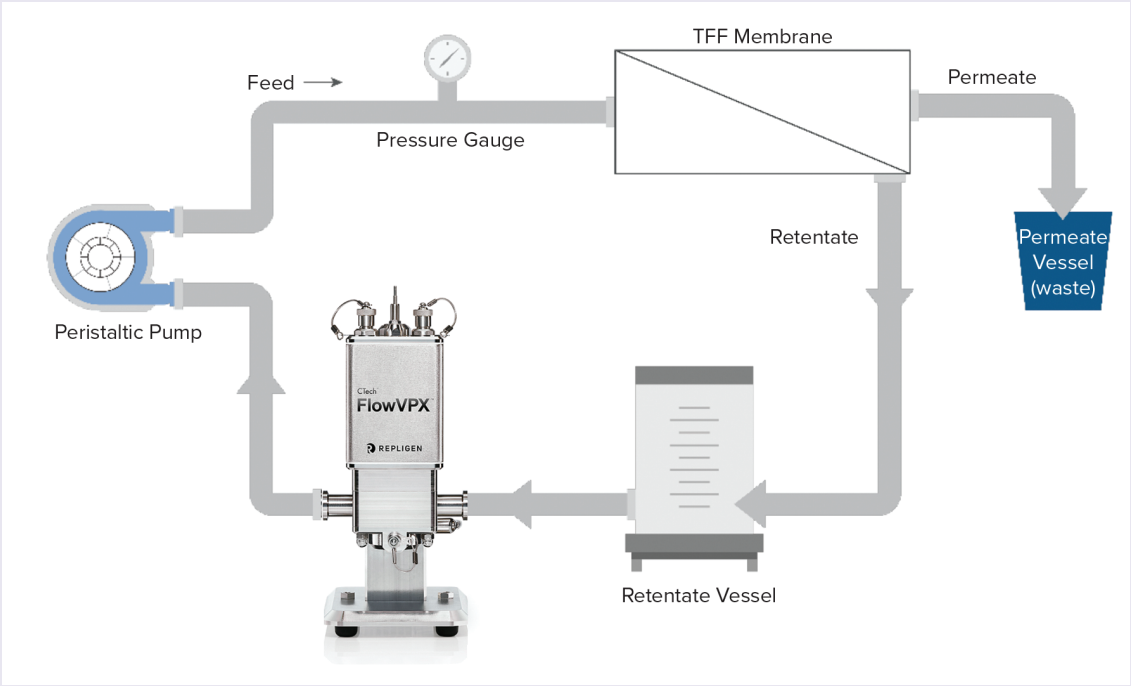
Partial Least Squares (PLS) Models — Polishing Step: Jürgen Hubbuch's team at the Karlsruhe Institute of Technology has expanded VPT by applying it alongside PLS models, with the goal of achieving selective quantification of MAb monomers and high molecular weight variants (HMWs) (**9**). The team developed an automated system for the preparative-chromatography polishing step to demonstrate the usability of the approach for in-line control (performed as part of the Horizon 2020 project). They showed that a combined VPT-PLS approach enables selective determination of proteins: The total column loading was 92 g/L for the separation of lysozyme and cytochrome with a root mean square error (RMSE) of 1.07 g/L. For the MAb polishing step, loading was 40 g/L, with an RMSE of 0.42 g/L (Figure 5). The results obtained indicate the prospective application of a combined approach for in-line monitoring and process control for the selective purification of individual protein species in preparative chromatography.

Ultrafiltration/Diafiltration

Ultrafiltration and diafiltration (often referred to as UF/DF) are common processes in biopharmaceutical manufacturing. Typically, UF/DF occurs in three stages: the first UF run increases the initial product concentration after the polish chromatography step; the subsequent DF step reduces the solution's ion strength or substitutes the initial buffer with another one that is more suitable for final manufacturing operations; and finally, a second UF run is used to maximize product concentration. This combined process can present a number of complications when using traditional UV measurement methods:

- Dilution may be necessary because of the high working concentration of a product in solution, which can lead to measurement errors.
- Considerable wait times may lead to production delays.
- Large delays between off-line measurements can reduce the amount of data generated and, in turn, compromise process understanding.
- Sparse, off-line analytics, often combined with auxiliary measurement methods such as retentate/permeate weight determination, consume resources and time.

Figure 6: A FlowVPX system in an ultrafiltration/diafiltration (UF/DF) setup using tangential-flow filtration (TFF)



The results obtained indicate the prospective application of a COMBINED APPROACH for IN-LINE MONITORING and PROCESS CONTROL for selective purification of individual protein species in preparative chromatography.

• A unit operation may occur during a third shift when quality-control (QC) personnel are unavailable for off-line testing.

• An operator may be unable to monitor process data remotely in real time, which can lead to adverse deviations.

The FlowVPX System, as an in-line analytical instrument in the UF/DF step, effectively addresses that latter issue and successfully overcomes the other problems mentioned above. Measurements are taken directly in the production flow, and high data frequency provides real-time product concentration values, helping operators to make rapid process decisions. In a UF/DF setup, the FlowVPX system typically is positioned after the retentate vessel and before the pump in the UF skid on the feed line (Figure 6). For single-pass tangential-flow filtration (SPTFF), the FlowVPX instrument should be placed directly after the membrane on the retentate line (Figure 7).

The broad detection range of the FlowVPX system (0.1 mg/mL to ›300 mg/mL) makes it especially suitable for UF/DF. For example, the device can measure protein concentration in UF/DF at values in which conventional UV sensors would be saturated (280 mg/mL in this particular case), as seen in Figure 8. The high accuracy and precision of FlowVPX measurement were confirmed by parallel comparisons with a standard off-line A_{280} method using gravimetric dilution and the SoloVPE instrument in a UF/DF run (**10**).

Dilution/Compounding/Fill and Finish

The drug manufacturing process continues with the dilution/compounding stage. A final bulk drug substance is diluted to its target concentration and mixed with excipients: buffers, salts, surfactants, polyols/disaccharides/polysaccharides, amino acids, antioxidants, and so on. The final filtration occurs, and the product is filled in primary containers (**11**). Concentration control is demanded both in final formulation and in each of the primary containers because it is one of the most important critical quality attributes (CQAs) of a product and is crucial for patient safety. To ensure safety and efficiency, the analytical methods in the

Figure 7: A FlowVPX system in a single-pass tangential-flow filtration (SPTFF) skid

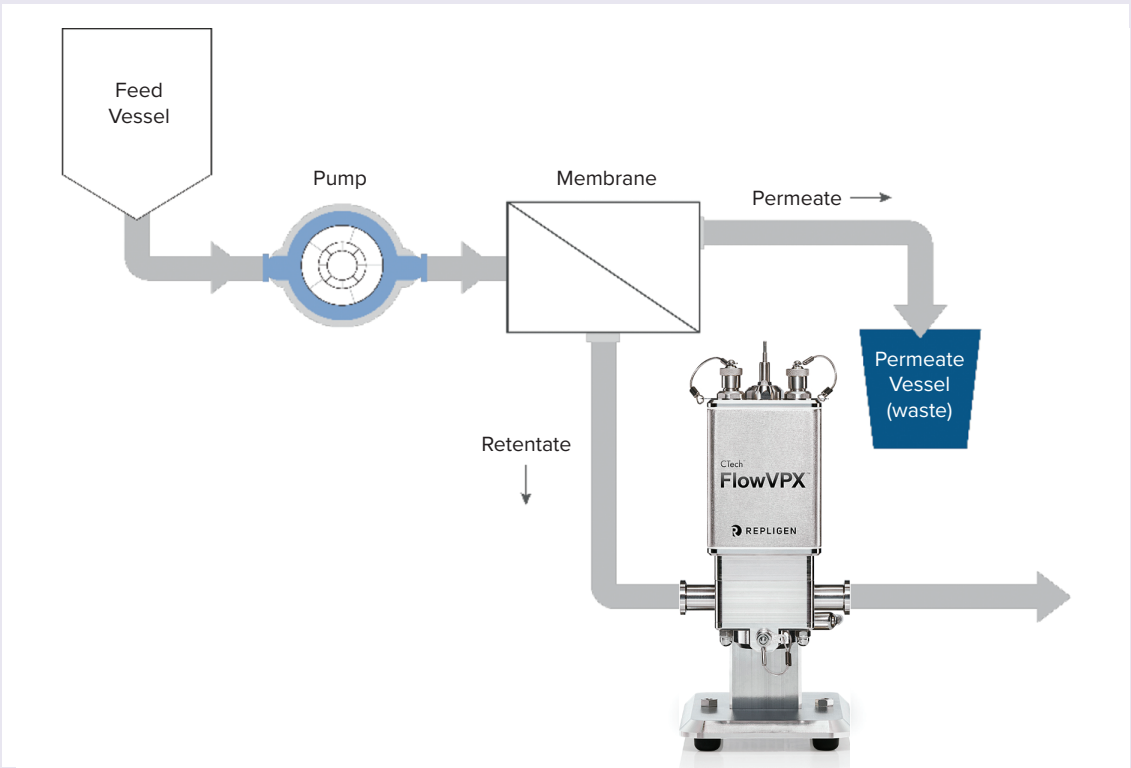


Figure 8: FlowVPX system in the UF/DF when compared with A_{280} (gravimetric dilution) and SoloVPE system; blue rounds = FlowVPX, red rounds = SoloVPE, black rectangles = A_{280}

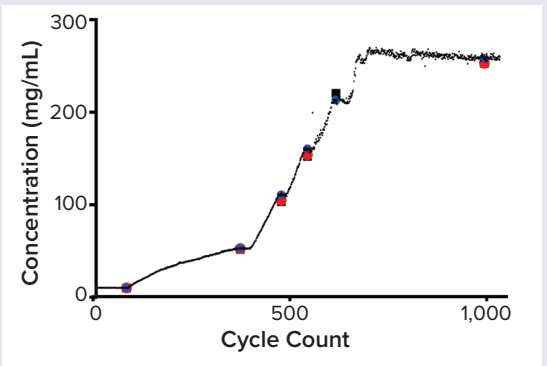
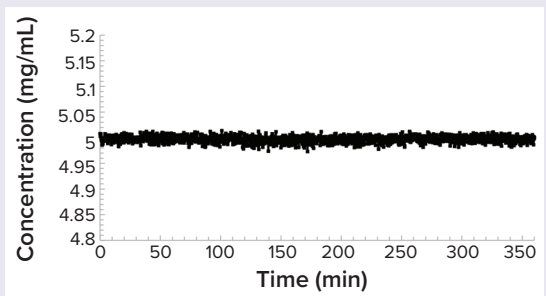


Figure 9: VPT application for in-line concentration control in primary containers (fill and finish step, **12**)



aforementioned steps should be accurate, fast, and preferably in-line. They also should be able to quantify a target substance within a large concentration range.

Proper implementation of a VPT instrument in a filling process line enables concentration measurement in every primary container. Automation strategies such as that allow for concentration control on each unit in a batch instead of sampling only a limited number of units (Figure 9). Application of the instrument during this step also may enable implementation of real-time release testing (RTRT). The many benefits of RTRT include increased manufacturing efficiency through reduced inventory and lower laboratory costs, as well as the potential to provide increased assurance of product quality (**13**). Continuous measurement also enables estimation of concentration-measurement accuracy throughout an entire batch and use of data for QC.

GMP Implementation

GMP compliance is critical for implementing an analytical instrument into a biopharmaceutical workflow. The FlowVPX system is designed for complete GMP compliance in terms of build,

Figure 10: CTech ViPER Anlytx software platform user interface

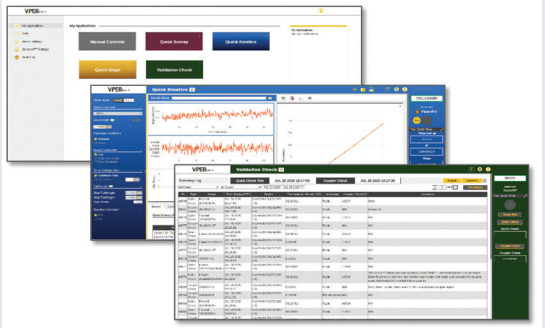


Figure 11: Single-use one-inch flow cell for FlowVPX system

Table 1: Flow cell total organic carbon (TOC) testing

Sample Description	Swab Location	NaOH Wash Run Time (min)	TOC Content (ppb)
FlowVPE 10 mm Flow Cell	Piping	30	285
	Window	30	205
	Flow fibrette tip	30	172
FlowVPE 10 mm Plastic Flow Cell	Piping	30	113
	Window	30	78
	Flow fibrette tip	30	149
FlowVPE GxP 10 mm Flow Cell	Piping	30	153
	Window	30	168
	Flow fibrette tip	30	152
FlowVPE GxP 10 mm Flow Cell	Piping	60	107
	Window	60	86
	Flow fibrette tip	60	96
FlowVPE GxP 22 mm Flow Cell	Piping	30	184
	Window	30	114
	Flow fibrette tip	30	64
FlowVPX 3 mm Flow Cell	Window	30	144
	Flow fibrette tip	30	123

functionality, and software complementarity. The instrument has a 316-L stainless-steel body with a fully enclosed design to withstand the harshest manufacturing environments, and it has received IP65, C1D2, and CE certification.

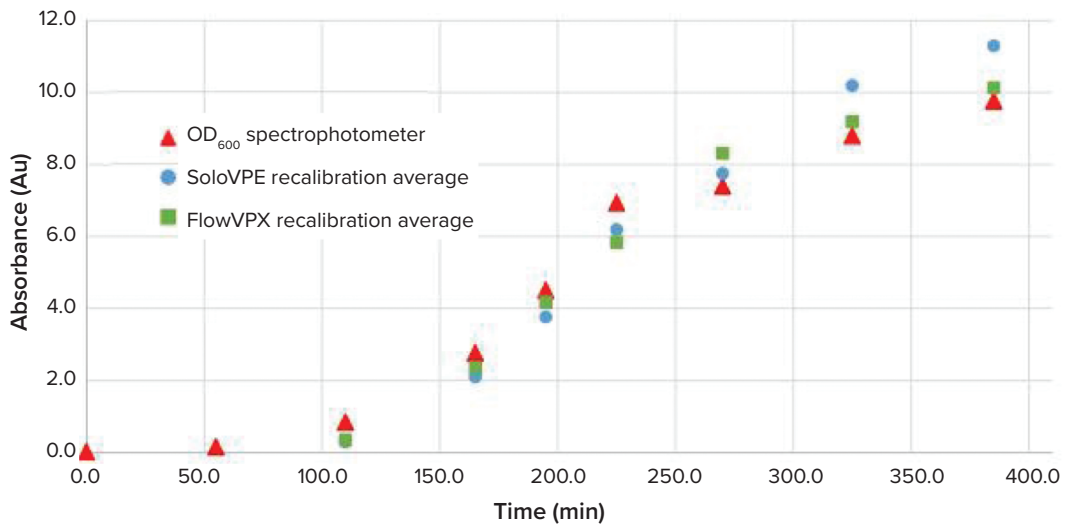
The device was designed to conform to GMP requirements. Its features include an ethylene propylene diene monomer (EPDM) diaphragm seal that is validated to withstand clean-in-place (CIP) procedures. CTech ViPER Anlytx software, which acts as an interface for the FlowVPX system and other VPT devices, supports GMP compliance and computer software validation. Its key features include database architecture management, lightweight directory access protocol (LDAP) integration, analog inputs/outputs, built-in support for OPC unified architecture (UA), 21 CFR Part 11 compliance for electronic records and signatures, and flexible report generation suitable for audit trails (Figure 10) (**14, 15**).

The cleaning validation procedure is an important part of GMP implementation. The flow cell is the only part of the FlowVPX instrument that has contact with a product. An internal cleaning study was performed for different flow cells to determine the effectiveness of standard CIP procedures on different flow cells. Those experiments yielded the results shown in Table 1.

The test was performed by filling flow cells with 100 mg/mL solution of human immunoglobulin G (IgG), leaving them for one hour, emptying them, and leaving them uncleaned for 24 hours. Then the flow cells were cleaned for 30 or 60 minutes with 0.5 M sodium hydroxide at a flow rate of 40 mL/min, then rinsed and swabbed at different locations for total organic carbon (TOC) determination. TOC values must be lower than 500 ppb to fulfill the official requirements of the European/United States/Japanese pharmacopoeias (EP/USP/JP).

It should be noted that CIP is not the only effective cleaning method for flow cells. The flow cells also were validated internally for multiple autoclave cycles. The FlowVPX system's scalability, efficiency, and ease of cleaning make it uniquely fit to address the parameters set forth in GMP

Figure 12: Comparison of in-line and off-line measurements with a standard spectrophotometer and VPT for each sampling time point



regulatory documents for method validation. The same is true for the system's specificity, working range, accuracy and precision, and robustness (**16**), which are evidenced by multiple successful GMP implementations at different process stages worldwide (**17**).

Process Analytical Technology: A major trend in CGMP analytics is application of process analytical technology (PAT), which also plays an important part in the concept of Bioprocessing 4.0 and the associated digitalization and automation of the biomanufacturing industry. In 2004, the US Food and Drug Administration (FDA) defined a *process analytical technology* as "a system for designing, analyzing, and controlling manufacturing through timely measurements (e.g., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality" (**18**). The biopharmaceutical industry's PAT initiative was introduced as a response to regulatory demands to increase process understanding and build quality into manufacturing processes — with an obvious emphasis on in-line monitoring and control (**19**).

Although the small-molecule pharmaceutical industry has implemented PAT comprehensively, application in the biopharmaceutical industry has been hampered by the complexity of both products and processes, as well as the high chemical similarity of impurities. The FlowVPX System offers several advantages that make it a strong candidate for PAT tool development:

- The FlowVPX system is the only UV-vis analytical instrument that can cope with the broad range of concentrations that is intrinsic to modern biopharmaceutical manufacturing.
- The technology is based on a relatively basic analytical principle and doesn't demand development of additional models, calibrations, or excessive elaborations, as are needed for other prospective PAT tools (e.g., Raman and near infrared (NIR) spectroscopy).
- The method is operator-friendly, and the system provides an easy-to-use interface for manufacturing.
- The system has features such as OPC UA incorporation that provide for seamless integration into process monitoring and control systems.

Single-Use Systems: Application of single-use (SU) equipment and related manufacturing concepts is among the fastest and largest growing trends in the biopharmaceutical industry. SU now is used for more than 85% of precommercial activities, mainly preclinical and clinical biopharmaceutical manufacturing, and it is being adopted increasingly for commercial product manufacturing (**20**). In this vein, Repligen offers a line of SU flow cells (Figure 11) that are made of polyphenylsulfone (PPSU), an ideal polymer for medical applications because of its mechanical stability, heat resistance, and resistance to high-energy radiation.

FlowVPX SU flow cells provide all of the advantages of SU systems in terms of cleanliness and efficiency. They fulfill the requirements of SU

Bacterial cell growth can be monitored with high **ACCURACY** and **REPEATABILITY** using the in-line FlowVPX system, with results comparable to OD_{600} data from a standard spectrophotometer.

approaches for process skid development, while providing measurements comparable with those from their stainless-steel counterparts.

Prospective Applications

As mentioned above, applications of the FlowVPX system are not limited to chromatography, UF/DF, and compounding steps. Other applications that are in development are touched on below.

Adeno-associated viruses: Adeno-associated viruses (AAV) are the most popular viral vector for gene therapy. VPT has proven to be a viable method for determining AAV concentrations. The ratio between full and empty AAV capsids was determined at 280 nm and 260 nm, respectively, which is an important QC parameter in AAV manufacture. The AAV titer during the UF/DF step was determined as well, with average values of 7.2×10^{12} vg/mL.

Oligonucleotides: In collaboration with Ionis Pharmaceuticals, Repligen assessed the feasibility of the FlowVPX system for determining antisense oligonucleotide (ASO) concentrations in highly concentrated eluate obtained from a reversed-phase chromatography process (**21**). In this study, readings from the VPT device were compared with a conventional ultraviolet absorbance chromatogram. The FlowVPX system measured ASO concentrations of more than 80 mg/mL, an amount that could not be measured by conventional UV sensors.

Microbial Growth: Optical density at wavelength 600 nm (OD_{600}) is a standard measurement method for determining microbial growth in the process of fermentation/cultivation. With most conventional UV-vis spectrophotometers, this method must be performed off line, and it requires a dilution after a certain growth period. Available in- and off-line sensors do not provide reliable data at high cell densities.

The Biofactory Competence Center (BCC) and Repligen tested the FlowVPX system for its ability to measure *Escherichia coli* growth during fermentation in a laboratory-scale reactor (Figure 12) (**22**). Those experiments showed that bacterial cell growth can be monitored with high accuracy and repeatability using the in-line FlowVPX system, with results comparable to OD_{600} data from a standard spectrophotometer.

Essential Advantages

The FlowVPX system is an in-line analytical instrument for real-time UV-vis measurements based on VPT. Compared with other instruments, it provides a number of advantages for bioprocessing applications, including

- fast, reliable measurement capabilities that provide for real-time monitoring and control
- a large concentration range (from 0.1 mg/mL to more than 300 mg/mL) that enables users to leverage all the benefits of an in-line approach
- high linearity, accuracy, precision, selectivity, and robustness of measurement
- support for full GMP compliance, allowing for successful validation and implementation in biopharmaceutical manufacturing
- reliance on UV-vis data, eliminating the need to develop additional and complicated models such as those needed for Raman or NIR spectroscopy
- ability to operate at flow rates as high as 160 L/min, enabling successful scale-up of a process from laboratory through manufacturing stages
- good resolution at high speeds, providing users quickly with larger amounts of process information than what can be obtained using conventional measuring techniques
- robust collection of process data, enabling development of data-intensive models (e.g., PLS methods) that would be impossible to create using off-line methods.

Such advantages make the FlowVPX system a well-established analytical instrument for chromatography, UF/DF, and compounding/fill–finish steps of recombinant-protein manufacture. The instrument also can be used to measure parameters for other biological modalities — such as for determination of AAV, ASO, and antibody–drug conjugate (ADC) concentrations, as well as for measurement of microbial growth (OD_{600}). Many other applications are under development. Compatible with initiatives toward automation and digitalization, the FlowVPX system can meet current and future needs for scalability and process control.

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In Memory of Ramsey Shanbaky (2 August 1978–16 July 2022)

Ramsey Shanbaky was an extraordinary member of our Repligen family. His larger-than-life personality manifested itself through his passion for living and through an equally large heart. Ramsey was comfortable speaking about laser systems, medical devices, bioprocessing workflows, and of course Slope Spectroscopy techniques, but in many ways his personality was his superpower. Curious, creative. and genuine to his core, Ramsey was able to forge strong, collaborative relationships with his colleagues, customers. and anyone else who had the good fortune to meet him.

Ramsey was part of the team that brought the world variable pathlength technology and the Slope Spectroscopy method. He spent years traveling the world to educate biotechnology and pharmaceutical scientists on the technique and explain its numerous benefits. He started with the SoloVPE system and then became the champion of in-line solutions such as the FlowVPX system. Ramsey never lectured. He merely engaged in conversations, asking at least as many questions as he answered and learning as much as he taught. For Ramsey, it was fun. For his customers, it was empowering and valuable.

Ramsey earned a reputation for being a bit of a cowboy. His confidence in the technology was so great that he was willing to try new things in the middle of demos and presentations. He did this with a calm confidence that could drive his fellow engineers a little crazy. But all were thankful that Ramsey was on their team.

Ramsey's passions certainly did not begin and end with his professional endeavors. The greatest priority in his life was his family. He would light up when talking about kite surfing with his wife or playing Minecraft or snowboarding with his son. His engineering and analytical talents were not directed solely toward spectroscopic endeavors. His home-brewed coffee roasting system and process-control techniques produced coffees that were fought over whenever those precious vacuum-sealed bags made their way into the office.

Ramsey's enthusiasm, energy, ideas and friendship are dearly missed. He was a bright light, gone too soon.