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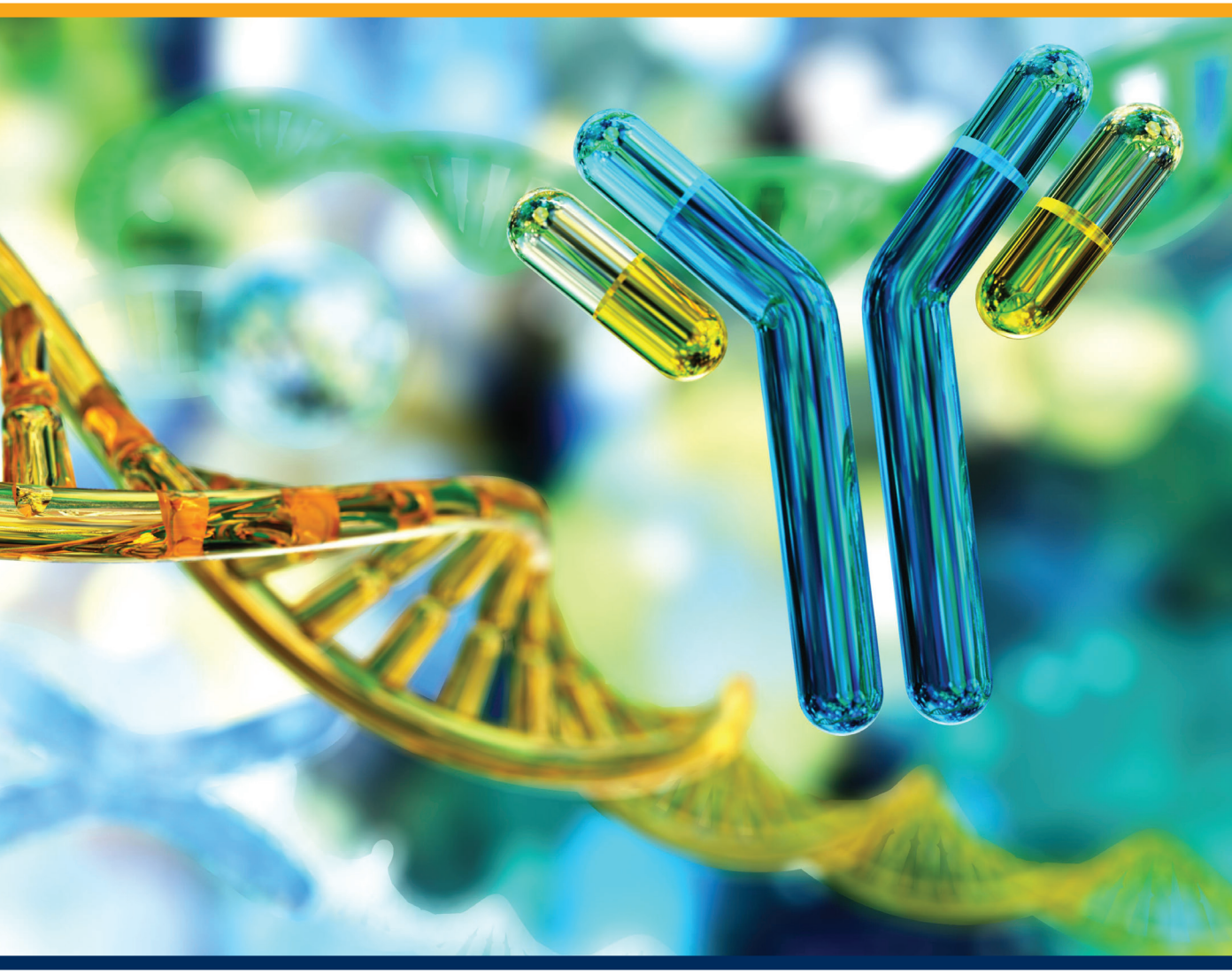
SPECIAL REPORT

Optimizing Efficiency in High-Concentration Biopharmaceutical Production



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Optimizing Efficiency in High-Concentration Biopharmaceutical Production

In-Line, Concentration-Controlled Tangential-Flow Filtration

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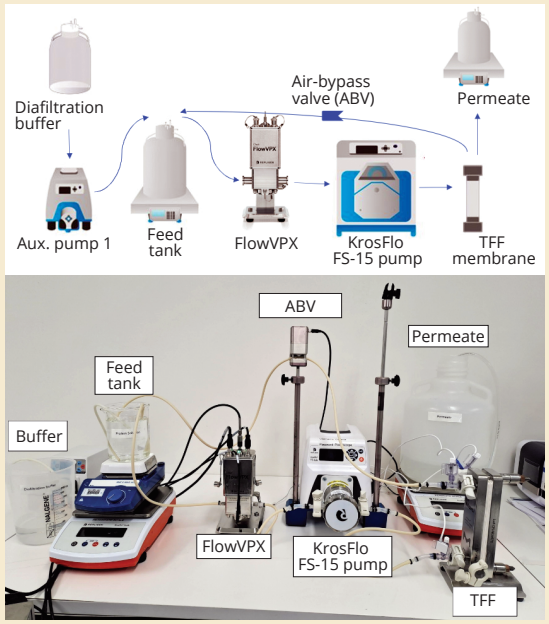
Membrane technology plays a critical role in biopharmaceutical purification, guiding manufacturing processes seamlessly from cell-culture clarification, through purification and polishing chromatography steps, and finally to fill–finish (**1**). Ultrafiltration (UF) and diafiltration (DF) precede the fill–finish stage. Often, those steps are integrated into a single process (UF/DF) that concentrates a drug substance and exchanges the buffer with a final formulation buffer. UF/DF operates on cross-flow filtration, also known as *tangential-flow filtration* (TFF), in which feed flows tangentially to a membrane's surface rather than through it (**2, 3**). Traditional UF/DF systems comprise a feed/retentate tank, permeate tank, membrane holder, and a main pump that controls feed flow toward the TFF membrane (**4**).

UF/DF processes typically involve mass-balance monitoring using scales or flow meters, requiring at-line samples for verifying protein concentration and other process parameters. However, that approach has drawbacks, including the need for detailed planning based on measurements of hold-up volumes, mass, and protein concentration. Variability across runs is a concern because UF/DF operation relies on a single variable (mass), necessitating pre- and post-run concentration measurements. Regular sampling introduces potential for human errors, material depletion, and contamination (**5**).

The therapeutic-antibody market continues to grow significantly, with 122 and 114 therapies having received US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval, respectively, as of June 2022 (**6**). Especially for subcutaneously administered therapeutic proteins, developers increasingly are selecting high-concentration formulations to work with delivery devices' restricted injection volume (1–1.5 mL), which is designed to minimize backpressure and injection pain (**7**). That trend has led to development of formulations that are concentrated enough to deliver the required amount of drug within the limited injection volume. Such formulations hold promise for simplifying drug-product storage and handling, but their manufacture is not without significant challenges (**8, 9**). During UF/DF processes, *high-concentration antibody products* — classified as injectable monoclonal or polyclonal antibody (mAb, pAb) therapies with concentrations ≥100 mg/mL — raise concerns for viscosity, pressure, cross-flow flux, and membrane fouling (**8, 10**). Moreover, protein–protein intermolecular forces increase solution opalescence, complicating concentration and turbidity determination (**11, 12**).

Increasing demand for high-concentration mAb drugs places a significant burden on development teams to design and optimize process steps with increased protein content while maintaining critical quality attributes (CQAs). Concerns relating to stability, aggregation, and overall product quality

Figure 1: (TOP) Schematic of a CTech FlowVPX system connected in line (on the feed line) with a KrosFlo FS-15 RPM tangential-flow filtration (TFF) system; (BOTTOM) laboratory setup for the connected systems



intensify with increased protein content (**8, 10**). Traditional analytical methods sometimes cannot provide timely insights about such parameters, creating industry need for advanced solutions that generate and analyze data in real time.

Defined by the FDA as means for designing, analyzing, and controlling pharmaceutical manufacturing processes, *process analytical technologies* (PATs) offer a framework for continuous, real-time assessment of critical parameters during biopharmaceutical development and manufacturing (**13–16**). Well-known PAT methods include in-line Raman spectroscopy and near- and mid-infrared (NIR, MIR) spectroscopic analyzers (**14, 17–19**). Although such instruments enable comprehensive monitoring of multiple process parameters (e.g., protein concentration, aggregation levels, and excipient concentrations), the resulting data are not readily interpretable (**19, 20**). Fully leveraging vibrational spectroscopic techniques requires that analysts apply specialized skill sets to process and make sense of collected data. Moreover, to extract valuable information in the form of concentration levels, analysts need additional skills in chemometrics and mathematical modeling. Thus, implementing PAT into operations often is difficult and time-consuming (**21**).

An alternative method for in-line, real-time monitoring of protein concentrations is variable-pathlength technology (VPT) based on ultraviolet–visible (UV-vis) spectroscopy and, accordingly, the Beer–Lambert law (**22–24**). Unlike traditional fixed-pathlength sensors, the CTech FlowVPX spectrophotometry system enables dynamic pathlength adjustment, compensating for the high absorbance of concentrated samples and providing accurate concentration measurements within seconds (**25, 26**). The resulting real-time data inform an automated control system that reacts promptly to deviations, ensuring that a mAb formulation process stays within a predefined design space.

In a traditional mass-balance UF/DF process, deviations in concentration often are detected after they have occurred, diminishing overall process efficiency. By contrast, the automated KrosFlo FS-15 TFF system provides for proactive control based on real-time concentration measurements with a VPT device (**25, 27**). Such capability can reduce the likelihood of quality deviations and enhance the robustness of an entire downstream process.

Herein, we present an in-line, automated, and concentration-controlled TFF process for high-concentration mAb formulations. We show that a holistic approach combining VPT (in the FlowVPX system) with capabilities for automated TFF and in-line concentration control addresses challenges posed by traditional mass balances during downstream development (**27**). To demonstrate how such an approach enhances process control, scientists from our team conducted experiments with samples representing a broad array of high-concentration products (90–250 g/L). Our results indicate that automated TFF significantly improves process efficiency, reducing the need for corrective actions after process completion, eliminating human error, and enhancing overall product quality.

Materials and Methods

Materials: UF/DF studies used immunoglobulin G (IgG) mAbs derived from a Chinese hamster ovary (CHO) cell culture (Alvotech). Before the experiments, mAb samples underwent a series of clarification and processing steps, including depth filtration of harvest, protein A affinity chromatography, viral inactivation, polishing chromatography, and nanofiltration. The equilibration buffer (40 mM of excipient 1 and 135 mM of excipient 2, pH 6.0) was exchanged with DF buffer (5 mM of excipient 3 and 240 mM of excipient 4, pH 6.0) for final formulation of the protein material.

Equipment and Experimental Setup: Our team connected the 3.0-mm flow cell of a CTech FlowVPX system (Repligen) to a KrosFlo FS-15 RPM TFF system (Repligen) using Luer connectors (Kemía ehf, EW-45512-04) and 1.6-mm tubing (Masterflex L/S 16 precision pump tubing, EW-96410-16). The flow cell was connected on the feed line between the retentate vessel and pump (Repligen, 999460). A magnetic stir plate (IKA C-MAG MS 4) operating at 100–200 rpm ensured consistent mixing in the retentate tank.

Mirroring traditional UF/DF processes, an auxiliary pump (Repligen, F20000655), permeate scale (Repligen KrosFlo, ACSS-20K), and feed scale (Repligen, KrosFlo, ACSS-20K) regulated the DF phase along with a predefined number of diavolumes (DVs). Concentration phases relied on in-line measurements from the FlowVPX system, predefined in the RPM software as the endpoint values determined by the VPT device.

The filtration systems used TangenX SIUS PDn HyStream (LP screen) single-use cassettes with surface areas of 0.01, 0.02, and 0.1 m^2 (Repligen, XP030MP2L, XP030MP1L, XP030M01L). Those cassettes feature a filter capacity of <500 g/m^2 and a molecular-weight cut-off (MWCO) of 30 kDa. Membranes were installed in a Pellicon cassette holder (Millipore, XX42PMINI) with the manufacturer-recommended torque setting of 180 lb and using the provided gaskets to prevent leaks. Figure 1 depicts the entire setup.

Figure 2: Real-time protein concentration trend from a concentration–diafiltration–concentration (C/D/C) run controlled by RPM software (initial concentration to 40 g/L and final concentration to 90 g/L); the dotted line represents the target concentration as determined by the software.

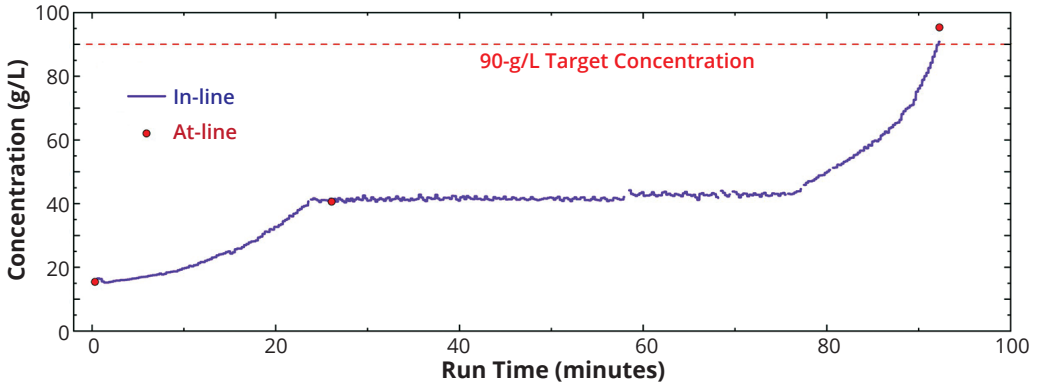


Table 1: Comparison of in-line (FlowVPX system) and at-line (SoloVPE system) protein-concentration measurements for each run at different process steps

	Starting Concentration (g/L)			Initial Concentration (g/L)			Final Concentration (g/L)		
Run	SoloVPE	FlowVPX	%Diff	SoloVPE	FlowVPX	%Diff	SoloVPE	FlowVPX	%Diff
1	15.2	14.7	2.9	40.7	42.5	4.2	93.4	91.5	2.0
2	15.4	15.6	0.9	40.6	41.2	1.6	95.3	90.8	4.7
3	15.2	14.9	2.0	42.5	41.8	1.5	146.7	149.4	1.8
4	15.4	15.1	2.1	41.7	41.9	0.6	214.0	203.9	4.7
5	15.1	15.3	0.7	40.9	40.8	0.2	212.9	215.1	1.0
6	10.4	10.7	3.0	42.5	42.5	0.0	215.1	218.8	1.7
7	10.4	10.1	2.5	42.8	42.4	0.9	250.5	251.9	0.5

Our study comprised seven independent runs. Before each run, the system underwent preconditioning with a 10-L/m^2 water flush followed by a 10-L/m^2 flush with equilibration buffer (40 mM of excipient 1 and 135 mM of excipient 2, pH 6.0) to saturate it with a buffer optimal for the mAb. Starting protein solutions had concentrations of 10–16 g/L depending on the batch source. For all runs, a solution was concentrated to 40 g/L. The equilibration buffer was exchanged with a final formulation buffer (5 mM of excipient 3 and 240 mM of excipient 4, pH 6.0). Then, the solution underwent a second concentration step to a target value. Final concentrations ranged from 90 to 250 g/L, representing a broad distribution. The flow rate differed by membrane area but was maintained at 3 L/m^2/min, while transmembrane pressure (TMP) was regulated at 1.0 ± 0.3 bar using an air-bypass valve.

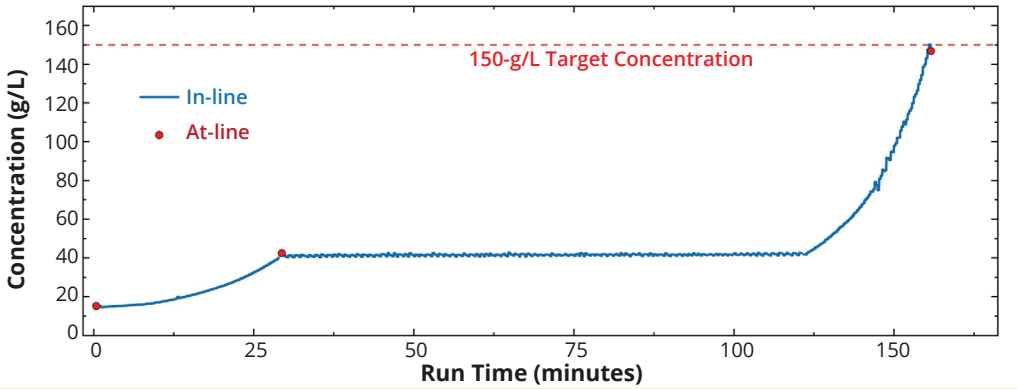
Protein-Concentration Measurements: UF/DF experimental protocols were established in the RPM software using the concentration–diafiltration–concentration (C/D/C) approach, with concentration endpoints based on in-line measurements from the FlowVPX system. The DF endpoint was governed by the number of DVs, as regulated by scales. Operating in Quick Slope mode, the VPT device captured seven data points at 280-nm wavelength for each concentration measurement with a known extinction coefficient.

A VPT-enabled CTech SoloVPE Protein A280 system was operated in Quick Slope mode to take at-line measurements for comparison, capturing either 10 or six data points at a wavelength of 280 nm for each concentration measurement, using a known extinction coefficient. The expected protein-concentration range determined the requisite number of data points. For samples with expected concentrations exceeding 100 g/L, we used six data points to ensure linearity in measurement. Samples for at-line measurement were collected for each UF/DF run, including samples from the beginning of the process and from before each concentration step. Table 1 presents those collective measurements.

Results and Discussion

The KrosFlo FS-15 RPM TFF system served as an automated platform for monitoring and managing high-concentration UF/DF runs. Our experimental design followed a C/D/C approach, using in-line protein concentrations as endpoints for both the initial and final concentration phases. Unlike a traditional mass-balance approach, formation of the

Figure 3: Real-time protein concentration trend from a concentration–diafiltration–concentration (C/D/C) run controlled by RPM software (initial concentration to 40 g/L and final concentration to 150 g/L); the dotted line represents the target concentration as determined by the software.



recipe in the RPM software did not involve extensive calculations dependent on starting protein-solution concentrations and volumes of material in the feed/retentate tank. Instead, the UF/DF process continued automatically to the next setpoint once FlowVPX system readings reached a target value.

To showcase the precision of automated TFF across a broad concentration spectrum, we conducted multiple runs with endpoints ranging from 90 to 250 g/L. Figure 2 plots data from the UF/DF run with a final concentration of 90 g/L, which is a common target for mAbs in final formulation intended for 50–60 g/L during fill–finish (**28, 29**). In the figure, the in-line concentration trend mirrors all phases of the UF/DF process, including initial concentration, DF, and final concentration, with at-line values overlaid for comparison. Throughout the run, automated control maintained key process parameters with a consistently low differential pressure across the membrane. The recipe ultimately achieved the predetermined protein concentration and DV setpoints without issue. In-line concentration values also corresponded closely with at-line values (Table 1).

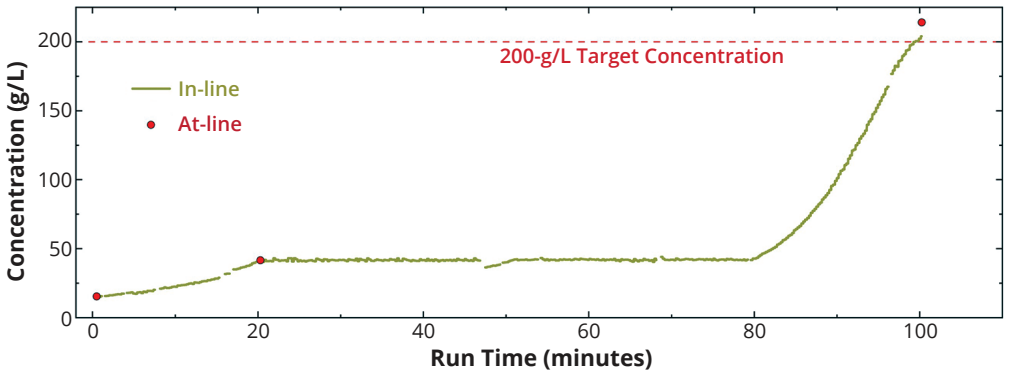
In-line concentration measurements represent a significant advancement in bioprocessing, enabling continuous feedback in real time as compared to sporadic off-line measurements taken at specific time intervals. High-frequency collection of concentration data during different UF/DF stages provides analysts with a comprehensive and immediate overview of a protein product's behavior. That allows for quick detection of deviations, immediate adjustments, and proactive responses to potential issues. Such adjustments are impossible with a traditional mass-balance approach, in which any manual intervention requires process shutdown or a switch to the next setpoint. Moreover, the constant process overview afforded by in-line measurement reduces risks for needing an additional "overconcentration" step if a target concentration is not met initially in the mass-balance–based recipe — a common challenge with highly concentrated formulations.

A run targeting 150 g/L served to investigate the accuracy of in-line, concentration-controlled TFF at a greater concentration (Figure 3). The run exhibited a consistent trend with no significant errors, and in-line and at-line concentration values correlated closely, with a 1.8% difference between the final concentration values. Across all runs, in-line and at-line measurements differed most for starting concentrations. Such discrepancies arise because at-line samples come directly from the starting material, whereas initial in-line samples experience slight dilution from residual equilibration buffer in the tubing used to precondition the membrane. To enhance precision between in-line and at-line measurements, analysts can circulate protein solution in the flow path to adapt the concentration or compare FlowVPX system values after collection of four or five data points. Regardless, the maximum difference between initial FlowVPX measurements and at-line values is 3%, which is negligible (Table 1).

Increasing demand for high-concentration mAb drug products (with final formulations exceeding the 150-g/L threshold) now poses significant bottlenecks for TFF operations (**28, 30**). Achieving final UF/DF concentrations of 200–250 g/L is challenging because of pressure concerns, the need to maintain optimal process conditions, and the work required to support the process. Thus, automating TFF is increasingly attractive to downstream development teams. To assess the KrosFlo system's performance at extreme mAb concentrations, we conducted a number of runs with the goal of surpassing the 200-g/L threshold.

Figure 4 presents data from a run with an endpoint of >200 g/L, as defined in the RPM software. In late

Figure 4: Real-time protein concentration trend from a concentration–diafiltration–concentration (C/D/C) run controlled by RPM software (initial concentration to 40 g/L and final concentration to 200 g/L); the dotted line represents the target concentration as determined by the software.



stages, the protein solution became highly viscous and decreased significantly in volume, complicating operation below the maximum feed pressure (3.0 bar) required toward the end of the process. That necessitated a manual flow-rate reduction. The process terminated when the in-line protein concentration surpassed 200 g/L. However, the at-line reading showed 214 g/L, indicating a 4.7% difference in measurement. The discrepancy suggests that the at-line sample might not have represented the overall solution concentration accurately, emphasizing the need for more samples at higher concentrations to ensure result accuracy. However, for process-development (PD) purposes, the observed 4.7% difference between in-line and at-line measurements fell within an acceptable range given typical specifications for the final concentration step in our UF/DF process, allowing for further processing and adjustments.

To validate the consistency between in-line and at-line measurements at high concentrations, we performed two additional runs (5 and 6 in Table 1). Results showed differences in measurement of 1.0% and 1.7%, respectively. Those findings underscore both the importance of well-mixed solutions for at-line measurements and the need to take more samples at high concentrations. Moreover, inadequate in-process mixing at low volumes — especially during PD — can skew in-line readings and prematurely end a process. RPM software is programmed to stop a process after the first reading above a target value, posing problems for high-concentration solutions at low volumes. For runs aiming at 200 g/L, we adjusted the setpoint to >215 g/L. To ensure proper mixing and attainment of a target concentration, it is critical to obtain at least five consistent readings at a target value. We recommend selecting a setpoint of ≥5 g/L above the target in the RPM software to ensure the expected outcome and minimize need for manual intervention.

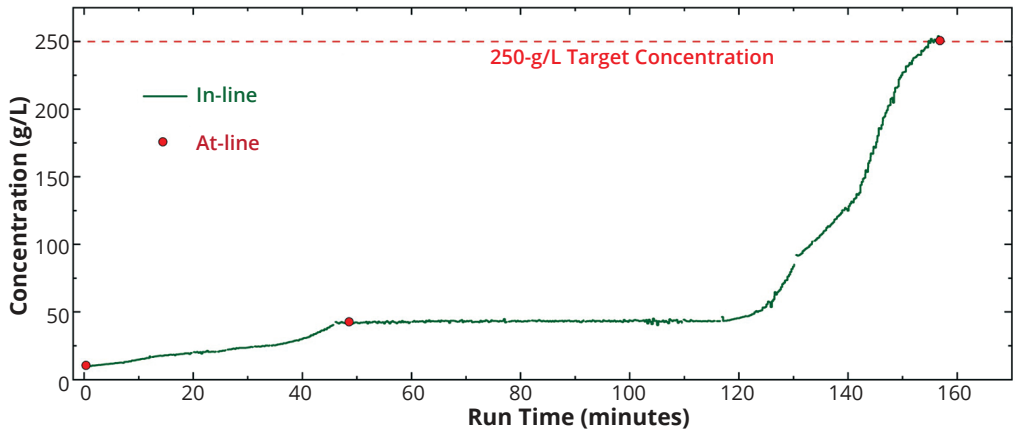
Considering the challenges that we encountered during those high-concentration runs — particularly with low solution volumes, high feed pressures, and insufficient mixing — we implemented adjustments in a final run targeting a protein concentration of 250 g/L. To enhance efficiency and facilitate process automation, we introduced two key modifications. The first was to increase the starting volume significantly by adopting the constant-feed concentration–diafiltration–concentration (CFC/D/C) mode, departing from the traditional C/D/C approach. The CFC method incorporates an additional auxiliary pump for consistent replenishment of starting material to the feed tank throughout the first process phase. That change was crucial to ensuring a large enough volume of material in the final UF/DF phase. For the second adjustment, we set the final concentration endpoint to 270 g/L instead of 250 g/L. That served to secure a sufficient number of data points beyond the target, thereby enhancing result accuracy. Those adjustments were implemented to enhance control, efficiency, and automation during high-concentration UF/DF.

Figure 5 represents data from the run targeting 250 g/L, with overlaid at-line measurements. The final concentration determined by the FlowVPX system was 250.5 g/L, and the SoloVPE system recorded 251.9 g/L, resulting in a 0.5% difference between the two measurements. Our adjustment played a critical role in attaining process targets while minimizing human intervention during the process. Such implementation of automation not only will enhance overall process efficiency, but also will enable real-time process monitoring and control.

Conclusions

We applied a KrosFlo FS-15 RPM TFF system along with a VPT device for monitoring and automated management of high-concentration UF/DF runs. Using

Figure 5: Real-time protein concentration trend from a constant-feed concentration–diafiltration–concentration (CFC/D/C) run controlled by RPM software (initial concentration to 40 g/L and final concentration to 250 g/L); the dotted line represents the target concentration as determined by the software.



a C/D/C approach with in-line protein concentrations as endpoints, the system demonstrated precision across a wide concentration spectrum. The automated control consistently maintained important process parameters, ensuring achievement of predetermined protein-concentration and DV setpoints. Our team also investigated the accuracy of in-line, concentration-controlled TFF, observing consistent process performance even at high concentrations.

Highly concentrated mAb products (>150 g/L) create challenges for the TFF steps preceding formulation. High concentration changes a protein solution's physical properties — e.g., by increasing its viscosity, density, and aggregation propensity. In traditional UF/DF setups, such changes diminish process control and complicate maintenance of CQAs within accepted ranges. TFF automation is becoming essential for downstream development because it can help to mitigate some of those issues. As demonstrated in runs surpassing the 200–250-g/L threshold, with operational adjustments for improved mixing and measurement, the automated procedure facilitated by RPM software proved to be highly effective in maintaining process parameters. The process also demonstrated good comparability between in-line and at-line measurements.

Implementing the system for high-concentration UF/DF runs can reduce risks associated with traditional methods — e.g., process and setup errors stemming from human factors and the need for complex calculations. Moreover, the system addresses limitations with conventional analytical methods, enhancing measurement accuracy. Such advantages can boost process-development efficiency significantly by providing real-time insights and the capacity to respond promptly to dynamic process changes.

Our work sheds light on optimization of high-concentration biopharmaceutical production through in-line, concentration-controlled TFF. Incorporation of automated systems, coupled with strategic adjustments, proved to be instrumental in improving process efficiency, paving the way for future advancements in biopharmaceutical manufacturing.

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Conflict of Interest

Research detailed in this study received technical support from Repligen. The authors affirm that Repligen's involvement in the research did not influence the presentation of results or the conclusions drawn in this manuscript.

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