Leveraging real-time concentration data for automated UF/DF in cGMP manufacturing

Steven Cates • Ing Kathleen Mihlbachler

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Introduction

The KrosFlo® RS 30 is a fully automated tangential flow filtration (TFF) system designed for use in a cGMP environment for concentration and diafiltration of biologically derived proteins and antibodies used in the production of clinical and commercial materials. The RS 30 is designed to concentrate final product as well as carry out diafiltration (buffer exchange) procedures with automated transitions between processes based on in-process metrics. Traditional TFF systems rely on volume concentration factor (VCF) to estimate product concentration and achieve the target value. In this approach, the starting and target product concentrations are used to calculate the amount that the initial feed volume must be reduced, which is monitored using the weight measured by the feed scale as well as flow sensors. However, this reliance on estimations and calculations is prone to error and can lead to deviation from the intended target concentration.

To reduce the risks associated with VCF calculations, the CTech FlowVPX® spectrophotometer with variable pathlength technology was integrated with the KrosFlo RS 30 system, providing real-time, in-line concentration measurement within the TFF recirculation loop. This enables automatic process transitions using actual product concentration values rather than estimates based on VCF. This application note examines an ultrafiltration/diafiltration (UF/DF) process using bovine serum albumin (BSA) to assess the capabilities of the KrosFlo RS 30 and CTech FlowVPX integrated system.

Materials and Methods

Preparation of BSA Solution

500 g of BSA (Sigma-Aldrich, St. Louis, USA) was added to 50 L acetate buffer (50 mM sodium acetate, 10 mM sodium chloride, pH 5) and mixed thoroughly to dissolve the powder. The solution was filtered through a 0.2 µm Opticap XL150 filter (Millipore Sigma, Burlington MA, USA) and initial concentration was measured with CTech SoloVPE® System.

System Setup

A 50 L single-use (SU) Tulip tank bag, ProConnex® flow path, and 0.5 m2 30 kD TangenX® PES SIUS cassette were installed according to the manufacturer's recommendation (Figure 1). The FlowVPX instrument with the CTech Beams 280 nm light source was installed in the flow path in between the Tulip tank and the diaphragm pump. The filter membrane was flushed with reverse osmosis deionized water (RODI) to remove residual sodium hydroxide storage solution. The system was drained completely, and an air diffusion integrity test was carried out to confirm the integrity of the membrane and TFF assemblies. The system was then primed with phosphate buffer (100 mM sodium phosphate, 100 mM sodium chloride, pH 7) before introduction of BSA solution.

Ultrafiltration/Diafiltration

BSA solution was transferred to the 50 L SU Tulip tank bag via the peristaltic addition pump and circulated by manual operation of the system.
The TFF run was controlled using the FlowVPX instrument to monitor product concentration and automatically trigger process transitions. Wavelength and extinction coefficient values were entered in the software, which automatically logged in-line absorbance (A280) data measured by the FlowVPX device. The UF/DF was performed at constant transmembrane pressure (TMP) of 0.5 bar and a flux of 330 LMH. The solution was initially concentrated to 100 g/L, followed by 6 diafiltration volumes (DVs) of Phosphate buffer. Lastly, the solution was concentrated to 200 g/L. Concentrated BSA was recovered via the feed pump, and the system was flushed with phosphate buffer to rinse remaining BSA in the system. Samples were collected from the Tulip tank throughout the process for off-line concentration measurement with the SoloVPE System.

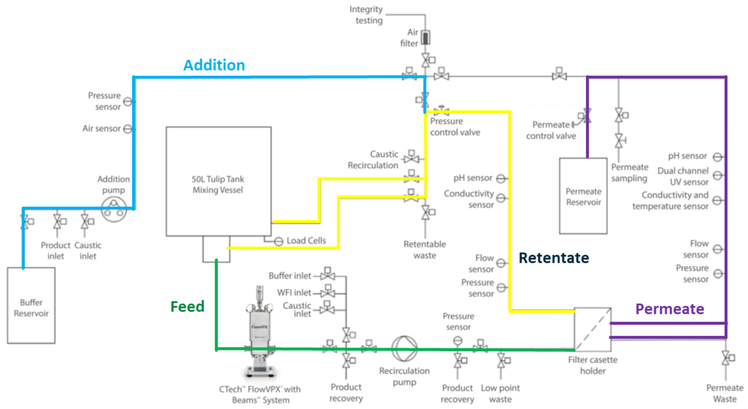


Figure 1. RS 30 System Setup

Results

The FlowVPX spectrophotometer was able to continuously monitor concentration accurately in real-time during all stages of the TFF process and enabled the KrosFlo RS 30 system to achieve the target product concentration without deviating or overshooting. Figure 2 shows the following metrics plotted versus time during the TFF run: feed weight (green line), FlowVPX in-line concentration measurement (blue line), concentration calculation using VCF (yellow line), and SoloVPE off-line concentration measurement (red crosses). The graph shows three distinct regions corresponding to the initial concentration phase, followed by the diafiltration phase, and then the final concentration phase.

During the initial concentration phase, the BSA concentration increased from 8.9 g/L to 100 g/L as the feed weight decreased from 49 kg to 3.89 kg, corresponding to a concentration factor (CF) of 11.4 and VCF of 12.6. At the start of the diafiltration phase, the concentration dropped to 78 g/L as the initial addition of diafiltration buffer diluted the sample. VCF calculations did not reflect this change, as the feed weight changed minimally, while the integrated FlowVPX device was more sensitive to the change in product concentration. The concentration and feed weight overall remained constant throughout the diafiltration period. During the final concentration phase, the concentration increased sharply to 201 g/L as the feed weight was reduced to 1.6 kg.

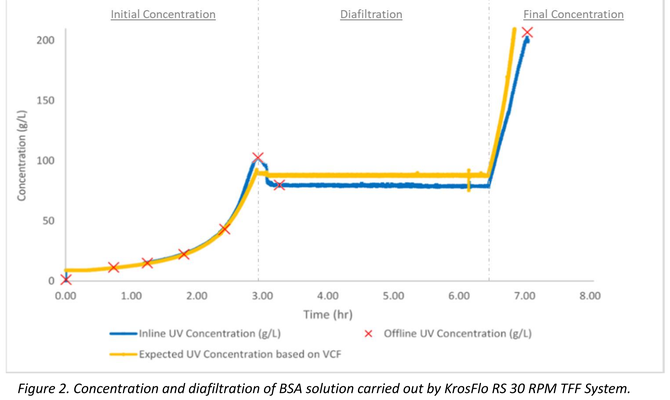


Figure 2. Concentration and diafiltration of BSA solution carried out by KrosFlo RS 30 RPM TFF System.

Table 1 shows concentration data at critical process stages. The in-line FlowVPX System measurements correlated extremely closely with the off-line SoloVPE System measurements at all stages of the UF/DF process, with an error consistently less than 1.5%, indicating that the FlowVPX instrument reliably monitored the concentration throughout the whole UFDF process. The expected concentration based on VCF calculations aligned with the in-line concentration measurement for the beginning of the initial concentration phase but deviated after reaching VCF 5.4, falling short of the actual concentration achieved at the end of the initial concentration phase by 10%. In addition, the VCF method did not predict the drop in concentration that occurred at the start of the diafiltration phase, leading to an overestimation of BSA concentration during diafiltration by 12%. The deviation was even larger during the final concentration stage, resulting in an overestimation of the final BSA concentration by 34%. These deviations reinforce the value of CTech FlowVPX instrument for reliable, real-time, direct concentration measurement and process control during all stages of TFF.

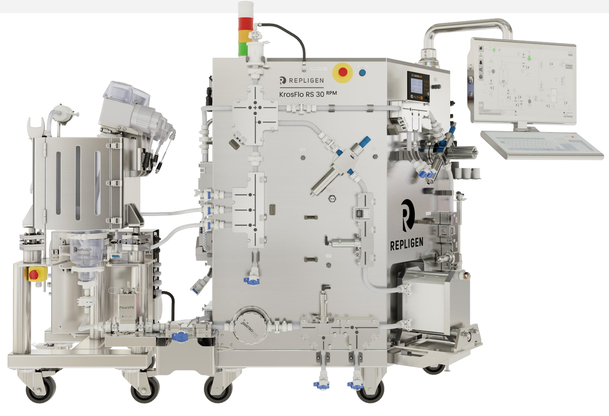
Measurement	C1 Conc.	C1 Error*	Conc. during D1	Conc. Error* during D1	C2 Conc.	C2 Error*
VCF Estimate	90 g/L	10%	87 g/L	12%	269 g/L	34%
FlowVPX In-line Analytics	101 g/L	1.0%	79 g/L	1.3%	200 g/L	0.5%
SoloVPE Off-line Analytics	100 g/L	–	78 g/L	–	201 g/L	–

**Concentration error was calculated with respect to the SoloVPE off-line measurements.*

Conclusion

The strong correlation between the in-line and off-line data confirmed the accuracy and reliability of the integrated CTech FlowVPX instrument for providing real-time concentration measurements. The low holdup volume of the KrosFlo RS 30 and the low minimum working volume of the 50 L tulip tank (1.6 kg) enabled significant volume reduction while simultaneously reaching the highest product concentration possible. The integrated CTech FlowVPX System successfully monitored concentration for the full process duration without the need for off-line measurement and sample dilution.

The KrosFlo RS 30 equipped with the CTech FlowVPX effectively enables the control of precise phase transitions within a UF/DF process. Through the in-line concentration measurement capabilities of the FlowVPX instrument, the RS 30 System adeptly utilizes live concentration data to execute process transitions. This integration of real-time data insight not only allows for accurate timing but also significantly enhances the overall precision of the UF/DF process.



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