WHITE PAPER

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REAL-TIME, MODEL-FREE NEAR-IR QUANTITATION OF ULTRAFILTRATION/DIAFILTRATION TFF PROCESSES

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BACKGROUND

Ultrafiltration/diafiltration (UF/DF) via tangential-flow filtration (TFF) is a critical step in biopharmaceutical manufacturing, requiring careful optimization of membrane efficiency, pressure setpoints, flux, and recovery. While pH and conductivity are standard real-time measurements, they provide minimal insight into a process. UV-Vis, though increasingly used, only quantifies protein concentration, offering no information on excipient clearance or buffer exchange. Without a more comprehensive real-time monitoring solution, critical process parameters remain unmonitored and uncontrolled.

Real-time process analytical technology (PAT) offers a solution, but traditional spectroscopic methods require extensive chemometric modeling and struggle with accuracy at high protein concentrations. Near-Infrared (NIR) spectroscopy provides a rapid, non-invasive alternative, capable of real-time, multi-analyte quantification without complex modeling.

In this study, two TFF systems performing the same UF/DF operations were analyzed in real-time with the Atlas Flow NIR system to evaluate real-time system performance and process outcomes. The Atlas™ Flow system quantifies both protein and excipient concentrations with a single scan.

This study demonstrates how the NIR-based, Atlas™ Flow system enables continuous UF/DF monitoring by analyzing feed-line samples in real-time with no multivariate modelling or training data required. The Atlas Flow system joins the next generation of PAT tools, with simple and robust operation allowing for bioprocessing efficiency at the right time and with the right data.

METHODS

Sampling Workflow

Two TFF systems (TFF System vendor 1, and TFF System vendor 2) were set up with the same process conditions. Identical 0.0186m^2, 10kDa flat sheet membranes were attached to each system.

The Atlas™ Flow system was attached to the feed line on each system, as shown in Figure 1. The adjustable scan interval was set to 10 seconds for these experiments.

Compared to traditional UF/DF sampling, this allows for a homogenous reading of sample from the retentate pool. Most systems are only able to sample from the unpressurised retentate line, as to not introduce bubbles over the TFF membranes. By sampling continuously before the filter, the Atlas™ Flow can analyze the most relevant process point for automation and process insights. In

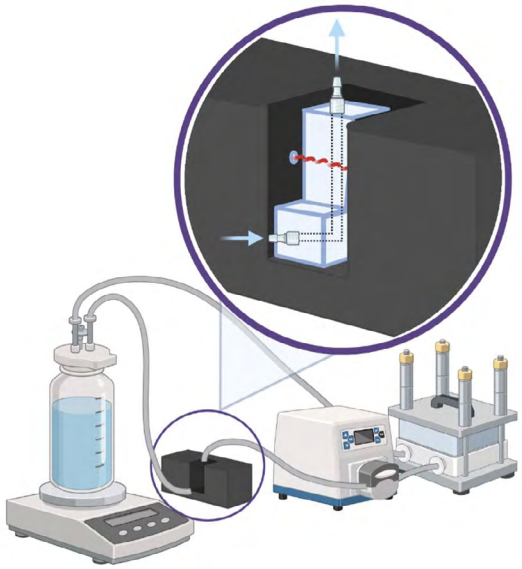

Figure 1: Atlas™ Flow NIR system on a TFF setup (simplified). Highlighted is the removable flowcell design and laser path (red) through the sample via optical windows on the flowcell.

addition, the design of the flow path mitigates bubbles, and is large enough to handle standard flow rates and pressures for a standard TFF process, allowing for easy use and unparalleled flexibility.

Analyte Library Creation

Samples of each individual analyte (e.g., BSA, Sucrose and Histidine) were first measured on an Atlas™ at-line system, and "library component spectra" generated. These library components were then transferred to the Atlas™ Flow system. The at-line system was used to build libraries for these experiments to reduce sample waste, although libraries can be created with any Atlas™ system.

The no-model analytics of the Atlas™ Flow and high precision wavelength referencing allows this library building process to only happen once – reference libraries are transferrable between systems and processes.

In addition, unlike traditional modelling approaches, no training data is needed to implement new analytes into existing processes. Each analyte can be quantified without calibration within a matrix.

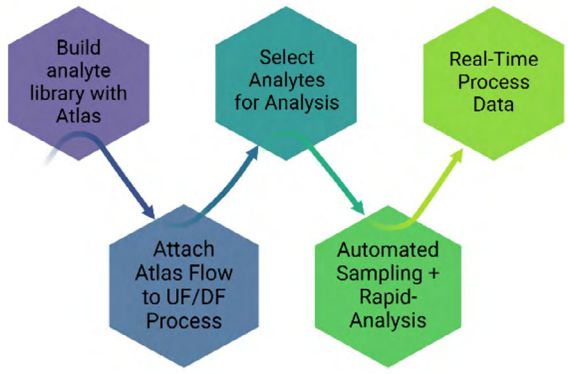

Figure 2: Atlas™ Flow Setup Workflow.

Analysis Workflow

Prior to the start of the run, analytes for measurement (from the reference sample library) are selected through the Atlas™ Flow analysis software. As the UF/DF run proceeds, samples are automatically scanned every 10 seconds and immediately analyzed for analyte concentrations.

For this demonstration, the only automation used was onboard the TFF systems, however, the rapid scan and analysis speeds from Atlas™ Flow enable simplified, real-time process control.

RESULTS

Real-Time Monitoring of UF/DF Runs Using NIR Spectroscopy

To evaluate the performance of real-time NIR monitoring in ultrafiltration/diafiltration (UF/DF) processes, three runs were performed across two TFF systems under varying process conditions (Table 1).

Run #	TFF System	BSA Load	Unit Ops
1	System #1	27 g/m^2	6x Diafiltration + Concentration
2	System #2	27 g/m^2	6x Diafiltration
3	System #2	13.5 g/m^2	Concentration

The results demonstrate the capability of the Atlas™ Flow with NIR spectroscopy to monitor protein and excipient levels in real-time, detect process deviations, and compare system performance.

Run Performance Summary

Run 1: System 1 – 27 g/L BSA, 6x Diafiltration + Concentration

The Atlas™ Flow data captured an unintended concentration event during diafiltration due to poor permeation control in System 1. While the expected excipient clearance pattern was observed, the system's inability to maintain a stable transmembrane pressure (TMP) resulted in increased BSA concentration during diafiltration (Figure 1). Despite this, the concentration step performed well, quickly reaching the target BSA concentration.

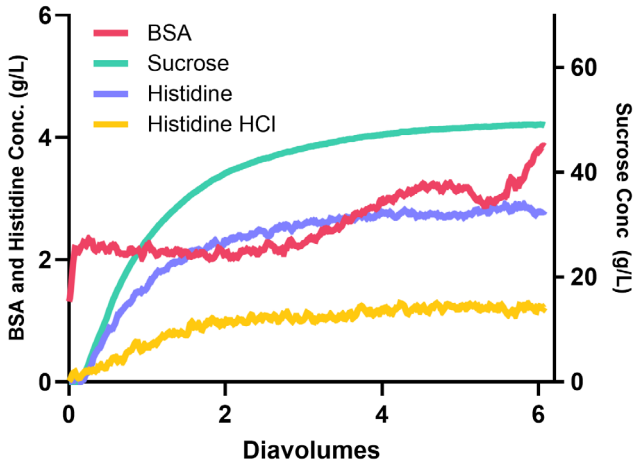

Figure 3: Excipient and Protein Data from Run 1 Diafiltration. Data is normalized to calculated diavolumes by permeate weight.

Diafiltration Comparisons: Run 1 vs Run 2

Compared to Run 1, Run 2 exhibited excellent control of diafiltration, maintaining stable BSA and excipient concentrations throughout the process. The Atlas™ Flow data confirmed that excipients were efficiently removed while protein concentration remained steady (Figure 4). The protein load was lowered in Run 2 to evaluate if this caused varied BSA concentrations, but review of Run 1 data confirmed variable addition control was off during the run. No unintended concentration was observed for Run 2, highlighting the superior permeation and buffer addition control of this system.

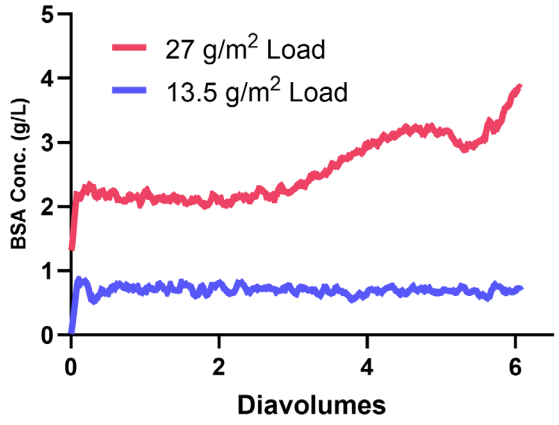

Figure 4: Atlas™ Flow BSA concentration data comparison of Run 1 and Run 2 diafiltration steps.

Concentration Comparisons: Run 1 vs Run 3

This run, which focused solely on concentration, exhibited a prolonged lag phase (Figure 5). Despite system-calculated concentration factors suggesting that the final BSA concentration should have been reached, the Atlas™ Flow data indicated that the process failed to achieve the expected BSA levels.

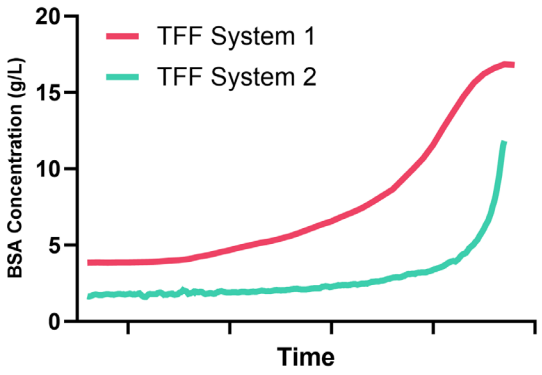

Figure 5: Atlas™ Flow BSA data from Run 1 and Run 3 concentration. Data is normalized to start and end points of concentration, as determined by retentate weight.

The discrepancy was attributed to inaccuracies in the system's weight-based monitoring method, which struggled to account for the density differences between the formulation buffer and concentrated BSA solution.

Gibbs-Donnan Effect Considerations

Notably, no unexpected retention of excipients was observed in any run, suggesting that the Gibbs-Donnan effect did not significantly impact the UF/DF process under these conditions. This aligns with expectations, as the isotonic nature of the formulation buffer and the balance of ionic species (150 mM NaCl) likely mitigated the charge-driven retention effects that can occur during diafiltration. The ability of the Atlas™ Flow to verify real-time excipient clearance further supports this conclusion (Figure 6).

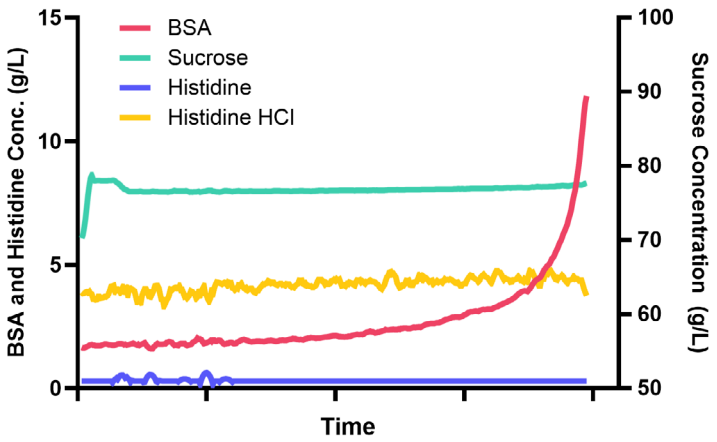

Figure 6: Excipient and Protein results from Run 3 Concentration. Data is normalized to start of concentration, as determined by retentate weight.

DISCUSSION + CONCLUSION

The Advantages of a Training Data-free, Atlas™ NIR Approach for UF/DF

Currently, traditional PAT methods for UF/DF monitoring have significant limitations. UV-Vis can measure only protein concentration, while other spectroscopic methods require extensive chemometric modeling for each excipient under varying process conditions. These approaches require long setup times and lack the flexibility needed for dynamic bioprocessing environments.

The Atlas™ Flow NIR system approach eliminates the need for complex modeling, using pre-built, unit-to-unit transferable, spectral libraries based on pure component spectra. Unlike other spectroscopy-based models, these libraries remain valid across different UF/DF processes and

do not require re-characterization. Additionally, at-line spectra can be seamlessly transferred to inline monitoring, reducing setup time while maintaining robust multi-analyte tracking.

TFF System Comparisons & Real-Time NIR Insights

Beyond its flexibility, the Atlas™ Flow system revealed key differences in TFF system performance. System 1 exhibited poor permeation control, leading to unintended concentration during diafiltration, while System 2 maintained stable diafiltration but struggled with accurate concentration tracking due to density-related errors in weight-based measurements. The ability to detect these differences in real time underscores the power of NIR-based Atlas™ Flow in TFF system selection and process optimization.

Implications for UF/DF Process Optimization

By implementing data from an Atlas Flow system into existing process development, scientists gain the advantage of optimizing based on all process conditions, instead of only protein concentrations or bulk properties. These findings demonstrate the Atlas™ Flow NIR system as a robust, scalable PAT tool for UF/DF. It enables real-time, multi-analyte measurements without extensive model development, making it ideal for process development, tech transfer, and manufacturing control. Future applications could extend to automated feedback control, dynamically optimizing diafiltration and concentration steps to enhance bioprocess consistency and efficiency.