Real-time Monitoring and Control of Bioprocess Ultrafiltration/Diafiltration via In-line Tunable NIR Spectroscopy

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Abstract

In downstream bioprocessing, multi-attribute process analytical technology (PAT) tools have been utilized more frequently for maintaining precise control over ultrafiltration/diafiltration (UF/DF) processes. **NIR spectroscopy has emerged as a powerful tool for monitoring bioprocesses, through measurement of protein and excipient concentrations.** NIR provides advantages such as high signal-to-noise ratio, rapid quantification, simple in-line and at-line implementation, and high signal specificity for each analyte of interest. These advantages of NIR overcome disadvantages seen in techniques like Raman spectroscopy, which are growing in popularity but is still limited by the high material and labor requirements of developing and validating statistical models.

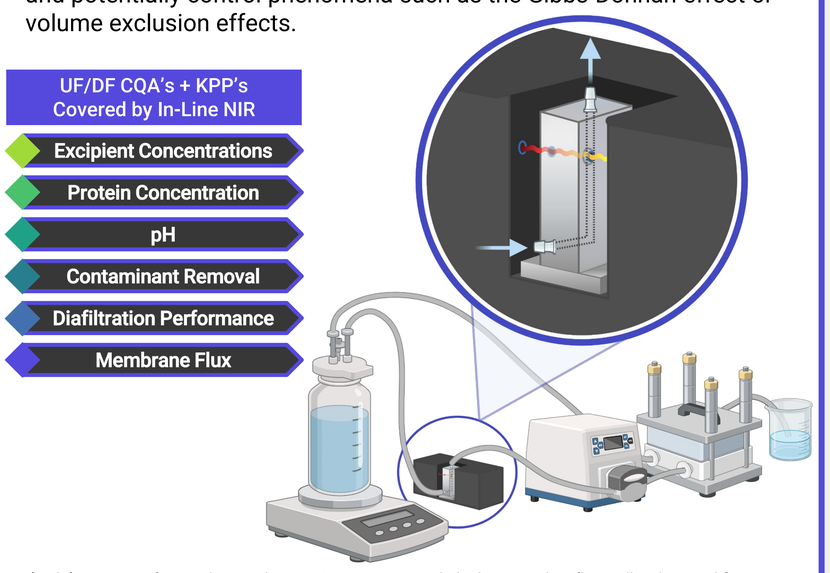
An implementation of the Nirrin Atlas system was developed for in-line measurements. The at-line Atlas which uses **high-precision, tunable NIR spectroscopy** (NIR-HPTLS) for measurement, was utilized **to develop and validate analyte-specific spectra, which were then transferred to the in-line system to reduce development time.** Previous validation was done to ensure no instrument dependence in the analyte-specific library spectra that were created and transferred.

Successful implementation of the NIR in-line approach **is demonstrated here for UF/DF runs throughout a UF/DF optimization process**. This run data demonstrated accurate and rapid quantification of protein concentrations and all excipient concentrations during the UF/DF operation.

By utilizing transferrable at-line tools, this method was able to reduce the development and implementation time of a PAT tool for UF/DF monitoring from months to under 3 hours. Overall, **the in-line NIR spectroscopy data proved the capability of this tool for monitoring and controlling the UF/DF step and enabling of process automation through access to data that was previously unavailable for in-line implementation.**

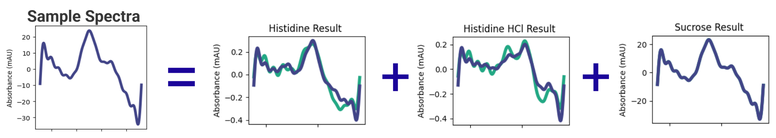
Background

- NIR-HPTLS measures the combination band region of the infrared spectra – in this region all analytes have unique chemical signatures, which makes spectral signals with high specificity and repeatability.
- High precision tunable NIR spectroscopy is well-suited for multi-analyte measurements, at multiple process stages and development
- Ultrafiltration/ Diafiltration (UF/DF) is done to concentrate and exchange buffer conditions for a biological product, to a condition that aligns with final formulation and fill/finish requirements.
- Careful monitoring of product and excipient concentration is needed in UF/DF process steps, not only to optimize parameters, but to measure and potentially control phenomena such as the Gibbs-Donnan effect or volume exclusion effects.



(Right) Diagram of NIR in-line implementation in a UF/DF skid. The NIR in-line flow cell is designed for sampling pre-filter, for the most accurate excipient and product quantification. (Left) List of Critical Quality Attributes (CQA's) and Key Process Parameters (KPP's) for UF/DF that can be measured by NIR. Highlight regions shows principle of measurement for the NIR flow cell design.

- To eliminate complex chemometric modelling, Nirrin developed analysis algorithms wherein individual analytes are measured against component calibration library spectra.
- Each calibration library includes a linear and nonlinear term, which allows for correction of analyte behavior at high concentration.
- **Independent analyte calibrations allow for high repeatability and simple transferability between units.**



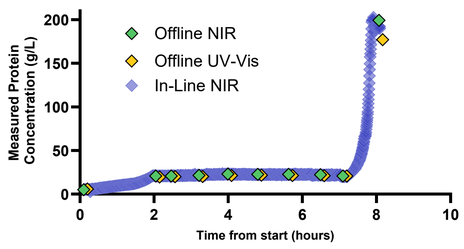
(Above) Example of simultaneous excipient quantification, via Iterative Optimization Technology (IOT) for spectral deconvolution.

RESULTS & DISCUSSION

Real-time Protein Quantification

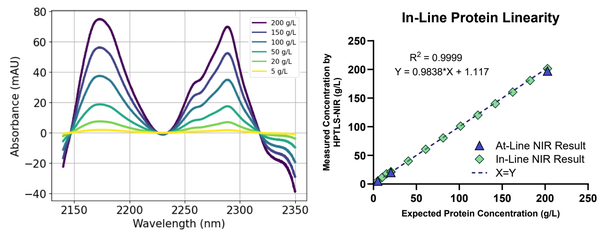
- Main data shown is from the final process run, done to confirm the individually tested parameters for filter load, pressure setpoint, and crossflow rate.
- The process consists of a continuous feed concentration (4x CF), followed by diafiltration (8x DV), and then a further 10x CF concentration.
- Initial buffer is 20mM Acetate, pH 5.51. The final formulation buffer is 20mM Histidine, 37.5mM Arginine, 25mM Methionine, 3% Sucrose, pH 5.54.

Offline vs In-Line Protein Concentration



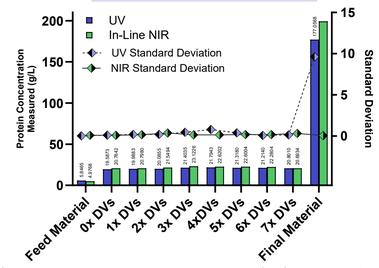
(Left) Comparison of in-line NIR, offline (at-line) NIR, and offline UV-Vis results for protein concentration during the full UF/DF run. Results show good alignment between UV-Vis and NIR, at all stages.

NIR Linearity



(Middle) Linearity of in-line and at-line NIR for measurement of protein concentrations. This data indicates a linear response for protein in the NIR region. This extends beyond the linear range of proteins for most UV-Vis methods, indicating that NIR is a suitable replacement for UV protein concentration measurements at high titers. This is especially true for in-line quantification, where the linearity observed for the in-line NIR-HPTLS system yields an R^2 of 0.9999.

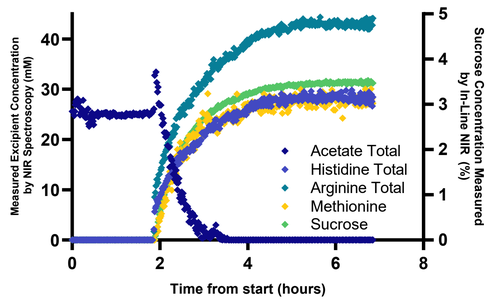
Offline NIR vs. UV-Vis



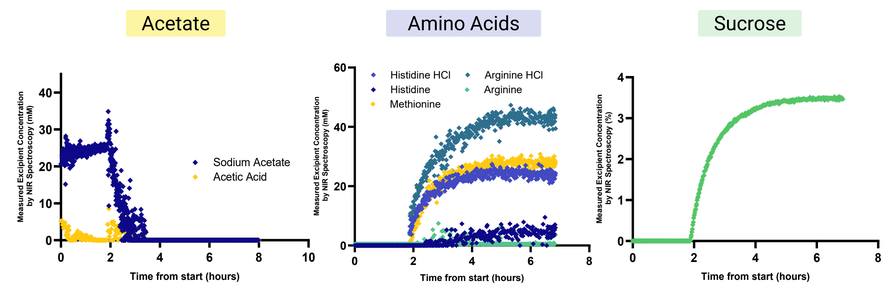
(Right) In-line NIR protein concentration results (g/L) compared to off-line UV-Vis measurements with samples taken throughout the UF/DF process. The NIR method shows high repeatability for samples during the diafiltration step, and accuracy for samples at both the high and low concentration levels. Standard deviations from three replicates are also plotted, showing that UV–Vis has higher variability, both during diafiltration and in the final high-titer material.

Real-time Excipient Quantification Using In-Line NIR

- The Nirrin IOT spectral deconvolution method enables the NIR in-line system to quantify excipients simultaneously with proteins.
- By quantifying excipients in real time, important process information can be gathered and used to optimize process steps, without the need for multiple characterization runs and offline analysis for retrospective changes.



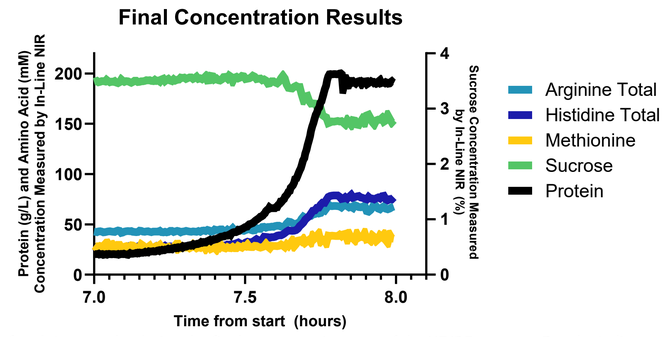
(Left) Real time measurements of all excipients during the first concentration step and the diafiltration step. Diafiltration was performed for the standard 8x diavolume length, by the end of which all monitored excipients were fully exchanged and had reached stable concentrations.



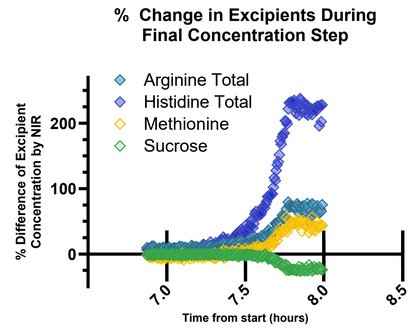
(Right) Individual plots for all excipients analyzed, during the diafiltration step. NIR-HPTLS is able to resolve the spectral differences between protonated and deprotonated forms of amino acids, and other buffer components, enabling the components to be analyzed independently. This allows for potential pH measurements by examining the ratio of acidic and basic species. The data shows sodium acetate and acetic acid were fully removed before the halfway point of the diafiltration – this suggests alterations to the method can be made for a shortened run time, as the acetate removal is non-limiting.

Gibbs-Donnan Effect Measurement

- During the final concentration step and recovery step, excipient and protein concentrations were closely monitored by the NIR in-line system.
- Results show an enrichment in amino acids during this step, as well as a reduction in the sucrose concentration. This data shows the real-time measurement of this phenomena, known as the Gibbs-Donnan effect.



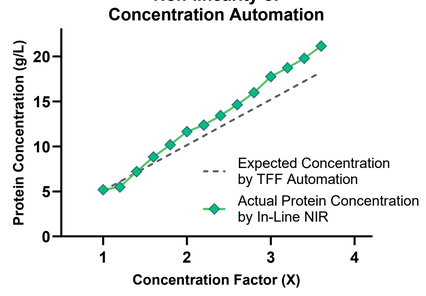
(Left) Plot of real-time results during the final concentration step of the UF/DF process. Results indicate while good protein concentration is achieved, excipient concentration changes observed. Future iterations of the in-line NIR system can potentially be used to directly control UF/DF processes for concentration end points or excipient additions. In addition, no protein increase was seen during the recovery step, indicating that removal of this step would not decrease overall process yield.



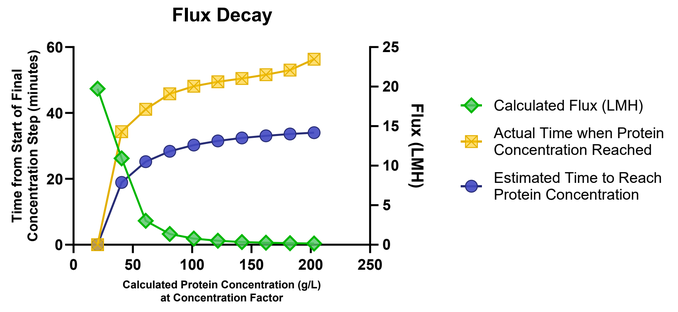
(Right) Percent change of each excipient concentration from the value measured prior to the start of the concentration step. Trends indicate that sucrose saw no permeation until approximately the 7.7-hour mark. These results potentially suggest that concentration of the other excipients impacts the permeation rate of sucrose.

Real-time Process Control and Monitoring

- Concentration of the protein and excipients were used to calculate process parameters and outcomes in real time.
- The reduced permeation rate seen during the final concentration step (right) indicated that the setpoint for 3psi of permeate backpressure, meant to increase filter permeation, was ineffective with increasing protein concentrations during the final stage.



(Left) Expected vs measured protein concentration during the first concentration step. This data indicated that onboard software used by the TFF system did not account for higher or variable protein permeation rates, that were incongruent with scale readings due to changing density of the product. This led to an overconcentration in the initial step, and thus a higher concentration in the diafiltration step, which impacted excipient permeation rates.



(Right) Real time permeate flux rate, as determined by rate of change of protein concentration as measured by the in-line NIR system. This showed a viscosity or protein concentration impact on permeation – the effect is most notable at concentrations above 50g/L.

Conclusion

This study confirms the effectiveness of the Nirrin Atlas NIR-HPTLS platform for accurate, in-line, quantification of both proteins and excipients throughout Ultrafiltration/ Diafiltration (UF/DF) processes. The ability the quantify individual excipients in real time enables new strategies for controlling UF/DF steps, and for monitoring important CQA's, KPP's, and phenomena such as the Gibbs-Donnan effect.
By reducing modelling and characterization requirements through independent analyte calibrations, the in-line tunable NIR spectroscopy system can enable real-time process monitoring and control, with reduced material and labor costs for implementation.

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