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Research Article

A Novel Approach for Continuous Inline Monitoring in Ultra-and Diafiltration by Mid-Infrared Spectroscopy

Camilla Marasca[1]*, Stefan Werner[2], Niklas Zell[1], Alexander Graf[2], Alexander Geissler[1],

[1]IRUBIS GmbH, Bayerstraße 85a 80335, Munich, Germany
[2]Rentschler Biopharma SE, Erwin-Rentschler-Str. 21, 88471 Laupheim, Germany

***Corresponding Author:** Camilla Marasca, IRUBIS GmbH, Bayerstraße 85a 80335, Munich, Germany.

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Abstract

TProcess Analytical Technology (PAT) is becoming increasingly important in biopharmaceutical manufacturing, especially as the industry shifts toward continuous bioprocessing. It enables real-time process control, supports product quality, improves efficiency, and enhances scalability.

While spectroscopic methods are well established in upstream processing, their use in downstream applications remains challenging because of rapid composition changes and short processing times. These limitations are especially critical during ultrafiltration and diafiltration (UF/DF), key final downstream steps used to achieve the target product concentration and buffer composition before fill-and-finish.

This study presents a novel approach for integrating the mid-infrared spectrometer Monipa into UF/DF using a single-use flow cell. Calibration models were developed for real-time monitoring of protein concentration and buffer exchange, reducing reliance on time-consuming offline analyses. Performance was evaluated by comparing real-time measurements with offline reference methods. The system achieved a Mean Absolute Percentage Error (MAPE) of 6.3% for protein concentrations between 11.4 and 120 g/L and successfully tracked buffer exchange during diafiltration. These results highlight the potential of mid-infrared spectroscopy as a valuable PAT tool for more efficient and reliable downstream bioprocessing.

Keywords: Bioprocessing, Process Analytical Technology (PAT), Mid-infrared spectroscopy (MIR), Ultrafiltration/diafiltration (UF/DF), Downstream Processing, Analytics.

Introduction

Monoclonal antibodies (mAbs) are central to modern biopharmaceuticals and are widely used to treat cancer, autoimmune diseases, and infections. Their importance is reflected by the FDA approval of the 100th mAb in 2021 [1,2]. However, developing a new biologic drug remains costly and time-consuming, often taking 15 years and exceeding $2 billion. This creates strong pressure to improve manufacturing efficiency, particularly by increasing product yield. Even a modest 10% yield increase in a typical production setup can generate substantial annual profit [3].

Although upstream processing has improved significantly over recent decades, downstream processing still accounts for about 60% of total production costs and remains a major bottleneck. This challenge is intensified by the growing demand for high-concentration antibody formulations, which are more difficult to purify and handle [4].

A key downstream operation is ultrafiltration/diafiltration (UF/DF), the final formulation step before drug product completion. UF/DF generally includes an initial ultrafiltration step (UF1), which concentrates the protein in a primary buffer, followed by diafiltration (DF), which exchanges the buffer while maintaining protein concentration. The final formulation buffer is carefully designed and often contains excipients such as sugars, surfactants, polyols, and amino acids to ensure

protein stability during storage and use. A second ultrafiltration step (UF2) then raises the protein concentration to its final target [5].

Achieving the desired final concentrations of both protein and excipients is difficult. Effects such as volumetric exclusion and Donnan effect can cause significant deviations from target excipient concentrations, complicating formulation and often requiring repeated process adjustments [6].

At present, buffer exchange during UF/DF is typically monitored using mass balance calculations. While useful in some cases, this method has limited precision, sensitivity, and suitability for real-time monitoring, especially in large-scale or complex processes [7]. In practice, protein and excipient concentrations are often measured only from samples taken at the end of the process. This delayed feedback frequently leads to multiple iterations, increasing both time and resource consumption [8].

Inline Process Analytical Technology (PAT) offers a promising way to address these issues. By enabling real-time monitoring and control of critical process parameters, PAT can improve efficiency, reduce reliance on sampling and offline analysis, and lower costs, particularly when expensive buffers are involved. Faster monitoring also reduces product stress and may decrease the risk of protein aggregation [9].

Several spectroscopic PAT methods have been investigated for UF/DF, including Raman [7], near-infrared (NIR) [10], and mid-infrared (MIR) spectroscopy [11]. Compared with conventional UV methods, vibrational spectroscopy can monitor multiple parameters simultaneously because of its broader spectral information. However, these techniques also present challenges, especially the need for extensive calibration work and the trade-off between acquisition speed and sensitivity [12].

UV-Vis spectroscopy is widely used for inline protein concentration monitoring because it is robust and reliable. Its main limitations are reduced performance at high protein concentrations and its general restriction to monitoring a single parameter [13]. Raman spectroscopy allows simultaneous monitoring of multiple components, but fluorescence interference, light loss through probes, and the complexity of the signal can reduce sensitivity and require time-intensive model development [14].

MIR spectroscopy offers strong absorption signals, rich molecular information, and the ability to monitor several parameters inline over a broad concentration range. It can be implemented through transmission or attenuated total reflection (ATR). Transmission measurements are often limited by long optical path lengths, flow-cell constraints, and water interference. ATR overcomes many of these issues by shortening the effective path length and focusing on surface interactions, making MIR more robust and practical for inline biopharmaceutical applications [15].

This study introduces Monipa, an ATR-based mid-infrared spectrometer developed for inline monitoring of protein concentration and buffer exchange. The system combines rapid measurement, low water interference, and straightforward integration with a single-use flow cell. Its performance was evaluated against offline UV measurements and mass balance calculations, showing comparable accuracy. These results suggest that Monipa is a valuable tool for real-time monitoring and can help make UF/DF processes more efficient and cost-effective in biopharmaceutical manufacturing.

Material and Methods

Mid-Infrared Spectroscopy

For inline monitoring, the mid-infrared spectrometer Monipa 1 (IRUBIS GmbH, Munich, Germany) was integrated into a feed-bypass via a 3D printed single-use flow cell equipped with a silicon attenuated total reflection (ATR) crystal (IRUBIS GmbH, Munich Germany). Spectra were continuously measured, with each data point representing the average of spectra acquired over 15 seconds at a scan rate of 5 Hz. The MIR spectra were continuously collected in the wavelength range of 2.5−12.5 µm (4000−800 cm−1) at a nominal wavenumber resolution of 4 cm−1 and used for further data analysis.

UF/DF Setup

A custom-made setup was prepared by Rentschler Biopharma to monitor protein concentration during a UF/DF/UF process (Fig. 1).

Tangential flow filtration (TFF) was carried out using a Pellicon® 3 C-Screen cassette with 30 kDa cutoff and a filtration area of 88 cm² (Merck Millipore, Billerica, MA, USA), positioned in a Sartocon® Slice Holder (Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany). A constant feed stream of 350 LMH was generated using a Quattroflow QF30 membrane pump (ALMATEC Maschinenbau GmbH, Duisburg, Germany), while the transmembrane pressure (TMP) was manually regulated to approximate 1.2 bar using a manual fine control valve (EM Technik, Maxdorf, Germany). Single-use pressure sensors (PendoTech, Plainsboro, NJ, USA) were mounted at the feed stream inlet, the retentate, and the permeate outlet of the TFF membrane. The volume flow of the retentate stream was measured with a Proline Promag H100 electromagnetic flowmeter (Endress+Hauser, Reinach, Switzerland), and the permeate weight was determined using a scale (Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany). The silicone tubing (Silicone Altimex Ltd., Stapleford, UK) connecting the components had an inner diameter of 1.6 mm. The signals of all sensors and the scale were captured with an ExpertLogger400 datalogger system (Delphin Technologies AG, Bergisch Gladbach, Germany) controlled by ProfiSignal5 Software (Delphin Technologies AG, Bergisch Gladbach, Germany).

The entire setup was initially flushed with an equilibration buffer at pH 6.0. The experiment was conducted at a membrane

loading of 325 g/m². The starting material, consisting of 11.4 g/L monospecific antibody in buffer without sucrose, was first concentrated to approx. 50 g/L (UF1). Subsequently, the buffer exchange was performed with a solution containing 290 mM sucrose and 10 mM histidine at a pH of 6.0, for a total of 5 DF volumes. The diafiltration buffer was introduced to the via tank using a peristaltic pump, with its flow rate manually adjusted to ensure equal transfer and permeate flow. Finally, the protein concentration was increased from 50 to 120 g/L (UF2). The recorded MIR spectra were used to track changes in protein concentration and buffer exchange over time.

Offline Analytics

MIR spectral results were compared to those obtained by a A280 –A310 UV Lunatic system (Unchained Labs, Pleasanton, CA, USA) with offline samples being collected at specific time points throughout the run. According to the manufacturer, the Lunatic system achieves a measurement precision with a coefficient of variation (CV) below 1% and an accuracy within 2% for IgG quantification across a broad dynamic range.

Data Analytics

Monipa's analytics utilize pure component modeling (PCM), a chemometric technique designed to quantify individual chemical components within a complex mixture, even when their spectra overlap. PCM is based on obtaining each pure component's spectrum separately and then combining them mathematically to model the acquired mixture spectra adequately [16].

A reference spectrum of the protein was derived from a previous UF/DF run as the difference spectrum between two time points, for which the reference concentrations had been determined with UV-Vis spectroscopy. This spectrum was uploaded to Monipa and used for the PCM analysis of all the spectra collected during the monitored UF/DF run. In the MIR region, proteins exhibit characteristic peaks in the wavenumber ranges of 1450 to 1580 cm−1 (Amide II) and 1600 to 1700 cm−1 (Amide I). Prior analysis, the raw absorbance spectra were smoothed with a Savitzky-Golay filter with a window size of 15 and polynomial degree of 2. Protein concentrations were then calculated from these smoothed spectra using PCM. For this calculation, PCM was performed via a linear regression of the first-order derivative spectra against the derivative spectrum of the protein reference. The resulting slope provided the protein concentration. Additionally, prediction accuracy was assessed in terms of the Mean Absolute Percentage Error (MAPE), the coefficient of determination (R^2), and the Root Mean Squared Error (RMSE) between the predicted and the offline reference concentrations. For these metrics, the definitions of the Open-Source Python package scikit-learn were used:

$$\mathrm{MAPE}(y,\hat{y}) = \frac{1}{n}\cdot\sum_{i=1}^{n}\frac{|y_i-\hat{y}_i|}{\max(10^{-7},|y_i|)}$$

$$R^2(y,\hat{y}) = 1-\frac{\sum_{i=1}^{n}(y_i-\hat{y}_i)^2}{\sum_{i=1}^{n}(y_i-\bar{y})^2}$$

$$\bar{y} = \frac{1}{n}\cdot\sum_{i=1}^{n}y_i$$

$$\mathrm{RMSE}(y,\hat{y}) = \sqrt{\frac{1}{n}\cdot\sum_{i=1}^{n}(y_i-\hat{y}_i)^2}$$

Here, "y" _"i" and "y" ^_i denote the concentration of the i-th sample obtained from the reference and the PCM-method, respectively. For the MAPE, the constant 10-7 is added to avoid and infinite relative error when "y" _"i" is zero [17].

To monitor the buffer exchange, a process change criterion was defined to identify the start and end of the buffer exchange. During this process, the concentration of individual components may either increase or decrease until an equilibrium is reached. However, the direction of the concentration changes is not relevant for the identification of the steady state. Therefore, a modified definition of spectral complexity, originally proposed by Friese & Banerjee, was defined as follows:

$$\delta_{change} = \int_{\tilde{\nu}_1}^{\tilde{\nu}_2}\left|\frac{d^2\left(A(\tilde{\nu})-A_{blank}(\tilde{\nu})\right)}{(d\tilde{\nu})^2}\right|$$

This expression integrates the absolute area underneath the second-order derivative of the difference spectrum between the current and the blank spectra [18]. Thus, only the qualitative spectral changes are captured, regardless of their direction. The blank spectrum was obtained after the completion of UF1 by recirculating the pre-concentrated solution until the spectra stabilized. The integration region was chosen to cover the fingerprint region, defined as ν̃_1=900 cm^(-1) and ν̃_2=1800 cm^(-1). Due to the absolute value operation, both negative and positive spectral changes result in a positive δ_change, thus measuring only the magnitude of the spectral changes. To determine the start and end of the DF phase, the slope of δ_change over time was analyzed using an outlier-robust Theil-Sen regression. The start of the DF was detected when five consecutive slopes were positive with 99% confidence, indicating systematic spectral changes that were distinct from random noise, which would otherwise show alternating positive and negative slopes. Similarly, the steady state at the end of DF was identified when five consecutive slopes fell below an empirical threshold, set to 0.1% of the maximum slope observed at the beginning of the DF.

Results

The protein concentration monitored by Monipa during the UF/DF/UF process is shown in Fig. 2, alongside the reference concentrations obtained from the offline samples.

Monipa was initially blanked using the starting material (monospecific antibody in equilibration buffer, pH 6.0). During UF1, the protein concentration was increased from 11.4 g/L to approximately 50 g/L. As depicted in Fig. 2(a), the spectra acquired by Monipa show a continuous increase in absorbance, most prominently in the protein regions at 1450 to 1580 cm−1 (amide II) and 1600 to 1700 cm−1 (amide I) [19]. Fitting these spectra with the protein reference spectrum enabled quantitative monitoring of the protein concentration.

During the DF, the protein concentration remained constant while the concentrations of excipients changed. Therefore, Monipa was blanked so that the resulting spectra only show the change in the excipient concentrations as depicted in Fig. 2(b). Although the excipients were not included into the PCM regression model, their presence did not negatively affect the predicted protein concentrations. After the DF, Monipa was blanked again to follow only the second change in protein concentration in the course of UF2. This allowed for monitoring the concentration increase from approximately 50 g/L to 120 g/L, as presented in Fig. 2(c).

For a quantitative comparison with the offline reference concentrations, the continuously monitored predictions from Monipa were interpolated to the respective offline sampling timestamps. The highest measured protein concentration using the UV reference method was determined through four independent dilution series, with three replicate measurements per dilution. The resulting average concentration was 121.38 g/L ± 4.87 g/L (mean ± standard deviation), which represents a maximum coefficient of variation of approximately 4% for UV measurements including sample dilution. In direct comparison, Monipa demonstrated excellent agreement with the offline reference values across the entire concentration range from 11.4 to 120 g/L. The calculated Mean Absolute Percentage error (MAPE) was 6.3% with a coefficient of determination (R2) of 0.996, and a Root Mean Squared Error (RMSE) of 2.27 g/L.

Real-time monitoring of the buffer exchange was conducted between UF1 and UF2 as shown in Fig. 3. The algorithm used is reference-independent, making it adaptable to any combination of buffers and proteins.

The differences in the spectra are shown in Fig. 2(b), where the protein was blanked out, leaving peaks corresponding to the sucrose/histidine buffer. Spectral changes in the excipient region were tracked over time using the process change criterion δ_change, which resulted in an exponential saturation curve. The final plateau of this curve indicates the end of the diafiltration process. Due to stagnation in the buffer feed control, the algorithm prematurely identified the end of the buffer exchange, as no changes were detected, except for background noise. Nevertheless, δ_change continued to be monitored until the actual (quasi-) stationary state was reached.

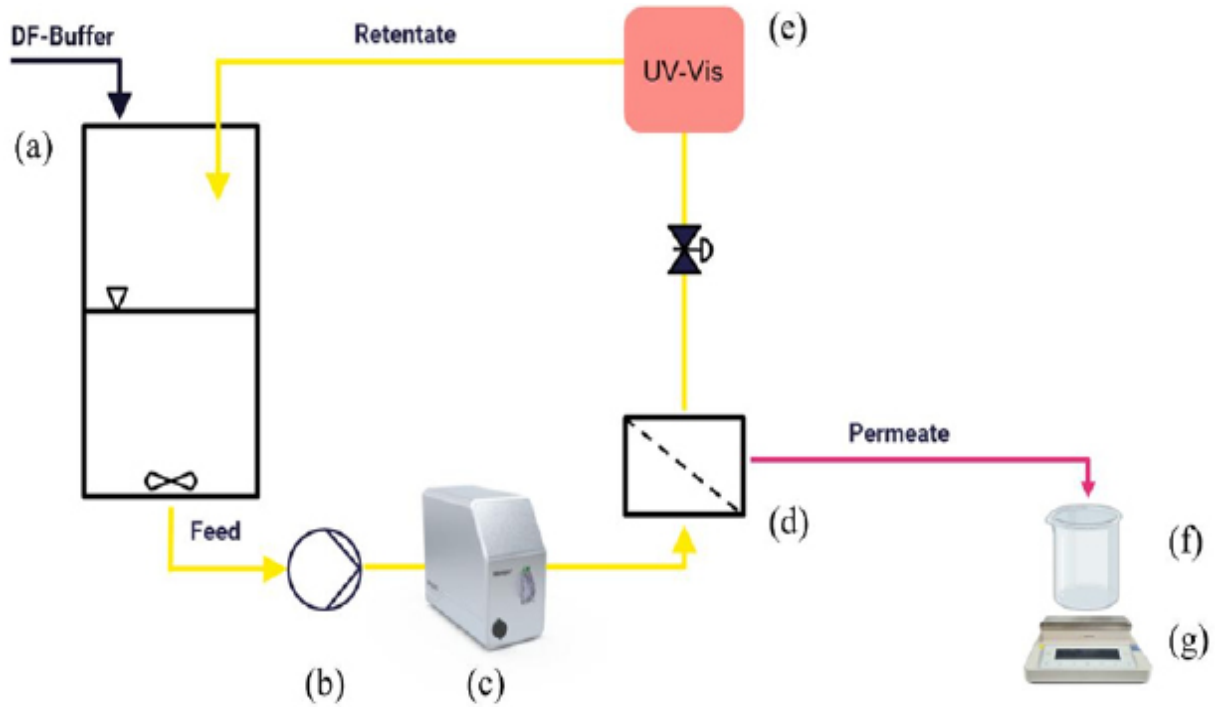

Figure. 1 Custom-made UF/DF setup: (a) Recirculation tank, (b) Pump, (c) Monipa 1, (d) Membrane module, (e) UV-Vis, (f) Permeate collection, (g) Scale

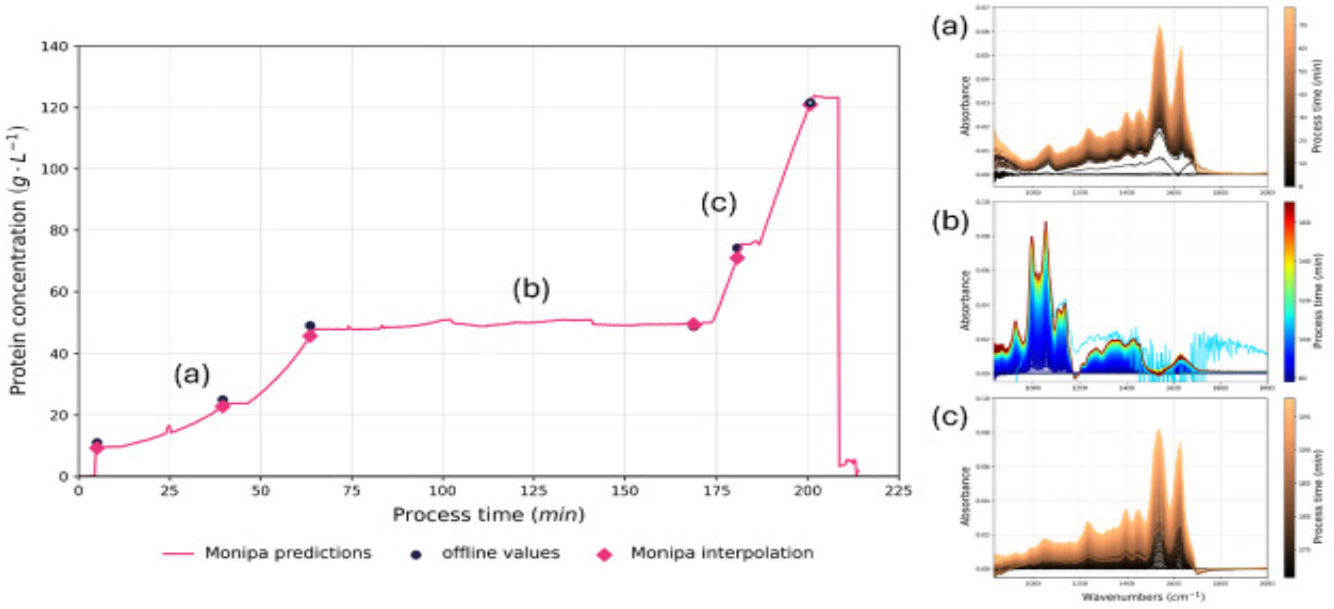

Figure. 2 Monitoring of the UF/DF/UF run with Monipa: overlapping of MIR spectra during (a) UF1 (b) DF (c) UF2

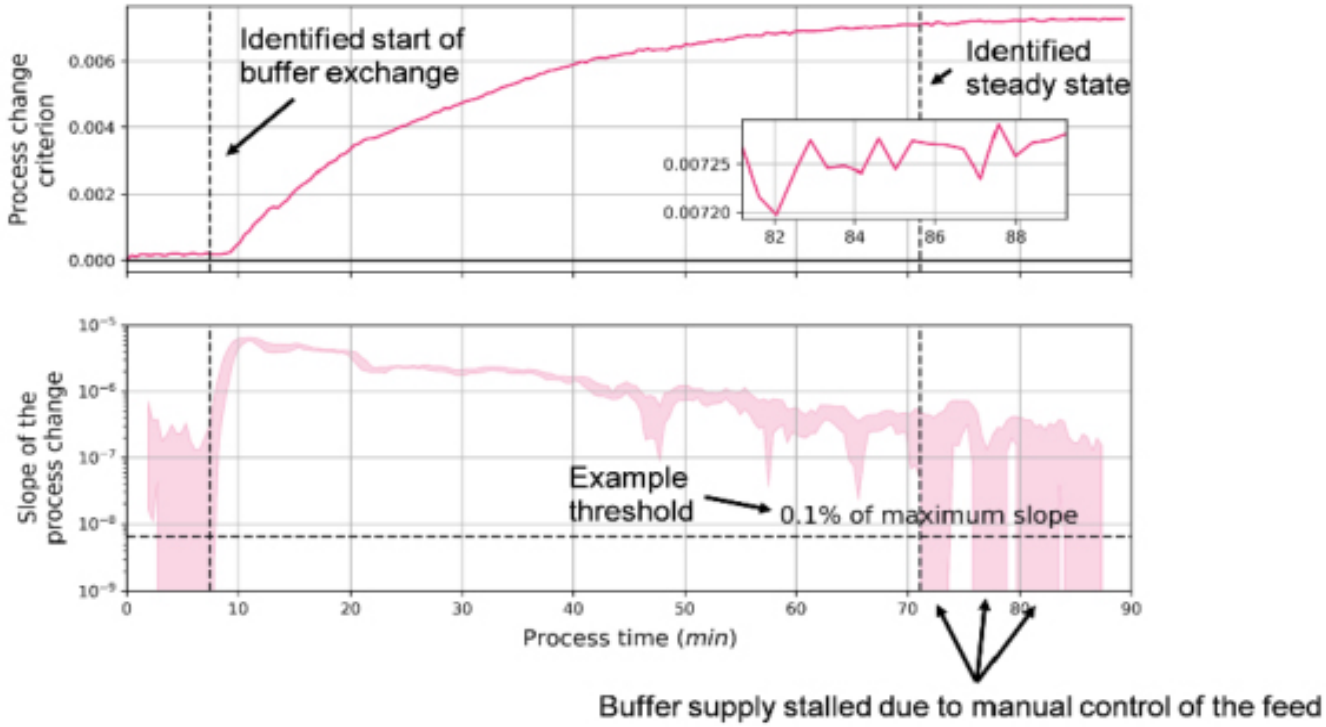

Figure. 3 Real-time tracking of the buffer exchange based on change criterion δ_change (top) and its change over time visualized in terms of the lower and upper 99%-confidence intervals of the slope of a moving Theil-Sen regression (bottom)

Conclusion

In this study, the inline mid-infrared spectroscopic device Monipa was assessed to monitor a UF/DF/UF process. The device was integrated into a feed-bypass via its single-use flow cell to assess the potential of mid-infrared PAT for tracking key process parameters, such as protein concentration and buffer exchange. The results obtained were compared

with offline data to verify the performance of the system.

Monipa successfully provided accurate real-time quantification throughout the entire process over a concentration range of 11.4–120 g/L using a PCM algorithm tailored for the mAb of interest. Considering the reduced precision of the UV reference method at higher concentrations due to dilution steps, the MAPE of 6.3% and RMSE of 2.27 g/L underscore the overall accuracy of Monipa's protein concentration predictions across a wide concentration spectrum, while offering a more dynamic and efficient alternative to conventional offline mass balance regulation methods. These outcomes are in good agreement with results found in the literature for orthogonal methods, Raman and variable path-length UV [7].

For a robust model, an individual reference spectrum is required for each specific protein under investigation given that different protein structures result in different spectra – even between different mAbs. Regarding the batch-to-batch variability of the same target mAb, an average of multiple spectra can be used as a reference. This gives a good tradeoff between robustness and accuracy.

The presence of excipients that were not included in the pure component modeling did not affect the protein predictions due to the low spectral overlap, which was further reduced by the use of derivative spectroscopy. However, for more complex buffer-protein combinations exhibiting significant spectral overlap, it is necessary to simultaneously account for protein and excipient components in the PCM.

The algorithm developed for diafiltration tracked the achievement of the steady state of buffer exchange, independently of the excipients in the buffers. While this method provides qualitative tracking of excipients during diafiltration, a different approach, such as PCM, would be necessary to monitor excipient concentrations during UF/DF.

Future development of the Monipa device aims to integrate the monitoring of additional process parameters, specifically excipient concentrations, to facilitate their accurate quantification, ensuring consistent product quality and reliable process control. This progress supports the biopharmaceutical industry's ambitions of developing PAT tools for real-time release capabilities [20].

In conclusion, the development and implementation of advanced analytical technologies, such as the mid-infrared spectrometer Monipa, are an important step in overcoming the challenges of biopharmaceutical manufacturing. By enabling real-time monitoring and control, these technologies can enhance process efficiency, reduce costs, and improve product quality. As the demand for monoclonal antibodies and other biological drugs continues to grow, PAT innovations like Monipa will play an increasingly important role in optimizing manufacturing processes to answer the needs of patients and healthcare providers worldwide.

Conflict of Interest: Author Alexander Geissler is shareholder of IRUBIS GmbH.

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