Application note

Using Raman Spectroscopy as a Process Analytical Technology Tool in a 50-Day Continuous Perfusion Run

Authors

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Highlights

- Implementation of Process Raman as a PAT tool for in-line and real-time monitoring of critical process parameters, including titer, cell densities, and nutrient concentrations in mAb-producing, high-cell-density perfusion CHO cell culture, is discussed.
- The Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer with Thermo Scientific™ Lykos™ PAT Software, with 21 CFR Part 11 compliance, is used for in-line, real-time, *calibration-free* monitoring of a bioreactor during a 50-day continuous perfusion run.

Summary

The biopharmaceutical industry is driven by the need to increase production and reduce costs while maintaining product quality. One effective way to achieve this goal is to streamline the monitoring process of biologics production, thus allowing more effective control of production parameters. In this application note, we introduce a case study using Process Raman for in-line, real-time monitoring of specific critical process parameters (CPPs) in high-density mammalian perfusion cell cultures reaching 100–130 million cells mL^{-1}. The Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer System is shown to enable in-line measurements of CPPs, including glucose, lactate, ammonium, product titer, and cell viability, over the course of a 50-day continuous perfusion bioreactor run.

Background

In recent years, Raman spectroscopy has gained popularity as a process analytical technology (PAT) tool that enables real-time monitoring and control of critical bioprocessing parameters that are key to the successful production of therapeutic drugs. The product portfolio of biologics is broadening, and implementation of different spectroscopic PAT tools can address the limitations of traditional off-line analytical methods for such products. The production of biotherapeutic products requires high process efficiency while ensuring product quality and minimized manufacturing costs[1]. Implementing PAT tools in biopharmaceutical manufacturing is a critical priority identified by the FDA, with the goal of allowing rapid development and access to novel therapeutics and existing medications without compromising product quality[2,3].

Cell culture processes are labor intensive due to the frequent sample analyses required. When operating bioreactors in fed-batch mode, nutrients are periodically supplemented via a bolus feeding strategy using predetermined volumes of concentrated nutrients. While batch production is a well-vetted manufacturing method, this production strategy can be slow and inefficient. Recent advances in biomanufacturing processes involve continuous processing. Continuous bioprocessing technologies, whether upstream or downstream, can increase speed while decreasing the costs of producing these essential biologics[5]. Perfusion cell culture is a process that uses filters to keep cells in a bioreactor while continuously exchanging culture medium. Fresh medium replenishes nutrients and carbon sources, while cellular waste and medium depleted nutrients are removed. Key advantages of bioreactors operated in perfusion mode include flexibility, lower cost, improved quality, and greater speed.

In this study, we focused on the continuous perfusion operation mode for suspension cell culture. The goal of continuous perfusion is to develop a process that maintains a steady state in which productivity and product quality can be sustained long-term with minimal variability, with bioreactors running for 30 – 90 days.

Raman spectroscopy is a laser-based method for generating a chemical fingerprint of a sample[2,3]. A key advantage of Raman spectroscopy is its ability to measure numerous analytes in a non-destructive manner, *in situ*, and with low interference from water. Fortuitously, numerous analytes with distinct Raman fingerprints enable monitoring of CPPs such as nutrient feed levels, cell metabolites, cell growth profiles, product levels, and product quality attributes[1,2]. The MarqMetrix All-In-One Process Raman Analyzer with Thermo Scientific Lykos PAT Software is designed to offer accurate, reliable, real-time identification and quantification of numerous CPP analytes. Process Raman is extremely advantageous when adopted into a continuous perfusion culture system. Utilizing an immersed Raman optical sensor provides real-time process information about the CPPs, unlike traditional monitoring systems, which require manual sampling and off-line analysis[1,2]. Noted advantages include an increase in data acquisition frequency, the opportunity for rapid correction of any detected process parameter deviations, and reduction of contamination risk due to a reduced offline sampling.

This application note describes the integration of the MarqMetrix All-In-One Process Raman Analyzer with the 50L Thermo Scientific™ DynaDrive™ bioreactor to perform in-line measurements of CPPs in a continuous perfusion run (See Figures 1-3). Here, we highlight the integration of the MarqMetrix All-In-One Process Raman Analyzer System to perform in-line measurements of glucose, lactate, ammonium, viable cell density (VCD), total cell density (TCD), and titer. This PAT tool provides accurate prediction models for several parameters and metabolites and shows high correlations with offline measurements. The simultaneous measurements of metabolites, product titer, and protein concentration allow for real-time process control, demonstrating the effectiveness of process Raman spectroscopy as a PAT tool in biopharmaceutical industries.

Materials and methods

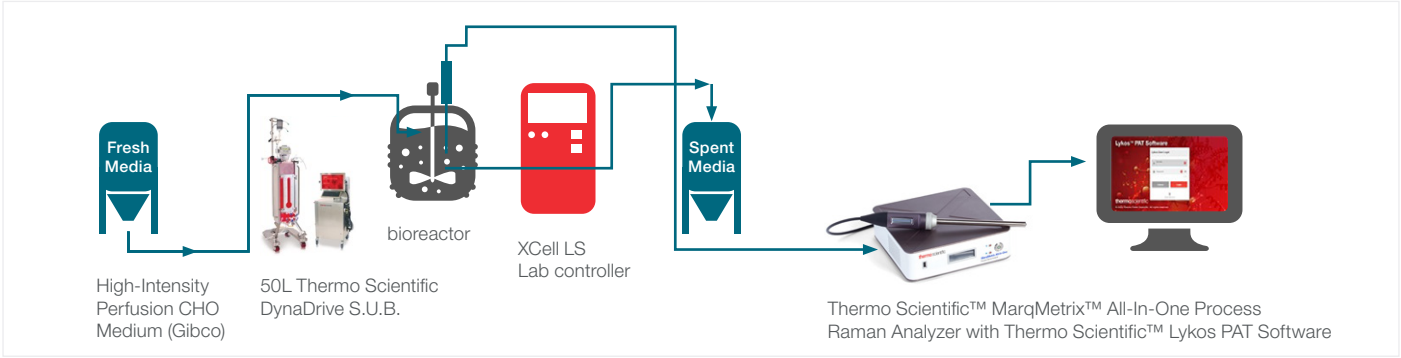

Figure 1. Scheme of perfusion process configuration and integration of an in-line Raman sensor (MarqMetrix All-In-One Process Raman Analyzer with Lykos PAT Software).

Cell line and medium

For the two 50 L perfusion cultivations, a trastuzumab-producing CHO K1 cell line was used. The basal medium was 0.66x concentrated High-Intensity Perfusion CHO medium (Gibco™), whereas 1x concentrated medium was used as the feed medium. Both media were supplemented with 4 mmol L^{-1} l-glutamine (Gibco™) and 1% Anti-Clumping Agent (Gibco™).

Inoculum production

Inoculum production was carried out over a period of 10 days. The first three passages were carried out at a shake flask scale. A 10 L wave-mixed bioreactor (Cellbag 10 L, Cytiva) with a working volume of 5 L was used for the last passage.

Perfusion cultivations at 50 L scale

For the 50 L perfusion cultivations, the ATF version of the DynaDrive S.U.B. (Thermo Scientific) with the corresponding G3Pro Bioprocess Controller was used. Cell retention was realized with Repligen's XCell ATF6 single-use version and the corresponding XCell LS controller at an average ATF flow rate of 17 L min^{-1}. The cultivation was started with a VCD of $3x10^5$ cells mL^{-1}. Perfusion was started after an initial 3-day batch phase. The perfusion rate was limited to 1 d^{-1}. The bleed was controlled with the permittivity probe IncyteArc (Hamilton Bonaduz) to keep a viable cell volume of 100–130 mm^3 mL^{-1} constant. To keep the bioreactor volumes constant at 50 L, the harvest pump was controlled by the bioreactor weight. Glucose concentration was either kept constant at 2 g L^{-1} with a CIT Sens Bio glucose sensor (C-CIT) for the first run or was manually controlled for the second run. To prevent foaming, a 1:10 diluted antifoam C emulsion (Sigma Aldrich) was added quasi-continuously. In all cultivations, the pH was controlled to a value of ≤7.15 by adding CO_2 via the drilled hole sparger. DO was controlled at 40% by adding N_2, air and O_2 using the corresponding sparger. An overlay gassing rate of 0.05 vessel volumes min^{-1} with air was chosen. The stirrer speed was set to 136.5 rpm (corresponding to 40 W m^{-3}) in the DynaDrive.

Analytics

Once a day, cell-specific parameters, such as the VCD, viability, and cell diameter, were determined with a Cedex HiRes analyzer (Roche Diagnostics, Basel, Switzerland). The Cedex Bio analyzer (Roche Diagnostics) was used to check the substrate and metabolite concentrations of glucose, lactate, glutamine, and ammonium as well as the IgG titer.

Figure 2. 50L Thermo Scientific DynaDrive S.U.B. for cell culture applications.

Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer Measurements

Measurements were performed using the MarqMetrix All-In-One Process Raman Analyzer System, with the optical Bioreactor ball probe directly immersed in the bioreactor (50L). Each Raman spectra resulted from an average of 60 measurements, with an integration/exposure time of 1 sec and a laser power setting of 450 mW. The total acquisition time per data spectra was 2 minutes, with a timestamp matched between the MarqMetrix All-In-One Process Raman Analyzer and offline instrument analysis to build the model.

Figure 3. MarqMetrix All-In-One Process Raman Analyzer with optical MarqMetrix Bioreactor BallProbe™ immersed in a 5L glass bioreactor.

Chemometric Model building

Independent data from multiple MarqMetrix All-In-One Process Raman Analyzers and numerous bioreactors were used to create models for each analyte. The training datasets were collected from 12–24 samples per bioreactor to create each chemometric model. In-line and at-line measurements were aligned using timestamps between the MarqMetrix All-In-One Process Raman Analyzer and the at-line instrument, the Nova Flex II. All data was reviewed before building the models. In addition, an algorithm was implemented to remove data spikes in the spectra caused by cosmic rays. The spectral region of interest was selected, and multiple spectra were averaged to increase signal-to-noise ratios such that each measurement corresponded to a ten-minute read-time. The spectra were pre-processed to remove differences in the baseline due to fluorescence and other effects. Spectra were also normalized to remove differences in absolute intensity between various bioreactor types. Partial Least Squares (PLS) models were created for each analyte of interest, and leave-out-one-run cross-validation was performed to test the optimization of each model. Analytes of interest include glucose, lactate, ammonium, VCD, TCD, and titer.

Additionally, an augmentation approach was used to improve the models. Data from two ZHAW perfusion runs was collected. One run was used to augment training data to improve the prediction accuracy for the other. The run used to augment the training data was weighted equally to the rest of the training data despite having far fewer samples because this data was assumed to have greater similarities to the prediction run. In this manner, the model was able to learn from the bulk data in the regular training set but was fine-tuned specifically to give the best predictions on ZHAW data. This approach optimizes the benefits of using both a broad general dataset and a specifically targeted but much smaller dataset.

Results

To analyze the chemometric modeling results, let us first define the key figures of merit: bias and RMSEP. Bias is the average difference between predicted values and reference values. The Root Mean Squared Error of Prediction (RMSEP) is the combined error of bias and precision, where precision is the randomness (noise) around the mean of the predicted values, assuming there is no bias. Furthermore, the quality of a chemometric model is typically evaluated using the Q-residuals and Hotelling's T-squared values. The Q-residuals are used to quantify how well the model fits the raw data. Q-residuals should typically be less than 1, which indicates that the model accounts for all the variance in the spectra. A high Q-residual value indicates an observation not well explained by the model, suggesting it may be an outlier. Hotelling's T-squared values measure the distance of each observation from the model's center in the space of the retained components. A high T-squared value expresses an observation far from the model's center, which could also suggest it as a potential outlier. Both Q-residuals and Hotelling's T-squared values are important tools for model diagnostics in chemometrics.

While the results of applying generalized chemometric models, based on bolus-fed CHO cell lines, produced prediction errors of approximately 1 g L^{-1} for glucose and lactate, these generalized models produced poor results when predicting ammonium, titer, VCD and TCD. In contrast, the augmentation of the generalized models using one ZHAW run to predict the other ZHAW run resulted in prediction errors of 0.36 g L^{-1} for glucose and 0.37 g L^{-1} for lactate. Furthermore, the augmentation of the generalized model enabled accurate predictions for ammonium (RMSEP = 0.95 mmol L^{-1}) and titer (RMSEP = 0.36 g L^{-1}) while also providing prediction accuracy for VCD & TCD of ≈10 million cells mL^{-1}, which translates to +/- 10% of the stationary phase concentrations of ≈100 million cells mL^{-1}.

Figures 4-9 show the correlation between offline data and predictions made by applying the chemometric models to the spectra collected using the Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer. In each Figure, the predicted vs. offline data is plotted in panel A. These figures demonstrate the ability to monitor changes in various CPPs in real-time with a high degree of accuracy. Panel B in each figure speaks to the quality of the chemometric model, as the Q-residuals and Hotelling's T-squared values for each analyte model exhibit very low values; this indicates a good fit of the model with negligible unaccounted variance or outliers.

Panel C in each figure demonstrates the linearity of the CHO-cell training data, augmented with a weighted ZHAW run (green) and a ZHAW run used as a prediction data set (purple). One reason for applying the weighted-ZHAW augmentation to the independent training data set was the very small linear range for many analytes in these perfusion runs. In contrast to the small linear range of CPPs such as glucose, lactate, ammonium, and titer, the cell density values, VCD and TCD, exhibited a wide range from 20–130 million cells mL^{-1}. The results demonstrate excellent correlations between the model prediction data obtained from the Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer and the offline data for numerous CPPs (Table 1).

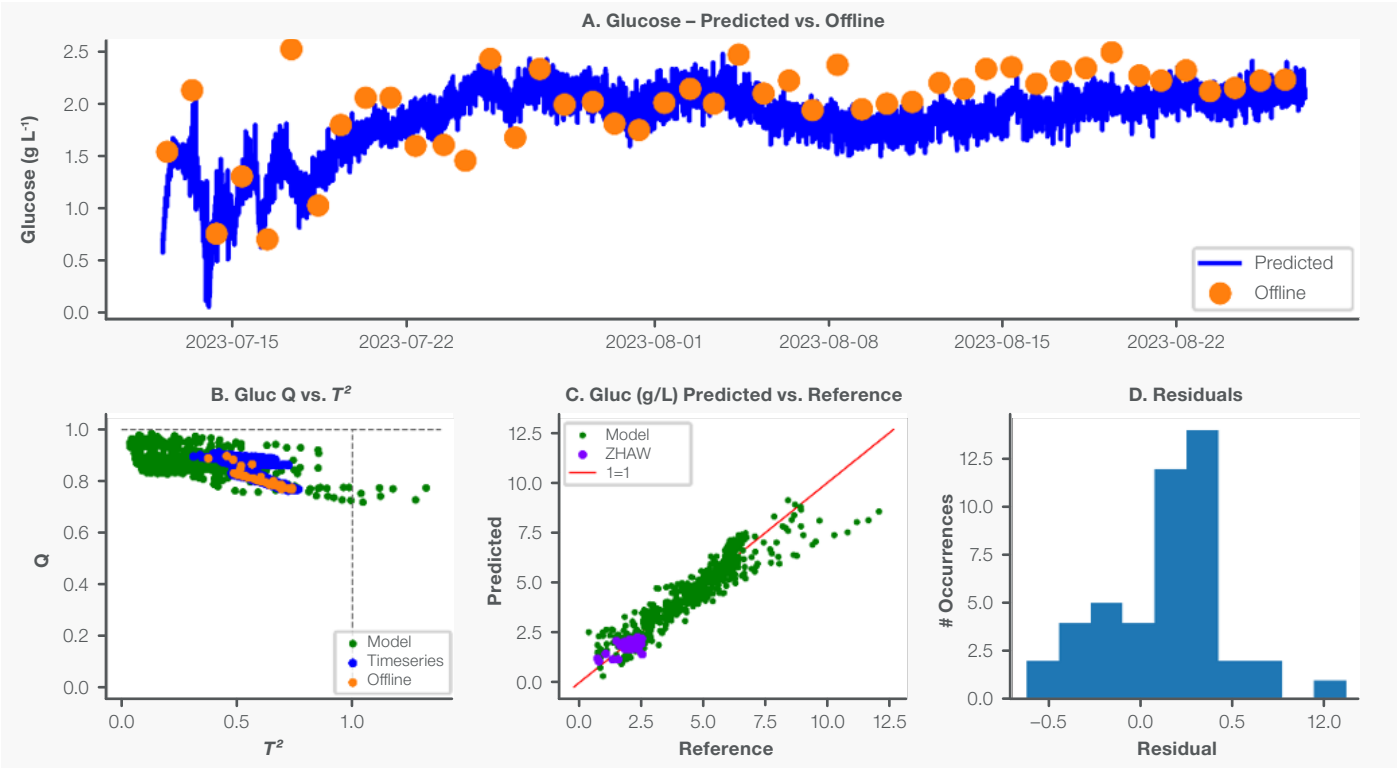

Figure 4. Modeling results for glucose monitoring in the 50-day perfusion run of the bioreactor. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

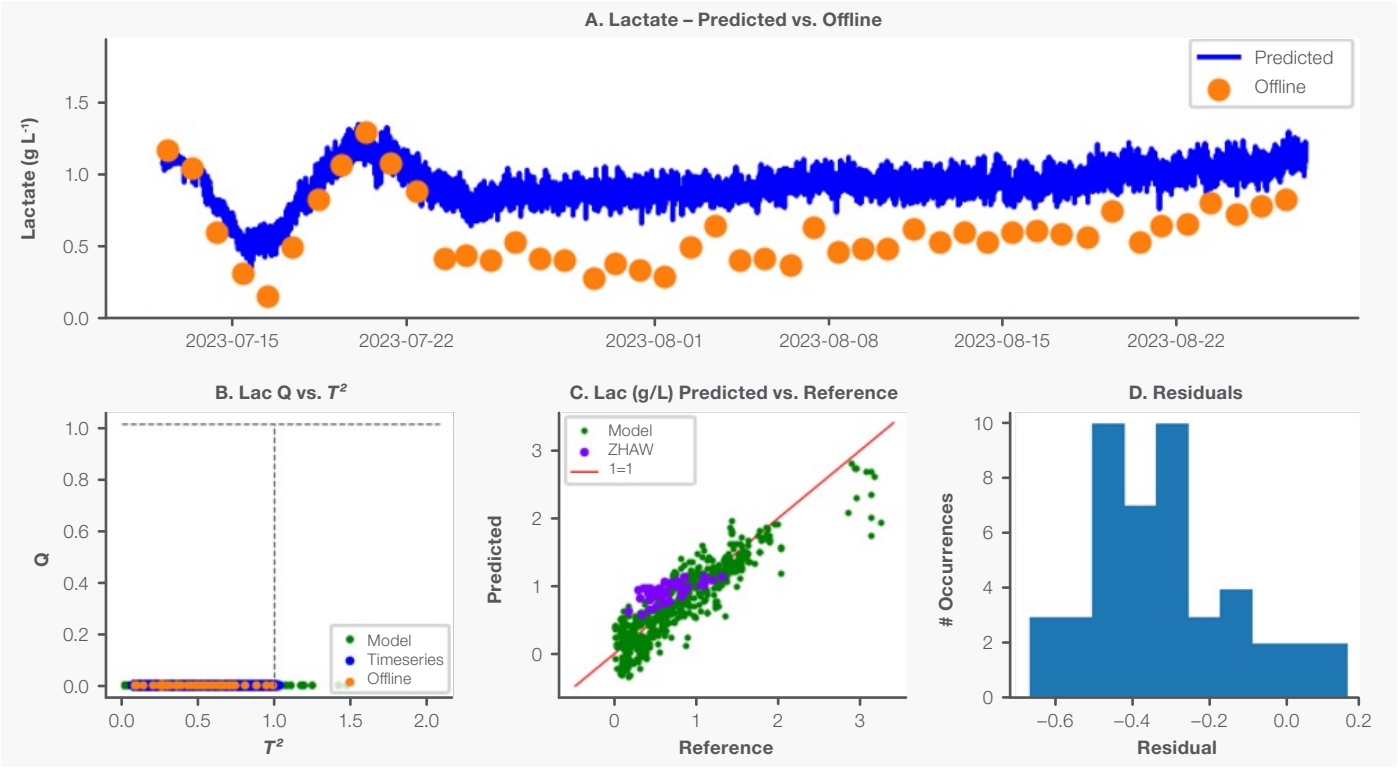

Figure 5. Modeling results for lactate monitoring in the bioreactor's 50-day perfusion run. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

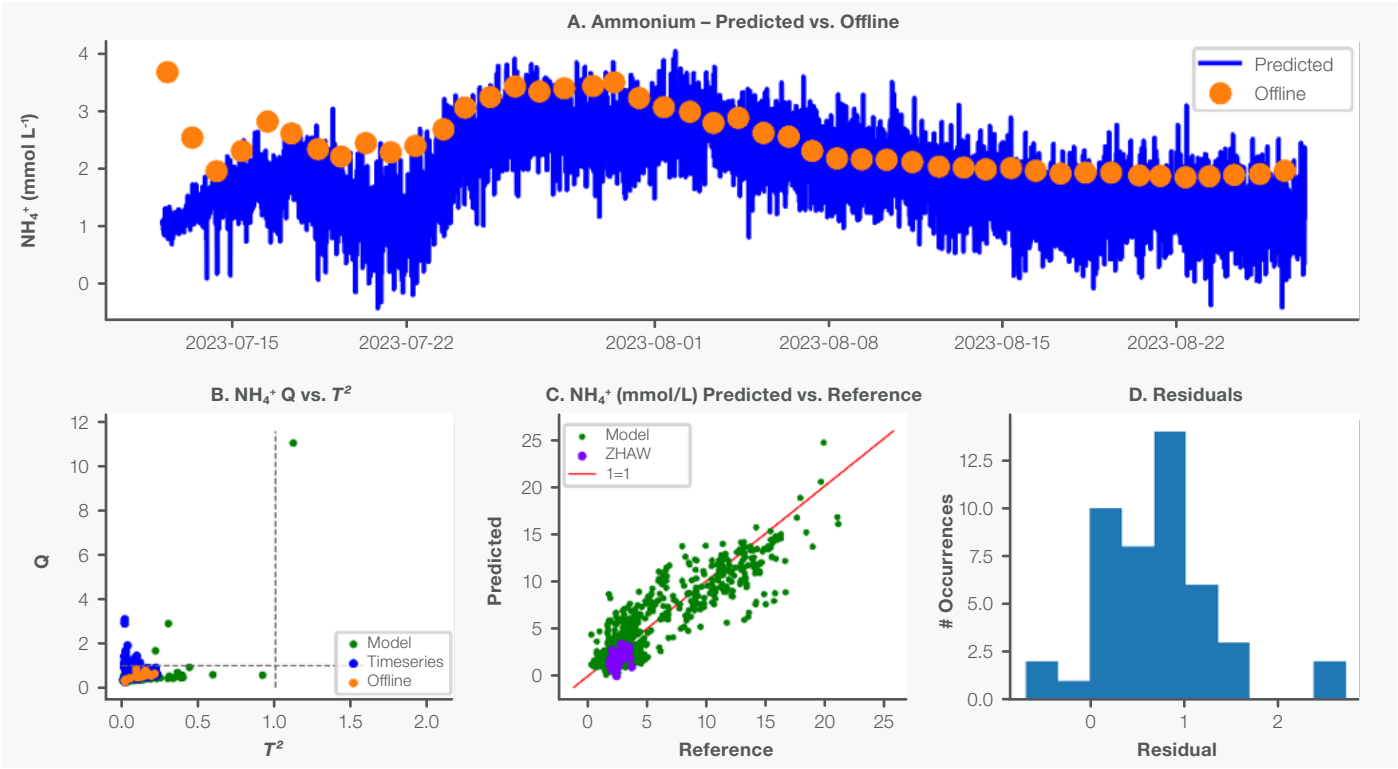

Figure 6. Modeling results for ammonium monitoring in the bioreactor's 50-day perfusion run. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

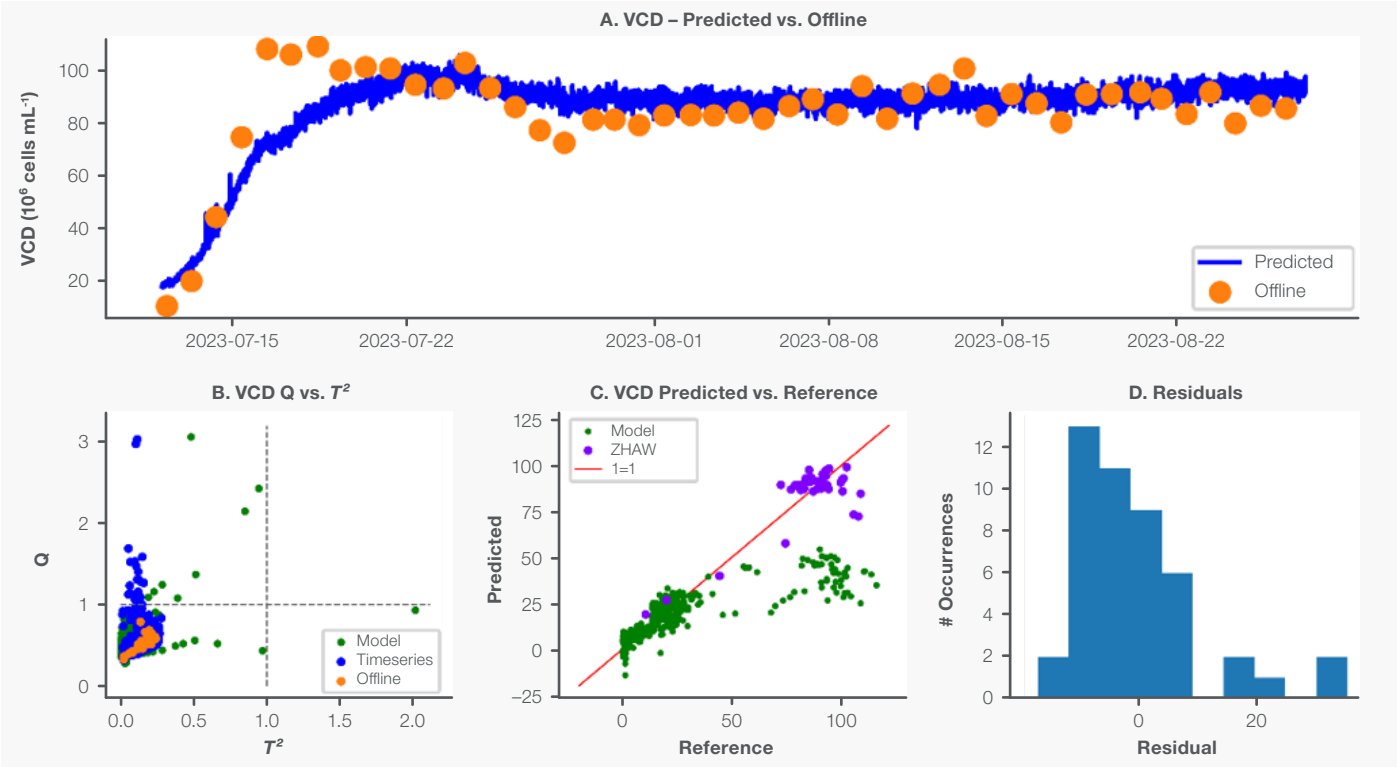

Figure 7. Modeling results for Viable Cell Density (VCD) monitoring in the 50-day perfusion run of the bioreactor. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

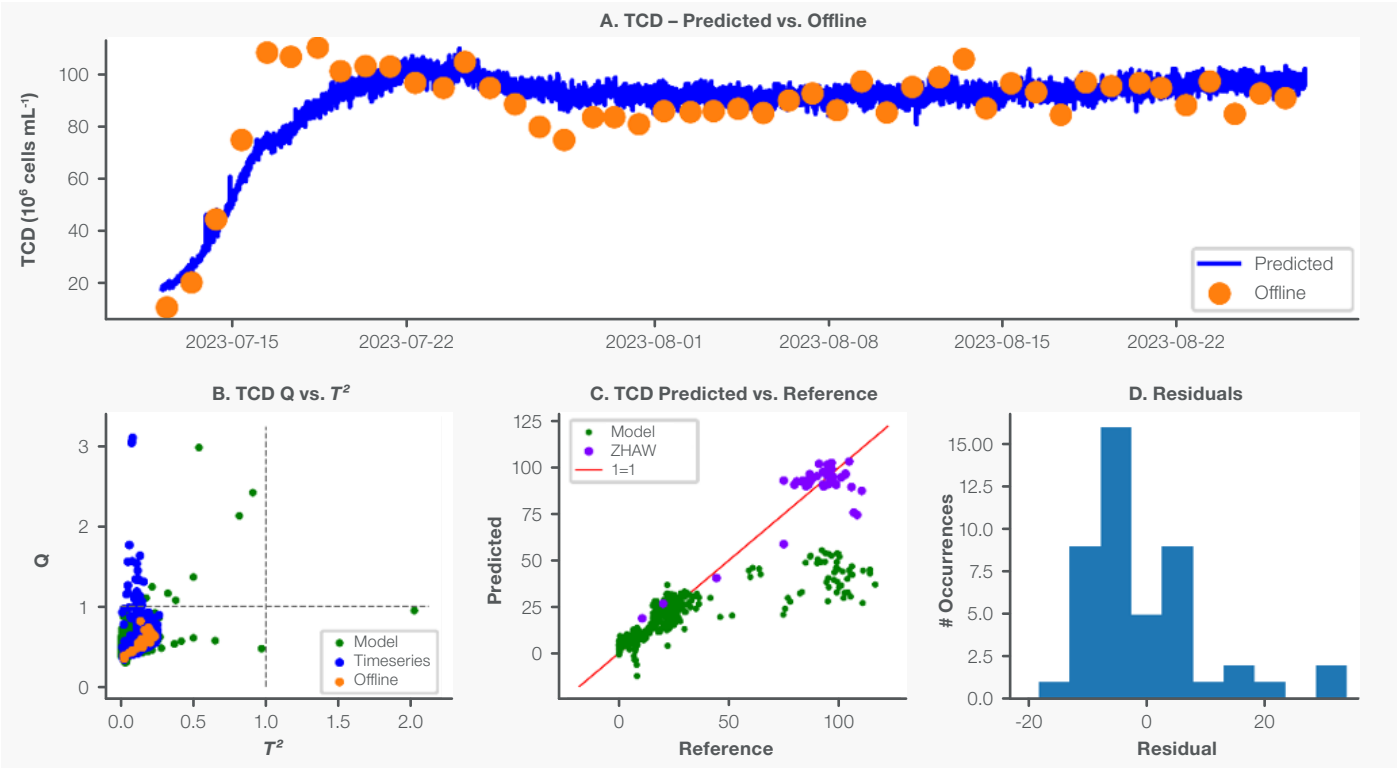

Figure 8. Modeling results for Total Cell Density (TCD) monitoring in the bioreactor's 50-day perfusion run. (A) shows the modeling results generated from the process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

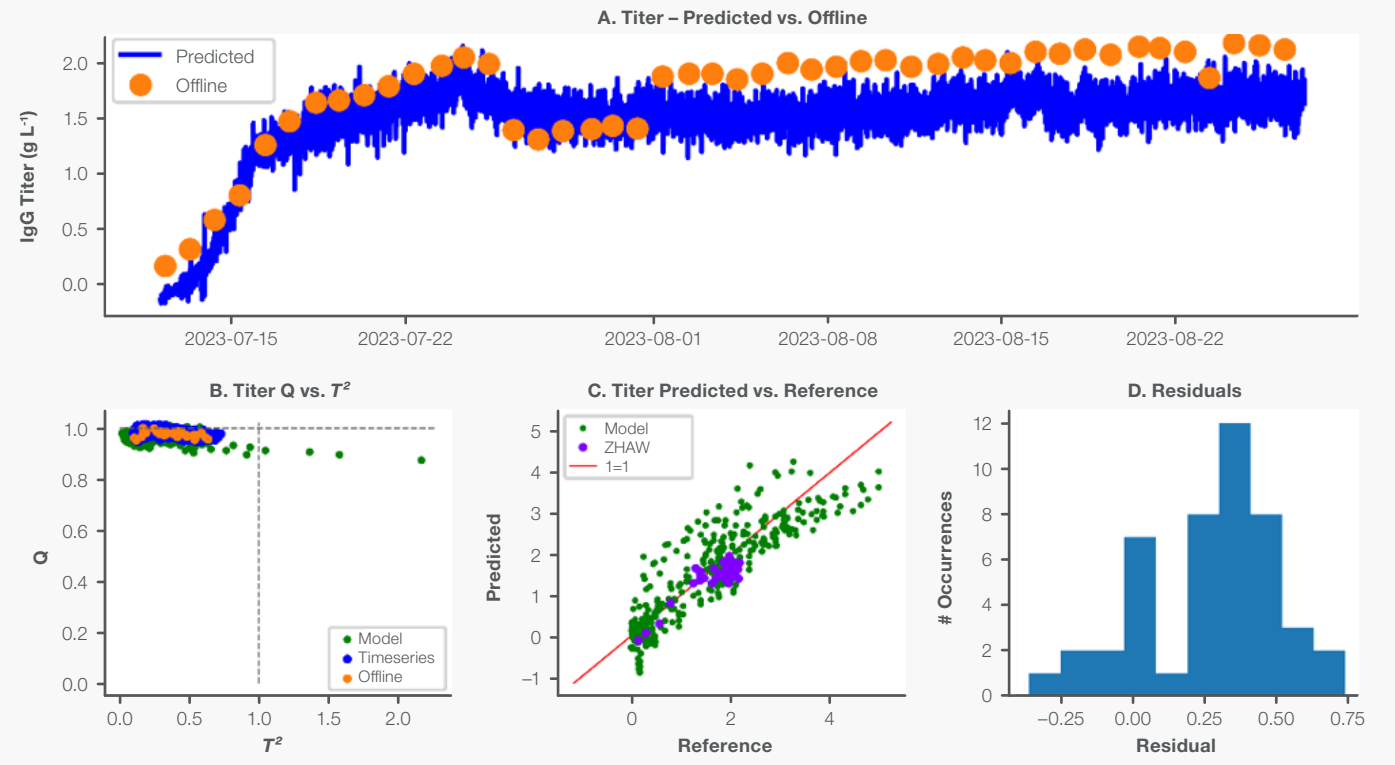

Figure 9. Modeling results for titer monitoring in the 50-day perfusion run of the bioreactor. (A) shows the modeling results generated from the Process Raman spectra compared to the results of offline analysis. (B) shows the Q-residuals vs. Hotelling's T^2 values. (C) shows the linearity of the predicted vs. offline values. (D) shows the histogram of residuals between predicted and offline values.

Analyte	Units	RMSEP	Bias
Glucose	g L^{-1}	0.36	-0.15
Lactate	g L^{-1}	0.37	0.32
Ammonium	mmol L^{-1}	0.95	-0.71
VCD	million cells mL^{-1}	10.78	0.47
TCD	million cells mL^{-1}	10.59	0.62
Titer	g L^{-1}	0.36	-0.27

Table 1. Summary of Chemometric Modeling Results for this 50-day Perfusion Run.

Conclusion

The study in this application note highlights an approach to using Raman spectroscopy, specifically Process Raman, for in-line, real-time monitoring of a high cell density mammalian cell culture run in a bioreactor operated in perfusion mode The MarqMetrix All-In-One Process Raman Analyzer provides exceptional data quality, which, when combined with multivariate PLS modeling, enables in-line, real-time monitoring of CPPs glucose, lactate, ammonium, titer, and cell density measurements. The ability to obtain accurate cell density measurements in a wide range from 20-130 million cells mL^{-1} showcases the robustness and utility of these chemometric models and the efficacy of utilizing the MarqMetrix All-In-One Process Raman Analyzer to collect high-quality spectral data. Great data lead to robust and accurate chemometric models, thereby unlocking the potential of Process Raman as a critical PAT tool in biopharmaceutical processes.

The simultaneous measurements of metabolites and product titer are of special interest as they will allow for greater process control of cultivation and purification parameters within continuous biomanufacturing processes. Additionally, Process Raman greatly enhances the operator's understanding of the CHO cell perfusion process as these CPPs are measured repeatedly in short intervals in a non-destructive manner. This study demonstrates the capability of Process Raman spectroscopy as a PAT tool that can pair seamlessly with automation systems to improve yields and enhance product quality.

The results of the perfusion cultivations discussed in this application note have been published in detail, https://www.mdpi.com/2227-9717/12/4/806.[6]

References

1. Research, C. for D. E. and. *PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance*. U.S. Food and Drug Administration. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pat-framework-innovative-pharmaceutical-development-manufacturing-and-quality-assurance (accessed 2023-03-21).
2. Real time monitoring of multiple parameters in mammalian cell culture bioreactors using an in-line Raman spectroscopy probe. Nicholas R Abu-Absi [1], Brian M Kenty, Maryann Ehly Cuellar, Michael C Borys, Sivakesava Sakhamuri, David J Strachan, Michael C Hausladen, Zheng Jian Li. Biotechnol Bioeng. 2011 May;108(5):1215-21. doi: 10.1002/bit.23023. Epub 2010 Dec 22
3. Quick generation of Raman spectroscopy based in-process glucose control to influence biopharmaceutical protein product quality during mammalian cell culture. Brandon N Berry [1], Terrence M Dobrowsky [1], Rebecca C Timson [1], Rashmi Kshirsagar [1], Thomas Ryll [1], Kelly Wiltberger [2] Biotechnol Prog 2016 Jan-Feb;32(1):224-34. doi: 10.1002/btpr.2205. Epub 2015 Dec 21.
4. Modernizing the Way Drugs Are Made: A Transition to Continuous Manufacturing | FDA, Sau (Larry) Lee, Ph.D., Deputy Director of the Office of Testing and Research, and Chair of the Emerging Technology Team, Office of Pharmaceutical Quality, CDER
5. Integrated Continuous Pharmaceutical Technologies – A Review. András Domokos, Brigitta Nagy, Botond Szilágyi, György Marosi, and Zsombor Kristóf Nagy. *Organic Process Research & Development* **2021** *25* (4), 721-739 DOI: 10.1021/acs.oprd.0c00504
6. Scaling Fed-Batch and Perfusion Antibody Production Processes in Geometrically Dissimilar Stirred Bioreactors. Vivian Ott, Jan Ott, Dieter Eibl, Regine Eibl. Processes. 2024, 12(4), 806.