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In-Line Permeate Monitoring and Model Transfer With Raman Spectroscopy and BioPAT® Spectro Flow Cell

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Abstract

Monitoring process conditions such as productivity and metabolite levels is essential for ensuring quality, consistency, and cellular health in bioprocessing. Raman spectroscopy is a proven tool for this purpose, but its effectiveness depends on consistency between the calibration and deployment environments.

The BioPAT® Spectro Flow Cell provides a single-use flow-through interface for Raman measurements in tubing lines, extending the BioPAT® Spectro platform beyond in situ bioreactor monitoring. In this application note, the BioPAT® Spectro Flow Cell was used for in-line permeate monitoring in perfusion bioreactors, where reduced cell material can result in improved spectral quality. Multivariate models were developed using 2 L Univessel® bioreactor data and evaluated for transfer to a 50 L Biostat STR® bioreactor running the same process. The results demonstrate how permeate-line Raman monitoring can support real-time process visibility, model transfer across scales, and broader use of Raman modeling in biopharmaceutical manufacturing.

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Introduction

Process analytical technology (PAT) has attracted growing attention from the biopharmaceutical industry over the past 20 years. This growth followed guidance issued by the U.S. Food and Drug Administration (FDA) in 2004, which positioned PAT as a tool for achieving quality by design (QbD) in drug manufacturing.[1,2] Previous studies have shown successful deployment of PAT for real-time monitoring of process performance, critical process parameters, critical quality attributes, and other key performance indicators (KPIs).[3-6] Among these technologies, Raman spectroscopy has become widely used as a non-invasive, non-destructive approach for generating real-time on-line or in-line predictions of these process metrics.[3]

Multivariate data analysis (MVDA) transforms Raman spectroscopy's chemical fingerprint spectra into actionable information for real-time prediction. Algorithms such as orthogonal partial least squares (OPLS®) can be used to build a multivariate regression model that predicts concentrations of media components such as glucose and glutamine, KPIs including titer, and other process variables. While building such models is straightforward, it can be challenging to acquire sufficient data to train them appropriately. Additionally, models must be trained using spectra collected with the same measurement interface to ensure conformity between training and prediction spectra.

BioPAT® Spectro was designed to provide a consistent interface for collecting Raman spectra across bioreactor scales, supporting the development and transfer of in situ models of bioreactor conditions.[3] However, interest in Raman monitoring extends beyond in situ bioreactor conditions. To address this need, the platform has been expanded to include the new BioPAT® Spectro Flow Cell, which enables on-line or in-line measurements in any tubing line. Figure 1 shows how the BioPAT® Spectro Flow Cell makes the BioPAT® Spectro interface compatible with different tube diameters, enabling its use in tubing lines from permeate monitoring to post-chromatography product streams.

In this application note, the BioPAT® Spectro Flow Cell was used to monitor permeate lines of a perfusion bioreactor. Compared with in situ Raman measurements, the permeate contained almost no cellular material, resulting in lower fluorescence, improved spectral quality, and a shorter acquisition time per data point. The resulting models showed excellent predictive performance across a range of media components and KPIs, with the uniform Raman interface supporting successful model transfer between process scales when analyte trends were comparable. These capabilities support the broader application of Raman modeling across biopharmaceutical manufacturing processes.

Figure 1: The BioPAT® Spectro Flow Cell with a variety of connection sizes (⅛" to 1" inner diameter)

Materials & Methods

Three perfusion experiments were conducted in 2 L Univessel® bioreactors, each lasting approximately two weeks. In these experiments, an industrially relevant CHO DG44 cell line expressing an IgG1 monoclonal antibody (mAb) was cultured with the 4Cell® SmartCHO media system. Samples were taken from the permeate line and analyzed offline with a BioProfile® FLEX2 system for various cell culture parameters such as glucose and lactate concentrations. Titer data were collected via offline high-performance liquid chromatography (HPLC). A final perfusion experiment was performed in a 50 L Biostat STR® bioreactor using the same cell line and media strategy.

BioPAT® Spectro Flow Cell was connected to the perfusion line of each experiment to enable continuous Raman spectra collection. Spectra were acquired using a HyperFlux™ Raman spectroscopy system (Bruker) with identical acquisition settings, averaging five 12-second spectra at intervals ranging from 2 to 10 minutes, depending on the experiment. Figure 2 shows representative raw spectra over the course of an experiment.

SIMCA® was used to analyze the Raman spectra and build OPLS® regression models from the 2 L bioreactor data. Preprocessing consisted of first derivative (21 pts) followed by standard normal variate (SNV), applied to the combined fingerprint and C-H stretch regions of the Raman spectra. Representative transformed spectra are shown in Figure 3.

Models were built for glucose, lactate, glutamine, glutamate, and ammonium concentrations, as well as titer. Model performance was evaluated both by self-testing, using root mean square error of cross-validation (RMSECV), and prediction testing, using root mean square error of prediction (RMSEP). The models developed were then applied to the 50 L bioreactor data, allowing prediction errors to be reported for a withheld dataset. The models could then be deployed directly to the spectrometer for future experimental use.

Figure 2: Raw spectra from the perfusion line of three 2 L bioreactor experiments, colored by experiment

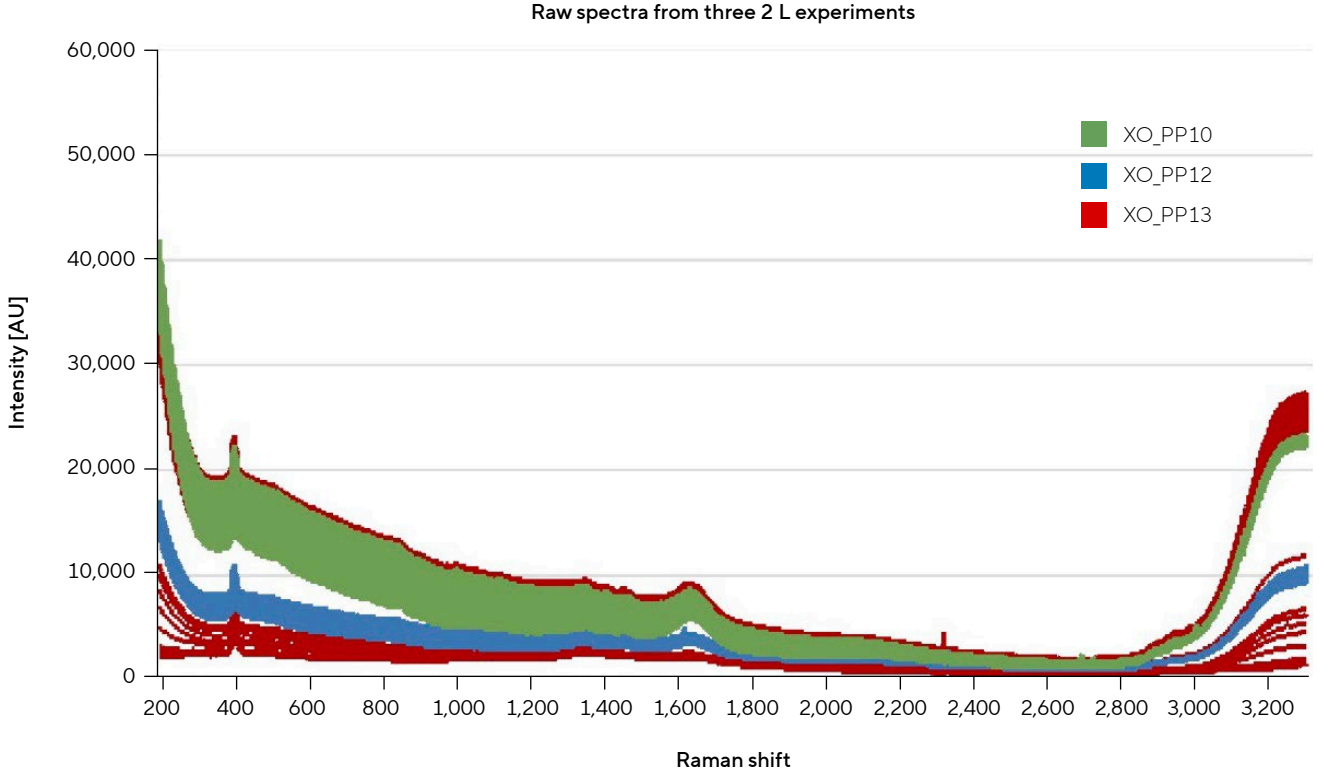
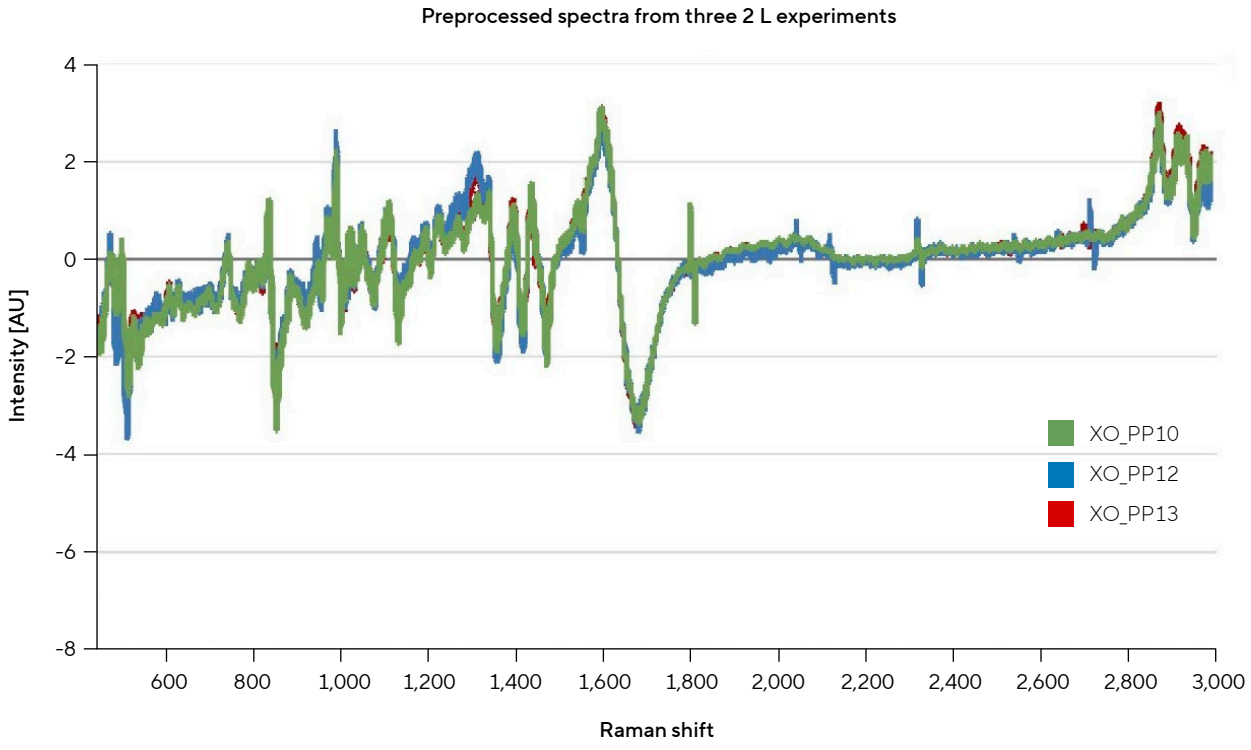


Figure 3: Spectra after first-derivative and SNV preprocessing, colored by experiment.



Results

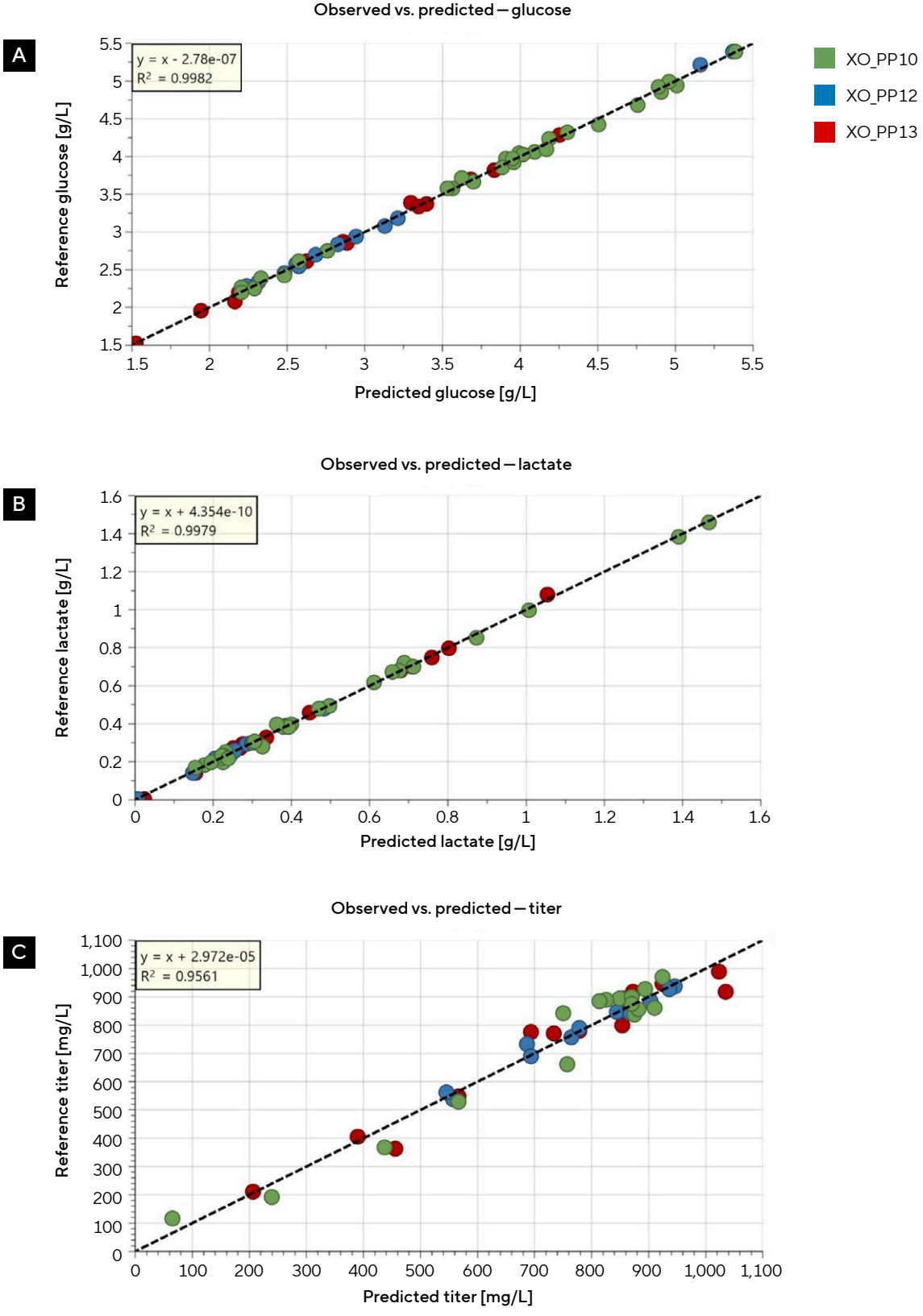
Using data from the three 2 L Univessel® experiments, multivariate regression models were developed in SIMCA® for glucose, lactate, and titer. Figure 4A–C contains the actual vs. predicted plots for these models, with good fit shown for each. R^2 values are uniformly high, and RMSECV values indicate strong predictive performance.

The models were then used to predict analyte concentrations in a 50 L Biostat STR® bioreactor running the same process. Figure 5A–C shows concentration predictions performed every 10 minutes (black lines), alongside offline measurements taken by reference methods (blue squares). A process deviation is observed in the real-time trace on day 5, when a foam-out event occurred and the process entered an emergency stop before manual reset by an operator.

The glucose feed remained active during the stop, resulting in a spike in glucose levels captured by the real-time predictions but not by offline measurements. Strong predictive performance was observed for all analytes. Titer predictions showed slight deviations during a period of low titer and rapid growth, potentially due to limited training data in this range, but later returned to agreement with offline measurements.

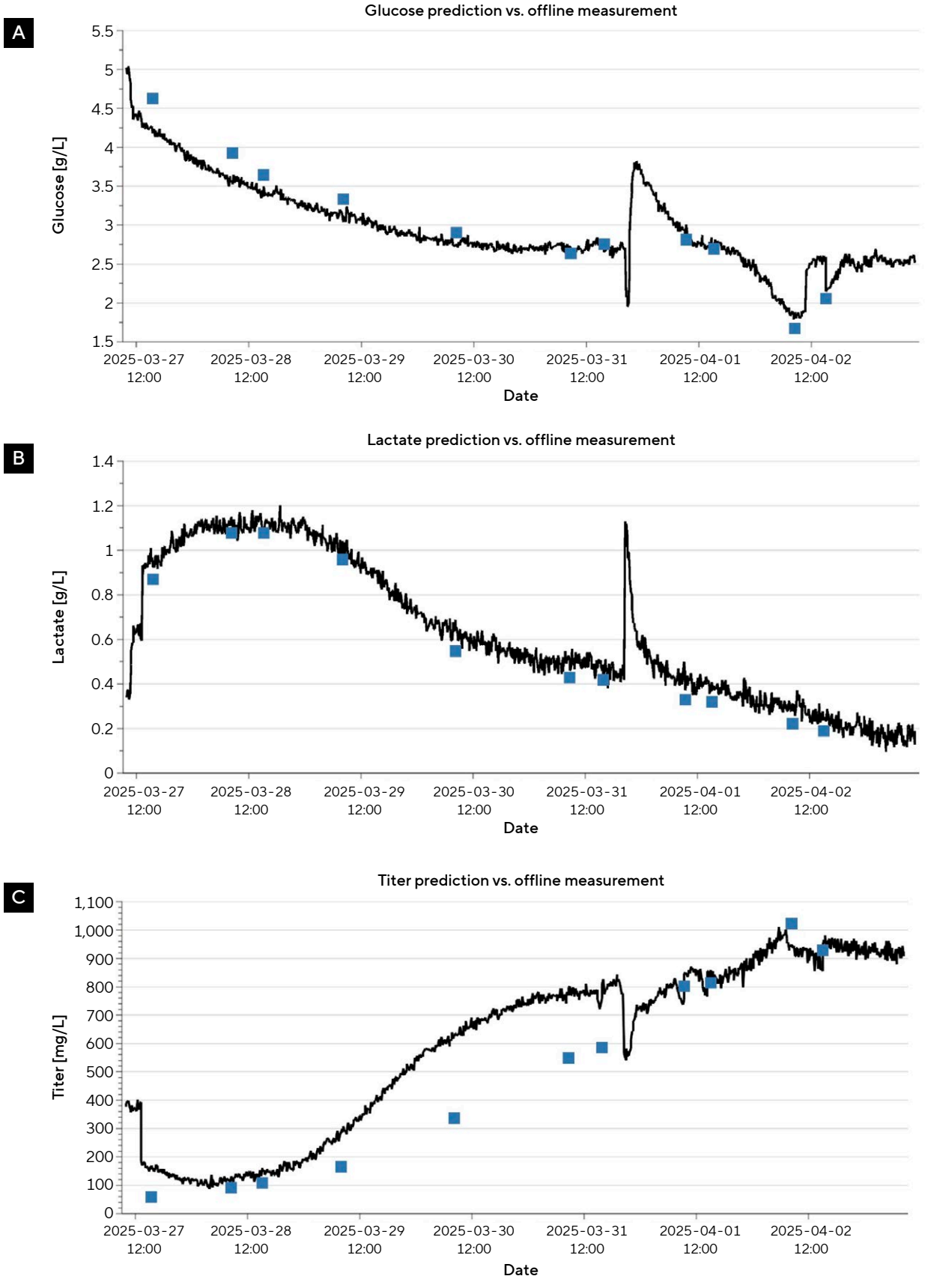
A key advantage of in-line Raman monitoring is the continuous prediction of process performance. While offline titer measurements provided 11 reference points over the multiday experiment, the Raman system generated thousands of predictions and captured fast-changing events such as those seen in the foam-out (Figure 5). This real-time information on process trends supports active decision-making based on timely data in critical development and manufacturing workflows.

Figure 4: Observed vs. predicted concentration plots for glucose **(A)**, lactate **(B)**, and titer **(C)**, colored by experiment. R^2 values are provided on each plot.



Note. RMSECV values were 0.106 g/L for glucose, 0.072 g/L for lactate, and 71 mg/L for titer.

Figure 5: Predicted concentration (black) and reference concentration (blue) over the course of a 50 L bioreactor experiment



Conclusion

With the BioPAT® Spectro Flow Cell, Raman spectroscopy can be used for real-time process monitoring through in-line measurements enabled by a uniform optical design. Leveraging real-time biological and chemical measurements in a manufacturing process can unlock significant value by increasing process visibility, which improves understanding and informs decision-making. Additionally, SIMCA®-Q can be integrated with spectrometers or automation systems, allowing model predictions to be incorporated into control software and supporting model-based predictive control strategies.

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