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BioPAT® Spectro: Enabling Transfer of Raman Calibration Models Across Bioreactor Scales

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Abstract

Over the past two decades, the biopharmaceutical industry has pursued the implementation of process analytical technology (PAT) to achieve quality by design in biological drug manufacturing. Raman spectroscopy has been demonstrated to offer non-invasive, real-time measurement of critical process parameters and quality attributes, revolutionizing bioprocess monitoring and control. Calibration models using advanced statistical methods are essential to translate spectral data into analyte concentrations. However, the development of these models is time-consuming, costly, and difficult at the manufacturing scale. Thus, the industry is shifting towards high-throughput small-scale bioreactor platforms, which enable automated generation of data for Raman calibration modeling. The main challenge with this approach is to ensure model transferability across bioreactor platforms and scales.

Here, we illustrate how Sartorius' BioPAT® Spectro addresses this issue with identical flow cell design for both small- and commercial-scale bioreactors, alongside SIMCA-Q® integration in the spectrometer software. In addition, we demonstrate the seamless transfer of Raman calibration models built in small- (Ambr® 250 High Throughput) to benchtop- (Univessel®) and manufacturing-scale (Biostat STR®) bioreactors. Despite different probe formats, this transfer can be achieved thanks to the comparable optical properties between BioPAT® Spectro and used immersion probe.

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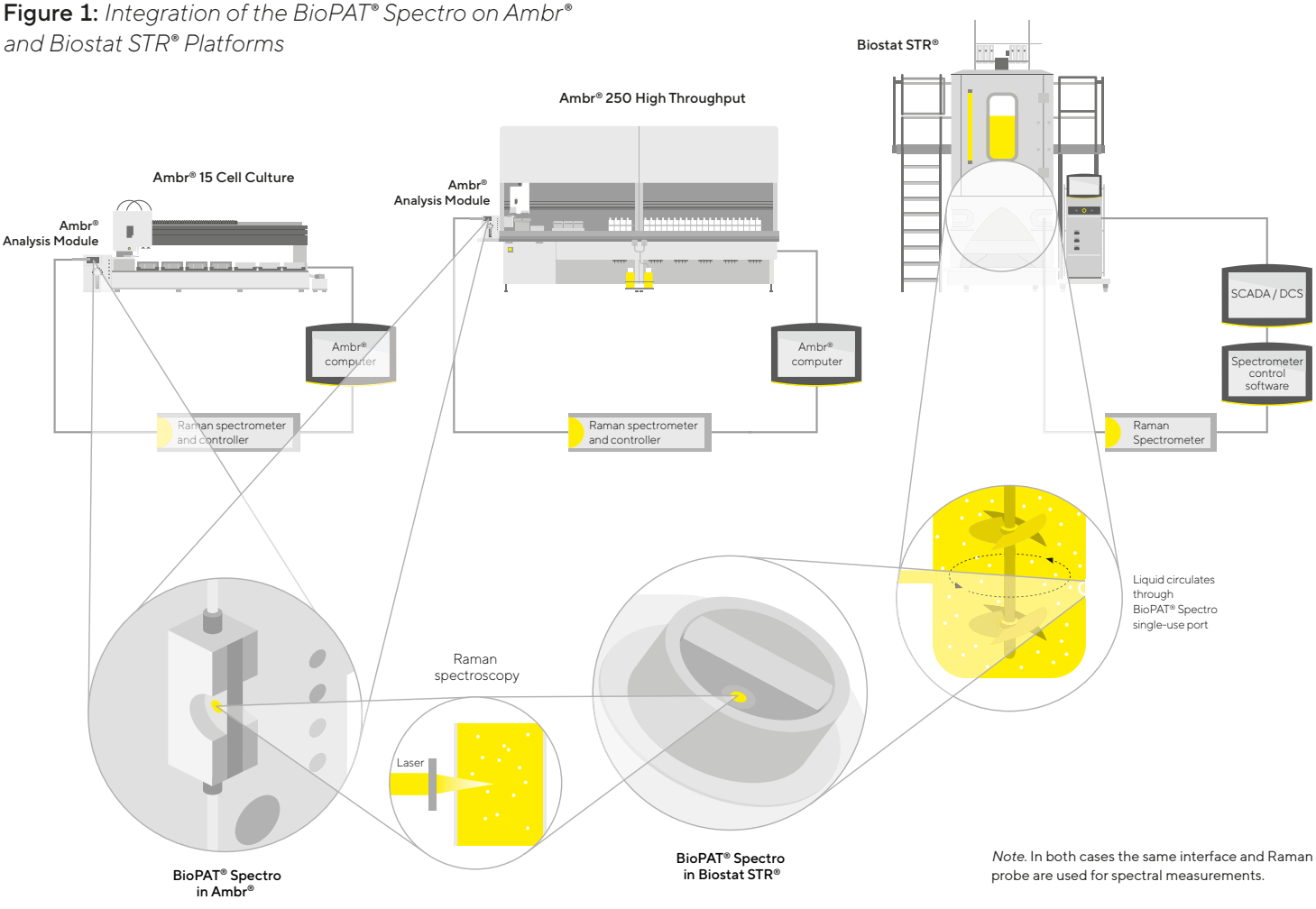
Introduction

Following the U.S. Food and Drug Administration's (FDA) 2004 guidance[1], the biopharmaceutical industry has been continuously striving for the implementation of PAT as a means to achieve quality by design (QbD)[2] in biological drug manufacturing. This endeavor requires technologies that can be implemented in an in-line or on-line fashion to enable real-time monitoring and process control of critical process parameters (CPPs)[3], critical quality attributes (CQAs)[4], and other key performance indicators (KPIs), e.g., protein titer.[5] Raman spectroscopy enables continuous, real-time monitoring of the manufacturing process by providing a non-invasive, in-line chemical fingerprint of the media composition, empowering scientists to make crucial process decisions while minimizing the dependence on results from offline analytics.

Calibration models created through multivariate data analysis (MVDA) are essential for Raman-based monitoring and control in bioprocess applications. These models are needed to translate spectral data into analyte concentrations, using advanced statistical methods like orthogonal projection to latent structures OPLS® regression.[6]

However, calibration model development is costly and time-consuming as it requires running multiple cell cultivation experiments under varying conditions to adequately cover process variability. Generating this data at manufacturing scale is usually not feasible due to the high costs associated with culture media and reagents. Therefore, the industry has shifted towards high-throughput small-scale bioreactor platforms such as the Ambr® 15 and Ambr® 250, which facilitate design of experiment (DoE)-based automated data generation for Raman calibration modeling.[7, 8] The main challenge with this approach is to ensure model transferability across bio-reactor platforms and scales: technical or optical differences in Raman interfaces such as flow cell and probe designs can contribute to unwanted variation in the Raman spectra at different scales, hindering the seamless and efficient transfer of calibration models. The Sartorius BioPAT® Spectro platform (Figure 1) solves this issue by using an identical design for both small (Ambr®) and manufacturing scale (Biostat STR®) bioreactors' Raman flow cells, enabling the use of compatible Raman probes (i.e., Endress+Hauser and Bruker's Tornado Spectral Systems) across process scales.

Figure 1: *Integration of the BioPAT® Spectro on Ambr® and Biostat STR® Platforms*



Note. In both cases the same interface and Raman probe are used for spectral measurements.

In this study, we show that an Ambr® 250 High Throughput (HT) system can automate the generation of Raman data in a CHO cell fed-batch process, enabling streamlined development of OPLS® calibration models for glucose, titer, and lactate using the software SIMCA® 18. The transferability of these models to larger scales is demonstrated successfully with the bench-top Univessel® Glass 2 L bioreactor equipped with an optically similar immersion probe, and with the Biostat STR® 50 L single-use bioreactor system with integrated BioPAT® Spectro.

Methods

Automated Generation of Raman Calibration Data in the Ambr® 250 HT System

The Ambr® 250 HT system, equipped with the Ambr® Analysis Module and BioPAT® Spectro, was utilized to perform cell cultures under varying conditions. An industrial relevant CHO DG44 cell line (Sartorius) expressing a monoclonal antibody (mAb, IgG1) was cultured with the 4Cell® SmartCHO media system (Sartorius). The culture conditions were established according to the DoE framework using the MODDE® software, where two factors with two levels each were covered: feeding strategy (FMA | FMB at 4% | 0.4% and FMA | FMB at 2% | 0.2%) and inoculation density (0.3×10^6 cells/mL and 0.6×10^6 cells/mL), with two experimental points duplicated, resulting in six fed-batch cultivations in total.

Samples were taken from the cultures (two times per day) and analyzed offline with the integrated BioProfile® FLEX2 system (Nova Biomedical Corporation) for various cell culture parameters along with the Cedex® Bio HT analyzer (Roche CustomBiotech) for titer measurements.

Bench-Top and Large-Scale Cell Culture in Univessel® and Biostat STR®

Two cell culture runs were performed in parallel using the Univessel® Glass and Biostat STR® systems at 2 L and 50 L, respectively. The same CHO DG44 cell line was cultured in fed-batch mode using the 4Cell® SmartCHO media system and an inoculation density of 0.3×10^6 cells/mL. The BioProfile® FLEX2 system (Nova Biomedical Corporation) was used to analyze various cell culture parameters along with the Octet® R8 system (Sartorius) for titer measurements.

Raman Spectral Data Acquisition Across Bioreactor Platforms

BioPAT® Spectro was used for Raman spectral measurements in the Ambr® 250 HT (Analysis Module) and the Biostat STR® single-use bioreactor. Spectral data was acquired using the Rxn-46 probe with the Raman Rxn2 analyzer (both Endress+Hauser Optical Analysis).

In the Univessel® Glass, an Rxn-10 probe with immersion optic (bIO-Optic, Endress+Hauser Optical Analysis), which has similar optical properties compared to the Rxn-46 probe, was used. Spectral data acquisition in Univessel® Glass and Biostat STR® cultivations was performed in parallel through the multiplexing functionality of the Raman Rxn2 analyzer.

Raman Calibration Modeling and Model Transfer Between Scales

For data analysis and model building, SIMCA®18 software (Sartorius) was used. The Raman spectra were pre-processed using 1st derivative and Standard Normal Variate (SNV) filters. After initial visual inspection of the spectra in SIMCA®, outliers were removed using Hotelling's T2 and DmodX detection tools. OPLS® regression models for prediction of glucose and titer were developed based on the Raman shift ranges of 450 – 1,800 cm^{-1} and 2,800 – 3,050 cm^{-1}, while the lactate model was based only on the 450 – 1,800 cm^{-1} range.

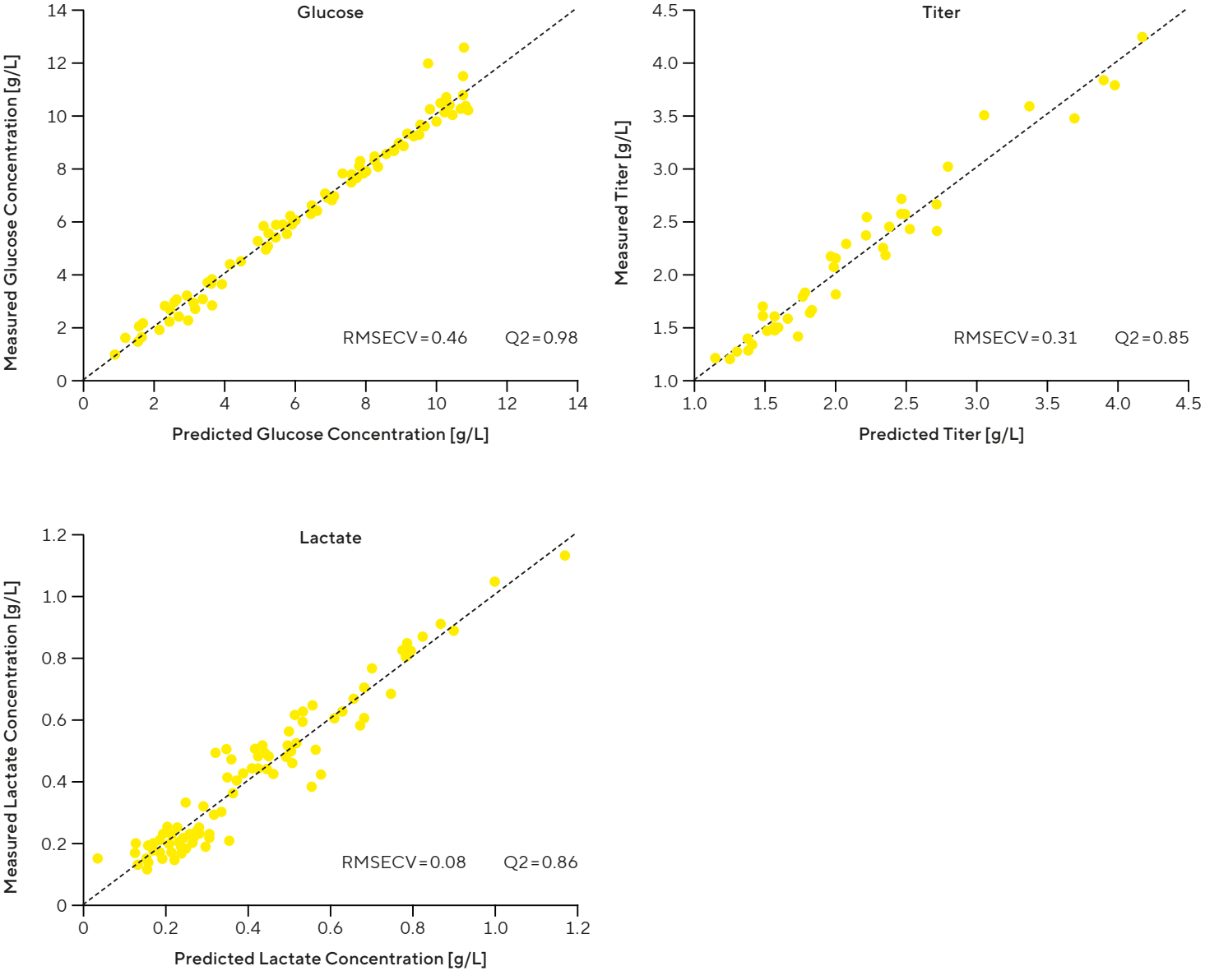
The transferability of the Raman calibration models developed from Ambr® 250 HT data was assessed by directly predicting the compound concentration of spectral data obtained from Univessel® Glass and Biostat STR® cultivations. The prediction accuracy of the models was evaluated by comparison to the offline measurements.

Results

Raman Calibration Modeling in Ambr® 250 HT Cultivation
An Ambr® 250 HT run of six individual cultivations was performed to generate calibration data for Raman-based glucose, titer, and lactate models developed in SIMCA®.

All models showed a good fit, as demonstrated by the correlation coefficients (R2) between observed and predicted values greater than 0.93 (Figure 2). These results demonstrate the efficacy and reliability of the Ambr® 250 HT and BioPAT® Spectro systems for robust generation of Raman data for calibration model development.

Figure 2: *Observed vs. Predicted Plots of Raman Calibration Models for Glucose, Titer, and Lactate Developed From Ambr® 250 HT Data*



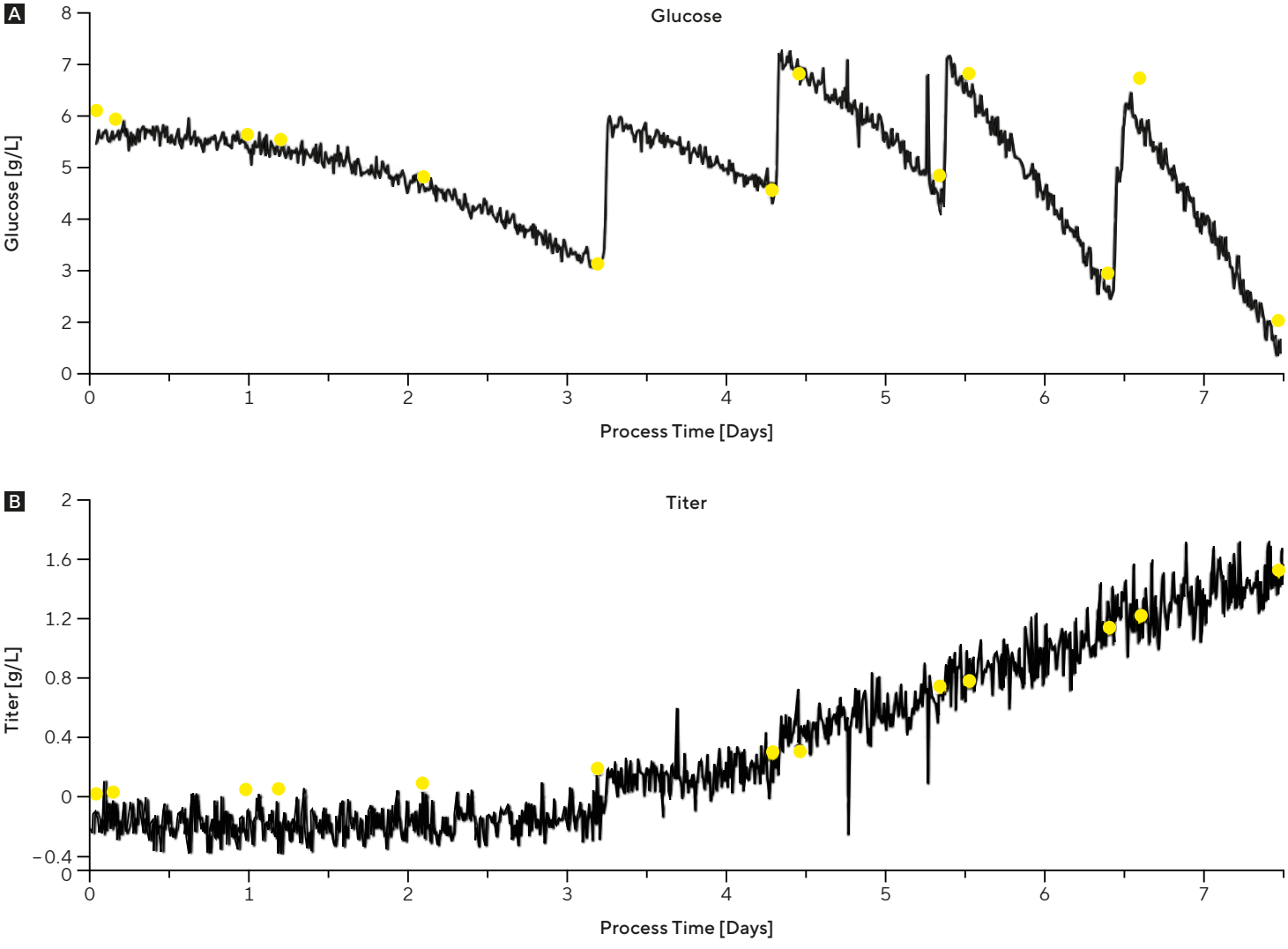
Note. The identity line (x=y), representing perfect predictions, is displayed as a dashed line. RMSECV—Root Mean Square Error of Cross-Validation.

Transferability of Raman Calibration Models From Ambr® to Biostat STR® Using BioPAT® Spectro

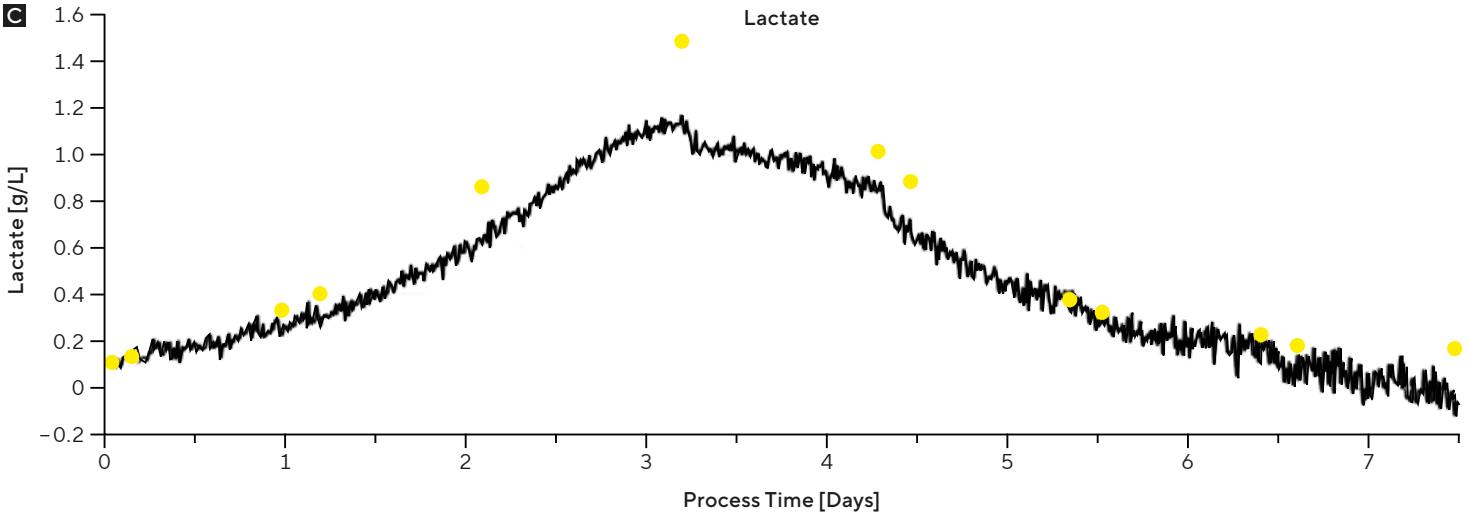
To assess model transferability between bioreactor platforms and scales, the calibration models developed from Ambr® 250 HT data were applied to spectral data obtained in Biostat STR® cell culture (50 L) with BioPAT® Spectro employing identical flow cell designs. As can be seen in Figure 3, predictions from the Raman calibration models showed good alignment with the offline measurements. The root mean square errors of prediction (RMSEPs) for glucose, titer, and lactate were in strong agreement with the calibration model's internal prediction errors (Table 1). This shows that Raman-based calibration models developed on the Ambr® platform can be transferred to Biostat STR® cultivations without losing prediction accuracy.

Lactate prediction in the Biostat STR® resulted in a higher error than the root mean square errors of cross-validation (RMSECV) for the lactate model (0.16 g/L vs. 0.08 g/L), especially for high concentration measurements. This is likely because the Ambr® 250 HT lactate model contained only a few observations at higher lactate concentrations, leading to increased prediction errors for these concentrations in the Biostat STR® culture. This was especially pronounced at the maximum lactate level where model extrapolation was required to obtain a prediction. Such limitations may be overcome by including analyte spiking experiments during calibration data generation, a process that can be automated in Ambr® systems.[7,8]

Figure 3: *Raman-Based Predictions of* ***(A)*** *Glucose,* ***(B)*** *Titer, and* ***(C)*** *Lactate Concentrations Compared With the Offline References Across Biostat STR® 50 L Cultivation. The Data Presented Is Limited to Day 7 Due to Technical Issues.*



Note: Black lines indicate Raman-based predictions and yellow circles indicate offline references. Predictions were obtained from Raman calibration models developed in Ambr® 250 HT. The process is only shown until day 7 due to technical issues.



Note: Black lines indicate Raman-based predictions and yellow circles indicate offline references. Predictions were obtained from Raman calibration models developed in Ambr® 250 HT.

Table 1: *Comparison of Glucose, Titer, and Lactate Prediction Accuracy of the Ambr® 250 HT Raman Calibration Model*

	Glucose	Titer	Lactate
Ambr® 250 HT Model RMSECV (g/L)	0.46	0.31	0.08
Biostat STR® RMSEP (g/L)	0.48	0.14	0.16
Univessel® Glass RMSEP (g/L)	0.4	0.31	0.14

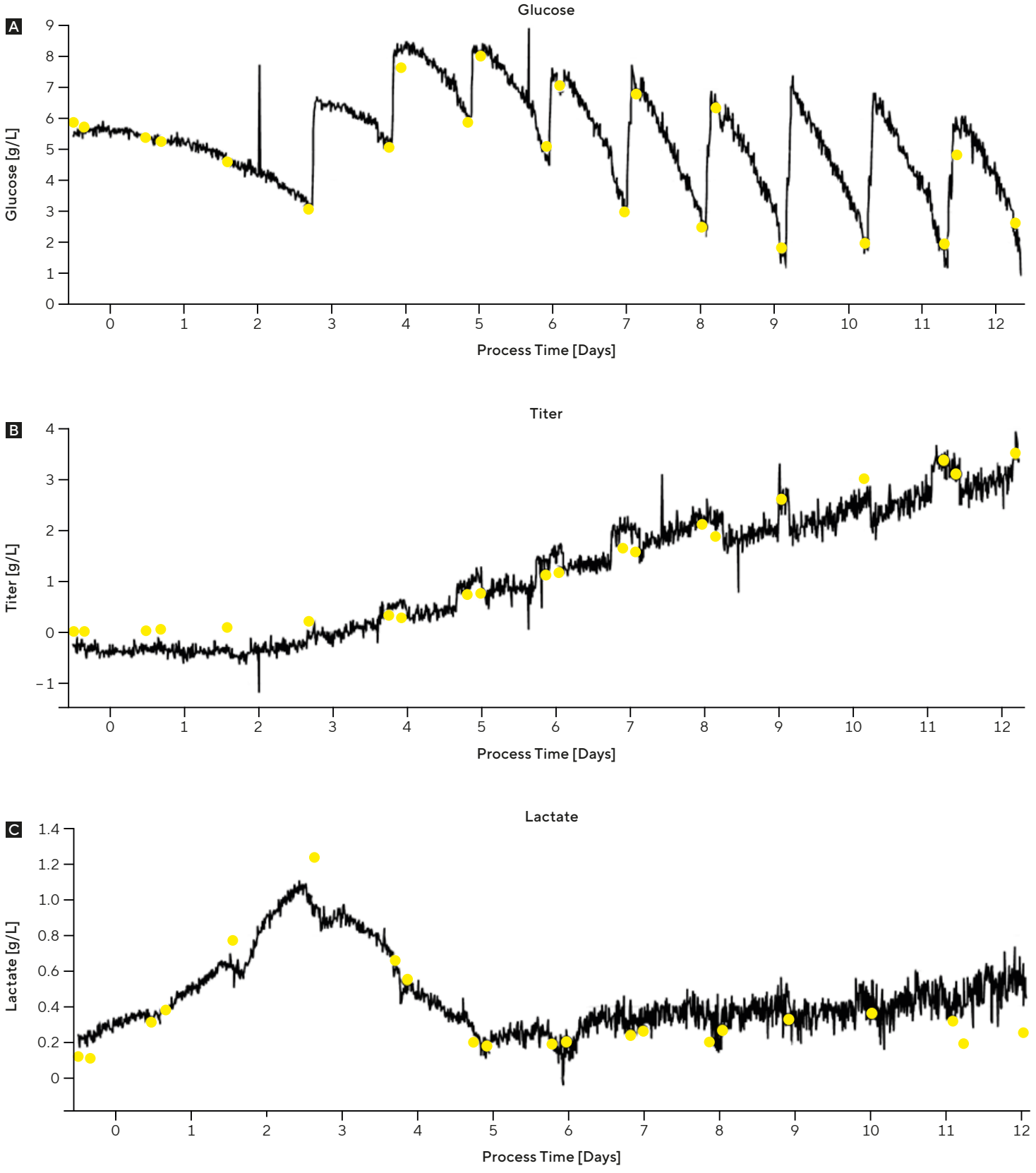
Note. Comparison of root mean square errors of cross-validation (RMSECV, internal model error) with the root mean square error of prediction (RMSEP, external prediction error) when the model was used for prediction in Biostat STR® and Univessel® cultivations.

Transferability of Raman Calibration Models From Ambr® to Univessel®

The calibration model transferability between Ambr® 250 HT and Univessel® Glass (2 L) was also assessed using an immersion probe with similar optical properties as BioPAT® Spectro. Although not directly related to the BioPAT® Spectro platform, this study represents a situation in which users do not or cannot transfer the process directly from Ambr® to large-scale single-use bioreactors like Biostat STR®.

The strong overlap between continuous Raman predictions and offline reference values for glucose, lactate, and titer over process time is shown in Figure 4. As for the model transfer to Biostat STR®, the calibration model RMSECVs and RMSEPs were highly similar. This demonstrates that Raman calibration models developed on the Ambr® 250 HT with BioPAT® Spectro can be deployed reliably with the Raman immersion probe used in bench-top bioreactors (Table 1). As before, increased prediction errors were observed at higher lactate concentrations, in the range outside of the Ambr® 250 HT calibration model.

Figure 4: *Raman-Based Predictions of* ***(A)*** *Glucose,* ***(B)*** *Titer, and* ***(C)*** *Lactate Concentrations Compared With the Offline References Across the Univessel® 2 L Cultivation*



Note: Black lines indicate Raman-based predictions and yellow circles indicate offline references. Predictions were obtained from Raman calibration models developed in Ambr® 250 HT.

Conclusion

The results presented above show that small-scale Raman models developed in the Ambr® system can be used for online analyte monitoring in large-scale Biostat STR® cultivations thanks to the identical optical design of the BioPAT® Spectro flow cells and the integration of SIMCA-Q® in the Raman spectrometer. The OPC connectivity further allows for seamless integration of the model predictions into the BioPAT® MFCS control software, opening the possibility for predictive model-based control strategies.

Transferability of the Raman models from the BioPAT® Spectro platform to systems using immersion probes depends on the similarity of optical properties between solutions. In our case, due to similar optical properties between the BioPAT® Spectro flow cell and the immersion probe from Endress+Hauser, models developed in Ambr® could be directly used in the bench-top scale Univessel® cultivations. Additional experimental effort and model adjustment may be required if the optical properties of the immersion probe differ too much. However, even in such cases, the data acquired in a small-scale high-throughput system can be used to generate Raman models for larger scales more efficiently.[8,9]

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