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Preparation, Separation,
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Application Note

# Real-time and In-line Raman Spectroscopy: A Window into Glycosylation Quality Analysis


## Abstract

This case study outlines the implementation of in-line Raman spectroscopy focusing on the ability to monitor monoclonal antibody (mAb) glycan profiles in the production bioreactor. This study opens the door to the use of Raman technology in process control automation and tailoring with regards to glycosylation patterns during monoclonal antibody production.


**Highlights**

- Raman spectroscopy provides advantages to bioprocess engineers in terms of the elimination of sample preparation steps and faster measurement time compared to traditional methods for glycan analysis.
- The technology offers the ability to determine the abundance of specific glycan structures within complex biological samples in-line and in real time over the process.
- Raman spectroscopy holds great promise to facilitate better control over processes in real time, maintaining product quality consistency in manufacturing and bioprocess research.

## Introduction

In bioprocessing applications, failing to control glycosylation profiles can lead to risks such as significant modifications of biological activity, pharmacokinetics, immunogenicity, and cellular recognition of proteins. Thus, bioprocess engineers need to ensure the efficiency, safety, and regulatory compliance of the monoclonal antibodies that are used to treat patients. In recent years, Raman spectroscopy has demonstrated its noteworthy ability to offer non-invasive and real-time information, making it an attractive tool for quantitative glycan analysis. Furthermore, in the future, it holds great potential for automated process control in glycan analysis.

This application note describes the use of the ProCellics™ Raman Analyzer to assess and to predict the glycan profile evolutions during fed-batch processes.
By modeling the Raman spectra collected over different batches, robust predictive models were developed to monitor glycosylation profiles. The workflow and the results of this study demonstrate the effectiveness of the Raman sensor in providing continuous in-line monitoring capabilities for glycan patterns of biopharmaceutical products in upstream processing.



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## Benefits

Monitoring glycans in real time with Raman spectroscopy offers multiple advantages for the bioprocessing industry at the process development and manufacturing scales:

- **Streamlined Research and Development:** Raman real-time monitoring provides insights into process dynamics. Continuous monitoring provides information into how different process parameters affect glycan profiles. Understanding these dynamics helps to optimize and fine-tune processes to achieve desired glycan structures.
- **Time Efficiency:** Raman spectroscopy enables real-time monitoring, allowing for instant analysis without requiring lengthy sample preparation or waiting for results from traditional methods. This swift analysis can significantly save time in research, manufacturing, and quality control processes.
- **Enhanced Product Consistency:** Real-time monitoring using Raman technology ensures that the glycan profiles remain consistent, minimizing batch-to-batch variability and ensuring product reliability during the production process.
- **Faster Decision-Making:** Immediate access to real-time data allows for prompt decision-making. If any adjustments are needed in the production process to maintain glycan consistency, decisions can be made quickly, minimizing potential risks or delays.

## Process setup and operation

To evaluate the effectiveness of Raman technology as a glycan sensor, standard fed-batch cell cultures (n=5) were performed in Ambr®250 Modular with a CHOZN® GS -/- backbone cell line (MilliporeSigma) producing an IgG1 using EX-CELL® Advanced CHO fed-batch medium (MilliporeSigma). Multiple off-line samples were collected over the process time and then analyzed by a 2D-LC-MS off-line analyzer and by Raman spectroscopy using the ProCellics™ Raman Analyzer (**Figure 1**).

Alternatively, the Raman probe tube of the ProCellics™ Raman Analyzer can be integrated directly in the Ambr®250 Modular bioreactor for in-line and real-time monitoring.

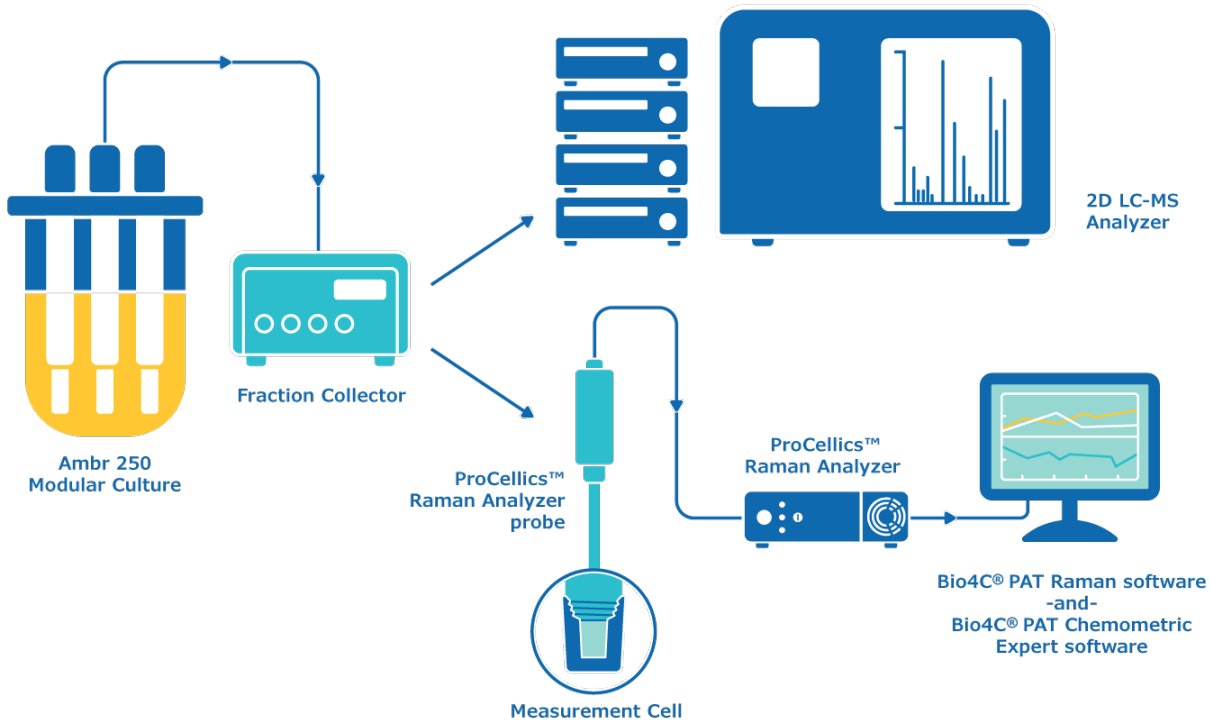


**Figure 1**: Fed-batch process configuration (2D LC-MS analyzer) and integration of the Raman sensor (ProCellics™ Raman Analyzer) for spectral acquisitions.

## Evaluation of the Utilization of Raman Technology for Glycosylation Monitoring

**Figure 2** showcases the regression lines of four calibration models for specific parameters, namely 2*G0F (**Figure 2a**), G0F + G1F (**Figure 2b**), G0 + G0F (**Figure 2c**), and G1F + G2F (**Figure 2d**). These models were developed using data obtained from four separate fed-batch cultures conducted over a period of 10 days of cultivation in Ambr®250 Modular bioreactors. The four calibration models demonstrated a good coefficient of correlation ($R^2$) higher than 0.90.

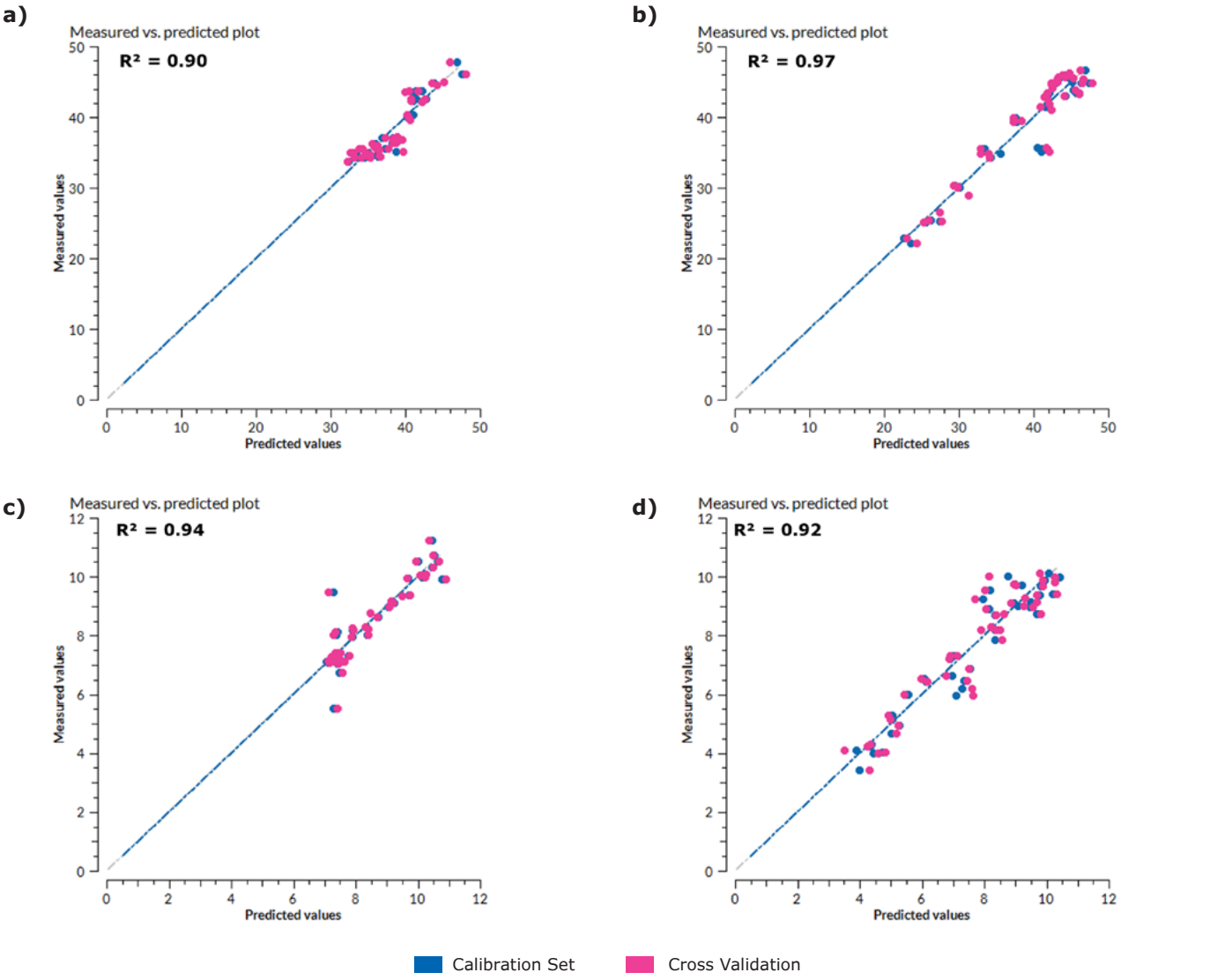


**Figure 2**: Partial least squares (PLS) calibration curves that predict the different glycans: a) 2*G0F, b) G0F + G1F, c) G0 + G0F and d) G1F + G2F.

An independent batch was used as a validation set to evaluate the model performance of each of the four parameters, in order to ensure the robustness and specificity of each model (**Figure 3**). The evaluation of the RMSEP (Root Mean Square Error of Prediction) and the relative errors from the validation set provide valuable insights about the capabilities of Raman spectroscopy to monitor glycosylation profiles in bioprocesses. The 2*G0F, G0F + G1F, G0 + G0F and G1F + G2F glycans present a low relative error percentage (below 10%), calculated based on the RMSEP values. The obtained results demonstrate a strong model fit, indicating high accuracy and reliability of the measurements for the assessed parameters during bioprocess monitoring. This serves as an affirmation of the effectiveness and robustness of the Raman technology in capturing and analyzing the desired parameters. **Figure 3** illustrates the use of these Raman models to predict the main target parameters. Despite a slight gap at the beginning of the process, the overall glycan concentration trend is well monitored, and the predictive measurements are consistent with the off-line data throughout the duration of the cell culture. Thus, the in-line and real-time monitoring using ProCellics™ Raman Analyzer provides valuable insights into the CQA glycan profiles. This information strongly contributes to process optimization and product quality assessment.

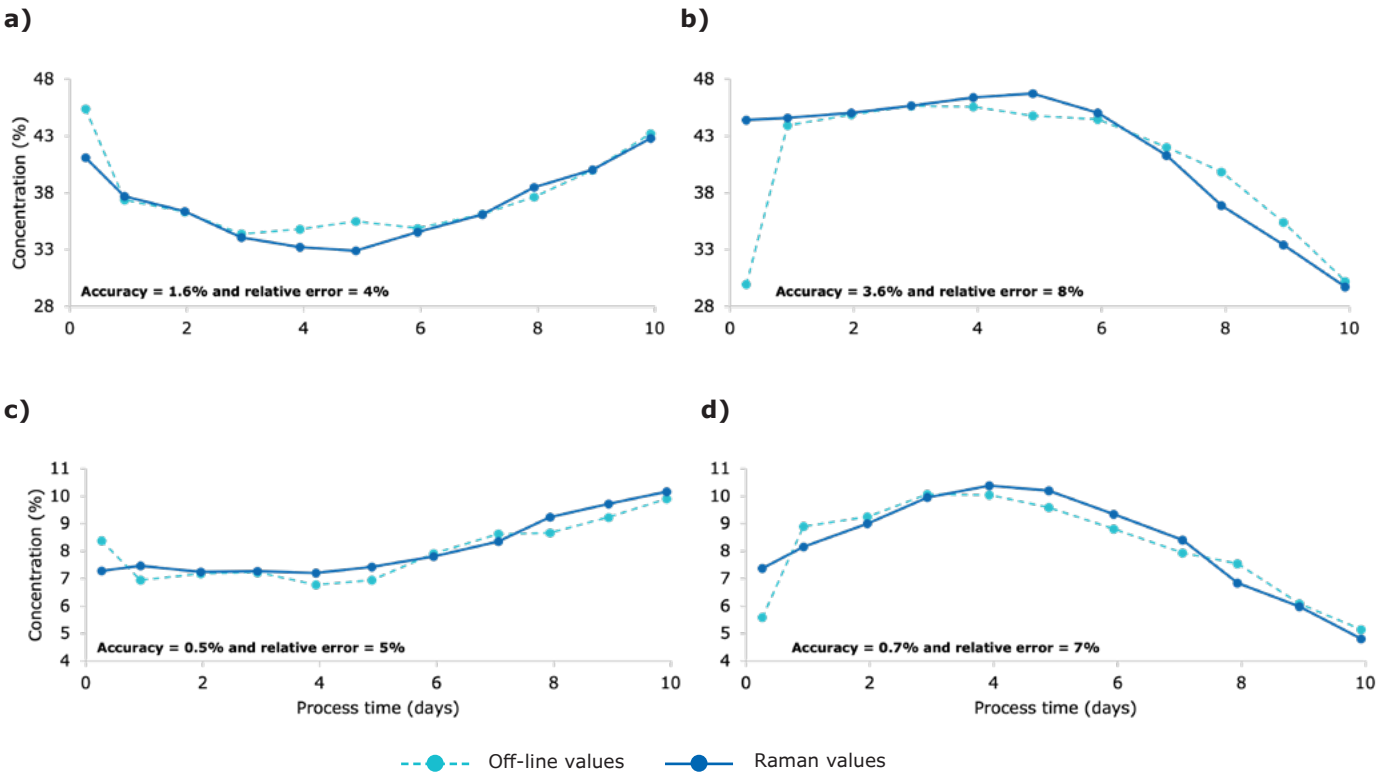


**Figure 3**: Comparison of off-line measurements and prediction values over the process time obtained from the PLS models of the four main glycans: a) 2*G0F, b) G0F + G1F, c) G0 + G0F and d) G1F + G2F. Accuracy* (%) and the relative error (%) are expressed for each glycan.

*Accuracy refers to RMSEP related to the difference between the reference value obtained with off-line analysis and estimated compositional value obtained with Raman.

## Conclusion

This application note provides a thorough characterization and assessment of targeted glycans using a straightforward modeling approach utilizing Raman spectroscopy. The ProCellics™ Raman Analyzer successfully monitored an accurate quantitative profile for the key glycan parameters in the process.

This innovative approach offers significant advantages, including time-saving analysis, real-time process control, consistent product maintenance, and enhanced quality assurance in both manufacturing and bioprocess research.




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