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Application Note

Enabling accelerated Raman model calibration for seamless and reproducible real-time monitoring by combining Raman and automated sampling technologies

Introduction

In bioprocessing, Raman spectroscopy is recognized as an integral part of a process analytical technology (PAT) strategy to manage process performance and ensure a pre-defined final product quality. However, Raman calibration requires the recurrent presence of operators for manual sample collection introducing an increased risk of bioreactor contamination, in addition to being time- and resource-consuming.

In this case study, we assess an innovative workflow aimed at expediting the integration of an in-line Raman analyzer for a bioreactor application. This is achieved by leveraging an on-line technology for automated sampling and data analysis. The approach combines the utilization of the ProCellics™ Raman Analyzer with the MAST® Autosampling Solution. It accelerates the Raman model building phase, facilitating in-line, real-time monitoring of critical process parameters (CPPs) and critical quality attributes (CQAs) during the manufacturing process.

Experimental Design

Intensified seed train cell cultures (N-1) (n=4 runs) were performed in four 3 L glass stirred tank bioreactor systems in parallel for 11 days. To enable perfusion operation, the Cellicon® Filter provided cell retention and spent media removal in conjunction with the Cellicon® Perfusion Controller (Merck). Perfusion rate was adjusted daily based on a desired constant cell specific perfusion rate (CSPR) of 25 $pL.cell^{-1}.day^{-1}$ and the predicted increase of cell density.

MAST® Autosampling Solution

Each bioreactor was connected to the MAST® Autosampling Solution by aseptically welding the tubing from the sterile bioreactor sample line to the tubing of the autoclaved Sample Pilot. The MASTconnect software was programmed with a sample strategy for the duration of the experiment. The samples were then drawn from each of the four bioreactors and delivered directly to the bioanalyzer (BioProfile® FLEX2 Analyzer, Nova Biomedical Ltd.) where nutrients, metabolites, and osmolality were measured. Viable and total cell density (VCD and TCD) were determined using an automated cell staining and counting analyzer system (Vi-CELL XR, Beckman Coulter).

Secreted antibody in the culture broth was quantified using a High-Performance Liquid Chromatography (HPLC) system (Agilent 1100, Agilent Technologies) with affinity-based separation using a bind-elute cycle in a small-scale protein A column (0.1 mL). The on-line sample measurement frequency in this study was designed to draw bioreactor samples every six hours, totaling four samples per 24-hour period, and 48 total samples from each bioreactor.

Raman PAT Platform

The ProCellics™ Raman Analyzer multi-channel unit, comprised of four probes, was employed in this study. Each bioreactor was equipped with one probe, enabling in-line spectral measurements to be taken every 10 minutes in a sequential manner. The Bio4C® PAT Raman Software and Bio4C® PAT Chemometric Expert Software were utilized to build the Raman calibration model for mAb titer, glucose, lactate, and cell densities. These models underwent validation and were utilized for monitoring the CPPs and CQAs in real time.

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Combined Solution: MAST® Autosampling Solution + ProCellics™ Raman Analyzer

This case study explores the integration of the ProCellics™ Raman Analyzer and the MAST® Autosampling Solution to enhance the Raman model building phase (**Figure 1**). By automating sample collection and analytics, the MAST® Autosampling Solution significantly speeds up the traditionally off-line process. The expedited data is then input into the Bio4C® PAT Raman Software, facilitating the implementation of an in-line, real-time Raman sensor in a bioreactor application.

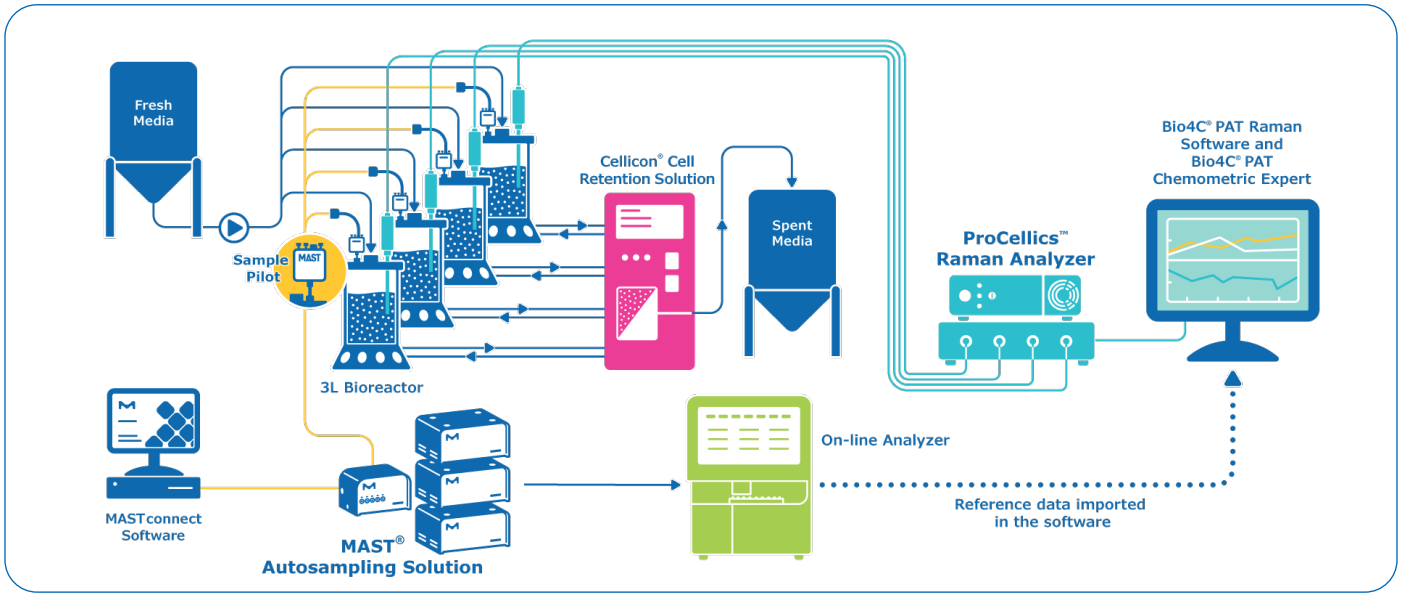

Figure 1: Schematic of a perfusion process configuration (bench-scale stirred tank bioreactor and Cellicon® Cell Retention Solution) integrating the in-line ProCellics™ Raman Analyzer and the MAST® Autosampling Solution.

Results and Discussion

By combining the MAST® Autosampling Solution and ProCellics™ Raman Analyzer, it becomes possible to generate calibration Raman models that exhibit comparable performance to the traditional approach (see 'Implementation of Raman Spectroscopy for in-line monitoring of critical process parameters of CHO cell perfusion cultures' application note). The five calibration models demonstrated a coefficient of correlation (R^2) higher than 0.98, as well as satisfactory performance during cross-validation (**Table 1**).

An independent batch with 38 sampling points was used as a validation set to evaluate the performance of each of the five parameters, ensuring the robustness and specificity of each model (**Table 1**). The evaluation of the RMSEP and the relative errors from the validation set provides valuable insights into the capabilities of Raman spectroscopy for monitoring CPPs and CQAs.

Table 1: Summary table of the calibration model performance and monitoring results for each parameter

		Raman calibration models						Raman Monitoring			
Parameter	**Units**	**Sample Size**	**Range Value Min -Max**	**Latent Variables**	**R^2**	**Q^2**	**RMSEcv**	**Sample Size**	**Range Value Min -Max**	**RMSEp**	**Relative error (%)**
VCD	10^6 cells.mL^{-1}	131	0 – 172.8	4	0.99	0.98	6.40	38	0 – 169.7	7.08	4%
TCD	10^6 cells.mL^{-1}	131	0 – 177.8	4	0.99	0.98	6.12	38	0 – 171.6	7.18	4%
Glucose	g.L^{-1}	131	0 – 10.6	4	0.99	0.99	0.31	38	1.1 – 10.4	0.37	4%
Lactate	g.L^{-1}	131	0 – 5	5	0.98	0.98	0.20	38	0 – 3.1	0.23	7%
Titer	mg.L^{-1}	38	88 - 1781	4	0.98	0.98	74.7	11	118 – 716	67.9	9%

Benefits of Combining Automated Sampling with Raman

This optimized process development set-up consisting of four bioreactors, each equipped with autosampling and Raman sensor capabilities, provides the following benefits:

1. **Accelerated Raman deployment:** Raman calibration incorporating the MAST® Autosampling Solution is four times less time-consuming compared to traditional approaches. In the same amount of time, the bioprocess engineer can run four experiments in parallel. Combined with the MAST® Autosampling Solution, the increased data volume plays a vital role in the construction and validation of the Raman calibration model. As a result, scientists can expedite process scaling by utilizing in-line monitoring, thanks to the faster and more reliable model development achieved.
2. **Enhanced efficiency and consistency:** The utilization of the MAST® Autosampling Solution not only enables a more comprehensive mapping of the design space through the acquisition of a larger dataset but also captures more complete batch kinetics during the process. This comprehensive dataset facilitates a refined understanding of process variations over time. In contrast, relying on traditional manual sampling methods may lead to process knowledge gaps, obstructing a comprehensive understanding of the process dynamics. Combined with the ProCellics™ Raman Analyzer, robust Raman calibration models can be generated with a minimum number of experiments, covering the kinetics design space for each CPP and CQA.
3. **Ensured reliability and reproducibility:** The automation of sample collection, along with the integration of four Raman probes from the same analyzer, enables the simultaneous monitoring of four bioreactor batches, eliminating the need to run batches sequentially. This approach ensures consistent and reproducible sampling and spectral data collection. Implementing robust Raman models on the ProCellics™ Raman Analyzer is crucial for confident and high-quality monitoring of CPPs and CQAs across the entire process - from development to manufacturing.
4. **Contamination prevention:** The MAST® Autosampling Solution, connected to the analyzers in a closed and aseptic manner, along with the implementation of the Raman probe directly in-line with the process, eliminates the contamination risks typically associated with manual sampling.
5. **Autonomous 24/7 operation:** The combination of the MAST® Autosampling Solution and ProCellics™ Raman Analyzer enables continuous operation, even during weekends and holidays, without requiring human interaction, significantly enhancing productivity and efficiency.

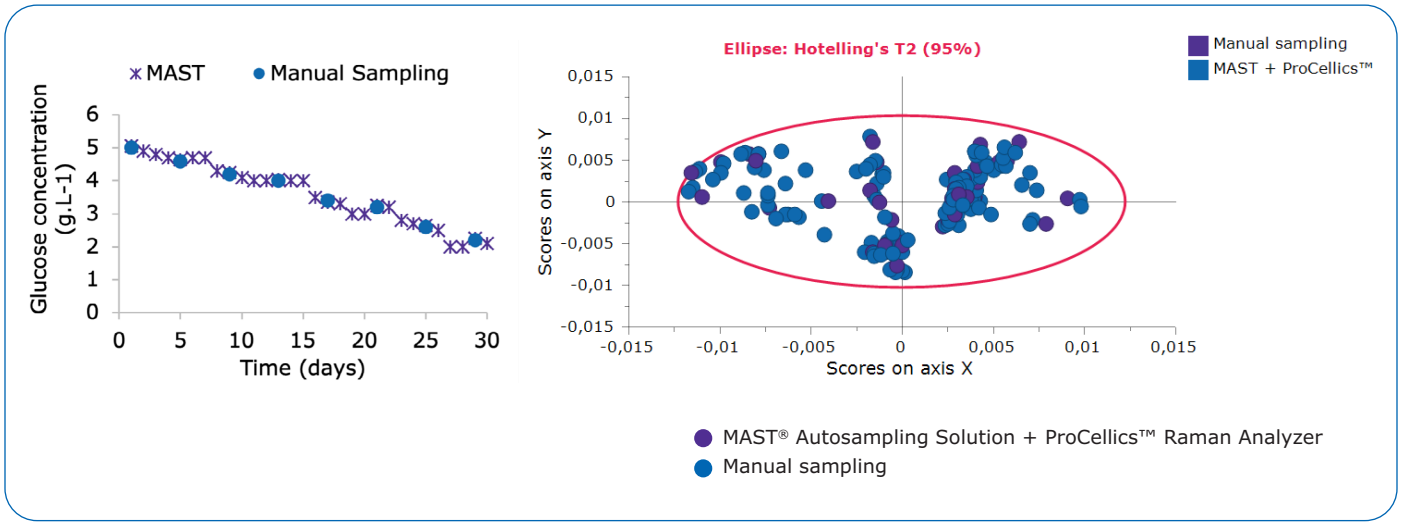

Figure 2: Design space of the Raman calibration models with off-line sampling versus automatic sampling

Conclusion

In conclusion, this innovative platform offers a highly efficient, reliable, and reproducible approach to spectral data acquisition, sampling, and analysis. By accelerating spectral data acquisition, automating sample collection, and streamlining the analysis process, the platform significantly reduces the time needed for Raman model development and validation. As a result, it expedites the implementation of Raman spectroscopy from process development to the manufacturing floor, playing a pivotal role in supporting the timely delivery of new molecules to the market.

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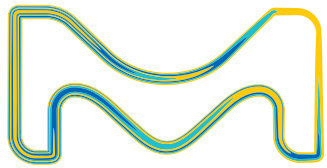

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