Application note

Use of Lykos and TruBio Software Programs for Automated Feedback Control to Monitor and Maintain Glucose Concentrations in Real Time

Authors

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Summary

In this study, the Thermo Scientific™ MarqMetrix™ All-In-One Process Raman Analyzer with Thermo Scientific™ Lykos™ PAT Software and Thermo Scientific™ TruBio™ 6.0 Bioprocess Control Software was used to provide in-line feedback control of glucose in both Fed-Batch and Perfusion CHO cell culture processes without the need for operator intervention. This ability to monitor and maintain the desired glucose concentration in real time leads to improved process consistency and product quality, supporting crucial mAb post-translational modification of the product (e.g., glycosylation). An OPC-UA (Open Platform Communications United Architecture) connection between the Lykos PAT software and the TruBio 6.0 bioprocess control software was used for feedback control of one of the integrated pumps on the G3Lab controller supplying a concentrated glucose solution. Both Fed-Batch and Perfusion cultures were maintained automatically without operator process intervention.

Introduction

Process analytical technology (PAT) enables manufacturers to measure and control a process based on the product's critical quality attributes (CQAs) in real time. Enhanced control of critical process parameters (CPPs) optimizes quality while reducing the cost and time of product development and manufacturing. With PAT, these CPPs and, in some instances, CQAs can be measured in real time, therefore leading to gains in essential real-time process information and the ability to create a quality-by-design workstream. In recent years, process Raman spectroscopy has gained popularity as a PAT tool that enables real-time monitoring and control of critical bioprocessing parameters which are key to the successful production of therapeutic drugs. Successful production implies high process efficiency, high and consistent product quality, and minimized manufacturing costs[1]. Implementing PAT tools in biopharmaceutical manufacturing continues to receive much interest, allowing rapid development and access to therapeutics and existing medications without compromising high quality[5,6].

Complex, multivariate, and univariate instrument data are interpreted, and based on this information, critical process parameters are predicted and, where necessary, adjusted to optimize the outcome of the process. Analytical results make it possible to predict the quality of the end material and understand how altering CPPs will affect the process and product. In turn, by executing experiments with real-time quality predictions, the relationships between the CPPs and CQAs can be established to develop true process understanding. Armed with this knowledge, it is possible to 'close the loop' and control the process using those quality predictions. Integration of continuous monitoring into a bioprocess and the application of analytical data is crucial for understanding the process and proactively addressing challenges. A key challenge is in-line monitoring of critical quality attributes such as glycosylation that affect the bioproduct's stability, immunogenicity, safety, and potency. Maintaining the glucose concentration at a steady level in the bioreactor is extremely important to ensure consistent glycosylation of the product while optimizing product yields[1,2]. This online continuous monitoring and control also allow for the reduction of manual sampling and feeding of the bioreactor, which are costly and inefficient and increase the risk of contamination each time the sterile system is accessed.

This application note describes the use of the MarqMetrix All-In-One Process Raman Analyzer with Lykos PAT Raman Software to monitor glucose levels in a bioreactor and to trigger automated addition of feed so as to maintain the desired concentration. Communication between Lykos and the TruBio 6.0 control software was executed using OPC-UA[9]. A system that incorporates a sensitive Raman Process Analyzer and an established complex feedback loop allows for real-time monitoring of a critical parameter while maintaining stable concentrations. This system frees the operator from manual sampling and feeding, thereby eliminating the risks of contamination to the health of the cells.

Materials & Methods

Bioreactor Run Summaries	
Run Mode: Fed-Batch	**Run Mode: Perfusion**
• 1 Bioreactors (5L glass vessel)	• 1 Bioreactor (3L glass vessel)
• Inoculation: $0.6*10^6$ cells ExpiCHOs	• Inoculation: $0.6*10^6$ cells ExpiCHOs
• Initial media: ExpiCHO SPM	• Initial media: HipCHO
• Feed media, EFC+	• Feed media: HipCHO (perfusion)
• Run time: 14 days	• Run time: 10 days

Table 1. Media and Cell Line Summaries.

Bioreactor Control System and Process Parameters

Process monitoring and control were performed using the MarqMetrix All-In-One Process Raman Analyzer and TruBio 6.0 Bioprocess Control Software powered by the Emerson DeltaV Distributed Control System. Cell culture was performed in a HyPerforma Glass reactor and a HyPerforma G3Lab Controller.

Fed-Batch strategy

A 3L reactor was prepared with an initial working volume of 1.5L of SPM (ExpiCho stable production media + 6mM glutamine + 1 g/L pluronic) culture medium and inoculated with ExpiCho cells at $0.6*10^6$ cells/mL. Bioreactor environmental control parameters were set at a temperature of 36.5°C, a pH of 7.0, and a dissolved oxygen content of 40% air saturation. The pH level was controlled as needed using CO_2 gassing and 1M sodium carbonate additions.

Feeding Strategy

The cells were grown in a chemically defined medium and were fed using a continuous feeding process starting on day 3. The feed media (2X EFFICIENT FEED C+) was added at 3% reactor weight daily. The run was terminated after 14 days. The bioreactor was covered to protect it from stray light.

Perfusion strategy

Fresh basal medium addition was initiated on day 3 at 0.5 reactor volumes/day while spent media was removed through a proprietary perfusion device at the same rate, thereby maintaining the reactor volume at a constant level. The perfusion rate was increased stepwise up to 2 volumes per day by day 5 and held at that rate for the duration of the culture.

Glucose control Strategy

Glucose feedback control was initiated on day 3 for the fed-batch culture and day 0 for the perfusion culture, using the glucose value provided by Lykos to the TruBio 6.0 Bioreactor Control Software, to control the glucose concentration in the bioreactors at 3.0 g/L for the fed-batch and 4.0 g/L for the perfusion cultures.

MarqMetrix All-In-One Process Raman Analyzer Monitoring

Measurements were performed using the MarqMetrix All-In-One Process Raman Analyzer, with the optical BioReactor Ball Probe of the MarqMetrix All-In-One Process Raman Analyzer directly immersed in the bioreactors[7,8]. Each Raman spectrum was the result of an average of 20 measurements with an integration/exposure time of 3000 milliseconds and laser power set at 450 mW. The total acquisition time per data spectra was 2 minutes (1 min dark spectrum correction and 1 min sample spectra) with a timestamp matched between the MarqMetrix All-In-One Process Raman Analyzer and offline instrument analysis to align the online and offline results. Measurements were taken every 2 minutes, and based on these measurements, the pump rate was adjusted automatically.

LyKos PAT Software

The Lykos provided user access to the MarqMetrix All-In-One Process Raman Solution and data access for the stored Raman spectra. As part of this application note, two new capabilities were developed: A process analysis engine that applies a chemometric model to generate a process value, and an OPC-UA server which allows automated data access.

Process Control Integration

Glucose addition rate control by the TruBio 6.0 Process Control System was based on process measurements from the Lycos PAT Software via OPC-UA.

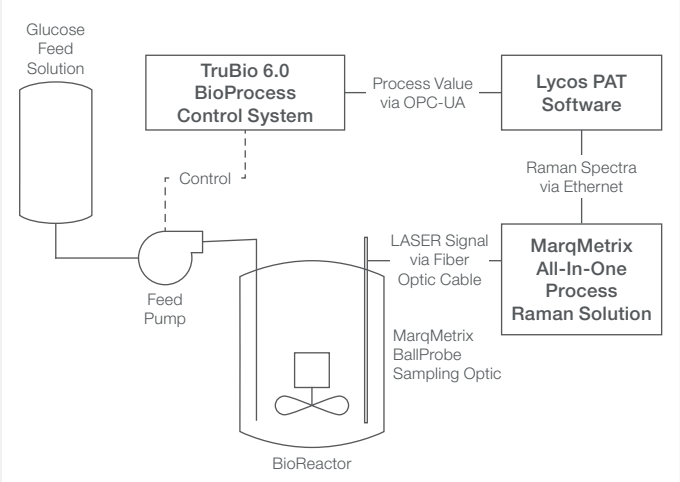


Figure 1A. Process Control Diagram.

Chemometric Model building

Independent data from multiple MarqMetrix All-In-One Process Raman Analyzers and 3 bioreactors were used to create models. 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 MarqMetrixAll-In-One Process Raman Analyzer and the at-line instrument, a Flex2 cell culture analyzer from Nova Biomedical. 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 each measurement corresponded to ten minutes. The spectra were pre-processed to remove the baseline and, maximize the signal-to-noise ratio, and correct for path length differences. Partial Least Squares (PLS) models were created for each property of interest, and leave-out-one-run cross-validation was performed to test the optimization of each model. Properties of interest included glucose, lactate, and titer generated during the bioreactor culture run.

Lykos PAT Software communicated directly with the bioreactor-controlling software to send the glucose concentration measured in the bioreactor. A MarqMetrix All-In-One Process Raman Analyzer and Lykos PAT Software were integrated with TruBio 6.0 Biorprocess Control Software (powered via the DeltaV Distributed Control Platform from Emerson). Raman spectroscopy was used to determine the glucose concentration in the cell culture.

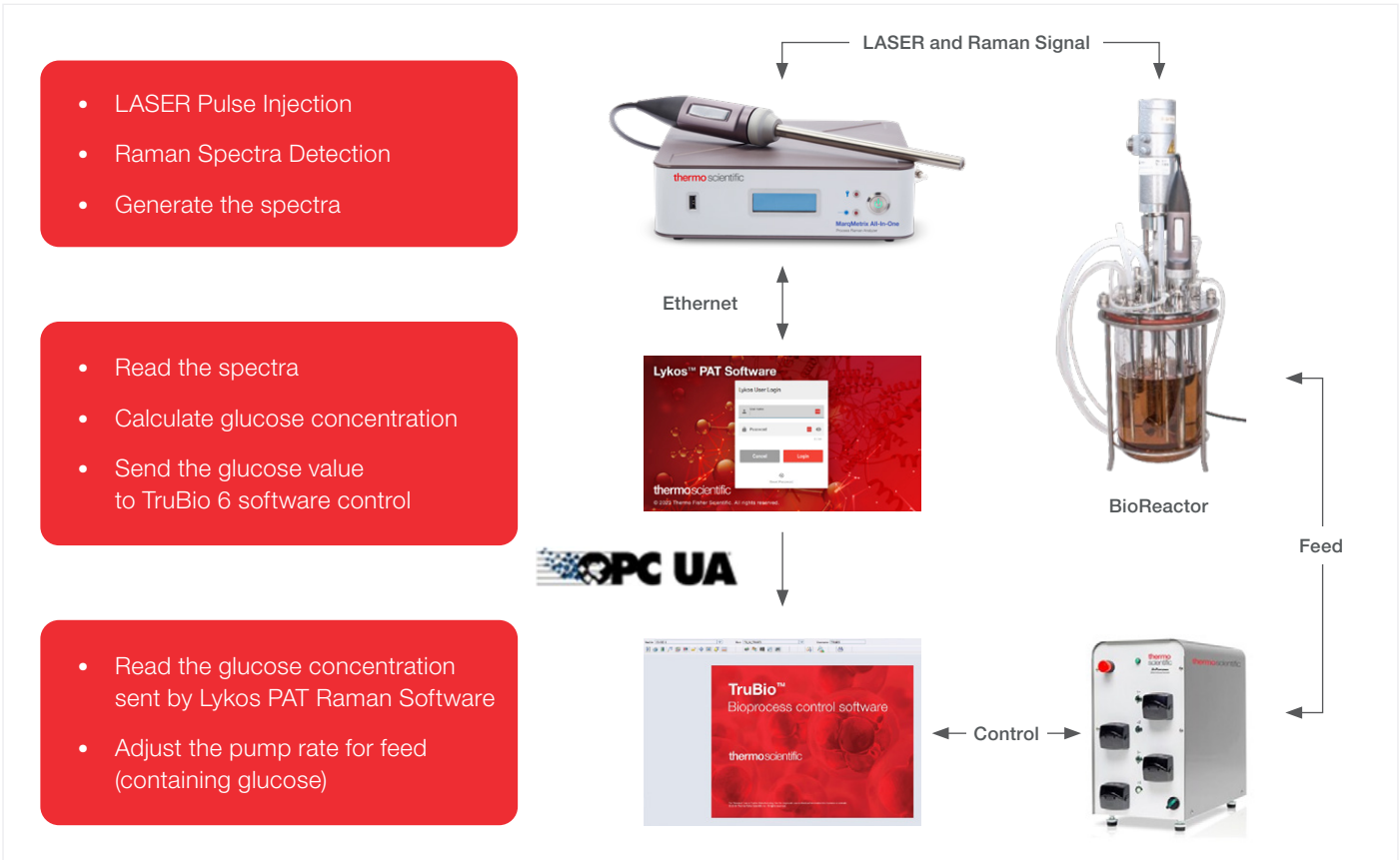
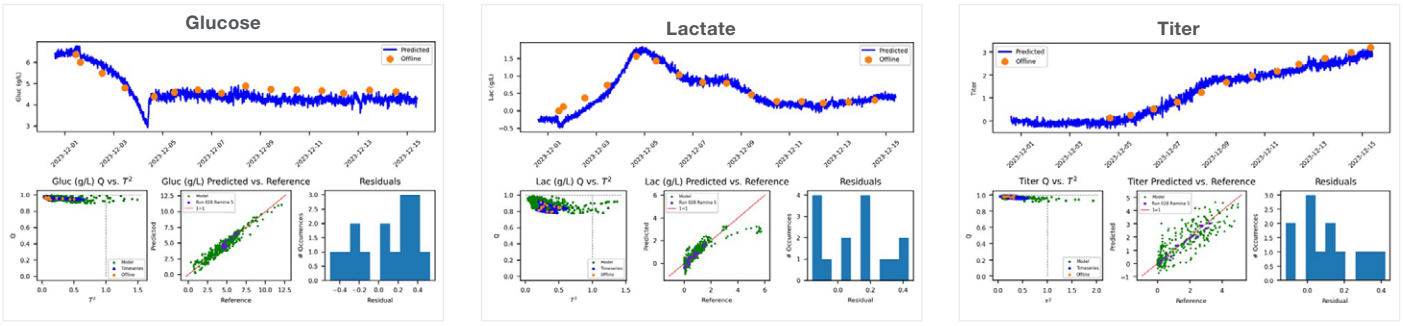


Figure 1B. Process Monitoring & Control.

Results

During the cell culture process, glucose was consumed by the cells, and concentration levels stabilized at around 3 g/L for the fed-batch culture and around 4 g/L for the perfusion reactor. Figures 2A & B show that once the target glucose concentration was reached, the glucose concentrations were precisely maintained at 3 g/L and 4 g/L for the fed-batch and perfusion cultures, respectively. Traditional offline samples were collected to measure the glucose and lactate concentrations as controls to compare the accuracy of the Raman analyzer measurements.

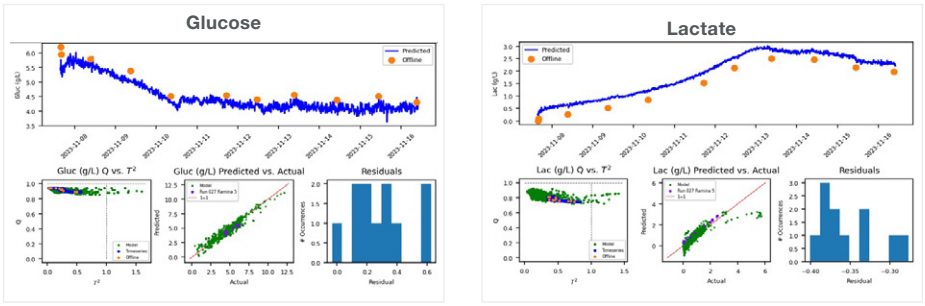


Correlation of Model Prediction with Offline Data

Metabolite/Variable Predicted	R^2 Predicted	RMSEP
Glucose (g/L)	0.890	0.315
Lactate (g/L)	0.929	0.223
Titer (g/L)	0.980	0.200

Figure 2A. Fed-Batch

Data from real-time control of glucose and monitoring of lactate and titer for a fed-batch culture are presented here.



Correlation of Model Prediction with Offline Data

Metabolite/Variable Predicted	R^2 Predicted	RMSEP
Glucose (g/L)	0.95	0.34
Lactate (g/L)	1.00	0.36

Figure 2B. Perfusion

Data from real-time control of glucose and monitoring of lactate for a perfusion culture are presented here.

Discussion

In the above-described experiments, process data was monitored by Lykos PAT software and easily integrated with TruBio bioprocess control software via an OPC-UA protocol. The control loop demonstrated a very stable glucose concentration, which was achieved along with accurate measurements by the MarqMetrix All-In-One Process Raman Analyzer. Automating the process, as demonstrated, significantly reduced the intervention of the operator, who typically must sample the bioreactor for offline or at-line analyses. Because of this automation, the risk of contamination and batch rejection relating to operator error and other deviations is significantly reduced. In this application note we demonstrated a culture environment that can ensure maximum productivity in a reproducible manner using an automated feedback control loop. With real-time process monitoring by Thermo Fisher Scientific's MarqMetrix All-In-One Process Raman Analyzer with Lykos PAT Raman Software and feedback control enabled by TruBio 6.0 Bioprocess Control Software, we have demonstrated a real-world application of PAT and the ability to implement a process developed with a quality-by-design approach.

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