Application note

Process Raman analysis

Real time metabolite monitoring using the MarqMetrix All-In-One Process Raman Analyzer and the 500L Dynadrive Single-Use Bioreactor (S.U.B.)

Authors

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Background

The capability of Raman spectroscopy to reflect small changes in complex aqueous systems has expanded the application of this technique to analyze biopharmaceutical processes such as cell growth in bioreactors. When the Raman spectrometer is utilized as a continuous process analyzer of these complicated chemical systems, this technology can monitor biopharmaceutical production processes real-time, in-situ and non-destructively. The ability of Raman Spectroscopy to detect changes of numerous metabolites during bioreactor processes has elevated this technology to a robust Process Analytical tool.

Thermo Scientific™ MarqMetrix™ Single-Use Bioreactor BallProbe™ Sampling Optic with TouchRaman™ immersion technology

Reusable Raman Spectroscopy analysis optical probes offer benefits such as improved process repeatability and reliability by reducing run-to-run variability. With the MarqMetrix All-In-One Process Raman Analyzer, there are a wide range of probes available. The MarqMetrix Single-Use Bioreactor BallProbe Sampling Optic is designed to meet the requirements of the bioprocess industry and can be used with the MarqMetrix All-In-One Process Raman Analyzer. These probes are quick and easy to swap and connect, are durable and can handle sterility practices including offline autoclaving.

Thermo Scientific™ DynaDrive™ Single-Use Bioreactor (S.U.B.), for perfusion cell culture applications

The DynaDrive Single-Use Bioreactor (S.U.B.), the latest advancement in S.U.B. technology, offers improved performance and scalability for large volume bioproduction. The cuboid-shaped tank offers several key advantages over legacy S.U.B. designs including superior mixing and mass transfer capabilities as well as improved scalability.

This application note describes the integration of the MarqMetrix All-In-One Process Raman Analyzer with the 500L DynaDrive Single-Use bioreactor to perform in-line measurements of critical process parameters (CPPs). Utilizing continuously generated spectral data throughout a cell growth culture run, accurate prediction models for several parameters and metabolites were developed using this integrated system.

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Materials and methods

Cell culture and feeding strategy

Cell culture was performed in a 500L DynaDrive S.U.B, containing a working volume of approximately 320L of cell culture medium, and inoculated with 0.5*106 cells/mL at a temperature of 36.5 °C, pH= 6.9+/- 0.3, DO = 50%). The pH level was controlled by CO_2 gassing and sodium carbonate additions, as needed. The cells were grown in a chemically defined medium and fed daily with a two-step feeding process, starting at day 3. The first feed media was added at 4% by weight based on the starting volume and the second feed media was added at 0.4%. The temperature was shifted to 33 °C on day 6. The run terminated after 14 days. The bioreactor was covered to protect from stray light. After autoclaving, the MarqMetrix Single-Use Bioreactor BallProbe Sampling Optic was inserted into the DynaDrive S.U.B. during the run for in-line, real-time spectral Raman data generation.

Figure 1. 500L Thermo Scientific DynaDrive S.U.B. for cell culture applications.

MarqMetrix All-In-One Process Raman Analyzer measurements

Measurements were performed using the MarqMetrix All-In-One Process Raman Analyzer, with the MarqMetrix Single-Use Bioreactor BallProbe Sampling Optic of the MarqMetrix All-In-One Process Raman Analyzer directly immersed in the bioreactors (500L) D. Each Raman spectra was the result of an average of 20 measurements with an integration/exposure time of 3 sec, and laser power setting at 450 mW. The total acquisition time per data spectra was 2 minutes, with a timestamp matched between the MarqMetrix All-In-One Process Raman Analyzer and off-line instrument analysis to build the model.

Chemometrics, model building

Independent data from multiple MarqMetrix All-In-One Process Raman Analyzer instruments, probes, and bioreactor types were used to create models. The training datasets were collected from 45 samples per bioreactor to create each chemometric model. The spectral data was reviewed, and outlier spectral spikes caused by cosmic rays were removed. The spectral region of interest was selected, and the spectra were pre-processed to remove the baseline and maximize signal to noise.

Figure 2. Thermo Scientific MarqMetrix All-In-One Process Raman Analyzer.

Many pre-processing techniques were tested, including the Savitzky Golay filter with derivatives, Automatic Whitaker Smoothing, Extended Multiplicative Scatter Correction, SNV, and mean centering. The best pre-processing techniques used varied, based on which specific parameter of interest was modelled. Partial Least Squares (PLS) models were created for each property of interest and cross-validation was performed to test the optimization of each model. Properties of interest included glucose, lactate, glutamine, glutamate, TCD, VCD, and other common metabolites generated during the bioreactor culture run.

Results

In this work, continuous in-line Raman spectroscopy was applied to a fed-batch CHO cell culture process. The in-line spectral data was correlated to the offline analytical data acquired for parameters of interest. The use of Raman spectroscopy to monitor process parameters first requires chemometric model building with an externally calibrated data set (independent offline data). To assess the accuracy of the MarqMetrix All-In-One Process Raman Analyzer predicted values, bioreactor samples were collected daily and analyzed for comparison. The root mean square error of calibration (RMSEC), root mean square error of cross validation (RMSECV) and root mean square error of prediction were calculated for each parameter (RMSEP). The error was averaged based upon the prediction of the model to identify the RMSECV which is used to construct the model. The RMSEP is used to test the model against “new” data that the model has not seen. The coefficient of variation, R^2, was recorded for each PLS model. The value is used to determine the amount of variation of the Y variable which the model predictors (X variables) can explain.

It is important to note that the combined use of several large, independent data sets from bioreactor runs of the same CHO culture process produced predictive chemometric models that are more accurate and robust. For this study, five independent datasets from previous bioreactor runs were combined to train a large chemometric model. The calibration model was then applied to the spectral data obtained during this DynaDrive S.U.B run. The data indicates that the model was able to accurately predict this new dataset, and that model predictions were highly correlated with data measurements collected offline for numerous metabolites as shown in Table 1.

Metabolite Predicted	R^2 Predicted	RMSEC	RMSECV	RMSEP
Glucose (g/L)	0.98	0.43	0.49	0.40
Lactate (g/L)	0.92	0.15	0.18	0.25
Glutamine (mmol/L)	0.92	0.42	0.48	0.58
Titer	0.92	0.21	0.25	0.37
Cell Viability (%)	0.94	1.72	2.29	1.83

Table 1. Correlation of model prediction with offline data analysis.

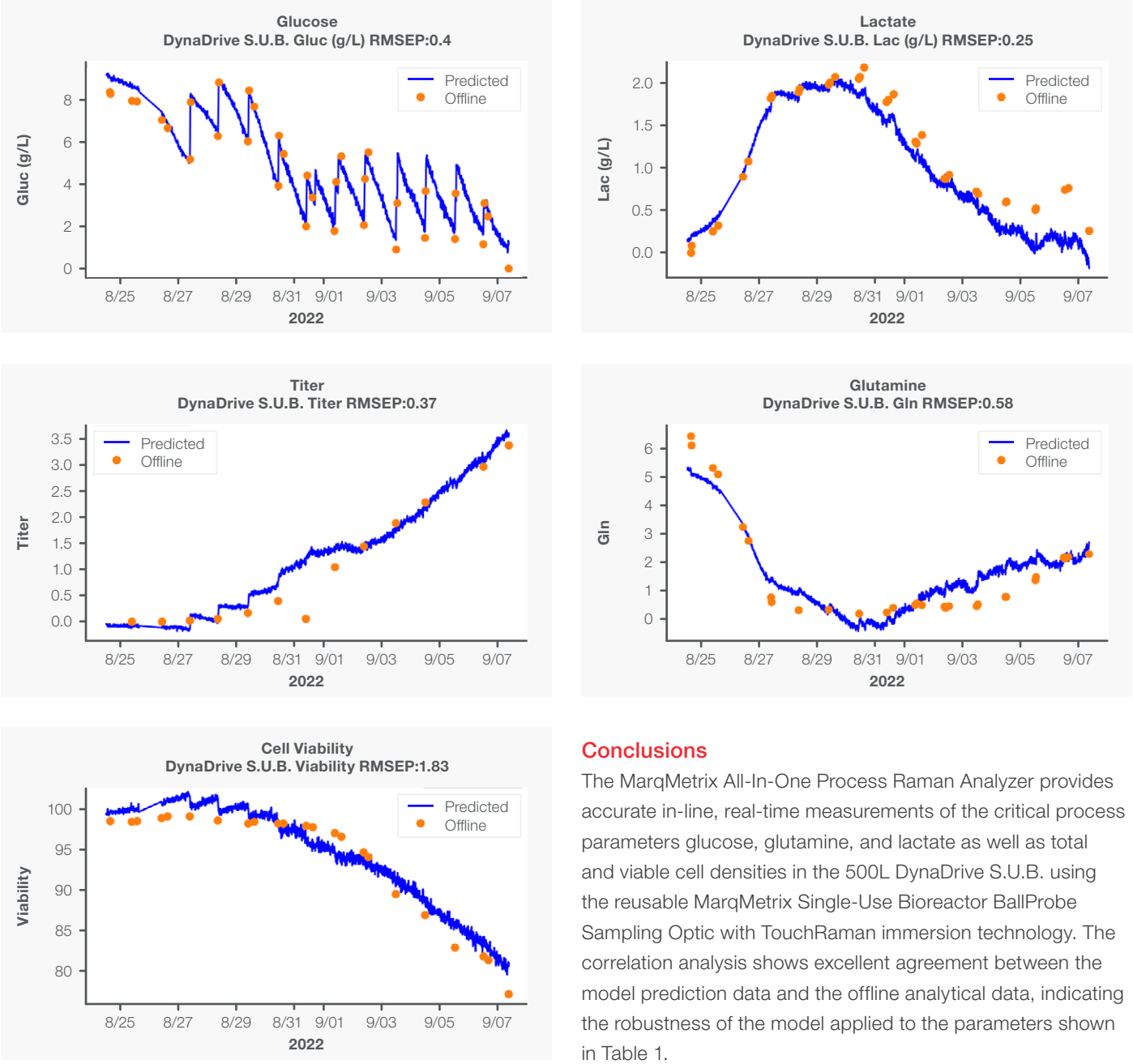

Figure 3. Thermo Scientific DynaDrive S.U.B. Chemometric Model Plots- Comparison of Raman Model vs Offline Analytical Data for Important Bioreactor Parameters.

Conclusions

The MarqMetrix All-In-One Process Raman Analyzer provides accurate in-line, real-time measurements of the critical process parameters glucose, glutamine, and lactate as well as total and viable cell densities in the 500L DynaDrive S.U.B. using the reusable MarqMetrix Single-Use Bioreactor BallProbe Sampling Optic with TouchRaman immersion technology. The correlation analysis shows excellent agreement between the model prediction data and the offline analytical data, indicating the robustness of the model applied to the parameters shown in Table 1.

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