APPLICATION NOTE

Enwave Optronics, Inc.

Keywords

- Cell culture
- Bioprocess
- Metabolites, glucose, and cell density
- CHO Cells

Techniques

- Raman spectroscopy
- Chemometrics
- Process analytical technology
- Fed-batch bioprocess

Applications

- On-line analysis of bioprocess
- Control strategy

Implementation of PAT should result in better understanding of process, but it requires affordable & reliable tool to gain the wellness of initial deployment

Raman Spectroscopy for Cell Culture Monitoring in Biologics Manufacturing

Upstream engineering, media optimization, process monitoring, and control strategies for cell culture productions have become crucial subjects to meet increasing demand for high value biopharmaceuticals. Raman Spectroscopy has gained great attention for process monitoring and control to ensure quality and efficiency in the biologics manufacturing.
In this note, we explore Enwave's CellProbe Raman analyzer as a Process Analytical Technology (PAT) tool for real-time determination of metabolites and nutrients in CHO cell culture process.

INTRODUCTION

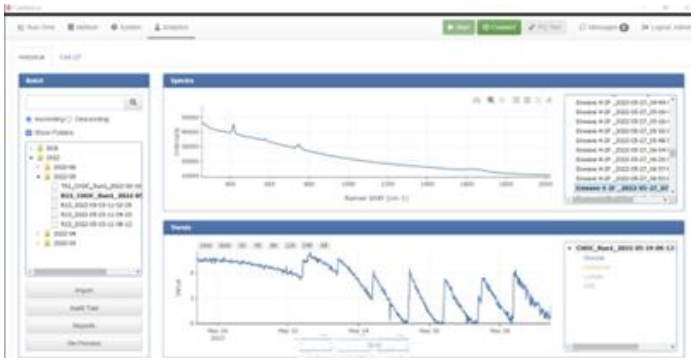
The manufacture of therapeutic proteins by mammalian cell culture-based processes is driving the development of a Raman spectroscopic based analytical methodologies. With the widespread use of efficient media in animal cell culture processes and the increasing emphasis on quality by design and PAT, the use of online process monitoring is becoming increasingly important. Traditional bioprocess monitoring requires multiple sensors and periodical sample extractions. The integration of these process analytical tools is essential to achieve real-time monitoring of cellular physiological status of biological processes and control of process to achieve product quality consistency. The new Raman spectroscopy sensor could produce multiple critical quality parameters with one immersion probe and becoming the preferred analysis tool in the bioreaction processes like fermentation and cell culture. The unique features of Raman technique provide rapid, non-destructive, reliable, and robust analytical method to ensure efficient and reliable process control, to improve fermentation performance and product quality, leading to overall improved cost and quality control.

EXPERIMENTALS

We monitor the cell culture process of a small-scale recombinant protein production using CHO cells in a 5 L bioreactor over 12-14 days fed batch runs. For each run, the process was sampled at pre-defined set time points over the course of the cell culturing. The fed-batch bioprocess was operated using proprietary basal and feed media formulations

Raman spectra were collected in situ using the CellProbe™ (Enwave Optronics, Inc.) analyzer with stainless-steel top mounted immersion probes fitted with standard adapter. The Raman probes were sterilized prior to being manually inserted into the bioreactor. Data acquisition used CellMetrics™ (Enwave Optronics, Inc.) process software and spectra were collected from bioreactor vessel every 15 minutes throughout the course of culture process.

Sequential Raman spectra generated from culture runs were aligned against the daily sampling times of the off-line metabolite measurements for comparison. Raman spectra were trimmed to keep only the relevant ranges (fingerprint 500-1800 cm^{-1}) for analyses. To minimize the effects of baseline drift, scatter effect due to turbid biological media and uncontrolled fluctuations, baseline features were eliminated by calculating the second-order derivative of the spectra prior to chemometric modeling. All calculations were performed using the CellQuanT chemometric software (Enwave Optronics, USA) for developing Partial Least Squares (PLS) regression models. The PLS model quality was assessed using a combination of parameters including root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP) for validation/test set, relative error of prediction, and the square of the correlation coefficient (R2) between predicted and measured titers for the validation set. The use of cross-validation (root mean square error of cross validation RMSECV) is the assessment of the performance of PLS calibration model to avoid potential overfitting,



The CellMetrics process software,

RESULTS

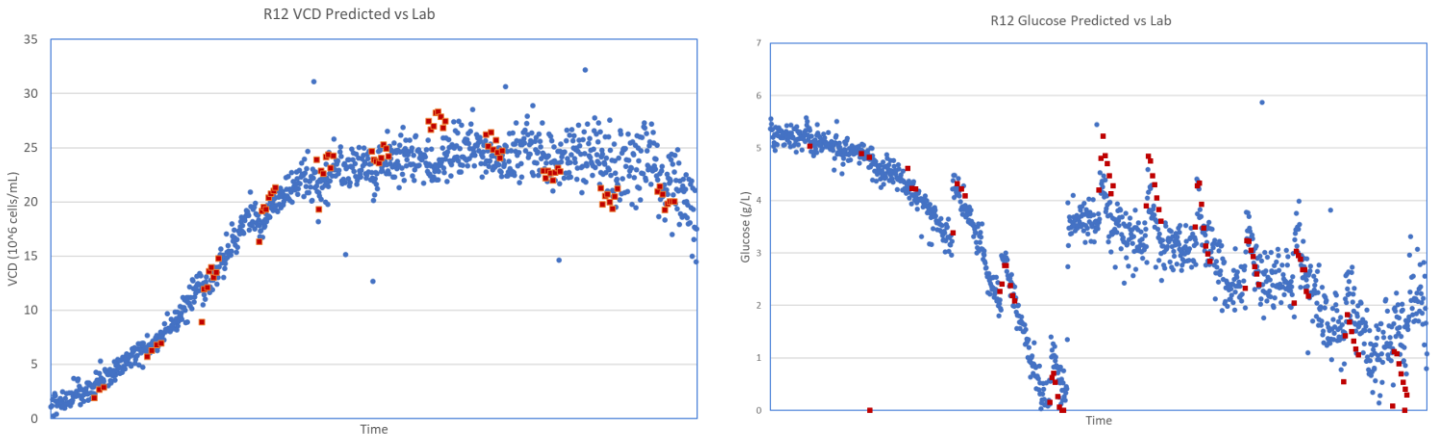
The spectral data used for model calibration and testing were collected from 5 different batches performed at pilot scale. The first 3 batches were used as a calibration data set for model calibration. Independent batches R211 and R12 were used as validation set. The calibration data set spans a concentration range of glucose from 0.12–5.44 g/L and cell density from 0.43-26.98 10^6 cell/L and is composed of 225 samples.
The performance of the model for calibration was assessed using root mean square error of evaluation (RMSEC) and cross-validation (RMSECV). Seven latent variables were chosen for the PLS regression mode for glucose, which provides a RMSEC value of 0.28 g/L and RMSECV value of 0.34 g/L, while latent variables were chosen for the PLS regression mode for cell density, which provides a RMSEC value of 1.20 (10^6 cell)/L and RMSECV value of 1.40 (10^6cell)/L, The closeness of RMSEC and RMSECV values indicates that a significant amount of variability in calibration data set was sufficiently captured by the five latent variables. Table 1 summarizes the calibration results of the model.

Table 1: Partial least squares (PLS) model summary

	Calibration Set (Raman Run 8, 10 & 11)			**Validation Set (R211)**	
Model	R2	RMSEC	RMSCV	R2	RMSEP
Cell density	0.969	1.20	1.40	0.871	2.99
Glucose	0.939	0.28	0.34	0.854	0.43

The built calibration model was used on the blind test to quantify the performance of the model so that the model was applied to the validating data se R211. The RMSEP values were 2.99 and 0.43 g/L for cell density and glucose, respectively. The reference measurements and component predicted time course concentration profiles obtained from the model on all the spectra acquired continuously during batch R12 were presented in Figure 1. As can be seen, the model accurately follows the real trend of cell density and glucose concentration with high accuracy throughout the batch R12. Overall, time course plots presented here demonstrate that the Raman model was capable of accurately predicting the concentration trend and values of cell culture and glucose in bioreactor every 15 min without sampling.

Figure 1. Time series plots of reference measurements and model predictions for CHO cell culture from B12 batch. The plot demonstrates PLS model prediction of cell density (Left) and glucose (right) on time frame of fed-batch cell culture process of R12 in 12 days. Blue dots represent the measured concentrations by Raman and the red dots represents the concentrations of reference method.



SUMMARY

In this application note, we demonstrate the usage of in-line Raman spectroscopy for real-time monitoring of cell density and glucose in bioreactor of CHO cell culture bioreactions. Chemometric model was developed using Raman spectroscopy and tested with two different batches which are not included in the calibration data set. The developed model estimates the concentrations of cell density and glucose in bioreactor with acceptable accuracy and can eliminate the need for offline sampling during the manufacturing process.

In summary, Enwave's affordable and reliable CellProbe Raman analyzer is an effective and suitable method for the quantitative determination of the critical quality parameters in CHO cell culture process. The results of this study represent evidence that the Raman technique can be implemented from R&D development, pilot study or manufacturing for real time continuous monitoring. The system allows faster decision making and creates new opportunities for downstream process intensification and control.

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