Cross-scale *in situ* Raman monitoring of a cell culture

Figure 1: Real-time, *in situ* probes, optimized for bioprocess conditions, enable measurements in laboratory (left) and manufacturing (right) with easy transfer of the analytical model.

Benefits at a glance

- Simultaneous in-process monitoring of nutrients, metabolites, and cell attributes with a single probe
- Raman measurements increased process understanding
- Real-time, Raman-based process control and model transferability
- Specific and quantitative process knowledge
- In accordance with the U.S. FDA Process Analytical Technology (PAT) and Quality by Design (QbD) initiatives

Introduction

In mammalian cell-based bioprocesses, the most common critical process parameters (CPPs) include physical parameters (temperature, agitation rate, and dissolved oxygen (DO)), chemical properties (pH, nutrients, and waste concentrations), and biochemical properties (cell count and viability). Careful management of these CPPs is necessary to ensure process quality and maintain the tight parameters on product variability demanded by the U.S. Food and Drug Administration (FDA) for cGMP manufacturing.

Monitoring of CPPs *in situ* has typically been limited to temperature, pressure, pH, and DO because sensors exist to measure these properties. Chemical and biochemical properties are typically measured off-line or at-line. However, the inherently time-consuming nature of off-line or at-line analyses are not compatible with real-time process control.

Raman advantages

Innovations in Raman spectroscopy enable chemical and biochemical information to be acquired easily. Raman has been widely applied for non-invasive, non-destructive process monitoring in several industries, including biopharmaceuticals, to enable real-time process control.[1,2]

The advantages of Raman for bioprocess monitoring and control include highly specific information within the spectrum, allowing cross-model scalability and simultaneous measurement of multiple chemical and biochemical parameters. Raman provides a non-invasive, non-destructive analysis without a need for sampling or additional reagents. Moreover, engineering and scientific advances in measurement and control have progressed to enable advanced process control strategies. Real-time Raman-based feed control is now widely used in biopharmaceutical companies from lab to cGMP. This work demonstrates successful process development and transfer from benchtop to manufacturing.

ⓘ All Raman analyzers and probes referenced in this application note are Endress+Hauser products powered by Kaiser Raman technology.

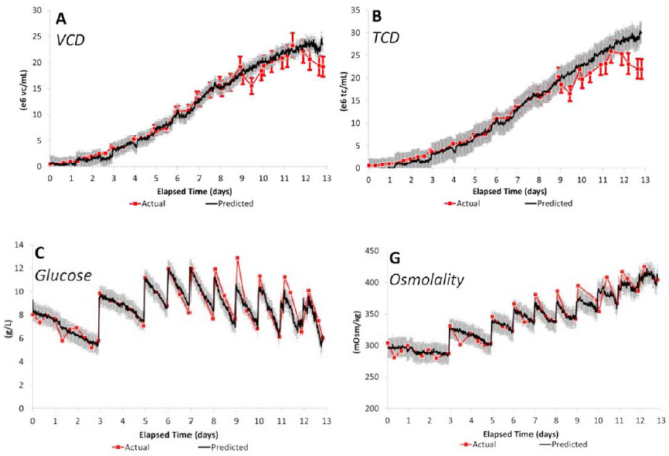

Figure 2: PLS model prediction results for major CPPs. Calibration data from batches at all three scales (3, 200, and 2000 L) were used to predict results from a batch at the 2000 L manufacturing scale. Reprinted with permission from Ref. 1. © 2014 American Institute of Chemical Engineers.

Experimental

Raman analyzers were used *in situ* to simultaneously quantitate the following CPPs: total cell density (TCD), viable cell density (VCD), glucose, lactate, glutamate, ammonium, and osmolality in a fed-batch bioprocess using a Chinese hamster ovary (CHO) cell line. Bioreactors at the 3 L process development scale, 200 L pilot scale, and the 2000 L manufacturing scale were examined *in situ*, and the spectral data were correlated with off-line reference data using both spectral preprocessing and partial least squares (PLS) regression.

A Raman analyzer (λ=785 nm) equipped with a Rxn-10 probe with a stainless steel immersion optic (for benchtop analysis) and a bioprocess probe (for pilot or production-scale runs) was used in this study (Figure 1).

Results

Calibration models were created for each of the CPPs under investigation: VCD, TCD, glucose, lactate, glutamate, ammonium, and osmolality. PLS model predictions were generated from batches at the process development (3 L), pilot (200 L), and manufacturing (2000 L) scales and were used to predict the results from a batch at the manufacturing scale. Figure 2 shows how Raman-predicted results corresponded closely to measured values.

Conclusions

The results from this work demonstrate that Raman spectroscopy can be used to simultaneously generate quantitative information on multiple CPPs. A bioprocess-compatible Raman analyzer can simultaneously measure multiple parameters and real-time quantitative information on multiple nutrients *in situ*.

The work further demonstrates that Raman produces reliable results at benchtop, pilot, and manufacturing scales and that these results can be used to develop robust process models for application in the manufacturing environment. The rich bioprocess information generated by Raman spectroscopy opens up promising new avenues of bioprocess understanding, enables QbD, and real-time control of the bioprocess to optimize cell viability and titer.

References

1. Berry et al. Cross-scale predictive modeling of CHO cell culture growth and metabolites using Raman spectroscopy and multivariate analysis. *Biotechn. Prog.* **2015**; *31(2)*, 566–577.

2. Abu-Absi et al. Real time monitoring of multiple parameters in mammalian cell culture bioreactors using an in-line Raman spectroscopy probe [Communication to the editor]. *Biotechnology and Bioengineering* **2010**; *108(5)*, 1215–1221.