Cross-scale In Situ Raman Monitoring of a Cell Culture Bioprocess

**Key Issues**
- **Simultaneous monitoring of nutrients, metabolites, and cell attributes**
- **Increased process understanding for QbD of cell culture bioprocess**
- **Real-time process control and model scalability**

**Introduction**

In mammalian-cell-based bioprocesses\(^1\)-\(^2\) the most common critical process parameters (CPPs) include physical parameters (temperature, agitation rate, and dissolved oxygen [DO] levels) as well as chemical properties (pH, nutrients, and waste concentrations) and biochemical properties (cell count and viability). Careful management of these CPPs is necessary to affect quality-assurance and maintain the tight parameters on product variability demanded by the FDA for GMP manufacturing.

Monitoring of these 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.

**Advantages of Raman Spectroscopy**

New 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, often enabling real-time process control.

The main advantages of Raman for process monitoring and control include highly specific information within the spectrum, enabling cross-model scalability and simultaneous measurement of multiple chemical and biochemical parameters. Raman provides a non-invasive, non-destructive analysis without need for sampling or additional reagents. Moreover, the delicate balance required between measurement and control has progressed to such a point that today real-time process control is widely available.

This note presents a cross-scale application as a follow-up to the process development study reported in Kaiser Application Note 322: “Analysis of a Mammalian Cell Culture.” The work here demonstrates successful process development from benchtop to manufacturing.

**Methods**

In this study, Raman spectroscopy was 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.

The Raman instrument used in this work was a bioprocess RamanRxn2™ Multi-channel analyzer from Kaiser with stainless-steel immersion probes (bIO-LAB-220 and bIO-PRO for benchtop and pilot or production-scale runs). The incident radiation was 785 nm near-infrared laser light. Raman spectra were collected every ten minutes by co-adding six hundred one-second scans.

**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. Figure 3 shows the close correlation between actual and predicted values for these CPPs.
Conclusion

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.

This work further demonstrates that Raman spectroscopy 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 modifications to the bioprocess to optimize cell viability and titer.

References:
