In Situ Monitoring of CHO Cell Culture Medium Using Near-Infrared Spectroscopy

Robert A. Mattes, Denise Root, David Chang, Mike Molony, and Mahalia Ong

Near-infrared (NIR) spectroscopy is an analytical technique based on absorption measured in the near-infrared region of the electromagnetic spectrum, between the visible and the midinfrared. The fundamental absorption bands of chemical functional groups occur in the midinfrared and are very strong, so dilutions and very small path lengths are usually required to bring such absorbances within the linear range of a detector. The overtone absorptions of those fundamental bands occur in the NIR spectral region and allow direct measurement without sample preparation because of the relative weakness of absorption. Fused silica fibers do not absorb strongly in the NIR region, so economic fiber-optic cables can be used to measure processes at relatively remote locations (from the analyzer) with probes inserted into processing equipment.

NIR spectroscopy (NIRS) has been used by the bioprocessing industry since the early 1990s for ex situ analysis, but it has potential for in situ bioprocess monitoring (**1**). In efforts to maximize antibody yield, it is important to monitor a bioreactor in situ during CHO cell culture for effective control and optimization of nutrient levels and ammonia production. Routine sampling for wet chemistry can introduce microbial contamination. Even when samples are taken hourly, results are not available for

Photo 1: Bioreactor with NIR process instrument

Photo 2: NIR Ingold probe installed in a bioreactor sample port

Photo 3: An Ingold probe for fermentation reactors

Figure 1: Calibration set of CHO cell culture raw spectra

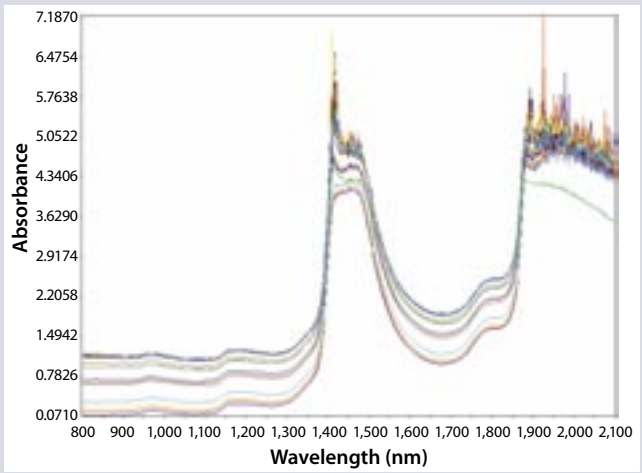
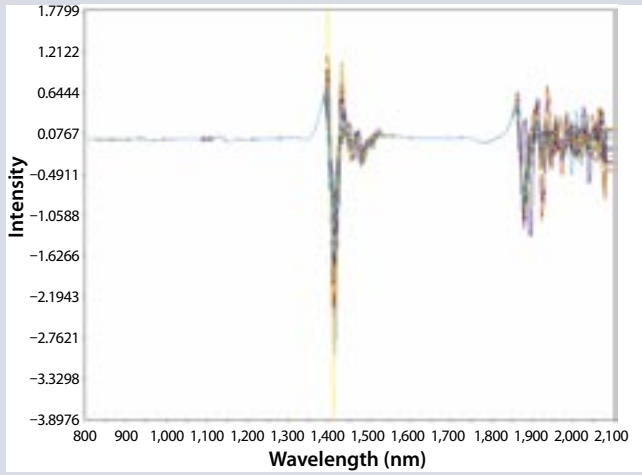


Figure 2: Second-derivative math pretreatment of calibration set spectra



hours or even days. NIRS using specialized transmission probes and fiber optics allows real-time in situ analysis of bioreactor feed stocks and product. With continuous NIR analysis, metabolic parameters and culture media constituents can be better monitored and controlled, which increases CHO cell life and lengthens the production phase. Each NIR spectrum can be used to predict as many as 30 fermentation parameters after prediction models have been developed.

Experimental

We set up a FOSS NIRSystems Process Analytics near-infrared spectrophotometer with a fiber-optic probe (using an Ingold port-adapted probe) installed into a 100-L bioreactor. Photos 1 and 2 show similar installations. The Ingold type immersion probe had a 4-mm pathlength and was similar to the one seen in Photo 3. This design allows good absorbance of constituents and high throughput while remaining within the dynamic range (0.0–6.0 AU) of the InGaAs detector. The probe was sterilized in place because it can withstand temperatures up to 300 °C and pressures up to 5,000 psi.

Before the probe was inserted into the bioreactor, an external reference of air was collected and adjusted to the internal reference fiber. We collected 32 coadded reference scans and 32 coadded sample scans to make each spectrum. The spectral range was 800–2,100 nm, with a data collection point every 2 nm. NIR spectra were collected every hour throughout a 12-day culture. The spectra can be collected as often as every 15 seconds. We also withdrew reference samples every 24 hours to provide wet-chemistry values for developing regression models using the Vision chemometric software that is provided with the FOSS instrument.

Discussion

Water absorbs strongly in the NIR (Figure 1), and our spectra saturated in absorbance at 1,400–1,500 nm and at 1,900–2,100 nm. Those bands, where water absorbs strongly, were notched out for this analysis because they exceed the linear dynamic range of the detector.

Empirical models must be developed for NIR to predict on unknown samples. We correlated NIR calibration spectra to the wet chemistry of samples withdrawn daily from the growth media. A blood analyzer was used to determine glucose and ammonia content, and HPLC was used to measure amino acid content. We developed a separate prediction model for each analyte using those wet chemistry results. Chemometrics (the application of mathematical and statistical methods to solve chemical problems) was used to develop the prediction models. We chose to use partial least squares (PLS) regression because of the complexity of the media matrix. The second derivative math pretreatment (with a smoothing segment of 10 and a gap of zero) was applied to the spectra to normalize the baseline and enhance spectral features (**2**). That led to inversion of absorption peaks as seen in Figure 2.

Glucose, ammonia, titer, and methionine prediction models were used for predictions on all the NIR spectra, which were collected hourly throughout our 12-day culture. They were plotted as the trend plots shown in Figures 3–6, with time in hours on the X-axes and the NIR predicted values on the Y-axes.

Glucose: Figure 3 is a trend plot of glucose concentration showing the NIR-predicted values for spectra in pink and the wet-chemistry calibration samples in blue. It appears that the initial glucose stock was depleted, an abundance was added, and an oscillation of feed and depletion caused a "ringing" control trend. Real-time NIR monitoring would enable reactor operators to control glucose and thus optimize cell productivity (**3**).

Ammonia: Figure 4 is a trend plot of ammonia concentration showing the NIR-predicted values for spectra in pink and the wet chemistry and calibration samples in blue. The trend is smooth and tight during the first third of the run but tends to scatter after 100 hours, as do the reference values. Cells respond quickly to their physicochemical environment, so operator control requires knowledge of such responses to optimize biological processes (**4**).

Titer: Figure 5 is a trend plot of titer concentration showing the NIR-predicted values for spectra in pink with the wet chemistry calibration samples in blue. NIR titer predictions track the reference chemistry well. The discontinuity around 190 hours (added media causing the titer concentration to drop temporarily) is not reflected in the wet chemistry data.

Methionine: Figure 6 is a trend plot of methionine concentration showing the NIR-predicted values for spectra in pink with the wet chemistry calibration

samples in blue. The trend plot tracks well with the chemistry, although the spike around 150 hours is not seen in the reference data. It should be noted that the methionine was depleted toward the end of the cell culture run. With real-time monitoring of this and other "limiting" nutrients, optimum levels could have been maintained throughout the run, resulting in better cell vitality and productivity.

Other Models: We also developed lactate, glutamate, and glutamine prediction models that predicted well, and their trend plots are shown in Figures 7–9. The first reference point in the glutamine plot does not predict well. Whether that is due to limited calibration data early in the culture or erroneous reference data is unknown. Future studies are planned to test these models with subsequent cell cultures.

Modeling for the Real World

Culture media constituents can be predicted in situ for a CHO-cell bioreactor

Figure 3: Glucose trend plot showing "ringing" oscillations

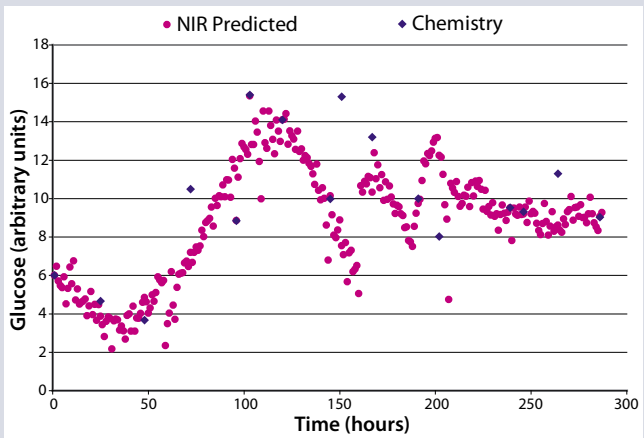


Figure 4: Ammonia trend plot

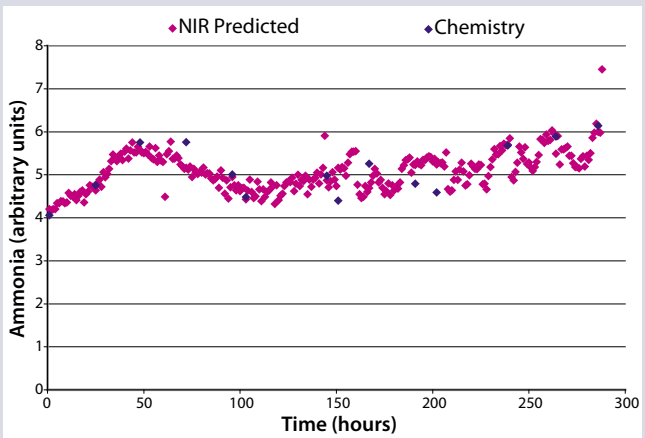


Figure 5: Titer trend plot

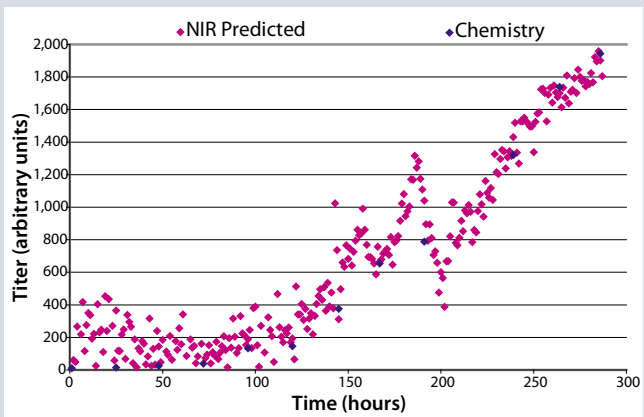


Figure 6: Methionine trend plot

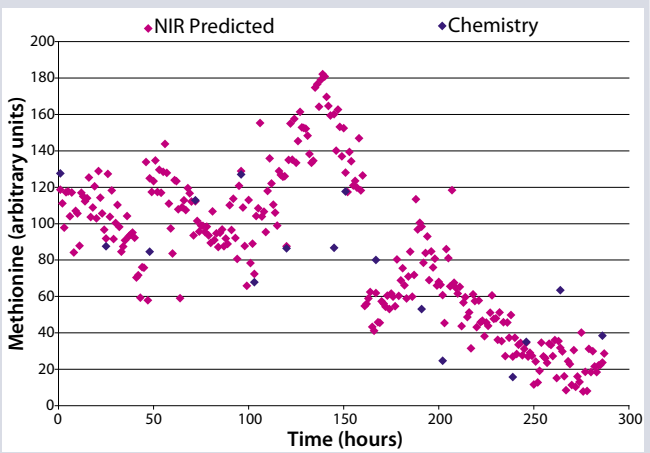


Figure 7: Lactate trend plot

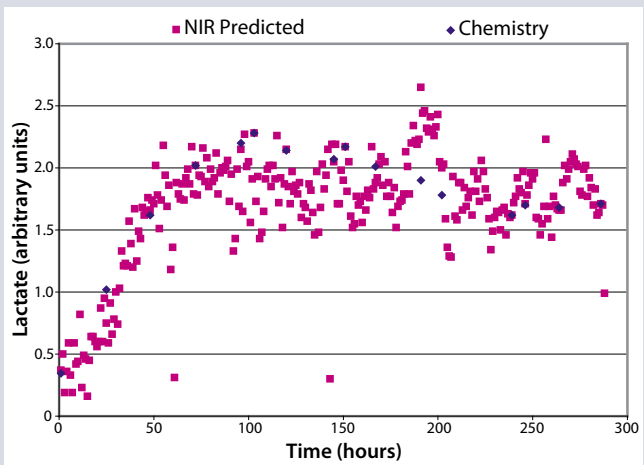


Figure 8: Glutamate trend plot

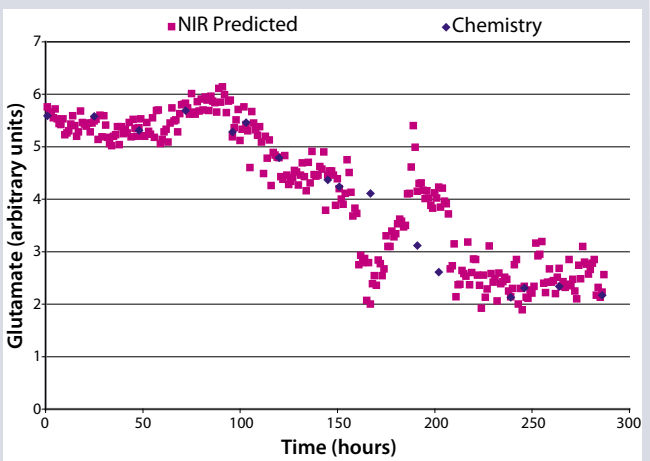
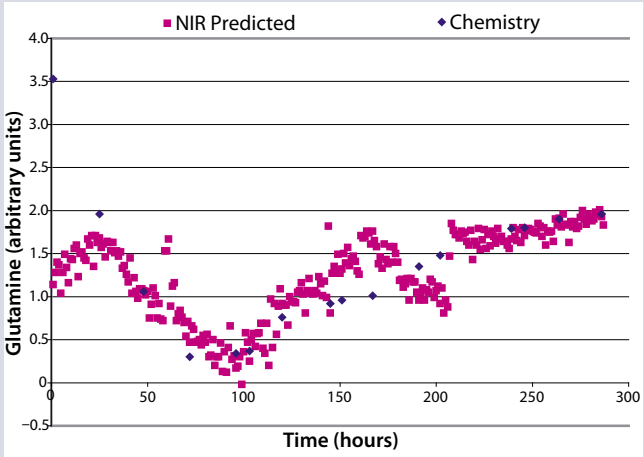


Figure 9: Glutamine trend plot



using near-infrared spectroscopy. Laboratory time can be reduced compared with that required to perform wet chemistry on periodic samples while providing real-time results that can be used to control and optimize antibody production during CHO cell culture. The NIR probe can be sterilized in situ, and measurements can be acquired continuously without risk of introducing microbial contamination. Models were developed that predicted trend plots of culture media constituents over time. The plots show smooth trends that pass through reference data, indicating good prediction model performance.

References

1 Arnold SA, et al. In-Situ Near Infrared Spectroscopy to Monitor Key Analytes in Mammalian Cell Cultivation. *Biotechnol. Bioeng.* 84(1) 2003: 13–19.

2 O'Haver TC, Begley T. Signal-to-Noise Ratio in Higher Order Derivative Spectrometry. *Anal. Chem.* 53(12) 1981: 1876–1878.

3 Arnold SA, et al. Employing Near-Infrared Spectroscopic Methods of Analysis for Fermentation Monitoring and Control, Part 1. *BioPharm* November 2002: 26–34.

4 Montigue G. *Monitoring and Control of Fermenters*. Institute of Chemical Engineers: Rugby, Warwickshire, UK, 1997.

Corresponding author ***Robert A. Mattes*** *is an applications scientist, and* ***Denise Root*** *is product manager at FOSS NIRSystems, Inc., 7703 Montpelier Road, Laurel, MD 20723; 1-301-680-7251; rmattes@foss-nirsystems.com.* ***David Chang***, ***Soheil Rahmati***, *and* ***Mahalia Ong*** *were all with Biogen IDEC, Inc. at the time of the work reported here.*