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Protein Concentration Prediction in Cell Cultures: The Next Stage in NIR Bioprocess Analysis

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Key Words

- Antaris
- Biopharmaceutical
- Bioprocess
- NIR
- Protein

Introduction

Biologically produced materials are an increasingly important aspect in many industrial processes including those related to pharmaceuticals, food, diagnostics, and fuels. Most of these biologicals are produced in fermentors and bioreactors where specialized cell cultures grow and manufacture the molecule of interest. Many different types of cells are used in culturing and producing biopharmaceutical products including genetically engineered bacterial and yeast cells. However a majority of the products are proteins cultured from mammalian systems such as Chinese hamster ovary (CHO), green monkey (VERO), or human embryonic kidney (HEK) cell lines. Many of these products are large complex proteins, hormones or polysaccharides that are impossible or difficult to manufacture in large quantities any other way. A recent survey of the U.S. Food and Drug Administration noted that there are over 350 biologicals approved for various uses including vaccines and diagnostic and therapeutically important antibodies.

Bioprocesses that produce the desired materials by nature rely on complex biological systems to synthesize their useful products. While typical chemical manufacturing processes have relatively little variability, the inherent complexity of biological systems makes a great deal of variability from batch to batch inevitable. As a consequence of the complexity and variability of the processes it has been estimated that 30% of the production batches need to be reprocessed for quality reasons which results in a 10-fold loss in profit. Industries that rely on these complex biological systems benefit greatly from closely monitoring the growth of their cell cultures and production of the target molecule. Process Analytical Technology (PAT) initiatives in bioprocesses improve the overall product quality and reduce waste by reducing and accounting for this inherent variability.

Monitoring and controlling cell culture conditions greatly reduces this variability and results in improved target protein production. Fourier transform near-infrared (FT-NIR) spectroscopy has proven to be a useful technology for monitoring and controlling manufacturing processes including more specific bioprocess applications. It is also part of PAT initiatives across many industries including bioprocessing. Previous work performed on cell cultures using NIR spectroscopy has usually focused on monitoring and controlling nutrients, waste products, cell densities and other parameters related to the health of the cell culture. While these parameters are useful for determining the relative health of the cell culture, the more important parameter of interest is the actual production and concentration of the target molecule. Very few NIR studies have determined and measured protein concentrations in actual cell culture conditions. In this application note, the feasibility of using the Thermo Scientific Antaris MX FT-NIR process analyzer (Figure 1) to predict protein concentrations at biologically relevant concentrations in dynamic cell cultures is demonstrated.

Figure 1: Antaris MX FT-NIR analyzer used for collecting the spectroscopic information from the cell cultures

NIR spectroscopy uses light between 10,000 and 4000 cm^{-1} to determine the identity and quantity of a variety of materials. Most molecules of interest absorb light in this region through combination or overtone vibrations. The advantage of performing spectral analysis on these absorption bands is that the light is able to penetrate more deeply into the material under analysis and doesn't require dilution or manipulation of the sample. Therefore NIR analyzers can be coupled directly into a process stream or tank where spectral analysis can be performed without human intervention. Fourier transform NIR has been implemented in many different industrial, pharmaceutical and other process settings for many years and has proven to be extremely valuable in collecting real-time analytical data automatically. When used in process environments, the Antaris™ MX process analyzer is easily coupled to process control computers where it is an integral part of maintaining optimal manufacturing conditions. Because of these advantages and the need to control the inherently variable biological systems found in cell culture technologies, NIR is an excellent choice for analyzing different components in bioreactors including proteins.

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Methods

Chinese hamster ovary (CHO) cell cultures were grown at optimal conditions until the cell concentrations reached approximately one million per milliliter. This represents a typical cell density for a young and growing culture. Samples of the cell culture were tested on a Nova BioProfile® analyzer to determine concentrations of glucose, glutamine, lactate, and ammonia. The concentrations of these materials changed throughout the experiment and accounted for some variability that might be encountered across multiple cultures. The concentrations were variously and singularly altered by spiking the samples with nutrients or waste products or diluting the samples with unaltered cell culture. Each of those four components was altered so that two or three different concentrations were represented for each. Table 1 lists the concentration ranges for the various nutrient, waste and protein components of the tested samples. This methodology also has the effect of removing covariance between the different components and protein present. Ultrapure bovine albumin protein was added to the solutions to represent target protein synthesized by the cells. Genetically modified cell cultures are designed to produce the target protein in large quantities almost exclusively to all other cellular proteins. As a result, the protein concentrations in the cell culture media will often approach and exceed 5.0 g/L and consist almost entirely of the target molecule. Albumin protein is an excellent mimic for recombinant proteins because it is available in extremely pure form and contains NIR active groups essentially identical to a typical target protein from a cell culture. In this case, purity is extremely important because any extraneous material present will also have a NIR signal and would lead to confounding results. The albumin protein material was carefully weighed and added to the cell cultures in concentrations ranging from 0.16 to 5.0 g/L. Over 35 different solutions were produced that had a range of nutrient and waste as well as protein concentrations. These varied solutions resulted in 54 spectra that were used to build the chemometric method and 20 spectra that were used to validate that method.

Component	Range (g/L)
Protein	0.16–5.00
Glucose	7.98–8.12
Glutamine	0.28–0.58
Lactate	0.45–0.90
Ammonia	0.05–2.39

Table 1: Concentration ranges of various components. The solutions represented over 35 different protein concentrations that also varied in concentrations of nutrient and waste components.

The cell culture samples were scanned with an Antaris MX process analyzer in the range between 10,000 and 4000 cm^{-1}. The analyzer was coupled to a transflectance probe with an adjustable pathlength. The gap distance was set to 1.25 mm for a total pathlength of 2.5 mm. Sixteen scans were averaged per spectrum and were collected using eight wavenumber resolution with a gain of 0.1. Sample time took approximately 15 seconds. Two spectra were collected per sample. Figure 2 shows images of the probe before insertion into a cell culture sample and during spectral collection.

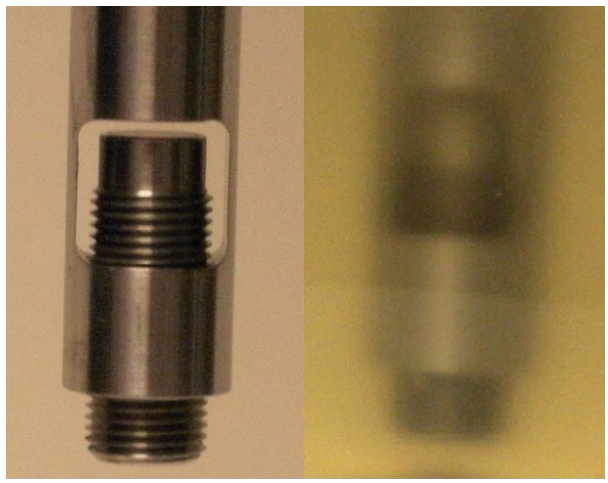

Figure 2: Transflectance probe used for data collection. Left panel shows the design of the probe with the adjustable pathlength. Right panel shows probe inserted into cell culture during data collection.

The sample spectra were loaded into Thermo Scientific TQ Analyst software for chemometric analysis using a Partial Least Squares (PLS) method with a constant pathlength. The spectra were analyzed in the first derivative using a Norris smoothing filter. Two regions were used for the analysis; 8910 to 5340 cm^{-1} and 4830 to 4340 cm^{-1}. These two regions collected information across a wide range of data points while avoiding the totally attenuating water peak centered around 5100 cm^{-1}. Figure 3 shows representative raw spectra and the first derivative spectra of the samples.

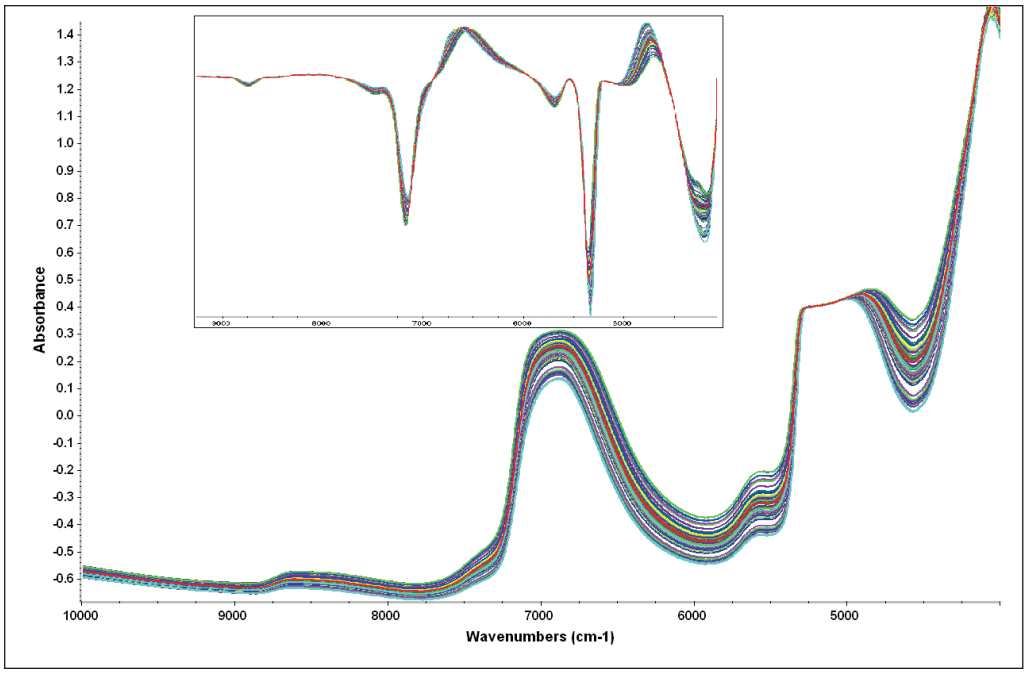

Figure 3: Representative raw spectra showing the variability present in the cell culture samples. Regions of analysis avoided the attenuated water peak at 5100 cm^{-1}. Inset shows the first derivative spectra used for the PLS chemometric method.

Results

Partial Least Squares analysis of the protein concentrations in the various cell culture samples revealed excellent predictive capabilities within the range of materials tested. The 54 spectra used to develop the PLS method are shown on a calibration plot (Figure 4) that compares the calculated protein concentrations versus the actual concentrations. The calibration plot can be used to determine how well the method predicts the actual protein concentrations in the samples. The plot developed by the chemometric method resulted in a correlation coefficient of 0.977. Root Mean Square Error of Calibration (RMSEC) was 0.33 g/L and the Root Mean Square Error of Prediction (RMSEP) calculated from the 20 validation samples was 0.31 g/L. Additionally, the Root Mean Square Error of Cross Validation (RMSECV) was 0.51 g/L. These errors indicate that the protein concentration in the cell culture samples can be predicted to 0.5 g/L or less. Approximately ½ of this error was attributed to the balance used to weigh the protein material.

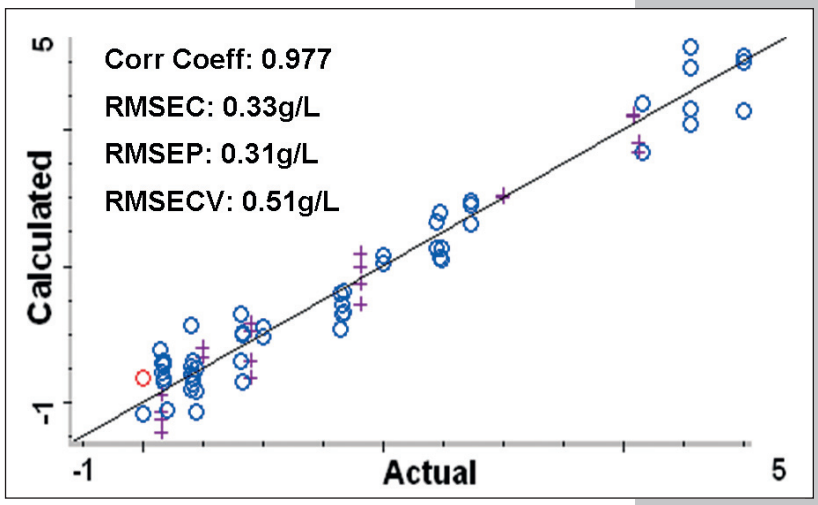

Figure 4: Calibration plot comparing the calculated protein concentrations to the actual concentrations from the PLS method. Root Mean Square Errors are approximately 0.5 g/L or less. Blue circles (o) represent spectra used to create the method, purple crosses (+) are spectra used to validate the method.

Conclusions

Measuring protein concentrations in living dynamic cell cultures was successfully performed with the Antaris MX process analyzer. Protein concentration is a critical parameter in determining the success and quality of a cell culture in manufacturing a viable end product. This NIR technique successfully demonstrates the ability to measure and monitor protein concentrations in real time at relevant concentrations. The method developed shows excellent correlation with actual protein concentrations between 0.16 and 5.0 g/L with errors of less than 0.5 g/L.

This application demonstrates the continued capability of the Antaris FT-NIR systems to be successfully used in bioprocess environments where it can safely, accurately and automatically monitor and control cell cultures. While previous NIR studies have monitored cell culture conditions to promote optimal protein production, few have actually monitored and predicted protein concentrations. This feasibility study shows the power of the Antaris FT-NIR analyzer to correctly predict target protein concentrations in a live and dynamic cell culture.

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