APPLICATION NOTE

No. AN51845

# Cell media dissolution by NIR: controlling biopharma variability

Todd Strother, Thermo Fisher Scientific, Madison, WI, USA

## Introduction

An increasingly important field of pharmaceutical research and development is the area of biologically derived molecules. These biopharmaceuticals include recombinant proteins, antibodies, hormones, cytokines, and growth factors that are produced using cell cultures. The cells used in producing the target molecules can be bacterial in nature or may be from various eukaryotic systems such as yeast, insect, avian, or mammalian lines. Unlike typical chemical manufacturing processes, there is potential for substantial batch-to-batch yield variation using these biological systems. Biological processes are complex by nature and are difficult to control, which results in variable yields. The sources of variability may be traced to raw materials, differences in starting cultures, and even variations in the growth medium. It is vital to control as many aspects of the process as possible in order to produce consistent amounts of a high-quality product.

One key source of variability that has not usually been the focus of control is in cell culture media production. Synthetic media is a complex solution of multiple components specifically designed for a particular cell line and process. Slight changes or differences in media composition can have dramatic consequences on cell health and activity and ultimately on final product quality. Components such as buffers, glucose, amino acids, and other nutrients are carefully weighed prior to dissolution, but the resulting media solution is rarely comprehensively checked for quality.

The chief variable in a properly prepared media batch is ensuring the materials are completely dissolved, and no residual solids remain. Media preparation laboratories may ignore this aspect completely or may have protocols in place that mix the media for an unnecessary and inordinate amount of time. Testing for total dissolution, if done, may


**Figure 1: Antaris MX FT-NIR Analyzer used for the collection of media dissolution spectra**

be limited to simple conductivity tests or measurements of pH. These tests are limited in scope and do not reveal a comprehensive view of the total dissolution of materials. Measurement of pH, for example, will not change appreciatively due to the inherent stabilizing effects of buffers. Alternatively, comprehensive testing may be done with a battery of off-line chromatographic, spectroscopic or wet chemistry techniques that require samples to be withdrawn and tested. These tests are labor-intensive and time-consuming, which adds to the expense and time delay of the media use. A preferred analytical technique would non-destructively and aseptically monitor total dissolution in real-time for each and every batch of media produced. For this application, the Thermo Scientific™ Antaris™ Fourier Transform Near-infrared (FT-NIR) Spectrometer is shown to be an effective means for performing this analysis and reporting important dissolution data.

ThermoFisher SCIENTIFIC

Near-infrared spectroscopy is a well-established technique used for many years in pharmaceutical, food, chemical, and other process environments. It measures the electromagnetic spectrum between 10,000 $cm^{-1}$ and 4000 $cm^{-1}$ which is the region responsible for setting up particular molecular vibrations in many compounds. These molecular vibrations are unique in that they allow excitation near-infrared energy to penetrate deeply into materials. This deep penetration allows for larger sampling volumes than other techniques such as mid-infrared spectroscopy. Additionally, near-infrared light is easily shunted through fiber optic cables and probes, allowing it to be used remotely and directly in the process stream without withdrawing or preparing samples.

## Methods

A Thermo Scientific™ Antaris™ MX FT-NIR Process Analyzer (Figure 1) was used to collect standard spectra from well-characterized and accepted standard media solutions. The Antaris MX Analyzer is designed to be used in process environments and can collect data simultaneously from four different processes. The analyzer was used in conjunction with an adjustable pathlength transflectance probe (Figure 2). Light traveling from the probe passes through the gap, where it impinges on a mirror and is reflected back into the probe. The pathlength can be altered for specific uses to accommodate a variety of solutions. Transflectance probes are advantageous in that they can collect spectra from solutions that change in opacity, such as media solutions or active cell cultures.

**Figure 2: Transflectance probe used in the analysis, showing detail of adjustable pathlength.**

A variety of media solutions were prepared, and dissolution was tracked with the Antaris MX Analyzer. The makeup of the solutions was empirically determined to have factors and nutrient concentrations optimized for the growth of specific mammalian cell lines. The specific cell lines and media components were proprietary.

Spectra were collected from fully characterized and accepted ideal solutions, which were used as standards to compare to subsequent production solutions. The standards spectra were collected over the range from 9880 $cm^{-1}$ to 4120 $cm^{-1}$ and were the result of 16 scans with 8 $cm^{-1}$ resolution. The spectra were ratioed against a water background so that the spectral features represented the influence of the dissolved solutes. These standards were included as part of a Similarity Match chemometric method generated in the Thermo Scientific™ RESULT™ and TQ Analyst™ Software package. Similarity Match is a chemometric algorithm where the spectrum from a sample is compared to the spectra from the set of ideal standards. The match score reported will indicate the percentage of variability in a sample that is described by the standards in the method. A perfect match will have 100% of the variability described by the standards and be given a Match Score of 100. The raw spectra were analyzed without any smoothing. No further spectral pretreatment was performed other than a linear removed baseline correction.

## Results

To demonstrate the usefulness of real-time quality assurance and variability control of cell culture media, new batches were mixed and dissolved using the established standard protocols. Each media batch was created by first filling a process tank with ultrapure water then inserting the probe and impeller. The spectral analysis was started by first taking a background spectrum from the water prior to the addition of media components. Figure 3 is an image of the process tank showing the probe and impeller in place before adding the media. After the addition of the components, spectra were automatically collected using the same parameters as the standards spectra collection. The spectra were collected approximately every 12 seconds and compared to the spectra generated from the standard media using the similarity match algorithm. Figure 4 shows the dramatic change in spectral character as the dissolution of the materials takes place. The features found in the spectra are the result of the increasing presence of solutes in the media as well as changes in water molecule vibrations.

As the dissolution takes place, the media reaches a point where it is spectroscopically indistinguishable from the ideal solution as determined by similarity match values approaching 100. At this point, the mixing and dissolution are complete, and the media is ready for use.


**Figure 3: Process tank for preparing media. Transflectance probe and impeller are in place prior to adding media constituents.**

The similarity match values were recorded and plotted as shown in Figure 5. This plot shows the characteristic and expected increase over time approaching 100 within 250 seconds. Dissolution kinetic studies were also performed on these test batches. Based on the curve from Figure 5, it was found that this dissolution approximates a first-order kinetics rate law. Figure 6 shows the results of the data graphed as a first-order kinetics plot. The residual variation was calculated by subtracting the values of each data point in Figure 5 from 100. The natural log of this residual variation was plotted versus time to develop the kinetics plot. The result of this plot is a straight line with a slope of -0.03 and intercept of 4.34 where the negative of the slope is defined as the rate constant k. From this information, the half-life of the dissolution reaction can be calculated from the equation $t_{1/2}$=ln(2)/k, and is found to be approximately 23 seconds.

## Conclusion

The results of this study show that NIR can be used to monitor media dissolution online in real-time without having to remove samples. This novel technique takes advantage of the unique characteristics of the Antaris MX FT-NIR Analyzer. New batches of media were created and spectrally compared to ideal standard media during the course of dissolution using a Similarity Match chemometric algorithm. The non-destructive and real-time nature of this analytical technique makes it a superior method for controlling batch-to-batch variability in media solutions. By controlling this variability, more consistent target molecule yields can be realized in cell cultures used by the biopharmaceutical industry.

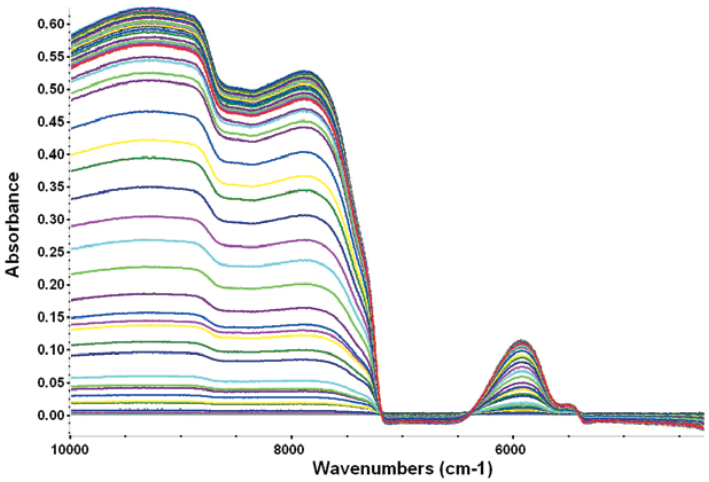


Figure 4: Spectra tracking dissolution of media over time. Spectra were ratioed against a water background.

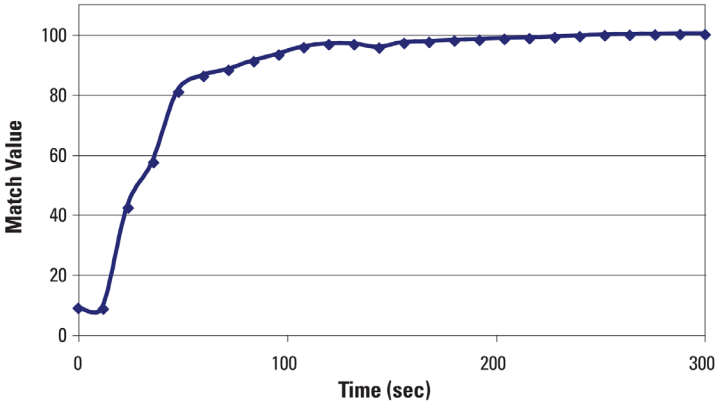


Figure 5: Match values plotted vs. time. The Similarity Match values increase over time and level off at 100% match within approximately 250 seconds.

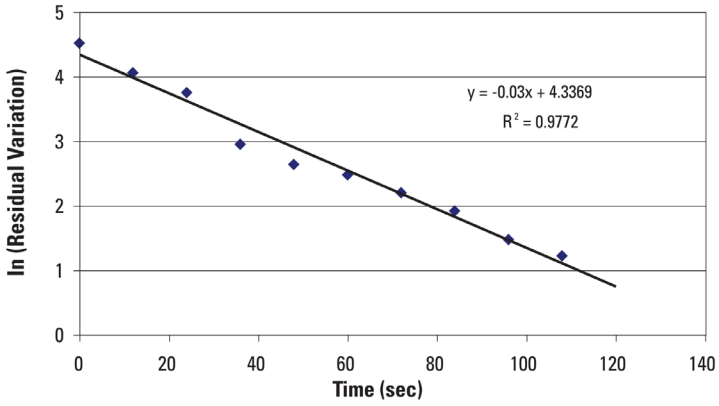


Figure 6: Kinetics plot showing the dissolution of the media follows a first-order rate law. Dissolution half life ($t_{1/2}$) is calculated as approximately 23 seconds.

Find out more at **thermofisher.com/nir**

ThermoFisher SCIENTIFIC