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Spectroscopy

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FTIR/NIR/Raman in Bio/Pharmaceutical Analysis

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Q&A: Protein Secondary Structure

Interview with Suja Sukumaran, PhD, Senior Applications Scientist, Thermo Fisher Scientific

The advantages of FTIR for protein analysis.

Spectroscopy and *PharmTech* sat down with Suja Sukumaran to discuss the advantages of Fourier transform infrared (FTIR) analysis of proteins, why secondary structure is important, upcoming trends, and more.

SPECTROSCOPY/PHARMTECH: **Which are protein FTIR applications?**

SUKUMARAN: Infrared spectroscopy has proved to be a very powerful tool for studying biological molecules. In general, it is used to characterize proteins, lipids, sugars, cellulose, and other bio-chemistries. With proteins specifically, FTIR is used for secondary-structure determination, structure-function correlation, protein stability, and protein folding and unfolding. Application of FTIR is common in the pharmaceutical and biotechnological industries, but it is also gaining momentum in the food industry.

SPECTROSCOPY/PHARMTECH: **How is secondary structure calculated from the spectra, and why is it important?**

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Protein Secondary Structure Elucidation Using FTIR Spectroscopy

SUKUMARAN: When determining protein secondary structure, spectroscopy provides a unique advantage over other methods such as X-ray crystallography, nuclear magnetic resonance (NMR), or reflection electron microscopy (REM) because it can dive into the secondary structure by itself, and it can do it very fast. When investigating secondary structure, the characteristic region the instrument looks at is the amide regions or the amide bonds. Protein secondary structure is composed of alpha, beta, and random structures.

In general, when a protein's secondary structure needs to be determined, a researcher analyzes spectrum from 400–4,000 wavenumbers. The key region they focus on is the amide I region, between 1,600–1,700 wavenumbers. Although other regions such as amide II and amide III are also used in the analysis, amide I is the key region for secondary-structure analysis. Once the spectrum has been collected, the broad peak is deconvoluted into individual components, which in turn is fitted to the original peak using the peak-fitting function in the software. Once the peak is fitted, you get the area under the peak information, which is used for calculating the percentage of alpha helix, beta sheet, and random structures. All this can be achieved with standard OMNIC software. There are other commercially available software that can do similar secondary-structure evaluation with our data.

SPECTROSCOPY/PHARMTECH: What are the advantages of FTIR analysis of proteins?

SUKUMARAN: FTIR is fast, non-destructive, and requires minimal sample preparation. The instrumentation required is compact and does not need any consumables. In many instances, precious purified protein material can be retrieved as well. In addition to pure protein analysis, FTIR also allows you to look at a mixture of proteins or proteins with additives and inhibitors, and then characterize these for their stability, kinetics, etc. Proteins can also be used in various forms—powders, liquids, gels, hydrogels, or deposited on a surface.

RECENT TRENDS

A recent trend in FTIR is to combine analytical techniques (e.g., Rheo-IR: rheology and FTIR). This combination analyzes the rheological property of your protein while you're observing how the chemistry or the secondary structure of your protein is changing.

An increasingly popular trend is to analyze protein powders and protein aggregates using FTIR microscopes. It is also used to study distribution of proteins and lipids in tissues, biofilms, and so forth.

Q&A: PROTEIN SECONDARY STRUCTURE

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Protein Concentration Prediction in Cell Cultures

Another advantage is the spectra obtained from FTIR—spectra are generated in a matter of minutes and are simple and easy to interpret. With all the recent technological advances in software and computing, these analyses have become very routine and fast.

SPECTROSCOPY/PHARMTECH: **What other information can we get from the analysis?**

SUKUMARAN: FTIR spectra of the protein not only provides insights into the protein secondary structure, but it can also enumerate details of amino acids. For example, insights into structure-function can be obtained by looking into the side chain protonation and deprotonation states of proteins.

You can also analyze most proteins as well as peptide samples in different environments (i.e., different buffer conditions, temperature conditions, and pH conditions). All of this contributes to a better understanding of protein stability.

SPECTROSCOPY/PHARMTECH: **Which analyzer and accessories are used in protein analysis and how?**

SUKUMARAN: Depending on the end goal, we use our entry-level instruments, iS5s and Summits, for QA/QC characterization on protein powders, protein solutions, lyophilized proteins, and so forth. To push the limits of detection, our higher end instruments such as the iS50 offer the highest sensitivity with the best signal-to-noise. Nicolet iS50R is used for step-scan measurements and for various fast kinetic measurements. There is a range of research instruments or entry-level instruments that can be used for FTIR analysis.

Accessories also depend on the end goal or the type of starting materials. For example, if you're starting with pure powders, lyophilized materials, or concentrated solutions, a single- or multi-bound attenuated total reflectance (ATR) measurement can be used. Single-bound ATRs are great for high-concentration materials and pure materials; multi-bounds ATRs are used for very low-concentration materials.

Proteins are also analyzed in transmission mode, which uses a small pathlength transmission cell ranging from 6 to 10 microns for most studies. There is increasing interest in analyzing proteins and peptides that are used as coatings. These coatings are either on a glass slide, a silicon wafer, or a polymer surface. These samples are analyzed with grazing-angle ATRs or grazing-angle specular reflectance accessories. At times, researchers want to characterize the orientation of proteins and peptides on the surface of a certain material. In these instances, we use polarized, light-based FTIR analysis.

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Q&A: Foreign Particulate Identification in Biopharmaceuticals

Interview with Bruno Beccard, EMEA Senior Applications Leader, FTIR and Raman, Thermo Fisher Scientific

An overview of the advantages of FTIR and Raman spectroscopy for identifying microparticles in pharmaceuticals and medical devices.

In this *Spectroscopy* and *PharmTech* interview, Bruno Beccard discusses microparticles in pharmaceuticals and medical devices, techniques to identify them, advantages of Fourier transform infrared (FTIR) and Raman spectroscopy, and more.

SPECTROSCOPY/PHARMTECH: **Why are microparticles so important in pharmaceuticals and medical devices?**
BECCARD: Microparticles are foreign matter—which nobody wants entering the body—that are due to mechanical properties, sizes, and chemical composition. They can be either toxic materials, carrying toxic compounds, or simply mechanical; they can block a vessel and cause a stroke, for example. So, it's important to control the small particles that could be in drugs, injectables, etc.

SPECTROSCOPY/PHARMTECH: **Are there regulations for particles in pharmaceuticals and medical devices?**
BECCARD: There are regulations on the number of particles by

size class, which is usually determined optically—a visual inspection using different optical microscopes. These regulations have been around for 10 to 12 years, and FDA and other regulatory bodies have been lowering the size and the total number of particles.

SPECTROSCOPY/PHARMTECH: **Why is it important to go further?**

BECCARD: There is a growing concern about smaller particles, two of which are really of interest: below 3 microns, because they can go through the intestinal barrier and into the blood and cause harm; and big particles, which can be incorporated in the blood and cause harm because they carry a lot of toxic compounds and can block larger vessels, which may be even more of a problem for strokes. These are more of a concern for injectables, perfusion, and dialysis.

SPECTROSCOPY/PHARMTECH: **What techniques can be used to ID these particles?**

BECCARD: Many techniques have been proposed for use by manufacturers, including electron microscopy with energy dispersive spectrometers (EDS) or wavelength dispersive spectrometers (WDS). These are very efficient for minerals and determining the elemental composition, but they won't work if you have an aggregate of an active pharmaceutical ingredient (API), an excipient pharmaceutical drug, or a polymer needing to be identified.

Some have proposed two microscopy techniques on top of optical microscopy: infrared microscopy and Raman microscopy. I don't like the term infrared microscopy because that usually means you are getting an image from infrared, which is not how infrared or Raman work. What you actually get is spectra, which are information about chemical composition. From those spectra, you derive a presence or absence, or a concentration and build an image from that.

At Thermo, we can provide scanning electron microscopy (SEM) as well as

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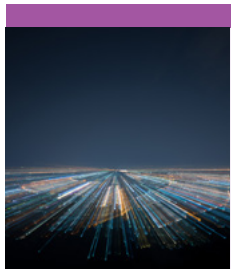

Raman and FTIR microscopes. For very small particles, some techniques burn those particles and then analyze the gasses using, for example, gas chromatography-mass spectrometry (GC-MS).

SPECTROSCOPY/PHARMTECH: **Is there a specific advantage of FTIR versus Raman or vice versa?**

BECCARD: It's not that simple. The advantage of Raman is that it uses shorter wavelengths, which means we get better spatial resolution, and we're able to analyze smaller particles than with FTIR. But Raman has two drawbacks: First, it uses a lot of energy, which can be a problem with fragile samples that can be destroyed; and second, fluorescence is way more efficient than Raman. There can be issues with identification because the Raman spectrum is hidden by fluorescence.

On the other hand, FTIR is faster and more sensitive, but it uses longer wavelengths and is more limited in terms of spatial resolution. FTIR can only analyze larger particles whereas Raman can go down to 0.5 micron and get very good identification. FTIR doesn't go much lower than 7 microns, but FTIR is faster than Raman.

"There can be issues with identification because the Raman spectrum is hidden by fluorescence."

In the case of analyzing filters with lots of particles, for example, which come from pharmaceutical manufacturing, many prefer FTIR because they can analyze more particles within the same amount of time.

SPECTROSCOPY/PHARMTECH: **Are there any additional benefits of identification of chemical nature?**

BECCARD: Yes, there are because if you can identify the particle nature of a polymeric material, for example, then you can go back to where those particles were introduced into the drug, the injectable, or during the manufacturing process—something could come from a pump or a container used for the drug. It can also come from an aggregate of an API or an excipient, and in that case, a different approach is used to solve the problem. So, getting the identification is important. Also, in some cases, knowing what the polymer is may point to a product that could be a heavy metal associated with a particular type of polymers.

SPECTROSCOPY/PHARMTECH: **Do you have any examples of applications?**

BECCARD: Yes, I have a number of examples such as, when dissolving tablets, we found polymer particles that traced back to the bags containing ingredients used for formulating the tablet. We also have

several cases with blood transfer or plasma separation machines in which particles were released and, in one case, we discovered those particles were technical polymers released by the pumping devices. In fact, a few hundred machines in Europe were stopped due to these findings.

We've also seen a lot of concern with injectables such as the COVID-19 vaccines. These vaccines have several doses in the same vial, so each time you go through the cap, there's concern whether any rubber was injected into the person.

Another example is a company that is building devices for mixing cancer treatments bedside. The company was examining the mixing process and making sure there weren't any particles because they are made of polyethylene terephthalate and various other polymers. On the tests we conducted, we didn't see any particles larger than our spatial resolution limit.

Bruno Beccard

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Enabling Real-Time Release of Final Products in Manufacturing of Biologics

Shaileshkumar Karavadra, David James, and Arnaud Di Bitetto

Important applications of Raman spectroscopy to the manufacturing and analysis of biopharmaceuticals.

Introduction

Biopharmaceuticals (or biologics) are manufactured using biological-expression systems (such as mammalian, bacterial, insect cells, etc.) and have spawned a large and growing biopharmaceutical industry (BioPharmaceuticals). The structural and chemical complexity of biologics, combined with the intricacy of cell-based manufacturing, imposes a huge analytical burden to correctly characterize and quantify both processes (upstream) and products (downstream). In small-molecule manufacturing, advances in analytical and computational methods have been extensively exploited to generate process analytical technologies (PAT) that are now used for routine process control, leading to more efficient processes and safer medicines.

Raman spectroscopy is a vibrational spectroscopy technique with several useful properties (non-destructive, non-contact, high molecular-specificity, and robustness) that make it particularly suited

"Furthermore, many clinical products are developed using a small number of batches resulting in a narrow range of raw material variation and thus a limited process understanding."

for PAT applications in which molecular information (composition and variance) is required.

Typical good manufacturing practice (GMP) operations involve performing an extensive set of tests according to approved specifications before the material is released to the market or for further processing. Recent ICH guidelines (ICH Q8, Q9, Q10, and Q11), however, suggest an alternative real-time release strategy to provide assurance of product quality prior to release. Real-time release testing uses the principles of the pharmaceutical Quality by Design (QbD) to optimize release and stability testing. A combination of manufacturing process understanding, process control, and product knowledge can be used to demonstrate that the material was made according to GMP.

The exact approach to real-time release testing (RTRT) will vary depending on the process requirements. The RTRT strategy may be based on control of process parameters, monitoring of product attributes, or on a combination of both at appropriate steps throughout the process. Critically, the RTRT strategy should be based on a firm understanding of the process and the relationship between process parameters, in-process material attributes, and product attributes.

Quality, cost, and speed are the major drivers for implementing in-line at-line monitoring and real-time release.

Here, we review some of the most important applications of Raman spectroscopy to the manufacturing and analysis of bio-pharmaceuticals. This article covers two aspects of the bio-manufacturing process, identity/variance testing of raw materials and cell culture media, to multi-attribute product testing of a biologic drug product, or final product testing of a biologic drug product.

Raw material characterization

Acceptance of raw materials today is often predicated on small-scale functional testing and/or limited analytical methods, which may not be representative of at-scale performance. This leads, in some cases, to fluctuating process outputs and, in extreme cases, not meeting predefined release criteria. Furthermore, many clinical products are developed using a small number of batches resulting in a narrow range of raw material variation and thus a limited process understanding. Especially in upstream cell culture, the unforeseen variability of various components of the cell culture media can impact a product's micro-heterogeneity and its critical quality attributes (CQA).

ENABLING REAL-TIME RELEASE OF FINAL PRODUCTS IN MANUFACTURING OF BIOLOGICS

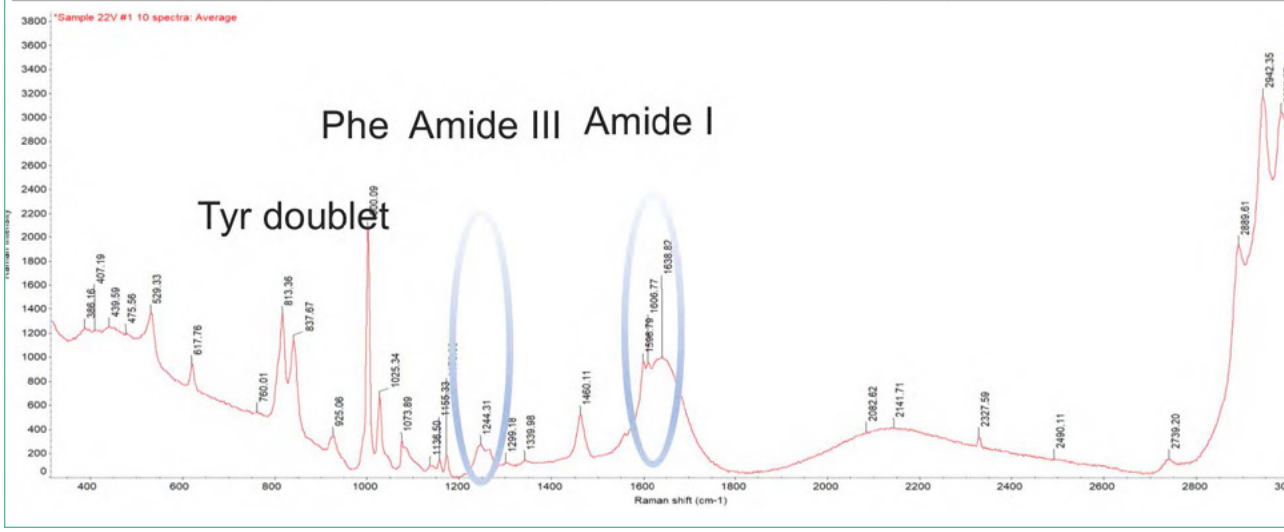
Multi-attribute tests for high-risk raw materials may include Identity test, quantitative test for the concentration of key ingredients in a raw material, batch-to-batch variability test, and degradation tests.

One such raw material encountered in biologics manufacturing is cell culture media. Identification of cell culture media samples by traditional LC methods, such as amino acid or vitamin analysis, has high costs and requires significant analytical expertise and laboratory space. Raman spectroscopy offers many potential benefits, such as low cost, portability, and potentially limited skill required to operate the instruments.

Buffers are another set of critical raw materials used in downstream manufacturing. Osmolality is a measure of concentration and is considered a critical

FIGURE 1: DXR3 SmartRaman spectrum showing characteristic bands of a biologic drug product

Band frequency (cm^{-1})	Region	Vibrational mode	Protein structure assignments
870-1150	Backbone, skeletal stretch	C_α-C, C_α-C_β, C_α-N	Secondary structure elements: α-helix,β-sheets, less-ordered structure
1200-1340	Amide III	N-H in-plane, Cα-N stretch	Hydrogen bonding, secondary structure
1400-1480	Side chain deformations	CH_2 and CH_3 deformations	Local environments, intermolecular interactions of side chains
1510-1580	Amide II	N-H deformations and C-N stretch (observed in UVRR and not conventional Raman spectra)	Local environments, intermolecular interactions of side chains
1630-1700	Amide I	C=0 stretch NH in-plane bending	Secondary structure elements: α-helix,β-sheet, less-ordered structure



quality attribute and critical process parameter in bioprocessing. The yield and quality of a biologic are highly dependent on the optimization of the downstream process. Identity testing along with Osmolality of buffers can be carried out using a multiattribute method based on Principal component analysis and Partial list squares. Rapid testing of buffers through single-use flexi bags can be carried out using the fiber optics probe of the Thermo Scientific™ DXR3 SmartRaman Spectrometer at the point of use with no need for sample preparation.

Final product identity testing

Final product identification of biologics pre- and post-shipment is another regulatory requirement. Product testing for identity test through different kinds of primary packaging (glass vials, syringes, glass bottles) pose a significant analytical challenge in the manufacturing of biologics. Fill finish sites may not have the necessary analytical expertise to carry out the tests and may have to send the samples to the parent site or external lab for testing, incurring time and money.

Moreover, biologics or small molecule drug products would also have to undergo retesting upon importation from a third country in the EU member state or the USA when drug products have been sent to the USA from other countries. A full list of tests is typically carried out, including final product Identity testing. For biopharma manufacturers, this involves either sending the samples back to the parent site for analysis or employ third-party labs in the country of import. This increases significant costs and delays in the delivery of highly needed drug products.

End product identity testing/final product identity testing of biologics after fill-finish or pre-shipping to the fill-finish line is carried out by a variety of analytical techniques depending on the molecule/registration dossier.

For example, the verification test for biologic proteins is peptide mapping—a long-established workflow for protein identification using LC/MS. This complex separation technique requires protein extraction and clean-up, enzyme digestion, one or more stages of liquid chromatography, and two phases of mass spectrometry before the final spectrum is matched against protein databases. Although a standard methodology, peptide mapping necessitates an analytical lab with qualified technical resources, entails extensive time for preparation, and introduces significant costs in solvents, columns, and analytical equipment.

The DXR3 SmartRaman Spectrometer, with its high sensitivity and resolution, allows characterizing the drug product by evaluating the fingerprint region of the molecule. Therefore, the DXR3 SmartRaman spectrometer's unique capability with sampling flexibility ensures repeatable measurements, and subsequent analysis allows rapid method development and deployment.

We ran a feasibility study for multinational drug manufacture whereby the primary goal was

to set up a rapid multi-attribute end product test to differentiate 15 different types of drug products and determine the concentration of the two preservatives in the drug products.

For this feasibility test we were given 15 different types of biologic drug products varying in concentration from 0.5 mg/ml to 6 mg/ml. Concentration of two preservatives A and B ranged from 0.85 mg/ml to 5.0 mg/ml and 0.42 mg/ml to 3.91 mg/ml, respectively.

These commercial drug products were supplied in their native glass vials varying in size and volume. A picture of such glass vials is shown below.

Characteristic Raman band assignment

A reversed-phase high-performance liquid chromatographic (HPLC) is currently used for the final product identity test and quantitative measurement of two preservatives in the final drug product.

DXR3 SmartRaman Spectrometer with universal sampling plate and 180-degree sampling module was used to acquire spectra of 15 drug products. 532nm laser with 40 mW power and 1 minute of scanning time was used to acquire each spectrum. Ten spectra were acquired for each sample to accommodate the variability of glass vials and scattering effects.

In addition, it offers excellent selectivity, repeatability, and full wavelength range to characterize biologics based on the characteristic band assignment.

FIGURE 2 shows the spectra of a sample containing a drug product against its placebo. It is imperative to establish that technique chosen for a feasibility study. In this case,

FIGURE 2: Raman spectra of drug product and its placebo and variance spectrum

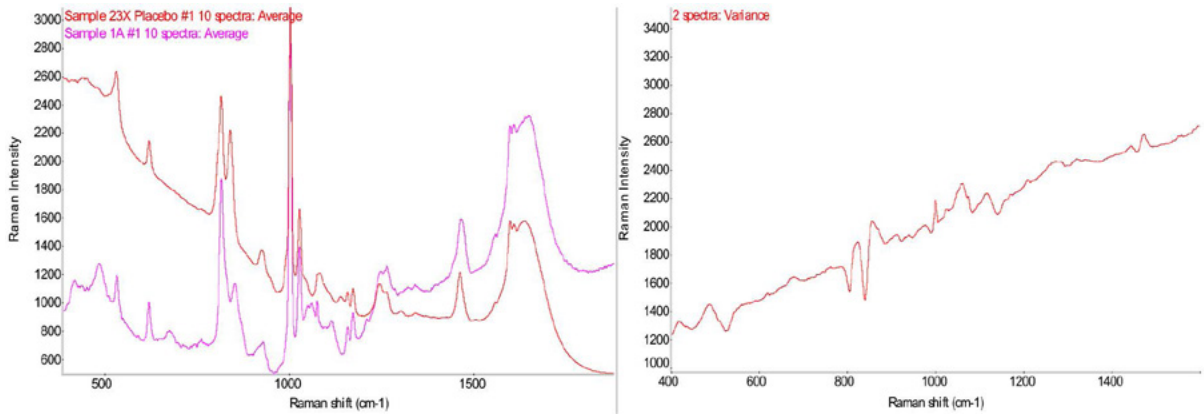
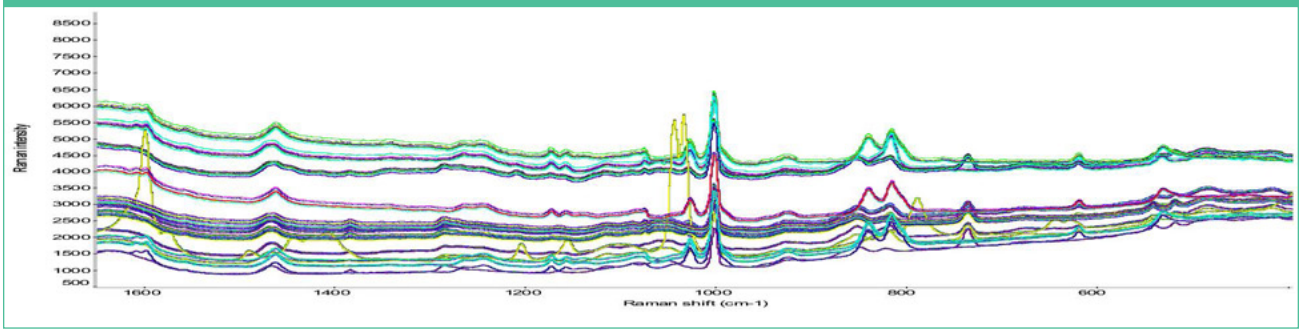


FIGURE 3: Raman spectra of different classes of drug products



Raman spectroscopy is sensitive enough to detect the differences between the drug product and its placebo. DXR3 SmartRaman Spectrometer offers high sensitivity to determine the significant differences between placebo and actual drug products.

FIGURE 3 is showing spectra of different classes of drug products. These spectra were utilized to build the discriminant analysis method on the Thermo Scientific™ TQ Analyst™ Software. TQ Analyst Software is a validated qualitative and quantitative method building software offering full compliance for pharmaceutical applications.

The discriminant analysis classification technique can be used to determine the class or classes of known materials that are most similar to an unknown material by computing the unknown's distance from each class center in Mahalanobis distance units. The discriminant analysis technique is typically used to screen incoming materials or final products to determine if they are compound/molecule a, b, or c.

Discriminant Analysis methods typically specify at least two classes of known materials, but the method also works with only one class. Multiple standards may be used to describe each class (at least one class must contain two or more standards). Multiple regions of the spectrum may be used for the analysis.

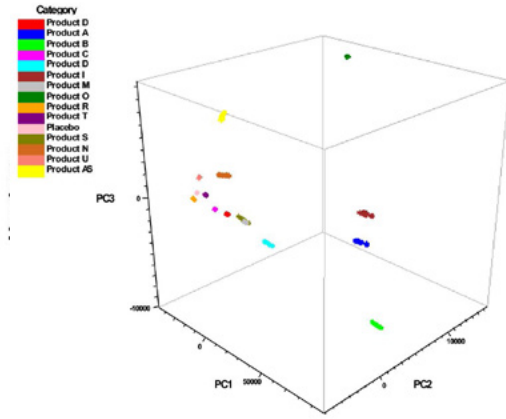
What does discriminant analysis do?

A discriminant analysis method applies the spectral information in the specified region or regions of an unknown sample spectrum to a stored calibration model to determine which class of standards is most similar to the unknown.

When the method is used to analyze an unknown sample or a class, the software performs a principal component analysis on the spectra of the standards and uses those results to determine score values for the

FIGURE 4:

Actual class	Mismatch	Calculated class	Calculated distance	Next class	Next distance
Product D		Product D	0.5809	C	4.5556
Product A		Product A	1.9869	I	12.9617
Product B		Product B	1.3796	E	25.1324
Product C		Product C	0.5417	D	3.8568
Product D		Product D	0.8466	M	9.0495
Product I		Product I	1.7709	A	13.9064
Product M		Product M	0.5284	S	3.3881
Product O		Product O	0.2244	X	17.3044
Product R		Product R	0.5419	C	4.4691
Product T		Product T	0.5944	X	2.3213
Product X		Product X	0.79	T	3.1646
Product S		Product S	1.1837	M	3.0829
Product N		Product N	1.0954	U	15.1798
Product U		Product U	0.1603	T	9.1738
Product S		Product S	1.8544	N	22.1624



unknown sample spectrum. The score plots are used to produce Mahalanobis distance values, which in turn are used to rank the classes.

The result of a discriminant analysis is the name of the class or classes that are most similar to the spectrum of the unknown sample. The Mahalanobis distance between the unknown sample and each reported class can also be reported. The closer each distance value is to zero, the better is the match.

After cross-validation principal component scores plot reveal the class differentiation and the report indicated that all the classes of the different products were correctly identified and there were no mismatches to indicate the false positives.

Quantitative analysis of biologics for preservative A and preservative B

As part of this feasibility study, our client also wanted to determine if the DXR3 SmartRaman Spectrometer test could be utilized to replace the HPLC test for measuring the concentration of two preservatives in their drug products, level of preservative A was 0.85 mg/ml to 5

FIGURE 5: Spectrum in blue is from pure preservative A and spectrum in red is from pure preservative B

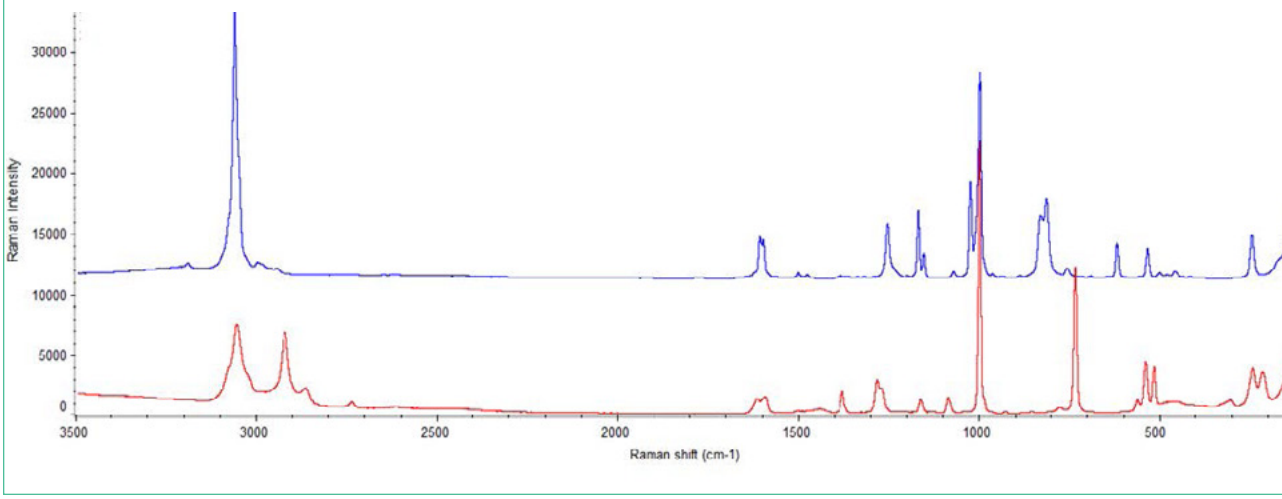


TABLE 1: Calibration and validation sample

	Preservation A (mg/ml)	Preservation B (mg/ml)
Standard 1 3 mL and 10 mL	0.85	0.42
Standard 2 3 mL and 10 mL	1.27	1.12
Standard 3 3 mL and 10 mL	1.57	1.75
Standard 4 3 mL and 10 mL	3.07	2.57
Standard 5 3 mL and 10 mL	5.07	3.91
Validation – 3 mL	1.57	1.75

mg/ml and that of preservative B was 0.32 mg/ml to 4.91 mg/ml.

Pure samples of preservatives A and B were acquired for reference and to ascertain their presence in the final drug formulation.

Samples of varying concentrations as per TABLE 1 were acquired using the same parameters as of spectra acquired for identity test through 3 ml vial. FIGURE 6 is showing the spectra of the drug product with the two preservatives.

Five standards with the reference values were supplied in 3 ml and 10 ml vials and a validation sample to test the model for 3 ml and 10 ml vials.

These varied solutions resulted in 15 spectra that were used to build the chemometric method. The final drug product samples were scanned with a DXR3 SmartRaman Spectrometer to acquire spectra Full spectral range of 3500 to 50 cm^{-1} and captured with a single exposure of the CCD, avoiding stitching artifacts. The sample time took approximately 1 minute. Three spectra were collected per sample. The sample spectra were loaded into Thermo Scientific TQ

FIGURE 6: Spectra showing varying concentration of preservatives in final drug product

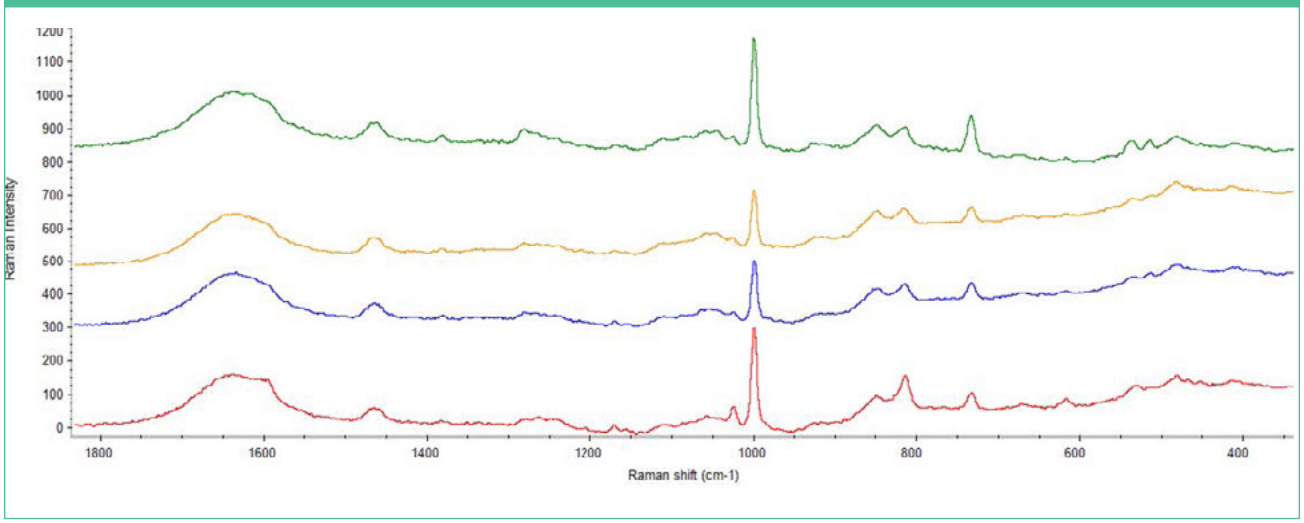


TABLE 2: Validation result for 3 ml sample

	Preservation A (mg/ml)	Preservation B (mg/ml)
Validation sample: 3 mL	1.58 actual 1.57	1.71 actual 1.75
Real sample in solution: 3 mL	1.56 actual 1.55	1.69 actual 1.77
Real sample in suspension: 3 mL	0.72 actual 0.69	1.23 actual 1.58

TABLE 3: Calibration and validation results for 3 ml 10 ml vials

	Preservation A (mg/ml)	Preservation B (mg/ml)
Validation sample: 3 mL	1.58 actual 1.57	1.71 actual 1.75
Real Sample in solution: 3 mL	1.56 actual 1.55	1.65 actual 1.77
Real sample in suspension: 3 mL	0.80 actual 0.69	1.21 actual 1.58
Real sample in suspension: 10 mL	0.73 actual 0.68	1.32 actual 1.57

Analyst Software for chemometric analysis using a Partial Least Squares (PLS) method.

Results

Partial Least Squares (PLS) analysis of the final drug product samples revealed excellent predictive capabilities within the range of materials tested. The 15 spectra used to develop the PLS method are shown on calibration plots (FIGURE 7 AND FIGURE 8) that compare the calculated preservative concentrations versus the actual concentrations. The calibration plot can be used to determine how well the method predicts the actual preservative concentrations in the samples. The plot developed by the chemometric method resulted in a correlation coefficient of 0.998 for preservative A. Root Mean Square Error of Calibration (RMSEC) was 0.0425 mg/ml, and the Root Mean Square Error of Prediction (RMSEP) calculated was 0.0372 for preservative A. Additionally method for preservative B resulted in a correlation coefficient of 0.999. Root Mean Square Error of Calibration (RMSEC) was 0.0316 mg/ml, and the Root Mean Square Error of Prediction (RMSEP) calculated was 0.0496.

When 10 ml vial calibration samples were added to the above PLS method, method performance remained the same and was able to accurately predict the 10ml validation sample.

FIGURE 7: Partial Least Square model result for preservative A

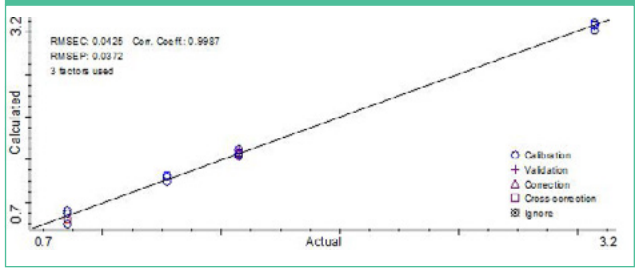
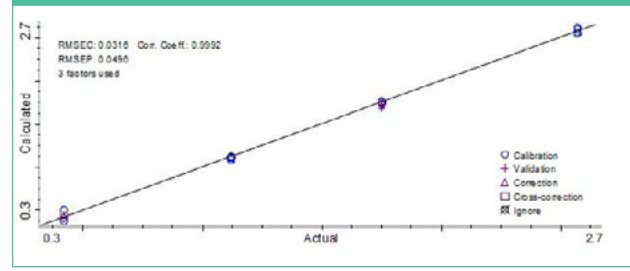


FIGURE 8: Partial Least Square model result for preservative B



Conclusions

A multi-attribute test to establish Final product identification and predicting concentrations of preservatives was done with the DXR3 SmartRaman Spectrometer by developing a discriminant analysis method and partial least squares method. The final drug product identification test is part of release testing and current methods used are time-consuming and laborious. This Raman technique successfully demonstrates the ability to measure and monitor preservative concentrations either in the lab environment or at the line. The method developed shows excellent correlation with actual preservative concentrations with errors comparable to the reference analysis method. This application demonstrates the continued capability of the DXR3 Raman Spectrometer to be successfully used in bioprocess environments for implementing multi-attribute final product testing of biologics. Apart from the examples shown here, DXR3 SmartRaman Spectrometer can be used to implement at-line control strategies to monitor protein concentration, excipients concentration, and critical quality attributes like Osmolality and pH. Many such examples are cited in the literature for Raman applications in biopharma manufacturing.

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On-demand webinar

Lean manufacturing with Raman spectroscopy

Biopharmaceutical manufacturers are increasing their testing regimes and increasing their efforts to raise current production levels. However, due to bottlenecks in current testing procedures, it's not always easy to shorten testing times.

Hear from Shailesh Karavadra, Thermo Fisher Scientific, addresses the role of Raman spectroscopy in the rapid release of raw materials through identity testing and real-time release testing of final products such as vaccines, hormones, and monoclonal antibodies. Gain further insight into how traditional testing can be replaced with Raman spectroscopy.

About our speaker

Shailesh Karavadra, Applications Manager
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Shailesh Karavadra is currently the Applications Manager for Vibrational Spectroscopy in EMEA. He has worked with the pharmaceutical, chemical, and food industries for over 15 years implementing vibrational spectroscopy in the QC labs and processes. He is currently responsible for the development of new applications with vibrational spectroscopy in the EMEA region.

Key learning objectives

- How to conduct raw materials testing and final product identification testing with Raman spectroscopy
- Learn about real-world examples of implementation of Raman spectroscopy for final product identification
- How Raman spectroscopy offers advantages over conventional analysis using wet ID methods

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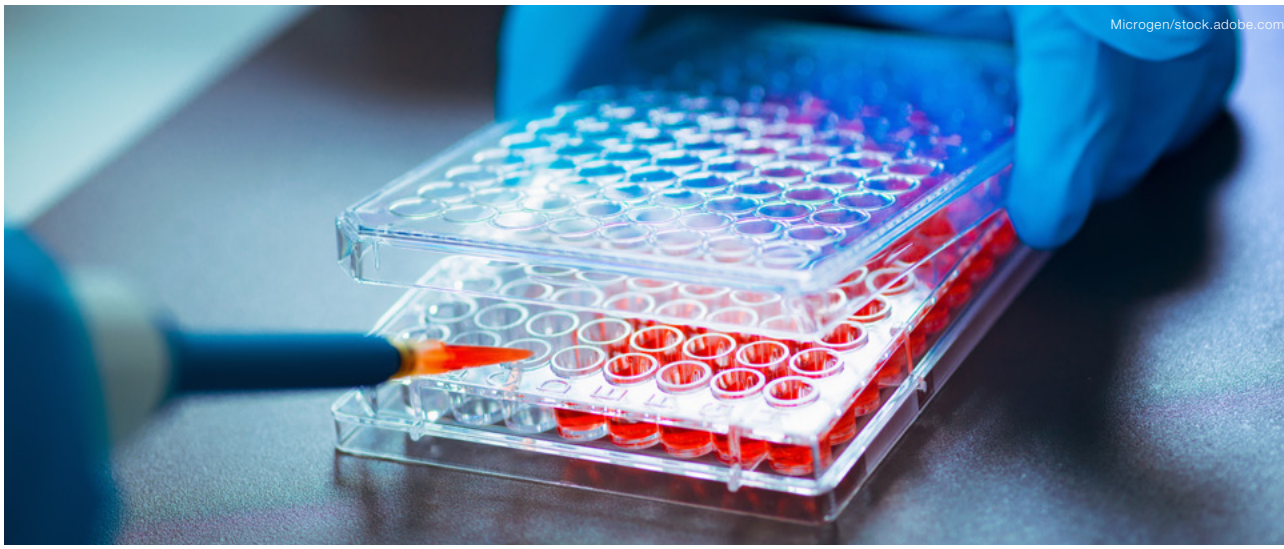


Cell Media Dissolution By NIR: Controlling Biopharma Variability

Todd Strother

Data exploring why Thermo Scientific Antaris FT-NIR Spectrometer is an effective means for performing cell media dissolution analysis.

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.

CELL MEDIA DISSOLUTION BY NIR: CONTROLLING BIOPHARMA VARIABILITY

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 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 offline 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.

“Alternatively, comprehensive testing may be done with a battery of offline chromatographic, spectroscopic or wet chemistry techniques that require samples to be withdrawn and tested.”

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

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

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

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.

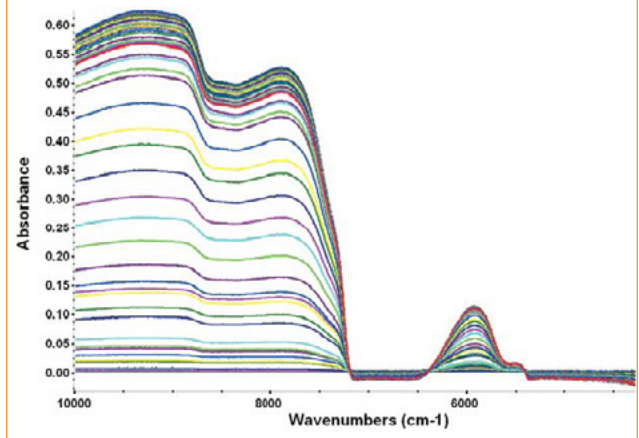
A variety of media solutions were prepared, and dissolution was tracked with the Antaris MX Analyzer. The makeup of the solutions was empirically determined to have factors and nutrient concentrations optimized for the growth of specific mammalian cell lines. The specific cell lines and media components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

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

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



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

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

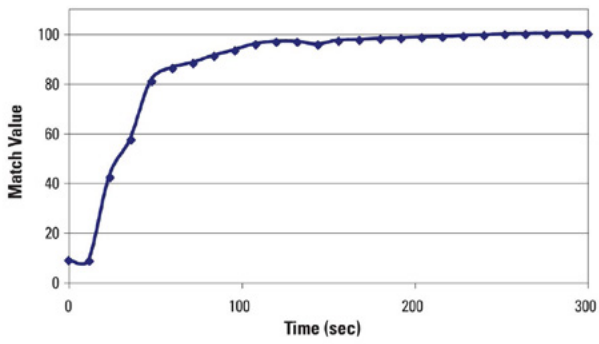
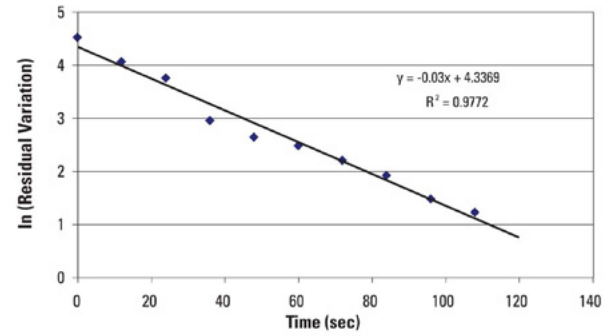


FIGURE 6: Kinetics plot showing the dissolution of the media follows a first-order rate law. Dissolution half life ($t_{1/2}$) is calculated as approximately 23 seconds.



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.

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 firstorder 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.

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On-demand webinar

Improving efficiency and return on investment by adopting molecular spectroscopy techniques in pharma manufacturing

Pharma and biopharma manufacturing are under constant pressure to improve efficiency, increase productivity, and reduce costs while still maintaining product quality.

Molecular spectroscopy (Fourier transform infrared [FTIR], near-infrared [NIR], and Raman) are versatile, non-destructive, and green techniques that enable rapid analysis of raw materials, allowing for processes to be monitored and final products analyzed with ease. Implementation of these techniques in manufacturing will ensure rapid return on investment and allow drug manufacturers (pharma, biopharma, parenteral, injectables) to easily implement quality by design (QbD) principles into routine practice.

About our speaker

Shailesh Karavadra, Applications Manager
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Shailesh Karavadra is currently the Applications Manager for Vibrational Spectroscopy in EMEA. He has worked with the pharmaceutical, chemical, and food industries for over 15 years implementing vibrational spectroscopy in the QC labs and processes. He is currently responsible for the development of new applications with vibrational spectroscopy in the EMEA region.

Key learning objectives

- Understand the differences between FTIR, NIR, and Raman spectroscopy
- How spectroscopy is applied in quality control labs
- Learn from real examples of spectroscopy techniques in pharmaceutical workflows (Raw materials analysis, process analytical technology [PAT], finished product testing, contaminant/foreign particle testing)
- Hear how the implementation of these techniques increased efficiency and minimized costs
- Ask our expert speakers questions and benefit from their knowledge and guidance

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