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How Raman ensures product quality from lab-to-process in biopharma

Using Raman spectroscopy for lab-to-process chemical composition measurement in biopharmaceutical manufacturing

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Executive summary

Overview of Raman spectroscopy as a key bioprocess measurement technology
Raman technology solves many process challenges for a wide range of upstream and downstream biopharmaceutical applications. This whitepaper discusses the role of Raman spectroscopy as an important optical analysis measurement tool to help biopharmaceutical manufacturers understand, measure, adapt, and control their chemistries to ensure product quality from lab-to-cGMP. As a valuable inline process measurement technology, today's advanced Raman systems improve process robustness, increase productivity, and speed time-to-market with bioprocess automation. In doing so, Raman aligns with key process analytical technology (PAT) and Quality by Design (QbD) industry initiatives.

What is Raman spectroscopy?
Raman spectroscopy is a measurement principle that uses visible or near-infrared light to measure chemical composition. As light interacts with molecular vibrations, it results in the light becoming inelastically scattered. When the energies of these transitions are plotted as a spectrum, changes in wavelengths can be observed which are very specific to each molecular vibration. This creates a powerful "molecular fingerprint" that can be used for the identification, quantification, and monitoring of particular chemistries.[1]

History of Raman in the biopharmaceutical industry

Background

While Raman spectroscopy has been used for measurements in laboratory academic settings for decades, it was not until the mid-to-late 1990s that Raman instrumentation became more user-friendly. This improvement, along with other technology advances, enabled the use of Raman across a wider range of industrial applications, such as the monitoring and control of crystallization and reaction endpoint processes.[2] Figure 1 shows an example of the specificity of Raman spectroscopy analysis for use in identifying application-specific molecules.

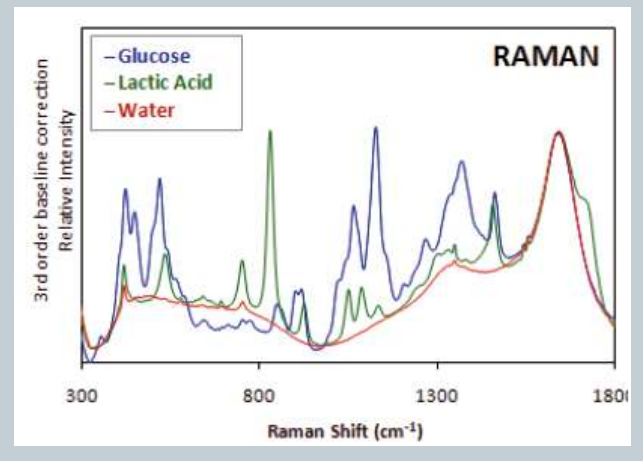

Figure 1: Example of Raman spectra identifying glucose and lactic acid in water (data collected by Endress+Hauser)

Because of its valuable measurement capabilities, the use of Raman spectroscopy can be found in many different applications in laboratory, portable, industrial, environmental, and clinical research settings today.[3] In fact, Raman's specificity enables the measurement of multiple components using a single fiber optic probe.

Possible bioprocess measurements that can be taken with one Raman probe:		
Cell Culture	Fermentation	Purification
Glucose	Glucose	mAb
Lactate	Optical density/biomass	Aggregation
Glutamine	Sugars and sugar alcohols	Structure and functionality
Glutamate	(MeOH, EtOH, Glycerol,	Sugar
Phenylalanine	Maltose, Maltotriose,	Buffer
Ammonium	DP4+, etc.)	Amino acids
Osmolality	Starch sum	
Viability	Other metabolites	
VCD		
Titer		and more…

Table 1: Example of Raman's specificity enabling multiple component measurement using a single probe (data consolidated by Endress+Hauser from measured components found in literature)

Raman's biopharmaceutical roots

Feasibility studies focused on the use of *in situ* Raman in cell culture arose during the late 2000s. Figure 2 depicts the growth of Raman spectroscopy in the biopharmaceutical industry over the last two decades. By 2010, some of the first industry publications emerged touting Raman as a reliable measurement technology for monitoring cell cultures during upstream bioprocessing. Since 2016, industry adoption of Raman has increased dramatically for both cell culture and fermentation. Raman technology products have likewise continued to develop, leading to proven model transfer, generic models, successful scale up into cGMP, and Raman-based process control. Today, hundreds of industry publications have been published on the benefits of Raman for upstream bioprocessing applications.[3]

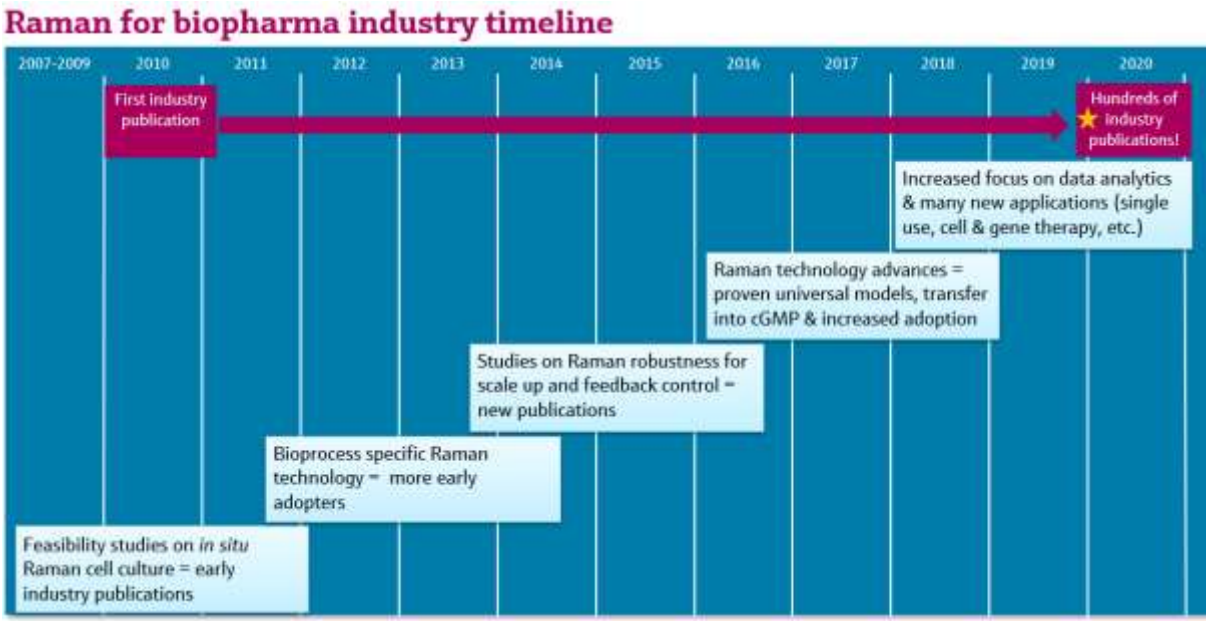

Figure 2: Timeline of Raman's use in the biopharma industry (collected using Endress+Hauser data)

A significant factor in Raman's growth over the last decade can be traced to pioneering work between a leading Raman instrumentation provider and early adopter biopharmaceutical manufacturers. That collaboration successfully proved out the benefits of early-generation Raman analyzers and probes in both laboratory and manufacturing environments. It also enabled real-world bioprocessing requirements to be directly incorporated into subsequent Raman products, leading the way for the more advanced Raman technology available in the market today.

Raman's role in large molecule development

In discussing the rise of Raman spectroscopy within the life science industry, it is important to differentiate between small molecule and macromolecule ("large molecule") development. Small molecule pharmaceuticals comprise most drugs developed for the broad population. They are primarily produced via traditional organic synthesis in a chemical lab.[2] While Raman

spectroscopy has been a valuable measurement tool for small molecule pharmaceutical applications, the scope of this whitepaper is focused on Raman's role in the large molecule development segment of the industry.

Because of their size and complexity, most large molecule therapeutics are developed by hijacking the protein production machinery of the cells of a living host. Monoclonal antibodies (mAbs) produced by mammalian cells are the main type of biopharmaceutical molecule used. However, other molecule types can be utilized such as amino acids, vitamins, and enzymes. Large molecule biopharmaceuticals target more specific population groups and are most commonly used to treat specific diseases. The sequence of complex processing steps used to develop these large molecule biopharmaceutical products is often known simply as "bioprocessing." Advances in bioprocessing have truly transformed the field of medicine. Hundreds of approved large molecule biopharmaceutical therapeutics are now commercially available.[4,5]

Biopharmaceutical manufacturing challenges

Bioprocesses are highly complex and expensive, requiring a long time to build process knowledge and optimize process productivity. It takes time to identify the molecule that works the best (sometimes from a pool of thousands), perform subsequent research studies, and then confidently move on to the next stage of analytical development. With traditional benchtop bioreactors, many off-line manual runs are required for knowledge buildup. Frequent, cumbersome in-process testing using multiple analytical techniques is needed to keep bioprocesses working within tight timeframes.

ⓘ Common bioprocessing challenges

- Too much time and expense needed to reprove out analytical methods and equipment for the process/ production environment after proven in the lab
- Non-representative sampling and inaccurate offline analytical methods
- Lack of transparency into what is happening real-time in bioprocesses
- Difficulty in monitoring and controlling processes/product quality parameters
- Inconsistent product quality
- Industry regulation compliance headaches

Lack of transparency into what is happening real-time throughout the course of the biopharmaceutical product lifecycle is an issue that hinders product quality. Offline manual sampling does not allow for tight monitoring and control of critical quality parameters, often resulting in inconsistent product quality. Because of these challenges, it is difficult to ensure product quality for short-run clinical batches and to comply with cGMP requirements. New approaches to Raman-based PAT in bioprocessing allow rapid knowledge buildup, supporting clinical and commercial manufacturing.[6]

How Raman solves bioprocessing challenges

Raman spectroscopy offers distinct advantages over traditional offline techniques because it acquires data quickly and continuously, has less contamination risk, and requires little or no sample preparation. A single Raman analyzer can be paired with multiple probes to deliver inline, real-time bioprocess monitoring, control, and optimization of multiple process streams. Some commercial Raman probes can even measure multiple, specific components in real-time for continuous, around-the-clock process feedback. In upstream development, for example, Raman accurately performs inline and simultaneous measurement of nutrients, metabolites, and cell viability. Raman spectroscopy is highly valued for its ability to improve product quality, speed cycle times, increase yields, comply with regulatory standards for process contact materials, and facilitate cross-scale method transfer from lab to manufacturing.[6]

ⓘ Benefits of Raman in bioprocessing

- Monitors multiple attributes with a single *in situ* probe around the clock
- Improves process transparency to gain advanced process control, adaptability, and optimization
- Increases productivity and time-to-market by speeding processing, reducing analytical wait times, and removing bottlenecks
- Maximizes return-on-investment from process automation, reduced product waste, and higher yields
- Ensures higher product quality and improves patient outcomes by reducing risk of contamination and minimizing process variability
- Facilitates scalability from lab to cGMP
- Reduces regulatory burdens

Raman's alignment with PAT and QbD

Raman spectroscopy embraces current industry initiatives by enabling PAT and QbD which helps to reduce a biomanufacturer's overall regulatory burden. PAT and QbD have greatly contributed to industry-wide improvements in bioprocess control. By allowing continuous inline bioprocess measurement and understanding, Raman empowers biopharmaceutical companies to optimize, adapt, and control their processes. This capability makes Raman a practical tool for PAT and aligns with the principles of QbD by allowing companies to achieve greater real-time quality assurance and better risk management throughout the biopharmaceutical process lifecycle.[1,3]

Recent Raman advances in bioprocessing

Biopharmaceutical manufacturers have a broad range of Raman spectroscopy products to select from in the marketplace today. However, standard Raman analyzers and sampling probes are often not fully capable of solving complex bioprocess measurement challenges. Therefore, many

biopharmaceutical companies seek out Raman technology products with more advanced features and functionality.

New Raman technology innovation

Raman spectroscopy is not a new measurement technique, but what has changed lately is greater automation and inline technology. Recently, there has been a new focus on data analytics, driving the emergence of new Raman applications including high throughput and automated modeling, single-use, perfusion, cell and gene therapies, downstream, and more. In just the last few years, advances in Raman analyzer and probe technology have enabled greater data integrity, smarter processes, and less manual intervention.

One of the most significant modern Raman innovations is lab-to-process scalability. The ideal Raman technology is one that is high performing regardless of whether a company chooses to leverage it just in the laboratory for research, development, or quality assurance purposes, in process for control or monitoring, or across all scales with transfer of knowledge as part of the technology's laboratory to manufacturing capability. For truly efficient bioprocess model development, many biopharma companies require high-resolution and high-quality data, but they also need model scalability, probe and instrument compatibility, and cross-product transferability. Raman systems are now commercially available with entire bioprocess product portfolios – analyzers, probes, software, and accessories – purposely designed with these capabilities to help companies smoothly scale from the laboratory to the process environment. As discussed further in this paper, lab-to-process scalability is expected to propel wider adoption of Raman spectroscopy as a principal bioprocess measurement technology.[2]

Optimized fiber optic sampling probes

Inline fiber optic Raman probes obviously play a crucial role in any Raman system, serving as an analytical "eye" directly into bioprocesses. Due to the sheer complexity of biopharmaceutical manufacturing requirements, general purpose Raman probes are not sufficient. Instead, Raman probes need to be optimized for bioprocesses, meaning that they have key characteristics designed to meet daunting industry requirements such as strict material standards, sterilization, port compatibility, and convenience. Bioprocess probes should feature a surface finish suitable for cGMP manufacturing (such as stainless-steel), fit the ports of small scale and large bioreactors, and offer compatibility with cleaning and sterilization protocols.

ⓘ Key characteristics of bioprocess probes

- High purity, low background windows
- Bubble shedding
- Fixed-focus design
- Port compatibility
- Sterilization
- Lab-to-process scalability

Raman probes that offer flexible sampling capabilities and high-quality window and contact materials are also extremely beneficial. As previously stated, it is advantageous if the same bioprocess probe technology can be used for many different bioprocess sampling points. This facilitates easy method transfer, maximizes

productivity, and speeds cycle times from the lab to the production environment.[6]

In addition, one of the more overlooked aspects of Raman spectroscopy is the importance of seamless analyzer and probe technology. It is highly recommended to invest in a partner that provides this unified Raman product portfolio design feature, rather than relying on a "mix and match" cross-vendor approach. Raman analyzers and probes that are specifically engineered to pair together offer maximum data accuracy, precision, scalability, and security of supply.

Advances in high throughput process development through single-use manufacturing

Newly created partnerships in the high throughput miniaturized bioprocess segment have made it possible for Raman spectroscopy analysis to be integrated into micro and mini bioreactor systems as well as single-use production bioreactors.[2] The resulting lab-to-process solutions offer the market an ideal interface to high throughput development through single-use commercial manufacturing. With these types of integrated systems, one run on an automated bioreactor platform can generate enough data to build highly robust models. This enables streamlined predictive Raman model building and real-time monitoring and control of many cell culture properties.[7] Many companies have moved to smaller, automated bioreactors for rapid process development since they are more efficient to run in parallel and to execute specific experimental conditions compared to larger, less integrated bioreactors.

ⓘ Value of recent Raman technology partnerships

- Enables faster, more price efficient, easier, and more robust model building
- Introduces Raman spectroscopy solutions to high throughput process development which supports companies' QbD efforts
- Provides a scalable approach and a more efficient transfer for single-use manufacturing
- Supports method transferability across bioreactor scales
- Delivers non-contact Raman collection so no cleaning, sterilization, or frequent probe maintenance is required

Raman as a first choice PAT

As Raman spectroscopy moved from early adoption to market acceptance, it has become a first choice PAT in the biopharmaceutical industry. For companies looking to better understand, control, and automate their bioprocesses, the value equation of Raman is very compelling from a risk management standpoint. Raman succeeds in achieving some high PAT objectives — minimizing process variability, waste, and development time, while maximizing data, productivity, process robustness, and product quality. As such, Raman spectroscopy is now a leading source of in-process data for the realization of PAT in the biopharma industry.[3]

The future of Raman in biopharmaceutical manufacturing

The future of Raman spectroscopy in biopharmaceutical manufacturing is very bright. As the industry moves to new frontiers in automation, single use, continuous manufacturing, and other applications areas, it becomes even more critical for biopharmaceutical manufacturers to select a Raman provider with a comprehensive Raman portfolio. Some Raman technology providers are better positioned than others to embrace key industry trends from development to manufacturing, traditional to single-use, and batch-to-continuous bioprocessing.

Single-use manufacturing

Over the next few years, utilization of Raman technology in single-use manufacturing is expected to continue to gain momentum. Advanced Raman analysis enables QbD and PAT methods that are scalable to all sizes of single-use bioreactors (SUBs). Raman probes compatible with several SUB vendors are available today. They are delivered to the end user pre-sterilized, ready-to-use, and fully qualified. Single-use Raman probe connections are preferable to reusable Raman probes for SUBs, since reusable probes must be sterilized by end-users before insertion and therefore pose a much higher risk for contamination.[2] As demonstrated in Figure 3 below, integrated multi-vendor Raman probes are commercially available to enable cross-scale and cross-platform Raman analysis.

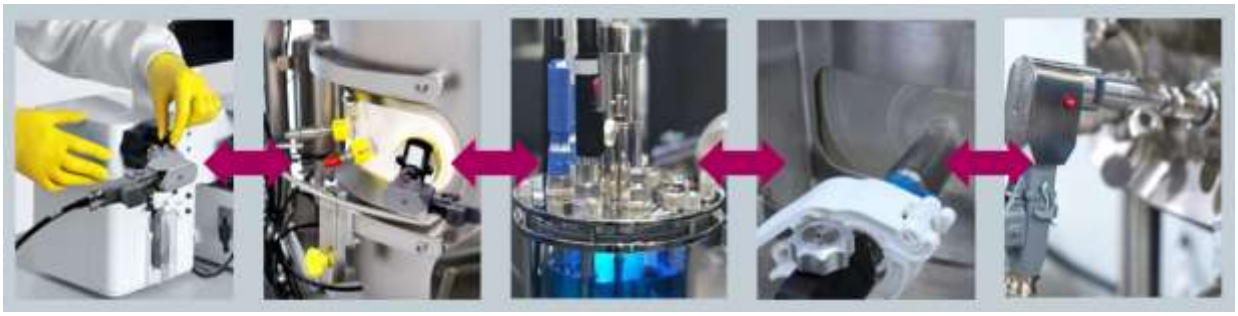

Figure 3: Example of cross-scale Raman analysis using analyzers and bioprocessing probes designed to facilitate scalability from micro and mini bioreactor systems through to single-use and traditional bioreactors of all sizes

Single-use Raman probes benefit biopharmaceutical manufacturers by offering greater ease-of-use, lower cleaning and maintenance burdens, and minimized risk without sacrificing performance. Raman spectroscopy is recognized as a scalable analytical approach with more efficient method transfer capability than alternative PAT tools and is one of the only multi-attribute single-use sensors available. In the future, additional partnerships will likely form to extend product compatibility as the single-use market continues to help bioproduction become more accessible and flexible around the world.[5, 6]

Continuous manufacturing

As some companies explore moving away from batch manufacturing, continuous manufacturing is fast becoming the name of the game. In the past, one of the challenges of continuous manufacturing has been the lack of inline process measurements to support data-driven control

strategies for continuous operations. In a recent paper from BioPhorum, the authors acknowledge that for continuous bioprocessing to be successful, a greater level of process monitoring is required compared to batch processes.[8] Reliable inline measurements provide the real-time process insight needed for biopharma companies to develop feedback and feedforward control strategies for continuous bioprocessing and move quickly from the development phase to clinical and commercial manufacturing. As such, Raman spectroscopy will continue to play a significant role in delivering the accurate, precise inline process monitoring and control capability required to make continuous manufacturing a reality in the years to come.[6, 8]

Cell and gene therapy

The trend of delivering more personalized medicine is also expected to gain speed in the years ahead as Raman's success in mAbs extends into cell and gene therapy development. Raman offers the opportunity to monitor donor specific cell behavior, nutrient consumption, and metabolite production in real-time for immediate process feedback. In cell and gene therapy development, minimizing time between research and development and patient treatment is critical. In such cases, inline measurement and process monitoring allow for faster knowledge buildup, more consistent product quality, and the ability to get production right the first time.[9] Raman technology will therefore continue to be a go-to bioprocess measurement technology in the field of cell and gene therapy. With such high stakes, biopharma companies in this niche are advised to invest in proven and reliable Raman systems with high quality data and instrumentation.

An upswing in downstream

The established history of Raman spectroscopy in upstream bioprocesses for molecular identification, quantification, and process monitoring lays the groundwork for the expansion of Raman spectroscopy's use in downstream applications. While downstream has been slower to embrace Raman than in the upstream application segment of biopharma, the benefits of Raman in downstream applications are just as compelling.[2] Several reported studies have recently emerged demonstrating the benefit of using Raman to measure downstream process attributes such as protein concentration, structure, crystallization, and aggregation, as well as buffer excipients and many others.[6] Measurement points reported include chromatography, purification, ultra/diafiltration, bispecific assembly reactions, and lyophilization. In general, the downstream segment of the market is ripe to become another high potential growth area for Raman spectroscopy.

Technology transfer

Technology transfer and associated lab-to-process scalability are significant challenges in biotherapeutic production because the manufacturing process not only needs to scale, so too does the analytical and quality measurement approach. To add to this challenge, each stage of a product's lifecycle—from research and development through clinical and commercial manufacturing—often takes place at different locations or organizations. As such, Raman-powered lab-to-process scalability will be a huge competitive differentiator in the years ahead as biopharma manufacturers look for ways to gain efficiencies, ensure consistent product

quality, and speed time to market. Raman spectroscopy is a critical ingredient in delivering these benefits because it can be implemented at every stage of a biopharmaceutical product's lifecycle (as shown in Figure 4 below) and used as a process quality fingerprint for technology transfer.

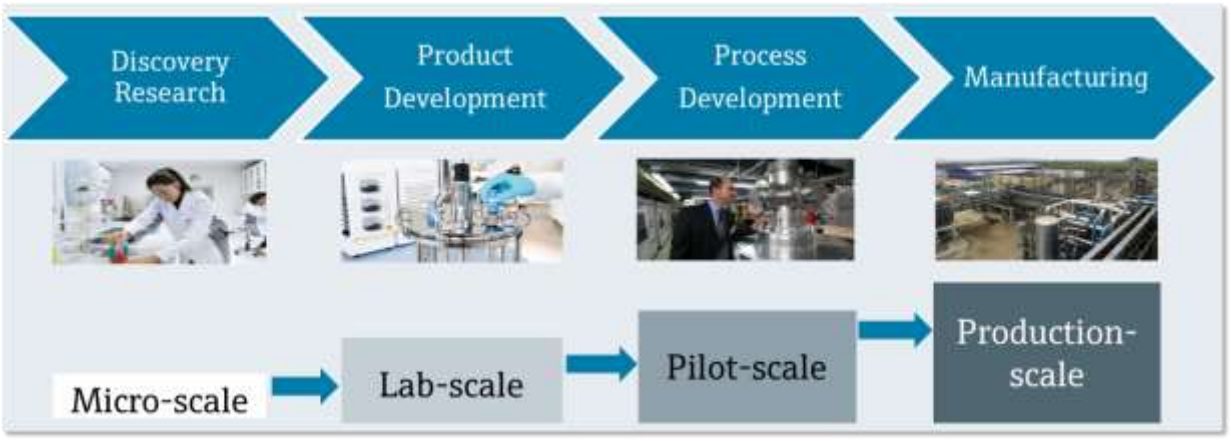

Figure 4: Raman's suitability for use across the biopharmaceutical product lifecycle

Finding the right Raman partner

Not every Raman instrumentation vendor has the experience, expertise, or comprehensive global service structure needed to support bioprocessing applications for the long haul. The right Raman partner can help biopharmaceutical companies deliver the most benefit from their Raman system now and in the future for maximum return on investment. With that in mind, several important factors should be considered when evaluating a Raman technology partner.

Well-established Raman expertise
It may go without saying that the optimal Raman technology partner has been an industry thought leader for decades with a proven track record of biopharma customer success. Raman providers that have led the way in Raman product innovation have undoubtedly recycled direct customer feedback and process requirements into their next gen Raman product portfolios. A good way to gauge a company's level of Raman expertise in biopharma is to research whether a Raman technology company and its products are documented in a range of customer publications and have received any awards in the biopharmaceutical industry.

cGMP and regulatory compliance experience
When assessing Raman providers, it is also crucial to find a Raman partner that offers a long history of data integrity and support of customer production environments, including cGMP manufacturing, with many proven successes. Look for those who have specific experience in cGMP, have led customers through the bioprocess PAT journey, and offer regulatory compliance, method transfer, maximum up time, and qualification expertise. Important criteria to ease cGMP regulatory compliance include ISO 9001:2015 certification, cGMP trained service

personnel, traceable calibrations performed onsite, automated calibration checks, and demonstrated experience hosting many successful audits by leading biopharmaceutical companies and vendors.

Industry 4.0 product strategy

As Industry 4.0 digitalization concepts continue to gain traction in the biopharmaceutical industry, it is advantageous to find a forward-thinking Raman technology partner with products that already embody IIoT strategy such as increased data security, system integration, and automated communication. One example of an Industry 4.0 concept is a Raman system featuring analyzer and control software in a fixed purpose device with built-in intelligence. For maximum return-on-investment, Raman technology should be able to communicate with external systems over network using standard automation protocols, thereby providing companies with reliable chemical composition measurements with secure 24/7 connectivity.

Proven lab-to-process scalability

As the biopharmaceutical industry continues to evolve, the Raman systems most likely to leap ahead of the competition will be those that are easily transferable across all scales from development to cGMP, small to large scale, and reusable to single-use. Raman products should speed analytical development and allow smooth transfer to process environments. When backed by demonstrated lab-to-process customer successes, a Raman partner with a scalable automation infrastructure that enables cross-scale model transfer is a PAT champion.

ⓘ Raman system features to look for to enable lab-to-process scalability

- Simplified equipment complexity
- A common software user-interface
- Identical materials and sampling areas across all bioprocess probes
- Easy method transferability
- Self-alignment and calibration
- Flexible analyzer installation options

Global service & support

As evidenced by many of the topics discussed in this paper, it is wise to look beyond “out of the box” Raman instrumentation when evaluating Raman technology partners. Finding a stable partner with an established track record of reliable global support is another critical factor to consider during an evaluation of any potential Raman partner. For example, be sure to ask Raman providers whether they offer onsite service and perform onsite preventative maintenance without requiring you to send back and then re-qualify the analyzer just for routine support.

The return on investment from a Raman system may hinge on finding a Raman technology partner that is experienced, responsive, and adaptive, with a demonstrated pattern of working with companies to solve their current and future application measurement challenges. In today’s global biopharmaceutical industry, the right Raman partner must have industrial process expertise and worldwide availability, along with an extensive local support network with service teams specifically trained in cGMP.

Summary

Biopharmaceutical companies are increasingly seeking new ways to understand, measure, adapt, and control their complex bioprocesses with accurate precision from the lab to the manufacturing floor. Full process transparency and system transferability are becoming prized as essential components for operational excellence and product quality. Today, advanced Raman spectroscopy is widely viewed as a highly reliable measurement technology to achieve these goals because of its inline, real-time measurement capability and its scalable hardware and software.

Demand for Raman spectroscopy as a key bioprocess measurement tool is expanding across multi-use to single-use platforms, and batch to continuous processing. Raman's value in the biopharma industry has also grown with the adoption of Industry 4.0 principles in PAT and QbD, increased automation, and greater integration of Raman into a wider range of process control applications. New partnerships and innovations in Raman are empowering biopharmaceutical companies to more easily scale-up and scale-out from product development to cGMP while complying with strict product quality standards. Still, the full potential of Raman spectroscopy throughout the biopharmaceutical product lifecycle has yet to be fully realized, so it will be exciting to see the new Raman frontiers that lie ahead.

Acknowledgement

We would like to thank Sean Gilliam, Ph.D., and Justin Moretto, Sr. Applications Scientists at Endress+Hauser, and the many other colleagues, collaborators, and customers who contributed to this work.

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WP01172C/66/EN/01.22