Solving the Mystery of Converting Data into Value

Real-world success and applications of Raman spectroscopy in bioprocessing

Justin Moretto
Life Science Product Manager
Endress+Hauser

INTRODUCTION

As optimized processes yield the greatest returns, any resources that facilitate improvements in manufacturing or development are decidedly beneficial. Process knowledge stems from the combination of technology and expertise, and ultimately leads to optimization. A multitude of peer-reviewed literature demonstrate the contributions of Raman spectroscopy to process understanding and enhancement, yet they often fail to include information on how the data have impacted their business as a whole. Recent case studies elucidate the ways in which Endress+Hauser's Raman systems have contributed to numerous industrial success stories in bioprocesses. Because of its value-adding nature and widespread applicability, the technology can benefit a variety of processes at any scale—from research, to development, to manufacturing.

RAMAN SPECTROSCOPY FUNDAMENTALS

In Raman spectroscopy, laser light of a specific wavelength interacts with molecules, producing elastic (Rayleigh) and inelastic (Raman) scattering in all directions. One out of every 10^8 photons experiences inelastic scattering, causing it to become frequency-shifted due to specific energy transfer. The back-scattered Raman photons are collected and separated based on small changes in energy levels and then counted on a charge coupled device (CCD) detector. The collected light is interpreted as a spectrum, which can be thought of as a "molecular fingerprint" of the material.

The minute changes in wavelength correspond to the vibrational modes of the material's chemical bonds that have very characteristic changes in energy, known as their Raman shift. For example, when a C-C single bond relaxes, the change in energy is different than the change observed with a C=C or C-O bond. The Raman shift is very sensitive to both the bond type and orientation of the bond, such that specific photons allow the detection of small chemical differences. The sensitivity provides enough resolution for both identification and quantification of a material's

components, even in complex solutions. A single probe can measure a wide range of analytes simultaneously.

Perhaps most importantly for process analytical technology (PAT) applications, monitoring the process with Raman at regular intervals enables batch evolution. This provides valuable insight into processes, such as cells in reactors. For example, Raman can discern the cells' metabolic response and growth after a feeding. Rather than setting everything up and testing periodically, real-time metabolite measurements provide a wealth of information to allow rapid process adjustments for improved outcomes. The monitoring and control are absolutely necessary for next-generation process development and manufacturing. **FIGURE 1** outlines various pharmaceutical and biopharmaceutical applications that are highly suitable to Raman-based measurement in the life science industry.

BENEFITS OF RAMAN FOR MANUFACTURING AND DEVELOPMENT

It can be difficult to define metrics for the success of Raman implementation, as different organizations have different perspectives. Obviously, the largest returns on investment (ROIs) will be realized in manufacturing because that is where the income is actually generated. Direct returns for a development organization are less concrete since the PAT value is effectively handed off to manufacturing. However, there are direct returns that can be realized nonetheless, such as dramatic reductions in analyzer consumables (as Raman has no consumables), increases in total data while reducing the human capital requirements for its collection, and a reduced risk of contamination associated with sampling. Still, the greatest value is what gets accomplished based on all of the data that is generated by the system. Real-time Raman measurements offer capabilities that are otherwise not available, including process characterization and feedback control, which are extremely valuable.

To quantify the value in development, it is helpful to ask questions such as: "How much does a single bench scale bioreactor run cost, all in?" "How many bench scale runs does it take to optimize a process?" What if the number of development batches needed to get to an optimized state could be reduced by 10% for both scales? What about pilot scale runs?" By addressing these questions using

FIGURE 1: Some of the applications suitable for Raman measurement in the life science industry.

- Pharmaceutical:
  - Reaction chemistry
  - Yield crystallization
  - Polymorph
  - Blending
  - Drying and other drug product unit operations
  - Granulations, tablets, and capsules
- Biopharmaceutical:
  - Bioprocess monitoring and control
  - PAT/QbD applications
  - Cell culture
  - Fermentation
  - Downstream operations
  - cGLP/cGMP

numbers specific to the organization, the savings can become substantial very quickly. The wealth of information from Raman accelerates achievement of the development goals and delivers significant savings. In a development organization, that can go quite a long way toward convincing people of its value. Even if automation is out of reach, considerable value can be realized through optimization alone. Simply increasing productivity to eliminate one batch can save a great deal of money.

BioPhorum (BPOG), a consortium of well-respected industry professionals, published a white paper in 2020 that included a cost benefit analysis for Raman implementation.[1] While it pointed out the expenses of licensing updates and filing costs, equipment-related expenditures, as well as inventory, training, SOPs and other transition costs, the benefits highlighted were numerous. Reduced testing and sampling, higher yields, faster process responses, fewer manual investigations, throughput increases, decreased discards, reduced equipment maintenance and calibration, and a reduction in inventory carrying costs made real-time monitoring with Raman very appealing.

Implementing the changes during development is much easier than in manufacturing. The benefits can be substantial, from cost savings in development to significant productivity increases and shorter time to market with real time release (RTR). A table presented in the BPOG white paper further enumerated the potential benefits of Raman, including "Better Yield" estimates that are likely dramatic understatements. Using one example of published data from a Raman system, an increase in productivity above 80% was achieved on a $2 billion-per-year product. That kind of productivity increase would reduce production costs by 40%, which puts the value of Better Yield closer to $60 million instead of $150,000 in savings per year for the life of the product. There are cascade benefits from this increased productivity as well, but they are more difficult to quantify outside of an organization. These could include the value of reduced duty cycle on the equipment, large savings on avoidance of facility expansion costs, or the ability to form strategic partnerships utilizing newly found excess capacity. The numbers may seem high, yet it should be noted that these are high value, high-cost projects so that little changes can yield big savings. Based on the white paper, there appears to be an agreed-upon financial narrative that demonstrates that value can be derived from this technology, but only if it can meet the demands that are outlined in the BPOG document.[1] Organizations considering Raman implementation are encouraged to read it in its entirety.

CASE STUDY: CELL CULTURE

True demonstrations of Raman's power can be found in peer-reviewed data. Biogen recently collaborated with Endress+Hauser in order to improve their mammalian cell culture process.[2] Matthews, T. et al. presented a study of a legacy process that suffered from a non-optimized feed strategy. There were serious issues with lactate and glucose accumulation late in the run, which often required early termination of batches and produced unpredictable Critical Quality Attributes (CQA). Viability for the batch was taken on the penultimate day; if it was below a certain threshold, the batch would be terminated. If it had not yet fallen, it would be run for an additional day. The process was not well-controlled, and it was believed that overfeeding of the glucose was the primary problem. Endress+Hauser's Raman technology-based control strategies included closed loop control of lactate via automated glucose feeding. Using pilot reactors, the cells were starved of glucose with the hopes that they would preferentially consume lactate. The plan was not without risk, because if the cells completely lost their carbon source before they adapted to their new menu item, they would enter a latent phase. In this event, productivity would collapse, and the batch would likely crash.

The experiment began with a very small existing data set, and a control loop was developed to keep glucose as close to zero as possible. The goal was to feed on demand and minimize waste in order to increase duration, viability,

productivity, and robustness. The error for the online glucose model (teal trace) was 0.25 g/L. The lower control band was set at 0.5 g/L, which was treated as zero. The cells were only fed if the online Raman measurement for glucose was below that value and the lactate, shown in red, was below 4 g/L. Once the lactate was above 4 g/L, it no longer received any glucose, even if the glucose was below the lower control band. By day eight, the lactate was on the lower end of the normal range. By day nine, it was fully separated from the historical range.

As a result of the successful online control strategy, the reduction in glucose and lactate accumulation led to significant process improvements. The harvest titer increased 85% over the historical process, while CQAs became predictable and consistent. Cellular health stabilized at high levels for the duration. In this case, the small process changes also led to a 35% reduction in the duty cycle, ultimately providing even more cost savings. Note that the batch was terminated on day 14 not because anything was wrong, but because the bioreactors were needed for other projects. Thus, it is unknown how long the high levels of viability and VCD could have been maintained.[2]

A subsequent study found that total glycation could be reduced by 50%.[3] In this case, tight glucose control allowed for significant manipulation of glycan profiles. In addition to reducing the glycation, it could have also been tuned to meet a specific target. The ability to tune the glycation to specific levels is quite valuable for hitting a target, matching an existing product for a biosimilar, or protecting against future biosimilars by making it more difficult to copy. Thus, the advanced control provided by the tunability is a real advantage.

In addition to process control, the ability to build models in development, where data points are less expensive, and then use that data to predict in manufacturing, is highly valued. In 2014, cross-scale predictive modeling was studied on CHO cell culture using Raman spectroscopy.[4] Data were compared across three scales: benchtop, pilot, and manufacturing. It was found that many parameters could be accurately predicted at any scale based solely on benchtop modelling. VCD and total cell density (TCD) were the only two variables that truly needed scale-appropriate data to predict accurately. Using the large development datasets and including limited manufacturing scale data yielded very positive results. The data was not only transferable to different scales but was transferable between different instruments. All of the data from the paper were collected with multiple instruments at different scales and different locations around the world, which demonstrated the excellent level of reproducibility of Endress+Hauser's instruments.

CASE STUDY: DOWNSTREAM PURIFICATION

Downstream applications for Raman spectroscopy have been growing in recent years. A presentation by Regeneron at the BioProcess International Conference in 2019 demonstrated its utility.[5] The primary goal was to measure protein concentrations and automate the concentration targets in order to reduce volumes. They were also able to condition the mAb, adjust pH, monitor excipients, and more. It took four runs to build models that achieved their predetermined 95% confidence interval with 90% of the data. After 15 runs, 100% of the data was falling within that confidence limit. In general, the highest error for the Gibbs–Donnan effect, at 3.1%, was found at the low end of the concentration range. The error decreased linearly across that range to 1.8% at the high concentrations. Specific real-time modeling errors were 1.9% for the primary concentration, 2.2% for diafiltration (DF), and 0.7% for the final concentration. These are very good values that were collected extremely fast. Specific amino acids were also monitored, and the errors were comparable to orthogonal methods. While monitoring high molecular weight species

during polishing, it was discovered that scan length influenced the error. The quality of predictions increased with shorter scans because it was in a flow cell, therefore a shorter snapshot yielded a better result, as less variation was observed in the rapid timeframe.

Scientists at Abbott have used Raman to test and release formulation buffers as well as monitor protein and excipient concentrations. As Ram, N. et al. presented at the BioProcessing International Conference in 2010, Raman can clearly differentiate between the sugars, the amino acids, the buffers, and surfactants used for the formulation, and therefore achieve excellent real-time predictions.[6] As such, this technology can be used to ensure that the right constituent is being introduced into the correct tank, that the mixture is happening properly, and that everything is at the proper concentration before it is used. It was reliably demonstrated with four discrete systems. Chemical contamination could be detected as well. Overall, it proved the advantages of rapid implementation for robust buffer testing and release.

Bristol-Myers Squibb used an Endress+Hauser Raman system to monitor soluble protein aggregation.[7] This was an early and interesting example of how greater process understanding leads to better processes. The experiment enabled the development of a calibration model for thermal aggregation of the target protein. While monitoring the aggregation, they discovered a strong correlation between higher temperatures and a decrease in Tryptophan C=C and C=N vibration intensities, a decrease in Tyrosine doublet intensities, and a significant reduction in the peptide backbone intensities. These phenomena would never have been observed without real-time data. They found an excellent correlation between measured and predicted aggregation. All of this information allowed them to modify their process, lower the range, and ameliorate some of the issues of thermal aggregation caused by the reduced instability of the bonds. The experiments demonstrated the strength of Raman spectroscopy as an in situ, non-destructive technique for protein stability studies, especially aggregation, due to its speedy delivery of valuable real-time information.[7]

For antibody bioprocess applications, a typical Raman spectrum of protein/mAb provides valuable insight into tertiary and secondary structure, hydrogen bonding networks, side chain interactions of the protein molecule, and intermolecular interactions. In 2017, Genentech presented results of a study for which the assembly of a bispecific antibody was monitored in real time.[8] Not only did in-line Raman spectroscopy allow them to monitor product generation, but it also furthered their understanding of reaction kinetics. Based on the process monitoring data, assembly conditions were modified to maximize quality and minimize waste.

ENDRESS+HAUSER RAMAN HARDWARE AND SOFTWARE

Endress+Hauser offers Raman analyzers for both laboratory and process environments. For more permanent production/cGMP installations, the Raman Rxn4 embedded analyzer is ideal for in situ real-time measurement and control. Conveniently, the human-machine interface (HMI) can be at the point of use or networked and operated remotely. Options for a rackmount or a NEMA 4X enclosure are available to suit the user's installation needs. The Raman Rxn2 analyzer is generally used in lab and pilot scale settings to support research, early process development, and scale-up. It can be cart-mounted or placed on a benchtop for location flexibility. Both of the analyzers easily communicate with distributed control systems (DCS), most commonly via open platform communications (OPC) unified architecture (UA) and data access (DA) standards.

In addition, Endress+Hauser's Raman analyzer systems now integrate with Sartorius's BioPAT® Spectro platform, which

FIGURE 2: Endress+Hauser's Raman Rxn-46 bioprocess probe integrated with the Ambr® system from Sartorius.

enables them to provide Raman solutions for the Ambr® and Biostat STR® bioreactor product lines. The enormous throughput of the Ambr® systems enables very rapid process optimization (**FIGURE 2**).

One of the most popular features of these Raman instruments is the calibration routine. Both the laser and wavelength calibrations are done internally, routinely, and automatically, without requiring any input or manipulation from the user. The only calibration step that requires user intervention is the intensity calibration, which consists of presenting a white light source to the optic in order to normalize parameters such as fiber length. A simple verification is performed to ensure that there is sufficient signal, and that the system is operating as expected.

Another critical feature of the Raman systems is their ability to maintain the sterile envelope. The probes meet all expectations for stainless steel (SS) reactors, and the robust proprietary window material avoids the 20% data loss from interference that is suffered with commonly used sapphire windows. Endress+Hauser Raman bioprocess probes have been designed to ensure compliance with industry norms for sterility and best practices in GMP manufacturing. Optimized for bioreactors and their standard interfaces, they are suitable for glass, disposable plastic, and 316 SS application platforms. All of the probes are optimized for turbid environments/high cell densities and there is no interference from bubbles or fouling. The Raman Rxn-10 probe plus bIO-Optic is designed primarily for benchtop reactors and autoclave sterilization, while the Rxn-45 probe was designed for stainless steel reactors and CIP/SIP. The Raman Rxn-10 probe offers flexibility for lab-scale process development, while the Rxn-45 probe offers a right-angle, robust, consistent connection to fit pilot and production scale reactor ports.

Single-use systems have become a critical tool for development as well as manufacturing. The biggest challenge measuring anything in real time is acquiring the information without breaking sterility. As seen in **FIGURE 3**, the Raman optic system for single use addresses this issue as it features a reusable optic and a disposable fitting for single-use bioreactors (SUB).

Essentially a ready-to-use gamma-sterilized window, it allows operators to get Raman data from a SUB without compromising the sterility, and with the same optical performance as standard Rama probes. Developed according to industry standards for single-use sensors, its materials of construction have been tested at an independent agency and Endress+Hauser's single use Raman offering has been tested and implemented at multiple biopharmaceutical companies.

FIGURE 3: Endress+Hauser's Raman optic system for single use.

GMP qualification is complete with one SUB vendor, and integration with additional vendors is underway.

CONCLUSION

Raman spectroscopy is uniquely suited for bioprocess PAT, with many benefits being realized by biopharmaceutical manufacturers. Endress+Hauser's Raman technology has proven success, from development to manufacturing, from traditional to single-use platforms, and from batch to continuous processes. Improved yields, predictable processes, consistent CQAs, faster duty cycles, fewer rejects, higher quality products, greater viability and VCD, tunable glycation, and cross-scale modeling are just a few of the ways in which real-time Raman measurements have added value to organizations.

Endress+Hauser's extremely stable, auto-calibrated suite of instruments work online without breaking the sterile envelope. In addition, their probes have been optimized for bioreactors and feature a proprietary window free from sapphire interference. New developments support the life science entity lifecycle and include both an integration to the BioPAT® Spectro platform by Sartorius and an automation interface to Emerson's DeltaV™, called Spectral PAT. Now one of the key drivers moving the industry toward realizing the benefits of PAT, Endress+Hauser Raman systems enable an in-line analytical platform technology to make quality biopharma products consistently and efficiently.

REFERENCES

1. In-line monitoring/Real-time release testing in biopharmaceutical processes – prioritization and cost benefit analysis (12 May 2020)
2. Matthews, T. et al. (2016) "Closed loop control of lactate concentration in mammalian cell culture by Raman spectroscopy leads to improved cell density, viability and biopharmaceutical protein production"
3. Berry, B. N., et al. (2016), Quick generation of Raman spectroscopy based in process glucose control to influence biopharmaceutical protein product quality during mammalian cell culture. Biotechnol Progress, 32: 224-234. doi:10.1002/btpr.2205
4. Berry, B., et al. (2014). Cross-scale predictive modeling of CHO cell culture growth and metabolites using Raman spectroscopy and multivariate analysis. Biotechnology Progress, 31(2), 566–577. doi:10.1002/btpr.2035
5. Passno, C. "Using Raman Spectroscopy for Monitor and Control of Critical Quality Attributes in Ultrafiltration/Diafiltration for Biotherapeutics," BPI East, Sept. 2019.
6. Ram, N. et al. (Sept 2010) Raman Spectroscopy for Purification Bioprocess Operations. BioProcessing International Conference
7. Mungikar, A. et al, "Non-destructive and Rapid Analytical Technique to Monitor Aggregation of a Therapeutic Protein ," in Amer. Pharm. Rev. Nov/Dec. 2010.
8. Maier, A. (April 2017) In-line Raman spectroscopy for bispecific assembly process monitoring. 253rd American Chemical Society National Meeting & Exposition