

Raman technology in bioprocessing

Scalable, *in situ* bioprocess
monitoring and advanced
process control





A partnership for success

Committed to helping you achieve operational excellence



Deliver consistent product quality from the beginning

The Endress+Hauser Raman bioprocess product portfolio, powered by Kaiser Raman technology, provides *in situ*, real-time analysis of nutrients, metabolites, product quality, and cell viability with a single probe. Our equipment helps you deliver higher yield and improved product quality with reduced contamination risk and greater efficiency.

Getting it right the first time

We understand continuous improvement is key in a dynamic business and regulatory environment. The trusted scalability and performance of Endress+Hauser analyzer systems simplify your process equipment complexity and ease method transferability. Our training, advanced analytics, support, and data modeling services allow you to focus on your core business while benefiting from our experience to ensure a rapid return on investment.

Complexity, simplified

We put our experience in instrument manufacturing, process analytics, data analysis, and life sciences into the design and manufacturing of our bioprocessing product line. You can easily integrate Raman technology for bioprocess advancement with full confidence in our robust and reliable analytical solutions.

Successes from process innovation to process automation

Endress+Hauser has an established installation base of more than 1500 units throughout the world in GMP manufacturing environments. Our continued growth speaks to how much biopharmaceutical industry leaders trust their products to Endress+Hauser.



Ensure process and product quality

Benefit from our leading bioprocess analytical solutions and comprehensive compliance offerings to get your lab-to-process analysis right the first time



Endress+Hauser Raman analyzer systems provide *in situ*, real-time sensing of multiple parameters for cell culture, fermentation, or downstream bioprocesses. Our offerings for *in situ* bioprocess analytics enable quality by design (QbD) and advanced bioprocess control. Our technology scales with you so that you can use Raman for traditional or single-use bioreactors, in batch or continuous mode, from lab to cGMP.

We capitalize on our experience in life sciences and closely work with industry leaders to provide a wide selection of bioprocessing analytical solutions. Ask our team of Raman experts about:

- Method development services
- Advanced training
- Service plans to help you reduce downtime, avoid instrument requalification and protect your process investment



Compliance with globally accepted standards & certifications

Endress+Hauser's compliance services help you to improve your process and ensure quality while maintaining cGMP. We carry a ISO9001:2015 certification, ensuring quality to our customers. Endress+Hauser has over 10 years of experience building ATEX, North American, and IECEx certified Raman spectroscopic analyzers for output into hazardous area installations. We also provide NEMA 4X enclosures for wash-down plant environments. Endress+Hauser is a Drug Master File holder with the U.S. Food and Drug Administration pertaining to reusable and single-use bioprocess Raman probes.

We provide a comprehensive portfolio of compliance services for cGMP applications including:

- Questionnaire and audit support
- Standard and customized IQ/OQ
- Factory acceptance testing
- In-house audits
- Supplier qualification
- cGMP trained service personnel

Trusted Raman life science expertise

30+ years in process Raman We understand the value of being a trusted partner. Endress+Hauser Raman analyzer systems, powered by Kaiser Raman technology, have an established history in the life sciences from our first feasibility studies in the 1990s to our first GMP installation in 2000. We worked with industry early to help solve their challenges and we continue to do so through our analysis products, compliance offerings, services, and support.

Our experience in life sciences From research to manufacturing, we have been a leading bioprocess monitoring and control solution provider for over 10 years. Our customers have shown that Raman can provide:

- Improved product quality
- Increased titer
- Higher volumetric productivity
- Closed-loop feedback control
- Cross-scale and cross-platform method transferability
- Automated feed control
- High density culture monitoring

Endress+Hauser is well known for our high-quality analyzer and bioprocess probe instrumentation, process automation knowledge, and cGMP expertise. Our strong support of the life sciences means that you can rely on us for your upstream and downstream bioprocess needs.

ASPEN AWARD



Our comprehensive approach Endress+Hauser offers an integrated Raman solution that is robust and reliable. It is comprised of high-performance analyzer equipment, user-friendly embedded software, and *in situ* sampling probes. Our Raman analyzers have the same internal hardware to provide consistent spectral response and high performance in location specific packaging. Ask about our benchtop, cart-mounted, rack-mounted, or process area enclosures.

Our optimized bioprocess probes Endress+Hauser's Raman sampling probes are designed to meet the specific application and installation needs of bioprocessing. We provide optimized probes from lab to manufacturing scale, single use or reusable, with our patented universal optical interface that deliver high quality spectra.

Our industry recognition In 2018, we received the industry-appointed Aspen Award for our contributions to Advancement of Upstream Bioprocessing. In 2020, our Rxn-46 bioprocessing probe was selected as a 2020 Pharma Innovation Award winner by Pharma Manufacturing Magazine.



Proven Raman applications in bioprocessing*

Cell culture	Fermentation	Downstream
Glucose	Glycerol	Aggregation
Lactate	Methanol	Protein crystallization
Glutamate	Ethanol	Formula stability
Amino acids	Sorbitol	Product CQA
Cell density	Biomass	Protein concentration
Titer		Buffer excipients

* Additional product and process-related parameters are possible

"I need full application and development support"

Our response:

- We are your solution from technology to implementation
- Our modeling, training, and advanced analytics services provide an opportunity for you to benefit from our experience in life sciences and expert application support so that you can focus on your core business

Raman in laboratory and process development

Endress+Hauser has the widest selection of Raman offerings with proven lab to cGMP scalability that is trusted by industry leaders

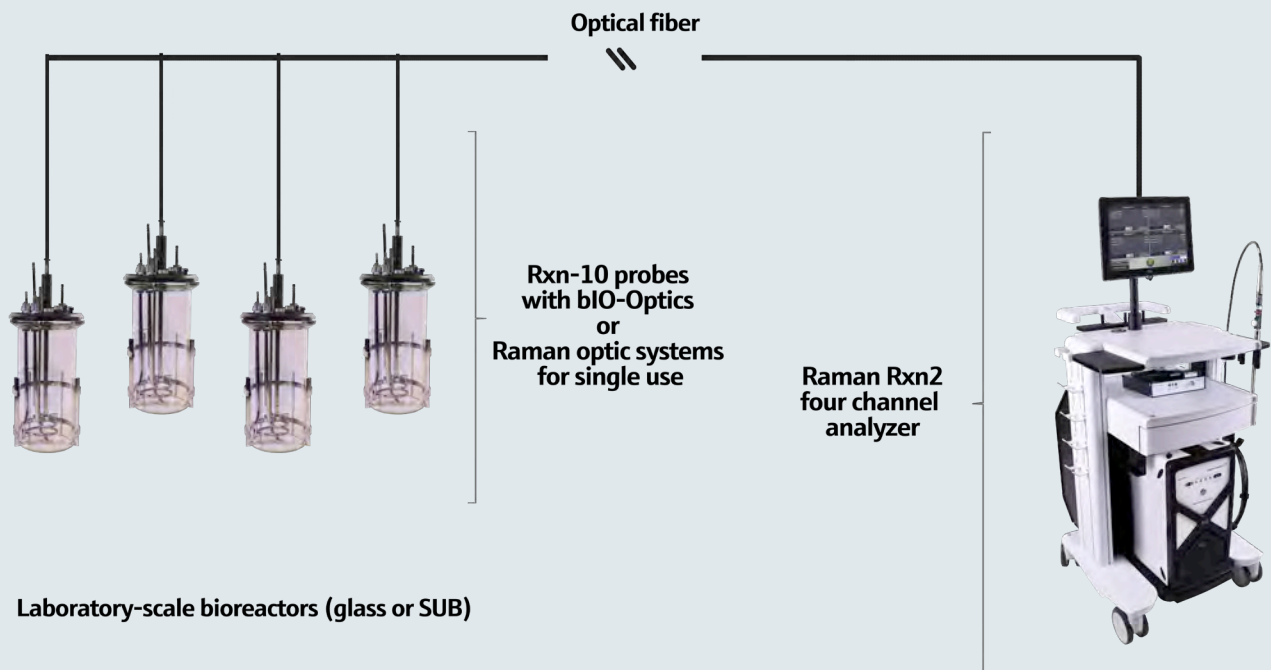
The Raman Rxn2 analyzer serves as an ideal bridge from lab to process. When combined with our phase-optimized sampling probes, the Raman Rxn2 offers high-resolution performance for *in situ*, real-time measurement and control.

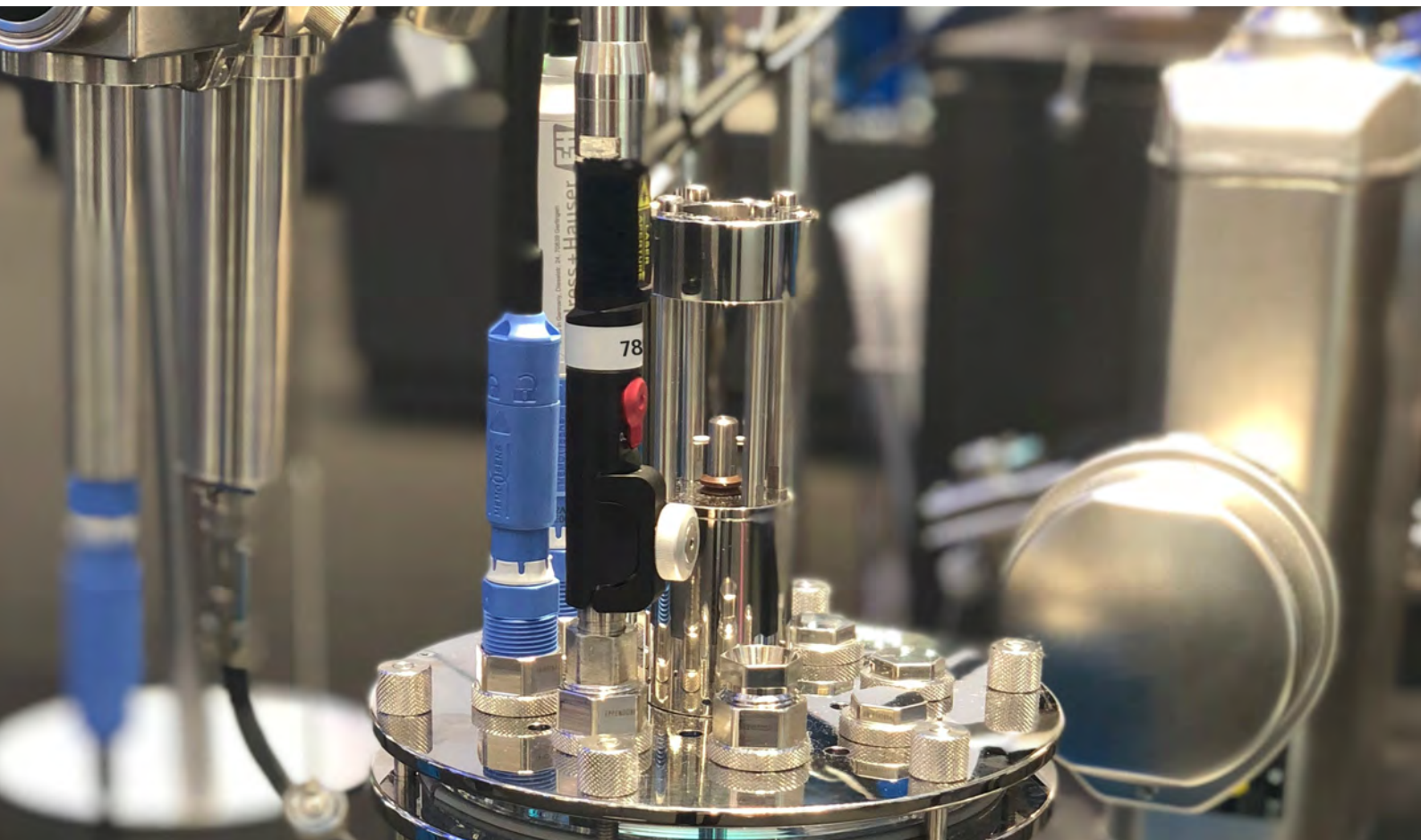
Raman Rxn2 analyzers feature fully embedded Raman RunTime software which ensures reliability and seamless connectivity while supporting the needs of Industry 4.0. Raman RunTime integrates the spectrometer functions into the analyzer electronics without needing a separate PC running proprietary software. Raman RunTime exports raw data and diagnostics using standard communication protocols to support data integrity assurance.

Available as a benchtop or cart-mounted analyzer, the Raman Rxn2 offers location flexibility for process development laboratories. With up to four probes per analyzer, measurement from four different bioreactors or sampling points is possible with a single system. The Raman Rxn2 has a unique analyzer self-monitoring system and is available in 532 nm, 785 nm, or 1000 nm wavelengths.



In-process Raman in laboratory and process development applications





Rxn-10 probe with bIO-Optic

- Compatible with bioprocess industry standard ports
- PG13.5 threaded connector
- 120, 220, 320 or 420 mm length
- Autoclavable



Rxn-10 probe with Raman optic system for single use

- Disposable fitting for single-use bioreactors (SUB), plus a non-contact reusable optic
- Developed to industry standards for single-use sensors
- Gamma sterilizable
- Tested and supplied by multiple SUB vendors



Raman RunTime

Lab-to-cGMP software for bioprocess monitoring and control

Benefits of lab and process development

- Intuitive touch screen interface
- Auto-calibration
- Compatible with multiple multivariate data analysis (MVDA) predictors
- OPC and Modbus enabled for integration with 3rd party control systems



i For information about robust model building via the integration of our Rxn-46 probe and Sartorius's Ambr[®] 15 and Ambr[®] 250 product lines, please see our "Raman bioprocessing solution compatibility with BioPAT[®] Spectro by Sartorius" brochure.

Raman in process and cGMP

Ensure your manufacturing project’s success with our comprehensive package of process analytical solutions

The Raman Rxn4 analyzer is a robust process Raman analyzer, delivering 24/7/365 performance and high precision that is essential for seamless calibration transfer.

Like the Raman Rxn2, the Raman Rxn4 analyzer features fully embedded Raman RunTime software which ensures reliability and seamless connectivity while supporting the needs of Industry 4.0. Raman RunTime integrates the spectrometer functions into the analyzer electronics without needing a separate PC running proprietary software. Raman RunTime exports raw data and diagnostics using standard communication protocols to support data integrity assurance.

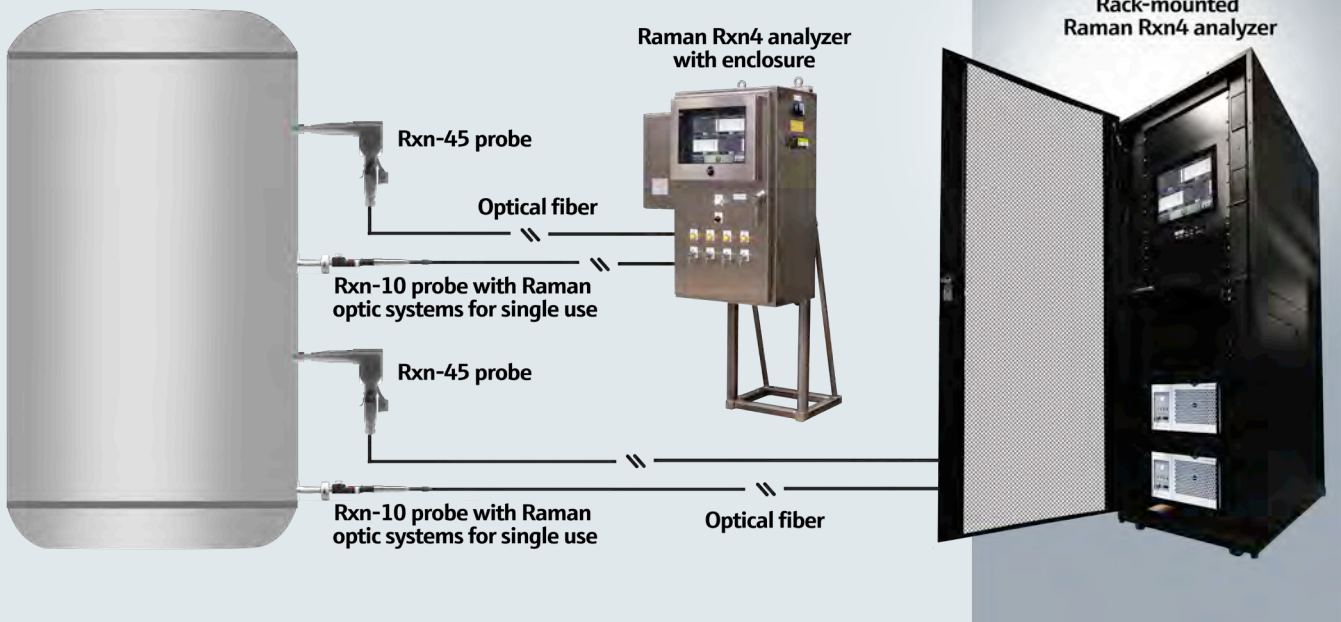
The Raman Rxn4 is available as a 532 nm, 785 nm, or 1000 nm rack-mounted analyzer for control room installations, or in a NEMA 4X enclosure for production floor installations. It has a unique analyzer self-monitoring system to ensure the validity of each measurement. The Raman Rxn4 analyzer is capable of self-calibration and utilizes self-diagnostics and self-correction methods.



In-process Raman in pilot and cGMP applications

Production floor

Control room or gray space





Rxn-10 probe with Raman optic system for single use

- Disposable fitting for single-use bioreactors (SUB), plus a non-contact reusable optic
- Developed to industry standards for single-use sensors
- Gamma sterilizable
- Tested and supplied by multiple SUB vendors
- cGMP qualified



Rxn-45 probe

- Compatible with bioprocess industry standard housings for 25mm side ports
- PG13.5 threaded connector with 120 mm probe length
- Wetted material surface finish Ra 15 with electropolish
- CIP/SIP compatible



Raman RunTime

Lab-to-cGMP software for bioprocess monitoring and control

Benefits for process and cGMP

- cGMP compliant ready
- Intuitive touch screen interface
- Auto-calibration
- Compatible with multiple MVDA predictors
- OPC and Modbus enabled for integration with 3rd party control systems
- Integrated to leading PAT management systems



i Please see the “Raman bioprocessing solution compatibility with BioPAT® Spectro by Sartorius” brochure for information about the integration of our Rxn-46 probe and Sartorius’s Biostat STR® single use manufacturing product line.

Application successes in upstream

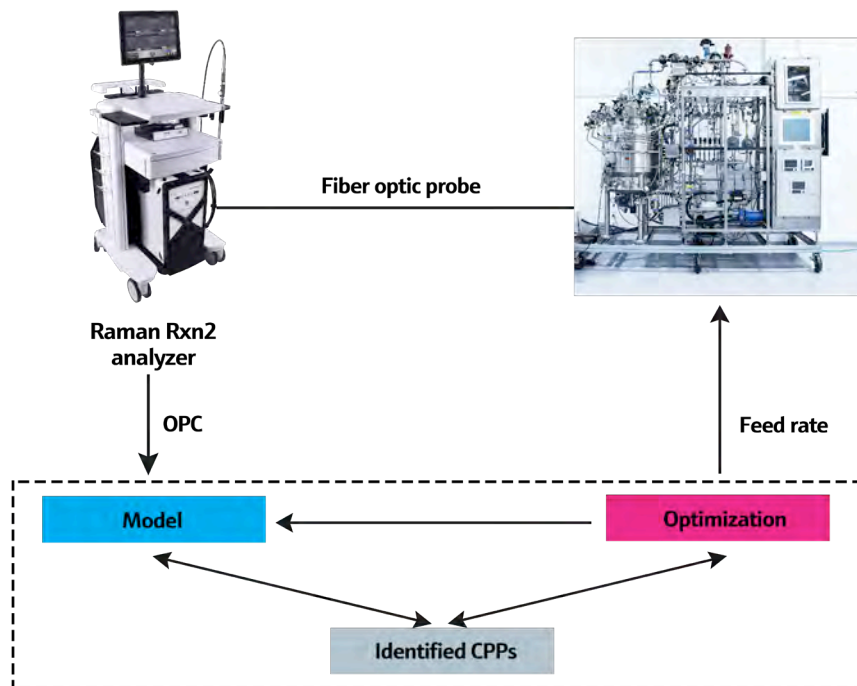
Increased titer up to 85% and improved product quality with Raman-based glucose or lactate control in a mammalian cell bioprocess

Customer successes with Raman applications

Cell culture and fermentation

- Real-time CPP and COA monitoring and control
- Increased productivity
- Improved product quality
- Cross-scale and cross-platform model transfer
- Automated feed control
- Advanced process control
- Raw material qualification
- Cell and gene therapies

Contact Endress+Hauser for technical references



Schematic of Raman-based feedback control

Production of protein therapeutics by mammalian cells is the most widely used bioprocess because of its ability to properly produce and fold a recombinant protein. 60-70% of biopharmaceuticals are produced in this way. Bioreactor parameters affect cell metabolic processes and detailed bioreactor knowledge is needed to achieve a balanced and consistent metabolic state of cultured cells.

Glucose is a critical process parameter because it affects the cell's metabolic profile, production of waste products and post-translational non-enzymatic glycation of proteins. Continuous glucose measurements, using Endress+Hauser Raman equipment, offer insights for important bioprocess understanding and enable feedback control from process development to manufacturing.

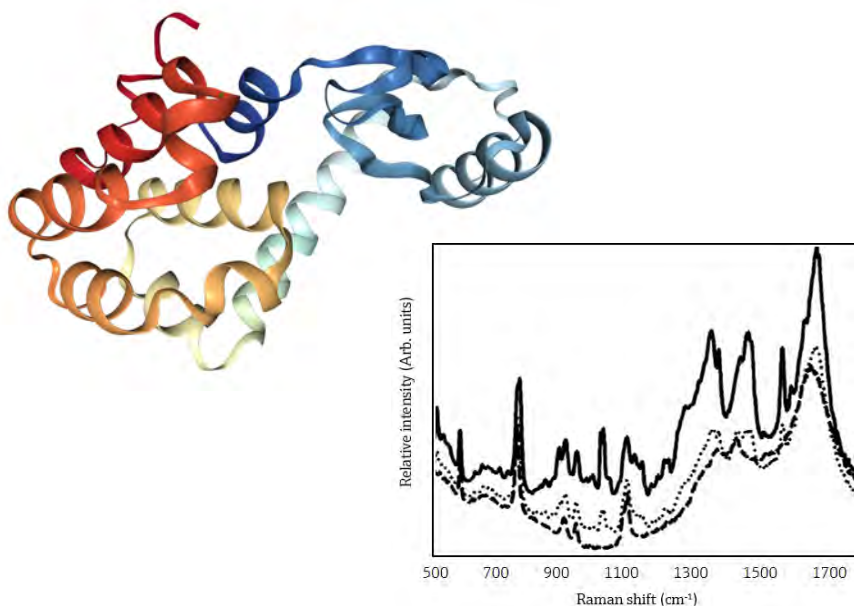
In studies performed by Berry et al¹ and Matthews et al², Raman-driven feedback control systems were rapidly implemented and demonstrated the ability to improve a biopharmaceutical protein's product quality as well as a yield increase of 85% over the historical process. Significant automated control was achieved after only two calibration steps and quickly integrated into process development work. Raman was able to support a targeted concentration condition or a stepwise condition, demonstrating Raman as a robust method to integrate into a controller of an industrially relevant bioprocess. Endress+Hauser's Raman technology is easily integrated into bioreactor controllers, and is a proven Process Analytical Technology (PAT) solution able to provide real-time process control and ensure biopharmaceutical product quality.

References:

1. Berry BN, et.al. (2016) Biotechnol Progress 32:224-234 . doi: 10.1002/btpr.2205
2. Matthews TE, et.al. (2016) Biotechnol Bioeng 113:2416-2424 . doi: 10.1002/bit.26018

Application successes in downstream

Rapid optimization of protein crystallization conditions



The protein structure of lysozyme (left) is affected by temperature, pH, precipitating agent, and crystallization time.¹ Raman spectra of lysozyme is depicted in the presence of acetate buffer at 90 mg/ml (right, top), 30 mg/ml (right, middle) and 0 mg/ml (right, bottom).

Customer successes with Raman applications

Cell culture and fermentation

- Flow cell sampling
- Protein and excipient quantification
- COA and aggregation monitoring
- Continuous chromatography
- Product concentration and buffer exchange – UF/DF, TFF

Contact Endress+Hauser for technical references

Raman spectroscopy is an established PAT for understanding crystallization in small molecule active pharmaceutical ingredients and protein therapeutics. Similar to small molecules, protein crystallization may be affected by temperature, pH, solvent, and concentration of species in the system. Raman spectroscopy was used to monitor a laboratory scale batch crystallization of lysozyme. *In situ* Raman spectroscopy was used to investigate the effect of temperature, concentration of precipitating agent, time of crystallization, and possible interactions between these factors.

The Raman spectra of a protein contains spectral contributions from the protein backbone and side chains. The amide III envelope at $\sim 1240\text{ cm}^{-1}$ and the amide I envelope at $\sim 1650\text{ cm}^{-1}$ provide higher order structure information such as the presence of α -helix, β -sheet or random coil. In the example by Mercado et al, bands at $750, 760$ and 2950 cm^{-1} yielded useful protein structure information, reporting on the chemical environment of tryptophan ($750, 760\text{ cm}^{-1}$) and CH_3 groups in aliphatic residues (2940 cm^{-1}).² Intensities of these bands, and the $760:750\text{-cm}^{-1}$ band area ratio, were sensitive to the effects of NaCl concentration, temperature, and time on lysozyme crystallization.

Raman-derived surface plots indicated that optimal conditions for lysozyme crystallization were within $35\text{--}40\text{ }^\circ\text{C}$ and $5\text{--}9\%$ (w/w) NaCl. These data could form the basis of a crystallization design space for scale-up, and process development studies. *In situ* Raman spectroscopy was effective in monitoring the effects of temperature, time, and NaCl concentration on the crystallization of a model protein. Raman spectra provided information on the protein backbone and side chains, which was used to generate quantitative process knowledge and determine optimal crystallization conditions. Extension of the technique to biopharmaceutical laboratory or process development environments can be achieved using the Raman Rxn2 analyzer platform, and in cGMP manufacturing environments using the Raman Rxn4 analyzer platform.

References:

1. Protein structure: 10.2210/pdb253L/pdb
2. Mercado, J. et al. "Design and In-Line Raman Spectroscopic Monitoring of a Protein Batch Crystallization Process." *Journal of Pharmaceutical Innovation*, December 2008, 271–279.

www.addresses.endress.com

IND1228C/66/EN/01.21