In-line Raman monitoring of protein crystallization

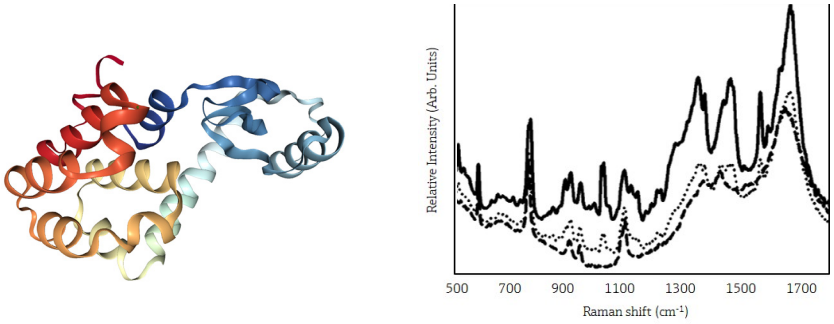

Figure 1: The structure of lysozyme (left) is affected by crystallization conditions. The Raman spectrum of lysozyme (right) measures protein structure changes. Reprinted with permission from Reference 1. © 2008 Springer.

Benefits at a glance

- Specific and quantitative process knowledge is in accordance with the U.S. FDA Process Analytical Technology (PAT) initiative
- Process conditions affect protein crystal structure
- Raman non-destructively measures protein crystal formation
- Protein crystallization conditions are rapidly optimized

Introduction

Optimizing crystallization conditions helps to ensure product quality. Real-time, in-process analysis of the crystallization process confirms crystal form and identifies impurities. Raman spectroscopy is well established as an analysis tool for crystallization because it is non-destructive and measures both chemical composition and molecular structure. On-line Raman can help to quickly understand how conditions, such as temperature, pH, agitation rate, or solvent, affect the crystallization process.

The goal of the study was to demonstrate the utility of Raman spectroscopy to enhance process understanding of protein crystallization using lysozyme as a model protein. *In situ* Raman spectroscopy was used to investigate the effects of temperature, concentration of precipitating agent, time of crystallization, and possible interactions between these factors.

Raman advantages

The Raman spectrum of proteins contains spectral contributions from the protein backbone and side chains. The amide III envelope at ~1240 cm^{-1} and the amide I envelope at ~ 1650 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 cm^{-1}) and CH_3 groups in aliphatic residues (2940 cm^{-1}).[1] Intensities of these bands, and the 760:750-cm^{-1} band area ratio, were sensitive to the effects of NaCl concentration, temperature and time on lysozyme crystallization.

This study reports the use of Raman spectroscopy in a Quality by Design (QbD) approach to optimizing a protein crystallization process. Lysozyme was chosen as a model protein because its crystallization has been widely studied, making it easy to validate the process monitoring approach.

ⓘ All Raman analyzers and probes referenced in this application note are Endress+Hauser products powered by Kaiser Raman technology.

Endress+Hauser
People for Process Automation

Experimental

A D-optimal experimental design was used based on three factors: concentration of precipitating agent (NaCl), temperature, and time. A Raman analyzer was used to monitor the process. The temperature used was 15 to 45 °C over 9 hours for each sample. Spectra were collected in twenty 10 second exposures after allowing 10 minutes of thermal equilibrium. Spectra of solid lysozyme were obtained using a 10× non-contact optic, and spectra from aqueous solution were obtained using a ¼" immersion probe.

Results

Figure 1 shows the effect of varying acetate concentrations on the Raman spectrum of lysozyme. Acetate concentrations: 90 mg/mL (solid line), 30 mg/mL (dotted line), and 0 mg/mL (dashed line). Useful peaks for quantitative analysis are found at 2940, 760, 750, and 155 cm^{-1}.

Figure 2 shows the relationship of lysozyme concentration in solution versus the concentration of NaCl as a precipitating agent. The lysozyme bands at 150 and 2940 cm^{-1} vary directly with NaCl concentration, whereas the shape of the overlapping 750- and 760-cm^{-1} region is more complex, with one decreasing when the other increases. The 2940-cm^{-1} band was the most useful for quantitative process monitoring in this study, but the 760/750-cm^{-1} ratio also had a high degree of validity.

Conclusions

Raman spectroscopy was shown to produce rich, quantitative process knowledge, enabling fast multivariate monitoring of a protein crystallization process with strong potential for real-time control of a scaled-up process. Extension of the technique to biopharmaceutical laboratory or process development environments can be achieved using a Raman Rxn2 analyzer platform, and in manufacturing environments using a Raman Rxn4 analyzer platform.

References

1. Mercado, J. et al. "Design and In-Line Raman Spectroscopic Monitoring of a Protein Batch Crystallization Process." *J. Pharma. Innov.* **2008**; 271–279.

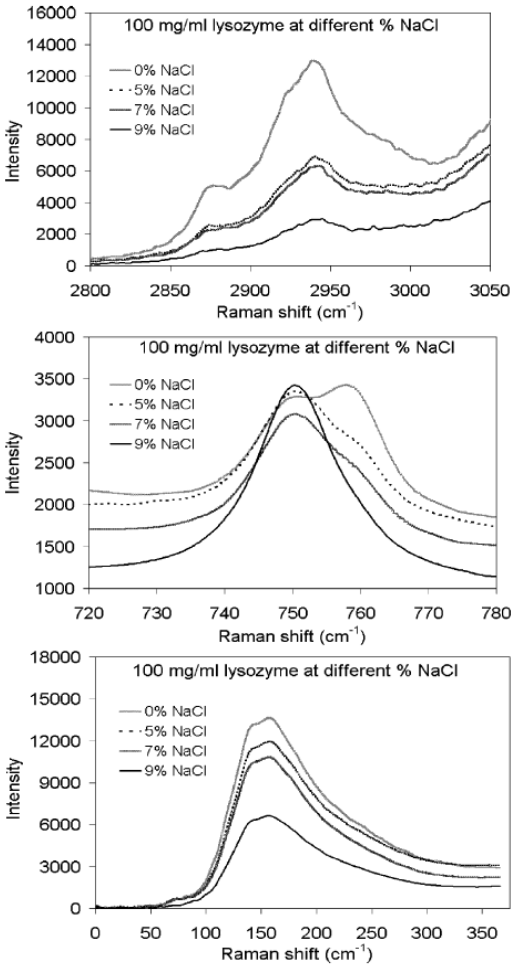

Figure 2. The Raman spectrum of lysozyme changes upon addition of sodium chloride salt, indicating increased protein crystallization.