In situ Raman spectroscopy enables rapid optimisation of protein crystallisation conditions

Raman spectroscopy is an established PAT for understanding crystallisation in small molecule active pharmaceutical ingredients (API). The Raman spectrum provides information on molecular structure and chemical composition which can be used to confirm API crystallinity, and identify polymorphs or amorphous content during API crystallisation. As an on-line PAT, Raman spectroscopy provides real-time information which can be used to understand how reactor conditions such as temperature, pH, agitation rate, or solvent affect the crystallisation process. On-line Raman allows for in-process corrections and a more thorough knowledge of the API crystallisation process.

Extension of Raman spectroscopy to biopharmaceuticals can impart many benefits. In situ Raman can improve process knowledge and ensure an efficient process that consistently makes quality product safely. Raman spectroscopy is an established in situ PAT of cell cultures and fermentations, enabling advanced feed control strategies. In downstream applications, Raman can be equally powerful. An application which has demonstrated feasibility in laboratory-scale studies is protein crystallisation. Similar to small molecules, protein crystallisation may be affected by temperature, pH, solvent, and concentration of species in the system. In this application note, we describe a study in which Raman spectroscopy was used to monitor a laboratory scale batch crystallisation of lysozyme. The goal of the study was to demonstrate a laboratory scale approach to enhance process understanding of protein crystallisation using lysozyme as a model protein.

In situ Raman spectroscopy was used to investigate the effect of temperature, concentration of precipitating agent, time of crystallisation, and possible interactions between these factors. Experimental

The effects of precipitating agent (NaCl) concentration, temperature, and time on lysozyme crystallisation were studied by in situ Raman spectroscopy. In situ Raman spectroscopy was performed using a RamanRxn1™ Raman analyser equipped with an Invictus™ 785nm laser (Kaiser Optical Systems Inc.) and a ¼” immersion probe placed directly into the solution. Crystallised lysozyme was examined by Raman spectroscopy for physiochemical properties, using the RamanRxn1™ analyser equipped with a non-contact optic, and polarised light microscopy for crystal habit and size.

Results

The Raman spectrum of proteins contains spectral contributions from the protein backbone and side chains. The amide III envelope at ~1240cm⁻¹ and the amide I envelope at ~1650cm⁻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 2950cm⁻¹ yielded useful protein structure information, reporting on the chemical environment of tryptophan (750, 760cm⁻¹) and CH₃ groups in aliphatic residues (2940cm⁻¹). Intensities of these bands, and the 760:750cm⁻¹ band area ratio, were sensitive to the effects of NaCl concentration, temperature and time on lysozyme crystallisation. Multivariate data from the experiments were used to generate contour plots of the intensity of the 2940cm⁻¹ band with respect to temperature and NaCl concentration. These data were combined with visual analysis of crystallised proteins using polarised light microscopy. Raman-derived surface plots indicated that optimal conditions for lysozyme crystallisation were within 35-40°C and 5-9% (w/w) NaCl. These data could form the basis of a crystallisation design space for scale-up and process development studies.

Conclusion

In situ Raman spectroscopy was effective in monitoring the effects of temperature, time and NaCl concentration on the crystallisation 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 crystallisation conditions. This and other studies establish a scientific basis for downstream processing, protein crystallisation, and formulation development Raman applications in an industrial setting. Extension of the technique to biopharmaceutical manufacturing environments can be achieved using currently available commercial instrumentation.

Reference


Further information: Kaiser Optical Systems, Inc. www.kosi.com