Key Issues

- **Real-time, in situ process understanding using a Raman-based PAT approach**
- **Testing and release of formulation buffers**
- **Quantitative monitoring of excipient and protein concentrations in downstream bioprocesses**

Introduction

The Process Analytical Technology (PAT) initiative of the U.S. FDA provides a framework for implementing principles of “Quality by Design” (QbD), which promotes quality assurance through understanding and controlling the critical process parameters that affect a biologic product's critical quality attributes. To this end, rapid, non-destructive spectroscopic methods offer significant monitoring and control options for bioprocess unit operations such as in situ monitoring of purification cycles, buffer identification, protein aggregation, product identification, and quality control.

Extending process understanding to downstream bioprocesses is vital to ensure high-quality biological products. Current research is focused on gaining a better understanding of biologic downstream steps by quantitatively measuring key analytes such as amino acids, sugars, and proteins. Similar technology has been used to trend nutrient and metabolite concentrations in situ and in real time during upstream bioprocessing stages. Raman spectroscopy is uniquely useful for biotechnology QbD applications because it enables fast, non-destructive monitoring and control. The work presented here investigates the development and application of new bioprocess analyzers in accordance with the aims of QbD.

Raman Advantages

A typical Raman spectrum consists of sharp, well-resolved peaks whose intensity is dependent on concentration. Raman can thus provide unequivocal compositional information about biological samples or a bioreactor’s contents. Raman spectroscopy is a type of vibrational spectroscopy, producing information similar to that from Fourier transform infrared (FTIR) spectroscopy. But unlike FTIR, Raman is well suited to monitor the solid phase in slurries, including those containing water. FTIR is essentially blinded by water’s strong IR absorption, but water produces only a weak Raman signal. In addition, Raman offers ease of use and flexible sampling benefits similar to near-infrared (NIR) spectroscopy, including remote location of the analyzer and use of non-contact sampling optics. The Kaiser Optical Systems Inc. (Kaiser) RamanRxn Systems™ suite of analyzers provide simple, turnkey solutions for true in situ monitoring.

Experimental

Two downstream bioprocessing applications were investigated at a leading biotech in New England: (1) testing and release of formulation buffers, and (2) monitoring excipients in ultrafiltration/ diafiltration (UF/DF) operations.

A RamanRxn2™ analyzer equipped with a 785-nm Invictus™ laser and a fiber-coupled probe fitted with a 304 mm in situ immersion optic (bIO) was used in this work. Raman spectra were collected by inserting the probe directly into each prepared sample and collecting Raman data for 30 seconds per sample. For the first application, mixtures of four components (amino acid, buffer A, sugar, surfactant) of a formulation buffer were prepared according to a DoE. Figure 1 shows the spectra of the pure components used to make the mixtures.

![Figure 1](image)

**Figure 1.** Raman spectra of four pure components of a buffer system. Zoomed to show regions of high spectral detail in (A) raw and (B) first-derivative forms.

To carry out the UF/DF application, protein was added to a three-component buffer system (amino acid, buffer B, sugar) in the range of 0 to 100 mg/mL according to a DoE. Figure 2 shows the protein spectra (in blue, labeled mAb) overlaid with other components of interest.
Results

Known concentrations for DoE samples from both applications were correlated to Raman spectra using partial least squares (PLS) multivariate calibration models. The results from Application 1 are summarized in Figures 3 and 4. Figure 3 shows the calibration models created for each constituent. These models were then used to predict concentrations of sample mixtures not included in the calibration set. Figure 4 displays the results of these predictions. The results from Application 2 demonstrated the ability to quantify protein in addition to excipients. The corresponding PLS results are summarized in Table 1.

Conclusions

Using Kaiser’s Raman technology, it has been shown that reliable testing and release methods for buffers can be developed. In the second application, monitoring and control of UF/DF operations was successfully accomplished. Raman offers simple and accurate analysis of aqueous-based systems and chemical specificity for both excipients and drug product. These results demonstrate the utility of Raman as a PAT tool to support a QbD manufacturing environment where real-time, in situ monitoring of downstream bioprocesses is important.

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Information withheld in compliance with project NDA.

References

