Downstream biopharmaceutical operations

Benefits at a glance

- Real-time, in-process understanding
- Testing and release of formulation buffers
- Quantitative monitoring of excipient and protein concentration using a single probe in ultrafiltration/ diafiltration operations
- Raman is a proven Process Analytical Technology (PAT)
- Real-time process and product quality assurance

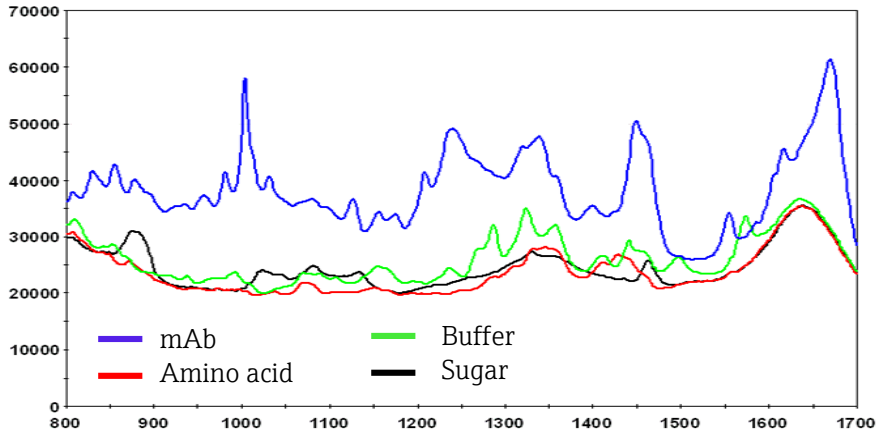

Figure 1: Raman provides quantification on multiple components during downstream operations using a single probe.

Introduction

The US Food & Drug Administration (FDA) 2004 process analytical technology (PAT) framework strongly emphasized a shift from tested-in quality after the drug product was produced, to building in quality throughout production with "continuous real-time quality assurance." The PAT framework also gives implementation 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.

Extending process understanding to downstream bioprocesses is vital to ensure high-quality biological products. Rapid and non-destructive spectroscopic methods offer monitoring and control options for bioprocess downstream operations, such as *in situ* monitoring of purification cycles, buffer identification, protein aggregation[1], product identification[2], and quality control.

Raman advantages

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[3]. 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 analytics in accordance with the aims of QbD. Raman offers ease of use and flexible sampling benefits, including remote location of the analyzer, non-contact sampling optics, and compatibility with flow cells.

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

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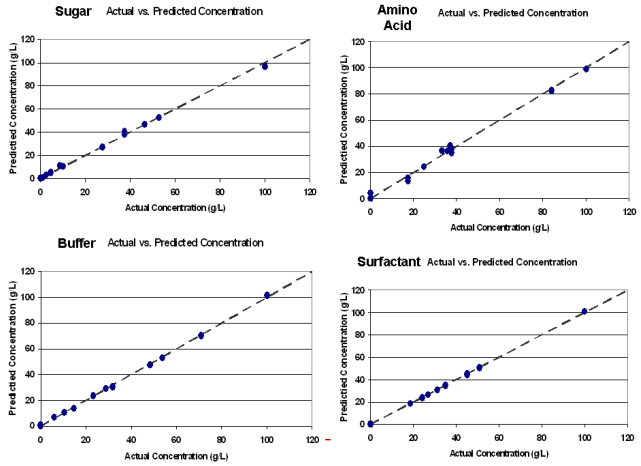

Figure 2: Raman spectroscopy provides quantification on process components similar to offline laboratory measurement, but in real-time and in-process.

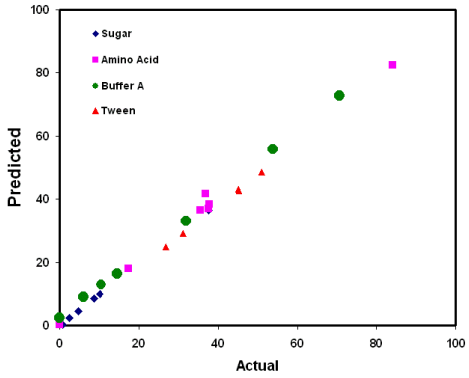

Figure 3: Raman analysis provides quantification on multiple process components during UF/DF operations.

Experimental

Two downstream bioprocessing applications were investigated at a leading biotech company: (1) testing and release of formulation buffers, and (2) monitoring excipients in ultrafiltration/ diafiltration (UF/DF) operations. A Raman analyzer equipped with a 785 nm laser and a fiber-coupled probe fitted with an *in situ* immersion optic 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 the design of experiments (DoE). 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. Known concentrations were correlated to Raman spectra using partial least squares (PLS) multivariate calibration models.

Results

Figure 2 shows the calibration models created for each buffer constituent. These models were then used to predict concentrations of sample mixtures in a new sample set. Figure 3 displays the results of predictions from the UF/DF application, which demonstrates the ability to quantify protein in addition to excipients.

Conclusions

Raman technology provides reliable testing and release methods for buffers, and for controlling UF/DF operations. 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 to support a QbD manufacturing environment where real-time, *in situ* monitoring of downstream bioprocesses is important.

References

1. Mungikar, A. et al. Am. *Pharm. Rev.*, **November/December 2010**, 78–83.
2. Wen, Z.-Q. et al. Am. *Pharm. Rev.*, **May/June 2010**, 46–53.
3. Abu-Absi, N. R. et. al. *Biotech. Bioengin.* **2011**, ***108 (5)***, 1215–1221.