High-Throughput Raman Process Monitoring of Downstream Protein Purification

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Raman Spectroscopy for Real-Time Bioprocess Monitoring

Raman spectroscopy is emerging as a powerful tool for in-line monitoring and downstream protein analysis. Enabled by HTVS™ technology, modern Raman systems deliver up to 30× higher signal, improving sensitivity, speed, and accuracy—even at low laser power. This allows real-time quantification of specific proteins and impurities during chromatographic separation, outperforming traditional UV-vis methods.

By providing actionable insights for process control, Raman supports PAT implementation, enhances product quality, and reduces downstream costs—unlocking significant gains in efficiency and profitability for biopharmaceutical manufacturing.

Methods

An ÄKTA™ Start system was integrated with a Bruker HyperFlux™ Pro Plus Raman spectrometer using a flow cell and immersion probe for in-line measurements Raman spectra were collected every 45 seconds (15 exposures, 3000 ms each), while UV absorbance at 260 nm correlated elution times.

Measurements:

Raman spectra every 45 sec; UV absorbance at 260 nm for correlation.

Chromatography:

HiPrep™ 16/60 Sephacryl™ S-200 HR column; buffer (25 mM sodium phosphate, 150 mM NaCl, pH 7.5).

Samples:

25 mg BSA + 12.5 mg Cytochrome C in 5 mL buffer; injected at 0.5 mL/min; full elution after 95 mL

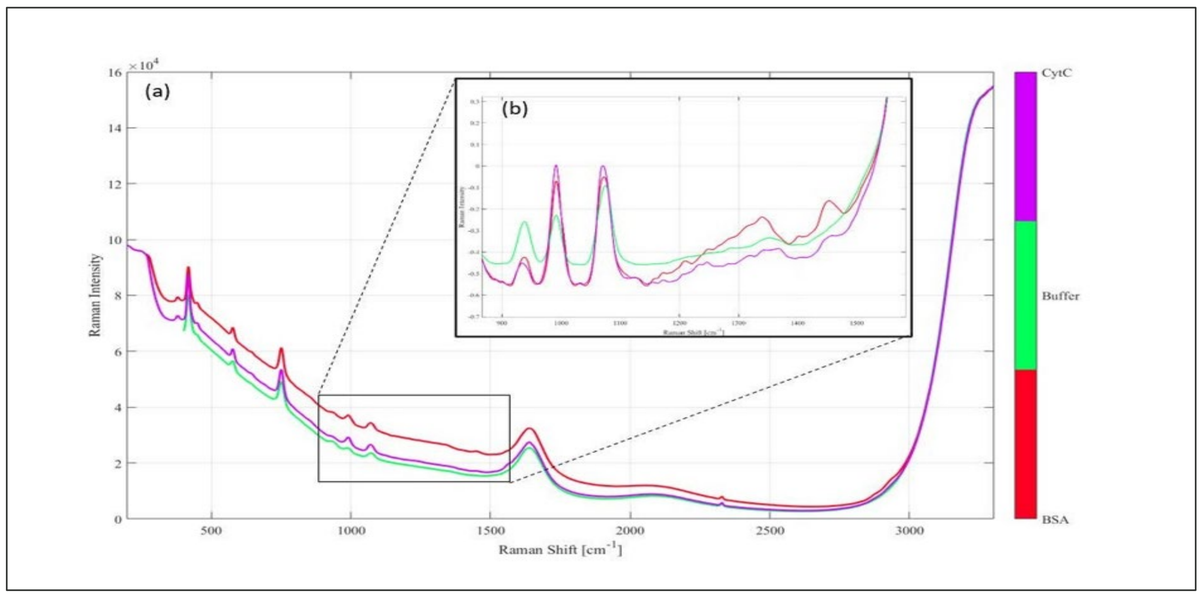

Fig. 2 (a) Raw Raman spectra of 5 mg/mL BSA in gel buffer (red), 2.5 mg/mL CytC in gel buffer (purple)and gel buffer (green).The region of interest (900-1500 cm-1) is further pre-processed and expanded in the inset (b), showing peak increases and shape changes in bands (amide II band ~1500 cm-1, amide III ~ 1300 cm-1) that are consistent with an increase of protein[6].

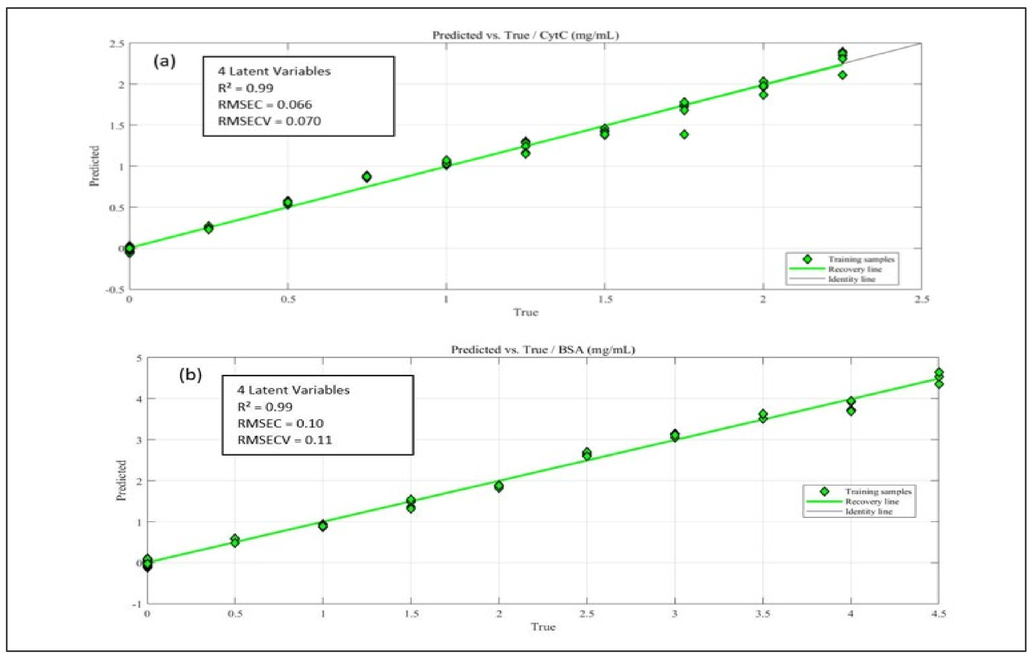

Fig. 2 (a) Correlation plot resulting from the PLS model for the prediction of Cytochrome C constructed using the calibration samples. (b) Correlation plot resulting from the PLS model for the prediction of BSA.

Results

- **Calibration Accuracy:** PLS model predicted BSA and CytC concentrations with errors of 0.109 mg/mL and 0.070 mg/mL (Fig. 2a & Fig. 2b).
- **Elution Tracking:** Raman identified BSA peak at ~50 mL and CytC at ~82 mL, matching UV chromatogram (Fig. 3).
- **Recovery**: Predicted totals closely matched injected amounts—93% for BSA and 89% for CytC (Fig. 4).
- **Specificity:** Near-zero cross-contamination confirms high selectivity for individual proteins.

Summary

Demonstrate HTVS™-enabled Raman spectroscopy for real-time, in-line quantification of individual proteins during downstream purification, compared to UV-vis

ÄKTA™ Start system integrated with Bruker HyperFlux™ Pro Plus Raman; monitored BSA and Cytochrome C elution using gel filtration and PLS calibration models (Fig. 1: Raman spectra; Fig. 2a & 2b: Calibration correlation).

Raman achieved high accuracy (errors: 0.109 mg/mL for BSA, 0.070 mg/mL for CytC), matched UV chromatogram, predicted recoveries of 93% and 89%, and showed strong specificity (Fig. 3: Elution spectra; Fig. 5: Predicted concentration profiles).

Enables faster, precise process control, optimizes protein collection, improves yield and purity, and supports PAT implementation for cost reduction and profitability

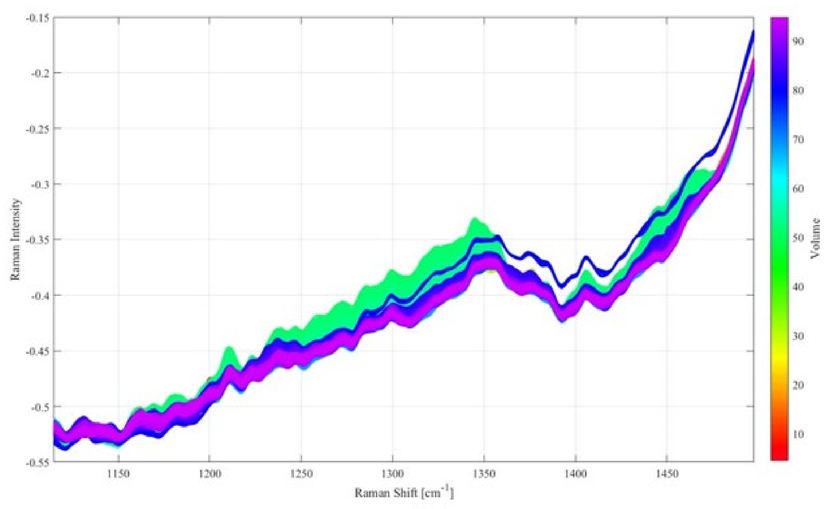

Fig. 3 (Left) Rubber band subtraction, 19-pt smoothing and SNV corrected spectra of the gel filtration process colored by progressive cumulative flow volume.

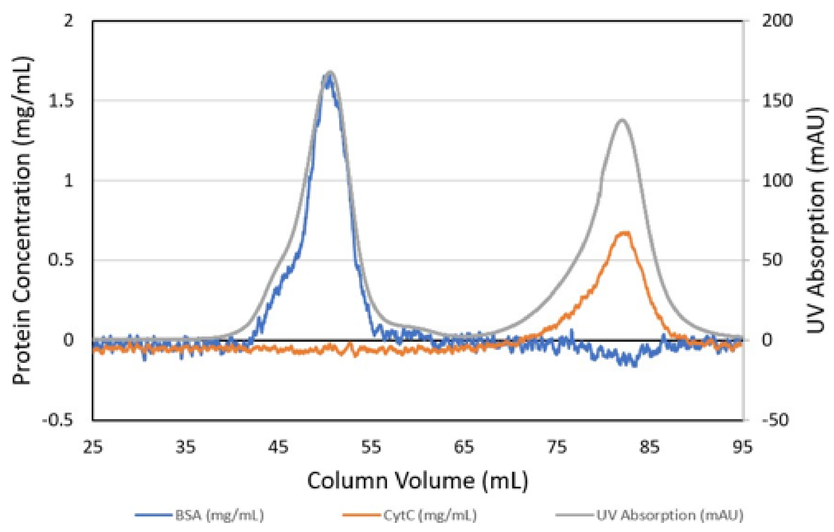

Fig. 4 (Right) Predicted concentration chromatogram of BSA and CytC by volume. On the secondary axis are the units for the chromatogram generated by UV detection.

Conclusion

- Raman predicts individual protein concentrations during purification.
- HTVS™ sensitivity enables fast, accurate measurements with superior SNR.
- Process Control: Optimizes collection, detects excursions, and improves yield.

Process Raman