MMS Evaluation of HOS for IgG Samples Spiked with BSA

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Abstract

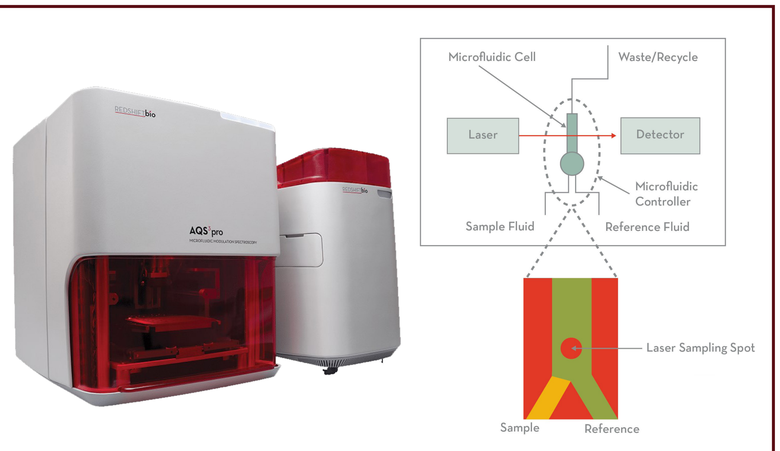
Microfluidic Modulation Spectroscopy (MMS) is a novel protein characterization technique that combines rapid modulation of sample and reference through a microfluidic flow cell with a tunable mid-IR Quantum Cascade Laser (QCL). Five key measurements are provided to assess the similarity, comparability, quantitation, denaturation, and aggregation of proteins by analyzing their higher order structure. A series of 20 mg/mL IgG samples (predominantly β-sheet) spiked with 20 mg/mL BSA (predominantly α-helical) was evaluated on the AQS³pro MMS platform at BSA concentrations of 0, 2, 4, 6, 8 and 10% to demonstrate the sensitivity of MMS for detecting small differences in secondary structure and measure the system Limit of Quantitation (LOQ). Differential absorbance spectra were collected, and second derivative spectra were calculated to evaluate similarity, comparability, and LOQ of MMS relative to FTIR. As BSA concentration was increased across the series, the α-helix absorbance peak at 1656 cm^{-1} increased and the β-sheet absorbance peak at 1637 cm^{-1} decreased accordingly. MMS was able to detect changes in secondary structure for the 2% BSA spiked samples with a calculated LOQ of 0.76% versus results from a similar FTIR study where structural differences were detected in the 8-10% BSA spiked samples with a significantly higher calculated LOQ of 22.7%. This study demonstrates the effectiveness of MMS as a powerful characterization technique for the analysis of protein secondary structure with an ability to generate accurate and high sensitivity HOS data using an automated IR platform.

Introduction

RedShiftBio, Inc. of Burlington, MA has developed the AQS³pro system powered by MMS as a new automated infrared spectroscopy tool for the analysis of protein secondary structure. This novel IR technology demonstrates significant increases in sensitivity, dynamic range, and accuracy when compared to conventional FTIR. The analyzer utilizes a tunable mid-IR Quantum Cascade Laser to generate an optical signal 100X stronger than the conventional sources used in FTIR spectroscopy. A brighter source also allows the use of a simpler detector without the need for liquid nitrogen cooling. Additionally, the protein sample solution and a matching buffer reference stream are automatically introduced into a microfluidic flow cell and are rapidly modulated up to 5 Hz across the laser beam path to produce nearly drift-free background-compensated measurements as a differential absorbance spectrum. This improved IR technology made it possible to analyze a series of IgG samples spiked with BSA resulting in a lower LOQ for the detection of small but critical changes in protein secondary structure.

Methods

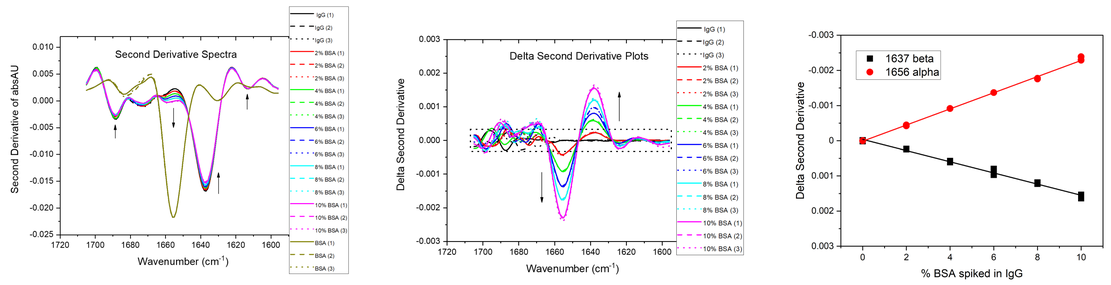
A series of spiked samples was created by mixing 20 mg/mL IgG with 20 mg/mL BSA at concentrations of 0, 2, 4, 6, 8, and 10%. Differential absorbance spectra were collected automatically at 1 Hz and 5 psi backing pressure on the AQS³pro MMS system. Absolute Absorbance, Second Derivative, Similarity (Area of Overlap), Delta, Quantitation, and HOS results were generated using the AQS³delta analytics processing software.



AQS³pro MMS System and Microfluidic Flow Cell
RedShiftBio, Inc.

Results

I. MMS Analysis: Second Derivative, Delta of Second Derivative, Stability, and LOQ* results for 20 mg/mL IgG spiked with 20 mg/mL BSA at 0, 2, 4, 6, 8 and 10%.

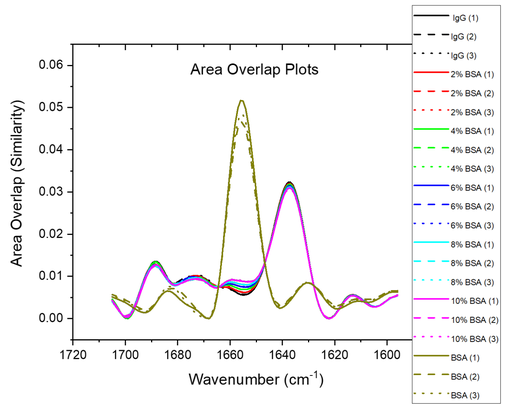


*Calculation of LOQ for MMS and FTIR was performed using:

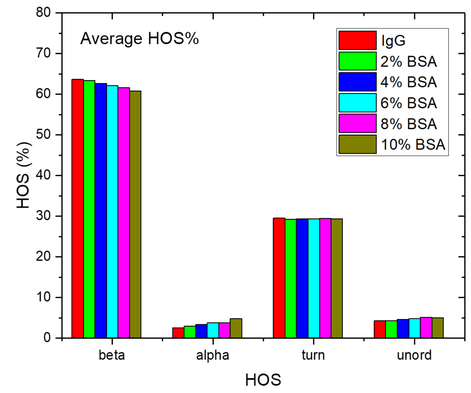
$$LOQ = \frac{10\,\sigma}{S} \qquad \sigma = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n-2}}$$

Figure 1: As BSA was increased across the spiked IgG series, the peak at 1656 cm^{-1} associated with α-helical structure increased, and the peak at 1637 cm^{-1} associated with β-sheet decreased as shown in the second derivative plot.

By tracking changes in the peaks at 1656 cm^{-1} and 1637 cm^{-1} using the Delta plot view, changes in secondary structure were detected with an LOQ* of 0.76%.



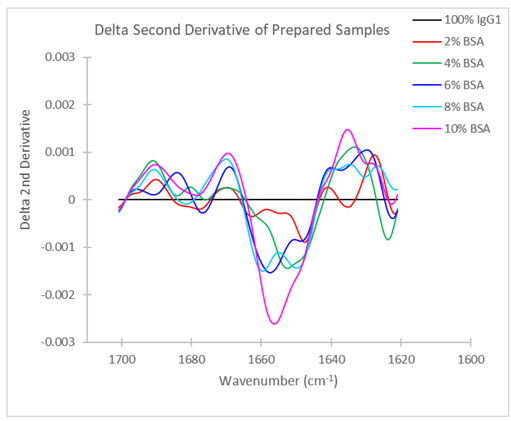
IgG Samples	Mean ± SD
0% BSA	99.61 ± 0.01
2% BSA	99.15 ± 0.03
4% BSA	98.43 ± 0.05
6% BSA	97.84 ± 0.06
8% BSA	97.19 ± 0.03
10% BSA	96.40 ± 0.15



IgG Samples	Beta (%)	Turn (%)	Alpha (%)	Unordered (%)
0% BSA	63.66	29.53	2.56	4.25
2% BSA	63.44	29.30	2.92	4.35
4% BSA	62.71	29.33	3.35	4.61
6% BSA	62.14	29.33	3.77	4.76
8% BSA	61.61	29.44	3.79	5.16
10% BSA	60.84	29.33	4.79	5.03

Figure 2: (above) The similarity results displayed as Area of Overlap indicate that MMS was able to successfully detect changes in secondary structure as low as 2% BSA in the spiked series. HOS analysis (right) confirms that an increase in the amount of α-helical structures and a decrease in β-sheet was measured in the 2% BSA spiked sample.

II. FTIR Analysis: Delta of Second Derivative and Quantitation/LOQ results for 20 mg/mL IgG spiked with 20 mg/mL BSA.



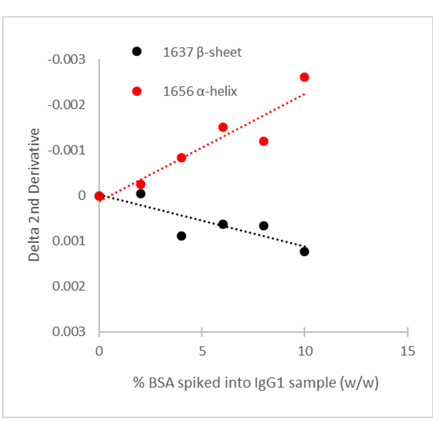


Figure 3: At 20 mg/mL, FTIR detected differences in secondary structure for the 8-10% BSA spiked samples. The LOQ using FTIR was calculated to be 22.7%.

Conclusions

- A series of 20 mg/mL IgG spiked with 20 mg/mL BSA at concentrations of 0, 2, 4, 6, 8, and 10% was evaluated using MMS and compared to the analysis of the same concentrations by FTIR (earlier study).
- As BSA was increased across the spiked series, the amount of α-helical structure signal at 1656 cm^{-1} increased and the β-sheet structure signal at 1637 cm-1 decreased.
- MMS accurately detected changes in secondary structure in the 2% BSA spiked sample with an LOQ of 0.76% versus FTIR, which detected changes in the 8-10% BSA spiked sample with an LOQ of 22.7%.
- The hands-on time for preparation and analysis using automated MMS was approximately 15 minutes to fill and load the plate; for FTIR it was approximately 5 hours in total.
- Compared to FTIR, MMS offers a powerful automated IR characterization technique for direct, label-free analysis of protein secondary structure through all phases of biologic drug development, from discovery through formulation, manufacturing, and quality control.