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Monoclonal Antibody Analysis by Microfluidic Modulation Spectroscopy in a Complex Formulation Buffer (1 to 150 mg/mL)

Introduction

Microfluidic Modulation Spectroscopy (MMS) is a powerful new infrared spectroscopy tool for protein structural analysis developed by RedShift BioAnalytics. This technology provides significant increases in sensitivity, dynamic range, and accuracy for determination of protein secondary structure relative to conventional mid-IR and far-UV CD techniques. The analyzer utilizes a tunable quantum cascade laser to generate an optical signal over 100x brighter than the conventional sources used in FTIR spectroscopy. Brighter sources also allow the use of simpler detectors without the need for liquid nitrogen cooling. Additionally, the sample (protein) solution and a matching reference buffer stream are automatically introduced into a microfluidic flow cell, and the two fluids are rapidly modulated (1-4 Hz) across the laser beam path to produce nearly drift-free background compensated measurements. In this note, a native monoclonal antibody was measured by MMS in a complex buffer formulation at concentrations ranging from 1 to 150 mg/mL.

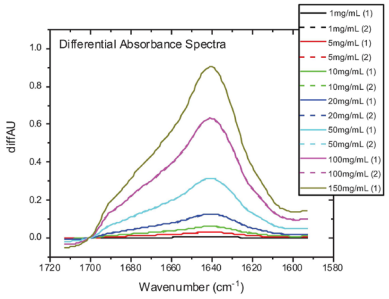
Methods

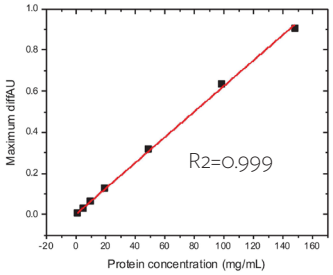
A monoclonal antibody (IgG1) was diluted in its formulation buffer (10 mM Histidine, 245 mM Trehalose, 10 mM Methionine, 0.05% PS-20, pH 5.2) to make a dilution series at concentrations of 1, 5, 10, 20, 50, 100, and 150 mg/mL. Differential absorbance spectra were collected automatically from a multi-well plate at a modulation rate of 1 Hz and a back pressure of 5 Psi on a RedShiftBio AQS³pro MMS system and data was analyzed using the AQS³delta Analytics software.

AQS³pro Microfluidic Modulation Spectroscopy System

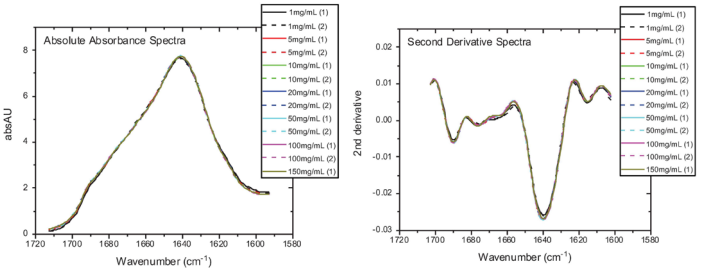
Results

I. Differential absorbance spectra (diffAU) and the linear concentration range. DiffAU of replicates at each concentration are very consistent indicating excellent repeatability and accuracy of the measurements. The plot of maximum diffAU against protein concentration is linear with an R2 value of 0.999 over the concentration range of 1 to 150 mg/mL.



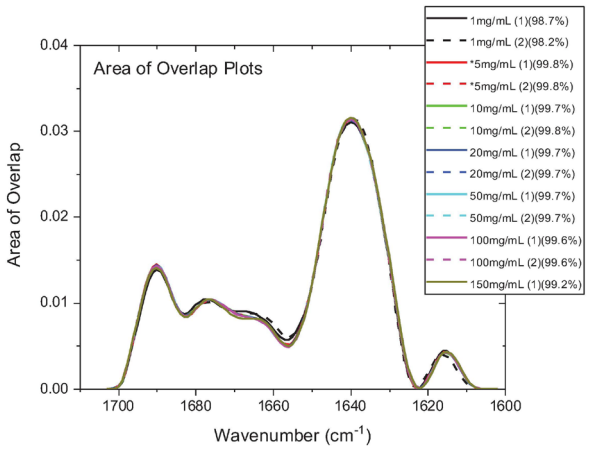


II. Absolute absorbance spectra (absAU) and second derivative spectra. The absAU spectra were calculated from diffAU spectra, with concentration normalization and protein displacement taken into account. The absAU spectra and the second derivative spectra of the antibody sample at different concentrations closely match each other indicating that the secondary structure of the antibody does not change upon dilution.



Results, continued

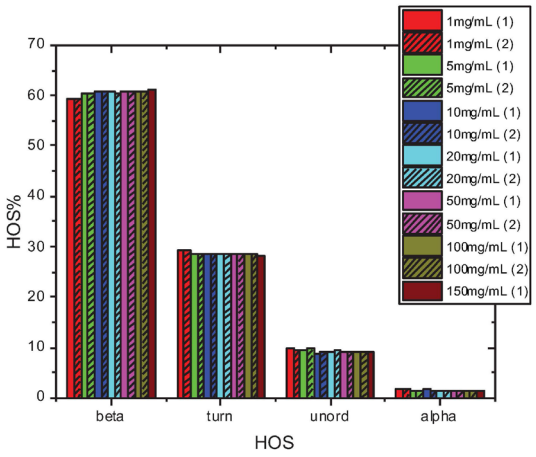
III. Area of Overlap (AO) and similarity comparison. When compared to the mean AO of *5 mg/mL sample replicates, samples at 1-150 mg/mL show >98% similarity over the entire concentration range. BSA denaturation at 20 mg/mL. Structural changes were similar to the results observed at 1 mg/mL.



Sample concentration	Similarity (%) of replicates		Mean±SD
1 mg/mL	98.74	98.22	98.48±0.37
*5 mg/mL	99.84	99.84	99.84±0.00
10 mg/mL	99.71	99.75	99.73±0.03
20 mg/mL	99.71	99.69	99.70±0.01
50 mg/mL	99.65	99.66	99.66±0.01
100 mg/mL	99.60	99.60	99.60±0.00
150 mg/mL	99.18	---	99.18

*The similarity data was obtained by comparing the Area of Overlap (AO) to the mean AO of 5 mg/mL replicates

IV. Higher order structure (HOS) analysis. HOS analysis (Gaussian curve fit) demonstrates that all samples have similar secondary structure across the entire concentration range.



Samples @	HOS% (Mean±SD) of replicates			
	Beta	Turn	Unordered	Alpha
1 mg/mL	59.38±0.15	29.14±0.02	9.67±0.33	1.81±0.20
5 mg/mL	60.31±0.07	28.69±0.04	9.57±0.21	1.43±0.18
10 mg/mL	60.76±0.16	28.62±0.01	9.05±0.38	1.57±0.21
20 mg/mL	60.61±0.14	28.63±0.04	9.24±0.21	1.52±0.11
50 mg/mL	60.78±0.05	28.52±0.01	9.30±0.04	1.40±0.00
100 mg/mL	60.82±0.04	28.54±0.01	9.11±0.06	1.53±0.03
150 mg/mL	61.04	28.24	9.16	1.56

Conclusions

- MMS enables quantitative analysis of monoclonal antibodies over a wide concentration range with high reproducibility and accuracy.

- MMS analysis did not require dilution of concentrated samples for analysis and there was no interference from the optically active formulation buffer components.

- MMS is a powerful and versatile tool for direct, label- free characterization of proteins through all phases of biologic drug development, from discovery through formulation and manufacturing.

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