Application Note
Oct 2020
AN-850-115

Secondary Structure Determination using Microfluidic Modulation Spectroscopy in the Presence of DMSO

- Biosimilars
- mAbs
- ADCs
- AAVs
- Ligand Binding
- ☑ Protein/Peptide Analysis
- VLPs
- Nucleic Acid
- Fusion Proteins
- ☑ Enzyme Analysis

- Aggregation
- Quantitiation
- ☑ Structure
- Stability
- ☑ Similarity

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Introduction

Characterizing biologic drug products is crucial in drug development and the presence of organic solvents and optically active excipients typically hinders traditional characterization techniques. RedshiftBio has developed the AQS^{3}pro, a novel characterization system powered by Microfluidic Modulation Spectroscopy (MMS) as a solution to overcoming these obstacles. MMS combines Infrared (IR) spectroscopy with automatic sample handling and a micro-flow cell for accurate measurements with no buffer interference. The system uses rapid modulation between a sample and its reference buffer to generate a differential spectrum for the most accurate buffer subtraction. This strategy results in the ability to successfully subtract any buffer including those which contain organic solvents and optically active excipients. The AQS^{3}pro uses a Quantum Cascade Laser (QCL) as the IR light source to monitor changes within the Amide I region of the IR spectrum. This laser generates an output which is 1000 times brighter than traditional FTIR light sources, resulting in a much greater signal-to-noise ratio and high sensitivity for accurate measurement of proteins ranging from 0.1 to over 200 mg/mL. In this application note, lysozyme was dissolved in a buffer containing 0, 5, and 10% DMSO, and the ability for MMS to successfully structurally characterize the protein is demonstrated.

Methods

Lysozyme from hen egg white (HEWL) was prepared in HPLC-grade water with 0, 5, or 10% DMSO at concentrations of 1 and 10 mg/mL. Samples were run in duplicate using a 1 Hz modulation rate with 5 psi backing pressure on the AQS^{3}pro. All data was processed using the AQS^{3}delta analytical software.

Results

I. Absolute Absorbance: Lysozyme spectra at 1 and 10 mg/mL with 0, 5, and 10% DMSO were collected in duplicate and the replicate averages are shown in the following figures. Figures 1 A/B show spectra which have been normalized for concentration and buffer subtracted. The spectra overlay very well, indicating similar secondary structure under all DMSO conditions. Additionally, the similarity between replicates at all DMSO conditions was measured at > 98% for 1 mg/mL and > 99% for 10 mg/mL (Table 1). This demonstrates the high level of repeatability of the system.

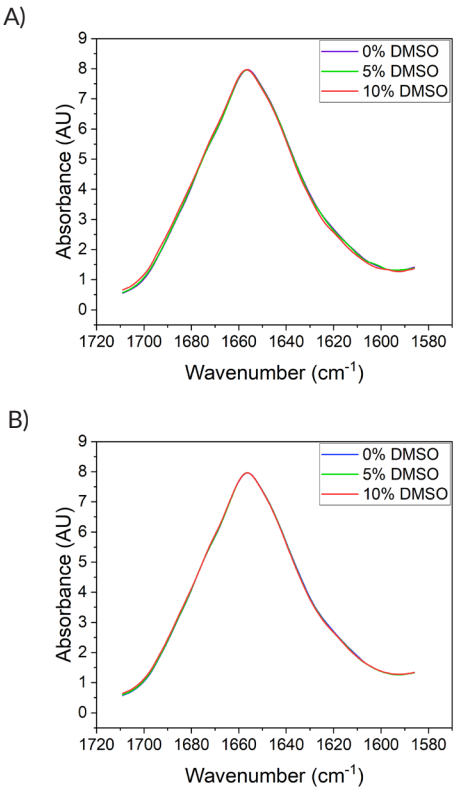

Figure 1. Absolute Absorbance spectra for replicates of A) 1 mg/mL and B) 10 mg/mL lysozyme with 0, 5, and 10% DMSO.

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Results, continued

Sample	% DMSO	Replicate Similarity	Percent Similarity *compared to 0% DMSO Sample
1 mg/mL Lysozyme	0*	98.8	100
	5	98.3	97.8
	10	98.7	96.7
10 mg/mL Lysozyme	0*	99.9	100
	5	99.9	98.8
	10	99.9	97.9

Table 1. The percentage of similarity (area of overlap) for each replicate calculated relative to the sample average and compared to samples with 0% DMSO*.

II. Second Derivative: Figures 2 A/B show second derivative plots which have been calculated and represent the major features making up the absolute absorbance spectrum. The most prominent feature for each lysozyme sample occurs at 1656 cm^{-1} and is representative of alpha-helical secondary structure.[1] Additionally, the Second Derivative plot is useful for observing small changes in structure across the Amide I band. At 10% DMSO, for example, a small deviation can be seen at 1675 cm^{-1} at each lysozyme concentration. In both plots, the average of replicates is used at each DMSO value.

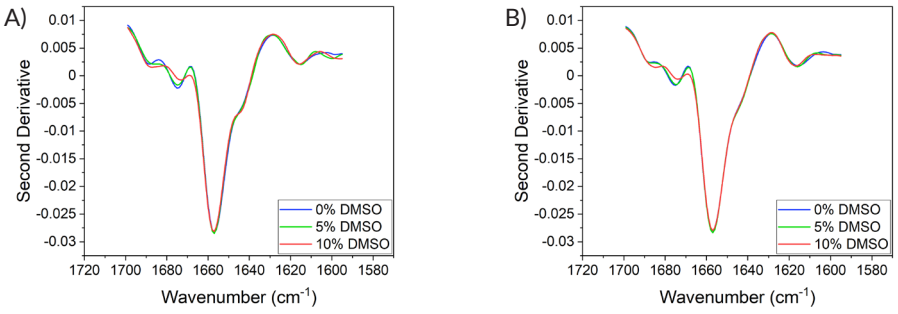
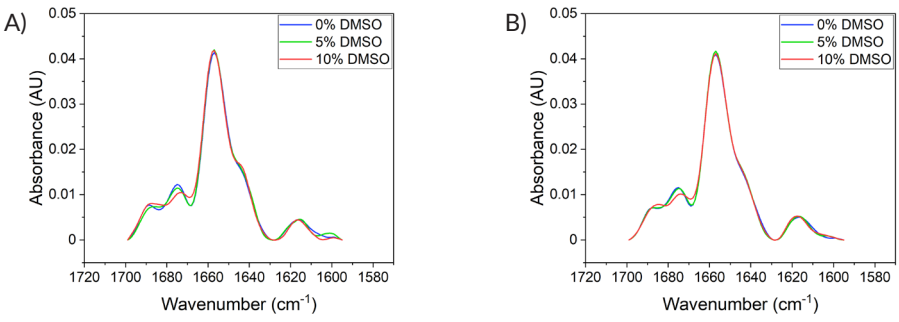


Figure 2. The Second Derivative Plots at A) 1 mg/mL and B) 10 mg/mL lysozyme with 0, 5, and 10% DMSO show the individual Amide I features.

III. Similarity: The Similarity Plots (Area of Overlap) shown in Figures 3 A/B are derived from the baseline-subtracted second derivative of the Absolute Absorbance Spectra. These plots show the area of overlap and how the percent similarity was calculated compared to the 0% DMSO sample (Table 1). The percent similarity decreases as the percent DMSO increases, showing that the presence of DMSO slightly alters the IR spectra and causes small but measureable structural changes in the lysozyme. This is meaningful considering the high data quality and replicate to replicate reproducibility, also shown in Table 1.



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IV. Higher Order Structure: Using the Similarity Plot, the Higher Order Structure (HOS) percentages were calculated by means of Gaussian Curve fitting. The HOS bar graphs are presented in Figures 4 A/B for both lysozyme concentrations. This data indicates that there are no significant secondary structural changes in the lysozyme resulting from the addition of up to 10% DMSO. The results of the HOS analysis are summarized in Table 2.

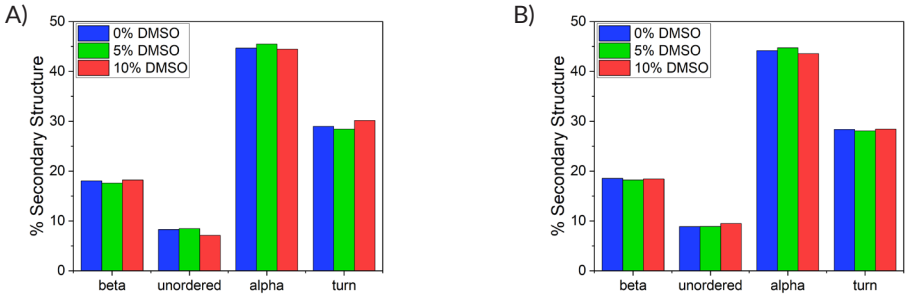

Figure 4. Lysozyme HOS bar graph at A) 1 mg/mL and B) 10 mg/mL lysozyme with 0, 5, and 10% DMSO.

Concentration (mg/mL)	% DMSO	Beta-sheet	Unordered	Alpha-helix	Beta-turn
1 mg/mL	0	18	8	45	29
	5	18	8	45	28
	10	18	7	44	30
10 mg/mL	0	19	9	44	28
	5	18	9	45	28
	10	18	10	44	28

Table 2. Percentage of Higher order structure for 1 and 10 mg/mL lysozyme with 0, 5, and 10% DMSO.

Conclusions

Organic solvents are very useful for increasing the solubility of certain biomolecules and their presence often complicates the characterization process. MMS, a novel technique for measuring small changes in secondary structure, is able to overcome the spectral challenges presented by the presence of organic solvents while maintaining high sensitivity and repeatability. In the presence of up to 10% DMSO, small changes in the structure of lysozyme were detected using MMS, but did not cause significant alterations in the overall higher order structure of the protein.

References

1. Dong, A.; Huang, P.; Caughey, W. S., Protein secondary structures in water from second-derivative amide I infrared spectra. Biochemistry 1990, 29 (13), 3303-3308.

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