Application Note
Dec 2020
AN-850-0117

Assessment of Quantitation Linearity for Bovine Serum Albumin Using MMS

- 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

Microfluidic Modulation Spectroscopy (MMS) is a revolutionary new technology capable of highly sensitive and reproducible measurements of protein secondary structure using IR absorbance in the Amide I band (1700-1600 cm^{-1}). MMS is performed on the AQS^{3}pro system from RedShift BioAnalytics, and it generates measurements over a wide range of complex backgrounds, including buffers that contain absorbing materials and scattering components such as reducing agents, adjuvants, surfactants, and other excipients. The AQS^{3}pro operates using a quantum cascade laser in combination with a microfluidic flow cell resulting in a wide dynamic range of 0.1 to >200 mg/mL for structural characterization, and further extended to 0.01 to >200 mg/mL for quantitation measurements. The system automatically acquires data from samples loaded into 24 or 96 well-plates, and data processing and customizable reports are performed using the AQS^{3}delta Data Analysis package included with the system.

In this study, BSA was prepared as a series and examined by MMS to measure the concentration linearity of the series. The MMS measurements were highly reproducible and quantitation was calculated as a linear regression fit with superior correlation. MMS differs from traditional benchtop secondary structure analysis techniques such as circular dichroism (CD) and Fourier Transform Infrared spectroscopy (FTIR) in that it can differentiate small changes in secondary structure across a wide comentration range without the interference of excipients or loss of linearity at the lowest and highest concentrations. It is also capable of delivering information about aggregation, stability, HOS, and similarity in addition to quantitation results.

Methods

Bovine Serium Albumin was prepared in PBS to create a dilution series at 0.1, 0.2, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0, 7.5, and 10.0 mg/mL. MMS was performed using an AQS^{3}pro system by RedShiftBio. The sample and buffer (PBS) were rapidly modulated through the microfuluidic flow cell (Figure 1) at a modulation rate of 1 Hz with 5 psi backing pressure. Three replicates at each concentration were analyzed and the differential absorbance spectra of each sample was collected, averaged, and processed using the AQS^{3}delta Data Analysis package.

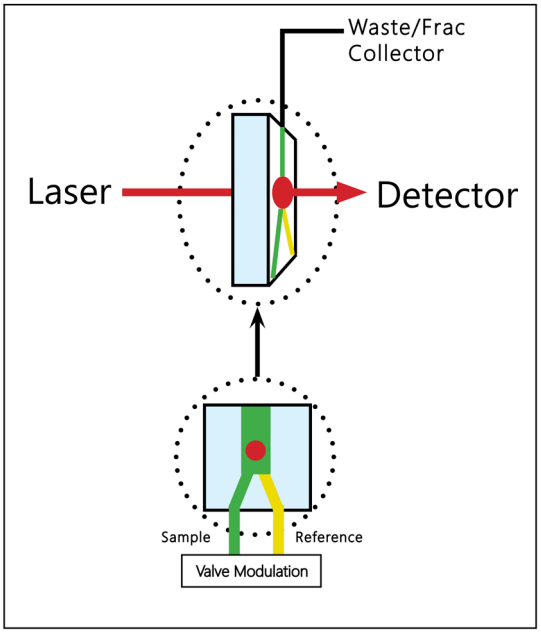

Figure 1. Schematic of an MMS microfluidic modulation spectroscopy flow cell and associated microfluidics showing sample and reference solution entry points.

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Results

I. Differential Absorbance: The differential absorbance (DiffAU) spectra are shown in Figure 2a, with one example spectrum at each concentration. The DiffAU spectra were then automatically background corrected and used to generate the quantitation results across the sample concentration range.

II. Quantitation: From the DiffAU results, the linearity of the BSA concentration series was automatically generated using the AQS^{3}delta data analysis package and these results are shown in Figure 2b. Each data point is the average of 3 replicates, and the linearity across the entire range resulted in R^2 fit values of >0.9999 for both regression methods reported.

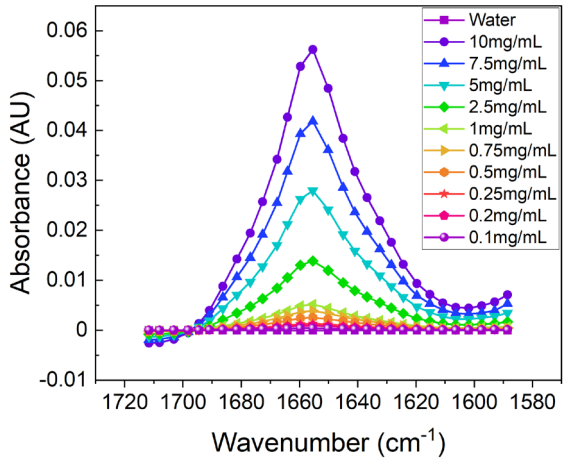

Figure 2a: Differential Absorbance Spectra of a BSA concentration series in PBS from 0.1 to 10 mg/mL.

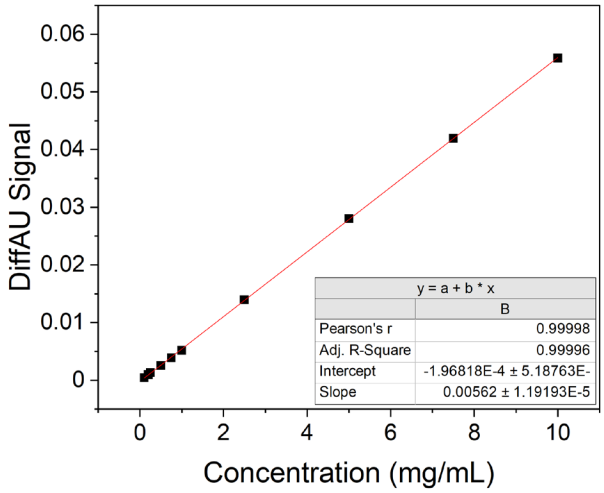

Figure 2b: Concentration linearity results of a BSA concentration series in PBS from 0.1 to 10 mg/mL.

Conclusions

The AQS^{3}pro MMS system from RedShiftBio demonstrated that it was able to automatically and accurately quantitate BSA across the sample concentration range of 0.1 to 10 mg/mL with resulting linearity R^2 values >0.9999. This ability to quantitate proteins as part of five key measurements supported by the AQS^{3}pro shows the value of adding the AQS^{3}pro MMS system to a protein characterization workflow through all stages of protein biotherapeutic development.

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