Microfluidic Modulation Spectroscopy of a Biotherapeutic at Low to High Concentrations without Interference from Formulation Excipients

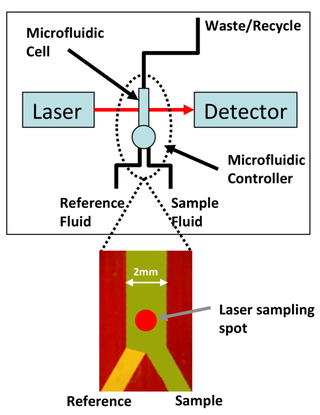
Libo Wang[1], Ioannis A. Papayannopoulos[2], Shannon Renn-Bingham[2], and Jeffrey Zonderman[1]
[1]RedShift BioAnalytics, Inc., 131 Middlesex Turnpike, Burlington, MA 01803
[2]Analytical Development, Celldex Therapeutics, 151 Martine Street, Fall River, MA 02723

Abstract

Microfluidic Modulation Spectroscopy (MMS), a novel protein characterization technique was used to measure the secondary structure of a monoclonal antibody on a RedShiftBio AQS^{3}pro instrument at concentrations from 1 to 80 mg/mL in both the formulation buffer (10 mM Histidine, 245 mM Trehalose, 10 mM Methionine, 0.05% PS-20, pH 5.2) and a pH 7.4 PBS buffer. Our results show that the absolute absorbance and the second derivative spectra of the mAb samples at 1-80 mg/mL in both buffers are closely matched suggesting very similar secondary structure profiles of these samples. There is no secondary structure changes of the mAb when diluted in the PBS buffer. When compared to the 5 mg/mL sample in the formulation buffer, the structure similarity is 98.5% for 1 mg/mL sample in the formulation buffer, and 99+% for all other samples in both buffers indicating all these samples are highly comparable. The mAb sample also displays great quantitation linearity of measurements at 1 to 80 mg/mL in both buffers with a R^2 value of 0.999, respectively. In addition, the secondary structure composition of these samples in both buffers is very consistent, i.e. 60-62% beta sheet structure, 29-31% turn structure and very small amount of alpha-helix structure. No dilution of high concentration samples is required for MMS measurements and there are no interferences from optically active excipients in the formulation buffer. Our results show that MMS is a powerful protein characterization technique providing comparability, similarity, quantitation linearity and HOS information of protein samples with high sensitivity and accuracy.

Introduction

RedShift BioAnalytics has developed a powerful new infrared spectroscopy tool for protein structural analysis based on Microfluidic Modulation Spectroscopy (MMS). This technology achieves significant increases in sensitivity, dynamic range, and accuracy for determination of protein secondary structure compared to conventional FTIR and far-UV CD methods. The analyzer utilizes a tunable mid-IR quantum cascade laser to generate an optical signal 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 buffer reference stream are automatically introduced into a microfluidic flow cell, and the two fluids are rapidly modulated (e.g. 1-5 Hz) across the laser beam path to produce nearly drift-free background compensated measurements. A simplified diagram of the instrument and a picture of a MMS instrument are shown below.

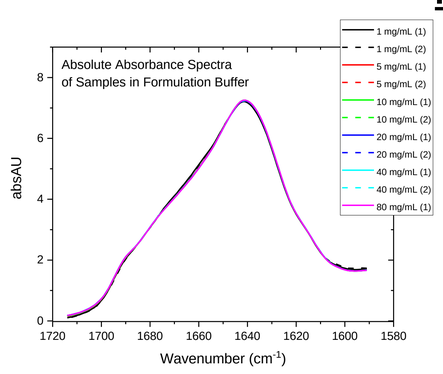


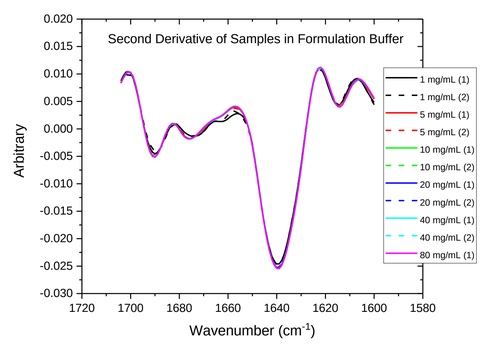
Method

A monoclonal antibody at 157 mg/mL was diluted in either the formulation buffer (10 mM Histidine, 245 mM Trehalose, 10 mM Methionine, 0.05% PS-20, pH 5.2) or a pH 7.4 PBS buffer to make two dilution series at 1, 5, 10, 20, 40 and 80 mg/mL, respectively. A RedShiftBio AQS^3 instrument was used to collect the differential absorbance spectra. Samples at 1-40 mg/mL in both buffers were tested at a modulation rate of 1 Hz and a back pressure of 5 psi. The 80 mg/mL sample in formulation buffer was tested at 1 Hz, 25 psi and the 80 mg/mL sample in PBS buffer was tested at 1 Hz, 10 psi due to the increased viscosity. All the data was analyzed using the proprietary analytical software, AQS^3 delta.

Results

I. mAb samples in formulation buffer



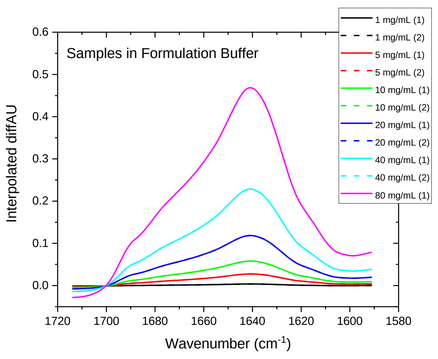


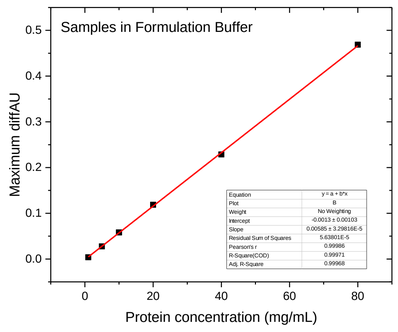
Similarity (%) of samples in formulation buffer

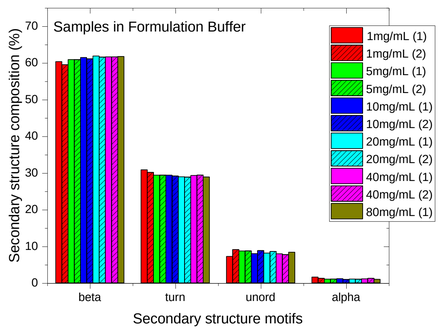
Conc. (mg/mL)	Similarity (%) of replicates	
1	98.19	98.54
5*	99.90	99.90
10	99.73	99.69
20	99.65	99.65
40	99.61	99.62
80	99.55	---

*similarity (%) was calculated by comparing to the mean of 5mg/mL sample replicates.

The absAU and the second derivative spectra of the mAb sample at different concentrations are closely matched indicating very similar secondary structure profiles. The similarity (%) data also shows that these samples in formulation buffer are very similar in structure.



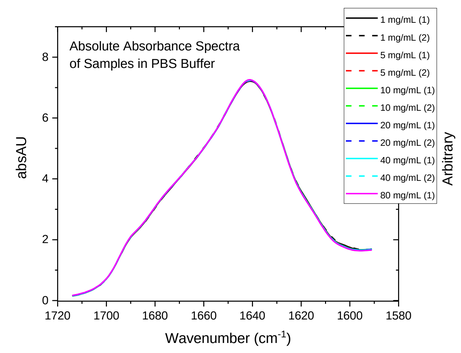


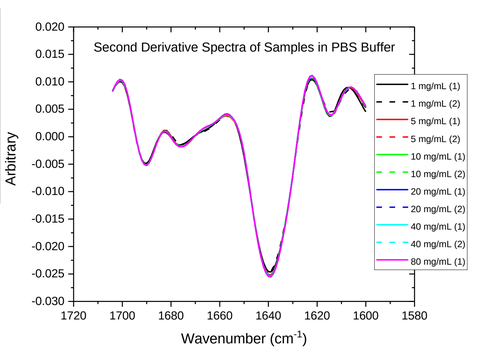


The diffAU are very consistent for each sample indicating an excellent data repeatability, and the sample shows a great quantitation linearity at the concentration range of 1-80 mg/mL.

HOS analysis shows that all these samples have similar secondary structure composition (%).

II. mAb samples in PBS buffer



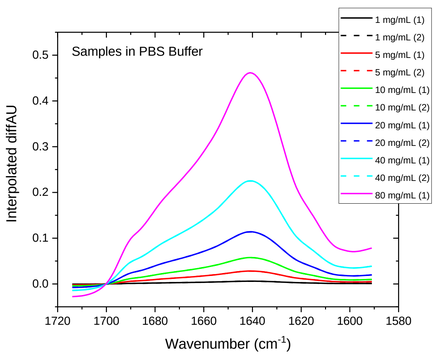


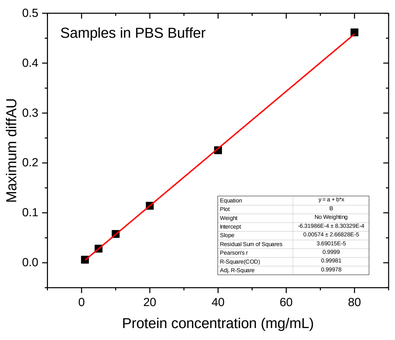
Similarity (%) of samples in PBS buffer

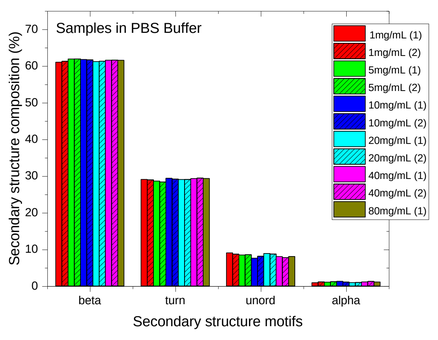
Conc. (mg/mL)	Similarity (%) of replicates	
1	98.98	98.99
5*	99.82	99.82
10	99.71	99.66
20	99.74	99.75
40	99.72	99.72
80	99.69	---

*similarity (%) was calculated by comparing to the mean of 5mg/mL sample replicates.

The absAU and the second derivative spectra of the mAb sample at different concentrations are closely matched indicating very similar secondary structure profiles. The similarity (%) data also shows that these samples in formulation buffer are very similar in structure.



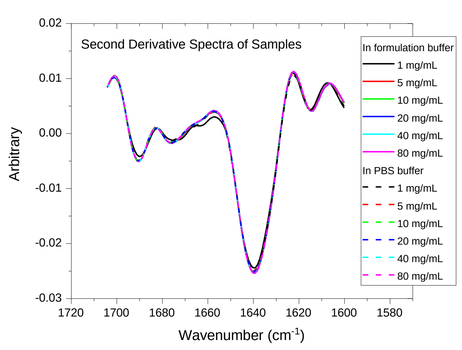


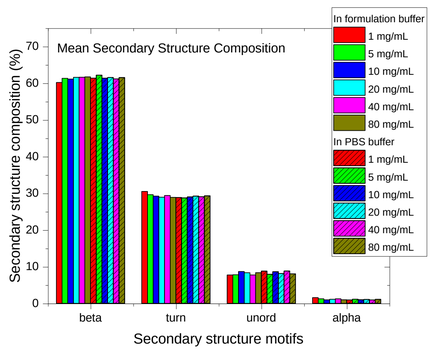


The diffAU are very consistent for each sample indicating an excellent data repeatability, and the sample shows a great quantitation linearity at the concentration range of 1-80 mg/mL.

HOS analysis shows that all these samples have similar secondary structure composition (%).

III. Comparison of samples in two different buffers





HOS analysis result

Samples	Conc. (mg/mL)	Mean secondary structure composition (%)			
		Beta	Turn	Unordered	Alpha
In formulation buffer	1	60.19	30.50	7.73	1.58
	5	61.31	29.63	7.81	1.25
	10	61.10	29.22	8.70	0.98
	20	61.59	28.94	8.36	1.12
	40	61.61	29.40	7.73	1.26
	80	61.73	28.88	8.39	1.00
In PBS buffer	1	61.38	28.85	8.81	0.96
	5	62.19	28.74	7.92	1.15
	10	61.37	29.01	8.63	0.99
	20	61.56	29.22	8.15	1.08
	40	61.15	29.07	8.82	0.96
	80	61.53	29.32	8.06	1.10

All the samples in both formulation buffer and PBS buffer have very similar secondary structure composition. PBS buffer does not change the secondary structure of the sample. There is no interference from the excipients in the formulation buffer.

Conclusion

Our data indicates that MMS is a powerful automated protein characterization tool for secondary structure assessment of biopharmaceuticals with high repeatability, accuracy and sensitivity, applicable to wide concentration ranges (1mg/ml to 80mg/ml) and buffers with various excipients, enabling structural characterization not achievable using traditional FTIR and far-UV CD methods.

For more information: Email Jeff Zonderman at jzonderman@RedshiftBio.com