Application Note
MAY 2023
AN-850-0134

Structure, Stability, Similarity, and Stress Characterization of Trastuzumab and Two Biosimilar Drugs Using Microfluidic Modulation Spectroscopy (MMS)

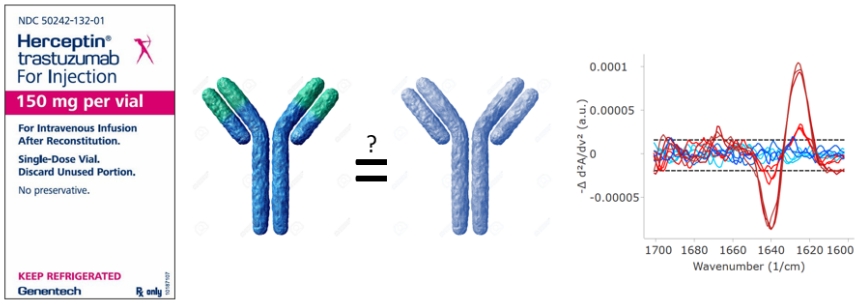
- [x] Biosimilars
- [x] mAbs
- [ ] ADCs
- [ ] AAVs
- [ ] Ligand Binding
- [ ] Protein/Peptide Analysis
- [ ] VLPs
- [ ] Nucleic Acid
- [ ] Fusion Proteins
- [ ] Enzyme Analysis

- [ ] Aggregation
- [ ] Quantitiation
- [x] Structure
- [x] Stability
- [x] Similarity

Abstract

Since biologic drugs are large and complex in nature, replicating them perfectly as generic drugs is nearly impossible. Therefore, if companies can show their product is highly similar to another FDA-approved biologic, this new drug can be considered a "biosimilar." Biosimilars must be consistent with the originator in structure, function, and safety, among other parameters. However, these similarity comparisons can only be as good as the tools used to make these measurements.

In this study, we used Microfluidic Modulation Spectroscopy (MMS), a novel and best-in-class structure characterization tool, to show the similarity between Trastuzumab (originator) and two research-grade biosimilar drugs. Additionally, we tested a few stress conditions, i.e., formulation buffer vs PBS and exposure to heat stress. All three molecules behaved very similarly under each buffer condition, however, the heat stress of 70°C for 30 mins had structural consequences on the Trastuzumab in PBS and much more subtle effects on the sample in formulation buffer. This shows the increased stability of Trastuzumab in formulation conditions. The heat-stress study was only completed on the Trastuzumab sample and future work will include replicating the heat-stress study with both biosimilars to understand their thermal stability.



Introduction

Trastuzumab (or Herceptin®) is a biologic used to treat certain types of cancers, specifically in patients that are HER2 positive.[1] There are multiple manufacturers that produce biosimilars of this drug for clinical and for research purposes. In order to qualify drugs as biosimilars, the drug must pass rigorous characterization that shows identical properties compared to the originator.[2] In this work, we tested the structure of the originator and two research-grade biosimilars using microfluidic modulation spectroscopy (MMS). Additionally, we tested the three samples under normal formulation conditions, in PBS, and after 30 mins of 70°C elevated temperature to gauge the stability.

MMS interrogates the amide I band of the IR spectrum to sensitively probe protein structure while modulating against the reference buffer for accurate, real-time background subtraction in aqueous-based samples. As it is so sensitive, this technique is particularly useful for quality control and is compatible with many different formulation buffers. The second-generation MMS system, Apollo, was used for this study and is equipped with a high-power quantum cascade laser that is significantly more intense than traditional FTIR light sources. The combination of more light and modulating background subtraction makes MMS about 30 times more sensitive than FTIR and 5 times more sensitive than CD to small changes in structure.[3]

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Methods

Trastuzumab (Herceptin®) was a generous gift from Dr. Shawn Owen from the University of Utah. The drug was received in its original packaging as a dry powder containing the salts of its formulation components. The pre-portioned powder was re-suspended with 7.4 mL HPLC water to bring up both the protein and buffer to the proper concentrations. A stock of matching formulation buffer was prepared with 18.4 mg/mL trehalose, 0.459 mg/mL L-histidine HCl monohydrate, 0.297 mg/mL L-histidine, 0.081 mg/mL PS20. Both biosimilars were graciously provided by IchorBio already dissolved in either formulation buffer or PBS. The three samples, Trastuzumab and both biosimilars, were split into two aliquots each, one was dialyzed in formulation buffer and the other was dialyzed in PBS to make sure each sample had a perfectly matching reference buffer and so the effect of buffer on the structure and stability could be assessed.

As the biosimilars were provided at 5 mg/mL and the Trastuzumab is formulated at 21 mg/mL, we ran the neat Trastuzumab in addition to a 5 mg/mL diluted sample to compare the effect of concentration.

Each sample was run in triplicate on an AQS^{3}pro equipped with sweep scanning capabilities and a flow cell with a pathlength of 22.3 µm. The backing pressure was 5 psi and the modulation speed was 1 Hz. Additionally, the Trastuzumab samples in each buffer were subjected to 30 min in a 70°C water bath and analyzed again in MMS after being cooled to room temperature to determine structural change and how the buffer impacts the effects of temperature stress. All results are shown as the averages of the three replicates.

Results

I. Comparing originator to two biosimilars:

Trastuzumab at the formulation concentration of 21 mg/mL, along with a diluted 5 mg/mL sample and two biosimilars also at 5 mg/mL were tested in the formulation buffer. Figure 1 shows the MMS results including absolute spectra (A), the second derivative spectra (B), the similarity plot (C), and the HOS fractional contribution profile (D). The absolute spectra were normalized for concentration and buffer contribution. The second derivative plot highlights regions of small change. By inverting and baseline-subtracting the second derivative plot, the similarity plot was generated and was then fit with Gaussian curves to quantitate the fractional content of HOS. Table 1 highlights the quantitative results using area of overlap of the similarity plot to determine repeatability of the replicates and compared to the control sample (Tras 5 mg/mL). Since the comparisons to the control all fall within the repeatability, it is evident that all samples in formulation conditions are equivalent in secondary structure.

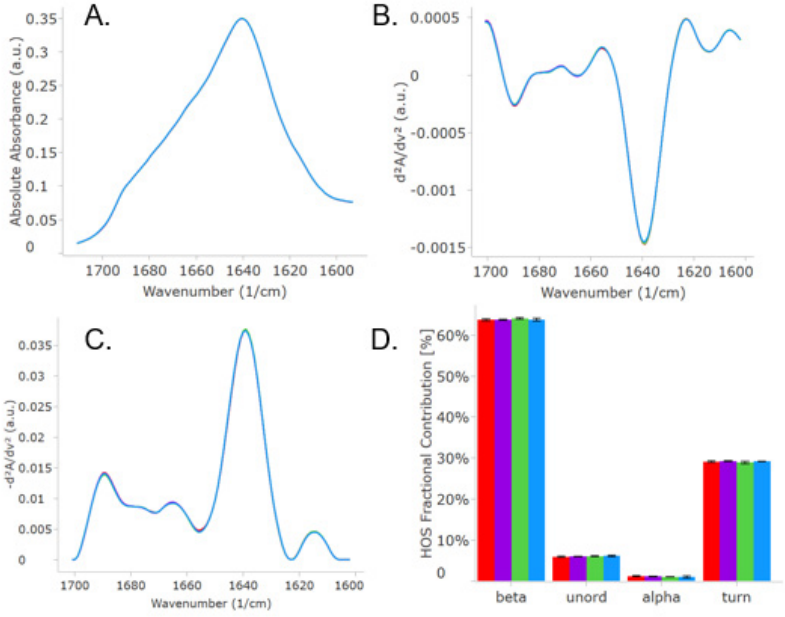

Figure 1. Absolute absorbance (A), second derivative (B), similarity (C), and HOS bar charts are shown above for the originator Trastuzumab at 5 and 21 mg/mL and two biosimilars each at 5 mg/mL. All spectra are well overlaid and appear as just one trace, but are actually four traces. The HOS bar chart highlights the similarities in structure. The colors correspond to: Trastuzumab 5 mg/mL is red, Trastuzumab 21 mg/mL is purple, biosimilar 1 is green, and biosimilar 2 is blue.

Sample	Conc (mg/mL)	Repeatability	Similarity
Trastuzumab	5	99.57	100
Tras (neat)	21	99.73	99.73
Biosim 1	5	99.65	99.40
Biosim 2	5	99.55	99.51

Table1. A quantitative analysis of concentration, repeatability, and comparison to Trastuzumab innovator.

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Results, continued

II. Formulation buffer vs PBS:

All samples were dialyzed in both formulation buffer and PBS in order to compare the effect of the buffer on the antibody structure. Figure 2 shows the second derivative (A), HOS bar graph (B), and the delta plot (C) for each sample in FB at 5 mg/mL and in PSB. The delta plot is a visual way to compare samples by subtracting the second derivative of each sample from a control. The dashed lines represent the replicate variation. Therefore, any traces that fall above or below those dashed lines reveal spectral change. In this study, none of the samples show change due to buffer. Table 2 quantitatively shows all samples are comparable as none of the similarities deviate by more than 1% compared to the control.

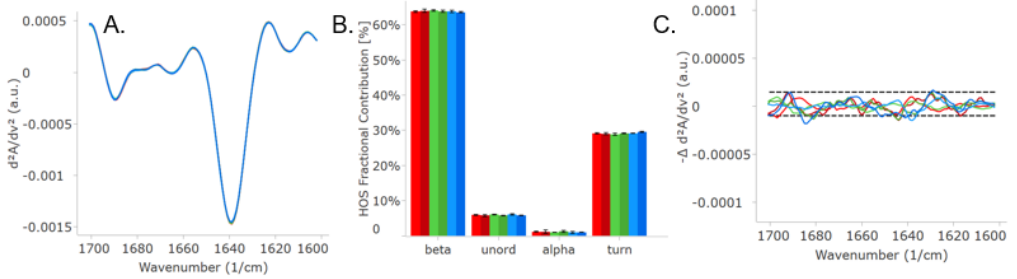

Figure 2. Second derivative plot (A), HOS bar chart (B), and delta plot (C) show each sample in PBS is highly similar to the samples in formulation buffer. The lighter shades are in FB and the darker shades are in PBS. The Trastuzumab samples are red, biosimilar 1 is green, and biosimilar 2 is blue.

Sample	Repeatability	Similarity
Tras FB	99.57	100
Tras PBS	99.35	99.45
Biosim 1 FB	99.65	99.40
Biosim 1 PBS	99.45	99.43
Biosim 2 FB	99.55	99.51
Biosim 2 PBS	99.48	99.34

Table 2. Quantitative measurements of area of overlap showing repeatability among replicates and similarity compared to the Trastuzumab in formulation buffer are all highly similar.

III. Heat stress qualitative results:

Trastuzumab in formulation buffer and in PBS were exposed to 70°C for 30 mins and cooled to room temp before running on MMS. Figure 3 shows the second derivative (A) and delta plot (B) where the RT in FB is light blue, the RT sample in PBS is dark blue, the heated sample in FB is bright red, and the heated sample in PBS is dark red. Both heated samples show an increase in the spectra around 1624 cm^{-1} and a decrease in the region at 1640 cm^{-1} (native beta-sheet). The results show only a slight structure change for the sample in FB and much larger changes for the sample in PBS. This implies that Trastuzumab is more resistant to temperature stress when in FB than when it is in PBS.

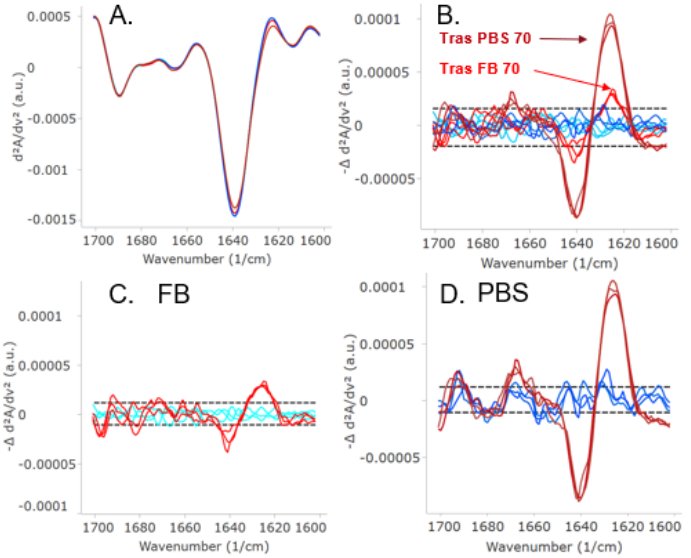

Figure 3. Second derivative plot (A) of Trastuzumab in FB at room temp (light blue) and after heat stress (bright red), and Trastuzumab in PBS at room temp (dark blue) and after heat stress (dark red). (B) shows the delta plot and highlights regions of change, and (C) and (D) are the delta plots separating out the samples in FB and in PBS, respectively.

III. Heat stress quantitative results:

Figure 4A shows the HOS bar graph for each sample. The heat stress did not result in major changes in the structure, however, Figure 4B clearly shows the trend that heat stress caused a decrease in beta-sheet content and an increase in turn structure. Additionally, the weighted spectral difference (WSD) was calculated and is shown as a graph in Figure 4C. Here the error bars represent +/- 2x the standard deviation among replicates. Both the WSD and the percent similarities show that the FB heated sample undergoes subtle structural change and the heated PBS sample undergoes more significant structural change. This result suggests a possible initial denaturing pathway that Trastuzumab undergoes when the stress temperature approaches its melting temperature (Tm1 = 71°C), that some beta sheets start to unfold and convert to beta turn structures.

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III. Heat stress quantitative results, continued:

This beta sheet to turn transition propensity is more pronounced in the sample in PBS compared to the formulation buffer, again showing the importance of formulation buffer in maintaining the antibody's stability.

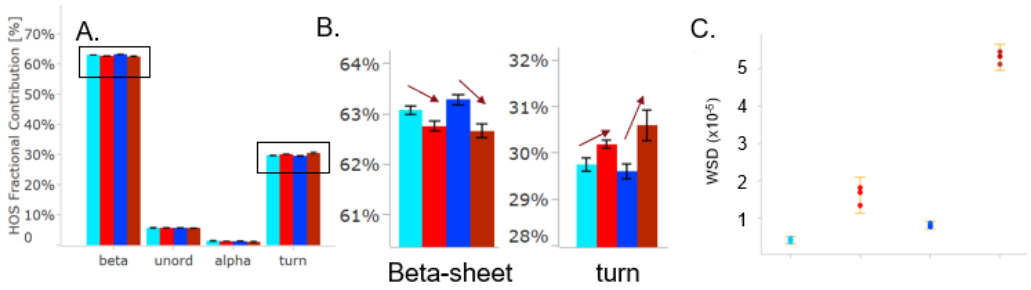

Figure 4. HOS bar charts for Trastuzumab in FB at room temp (light blue), heat-treated (bright red), and in PBS at room temp (dark blue), and heat-treated (dark red). Section (B) shows a more detailed view of the beta-sheet and turn structure changes and the trend that temperature stress has on Tras. The weighted spectral difference is shown in (C) and the change is clearly visible for both samples, however, the sample in PBS shows a much larger spectral change.

Table 3. Area of overlap calculated for the repeatability for each sample and comparison to the RT control in FB.

Sample	Repeatability	Similarity
Tras FB RT	99.62	100
Tras FB 70	99.61	99.23
Tras PBS RT	99.64	99.45
Tras PBS 70	99.58	97.75

Conclusions

Trastuzumab and both research-grade biosimilars were found to have very comparable secondary structures at both formulation concentration and diluted to 5 mg/mL as they maintain >99% area of overlap when compared to the control (Trastuzumab). Additionally, PBS did not affect the structure of the antibodies as determined using the delta plot and area of overlap percent similarities compared to the samples in formulation buffer. However, heat stress of 70°C for 30 minutes does affect the structure of Trastuzumab and the sample in PBS was more affected than the sample in formulation buffer. Heat stress caused a loss in beta-sheet content and a gain in turn structure, possibly indicating unwinding of the native structure. Overall, MMS was shown to be a robust and automated tool that can be used to quickly and easily analyze protein structure for biosimilar comparisons and determine change qualitatively, using the delta plots, and quantitatively using the percent similarity, HOS percentages, or the weighted spectral difference, simplifying the decision-making process when analyzing samples for comparability. Better biophysical characterization tools, like MMS, will enable better biosimilar drugs.

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2. Vulto, Arnold G., and Orlando A. Jaquez. "The process defines the product: what really matters in biosimilar design and production?." Rheumatology 56.4 (2017): 14-29.

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