Application Note
MAR 2026
AN-850-0154

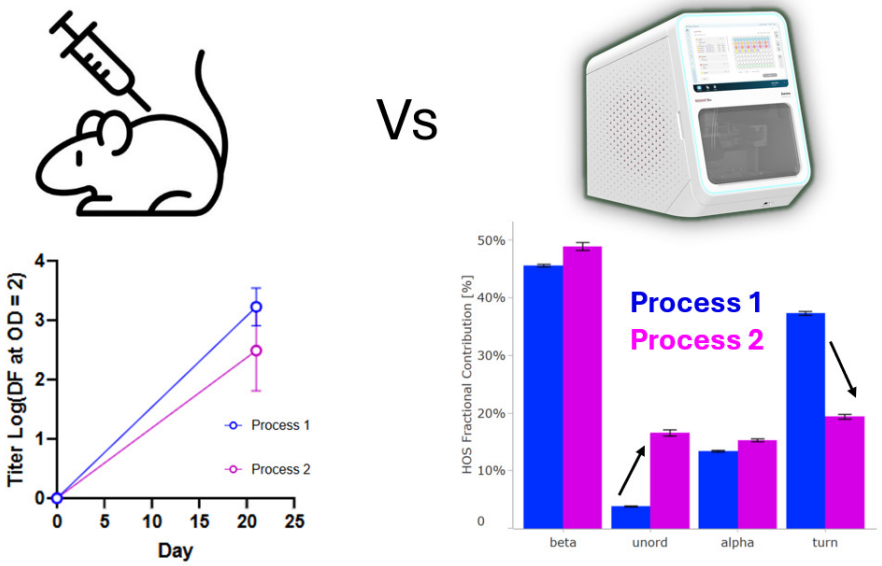
Using Microfluidic Modulation Spectroscopy for Peptide Quality Control

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

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

Abstract

Peptides are an increasingly popular and attractive therapeutic agent due to their specificity, versatility, and manufacturability. We've seen an increase in peptide therapeutic popularity since the introduction of the glucagon-like peptide-1 (GLP-1) for treating type 2 diabetes and obesity, but peptides can be used for a wide variety of diseases. In this study, we tested two lots of the same immunogenic peptide that went through different synthesis processes (Process 1 and 2) and were indistinguishable from each other by standard biophysical techniques but displayed different levels of efficacy in animals. We used Aurora TX to probe the native structure and stability of each peptide and found that the native structures are very similar, both mostly consisting of turn structure, yet the peptides from the two different processes show marked structural differences when formulated in common buffer systems. As this peptide is prepared as a pure solution for long-term bulk storage but formulated in buffers designed to mimic physiological pH and tonicity for use *in vivo*, we compared the samples in water and PBS to mimic conditions that they might experience in the animal testing. Both peptides showed a large increase in beta-sheet structure when dissolved in PBS, indicative of aggregation, but the peptide from Process 1 maintained more native turn structure than the Process 2 peptide and had much lower unordered structure in the stress PBS condition. These results indicated that the turn structure in these peptides may be important for potency in animal testing and demonstrates the utility of MMS as a peptide process development analytical tool.



TOC Figure: In this Application Note, we discuss the importance of peptide therapeutics and quality control. Small changes in process can have drastic effects on potency and immunogenicity but be hard to uncover with standard characterization methods without needing to repeat potency assays. MMS has potential to assess the quality of peptide lot-to-lot comparisons better than other standard techniques.

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Introduction

Peptides have come a long way in their development as therapeutic agents. Insulin was the first peptide to be used therapeutically back in the 1920's and involved treating a patient for type 1 diabetes with pancreas extracts from bovine sources.[1] Purification techniques were quickly developed and eventually recombinant insulin was introduced in the 1980's, eliminating the need for animal sources of insulin and greatly increasing the capability of large-scale production.[2] The first GLP-1 drug was approved in 2005 and became a blockbuster in 2017 with Semaglutide, a GLP-1 that only required weekly injection compared to twice daily for previous versions. Since then, there have been great strides in peptide design, synthesis, and application leading to peptides as an attractive therapeutic option for many different dieases.[3]

The peptide tested in this study is designed to be immunogenic, meaning it activates a therapeutic immune response. Immunogenicity is usually a negative side-effect for drugs, however, these peptides use the immune response to target neurodegenerative diseases like Alzheimer's and Parkinson's as well as cardiovascular and metabolic conditions like hypercholesterolemia and type 2 diabetes. Since this strategy uses the body's own immune system, the effects are long-lasting and can be very effective for these chronic diseases.

Although the peptides were designed to be potent, small changes in synthesis processes were found to greatly impact *in vivo* potency. Figure 1 demonstrates a loss of immunogenicity in rats for the Process 2 batch as compared to the Process 1 batch, even though they are identical peptides when characterized by CD (Circular Dichroism), FT-IR (Fourier Transform-InfraRed Spectroscopy), DLS (Dynamic Light Scattering), DSC (Differential Scanning Calorimetry), and SEC-MALS (Size Exclusion Chromatography-Multi-Angle Light Scattering).

In this Application Note, we explore Microfluidic Modulation Spectroscopy (MMS) as a technique for peptide process quality control to fill the structure gap left by other techniques to distinguish the difference between these two products without having to repeat expensive and slow animal testing. MMS interrogates the mid-infrared spectrum to probe carbonyl stretching vibrations that can be used to quantify secondary structural components in peptides, proteins, and nucleic acids. Its high sensitivity, reproducibility, and the ability to analyze samples in native and formulated conditions, positions MMS as an ideal technique for quality control applications.

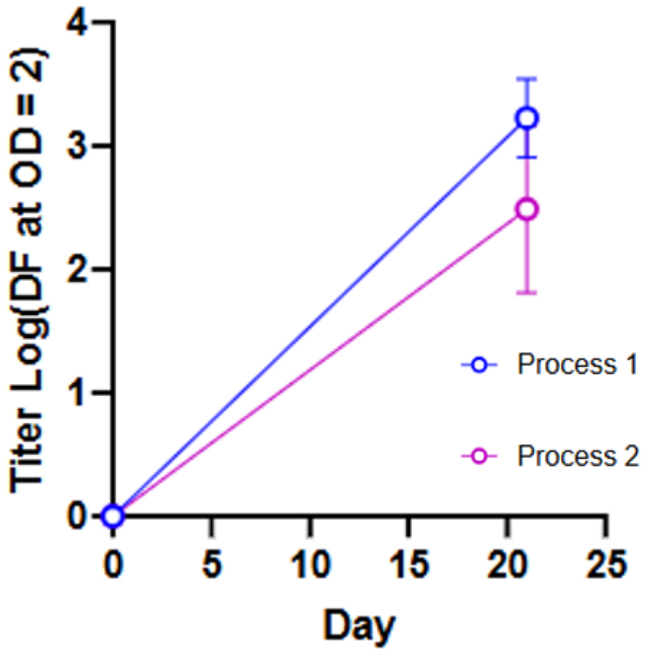

Figure 1: *In vivo* studies show Process 2 is less potent than Process 1.

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Methods

Peptides from Process 1 and Process 2 were supplied by the customer as liquids (pre-dissolved in water) or powders and were dissolved in water or PBS before testing. Measurements were taken on an Aurora TX at 1, 2, and 3 mg/mL each. The 1 mg/mL samples were stored in a 4°C fridge and repeated each day for 3 days to test short term stability. The next batch of samples was delivered as powder and dissolved in either water or PBS at 1 mg/mL and ran on Aurora TX immediately after dissolving. All samples were run in triplicate and we present the averaged data in each figure with error bars representing +/- the standard deviation. The data were processed on the RedShiftBio Analysis software. The absorbance data was converted into baselined-second derivative plots (similarity plots) and then Gaussian curve fitting was used to quantitate the percentages of higher order structure (HOS).

Results

To initially test for secondary structure differences, Process 1 and Process 2 peptide samples were tested via AuroraTX in water at 1 mg/mL each. The Similarity plots (Figure 2A) were derived from the baselined-second derivative plots and show both peptides have similar structures with peaks around 1646, and 1680 cm^{-1}. Using Gaussian curve fitting, we determined the native Higher Order Structure (HOS) of the peptides is mostly turn and beta-sheet structure (Figure 2B).

Additionally, we compared the samples at various concentrations (1-3 mg/mL) and fridge storage conditions (4°C for 1-3 days) to determine if there were conditions that could affect the secondary structure and ultimately, the function. Neither concentration nor fridge storage led to significant secondary structure changes indicating that in water, the two peptides are structurally indistinguishable.

We next subjected the peptides to formulation stress by dissolving the powdered peptides in PBS as opposed to its preferred solvent of pure water. Both Process 1 and Process 2 peptides showed signs of aggregation by the large increase in signal at 1630 cm^{-1}, corresponding to an increase in beta-sheet structure, and decrease of the native peaks at 1646 and 1680 cm^{-1}.

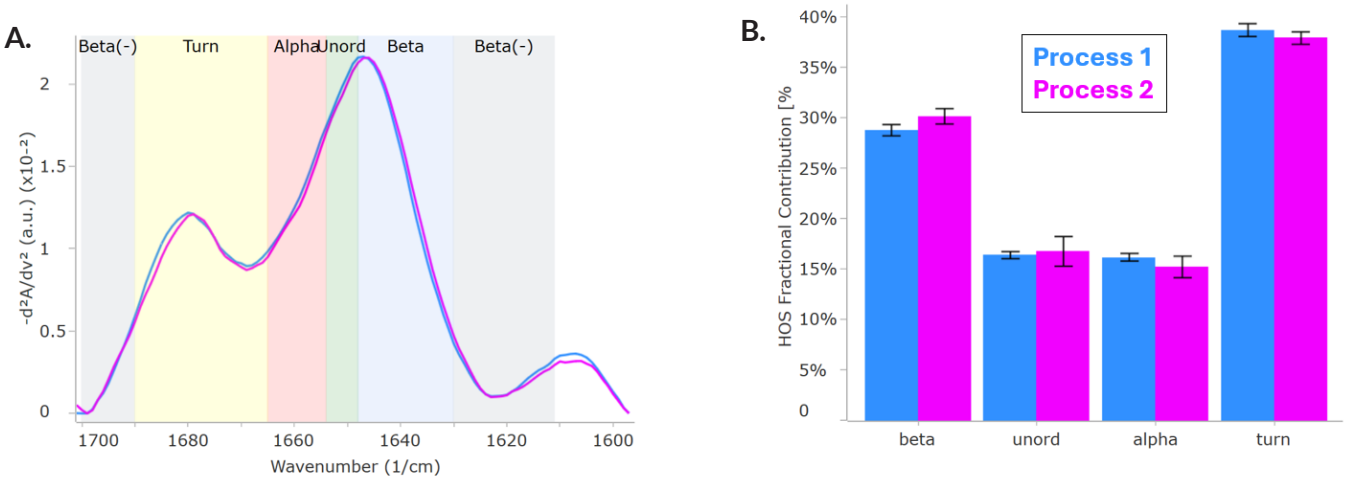

Figure 2: Peptides produced by Process 1 and 2 possess similar secondary structure determined by MMS. (A) Similarity plot of Process 1 and 2 showing the main peak occurs at about 1646 cm^{-1} with a distinct secondary peak at 1680 cm^{-1}. This results in a breakdown of HOS shown in (B) that is mostly turn and beta-sheet structure.

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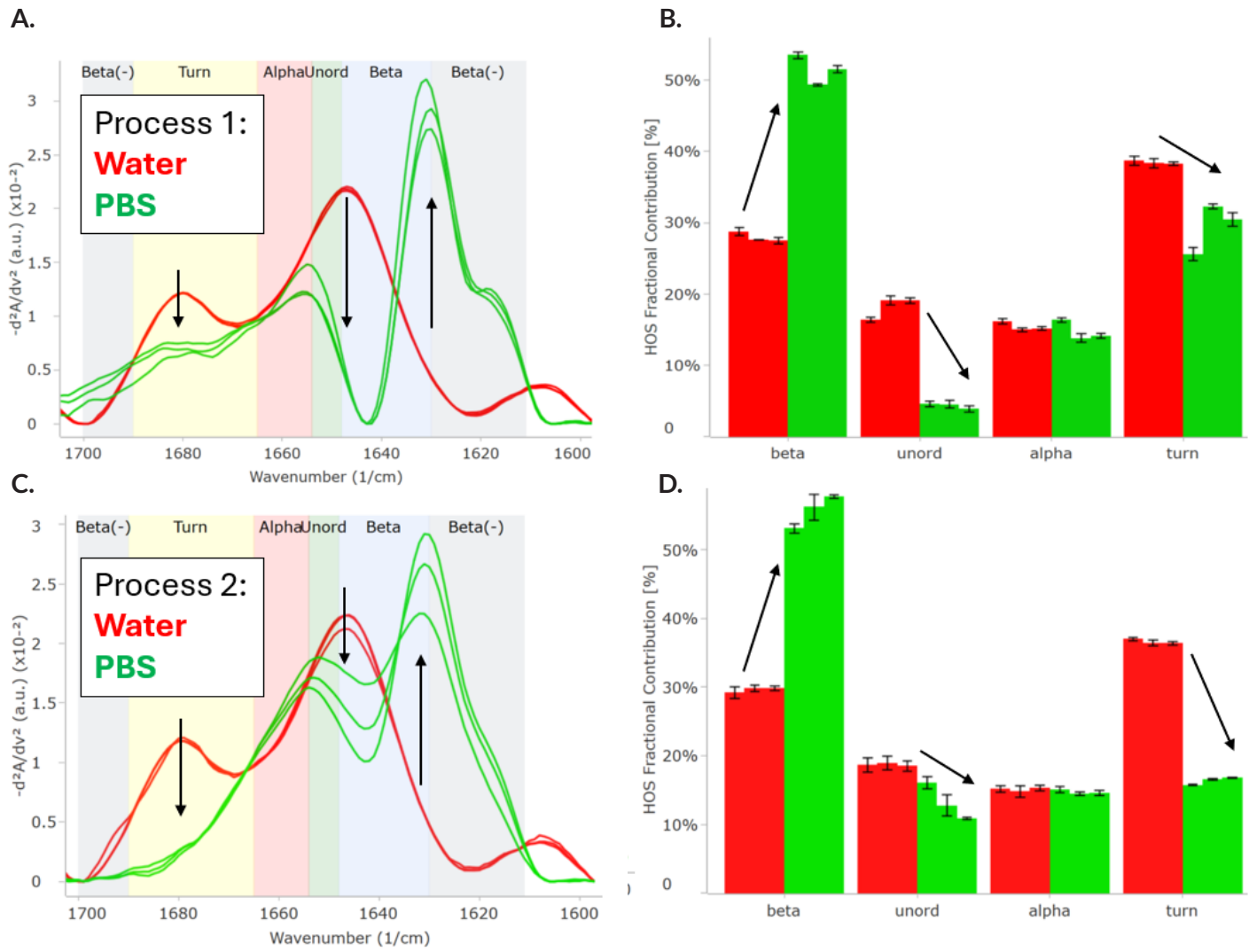

Figure 3: (A-B) Process 1 and (C-D) Process 2 in water and PBS. Both peptides show signs of aggregation in PBS due to the sharp increase in the peak at 1630 cm^{-1} corresponding to beta-sheet structure and loss of the native peaks at 1646 and 1680 cm^{-1}.

Figure 3 shows the spectral comparison along with the HOS of each peptide dissolved in water (red) compared to PBS (green).

Although both Process 1 and 2 showed aggregation when dissolved in PBS, they differ in the extent of change in unordered and turn structure. Process 1 maintained more of the native turn structure than Process 2. Figure 4 shows the direct comparison of Process 1 and 2 when dissolved in PBS.

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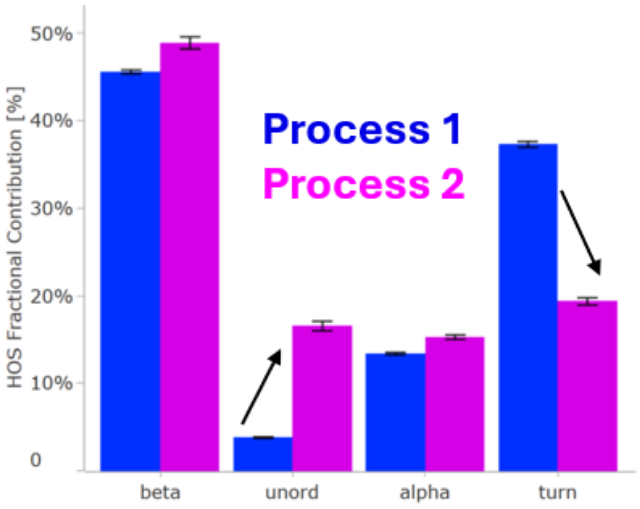

Figure 4: Process 1 and 2 in PBS. Process 1 has significantly less unordered structure and more turn structure upon dissolving into PBS.

The structural disparity in PBS between Process 1 and 2 correlates in the potency difference tested in rats that other biophysical tools missed. These results indicate that the structure in PBS maybe be similar to the structure in the *in vivo* experiments, and that this structure is important for maintaining potency.

Conclusions

In this Application Note, we've shown the capability of Aurora TX to quantitate the secondary structure of peptides from two different processes and distinguish differences in the structural response to physiological buffer conditions. The immunogenic peptides from Process 1 and 2 were indistinguishable via standard biophysical techniques such as CD, FT-IR, DLS, DSC, and SEC-MALS, but showed dramatically different potencies in rats. We propose the testing procedures laid out in this Application Note can be used for quality control purposes to test future batches and process to see if the peptides are more "Process 1-like" or "Process 2-like" as a screening tool for potency testing.

Contributors

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References

1. Banting, Frederick Grant, et al. "Pancreatic extracts in the treatment of diabetes mellitus." *Canadian Medical Association Journal* 12.3 (1922): 141.

2. Miller, W.L., Baxter, J.D. Recombinant DNA—A new source ofinsulin. *Diabetologia* 18, 431–436 (1980).

3. Wang, Lei, et al. "Therapeutic peptides: current applications and future directions." *Signal transduction and targeted therapy* 7.1 (2022): 48.