UNCHAINED LABS

Application Note

Formulations Development for a Biosimilar Using the HUNK

As the terminology suggests, the target product profile for a biosimilar will be similar to but not identical with the originator target product profile. The final drug product formulation may be one of those differences. Regardless of the final formulation, the suitability with regard to stability, compatibility and integrity of the active drug substance should be demonstrated. Any claims with regard to stability must be supported by data.

The optimal formulation is the one that provides optimal long-term stability

In terms of protein physical stability, aggregation depends on both the amount of protein present in forms prone to aggregation and the propensity of those forms (native or denatured) to aggregate. Only the HUNK data provides, for each formulation being investigated, quantitation of:

- **The protein conformational stability**
- **The amount of protein present in the denatured state**
- **The dominant mode and amount of aggregation**

In the example described below the addition of NaCl to the formulation of a biosimilar was found to significantly increase the protein conformational stability – by more than 10-fold. As illustrated in Figure 1, maximizing conformational stability is often very important in an effort to minimize denatured state aggregation. However, conformational stability is only part of the formulation optimization picture. The goal of formulations optimization, in support of optimizing long-term stability, is to minimize aggregation whether it originates from the denatured (or partially denatured) protein or the native protein. Figure 2 illustrates the ability to identify both native protein self-association and denatured protein aggregation from the HUNK data. As is sometimes the case in formulations optimization, and as will be seen in this case, changes in the formulation conditions can have opposing effects on conformational stability and propensity for aggregation. The HUNK is uniquely suited for the simultaneous and quantitative optimization of stability and minimization of aggregates.

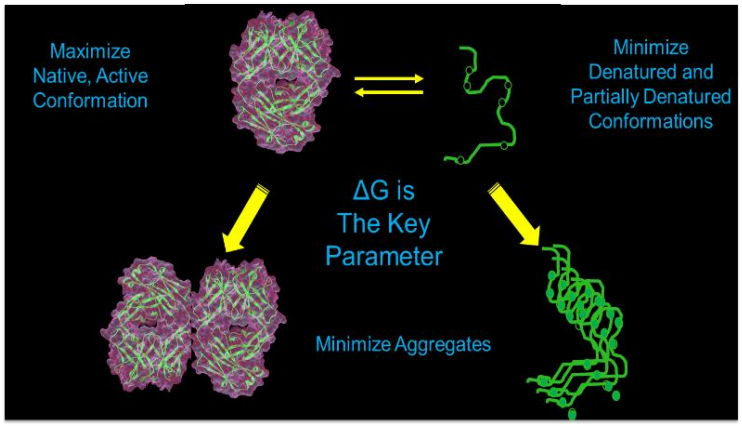


Figure 1. The goal of formulations optimization is to minimize aggregation that originates from either the native protein or the denatured protein. The HUNK data (specifically ΔG) identifies and quantitates these effects.

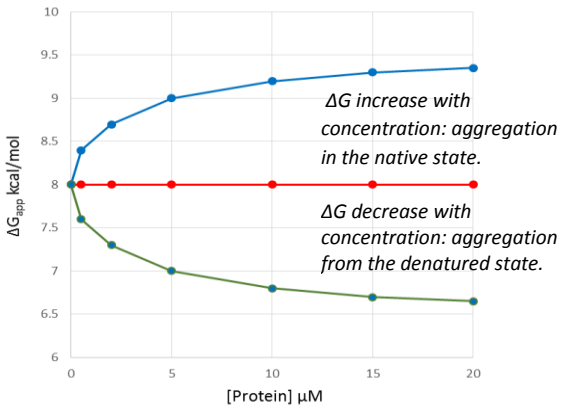


Figure 2. In the absence of aggregation, ΔG must be independent of protein concentration (red line).

In this example the application of NaCl as an excipient component of the biosimilar formulation was being investigated. Shown in Figures 3 and 4 are the HUNK denaturation curves for the 10 mg/mL solution of the Biosimilar at pH 6.80 in the absence of NaCl and in the presence of 100 mM NaCl.

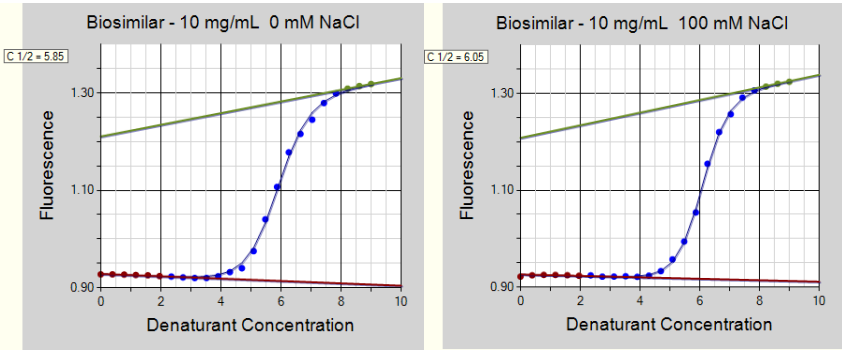
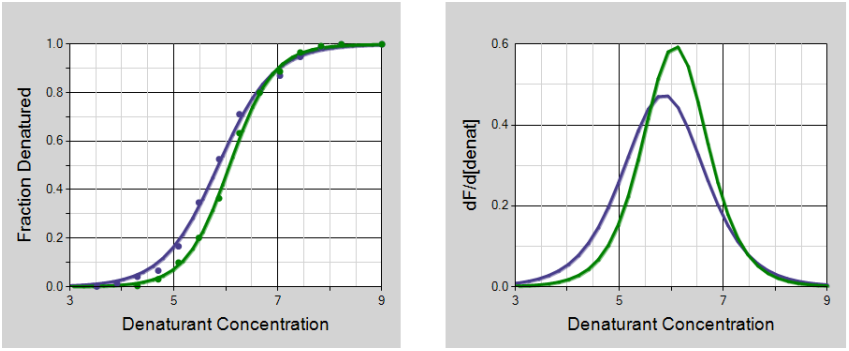


Figure 3. HUNK denaturation curves for a Biosimilar at pH 6.80 in the absence of NaCl (left) and in the presence of 100 mM NaCl (right). Adding 100 mM NaCl to the formulation increases the stability by more than 10-fold.

Analysis of these denaturation curves indicates that the stability is increased from 6.8 kcal/mol in the absence of NaCl to 8.7 kcal/mol in the presence of 100 mM NaCl. This 1.9 kcal/mol increase in stability with the addition of NaCl, all other things being equal, would be considered favorable since the amount of denatured protein in the formulation is reduced more than ten-fold from approximately 10 ppm to 0.4 ppm.

Figure 4. Overlay of the HUNK fraction denatured curves (left) and first derivative curves (right) in the absence of NaCl (blue) and in the presence of 100 mM NaCl (green) illustrates the increase in stability with addition of NaCl.



For this particular Biosimilar, the HUNK data (specifically, the protein concentration dependence of ΔG) indicated there was significant native protein self-association – ΔG increases with increasing protein concentration. As shown in Figure 5 the addition of 100 mM NaCl increased this self-association nearly 15-fold.

Conclusion

For this particular Biosimilar, the HUNK data indicated a significant increase in protein conformational stability with the addition of NaCl and also a significant negative impact on native protein self-association. From the HUNK data, the absolute conformational stability and the amount of denatured protein in the formulation were already known. This mAb was quite stable (ΔG = 6.8 kcal/mol) and the amount of denatured protein was quite small (10 ppm). Based on this knowledge, the developer was able to make the decision to eliminate NaCl from the formulation. The HUNK is uniquely suited for the simultaneous and quantitative optimization of stability and minimization of aggregation.

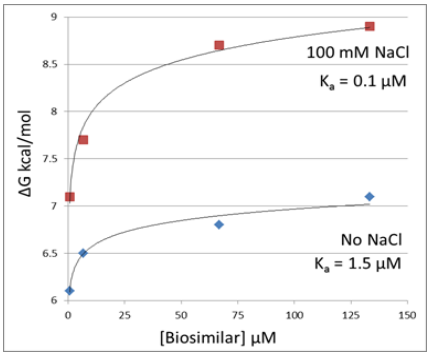


Figure 5. Addition of 100 mM NaCl worsens native protein self-association by 15-fold.

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