Table 6: HOS Characterization as a Tool in Method and Process Development

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SCOPE:
HOS characterization can be used to obtain a deeper and more comprehensive understanding of analytical methods and processes used in biologic drug development. For example, size exclusion chromatography multi-angle light scattering (SEC-MALS) may be used to determine the size or oligomeric state of aggregates that would be routinely quantified using SEC or analytical ultracentrifugation (AUC) may be used as an orthogonal approach to confirm SEC results. Furthermore, HOS is also commonly used to inform decisions and optimization of manufacturing and formulation processes. For example, additional characterization of protein structure and stability in different solution conditions may influence process and product design. In this session, we will discuss how HOS characterization may inform method development and optimization as well as process development for biologic therapeutics.

QUESTIONS FOR DISCUSSION:
1. How have you used HOS characterization to inform analytical method development and optimization? What techniques did you use? How was the method modified or improved? Was there any influence on sample handling or sample preparation?
2. How have you used HOS characterization to inform process development activities? What techniques did you use? How did the results impact upstream or downstream process development?
3. How have you used HOS characterization to inform drug product and formulation development? What techniques were most useful?

DISCUSSION NOTES:
- Use of AUC to confirm results by SEC-MALS is very common (perhaps expected) for biologic development; data likely not generated at IND but present in BLA filing
- Examples discussed where biophysical tools were used to determine optimal conditions to denature a protein for CE-SDS; not all proteins denature the same way; other methods may be used to determine the optimal concentration of denaturant, time and temperature required to denature
- Another example where protein aggregation occurred during cIEF analysis that was related to sample prep in a particular matrix. Some biophysical tools (i.e. DSF) were used to look at product stability in different matricies.
- Examples where loading a column with different sample concentration resulting in a different chromatographic profile could suggest an equilibrium is present and further analysis might be need to characterize this relationship (i.e. AUC?)
- Some participants commented that their analytical methods were well established and most of this work was done very early in development; others comments that particularly for novel molecules orthoganol work may come later in development
- There was some discussion about the use of NMR as a complementary tool and how to interpret data if a difference is observed in spectra between samples; would require deeper investigation and peak assignments would likely be required

- There was also some discussion about difficulties using SEC-MS as a complementary method; small amounts of impurities or adducts can be detected here that overlap with other peaks and were not detected by SEC-MALS; analysis can be challenging and hard define what is acceptable
- Some discussion about whether Tm values by DSC/DSF correlated with stabiluty; for some IgGs only Tm1 correlates with stability; discussion about whether measures of colloidal stability are more impactful (i.e. kD, B_{22})
- Some discussion about use of kD to optimize solution conditions for TFF to reduce viscosity of aggregation; Also use of biophysical characterization to support low pH viral inactivation steps (time, temp, etc.) or solubilization of proteins produced in inclusion bodies in bacteria (i.e. denaturant conc., time, temp)
- Some discussion around use of Cryo-EM – does it select for molecules that have symmetry and discount difference conformations? NMR may be a more reflective ensemble of everything in solution depending on Cryo-EM data analysis
- Discussed options for determine Kd (equil. Constant) – most people use ITC or AUC (could use NMR as well)