Table 3: Analytical Ultracentrifugation

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Scope:

Analytical Ultracentrifugation (AUC) is an excellent analytical tool for the detailed characterization of biophysical properties of proteins and the heterogeneity of their samples. Sedimentation Velocity (SV) is the most commonly used method for the characterization of protein biopharmaceutical products and their high molecular weight species. More recently, SV-AUC has been applied for the detailed identification of key critical quality attributes in adeno-associated viruses (AAV). This roundtable discussion focuses on highlighting best practices for carrying out successful SV-AUC experiments and common pitfalls and mistakes to avoid.

Questions for Discussion:

1. How do you prepare your samples for a successful SV-AUC experiments? How do you set up your data acquisition parameters?
2. How do you analyze your samples? What software do you use? How do you report your data?
3. Do you have any experience with the Optima AUC? What are some the advantages/drawbacks compared to the XLI/XLA?
4. Besides determination of aggregate level, what other applications of AUC do you seek?

Discussion Notes:

1. How do you prepare your samples for a successful SV-AUC experiments? How do you set up your data acquisition parameters?

   For interference measurements of complex buffers, meniscus-matching centerpieces are highly recommended. This is of increasing importance as AUC moves into the high-concentration space. Temperature equilibration time varies from lab to lab (30 min to 2h). A best-practice paper by a CASSS HOS AUC focus group will be covering that topic.

2. How do you analyze your samples? What software do you use? How do you report your data?

   Don´t limit yourself to one AUC analysis software package. Each of it has its strength and capabilities which are not available in the other packages. Sedimentation coefficients often reported up to three decimal places, aggregate values only one decimal place.

3. Do you have any experience with the Optima AUC? What are some the advantages/drawbacks compared to the XLI/XLA?

    Drawbacks:

    Convection is visible is most of the datasets collected with Optima AUCs but the extend varies from instrument to instrument. Worse than in XL-I due position of thermal elements. No live interference monitor available.

    Advantages:

    UV-optical system is much better although it is not a full multiwavelength optical system (spectrum at each radial point). Temperature setting closer to the true value.

4. Besides determination of aggregate level, what other applications of AUC do you seek?

    Determination of extinction coefficients and second virial coefficients (non-ideality terms k_S and k_D). Full/empty analysis of AAV triggered a more widespread use of the technique.