NovaSheet
Technology: AF4-UV/Vis
Application: Protein Aggregation Study BSA

Analysis Conditions / System Set-up

Solvent	: 0.1 M PBS Buffer, pH 7.4	- Channel Thickness: 250 µm
Injection Volume	: 5 µL	- Membrane: NovaRC PLGC 10 kDa
Sample Concentr.	: 4 mg/mL	- Channel Flow: 0.32 mL/min
Cross Flow	: 5.8 mL/min	- Tip Flow: 0.05 mL/min
Focus Time	: 3.5 min	
FFF System	: postnova AF2000 Focus Series	- Asymmetric Flow Field-Flow Fractionation
LS Detector	: postnova PN3000SLS/DLS	- Static/Dynamic Laser Light Scattering Detector
UV Detector	: postnova PN3240 UV/Vis	- 4-Channel UV/Vis Detector (Wavelength used: 280 nm)

Protein Aggregation has become an important issue in the development and QC of pharmaceutical formulations and novel drugs. The activity, biological availability and possible negative immune responses are directly connected with the existence and the generation of aggregates in such pharmaceuticals.

Thus not only the FDA, but also an increasing number of official public and private research institutions have shown growing interest in the characterization of these aggregates. The goal is to determine the exact conditions when and how much aggregation takes place in protein based pharmaceuticals and how to avoid this process.

However until now there were no or only insufficient analytical technologies available to handle this task and to provide these kind of information. Standard chromatography based approaches can not solve this questions, because of the following reasons:

1) Packed columns exert extensive shear forces which destroy or alter aggregates in their original form;
2) The sample experiences strong interaction with the column stationary phase;
3) Separations are too long to conduct and show little or even no reproducibility;
4) Special eluents and solvents have to be used for the separation process which have limited suitability for the samples under investigation.

These problems can be overcome with the unique FFF technology invented by Prof. Giddings, one of the founders of postnova/FFFractionation.

FFF is a chromatography-like separation technique, using a separation channel instead of separation column. No stationary phase is used inside the channel, which provides unique advantages, fast separation times, nearly interaction free sample characterization and the flexibility of a broad range of solvents which can be used.

Also FFF offers a wide separation range from 1 nm to 100 µm and from 1 kDa up to several billions of daltons. Thus, the technology can be used to get a complete view of the sample.

Polymeric and particulate species can be separated simultaneously at in the same run and no material is filtered out by the column material.

This application note describes results from the FFF characterization of BSA – Bovine Serum Albumin – as an example for a protein which is forming aggregates. The figure shows that the characterization is performed very fast and under gentle conditions with high resolution to get complete base-line separation of the BSA monomer and the corresponding aggregates.

BSA – Bovine Serum Albumin

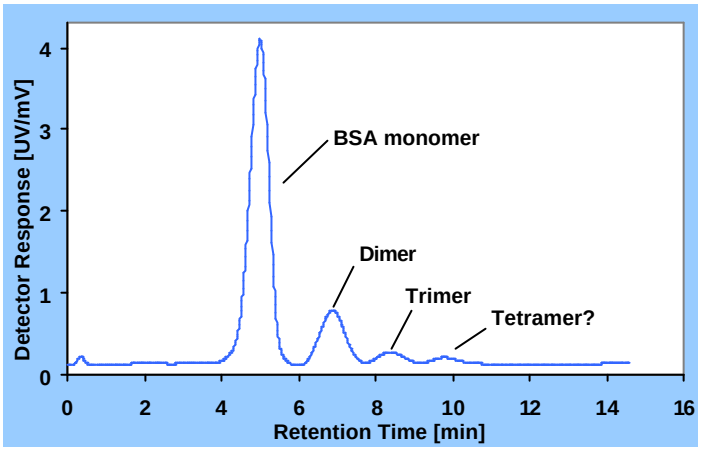

Fig. 1: AF4-UV Characterization of BSA and Aggregates.

Why use AF4-UV/SLS for the characterization of proteins and aggregates?

- ? High resolution separation of proteins and their aggregates.
- ? Fast, gentle and nearly interaction free separation without stationary phase.
- ? Separation of complex matrices without sample preparation or filtering.
- ? Easy direct coupling of AF4 with many powerful detection systems as LS, UV, RI, SEM, DLS, etc.

For further application information about the characterization of proteins and aggregates by FFF as well as more literature, please contact us directly at: info@postnova.com.