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

Analysis Conditions / System Set-up

Solvent	: 0.1 M PBS Buffer, pH 7.4	- Channel Thickness: 250 µm
Injection Volume	: 10 µL	- Membrane: NovaRC PLAC 1 kDa
Sample Concentr.	: 42 mg/mL	- Channel Flow Out: 1.1 mL/min
Cross Flow	: 3.6 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
RI Detector	: postnova PN3140 RI	- High Sensitivity RI Detector

Protein Aggregation has become an important issue in the development and QC of pharmaceutical formulations and novel drugs. The activity, the bio-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 an 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.

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 these 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 eluants 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 analytics and FFFractionation.

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

In this application note results from the characterization of Ferritin is presented, as an example for a protein which is forming aggregates. The different figures show that the characterization is performed fast, under gentle separation conditions, and with high resolution to get complete base-line separation of the protein and its corresponding aggregates.

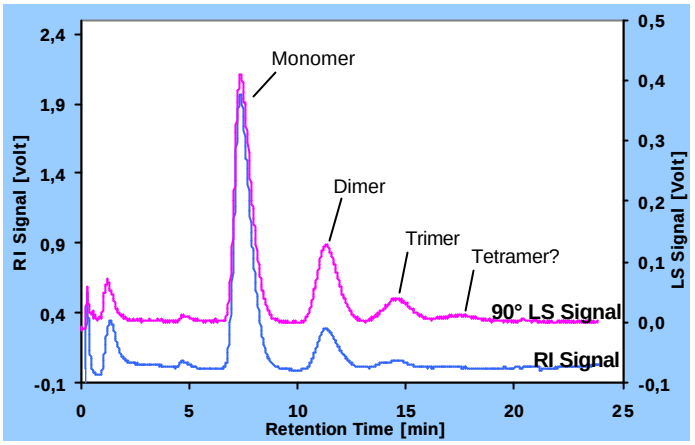

Fig. 1: AF4-MALS Characterization of Ferritin and its Aggregates.

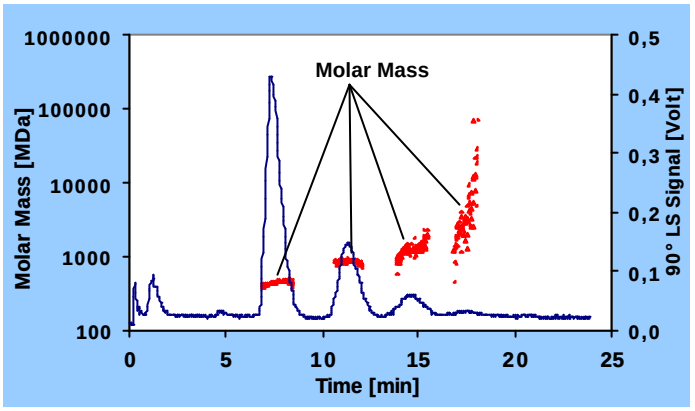

Fig. 2: Absolute Molar Mass Calculation of Ferritin and its Aggregates.

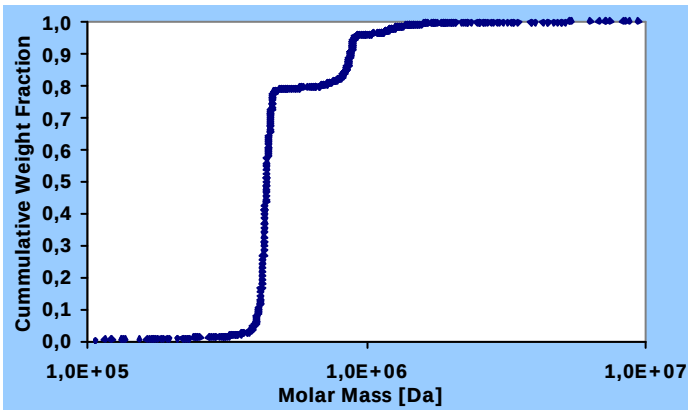

Fig.3: Cumulative Molar Mass distribution of Ferritin and Aggregates.

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