NovaSheet
Technology: AF4 - SLS
Application: Pharmaceutical Peptide

Analysis Conditions / System Set-up

Sample:	Aggregation of a Pharmaceutical Peptide
Solvent/Eluent:	De-ionised Water (pH 6.5)
Channel Flow:	0.5 mL/min
Cross Flow:	20% (0.125 mL/min) Constant Field
Separation System:	Postnova AF1000 Series Assymmetric Flow Field-Flow Fractionation
Detector 1:	Postnova PN3000 Laser Static Light Scattering Detector (SLS)
Detector 2:	Postnova PN3210 UV Detector (Wavelength 280 nm)

Aggregation studies of proteins and peptides are an increasing field of interest. Especially since FDA regulations require to monitor the exact amount of aggregates in a pharmaceutical active protein or peptide formulation. Classical column chromatography is limited to soluble analytes with small or medium molar mass. In contrast to that FFF and especially the AF4 technology has the advantage to cover a much broader size range of analytes from high molar mass analytes up to nano- and microparticles. Beside the monomer peptides and proteins also the corresponding aggregates and even suspended particles can be separated and characterized using the AF4 technology especially when coupled with Laser Light Scattering and UV detection.

In this application a Postnova AF1000 Series Asymmetric Flow Field-flow Fractionation system (AF4) coupled with a Postnova PN3000 Static Laser Light Scattering detector (SLS) and a Postnova PN3210 UV detector was used to perform an aggregation study on a pharmaceutical active peptide formulation. The time required for aggregate formation under different storage conditions was investigated. Fig. 1 and 2 show the separation of the peptide monomer and the corresponding monomers for fresh and at 40° C stored samples using UV detection (Fig. 1) and Static Light Scattering detection (Fig. 2).

The figures show that the peptide monomer and the newly formed aggregates can be separated easily, very fast (< 10 min) and gentle with a minimum of shear forces and interaction using the AF4 technology. Clearly the increased formation of aggregates and the decreased concentration of monomers can be observed in the older samples stored at elevated temperature for 90 days. Because of the unique nature of the basics of static light scattering, this detection shows a significant higher sensitivity to particles and aggregates than UV detection. But both together allow a multi-detection approach, where the UV signal gives a mostly mass dependent signal and the light scattering signal gives a mostly size dependent signal.

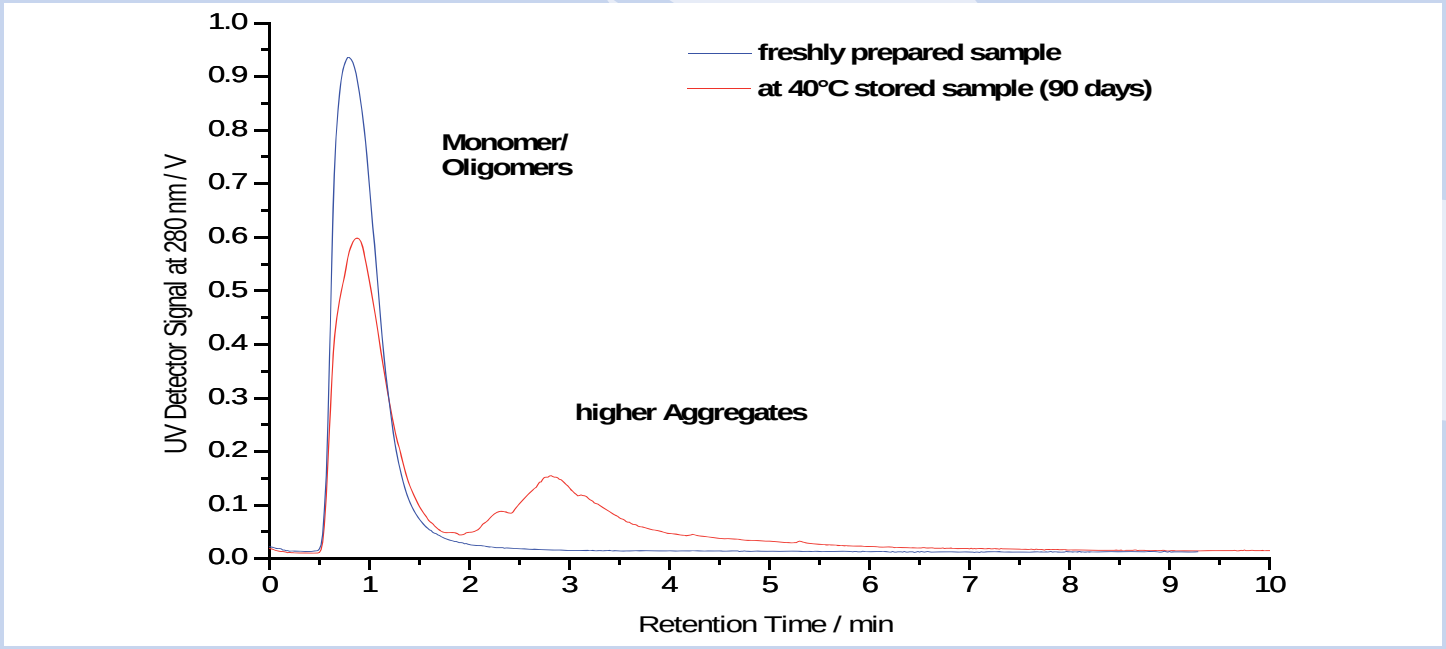

Fig. 1: Separation of peptide sample (fresh prepared and stored for 90 days) with UV detection.

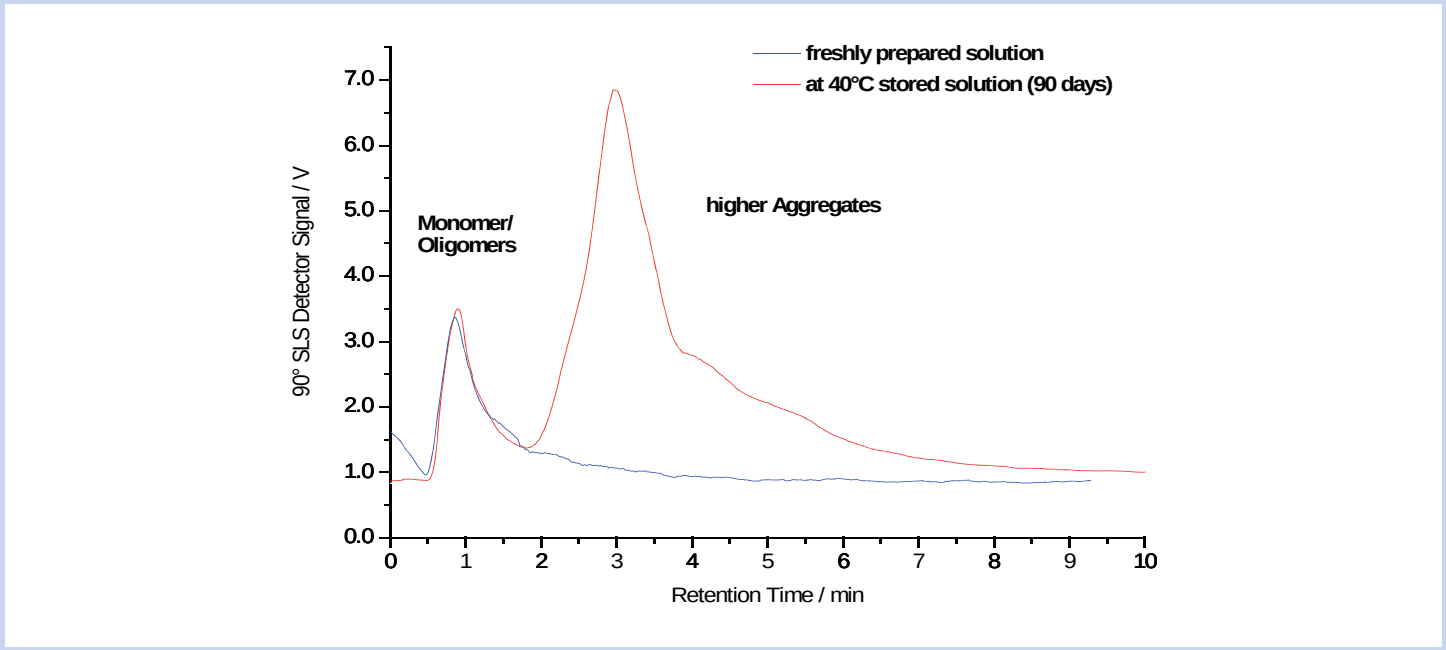

Fig. 2: Separation of peptide sample (fresh prepared and stored for 90 days) with SLS detection.

Why use AF4-SLS?

- When you need simultaneous and fast/gentle separation of small and big sized analytes or of sensitive samples conserving their bioactivity.
- To obtain high resolution separations of complex samples with broad and multi-modal distributions without cut-off limitations known from column chromatography.
- To set-up on- and off-line couplings with other analytical techniques as UV, RI, SLS, DLS, SEM, FTIR etc. to get multi-dimensional information.
- In general for bio/polymers, nanoparticles, proteins, viruses and liposomes in the size/ MW range of 1 kDa – 100 MDa and 1 nm – 100 µm.