NovaSheet

Technology: AF4-SLS/DLS

Application: Characterization of Virus and Proteins

Analysis Conditions / System Set-up

Solvent	: 100 mM PBS buffer, pH 7.4	
Injection volume	: 100 µL	
Sample Concentr.	: 0.5 mg/mL	
FFF System	: postnova AF10000 Series	- Asymmetric Field-flow Fractionation
LS Detector	: postnova PN3000DLS	- Static / Dynamic Light Scattering Detector
UV Detector	: postnova PN3240 UV/Vis	- 4-Channel UV/Vis Detector (Wavelength used: 210 nm)

Biotechnology has progressed in the last years as one of the most important disciplines to accelerate drug discovery in the pharmaceutical industry.

One important area of modern drug development is the use of viruses as drug-delivery systems and/or as highly efficient vaccines. But in order to be able to completely characterize these analytes and also to maintain a special level of quality, new high performance analytical techniques have to be used. Here not only the molar mass, but also the particle size and the existence and quantity of possible aggregates is in the focus of investigation, as these parameters have a strong influence on the activity an safety of such pharmaceutical products.

By using the traditional analytical separation technologies, as SEC and GPC, viral samples are difficult, if not impossible, to separate. The stationary phase with its large surface area hinders the efficient separation and results in long analysis times, non-ideal interactions and sometimes generation of artifacts.

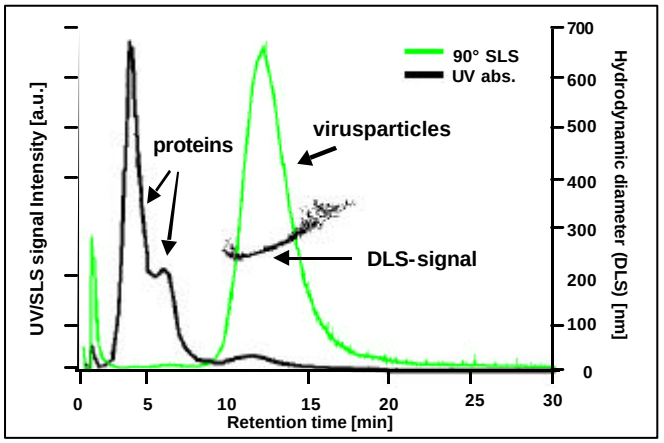

Fig. 1: Virus and Protein Characterization by AF4

But with AF4, viruses and other bioparticles can be easily separated and characterized in a fast and gentle way, without the use of a stationary phase. The combination of AF4 with different on- and off-line detection systems results in a very efficient analysis where even the most complex substances can be investigated.

Figure 1 shows such an example of a separation of a fermentation solution including proteins and virus particles. As detection UV at 210nm, Static Laser Light Scattering and Dynamic Laser Light Scattering was used. Also Dynamic and Static Laser Light Scattering (DLS, SLS) was applied as a powerful detection tool with the ability to measure particle size and MW simultaneously.

The sample includes proteins, (60 kDa/120 kDa) and virus particles with a diameter of about 300 nm. The use of different detection shows clearly shows the different strengths of the systems. UV detection is strongly sensitive to the protein population, while the virus particles produce only a weak signal. Light scattering detection on the other hand is much more sensitive in the bigger size range and is able to detect even small virus concentrations. With the integrated PN3000SLS/DLS detector, the molar mass and the radius of the proteins and the viruses can be determined simultaneously, fast and with high precision.

Why use AF4 for Virus/Protein Characterization?

- ? Fast, gentle and nearly interaction free separation without stationary phase.
- ? High resolution separation of proteins, viruses and aggregates simultaneously over a broad size range.
- ? Separation of complex mixtures without intensive sample preparation, filtering or centrifugation.
- ? Easy coupling with many other analytical detection systems as MALS, UV, RI, DLS, FT-IR, MS, etc..

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