

Characterization of Virus Like Particles by Asymmetrical Flow Field Flow Fractionation

Virus Like Particles (VLP) are of growing interest in pharmaceutical sciences. They are used for vaccination and immune stimulation. For quality assurance there is a tremendous need for a technique that allows detection and separation of different VLP fractions (fragments, monomers, dimers, trimers, aggregates) within a formulation. GPC, which is often the analytical standard for protein formulations, is not an ideal separation method due to the VLP particle sizes. Thus, Dynamic Light Scattering (DLS) is often used to investigate the quality of VLP formulations.

As a bulk method—without physical separation—DLS has strong limitations in distinguishing differing VLP species. In this study we compared Asymmetrical Flow Field Flow Fractionation (AF4) with DLS to elucidate whether AF4 renders a better insight into VLP purity.

A stressed VLP sample was subjected to DLS, using an RI dispersant 1.45, viscosity 0.89 cp and a wavelength of 633 nm. The polydispersity index (PI) and the hydrodynamic diameter were determined by using a NNLS (Non-Negatively Constrained Least Squares) fitting algorithm. An Eclipse AF4 (equipped with a DAWN and UV detection) was applied to separate the VLP fractions and to determine molecular weight (M_w), radius and amount of each VLP species.

The DLS measurements revealed one peak for the whole VLP formulation (Screen capture 1), with a hydrodynamic diameter of 30 nm. The PI of the formulation was below 0.12 typically referring to monomodal distributions. From this result it was assumed that only VLP monomers are present in the formulation. However, AF4 analysis revealed that this “monomodal” VLP formulation consists of *many* different VLP fractions like fragments, monomers, dimers, oligomers and aggregates (Screen capture 2). Furthermore, the M_w and the radius of the monomers, dimers and trimers and the proportions of all fractions could be determined from the Eclipse-MALS analysis (Table 1).

DLS as a standard analytical tool is not capable of resolving different VLP fractions. By contrast, the Eclipse is capable of separating VLP formulations into their fractions. Additionally, the M_w , size and amount of each fraction can be determined, thus making the Eclipse-MALS an indispensable system for the absolute characterization of VLP.

Table 1. Eclipse Results

VLP	Fragments	Monomer	Dimer	Trimer	Oligomers and Aggregates
amount [% of total]	0.8	89.0	6.5	1.4	2.3
M_w [Da]	n.a.	3.60E+06	6.60E+06	9.30E+06	1.3E+07 to 2.0E+08
diameter [nm]	n.a.	18.0	30.2	31.4	n.a.

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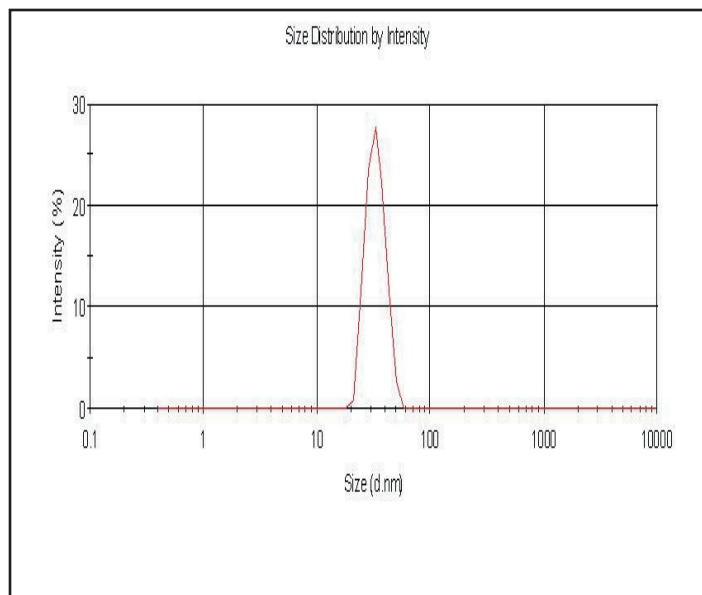


Figure 1. DLS analysis of the VLP indicating a monomodal distribution.

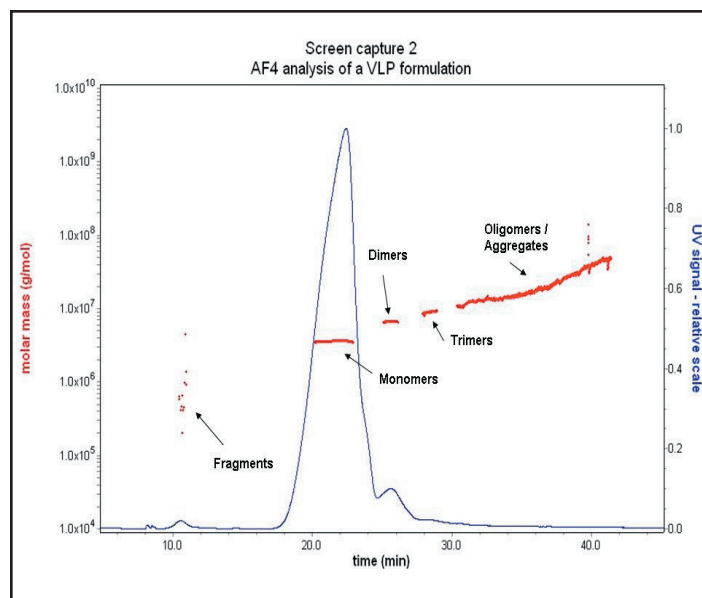


Figure 2. Eclipse separation and MALS detection of the oligomers of the VLP fraction indicating the sample is not monomodal.