APPLICATION NOTE

AN2614: Virus-like particle characterization using FFF-MALS

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Introduction

Virus-like particles (VLP), used for vaccination and immune stimulation, are of growing interest in the pharmaceutical sciences. In quality assurance there is a great need for techniques that characterize different VLP fractions (fragments, monomers, dimers, trimers, and aggregates). We have recently demonstrated that the separation and subsequent quantification of different VLP species is possible by field-flow fractionation combined with multi-angle light scattering (FFF-MALS). Common disadvantages of this technique, like long equilibration and analysis times, the need for high sample amounts and large eluent volumes, are overcome through the use of short channel geometries.

Materials and Methods

A stressed VLP sample was analyzed by an FFF-MALS system comprising an Eclipse™ FFF controller, standard HPLC modules such as pump, autosampler and online UV detector, and a DAWN® MALS detector. Either a Short Channel or a Long Channel was used, both with a 350 µm spacer height. MALS and UV data were analyzed to determine molar masses of the eluting fractions in order to assign identity of monomer, fragment, dimer, trimer or higher aggregate.

Results and Discussion

Comparative AF4 measurements of VLPs with the Long and Short Channels revealed similar peak heights when 20 µg of VLP were injected in the Long Channel vs. 10 µg VLP in the Short Channel (Figure 1). The increased peak heights obtained with the Short Channel for low sample injected masses are due to sharper peaks and the resolution is slightly better. At the same time, analysis duration and solvent volume were reduced significantly (Table 1).

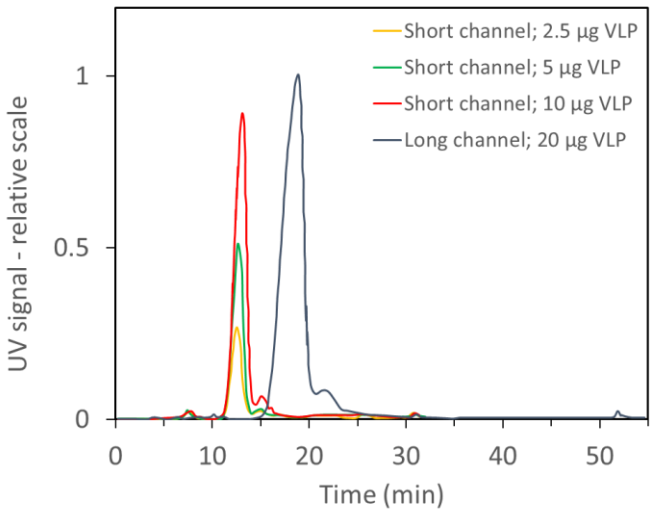

Figure 1. Comparison of peak heights, and therefore sensitivity, between Long and Short Channels.

Table 1. Comparison of duration, eluent volume and sample mass between the Long and Short Channels for equivalent sensitivity.

	Long channel	Short channel
Time/run	56 minutes	31 minutes
Eluent volume/run	159 mL	70 mL
Injection Amount	20 µg	2.5-10 µg

Using the Short Channel, analysis is possible with significantly less sample amount. In Figure 2 we see close correspondence, repeatability and accuracy of the elution times and molar masses of the various species despite quite different injected masses.

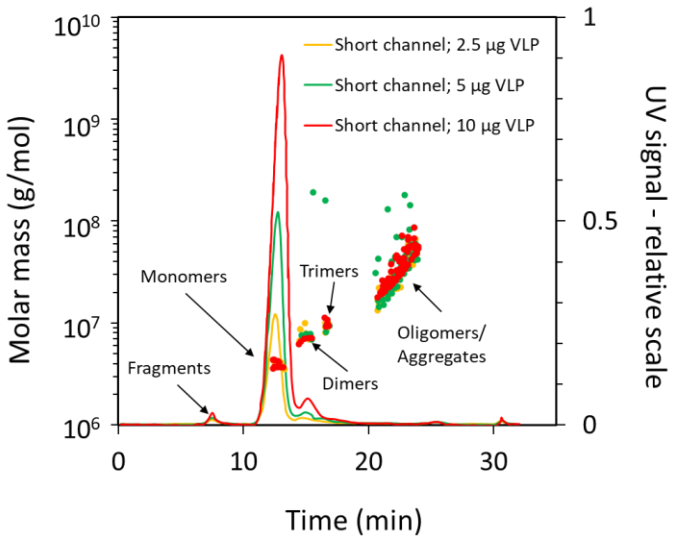

Figure 2. Different injection amounts compared, all using the Short Channel.

Conclusion

Traditional FFF long channels have limitations concerning sample amount and separation time. By contrast, utilizing Wyatt's Short Channel, analysis of far lower VLP amounts is possible in clearly shorter time and remarkably lower eluent volumes. Thus, it can be stated that the Short Channel is a clear improvement for VLP characterization as compared to the Long Channel.

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