Adenovirus Particle Characterization

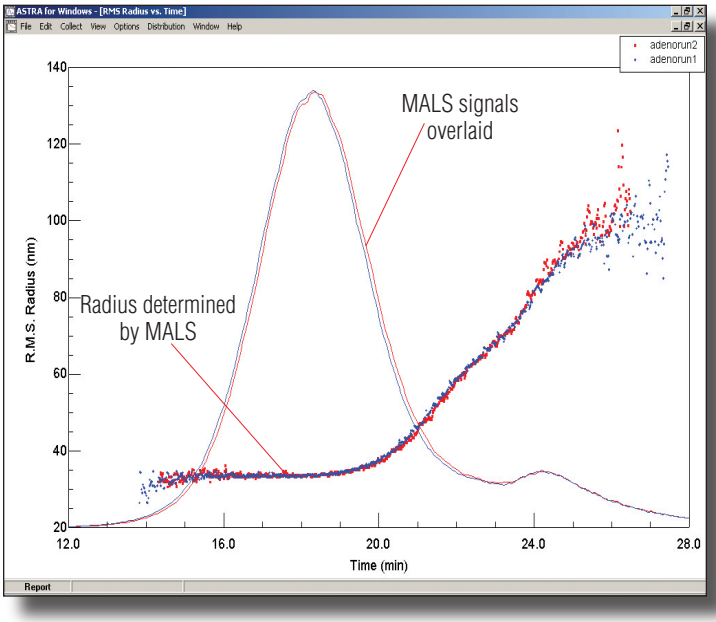
Adenoviruses are non-enveloped icosahedral virus particles. In the developing field of gene therapy recombinant adenovirus has been used as one of the most successful means of delivering genes of interest into target cells. As with any other biopharmaceutical, it is crucial to characterize the manufactured recombinant adenovirus in terms of its size distribution, aggregation, and chemical potency (such as concentration in terms of absolute virus particle counts per unit volume).

Because of the large size of adenovirus, SEC is not appropriate for providing information on the size distribution and aggregation of this virus. In this application note we report the results, in collaboration with the scientists at Biogen, of characterizing adenovirus particles using the Eclipse field flow fractionator (FFF) equipped with the DAWN EOS multi-angle light scattering (MALS) detector.

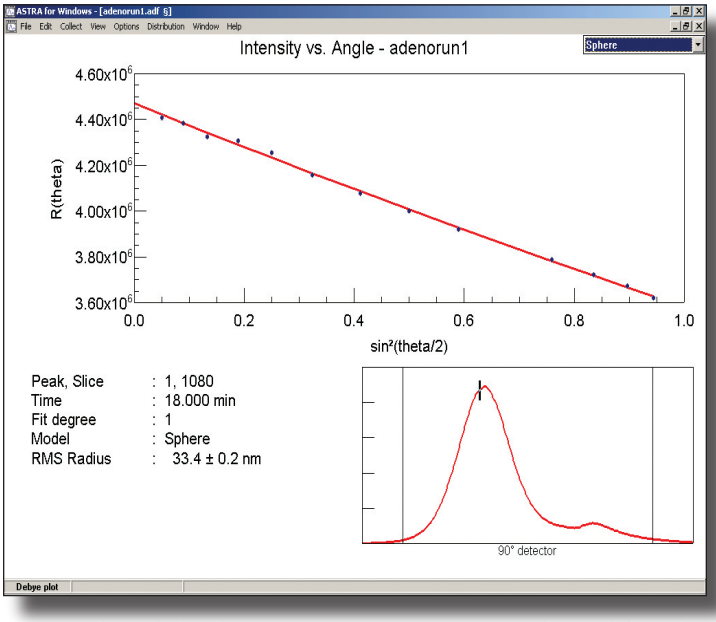
Figure 1 shows root-mean square (RMS) radius *versus* elution time in FFF of a Type 5 adenovirus sample run in its formulation buffer. The main peak eluting from 12 to 23 minutes contains mostly monomer virus having an RMS radius of 33.4 ± 0.2 nm. This RMS value translates into a radius for an equivalent homogeneous sphere[1] of 43.1 ± 0.3 nm, which is in *perfect* agreement with the recently reported mean particle value radius for a *single* adenovirus particle of 43.1 ± 5.4 nm, determined using field emission scanning electron microscopy by the Adenovirus Reference Material Working Group (ARMWG) and the U.S. Food & Drug Administration (FDA).

The peak from 23 to 28 minutes contains virus aggregates having RMS values approaching 100 nm. Results from two runs show excellent reproducibility of the FFF-MALS method. RMS radius of the adenovirus at each eluting slice is calculated from the initial slope of angular dependence of light scattering signals at angles from 15° to 165° as shown in Figure 2.

From the results demonstrated in this note, we can conclude that Eclipse FFF combined with MALS detector allows the detection of aggregation of the recombinant adenovirus sample as well as the measurement of its size distribution. This information is far more useful in characterizing and assessing virus aggregation than that achieved by measuring an average radius value on an unfractionated sample.



RMS radius versus elution time in FFF of a Type 5 adenovirus sample. Results from two runs show excellent reproducibility of the FFF-MALS method.



RMS radius of the adenovirus at each eluting slice is obtained from the initial slope of angular dependence of light scattering detectors placed at angles from 15 to 165°.

[1] RMS radius = $(5/3)^{1/2}$ R , Charles Tanford, "Physical Chemistry of Macromolecules", page 306.

[2] Product Information Sheet for VR-1516 Adenovirus Type 5 Reference Material from the American Type Culture Collection (www.atcc.org).

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