

Application Note

----- Size distribution analysis of Adenovirus ----- using the CPS Disc Centrifuge

Adenovirus for human gene therapy

Recombinant adenovirus is one of the primary vectors for human gene therapy. However, the aggregation of unstable virus has been a recurring problem during the production of purified virus for human therapeutics. To facilitate the development of a robust manufacturing process for recombinant adenovirus vectors, a convenient and reliable size distribution analytical assay is necessary and in this application note shows the CPS Disc Centrifuge is applicable to this purpose.

Instrument/Method description

The measurement range of the CPS Disc Centrifuge are particles in the range from 0.005 micron to 50 microns. The CPS Disc Centrifuge is most effective with particles between 0.005 and 20 microns. The analyzer measures particle size distributions using centrifugal sedimentation within an optically clear spinning disc that is filled with fluid. Sedimentation is stabilized by a density gradient within the fluid, and accuracy of measured sizes is insured through the use of a known size calibration standard.

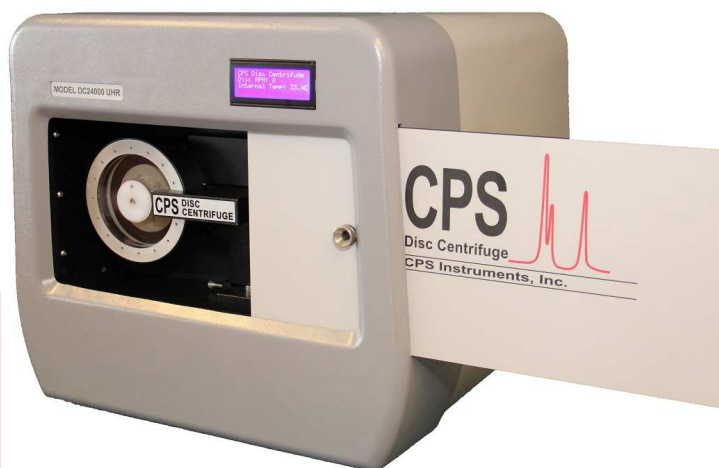


Figure 1 CPS DC24000 UHR with open door

For the measurements below the CPS DC24000 UHR was used. The DC stands for Disc Centrifuge, 24000 stands for the maximum speed (rpm in this case equal to 29000 g-force), and UHR stands for Ultra-High Resolution. This is the most advanced system CPS Instruments offers, but a DC12000 and a DC18000 are available when the ultra-high resolution and high resolution speed/g-force is not needed.

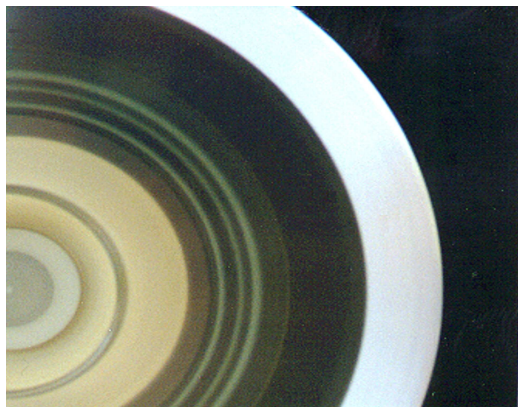


Figure 2 High concentrated sample with different particle sizes sediment in a disc

Results

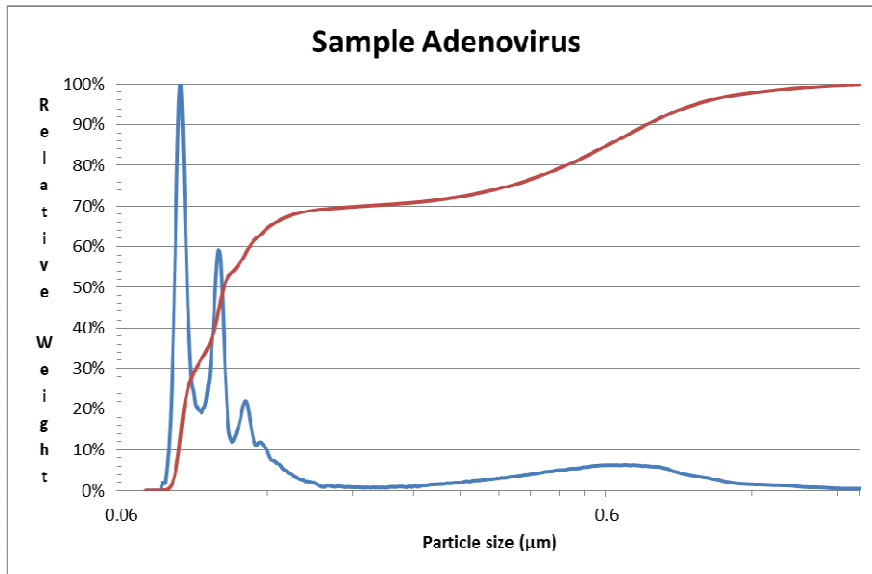


Figure 3 Weight distributions of an Adenovirus sample

consistent with a rigid sphere approximation for adenovirus, as well as a large aggregate of 0.65 µm. The appearance of viral aggregates is confirmed by increased light scattering based on A320:A260 ratios. The technique could be useful for monitoring the kinetics of aggregation for adenovirus and other DNA and RNA viruses in the submicron region.

Conclusion

The CPS Disc Centrifuge can be a critical tool for purification development of viral vectors for meeting therapeutic and research needs. The resolution, sensitivity, and run-to-run repeatability are unmatched by other particle sizing instruments.

For more information please contact your local representative or:

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