Adenovirus Particles

Monitoring the production and purification process

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Recombinant adenovirus is one of the primary vectors for human gene therapy. However, the aggregation of unstable virus has been a recurring problem during production and purification of virus for human therapeutics.

To facilitate development of a robust manufacturing process for recombinant adenovirus vectors, an analytical tool for measurement of extremely high resolution particle size distribution is necessary - the CPS Disc Centrifuge is ideal for this application. The CPS Disc Centrifuge can detect virus concentrations down to 0.01% (w/v) or 1 *106 particles per ml. The apparent hydrodynamic diameter of recombinant adenovirus was determined to be about 0.079µm. The disc centrifuge analysis was able to detect adenovirus aggregates (dimers, trimers, and tetramers) consistent with a rigid sphere approximation for adenovirus, as well as a large aggregate of 0.65µm (see Fig. 2 below).

The technique could be highly effective in monitoring the kinetics of aggregation for adenovirus and other DNA and RNA viruses in the sub micron region. Therefore the CPS Disc Centrifuge can be a critical tool for purification development of viral vectors for meeting therapeutic and research needs.

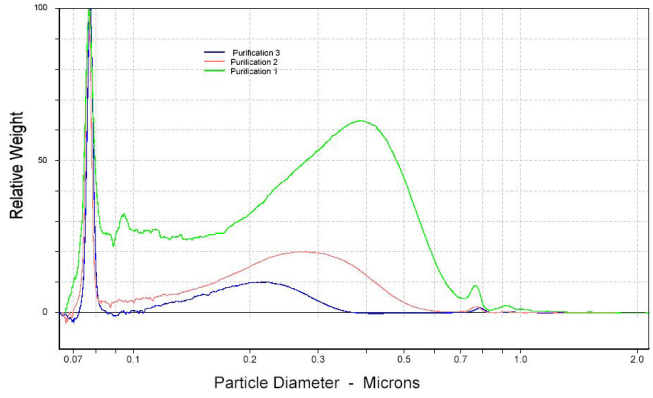

Fig.1 - *Measurements at different stages of an adenovirus purification process (overlaid).*

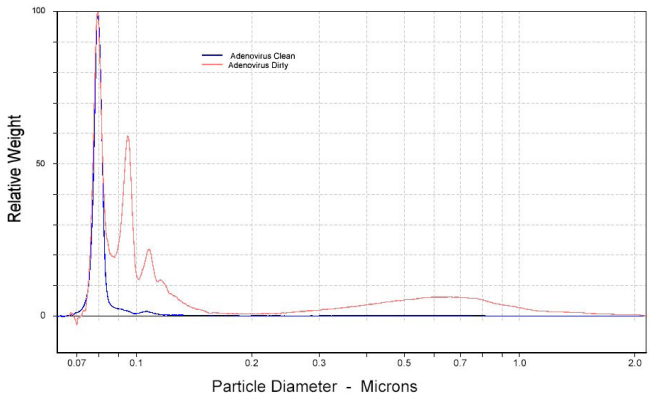

Fig.2 - *Measurements taken on clean and dirty adenovirus. Aggregations can clearly be seen in the dirty sample.*

An Introduction to Differential Centrifugal Sedimentation

Differential Centrifugal Sedimentation (DCS) is an innovative, yet simple technique that has been 'reborn' in recent years. Previous limitations and difficulties with the technique of sedimentation have been overcome with advances in technology and some smart thinking regarding instrumentation and disc design. DCS is now a powerful tool in measuring nano particle size distributions down to around 3nm.

With the unique ability to resolve very close multi-modal particle distributions, even within 2%, and to distinguish extremely small shifts and changes in particle size, DCS is once more becoming a valuable particle characterisation tool. The term 'high resolution' is a commonly used expression in the world of particle sizing, however DCS really does achieve unparalleled resolution as can be seen when used in characterisation of nano particle coatings covered earlier in this article. Practical range of the technique is from around 3nm right up to 80micron (exact range will

be dependent on density), however the real benefits over and above more traditional so-called nano particle sizing techniques are generally noticed below around 300nm.

These days, DCS has become fast, very simple to use, is highly accurate and reproducible. It can measure up to 40 samples on the same 'run', does 'speed ramping' for measurement of broad distributions in a single sample, and can even measure 'buoyant' or 'neutral density' particles (i.e. particles having a lower density to the medium in which they are dispersed). Due to the high resolution achievable DCS is ideal for resolving aggregates and agglomerates and to observe tiny relative shifts in peaks and tails of particle size distributions. It may also be used to measure absolute particle size too; however density of particle material must be known. It can even be used for quantitative measurements if optical property (refractive index) of the particulate is known. Number or weight distributions can also be easily calculated and displayed.

Fig.3

Want to find out more?

To learn more about high-resolution particle size characterisation using the CPS Disc Centrifuge UHR visit **analytik.co.uk/cps** (UK and Ireland) or visit **cpsinstruments.eu**.