Analytical Ultracentrifugation Assays for the Characterization of Purity in AAV Gene Delivery Vectors

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ABSTRACT

Gene therapy is emerging as a promising method for the treatment of numerous disease conditions. The FDA has not yet approved any gene therapy product for use in the US, yet hundreds of clinical trials are underway, with the aim of bringing gene delivery vectors into the biopharmaceutical market. Gene delivery vectors are often virus particles that are complex supra-molecular assemblies of capsid proteins that are designed to contain a nucleic acid in the core of the particle encoding the transgene.

In contrast to typical recombinant protein therapeutics such as antibodies, the availability of industry guidance on the characterization of gene delivery vectors is less well-defined. Gene therapy vectors are to be scaled up at the large scales of other biologics; than characterization of this class of product, in analytical laboratories, will have to evolve along with the same pace.

The goal of this work is to evaluate analytical ultracentrifugation (AUC) as a means of characterizing one particular type of gene delivery vector: recombinant adeno-associated virus (AAV). Reliable approaches need to be identified for assessing the polydispersity of recombinant AAV preparations, that allow for the quantitation of the amounts of full genome-containing capsids, and importantly the accurate, precise assessment of empty or partially-filled capsids in those same preparations.

AUC, as shown here, is an efficient and precise tool for the quantitation of purity in AAV drug products.

RESULTS AND DISCUSSION

AAV is a small, non-enveloped, icosahedral virus about 20 nm in diameter. The AAV capsids contain a linear, single-stranded DNA genome of about 4.8 kilo-base pairs. Wild type AAV is replication defective, and in the wild it is dependent on co-infection with a helper virus such as adeno-virus, in order to complete the AAV replication cycle. It should be noted, however, that when developed for use as a gene therapy vector, production of recombinant AAV can be accomplished by helper-free methods where the necessary helper factors are co-transfected into the production cell line.

In fact, the modes of production and purification employed in the manufacture of recombinant AAV can have an important influence on attributes that appear to be critical to the quality of the final product. One such critical attribute is related to the nucleic acid that is packaged within the capsids of recombinant AAV. When produced from transfected cell lines, it has been shown that recombinantly produced AAVs are not uniform with respect to the packaging of DNA into the core.

Currently, reliable analytical approaches are needed for assessing the polydispersity of recombinant AAV preparations, allowing for quantitation of full genome-containing capsids, and as importantly, the accurate and precise quantitation of empty or partially-filled capsids in those same preparations (and possibly other variants as well). AUC can provide such quantitation as shown below.

CONCLUSIONS

The ability to quantify the number of AAV species and their relative abundances, accurately and precisely, in a single assay is very useful in the development of this gene delivery product. AUC is an efficient and reproducible method for the evaluation of polydispersity in AAV samples, and is in a good position, compared to other methods that have previously been standards in AAV drug development.

REFERENCES

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