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

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1 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. If gene delivery therapeutics are to soon be produced at the large scales of other biologics[1], than characterization of this class of product, in analytical laboratories, will have to evolve along at 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.

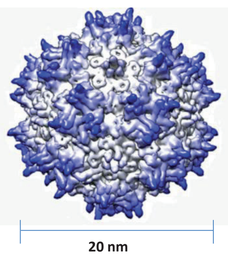
2 CONCLUSIONS

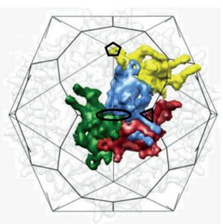
The ability to quantify the number of AAV species and their relative abundances, accurately and precisely, in a single assay is very useful in the development of this gene delivery product. AUC is an efficient and reproducible method for the evaluation of polydispersity in AAV samples, and is in a good position, compared to other methods that have previously been standards in quantifying AAV empty to full ratios. These are methods used extensively in the past, such as a combination of qPCR and ELISA assays to estimate the empty-to-full ratio, or optical density measurements that require accurate knowledge of capsid and DNA extinction coefficients. In this work we have demonstrated that AUC can reliably detect small changes in the size distribution profile that may correlate with varying amounts of species related to packed AAV capsids. This variability may impact the physiological behaviour of the product including its potency, and therefore is a valuable characterization tool in AAV drug development.

3 REFERENCES

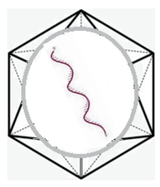
1. BioProces. J., (Winter 2014/2015) 47-54
2. Mol. Ther. Meth. Clin. Dev. (2014) 1, 9
3. Mol. Ther. (2014) 22, 1, 1

4 RESULTS AND DISCUSSION



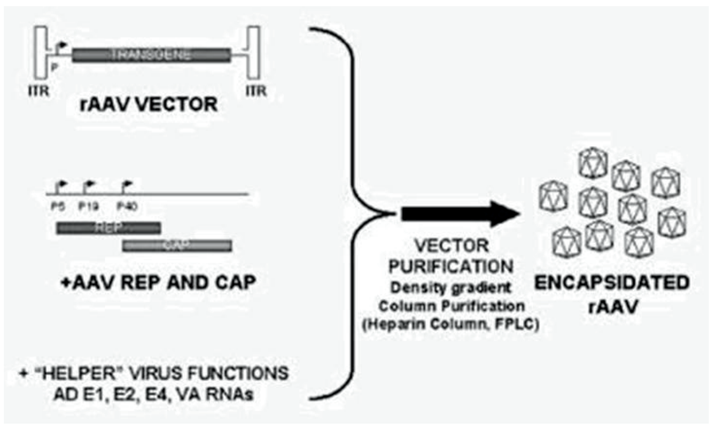


Three protein subunits VP1, VP2, VP3. About 60 proteins interlock to form the capsid of about 3.5 MDa.

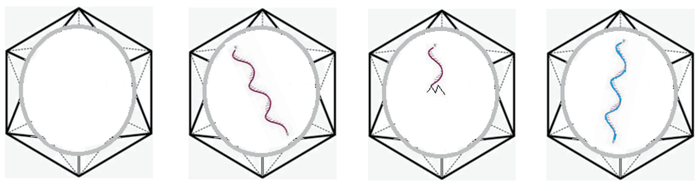


ssDNA of about 4.8 kbp. Wild type genome encodes proteins for replication and capsid structure. Replication defective.

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.

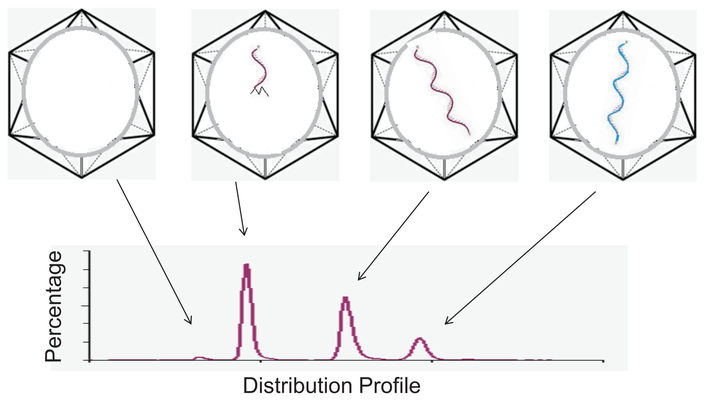


In fact, the modes of production and purification employed in the manufacture of recombinant AAV can have an important influence on attributes that appear to be critical to the quality of the final product. One such critical attribute is related to the nucleic acid that is packaged within the capsids 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.



Empty Capsids | Full Capsids | Fragment Capsids | "Other" Capsids

A high percentage of capsids may contain no genetic material at all, and would be termed "empty" capsids. Additionally, capsids have been shown to contain incomplete portions or fragments of the transgene-coding DNA, or even non-target, extraneous nucleic acid contaminants[2,3].



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.

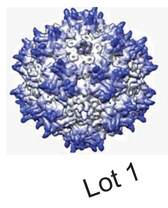
AUC Assay on a sample of AAV Serotype 2, "Lot 1"

Sample:

AAV Serotype 2

Sample ID: "Lot 1"

Stock Conc. = 1.2×10^{12} VP/mL in Formulation Buffer (FB)



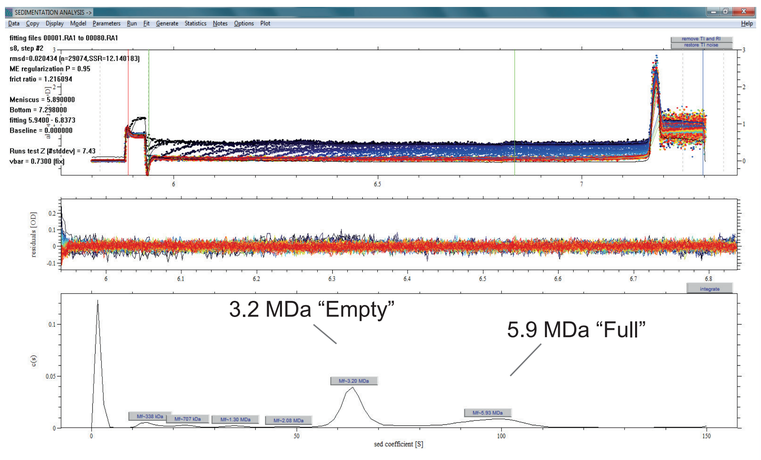
Lot 1

Sample Prep:

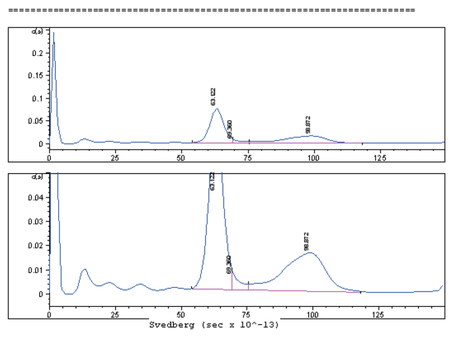
Sample was diluted with FB to 6.0×10^{11} VP/mL

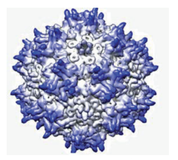
AUC Assay:

About 0.450 mL of sample or FB loaded into the sample/reference channels, respectively. After cell loading, alignment, and temperature equilibration, the sample was centrifuged at 20 ° C and 20,000 rpm until full sedimentation, or about 80 scans using UV absorbance detection at 220 nm. The raw data (sedimentation profiles) were analyzed by SEDFIT using a c(s) distribution model, to obtain the size distribution profile, which was integrated in Chemstation software.



Shown here are a series of sedimentation profiles that we obtained as the raw data from the AUC assay run as described above. After deconvolution, using the program SEDFIT, we see the size distribution profile (upper panel), comprised of two major peaks, and lower amounts of minor species, which, while in low abundance may be quite important to quantify. A mass estimate analysis of the major peaks, at ~60 S and ~99 S, suggests that they are likely to represent empty capsids and fully packaged capsids, respectively. The estimated masses associated with these species match up well based on what we know of the expected masses of the empty capsid structure and the capsid-plus-genome.

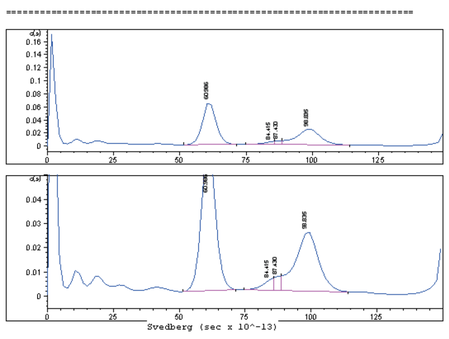


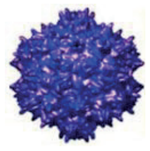


Lot 1

Signal: AIA1,Normalized SEDFIT c(s) Distribution				
Pk Type	S (secx10^-13)	Height %	Area Units	Area %
MF	63.122	78.047	27.933	59.455
FM	69.360	5.869	1.275	2.714
FM	98.872	16.084	17.774	37.831

Integration of the sedimentation coefficient distribution is shown above for the AUC assay of Lot 1 of some AAV material. A single replicate is displayed, however multiple replicates were performed with good reproducibility. This lot of AAV material is characterized by a majority of empty capsids over full capsids by a ratio of roughly 60/40. Minor amounts of other species may also be present as shown by the signals between 20-50 S.





Lot 2

Signal: AIA1,Normalized SEDFIT c(s) Distribution				
Pk Type	S (secx10^-13)	Height %	Area Units	Area %
MM	60.986	65.484	23.307	57.256
MF	84.415	4.305	1.514	3.720
FM	87.430	5.722	0.965	2.370
FM	98.835	24.490	14.921	36.654

In order to determine if the AUC method has the capability of resolving differences in the polydispersity profile of AAV preparations, a second lot of AAV was studied, which is of the same serotype and manufacturing process. The AUC assay for Lot 2 was conducted similarly to that for Lot 1. While some similarities between the two lots can be observed by comparing the respective profiles, an important difference is resolved around the region of the full capsid. The Lot 1 profile shows close to 38% of the full species around 99 S. This 99 S peak is broad, and has some asymmetry, with a larger leading edge than trailing edge. In contrast, the Lot 2 profile, in the same region, has slightly lower area for the "full" species at 99 S (37%). But more noticeably, the Lot 2 species has more definition around the leading edge side. More specifically, where the Lot 1 material has a broad signal in this area, the Lot 2 material has a clearly well-defined shoulder off of the leading edge of the 99 S peak. This result suggests the two lots have different abundances in the neighborhood of the full species that may correlate with variants of the full capsids. Although only one replicate is shown here, this difference was observed over multiple replicates of the two different lots, suggesting the difference is indeed sample related.

5 ACKNOWLEDGMENTS

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