

# MEASURING THE CONCENTRATION OF ADENO-ASSOCIATED VIRUS (AAV) WITH MULTI-ANGLE DYNAMIC LIGHT SCATTERING (MADLS)

## Introduction

For virus development and production, it is important to know the virus concentration at different stages of the process, to optimize the clone used as well as the production yields. For example, instances such as clone screening, multiplicity of infection (MOI) optimization and adaptations of cell culture methods are instances when virus concentration, or virus titre as it is also commonly called, is of interest.

[Dynamic light scattering \(DLS\)](#) can be used in virus development to measure drug substance or in a screening function to separate good and stable samples from those with contaminants or aggregates. With the new multi-angle DLS (MADLS) based particle concentration measurement available on the [Zetasizer Ultra](#), it is now possible to get both size and size distribution as well as particle concentration per population, within a few minutes.

In this application note, Allergan are kindly sharing data from their evaluation of the Zetasizer Ultra, examples of three adeno-associated virus (AAV) samples are shown. The concentration results are compared to results from capsid ELISA based virus titre assays.

## Method

Samples were measured using the low volume quartz cuvette (ZEN2112). The measurements were performed on a Zetasizer Ultra, using the particle concentration measurement, which gives both the MADLS size distribution and the concentration per peak. When this measurement is used, the following parameters need to be provided by the user:

Sample material; the measurements were done using protein as a material, as the virus capsid is made from a protein shell.

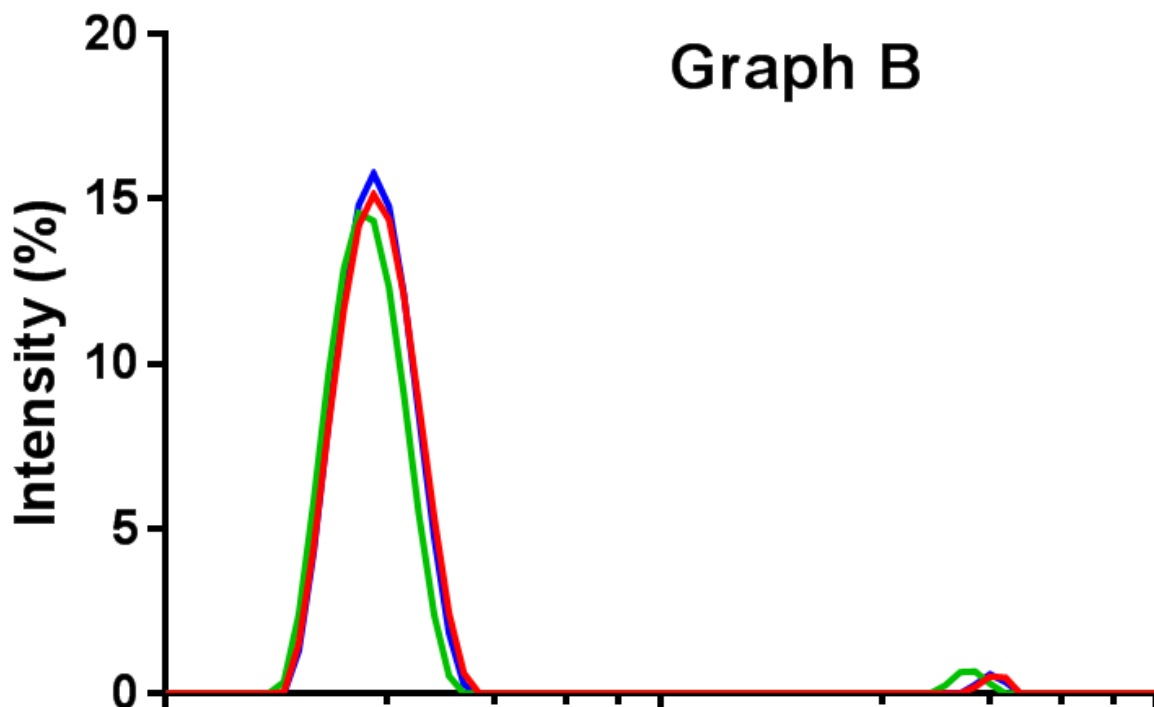
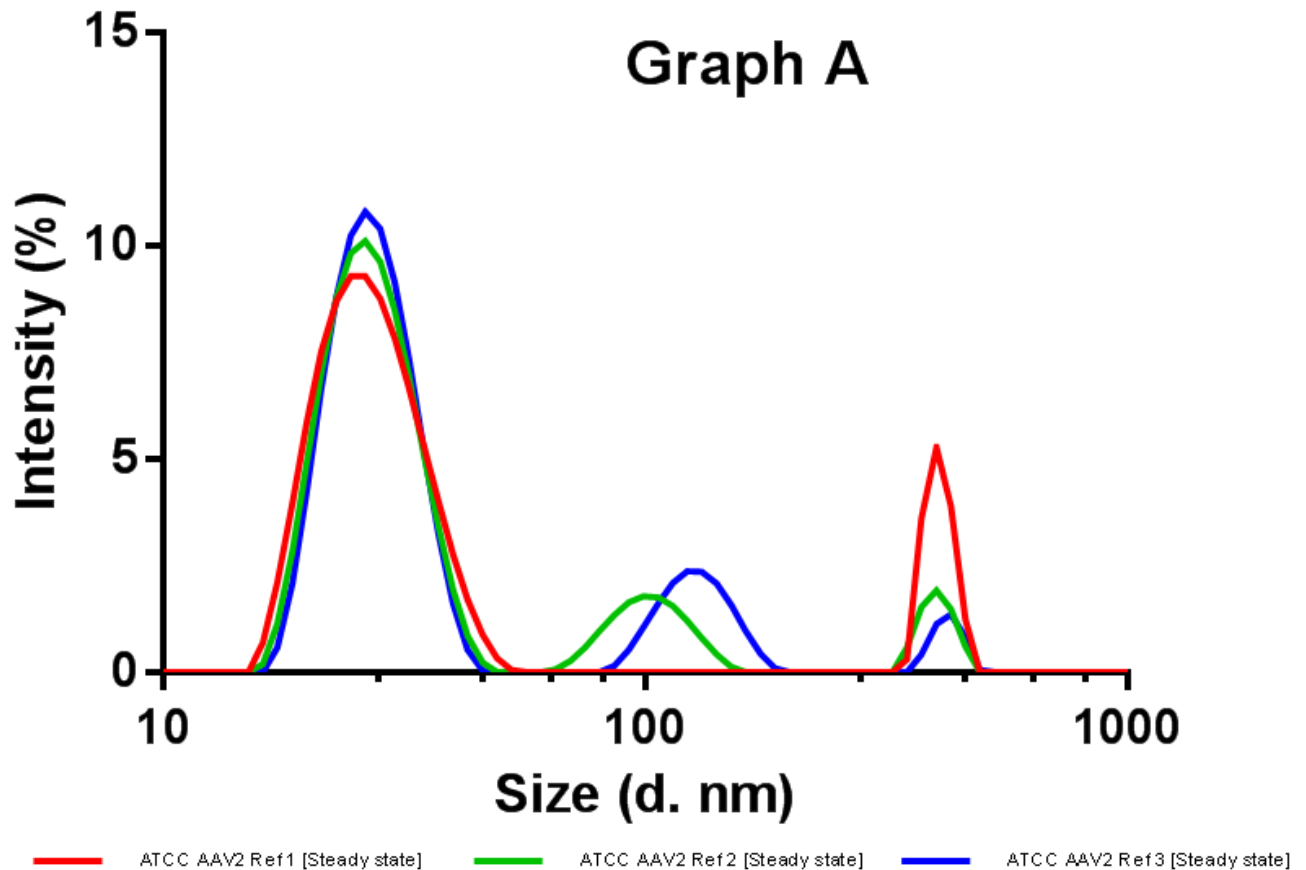
Dispersant material; the dispersant was initially set to water, but the viscosity was later corrected to obtain the correct size and concentration by editing the particle concentration result. The accuracy of the measured size is critical to the particle concentration calculation. The buffer viscosity was measured using bead lacing [1], where we recommend using a 60-200nm polystyrene latex bead. Note that larger beads scatter more and therefore it is not necessary to add as much to the sample.

Buffer scattering count rate (at backscatter angle); the buffer scattering intensity was measured in the same cuvette used for the measurement (ZEN2112), by simply setting up a single angle measurement using the backscatter angle and setting the measurement position to the centre of the cuvette. Run as a normal measurement, the derived count rate value is used in the 'Buffer Scattering' field for the particle concentration measurements.

The run duration for three repeat measurements is less than 4 minutes.

## Results

The size distributions by intensity of the three AAV samples are shown in Figure 1, graph A to C. The corresponding particle concentrations are shown in Figure 2, graph C to F. The ATCC AAV2 reference sample shown in Figure 1, graph A, contains aggregates, which isn't surprising as this sample was thawed from frozen, and due to the low volume available, was inserted directly into the cuvette without any pre-filtration. However, despite the presence of aggregates, the MADLS-based particle concentration gives a concentration value ( $1.12 \times 10^{12}$  particles/ml) that correlates well with the capsid ELISA assay ( $0.92 \times 10^{12}$  particles/ml). See Table 1 for further details on aggregate concentrations. For Allergan's two AAV samples, there are a lot less or zero aggregates detected and, as can be seen in Table 1, the concentration values for the virus peak in both samples correlates well with the capsid ELISA results.



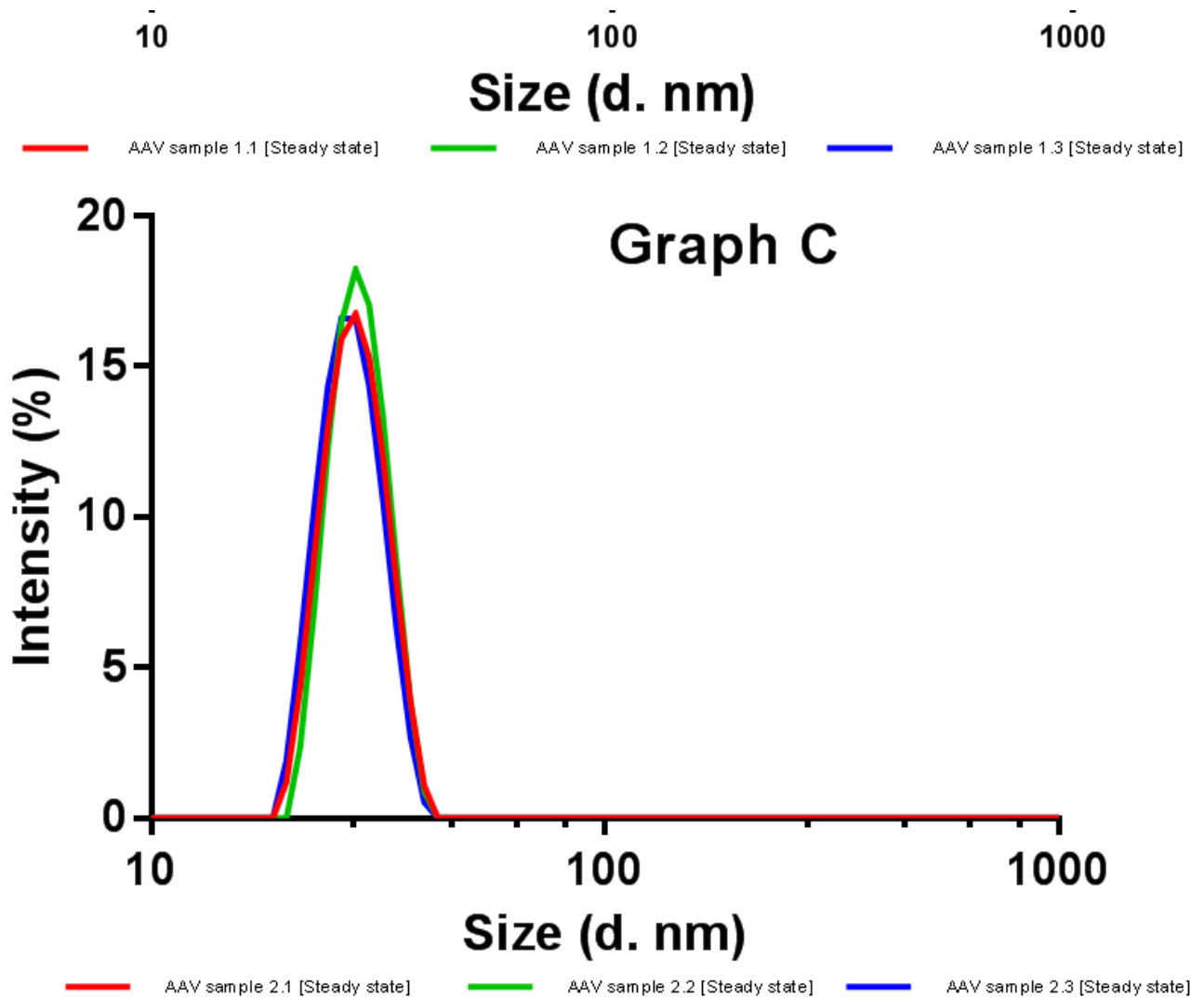
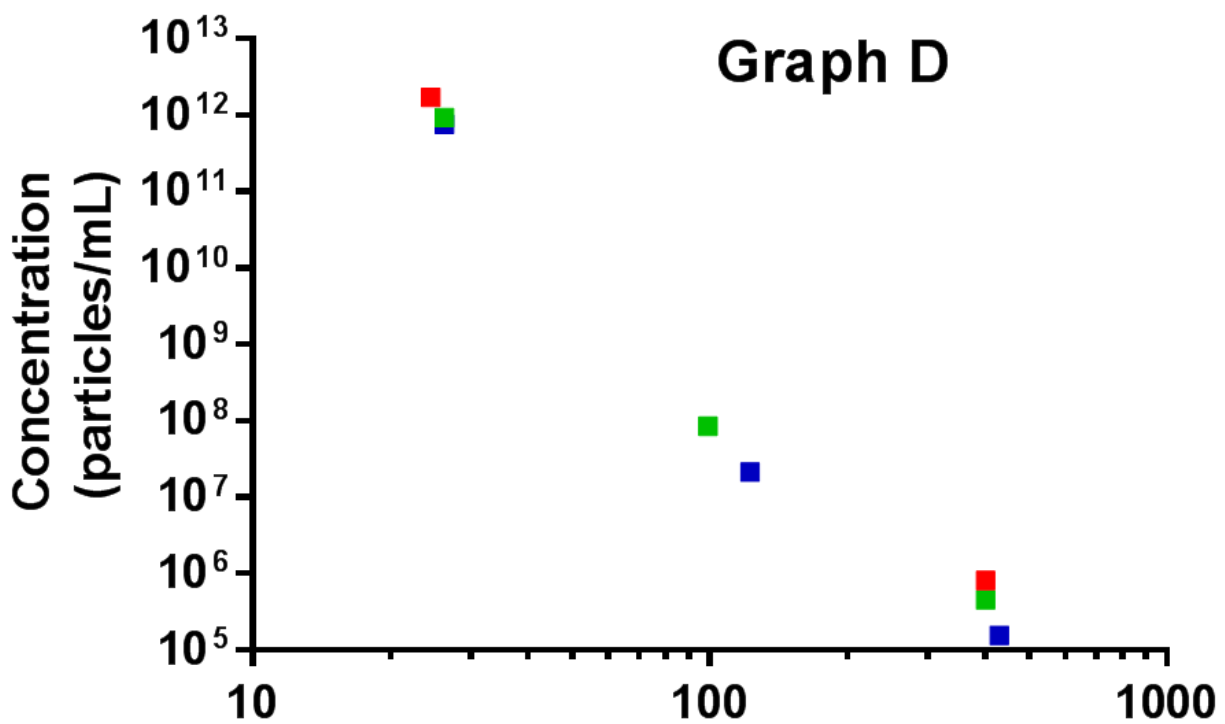


Figure 1: The three repeat measurement for each of the three samples are shown: In graph A - ATCC AAV2 reference sample, in graph B - Allergan AAV sample 1 and in graph C - Allergan AAV sample 2.



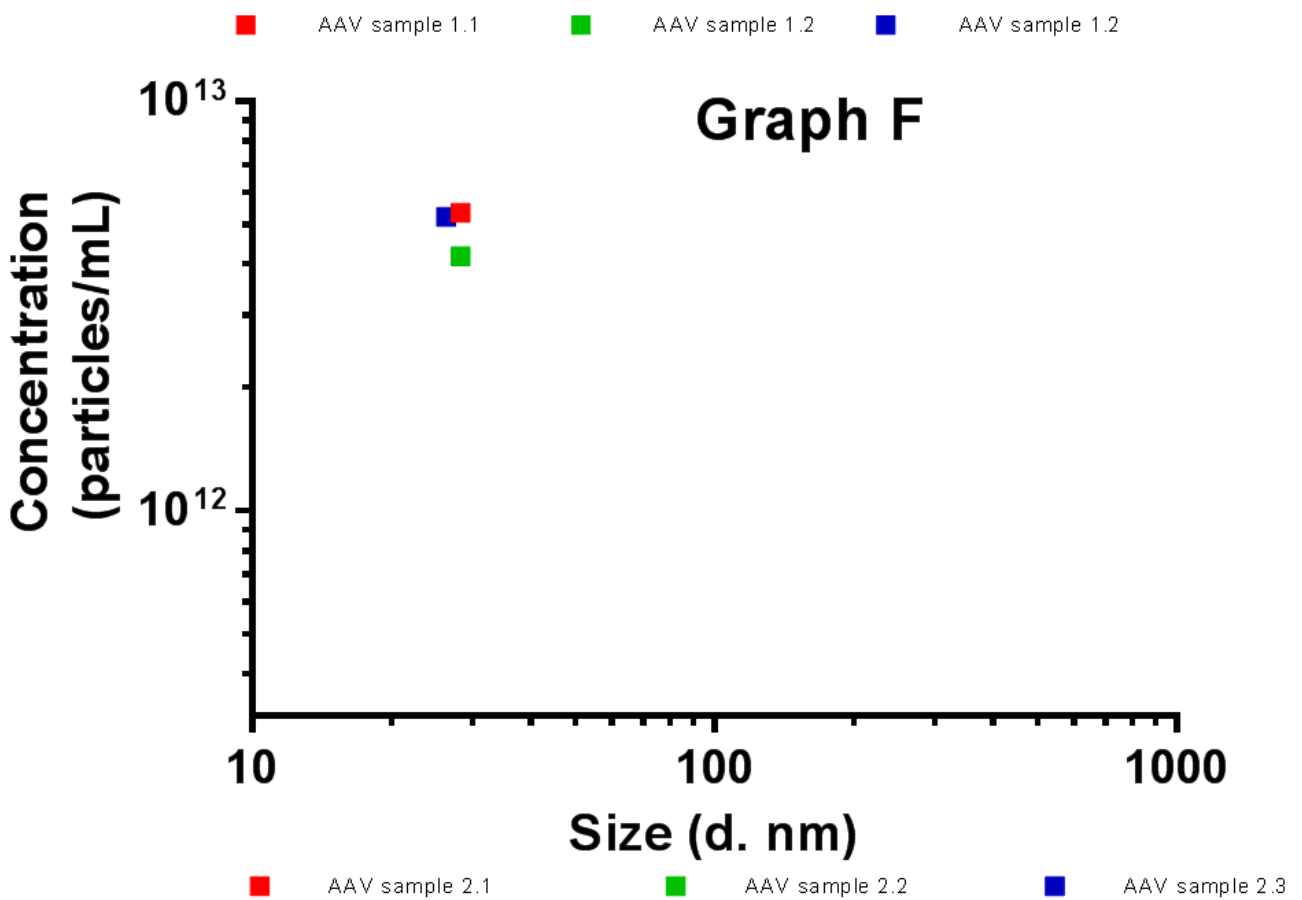
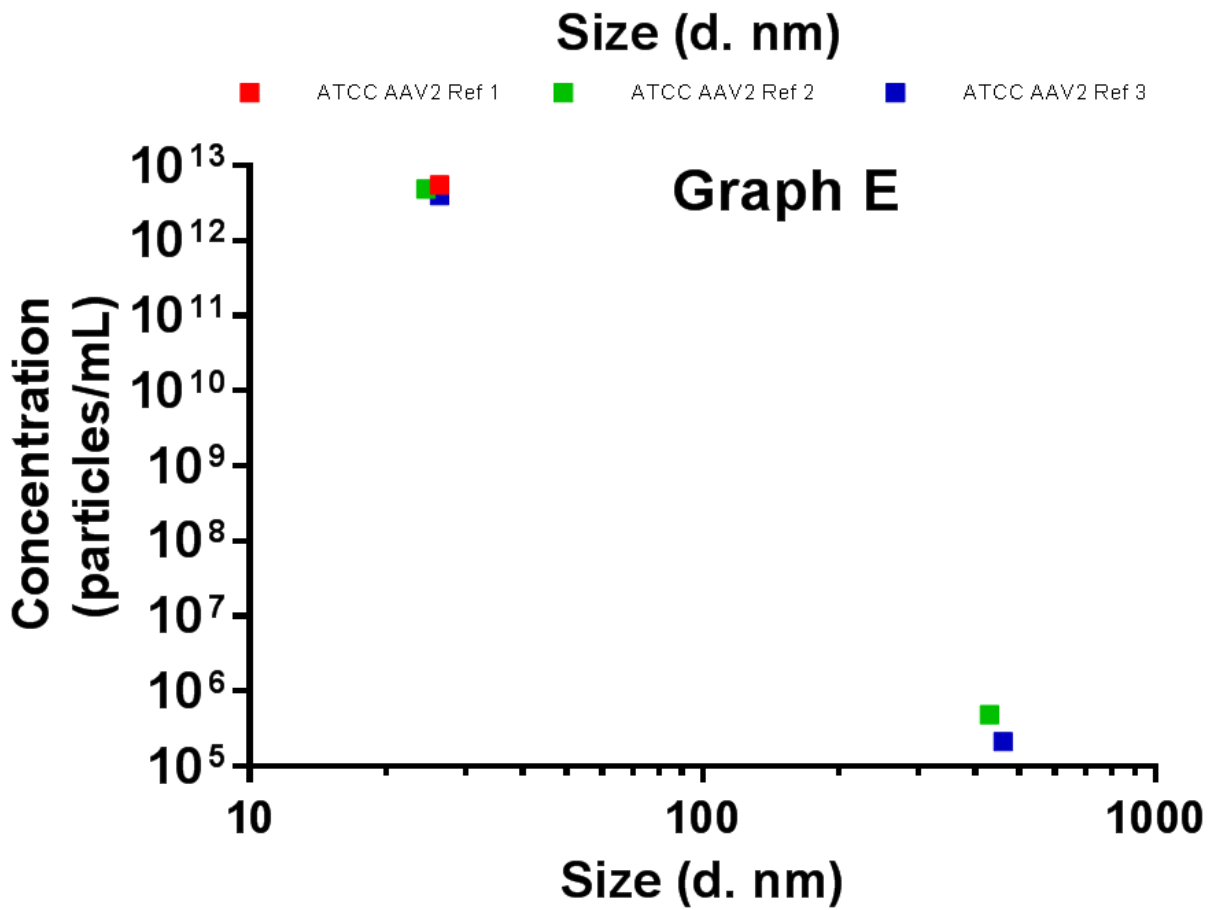


Figure 2: The distributed particle concentrations for the three repeat measurements of the same three samples as in figure 1: Graph C - ATCC AAV2 reference sample, graph E - Allergan AAV sample 1, and F - Allergan AAV sample 2.

In order to compare variation, %CV was calculated by taking the concentration result for each repeat measurement and calculate the standard deviation and mean value, and from this calculate %CV.

Sample	Capsid ELISA	MADLS Based Particle Concentration					
		Virus peak (n=3)	%CV	Aggregate peak 1	%CV	Aggregate peak 2	%CV
ATCC AAV2 reference sample	$0.92 \times 10^{12}$	$1.14 \times 10^{12}$	45	$5.30 \times 10^7$ (n=2)	84.6	$4.73 \times 10^5$ (n=3)	69.3
Allergan sample 1	$6.14 \times 10^{12}$	$4.82 \times 10^{12}$	15	$3.52 \times 10^5$ (n=3)	56	n/a	n/a
Allergan sample 2	$4.29 \times 10^{12}$ $2.83 \times 10^{12}$ (assay ran twice)	$4.92 \times 10^{12}$	13	n/a		n/a	

Table 1 Concentration data from capsid ELISA assay and MADLS-based particle concentration results shown for three samples; ATCC AAV2 reference sample, Allergan sample 1, Allergan sample 2. All concentrations are given as particles/ml. n= shows i how many of the three repeat measurements the peak was identified. Where there is no population peak present it states n/a as in not applicable.

Comparing the results in Table 1, the ATCC AAV2 reference sample shows the largest %CV for the virus population concentration value. This is due to the aggregates in this samples which are affecting how well the AAV peak can be determined. If you compare the size peak repeatability in Figure 1A, with either of the other virus population peaks in Figure 1B or 1C, you can see that the latter overlay better and are more repeatable. This will directly affect the repeatability of the concentration measurement. Both Allergan AAV samples showed peak repeatability of about 15%, in presence of no or a few aggregates in the sample.

### Conclusion

The data presented shows that the Zetasizer Ultra's MADLS-based particle concentration can provide both the size distribution of AAV virus samples and hence report on the sample aggregation state, as well as providing information on the concentration of the main virus population and for any aggregates present. Each repeat measurement takes less than 4 minutes.

The sample can be kept enclosed for the whole measurement inside the cuvette, so risk of contamination is minimal and risk of exposure to the operator is minimised.

As a final note, during testing it was identified that it is important to use the correct sample viscosity to ensure the most accurate measurement of the particle concentration. The dispersant viscosity can be measured using a viscometer or in the Zetasizer Ultra instrument itself using size reference beads [1].

### References

[1] [Determining Dispersant Viscosity Using Dynamic Light Scattering](#); Malvern Panalytical Application Note

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