MEASURING THE CONCENTRATION OF ADENO-ASSOCIATED VIRUS (AAV) WITH MULTI-ANGLE DYNAMIC LIGHT SCATTERING (MADLS) USING THE NEW ZETASIZER™ ULTRA

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Zetasizer Ultra

Launched in May 2018 Zetasizer Ultra is our most advanced system for the measurement of particle and molecular size. It represents the most intelligent and flexible instrument in the Zetasizer range and brings significant improvements including *two new and unique measurement types.*

- Size and size distribution measurements up to 3 times faster than in previous models
- Sample volume as little as 3 µL
- Multi-Angle Dynamic Light Scattering
- Particle concentration
- Particle charge
- Adaptive Correlation
- All-new data analysis method of Adaptive Correlation
- Tolerance to foreign or large aggregated species
- Measurement accuracy and repeatability
- ZS Xplorer software
- Machine-learning based expert advice system
- Ease of use, analysis speed and data confidence

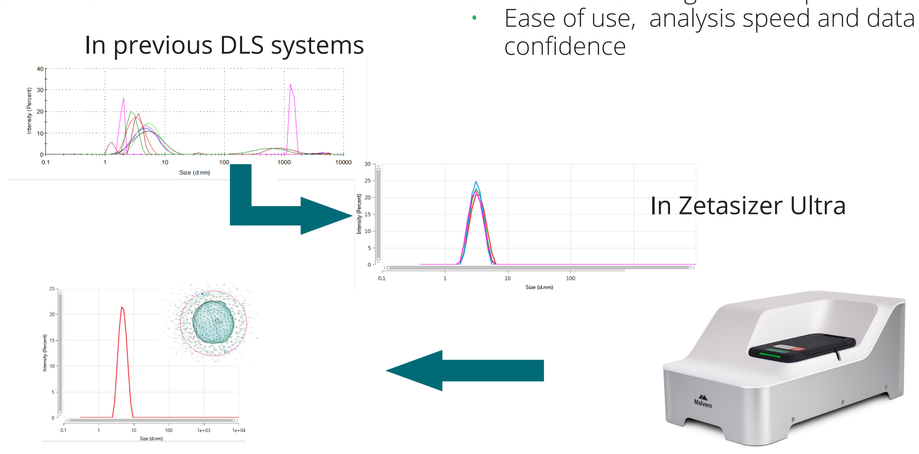

Figure 1 Zetasizer Ultra, and examples of data on size distributions

DYNAMIC LIGHT SCATTERING (DLS)

Correlation and Sizing

Dynamic Light Scattering is a useful biophysical tool to measure particle hydrodynamic diameter in solution. As light is shone on a sample, particles within it scatter light. We collect this light at particular angles and by correlating the signal as it changes due to particle movement over time we measure their coefficients of diffusion, and therefore hydrodynamic diameter.[2]

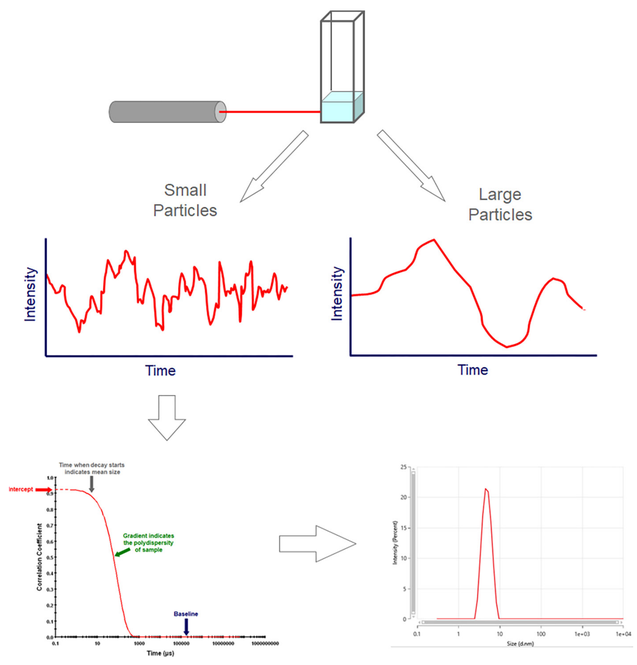

Figure 2 The amount of light scattered by a sample varies depending on the positions of particles within it. As they move under Brownian Motion the amount of detected scattered light changes (top and middle). By correlating the variation of collected scattered light from a sample over time we produce a correlogram (bottom-left). Subsequent analysis then results in an intensity-weighted size result (bottom right).

ELECTROPHORETIC LIGHT SCATTERING (ELS)

Zeta Potential

Zeta Potential (ZP) is key to understanding the stability of a colloidal system, and is measured in minutes using a combination of Electrophoresis and DLS. Hydrodynamic diameter and size distribution can also be determined in the same cell type. The Zeta Potential of a particle is its electrical potential at the Slipping Plane (red line, bottom right), the interface between two regions close to the particle surface named the Stern and Diffuse Layer. DVLO Theory[3] gives the aqueous stability dividing line as +/- 30 mV, between which a system is considered likely to be electrostatically unstable. Combination of this measurement with particularly three other types of stability metrics (T_{agg}, k_D and A_2,) provides powerful guidance in determining and rationally improving formulation conditions of your biomolecules.

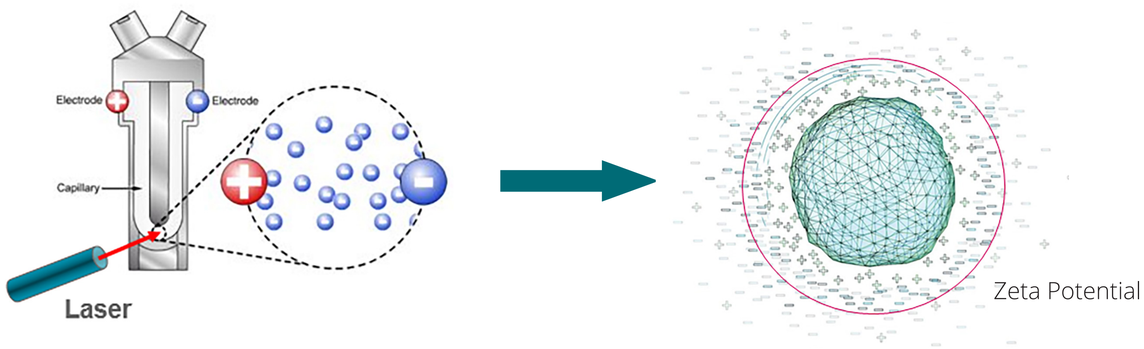

Figure 3 Zeta Potential measurements of particles in solution are made in folded capillary cells (left), and use a combination of electrophoresis and DLS to measure particle electrical potential at the slipping plane, otherwise known as Zeta Potential. Hydrodynamic diameter can also be measured using the same cell type.

References
1) Malvern Panalytical Application note: Measuring the concentration of Adeno-Associated Virus with multi-angle dynamic light scattering (MADLS).
2) A. Einstein (1926), Investigations on the theory of the Brownian movement. In: Fürth R., ed. Cowper, A.D., translator. Methuen, London,124.
3) B. Derjaguin & L. Landau (1941), Acta Physico Chemica, URSS, 14: 633.

ADENO-ASSOCIATED VIRUS (AAV)

Project introduction:1 Comparing AAV size and concentration measurements using the Zetasizer Ultra with concentrations determined using Allergan UK's established capsid ELISA assay method
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 production yields. For example, instances such as clone screening, multiplicity of infection optimization and adaptations of cell culture methods are instances when virus concentration, or virus titre as it is also referred to, is of interest.

Dynamic light scattering (DLS) can be used in virus development to measure critical biophysical parameters for a sample, or in a screening function to separate good, 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 measure not only hydrodynamic diameter and a size distribution of your samples (including aggregated species), but also particle concentration per population, within a few minutes.

In this study, Allergan UK 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.

AAV SIZE AND CONCENTRATION MEASUREMENTS

Using Multi-Angle Dynamic Light Scattering (MADLS) to Measure Size, Aggregation State and Particle Concentration in Minutes

By using the MADLS measurement on the Zetasizer Ultra, particle size distributions for three AAV samples kindly shared by Allergan UK were measured in triplicate, shown below. Low levels of aggregated species were observed, more so in the standard (A) than in their own AAV samples (B and C). By using the new Particle Concentration measurement, unique to the Zetasizer Ultra and calculated alongside the MADLS measurement, the concentration of AAV and each aggregated species was also measured (D-F) in minutes. Concentrations were compared to those recorded using Allergan's established capsid ELISA method ***(Table 1)***, showing less than 15% RSD for Allergan's samples and 45% RSD for the standard.

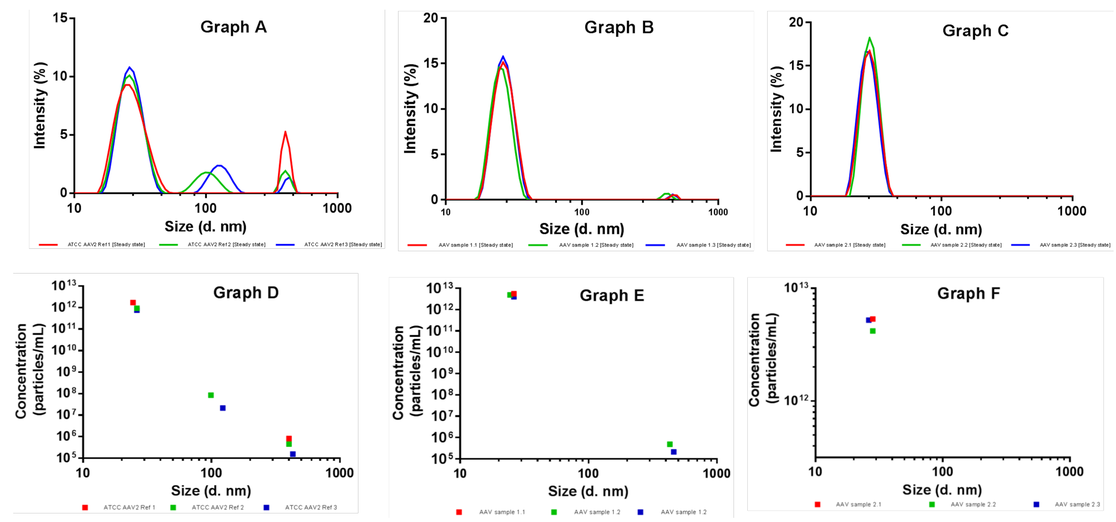

Figure 4 Intensity distributions of three repeat size measurements each of:
graph A - ATCC AAV2 reference sample, graph B - Allergan AAV sample 1 and graph C - Allergan AAV sample 2. Particle concentration measurements corresponding to the spectra directly above.

CAPSID ELISA MEASUREMENTS BY ALLERGAN UK

Using their established AAV capsid ELISA method

As described in the previous section, AAV Particle Concentration measurements made in minutes using the Zetasizer Ultra were compared to those recorded using Allergan UK's established capsid ELISA method. Results show little deviation to the more complex, time consuming and costly established method, demonstrating how the Zetasizer Ultra could be used reliably in such measurements.

Sample	Capside ELISA	MADLS Based Particle Concentration					
		Virus peak (n-3)	%CV	Aggregate peak 1	%CV	Aggregate peak 2	%CV
ATCC AAV2 reference sample	0.92×10^{12}	1.14×10^{12}	45	5.30×10^{7} (n=2)	84.6	4.73×10^{5} (n=3)	69.3
Allergan sample 1	0.14×10^{12}	4.82×10^{12}	15	3.52×10^{5} (n=3)	56	n/a	n/a
Allergan sample 2	4.29×10^{12} 2.83×10^{12} (assay ran twice)	4.92×10^{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 number of the repeat measurements the peak was identified in. Where there is no population peak present it states n/a as in not applicable.

- Non-destructive concentration measurements were made with no or minimal sample preparation, in minutes. Simultaneously the MADLS size distribution was recorded, which not only gave hydrodynamic diameters for particles of interest, but also described the populations of aggregates and other species present. Crucially the concentration of each population was also quantified.
- 15% or lower RSD was observed between the established Allergan UK capsid ELISA method and particle concentration measurements using the Zetasizer Ultra. 45% RSD was observed for a standard sample containing more aggregates.
- Contamination and user exposure risk is minimal as measurements are made in cuvettes that can be sealed during the course of experiments.

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