

Z-Average size

The Z-Average size or Z-Average mean used in dynamic light scattering is a parameter also known as the cumulants mean. It is the primary and most stable parameter produced by the technique. The Z-Average mean is the best value to report when used in a quality control setting as it is defined in ISO 13321 and more recently ISO 22412 which defines this mean as the 'harmonic intensity averaged particle diameter'.

The Z-average size will only be comparable with the size measured by other techniques if the sample is monomodal (i.e. only one peak), spherical or near-spherical in shape, monodisperse (i.e. very narrow width of distribution), and the sample is prepared in a suitable dispersant, as the Z-Average mean size can be sensitive to even small changes in the sample, e.g. the presence of a small proportion of aggregates. It should be noted that the Z-average is a hydrodynamic parameter and is therefore only applicable to particles in a dispersion or molecules in solution.

Cumulants analysis

This is a simple method of analysing the autocorrelation function generated by a DLS experiment. The calculation is defined in ISO 13321 and ISO 22412. As it is a moments expansion it can produce a number of values, however only the first two terms are used in practice, a mean value for the size (Z-Average), and a width parameter known as the Polydispersity Index (Pdl). The Z-Average is an Intensity-based calculated value and should never be confused with or directly compared to a Mass or Number mean value produced by other methods. The calculation is defined in the ISO standards, so all systems that use this calculation as recommended should give comparable results if the same scattering angle is used.

Polydispersity Index

This index is a number calculated from a simple 2 parameter fit to the correlation data (the cumulants analysis). The Polydispersity Index is dimensionless and scaled such that values smaller than 0.05 are rarely seen other than with highly monodisperse standards. Values greater than 0.7 indicate that the sample has a very broad size distribution and is probably not suitable for the dynamic light scattering (DLS) technique. The various size distribution algorithms work with data that falls between these two extremes. The calculations for these parameters are defined in the ISO standard document 13321:1996 E and ISO 22412:2008.

Polydispersity

In light scattering, the term polydispersity and % polydispersity are derived from the Polydispersity Index, a parameter calculated from a Cumulants analysis of the DLS-measured intensity autocorrelation function. In the Cumulants analysis, a single particle size mode is assumed and a single exponential fit is applied to the autocorrelation function and the polydispersity describes the width of the assumed Gaussian distribution. In terms of a protein analysis, a % polydispersity less than 20% indicates that the sample is monodisperse.

Diffusion Coefficient

Particles and molecules in suspension/solution undergo Brownian motion. This is the motion induced by the bombardment by solvent molecules that themselves are moving due to their thermal energy. If the particles or molecules are illuminated with a laser, the intensity of the scattered light fluctuates at a rate that is dependent upon the size of the particles as smaller particles are “kicked” further by the solvent molecules and move more rapidly. Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship. The diffusion coefficient, therefore, defines this Brownian motion of the analyte or particle in that particular solvent environment. The translational diffusion coefficient will depend not only on the size of the particle “core” but also on any surface structure, as well as the concentration and type of ions in the medium.

Hydrodynamic diameter

The hydrodynamic size measured by Dynamic Light Scattering (DLS) is defined as “the size of a hypothetical hard sphere that diffuses in the same fashion as that of the particle being measured”. In practice though, particles or macromolecules in solution are non-spherical, dynamic (tumbling), and solvated. Because of this, the diameter calculated from the diffusional properties of the particle will be indicative of the apparent size of the dynamic hydrated/solvated particle. Hence the terminology, Hydrodynamic diameter. The hydrodynamic diameter, or Stokes diameter, therefore is that of a sphere that has the same translational diffusion coefficient as the particle being measured, assuming a hydration layer surrounding the particle or molecule.

Correlation Curve – or correlation function

The measured data in a dynamic light scattering (DLS) experiment is the correlation curve which should be a smooth, single exponential decay function for a mono-size particle dispersion. Embodied within the correlation curve is all of the information regarding the diffusion of particles within the sample being measured. By fitting the correlation curve to an exponential function, the diffusion coefficient (D) can be calculated (D is proportional to the lifetime of the exponential decay). With the diffusion coefficient (D) now known, the hydrodynamic diameter can be calculated by using a variation of the Stokes-Einstein equation. For a polydisperse sample this curve is a sum of exponential decays.

Y-Intercept or Intercept

In DLS the Y-Intercept, or more simply Intercept, refers to the intersection of the correlation curve on the y-axis of the correlogram. The y-intercept can be used to evaluate the signal-to-noise ratio from a measured sample and thus is often used to judge data quality. It is usually scaled such that an ideal signal will give a value of 1, and a good system will give intercepts in excess of 0.6, and greater than 0.9 for the best systems.

Deconvolution or Deconvolution algorithm

An algorithm-based approach to resolving a mixture of exponentials derived from a polydisperse sample into a number of intensity values each associated with a discrete size band. The particle size distribution from dynamic light scattering (DLS) is derived from a deconvolution of the measured intensity autocorrelation function of the sample. Generally, this is accomplished using a non-negatively constrained least squares (NNLS) fitting algorithm, a common examples being CONTIN.

Count Rate or Photon Count Rate

In DLS this is simply the number of photons detected and is usually stated in a “per second” basis. This is useful for determining the sample quality, by monitoring its stability as a function of time, and is also used to set instrument parameters such as the attenuator setting and sometimes analysis duration. The count rate needs to be above some minimum value in order to have enough signal for analysis, however all detectors have a maximum count rate where the response remains linear, and if the count rate is not adjusted automatically, the manufacturer recommendations for adjusting the count rate must be observed.

Intensity Distribution

The first order result from a DLS experiment is an intensity distribution of particle sizes. The intensity distribution is naturally weighted according to the scattering intensity of each particle fraction or family. For biological materials or polymers the particle scattering intensity is proportional to the square of the molecular weight. As such, the intensity distribution can be somewhat misleading, in that a small amount of aggregation/agglomeration or presence of a larger particle species can dominate the distribution. However this distribution can be used as a sensitive detector for the presence of large material in the sample.

Volume Distribution

Although the fundamental size distribution generated by DLS is an intensity distribution, this can be converted, using Mie theory, to a volume distribution or a distribution describing the relative proportion of multiple components in the sample based on their mass or volume rather than based on their scattering (Intensity.)

When transforming an intensity distribution to a volume/mass distribution, there are 4 assumptions that must be accepted.

- All particles are spherical
- All particles are homogeneous
- The optical properties of the particles are known, i.e. the real & imaginary components of the refractive index
- There is no error in the intensity distribution

An understanding of these assumptions is particularly important since the DLS technique itself produces distributions with inherent peak broadening, so there will always be some error in the representation of the intensity distribution. As such, volume and number distributions derived from these intensity distribution are best used for comparative purposes, or for estimating the relative proportions where there are multiple modes, or peaks, and should never be considered absolute. It is therefore good practice to report the size of the peak based on an intensity analysis and report the relative percentages only (not size) from a Volume distribution analysis.

References:

- [1] International Standard ISO13321 Methods for Determination of Particle Size Distribution Part 8: Photon Correlation Spectroscopy, International Organisation for Standardisation (ISO) 1996.
- [2]] International Standard ISO22412 Particle Size Analysis – Dynamic Light Scattering, International Organisation for Standardisation (ISO) 2008.
- [3] Dahneke, B.E. (ed) Measurement of Suspended Particles by Quasi-elastic Light Scattering, Wiley, 1983.
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- [5] Washington, C. Particle Size Analysis In Pharmaceuticals And Other Industries: Theory And Practice, Ellis Horwood, England, 1992.
- [6] Johnson, C.S. Jr. and Gabriel, D.A. Laser Light Scattering, Dover Publications, Inc., New York 1981

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