Designation: E3247 – 20

Standard Test Method for Measuring the Size of Nanoparticles in Aqueous Media Using Dynamic Light Scattering[1]

This standard is issued under the fixed designation E3247; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method addresses the determination of nanoparticle size (equivalent sphere hydrodynamic diameter) using batch-mode (off-line) dynamic light scattering (DLS) in aqueous suspensions and establishes general procedures that are applicable to many commercial DLS instruments. This test method specifies best practices, including sample preparation, performance verification, data analysis and interpretation, and reporting of results. The document includes additional general information for the analyst, such as recommended settings for specific media, potential interferences, and method limitations. Issues specific to the use of DLS data for regulatory submissions are addressed.

1.2 The procedures and practices described in this test method, in principle, may be applied to any particles that exhibit Brownian motion and are kinetically stable during the course of a typical experimental time frame. In practice, this includes particles up to about 1000 nm in diameter, subject to limitations as described in the test method.

1.3 This test method does not provide test specimen preparation procedures for all possible materials and applications, nor does it address synthesis or processing prior to sampling. The test specimen (suspension) preparation procedures should provide acceptable results for a wide range of materials and conditions. The analyst must validate the appropriateness for their particular application.

1.4 This test method is applicable to DLS instruments that implement correlation spectroscopy. Analysts using instruments based on frequency analysis may still find useful information relevant to many aspects of the measurement process, including limits of applicability and best practices. On-line (flow-mode) DLS measurements are not treated here specifically and may have additional limitations or issues relative to batch-mode operation.

1.5 *Units*—The values stated in SI units are to be regarded as standard. Where appropriate, c.g.s. units are given in addition to SI.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1617 Practice for Reporting Particle Size Characterization Data

E2490 Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)

E2456 Terminology Relating to Nanotechnology

E3144 Guide for Reporting the Physical and Chemical Characteristics of Nano-Objects

E3206 Guide for Reporting the Physical and Chemical Characteristics of a Collection of Nano-Objects

2.2 *ISO Standards:*[3]

ISO 22412 Particle size analysis—Dynamic light scattering (DLS)

3. Terminology

3.1 *Definitions:*

3.1.1 *aliquot, n*—a representative portion of a whole, assumed to be taken with negligible sampling error. **adapted from ISO 11074**

[1] This test method is under the jurisdiction of ASTM Committee E56 on Nanotechnology and is the direct responsibility of Subcommittee E56.02 on Physical and Chemical Characterization.

Current edition approved Sept. 1, 2020. Published October 2020. DOI: 10.1520/E3247-20.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

3.1.2 *average hydrodynamic diameter,* $\bar{x}_{DLS}$, *n*—the ensemble average diameter that reflects the central tendency of the underlying population of particles as determined in dynamic light scattering. **adapted from ISO 22412**

3.1.2.1 *Discussion*—The average hydrodynamic diameter can be obtained from the size distribution calculated by different methods, including, for instance, the cumulants method combined with the Stokes-Einstein equation or any number of deconvolution algorithms that produce an intensity-weighted particle size distribution. Note that when obtained from the cumulants method the average hydrodynamic diameter is often referred to in the literature as the *z*-average diameter (*z-avg*), a legacy term. Enclosure by angular brackets $\langle x_{DLS} \rangle$ indicates the arithmetic mean derived from replicate measurements. Note this measurand can be scattering angle dependent and can be influenced by other factors besides size.

3.1.3 *baseline, n*—the measured far point of a correlation function, typically determined from one or more channels of the correlator positioned at very large delay times, τ.

3.1.3.1 *Discussion*—Essentially the square of the time-averaged scattered intensity; baseline can also be calculated from the square of the total measured photon counts over the course of the experiment divided by the total number of time intervals. In this test method, the baseline is the value of $[g^{(2)}(\tau)-1]^{0.5}$ determined from the far point channels (see *correlation function*, *correlogram*, and *signal-to-noise ratio*).

3.1.4 *combined standard uncertainty, n*—standard measurement uncertainty that is obtained using the individual standard uncertainties associated with the input quantities in a measurement model. **ISO/IEC Guide 99**

3.1.5 *correlation function (correlation coefficients), n*—the primary output or product of a digital correlator.

3.1.5.1 *Discussion*—Essentially, the raw data generated by a dynamic light scattering experiment that is subjected to analysis in order to derive a characteristic particle size or size distribution, or both. The correlator measures the intensity-intensity correlation, an exponentially decaying function of time with each individual data point referred to as a correlation coefficient. Also called the autocorrelation function because it compares photon counts from the same source at time *t* with those at time *t* + τ, where τ is the delay or lag time; it is typically baseline-normalized such that the value at very large τ approaches unity. By convention, upper case *G* is used to represent the unnormalized function, while lower case *g* indicates normalized values. The superscript 2, as in $g^{(2)}(\tau)$, indicates a second order (intensity) correlation function, from which the first order (electric field) correlation function is calculated according to: $g^{(1)}(\tau)\sqrt{\beta}=[g^{(2)}(\tau)-1]^{0.5}$, where $\sqrt{\beta}$ is the *y*-intercept of the function on the right-hand side extrapolated to τ = 0. For non-interacting Brownian particles, $g^{(1)}(\tau)$ is related to the translational diffusion coefficient, *D*, such that $\ln g^{(1)}(\tau)=-Dq^2\tau$ and *q* is the modulus of the scattering vector.

3.1.6 *correlator, n*—a digital correlator partitions time into a series of clock intervals of small (typically sub-microsecond) duration and constructs the sums of the products of photon counts registered at different intervals separated by increasing delay or lag time, τ.

3.1.6.1 *Discussion*—The raw product of the correlator is the intensity correlation function. Alternatively, a correlator can compare the signals arriving at identical times from two different sources, a method referred to as cross-correlation. Software aided computation of the correlation function is also feasible, where sufficient computational capacity exists.

3.1.7 *correlogram, n*—a graphical representation of the correlation function with delay time, τ, on the *x*-axis.

3.1.7.1 *Discussion*—The *y*-axis can be presented in one of several forms: (*1*) the normalized intensity autocorrelation, $g^{(2)}(\tau)$, for which the baseline value at τ = ∞ approaches 1 and the *y*-intercept at τ = 0 can obtain a maximum value of 2; (*2*) $g^{(2)}(\tau) - 1$, where the baseline value approaches 0 and the *y*-intercept has a maximum of 1; (*3*) $g^{(2)}(\tau) - 1^{0.5}$, sometimes denoted as $G^{(1)}(\tau)$, which also varies from 0 to 1; and (*4*) the normalized electric field correlation function, $g^{(1)}(\tau)$, with the same limiting values as the previous two functions, and the only function that is instrument-independent. Because of the exponential decay associated with correlation functions, natural logarithmic plots are occasionally used.

3.1.8 *count rate, n*—the average number of photons detected per unit time typically expressed in kilo-counts per second (kcps).

3.1.9 *cumulants, n*—a method for approximating the first order (electric field) autocorrelation function determined in a DLS experiment as a polynomial expansion in delay time, τ.

3.1.9.1 *Discussion*—As described in ISO 22412, cumulants produces an estimate of the mean scattered light intensity-weighted harmonic mean and width of the underlying particle size distribution.

3.1.10 *cumulative undersize distribution, n*—shows the relative amount at or below a specified particle size, where the value at 50 % represents the median size.

3.1.10.1 *Discussion*—Obtained by integration of the *differential (discrete) size distribution*. The percentile sizes derived from the cumulative distribution are commonly used in industry to specify product size characteristics. Typically presented as diameters, such as d_{10}, d_{50} and d_{90}, the percentiles d_x represent the value at which *x* % of the underlying population lies at or below size *d*.

3.1.11 *decay rate (decay constant) Γ, n*—the characteristic rate at which an exponentially decaying correlation function decreases toward its baseline, expressed in units of inverse time.

3.1.11.1 *Discussion*—The rate of decay is related to the translational diffusion coefficient of the particles, *D*, by the relationship $\Gamma=Dq^2$, where *q* is the modulus of the scattering vector.

3.1.12 *differential (discrete) size distribution, n*—shows the relative amount at each size value or interval.

3.1.12.1 *Discussion*—In dynamic light scattering, the differential or discrete distribution typically shows the % intensity on the *y*-axis and size on the *x*-axis, from which the mode and mean of each particle population can be identified. The distribution can be represented as a continuous function or as a histogram. In each case, the *y*-value yields the relative amount of the weighted population at the corresponding size or in the corresponding size bin.

3.1.13 *diffusion coefficient D, n*—mean squared displacement of a particle per unit time. **ISO 13099**

3.1.13.1 *Discussion*—Characterizes the random translational or "Brownian" motion of particles in a liquid medium. This quantity is used in the Stokes-Einstein equation to calculate the equivalent sphere hydrodynamic size. In DLS, the diffusion of non-interacting individual particles is observed under conditions where the concentration is sufficiently low. As the concentration increases, interparticle interactions increase, yielding a concentration-dependent or ensemble diffusivity. Non-spherical particles can also exhibit measurable rotational diffusion. These effects can lead to bias in the measurements.

3.1.14 *dynamic light scattering (DLS), n*—method in which particles undergoing Brownian motion in a liquid suspension are illuminated by a laser and the change in intensity of the scattered light is used to determine particle size. **adapted from ISO/TS 80004-6**

3.1.15 *expanded uncertainty, n*—product of a combined standard uncertainty and a coverage factor larger than unity.

3.1.15.1 *Discussion*—A coverage factor of two represents a confidence interval of approximately 95 %, assuming the degrees of freedom are sufficiently high (>10).

3.1.16 *high efficiency particulate air, HEPA, adj*—noting or using an air filter designed to remove at least 99.97 % of airborne particles greater-than-or-equal-to 0.3 µm in diameter.

3.1.17 *hydrodynamic diameter* d_H, *n*—the calculated diameter of a theoretical hard sphere that diffuses in solution at the same rate as the analyte particle.

3.1.17.1 *Discussion*—Hydrodynamic diameter is a generic term that is not specific to an analysis method or measurement technique.

3.1.18 *intensity-weighted size, n*—a distribution or mean where each particle is weighted by its light scattering intensity.

3.1.18.1 *Discussion*—DLS yields an intensity-weighted distribution or mean. Intensity is a higher order weighting relative to number or volume. For instance, using the Rayleigh approximation, the relative contribution for particles much smaller than the wavelength of light will be proportional to size raised to the 6th power.

3.1.19 *method validation, n*—the process used to confirm that an analytical procedure employed for a specific test is suitable for its intended purpose.

3.1.20 *modulus of the scattering vector q, n*—the absolute value of the momentum transfer or scattering vector, $q=\left(4\ \frac{\pi n}{\lambda_0}\right)\sin\left(\frac{\theta}{2}\right)$, expressed in units of inverse length, where θ is the scattering angle, n is the refractive index of the suspending liquid at the laser wavelength and λ_0 is the wavelength in vacuo.

3.1.21 *nanoparticle, n—for purposes of this standard*, implies that at least two external physical dimensions are smaller than about 100 nm (<10^{-7} m). **adapted from E2456**

3.1.21.1 *Discussion*—The length scale may be a hydrodynamic diameter or a geometric length appropriate to the intended use. With regards to the size range and the presence of size-related properties, this term is a subject of controversy in the field. The use of 100 nm as a reference point does not suggest that materials or products with dimensions above 100 nm cannot or do not exhibit dimension-dependent properties.

3.1.22 *number-weighted size, n*—a distribution or mean where each particle is given equal weighting irrespective of its size.

3.1.22.1 *Discussion*—Counting techniques, such as image analysis or resistive pulse measurement will yield a number-weighted distribution or mean.

3.1.23 *polydispersity index PI, n*—dimensionless measure of the broadness of the size distribution. **ISO 22412**

3.1.23.1 *Discussion—PI* refers in this standard to the value derived from a cumulants analysis of DLS data as defined in ISO 22412.

3.1.24 *qualification, n*—proof with reference material that an instrument is operating in agreement with the manufacturer's specifications. Also referred to as performance verification.

3.1.25 *relative refractive index, n*—ratio of the absolute refractive index (*RI*) of the particles to the real part of the suspending medium.

3.1.26 *sample, n*—a material or suspension from which test specimens or aliquots are obtained.

3.1.26.1 *Discussion*—In a DLS experiment, higher particle relative *RI* translates to greater light scattering intensity, subject to other properties such as particle size, shape, concentration and light absorption (imaginary part of particle *RI*).

3.1.27 *signal-to-noise ratio (S/N), n—in this standard*, defined for DLS experiments in the following manner: [(((*intercept* + 1)) / ((*baseline* + 1))] – 1, where *intercept* is the value of $[g^{(2)}(\tau)-1]^{0.5}$ extrapolated to τ = 0, and *baseline* is $[g^{(2)}(\tau)-1]^{0.5}$ determined from the far point channels of the correlator and should be close to 0 in magnitude.

3.1.28 *standard uncertainty u, n*—measurement uncertainty expressed as a standard deviation. **ISO/IEC Guide 99**

3.1.29 *test specimen, n*—an aliquot used for measurement purposes.

3.1.30 *volume-weighted size, n*—a distribution or mean where each particle is weighted by its volume.

3.1.30.1 *Discussion*—Equivalent to mass-weighting if the particle density is uniform. The relative contribution of each particle will be proportional to size-cubed.

3.1.31 *y-intercept (intercept), n*—the extrapolated τ = 0 value for a measured correlation function.

3.1.31.1 *Discussion*—The *y*-intercept value may be reported differently, depending on the specific form of the correlation function used in its determination. For instance, the *y*-intercept can be determined from a plot of the function $[g^{(2)}(\tau)-1]^{0.5}$ or $g^2(\tau)-1$, both of which yield a limiting *y*-intercept value of 1.

3.1.32 *z-average, n*—commonly used for the intensity-weighted average as applied to the diffusion coefficient or particle size isolated in a DLS experiment using the cumulants method of analysis.

3.1.32.1 *Discussion*—Abbreviated as *z-avg* this is a legacy