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.

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[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 $t + \tau$, 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 $\tau = 0$. 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 $\tau = \infty$ approaches 1 and the *y*-intercept at $\tau = 0$ 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

term commonly used to identify the intensity-weighted harmonic mean diameter derived from a cumulants analysis of DLS data as described in ISO 22412. This standard recognizes the common use of this term, but recommends that $\bar{x}_{DLS}$ be used for reporting DLS-based population mean size results, so long as the procedure used in its calculation is clearly identified (for example, cumulants, NNLS, or CONTIN).

4. Summary of Test Method

4.1 An appropriate volume and concentration of sample suspension is placed in a clean, optically transparent cuvette, well-plate or other instrument-specific sample holder appropriate for DLS measurements.

4.2 The intensity autocorrelation function is computed from the measured time-dependent fluctuation of scattered light using conditions set by the analyst or recommended in this test method. Calculations are based in part on the cumulants analysis. The mean intensity-weighted equivalent sphere hydrodynamic diameter is reported along with the polydispersity index as defined in the standard. Results obtained by application of a deconvolution analysis algorithm, such as a constrained inverse Laplace transform, are reported in a manner that enables all critical fitting parameters to be known and includes the original intensity-weighted distribution result. A representative normalized autocorrelation function in graphical format is reported for each aliquot. Appendix X1 provides a brief general overview of the DLS technique as applied in this test method. Appendix X2 provides general good practice guidance supplemental to Guide E2490. Appendix X3 lists currently available reference materials and quality control materials that might be applicable to DLS. Appendix X4 defines the application of a random effects ANOVA model to estimate the grand average measurand and the combined measurement uncertainty. Appendix X5 is a reporting checklist to aid the user in verifying compliance with this test method, and Appendix X6 is an example data report that meets the reporting requirements.

5. Significance and Use

5.1 Particle size is a key property of manufactured or engineered nanoparticles used in a wide range of applications. For purposes relevant to evaluations of safety, effectiveness, performance, quality, public health impact, or regulatory status of products, the correct measurement and uniform reporting of size and related parameters under use conditions, or during the manufacturing process, are critical to suppliers, analysts, regulators and other stakeholders.

5.2 This test method is intended principally for the analysis of nanoparticles in aqueous suspension with dimensions between about 1 nm and 100 nm, but may be applied to diffusive colloidal particles even if their dimensions fall outside the nanoscale range (up to 1000 nm).

5.3 For more detailed guidance on DLS measurements, including operational aspects, refer to Appendix X2 of this test method.

NOTE 1—The user is also referred to Guide E2490, which provides broad guidance for the application of DLS to nanomaterials. Guide E2490 is not required for the implementation of this test method.

6. Interferences

6.1 Interference can result from sample absorption at the wavelength of measurement. Absorption reduces scattered intensity and thereby reduces sensitivity. Absorption can also cause temperature increase, which alters the local viscosity affecting diffusivity. Refer to X2.1 for additional details and recommendations.

6.2 Fluorescence or autofluorescence by sample components is a potential interference if the excitation wavelength is at or near the wavelength of measurement. This interference might be difficult to identify without prior knowledge of the component excitation behavior.

6.3 Interference can result from number fluctuations (time-dependent variation of particle concentration in the scattering volume), particle-particle interactions (for example, at relatively high concentrations or when strong electrostatic forces are present) and multiple scattering (at relatively high concentrations – this effect increases with particle size). In this context, 'low' and 'high' concentration are situational (material and instrument dependent). Refer to X2.3 for further details and recommendations related to concentration effects and their mitigation prior to analysis (for example, conducting a dilution series analysis to identify an appropriate concentration range).

6.4 Salts, added deliberately or unintentionally (for example, via contamination of glassware), can significantly impact the measured size and stability of the analyte if the particles are electrostatically stabilized. Refer to X2.4 for further details and recommendations related to electrolyte concentration.

6.5 DLS cannot differentiate between the analyte of interest and contaminant particles that also scatter light. The presence of adventitious or undesired particles (for example, dust) can lead to measurement artifacts and bias. Proper handling of test specimens prior to analysis is key to avoiding contamination by adventitious particles and obtaining results that correctly reflect the properties of the analyte. Refer to X2.5 for further details and specific recommendations regarding particle contamination.

6.6 In general, DLS is highly sensitive to the presence of large particles, due to a strong size dependence of scattered intensity. However, this sensitivity depends on the scattering properties and concentrations of all particles present and on the instrument configuration used. For this reason, small numbers of very large particles (for example, adventitious particles, aggregates or agglomerates) within a population of very small particles, can effectively dominate the scattering signal. Commercial instruments typically use some form of "dust rejection" to mitigate this problem. On the other hand, if these large species are of practical concern for the intended use, then the analyst must be aware of any process their measurement system uses to reduce the effects of large particles. For instance, some instruments automatically reject a percentage of the measurement runs with the highest scattered intensity, the assumption being that runs affected by the presence of very large particles will be removed from analysis. The erratic

appearance of large size peaks in a calculated DLS size distribution, can indicate the presence of large particle contaminants, while the analyte peak should be relatively stable with respect to size and amplitude. Using a backscatter configuration also minimizes the effects of large particle scattering, which is strongly oriented toward forward (low) angles.

6.7 For particles much smaller than the wavelength of light (of order 60 nm or less for red lasers), scattering increases as the 6th power of size (Rayleigh approximation). This strong dependence on size continues above this threshold, but is more complex as particle size approaches the wavelength of light (Mie scattering). Therefore, polydisperse test specimens can yield results that do not accurately reflect the true distribution of sizes present.

6.8 Proteins, polymers and other macromolecular species, if present in significant quantities, can scatter sufficiently to affect the measured correlation function and thereby bias results. Just as with adventitious particles, DLS does not differentiate between types of scatterers. These species are generally small and are typically weak scatterers, so their impact will depend strongly on their concentration. At relatively low concentrations, their contribution can be insignificant, while at higher concentrations they can produce a bias in the results. The potential impact is also dependent on the size, concentration, and composition of the analyte itself.

6.8.1 Macromolecular species can also interact with the analyte to alter the analyte size or structure, thereby influencing the measured hydrodynamic size.

6.8.2 Dissolved macromolecular species, if present in sufficient quantity, can increase the apparent viscosity of the aqueous medium. If this effective change in viscosity is significant and not accounted for, it can yield a size that is larger than reality due to the inverse dependence of diffusivity on viscosity.

6.9 Air bubbles present a potential interference issue for DLS measurements. Degassing of aqueous suspensions occurs spontaneously when subjected to a temperature increase, thereby generating bubbles. If present, scattering from such large inhomogeneities can interfere with the intended measurement, leading to drastic and usually obvious impacts on the results (for example, highly atypical correlograms, extremely high baselines, very poor data quality metrics). Visual inspection of the sample cuvette is warranted in this case. Allowing sufficient time for test specimens or sample to equilibrate at or near the measurement temperature will also alleviate this problem.

6.10 The quality of the optical cell or cuvette can impact DLS results; surface scratches, imperfections or other sources of undesired scattering can produce measurement artifacts that significantly impact results and are difficult to isolate. Generally, quartz or optical grade glass cells yield the best results and are less prone to surface imperfections relative, for instance, to plastic disposable cuvettes or 96 well plates. This must be weighed against the convenience of disposable cuvettes and well plates for high-throughput applications. Refer to X2.6 for further details and specific recommendations on the choice and use of measurement cuvettes.

7. Apparatus

7.1 Commercial instruments are available from numerous manufacturers for the measurement of hydrodynamic size by photon counting and autocorrelation coupled with cumulants analysis and deconvolution algorithms for the analysis of polydisperse materials. Instruments typically operate at a fixed angle (for example, 90° or 173°), at multiple fixed angles (between about 13° and 173°) or use a goniometer to select angles over a broad range (from about 10° to 160°). Most have default settings or automated optimization of measurement parameters that require minimal input from the analyst. Manual operation is also an option provided on most instruments. The basic DLS instrument includes a coherent laser source (linearly polarized, visible wavelength), optics to focus and direct the laser beam and to detect scattered photons over very short time intervals, a sample chamber capable of maintaining constant temperature ± 0.2 °C, a photodetector (for example, avalanche photodiode), a correlator (either stand-alone or integrated into the control PC) and a PC with control software to perform measurements and to analyze data.

7.2 DLS instruments that use substantially different measurement principles or optical configurations are available, including frequency domain (power spectrum) analysis, cross-correlation spectroscopy and heterodyne detection; these alternative approaches are beyond the scope of this test method, but they should produce comparable results for optically dilute (transparent) nanoparticle suspensions. The cross-correlation configuration mitigates multiple scattering effects, while power spectrum analysis using heterodyne (reference beam) detection might improve sensitivity to very weak scatterers.

7.3 A HEPA-filtered hood or cabinet is useful for sample preparation and storage but is not required. Similarly, a centrifuge may be useful for separating the suspension medium (as supernatant) from analyte particles or to remove large adventitious particle contaminants; use is situational and must be fit for the intended purpose and clearly justified.

7.4 Appropriate filters for removal of unwanted or adventitious particles that are large compared with the analyte or for preparation of diluents; use is situational and must be fit for the intended purpose and clearly justified.

7.5 Appropriate syringes for filtration and transfer of samples and test specimens as needed.

7.6 Appropriate glassware and plasticware (for example, microtubes, transfer tubes, bottles) for transfer and storage of diluent, samples, and test specimens.

7.7 Sample cells or cuvettes, and other peripheral equipment as recommended by the manufacturer for the instrument used and fit for the intended purpose.

8. Reagents and Materials

8.1 Filtered deionized (demineralized) water for preparation of suspensions and solutions and for cleaning or rinsing glassware and measurement cuvettes.

8.2 Appropriate filtered aqueous solutions for dilution of concentrated samples if appropriate and fit for the intended use (for example, buffers, electrolytes, suspending medium).

9. Sampling and Test Specimens

9.1 It is important that the analyzed aliquot (test specimen) is representative of the larger sample or processing stream from which it is taken. The source material must be homogeneous before any sampling takes place; this can be achieved using procedures such as sample inversion, mild shaking or stirring, or vortexing. The procedure used to ensure homogeneity must be fit for purpose and appropriate for the target analyte, without altering the state of dispersion or introducing bubbles to the sample; generally, the least energetic approach shall be chosen that achieves the desired uniformity. Application of ultrasonic treatment or other high energy dispersive procedures (for example, intense shaking, high speed stirring, and excessive vortexing) is not appropriate for homogenization, where the goal is to achieve a uniform distribution of analyte, not to disrupt agglomerates or otherwise alter the native dispersion state. The procedure chosen should be included in the method validation process.

9.2 In general, stable suspensions of nanoparticles are "self-homogenized" due to Brownian motion, although some stratification can occur over long time periods (days to months) if left standing. Analysis and comparison of several independently sampled aliquots will provide validation that sampling is representative. If large (non-colloidal) particles or aggregates are present, they will sediment rapidly and are not appropriate for DLS analysis (which requires that the particles are predominately diffusive (colloidal) and do not settle significantly during the time-frame of the measurement). A series of replicate measurements on the same aliquot will provide confirmation that the sample is stable over the relevant time frame, if results vary randomly and a clear trend is not apparent. If following mild sample or test specimen agitation, sediment or stratification becomes visually apparent over the time span of several minutes, then the sample is not appropriate for DLS measurement and an alternative technique should be considered.

10. Preparation of Apparatus

10.1 Follow instrument manufacturer instructions for powering up device, and for time necessary to achieve laser and thermal stability necessary for analysis.

10.2 Clean glass or quartz cuvettes with filtered demineralized water and store dry. Periodic use of commercial cleaning agents formulated specifically for optical cells and components is recommended to remove difficult residues, but care must be taken to remove all traces of the cleaning detergent as this can impact analyte properties. Keep dry cleaned cuvette sealed/capped until needed. If available, store cuvette under HEPA filtered air (for example, in a clean bench).

10.3 Remove dust and other debris from plastic disposable cuvette or well-plate surfaces using a mild stream of clean (preferably filtered) compressed air or inert gas prior to use. Alternatively, disposable cuvettes can be rinsed with filtered demineralized water prior to use at the discretion of the analyst.

11. Performance Verification and Method Validation

11.1 DLS is a first-principles technique, and as such does not require calibration. However, analysts shall periodically measure appropriate reference materials or quality control materials in order to provide qualification (that is, verification) of correct instrument operation within manufacturer specifications. It is recommended, at a minimum, to verify instrument performance at the beginning of a new or substantially modified protocol as part of the method validation process, following lengthy periods of disuse, or after repairs or relocation of the device. Verification may be repeated at the end of a series of test measurements for additional assurance. In addition, annual performance certification by the instrument manufacturer is strongly recommended. Additionally, validation of the measurement method (including sample preparation) must be demonstrated as fit for purpose for all aspects of its scope. If a dilution series must be conducted to identify an appropriate concentration range to meet instrument requirements and avoid concentration-dependent effects, this test shall be included in the validation protocol. Validation is achieved using test materials equivalent (or similar) to the product or sample to be tested, which are processed and prepared in a similar fashion. Method validation must be performed only once unless some part of the procedure or product (test material) is changed, or major instrument operating conditions have been altered.

11.1.1 Correct calibration of the temperature sensor is necessary to convert the measured diffusion coefficient to size. This calibration is generally the responsibility of the instrument manufacturer. Temperature calibration issues should be considered if the basic instrument verification process fails.

11.2 For the purpose of instrument performance verification, polystyrene latex and silica reference materials with nominal diameters between (20 and 100) nm are available from commercial suppliers and government organizations. For qualification below 20 nm, and in the absence of available reference materials, proteins such as cytochrome c and bovine serum albumin are recommended. These materials are commercially available, are relatively inexpensive, and their hydrodynamic size has been thoroughly characterized and reported in the peer-reviewed literature. Polystyrene latex reference materials are also available in larger sizes, and these may be useful for applications involving polydisperse samples or samples containing particles larger than 100 nm. It should be emphasized that instrument performance verification does not necessarily require use of a reference material of the same or similar size to the analyte. However, use of two or more independent reference materials varying in size or composition, or both, is recommended as good practice, and using two that bracket the analyte size, if possible, provides the highest level of confidence. Refer to Appendix X3 for suggested reference materials and quality control materials.

11.3 The procedure for instrument performance verification using a reference material is adapted from ISO 22412, as follows:

11.3.1 Measure the reference material 5 times under repeatability conditions and calculate the average of the cumulants-based intensity-weighted average hydrodynamic diameters $\langle \bar{x}_{DLS} \rangle$ and their standard deviation u_{DLS} (in nm).

11.3.2 Divide the expanded uncertainty stated for the reference material mean size (in nm) by the coverage factor k to obtain the standard uncertainty u_{RM}; the value of k is typically 2, representing a 95 % confidence interval. If the stated uncertainty is expressed as a standard deviation (in nm), then conversion is not necessary, and the stated uncertainty then equals u_{RM}.

11.3.3 Calculate the tolerance u_{meas} (in nm) for the measured uncertainty by dividing u_{RM} obtained above by the stated mean diameter (in nm) for the reference material and then multiplying this value by $\langle \bar{x}_{DLS} \rangle$.

11.3.4 Combine the results of the previous two steps quadratically and multiply by a coverage factor $k = 2$ to obtain the expanded uncertainty U, where $U = 2\sqrt{{u_{RM}}^2 + {u_{meas}}^2}$.

11.3.5 Calculate the absolute difference between $\langle \bar{x}_{DLS} \rangle$ and the reference stated mean value (in nm). If this difference is $\leq U$, then the performance verification bias test passes.

11.3.6 If the relative standard deviation of the measurements $\frac{u_{DLS}}{\langle \bar{x}_{DLS} \rangle} \times 100$ % is < 2 % then the performance verification repeatability test passes.

11.3.7 Failure of verification tests indicate that a problem may exist with the instrument, the measurement cell, the reference material, or the preparation of the test specimen. Check that the reference material has not exceeded the stated expiration date. If the reference material is within its stated shelf-life, address other possible sources of error and contact the manufacturer if non-instrument issues prove inconsequential.

11.3.8 The above procedure presumes that the stated reference value is the hydrodynamic mean diameter measured by DLS or another appropriate technique.

11.3.9 In the absence of appropriate reference materials, one or more quality control materials with an established size (see for example, Appendix X3) may be used in order to verify correct performance over a specific size range. In this case, the comparison between measurement and known value must consider the material source and the method by which the known size was established.

11.4 For comparative purposes and instrument verification, particle size results obtained from DLS measurements often do not coincide with those obtained by other techniques (for example, electron microscopy). This is due to different method-defined measurands associated with different sizing techniques.

NOTE 2—For instance, differences arise from different weighted averages (for example, intensity versus number), as well as differences in the physical property that is actually measured (for example, particle diffusion versus projected particle area). For monodisperse hard core spheres larger than about 60 nm in diameter, these differences tend to decrease with increasing size; the presence of surface coatings (for example, ligands, polymers) can significantly alter this trend. Additionally, DLS is sensitive to the presence of small quantities of large particles or clusters of smaller particles, whereas electron microscopy-based results, for example, typically reflect the size of primary particles and, in combination with statistical counting limitations, may not represent a statistically relevant sampling of clusters or larger particles present (for example, agglomerates and aggregates, but also oversize primary particles).

11.5 For instrument performance verification, as part of method validation, preference should be given to reference materials that are similar to the target analyte or test material when available.

11.6 For regulatory purposes, analysts shall follow established guidance for method validation as appropriate for DLS analysis (see for example ICH (**1**),[4] FDA 1996 (**2**), and FDA 2015 (**3**)). Validation procedures written for analytical methods shall be adapted for DLS analysis, though some aspects and criteria might not be commutable (for example, limit of detection, limit of quantification, response linearity). Aspects relating to repeatability, reproducibility and robustness should, however, be fully commutable, and as such must be addressed during method development and validation.

11.6.1 The evaluation of robustness should be addressed during the method development phase. It should demonstrate the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are sensitive to variations in analytical conditions, the analytical conditions should be suitably controlled, or a precautionary statement should be included in the method.

12. Procedure

12.1 Always wear appropriate personal protective gear (for example, gloves, lab coat, goggles) and take appropriate precautions when handling nanomaterials.

12.2 The suspending medium (also known as, aqueous solution, diluent) shall be filtered *prior* to sample preparation using a 0.1 µm or smaller pore size membrane, and should be tested for scattering contributions to the measured signal (count rate) in the absence of the analyte. As a general rule, one should filter the medium to at least the nominal size of the analyte to be measured. This may not be practical for particles smaller than 20 nm; in this case, the count rate for the suspending medium should be measured separately and compared to the test specimen count rate to ensure the absence of, or to account for, interfering particles or other scattering entities present at significant concentrations. As a rule of thumb, the count rate for the aqueous medium should be less than about 10 % of the count rate for the test specimen (optionally, follow the manufacturer's recommendation).

12.3 If preparing a sample from dry material or a concentrate, the concentration shall be adjusted to accommodate the scattering properties of the sample and the optical requirements of the instrument (that is, according to instrument manufacturer specifications for acceptable count rate range). The presence of multiple scattering or particle interactions must be considered when preparing samples and test specimens for analysis (for recommendations, see 6.2 and X2.3). The presence of agglomeration should also be considered during sample preparation.

12.4 If a prepared sample (native product or material) is to be analyzed, the count rate must fall within the instrument manufacturer's specifications (generally, the linear range for

[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.

the detector). If it is too low, and the sample cannot be concentrated (for example, by centrifugation), then the sample is not appropriate for DLS analysis. If the count rate is too high, one option is to dilute the test specimen with an equivalent medium if available and if it does not alter the analyte state. The count rate can also be reduced to optimal levels by use of an attenuator located between the laser and the sample cell interface. Some instruments automatically determine and apply the required beam attenuation. It is also possible to reduce the laser power output by reducing the applied voltage, though this requires restabilization of the laser and is not common practice. In the absence of beam attenuation, and if dilution is not possible or desired, if the count rate exceeds the stated specifications, then the test specimen is not appropriate for DLS analysis on that system.

NOTE 3—Instrument-specific acceptability of a test specimen's scattered intensity (count rate) does not exclude the possibility of multiple scattering or particle interactions, both of which can bias the size measurement. Refer to X2.3 for further guidance on identifying and mitigating these concentration-dependent effects, and on the use of dilution and source attenuation.

12.5 Use appropriate sample cells for analysis (refer to instrument manufacturer recommendations). Quartz or glass cuvettes generally offer the best optical quality. Disposable plastic (for example, polymethylmethacrylate or polycarbonate) cuvettes offer convenience, but their use must be evaluated for each application as part of the method validation process. In addition, each plastic cuvette must be inspected visually for scratches and other visible quality defects prior to use; reject any cuvettes that are suspect. Choose the appropriate cell capacity depending on the available sample volume and instrument requirements. Ensure that cuvettes, cells, or well-plates are clean and dust-free prior to adding test specimen(s).

NOTE 4—Plastic disposable cuvettes and 96 well plates are convenient for high-throughput analysis, and can be a significant time-saver, as they can be used once and discarded. However, the optical quality of disposable cuvettes and well plates is generally less than that of quartz or optical glass, and therefore their appropriateness for a particular type and concentration of nanoparticles must be verified as part of the method validation process prior to use. Generally, disposable cuvettes and well plates become less prone to artifacts as the particle size, particle concentration or particle refractive index increase, or both (that is, they may not be suitable for some weak scattering analytes). Comparison of results obtained using disposable cuvettes or well plates with quartz or glass cuvettes is recommended during method validation. Plastic cuvettes also scratch easily, so care must be taken to avoid abrading the surface; do not wipe the surface of disposable cuvettes.

12.6 Test specimens that will be used to generate data for regulatory submissions shall not be filtered or otherwise modified without justification. Exceptions, for instance dilution to achieve a scattering intensity that is appropriate for the instrument used or to mimic in-use conditions, might be justifiable. Any test specimen modification shall be incorporated into the method validation protocol.

12.7 Non-regulatory samples (that is, for research and development), where the principal purpose of analysis is to characterize the primary analyte phase, can be filtered if necessary to remove dust and other large non-analyte particles if fit for purpose. The choice of pore size depends on the maximum dimension of the test particles and their tendency to adhere to the filter membrane. This is discussed along with non-filtration alternatives, in X2.5. If using a filter, pre-rinse the filter with at least 0.5 mL of the suspending medium (if available) before loading sample and reattaching filter cartridge; if suspending medium is not available, use deionized water. If a procedure is used to remove adventitious particles, it must be validated for the test material prior to measurement to ensure that the analyte is not being removed or otherwise modified by the process, and the procedure must be reported.

12.8 Include all relevant controls in both the validation and measurement processes and apply the same rules and recommendations to controls as to analyte.

12.9 *Loading Test Specimen:*

12.9.1 If using a syringe to load test specimen, allow the first 4 drops to go to waste. If possible, use the next 4 drops to pre-rinse the cuvette, and discard. The remainder can be used for measurement.

12.9.2 If using a pipette to load test specimen, fill the pipette once with sample medium or test specimen and eject to waste. Then refill the pipette with test specimen and use this suspension to load cell(s).

12.9.3 Load the test specimen into a measurement cuvette or cell using the minimum amount necessary to ensure the liquid level is above the entrance height of the laser beam for the instrument configuration being used. Refer to the instrument manual or contact the manufacturer to confirm beam height at the cell (typically 8.5 mm or 15 mm) or appropriate fill level. For microcuvettes with a sample-well insert, fill the well with test specimen, but do not fill beyond the well lip. Overfilling of a cuvette can lead to thermal gradients that will adversely impact measurement accuracy. If using a 96 well plate, fill the wells to the manufacturer's specification using clean pipette tips pre-rinsed with the suspending medium; the filling process is best conducted in a HEPA-filtered clean bench or biosafety hood.

12.9.4 Avoid touching the exterior surface of cuvettes or cells with bare hands. Wipe outside of quartz or glass cuvette with lens paper as needed.

12.9.5 Cap the cuvette or cell to prevent dust contamination and medium evaporation.

12.9.6 Inspect the cuvette to ensure that air bubbles are not adhering to a surface where they can interfere with the incoming laser beam or detected light. If necessary, *gently* tap cuvette on a non-metallic padded surface to release bubbles before placing cuvette in the sample holder. Never shake or vortex the cuvette, as this may introduce air bubbles or entrap air in the sample well of microcuvettes, which can adversely affect measurements. Place the cuvette correctly into the sample holder (for example, quartz windows should be facing the incident beam and detector). Refer to the instrument manufacturer's instructions for positioning of sample cuvettes. If using a 96 well plate, follow the instrument manufacturer's instructions for inserting the plate for analysis.

NOTE 5—Degassing the medium used to prepare test specimens (for example, by applying a vacuum) can help prevent the formation of air bubbles during analysis. Bringing the sample temperature close to the measurement temperature before filling the cuvette or well plate will also mitigate the formation of bubbles during analysis.

12.10 The necessary equilibration time for the test specimen to reach the measurement temperature will depend on the volume, the cell type and the difference between the initial and target temperatures. Larger test specimen volumes will require longer equilibration times. Performing measurements at the test specimen's temperature (for example, at room temperature at which sample has been equilibrated) will reduce any potential error. The required equilibration time must be evaluated as part of the method validation process. The measurement temperature in the instrument shall be controlled and measured with an accuracy of ± 0.2 °C or better for temperatures from (4 to 37) °C. At higher temperatures, accuracy will likely decline. Refer to the manufacturer's specifications for temperature accuracy over the applicable range.

12.10.1 The analyst may estimate equilibration time by running consecutive measurements at the target temperature and noting the initial temperature of the test specimen (if ambient, otherwise using a traceable thermometer, for example). When the measured size has reached a constant value, the equilibration is complete. This process is best performed using a reference specimen of known size.

12.11 Measurement duration should be set according to instrument manufacturer recommendations and will differ depending on particle size and scattering characteristics, as well as the optical characteristics of the instrument itself (for example, detector sensitivity, scattering angle). A *minimum* duration (measurement time) of 60 s shall be used for the analysis of nanoscale particles. Measurement duration shall be evaluated as part of the method validation process.

12.12 Perform at least 5 independent measurements per test specimen to establish measurement repeatability (precision).

12.13 Analyze at least 3 separate test specimens (aliquots) from the same source sample under identical conditions to evaluate heterogeneity and sampling precision.

12.14 Intermediate precision (also called within-lab reproducibility) assesses random effects that can vary over a longer timescale, such as different analysts, reagents, laboratory conditions, etc. Because more effects are accounted for by intermediate precision, its value, expressed as an absolute or relative standard deviation, is often larger than the repeatability standard deviation. If desired, perform identical measurements on fresh aliquots over several days to several weeks and vary the analyst if possible. Intermediate precision is not a requirement of this test method, but is recommended as good practice.

12.14.1 Inspect individual measurement results; if there is a clear trend in the data, consider whether the test specimen is stable and appropriate for DLS analysis and report observed trend if relevant.

NOTE 6—For example, if size is increasing, this suggests agglomeration. If intensity (count rate) is dropping, this suggests settling. If size is decreasing and intensity is decreasing, this suggests either dissolution or segregation of size populations due to settling.

13. Calculation and Interpretation of Results

13.1 A detailed description of data analysis will not be presented here; refer to the instrument manual or relevant standards and publications cited herein. There are essentially two approaches available to analyze the raw autocorrelation data from correlation-based DLS: cumulants analysis and deconvolution algorithms.

13.2 The recommended symbol for average hydrodynamic diameter obtained from DLS is $\bar{x}_{DLS}$, whether derived from cumulants or distribution analysis. Optionally, the symbol d_H may be used to specify an equivalent sphere hydrodynamic diameter obtained using DLS.

13.3 Presently, the only broadly recognized standard for the analysis of measured autocorrelation data is the cumulants method as stipulated in ISO 22412, Annex A. Cumulants analysis yields a mean intensity-weighted size, defined in ISO 22412 as the scattered light intensity-weighted harmonic mean (sphere-equivalent hydrodynamic) particle diameter, and a measure of the broadness of the distribution (polydispersity index, *PI*). Cumulants analysis does not generate a size distribution; it provides a mean value (central tendency) and the distribution variance. With the assumption of a single-mode Gaussian function, these values can be used to construct a hypothetical size distribution.

13.3.1 The results of cumulants analysis shall always be included with externally reported results, as the results can be indicative of sample quality and used to determine whether further analysis of DLS data using other methods is warranted.

13.3.2 While the cumulants method tends to produce a stable result and has been widely used, both for absolute determinations and for comparative purposes, its strict validity is limited to relatively narrow monomodal size distributions. It is generally not recommended for highly polydisperse or multimodal distributions, as indicated by a high value for *PI*. At high *PI* values, cumulants results might continue to be useful for relative comparisons (for example, in quality control applications), with the understanding that the reported results might not represent the true size and polydispersity of the test specimen particles.

13.3.2.1 As a rule of thumb, *PI* values smaller than about 0.05 indicate a tight monomodal distribution, often referred to as monodisperse. *PI* values greater than 0.05 suggest varying degrees of polydispersity and modality. For example, *PI* values up to about 0.2 are typically monomodal with relatively low polydispersity. For samples with *PI* values above about 0.3, cumulants analysis might not be appropriate (consider distribution algorithms) and for values over 0.7, samples might not be appropriate for DLS analysis (consider other techniques).

13.4 Alternatively, deconvolution algorithms (for example, regularization programs) are typically available on commercial instrument platforms, from which an equivalent sphere hydrodynamic particle size differential distribution can be extracted. Widely used methods include CONTIN and variations of Non-Negative Least Squares (NNLS). Basic guides to the theory and application of DLS and data analysis can be found in the literature (see for example, Morrison (**4**), Johnson and Brown (**5**), Brown (**6**), Finsy (**7**), Santos and Castanho (**8**), Pecora (**9**), Chu (**10**), Yin (**11**), and Stetefeld (**12**)). These algorithms are subject to the ill-posed inverse problem in light scattering and are not standardized in their implementation. If the analyst chooses to calculate a size distribution using one or

more of these mathematical transforms, the analyst must be aware of their limitations and the potential to generate artifacts and misleading results. In particular, these programs require high quality data, from an inherently low-resolution technique (DLS). Additionally, there are adjustable parameters that can significantly impact the results (for example, the regularization parameter, which puts limits on the amount of information permitted in the outcome, or the size range and binning used for fitting). Most algorithms constrain the outcome to positive values and use non-linear least squares methods to derive the best fit to the data.

13.4.1 The calculated intensity-weighted size distribution (that is, differential distribution) may be used to identify the central tendency (mean or mode) of each identified population in a multimodal mixture. As a rule of thumb, particle populations must be separated in size by a ratio of at least 5:1 in order to be adequately resolved by single-angle DLS. Note that the mean peak size derived from a deconvolution algorithm is generally an arithmetic average, while the cumulants mean is a harmonic average. If reporting calculated size distributions, the intensity-weighted differential distribution must always be included.

13.4.2 It is possible to transform an intensity-weighted distribution into a volume-weighted distribution if the *RI* value for the analyte is known. This conversion can be useful for comparative purposes in a research and development context, but this conversion shall always be conducted with great caution due to amplification of underlying uncertainties. Conversion should be avoided for particles that are known to deviate substantially from a spheroidal geometry. Additionally, the intensity-weighted differential distribution shall always accompany the volume-weighted distribution if the latter is used for external reporting.

13.4.3 Conversion of the size distribution from intensity to a number basis for external reporting purposes is deprecated in this test method due to the inherent errors associated with this transformation.

13.5 The cumulative form (that is, cumulative undersize distribution) of the intensity-weighted size distribution and its associated percentiles (for example, d_{10}, d_{50} and d_{90}), while not endorsed by this test method, may be used for external reporting purposes if justified and fit for purpose. It should be noted that cumulative distributions are not traditionally used to represent intensity-weighted distributions obtained by DLS.

13.5.1 Such cases must encompass the understanding that the reported results do not represent the true size and polydispersity of the test specimen particles.

13.5.2 Examples for such instances include application in quality control of batches or to identify processing issues, where the purpose of the percentiles is to alert the user to a change in the dispersion conditions.

13.5.3 In all cases, the use of percentiles should be carefully evaluated with representative data sets to demonstrate fit for purpose, including cross verification with complementary techniques, as needed.

13.5.4 Caution shall be used in reporting and interpreting this form of the size distribution obtained by integration of the differential (discrete) distribution obtained from application of a regularization program. The analyst should be aware that percentiles, based on a DLS-derived cumulative distribution, are of questionable meaning and utility. These percentiles can be useful for comparative purposes but should not be considered quantitative in nature. For instance, d_{50} represents the median hydrodynamic size below which 50 % of the scattered intensity occurs. Since scattered intensity is strongly size dependent, the practical meaning of d_{50} in this context is complex and unclear.

13.5.5 In this context, this test method emphasizes that size distributions obtained from regularization algorithms are highly dependent on the fitting parameters used, the quality of the underlying data and other factors that might or might not be obvious or available to the user. Any transformation of the intensity-weighted discrete distribution contributes additional uncertainty and potential bias. In particular, percentile sizes are extremely sensitive to the selection of regularization fitting parameters, especially at the extremes of the cumulative distribution; the same correlation data can produce an infinite number of solutions that meet the acceptability criteria and yet do not necessarily represent physical reality. The user must be fully aware of these issues when reporting such data externally or when using such data for internal purposes. Examining the dependence of the percentile sizes on user adjustable fitting parameters is strongly recommended.

13.6 Whichever model is used to analyze measured correlation data, the Stokes-Einstein equation will ultimately be employed to convert the measured diffusion coefficient, D, to the hydrodynamic diameter, d_H:

$$d_H = \frac{kT}{3\pi\eta D} \quad (1)$$

where k is the Boltzmann constant, T is the absolute temperature, and η is the absolute zero-shear viscosity of the suspending medium. Thus, it is important to input the correct temperature and viscosity values. Temperature is usually handled automatically by the instrument software, and good temperature control of the test specimen is critical for accurate measurement. On the other hand, viscosity is typically an analyst input value or determined by the instrument software based on identification of the medium (for example, water). One must therefore input correct values of viscosity for the particular medium used at the measurement temperature. Generally, for maximum accuracy, viscosity values must be accurate to within about 1 %. For solutions containing significant amounts of salt (for example, buffers, isotonic solutions), viscosity will be slightly dependent on salt concentration. For instance, use of water viscosity for a test specimen dispersed in phosphate buffered saline (PBS) results in an error of about 2 % in the viscosity at room temperature.

13.6.1 The influence of dissolved species (for example, proteins, polymers) on the effective viscosity must be considered if relevant to an analysis. In this case, it might be necessary to directly measure the viscosity of the medium and to use this value in place of the default value (typically water at the temperature of measurement). Methods for determining viscosity of complex solutions is beyond the scope of this

standard but must be included in the validation process for the application of this test method if utilized.

13.7 It is important to input the correct *RI* for the suspending medium, as this value is used in the primary calculations yielding the diffusion coefficient. *RI* values should be accurate to within about 0.5 %, or to two decimal places for water. There is a slight dependence of *RI* on salt concentration (and other non-light-absorbing soluble additives) in aqueous media, but the difference between PBS, for instance, and water, is less than 0.2 %. Similarly, the temperature dependence of *RI* is very weak over the normal measurement temperature range for water and varies by only 0.2 % from 20 ºC to 37 ºC. *RI* is also wavelength dependent, but for water this dependence is sufficiently weak over the normal range of wavelengths used in DLS instruments (typically in the 488 nm to 750 nm range), that values reported for the sodium D line (589.3 nm) are generally acceptable (with an error of less than 0.3 % over the entire range). The *RI* for pure water at 20 ºC is 1.332 (calculated based on IAPWS R9-97 (**13**)), and this value can be used for most dilute salt solutions over the temperature range from 20 ºC to 37 ºC.

13.7.1 The *RI* for the particle phase is only required for transformation of size distribution results from an intensity-weighted basis to a volume-weighted basis. *RI* does not impact the calculation of the intensity-weighted distribution, diffusion coefficient, cumulants mean diameter or *PI*. *RI* values for a range of materials can be found in the literature (for instance, see Palik (**14**)). Optically active analytes (for example, gold nanoparticles) will have a substantial and possibly ill-defined imaginary component of the *RI* that depends on size and other factors, making accurate transformation difficult if not impossible.

13.8 For the analyst's convenience, the table below provides recommended values for the absolute viscosity and *RI* as a function of temperature and suspending medium composition for several common aqueous suspending media. Viscosity is given in SI units of mPa·s, which are equivalent to the c.g.s. units of centi-Poise (cP). The base values for the viscosity of pure water are derived from the *NIST Chemistry WebBook* (**15**). The contributions of dissolved salt to the viscosity of pure water are assumed to be additive and are based on values interpolated from data available in the *CRC Handbook of Chemistry and Physics* (**16**) and fit using a 3rd order polynomial. Therefore, to calculate the viscosity of a salt solution presented in the table below, at a different temperature between 0 ºC and 100 ºC, simply subtract the value of pure water at 20 ºC from the corresponding value for the salt solution. This will provide the incremental increase in viscosity for a particular salt composition. The incremental value can then be added to the viscosity for pure water at temperatures not reported below, but available from sources such as the CRC Handbook or the NIST Chemistry WebBook.

Aqueous Medium	Absolute Viscosity (mPa·s)			Refractive Index
	20 ºC 293.15 K	25 ºC 298.15 K	37 ºC 310.15 K	
pure water	1.002	0.890	0.692	1.332
10 mmol·L^{-1} NaCl	1.003	0.891	0.693	1.332
isotonic saline (154 mmol·L^{-1} NaCl)	1.020	0.908	0.710	1.334
phosphate buffered saline (PBS)	1.023	0.911	0.713	1.334

14. Report

NOTE 7—The user is encouraged to consult Practice E1617 for general guidelines on reporting of particle size results. Similarly, Guides E3144 and E3206 provide guidance for reporting physical and chemical characteristics of nano-objects and their collections. These documents are not required for the implementation of this test method.

14.1 For reporting purposes in this test method, the following should be included in an analyst report or regulatory submission:

14.1.1 Analyst name, affiliation and date(s) of measurement.

14.1.2 Sample identification and source (for example, product name, manufacturer).

14.1.3 Analyte composition and mass concentration in sample (if known) and test specimens (if different from source sample).

14.1.4 Suspending medium composition (of sample and test specimen if different).

14.1.5 Test specimen preparation and transfer details if relevant (including any process that could potentially alter the physical state of the analyte or composition of the test specimen, such as dilution, filtration or centrifugation).

14.1.6 Any relevant supplementary information (for example, shape or size obtained by other means).

14.1.7 Instrumentation.

14.1.7.1 Manufacturer, model, and software version.

14.1.7.2 Laser wavelength (nm) and power (mW).

14.1.7.3 Angle of measurement (that is, scattering angle), if a fixed angle, otherwise angle for each set of measurements.

14.1.7.4 Date of most recent manufacturer certified operational verification.

14.1.7.5 Analyst conducted instrument performance verification date, procedure and results (refer to Section 11).

14.1.8 Measurement conditions and parameters.

14.1.8.1 Temperature of measurement in Kelvin (or °C if appropriate).

14.1.8.2 Cell type (standard macro, semi-micro, micro or well plate), material (quartz, glass, plastic disposable and type of plastic) and test specimen volume (µL or mL).

14.1.8.3 Viscosity of suspending medium (at measurement temperature) (mPa·s).

14.1.8.4 Refractive index of suspending medium.

14.1.8.5 (Optional) Instrument attenuation factor (relating fraction of light removed) or percent attenuation, if relevant and available to analyst.