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Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS)[1]

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1. Scope

1.1 This guide deals with the measurement of particle size distribution of suspended particles, which are solely or predominantly sub-100 nm, using the photon correlation (PCS) technique. It does not provide a complete measurement methodology for any specific nanomaterial, but provides a general overview and guide as to the methodology that should be followed for good practice, along with potential pitfalls.

1.2 *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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E 177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E 691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E 1617 Practice for Reporting Particle Size Characterization Data

F 1877 Practice for Characterization of Particles

2.2 *ISO Standards:*

ISO 13320-1 Particle Size Analysis—Laser Diffraction Methods—Part 1: General Principles[3]

ISO 14488 Particulate Materials—Sampling and Sample Splitting for the Determination of Particulate Properties[3]

ISO 13321 Particle Size Analysis—Photon Correlation Spectroscopy[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard*—Some of the definitions in 3.1 will differ slightly from those used within other (non-particle sizing) standards (for example, repeatability, reproducibility). For the purposes of this Guide only, we utilize the stated definitions, as they enable the isolation of possible errors or differences in the measurement to be assigned to instrumental, dispersion or sampling variation.

3.1.1 *correlation coefficient*, *n*—measure of the correlation (or similarity/comparison) between 2 signals or a signal and itself at another point in time.

3.1.1.1 *Discussion*—If there is perfect correlation (the signals are identical), then this takes the value 1.00; with no correlation then the value is zero.

3.1.2 *correlogram or correlation function*, *n*—graphical representation of the correlation coefficient over time.

3.1.2.1 *Discussion*—This is typically an exponential decay.

3.1.3 *cumulants analysis*, *n*—mathematical fitting of the correlation function as a polynomial expansion that produces some estimate of the width of the particle size distribution.

3.1.4 *diffusion coefficient (self or collective)*, *n*—a measure of the Brownian motion movement of a particle(s) in a medium.

3.1.4.1 *Discussion*—After measurement, the value is be inputted into in the Stokes-Einstein equation (Eq 1, see 7.2.1.2*(4)*). Diffusion coefficient units in photon correlation spectroscopy (PCS) measurements are typically $\mu m^2/s$.

3.1.5 *Mie region*, *n*—in this region (typically where the size of the particle is greater than half the wavelength of incident light), the light scattering behavior is complex and can only be interpreted with a more rigorous and exact (and all-encompassing) theory.

3.1.5.1 *Discussion*—This more exact theory can be used instead of the Rayleigh and Rayleigh-Gans-Debye approximations described in 3.1.7 and 3.1.8. The differences between the approximations and exact theory are typically small in the size

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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.

range considered by this standard. Mie theory is needed in order to convert an intensity distribution to one based on volume or mass.

3.1.6 *polydispersity index (PI)*, *n*—descriptor of the width of the particle size distribution obtained from the second and third cumulants (see 8.3).

3.1.7 *Rayleigh-Gans-Debye region*, *n*—in this region (stated to be where the diameter of the particle is up to half the wavelength of incident light), the scattering tends to the forward direction, and again, an approximation can be used to describe the behavior of the particle with respect to incident light.

3.1.8 *Rayleigh region*, *n*—size limit below which the scattering intensity is isotropic—that is, there is no angular dependence for unpolarized light.

3.1.8.1 *Discussion*—Typically, this region is stated to be where the diameter of the particle is less than a tenth of the wavelength of the incident light. In this region a mathematical approximation can be used to predict the light-scattering behavior.

3.1.9 *repeatability*, *n*—in PCS and other particle sizing techniques, this usually refers to the precision of repeated consecutive measurements on the same group of particles and is normally expressed as a relative standard deviation (RSD) or coefficient of variation (C.V.).

3.1.9.1 *Discussion*—The repeatability value reflects the stability (instrumental, but mainly the sample) of the system over time. Changes in the sample could include dispersion (desired?) and settling.

3.1.10 *reproducibility*, *n*—in PCS and particle sizing this usually refers to second and further aliquots of the same bulk sample (and therefore is subject to the homogeneity or otherwise of the starting material and the sampling method employed).

3.1.10.1 *Discussion*—In a slurry system, it is often the largest error when repeated samples are taken. Other definitions of reproducibility also address the variability among single test results gathered from different laboratories when inter-laboratory testing is undertaken. It is to be noted that the same group of particles can never be measured in such a system of tests and therefore reproducibility values are typically be considerably in excess of repeatability values.

3.1.11 *robustness*, *n*—a measure of the change of the required parameter with deliberate and systematic variations in any or all of the key parameters that influence it.

3.1.11.1 *Discussion*—For example, dispersion time (ultrasound time and duration) almost certainly will affect the reported results. Variation in pH is likely to affect the degree of agglomeration and so forth.

3.1.12 *rotational diffusion*, *n*—a process by which the equilibrium statistical distribution of the overall orientation of molecules or particles is maintained or restored.

3.1.13 *translational diffusion*, *n*—a process by which the equilibrium statistical distribution of molecules or particles in space is maintained or restored.

3.1.14 *z-average*, *n*—harmonic intensity weighted average particle diameter (the type of diameter that is isolated in a PCS experiment; a harmonic-type average is usual in frequency analyses) (see 8.9).

3.2 *Acronyms:*

3.2.1 *APD*—avalanche photodiode detector

3.2.2 *CONTIN*—mathematical program for the solution of non-linear equations created by Stephen Provencher and extensively used in PCS (**1**)[4]

3.2.3 *CV*—coefficient of variation

3.2.4 *DLS*—dynamic light scattering

3.2.5 *NNLS*—non-negative least squares

3.2.6 *PCS*—photon correlation spectroscopy

3.2.7 *PMT*—photomultiplier tube

3.2.8 *QELS*—quasi-elastic light scattering

3.2.9 *RGB*—Rayleigh-Gans Debye

4. Summary of Guide

4.1 This Guide addresses the technique of photon correlation spectroscopy (PCS) alternatively known as dynamic light scattering (DLS) or quasi-elastic light scattering (QELS) used for the measurement of particle size within liquid systems. To avoid confusion, every usage of the term PCS implies that DLS or QELS can be used in its place.

5. Significance and Use

5.1 PCS is one of the very few techniques that are able to deal with the measurement of particle size distribution in the nano-size region. This Guide highlights this light scattering technique, generally applicable in the particle size range from the sub-nm region until the onset of sedimentation in the sample. The PCS technique is usually applied to slurries or suspensions of solid material in a liquid carrier. It is a first principles method (that is, calibration in the standard understanding of this word, is not involved). The measurement is hydrodynamically based and therefore provides size information in the suspending medium (typically water). Thus the hydrodynamic diameter will almost certainly differ from other size diameters isolated by other techniques and users of the PCS technique need to be aware of the distinction of the various descriptors of particle diameter before making comparisons between techniques. Notwithstanding the preceding sentence, the technique is widely applied in industry and academia as both a research and development tool and as a QC method for the characterization of submicron systems.

6. Reagents

6.1 In general, no reagents specific to the technique are necessary. However, dispersing and stabilizing agents often are required for a specific test sample in order to preserve colloidal stability during the measurement. A suitable diluent is used to achieve a particle concentration appropriate for the measurement. Particle size is likely to undergo change on dilution, as the ionic environment, within which the particles are dispersed,

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

changes in nature or concentration. This is particularly noticeable when diluting a monodisperse latex. A latex that is measured as 60 nm in $1 \times 10^{-3}M$ NaCl can have a hydrodynamic diameter of over 70 nm in $1 \times 10^{-6}M$ NaCl (close to deionized water). In order to minimize any changes in the system on dilution, it is common to use what is commonly called the "mother liquor". This is the liquid in which the particles exist in stable form and is usually obtained by centrifuging of the suspension or making up the same ionic nature of the dispersant liquid if knowledge of this material is available. Many biological materials are measured in a buffer (often phosphate), which confers the correct (range of) conditions of pH and ionic strength to assure stability of the system. Instability (usually through inadequate zeta potential (**2**) can promote agglomeration leading to settling or sedimentation in a solid-liquid system or creaming in a liquid-liquid system (emulsion). Such fundamental changes interfere with the stability of the suspension and need to be minimized as they affect the quality (accuracy and repeatability) of the reported measurements. These are likely to be investigated in any robustness experiment.

7. Procedure

7.1 *Verification*:

7.1.1 The instrument to be used in the determination should be verified for correct performance, within pre-defined quality control limits, by following protocols issued by the instrument manufacturer. These confirmation tests normally involve the use of one or more NIST-traceable particle size standards. In the sub-micron ($< 1 \times 10^{-6}$ m) region, then these standards (e.g., NIST, Duke Scientific- now part of Thermo Fisher Scientific) tend to be nearly monodisperse (that is, narrow, single mode distribution, PI < 0.1) and, while confirming the *x* (size) axis, do not verify the *y* (or quantity axis). Further, there is a lack of available standards for the sub-20 nm region and therefore biological materials (e.g., bovine serum albumin–BSA, cholesterol, haem, size controlled dendrimers, Au sols) of known size (often by molecular modeling) can be utilized. Note that PCS is a first principles measurement and thus calibration in the formal sense (adjustment of the instrument to read a true and known value) cannot be undertaken. In the event of a "failure" at the verification stage, then the issues to check involve quality of the dilution water, state of dispersion and stability of the standard under dilution plus instrumental issues such as thermal stability, cleanliness and alignment of optical components. The raw correlogram data can be examined during and after acquisition. Such examination requires some experience and training. During data acquisition one looks for stable count level without jumps or leaps in the level of the scattering counts that could be produced by particles (of dust or contamination) falling through the measurement zone ('number fluctuations'). Ideally the form of the correlogram is an exponential decay to a flat baseline (approximating to the photon counts in the system without sample) and not rise again (again indicating number fluctuations in the data). Manufacturers also provide other means of assuring the reliability of the data and is recommended that these protocols are consulted, as appropriate.

7.1.2 Given the nature of the produced intensity distribution and the likelihood that the size standard has been certified by electron microscopy (number distribution) care needs to be exercised in direct comparison of the results. For a completely monodisperse sample, (every particle identical) then the number and intensity distributions are essentially identical. For the real-world situation where there is some polydispersity (width) to the distribution, then the number distribution is expected to be smaller than the produced intensity distribution; the greater the polydispersity, then the larger the differences between intensity, volume and number distributions. Note that verification of a system only demonstrates that the instrument is performing adequately with the prescribed standard materials. Practical considerations for real-world materials (especially 'dispersion' if utilized or if the distribution is relatively polydisperse) mean that the method used to measure that real-world material needs to be carefully evaluated for precision (repeatability).

7.2 *Measurement*

7.2.1 *Introduction*:

7.2.1.1 The measurement of particle size distribution in the nano- (sub 100 nm) region by light scattering depends on the interaction of light with matter and the random or Brownian motion that particle exhibits in liquid medium in free suspension. There must be an inhomogeneity in the refractive indices of particle and the medium within which it exists in order for light scattering to occur. Without such an inhomogeneity (for example, in so-called index-matched systems) there is no scattering and the particle is invisible to light and no measurements can be made by the PCS or any other light scattering technique.

7.2.1.2 For particles < 100 nm, as considered in this guide, several facts hold true:

(1) The amount of scattering is weak in relative terms and depends highly on the size of the particle. In the Rayleigh approximation region (typically $d < \lambda/10$ in which d is the diameter of particle and λ is the wavelength of light employed), then this intensity of scattering is proportional to r^6 – or $(\text{volume})^2$ or $(\text{relative molecular mass})^2$. With a commonly utilized helium-neon (He-Ne) laser (632.8 nm), then this limit is approximately 60 nm. This means, in practice, that a 60 nm particle scatters 1 million times as much light as a 6nm particle of the same composition. Thus, it is imperative that solutions are kept free of any contaminating particles, for example dust, that are often present in the local environment and is usually considerably larger than the material that requires measurement. This means filtering liquids used to contain or dilute the particles to a least the same level as the size of the particles that require characterizing. The very weak scattering means that conventional light detectors (e.g., silicon photodiodes) as used in other light scattering technique (for example, laser diffraction) cannot be used. The technique of correlating the signal with itself combined with photon counting techniques is thus employed; the principle being that the noise is random while the Brownian motion is fixed. Constantly subtracting the noise from the overall signal leaves the retained Brownian motion signal.