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Particle size analysis — Dynamic light scattering (DLS)

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National foreword

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Analyse granulométrique — Dispersion lumineuse dynamique (DLD)

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Foreword

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This document was prepared by Technical Committee ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization*.

This second edition of ISO 22412 cancels and replaces ISO 22412:2008 and ISO 13321:1996.

Introduction

Particle size analysis in the submicrometre size range is performed on a routine basis using the dynamic light scattering (DLS) method, which probes the hydrodynamic mobility of the particles. The success of the technique is mainly based on the fact that it provides estimates of the average particle size and size distribution within a few minutes, and that user-friendly commercial instruments are available. Nevertheless, proper use of the instrument and interpretation of the result require certain precautions.

Several methods have been developed for DLS. These methods can be classified in several ways:

a) by the difference in raw data acquisition (autocorrelation, cross-correlation and frequency analysis);

b) by the difference in optical setup (homodyne versus heterodyne mode);

c) by the angle of observation.

In addition, instruments show differences with respect to the type of laser source and often allow application of different data analysis algorithms (e.g. cumulants, NNLS, CONTIN, etc.).

INTERNATIONAL STANDARD ISO 22412:2017(E)

Particle size analysis — Dynamic light scattering (DLS)

1 Scope

This document specifies the application of dynamic light scattering (DLS) to the measurement of average hydrodynamic particle size and the measurement of the size distribution of mainly submicrometre-sized particles, emulsions or fine bubbles dispersed in liquids. DLS is also referred to as "quasi-elastic light scattering (QELS)" and "photon correlation spectroscopy (PCS)," although PCS actually is one of the measurement techniques.

This document is applicable to the measurement of a broad range of dilute and concentrated suspensions. The principle of dynamic light scattering for a concentrated suspension is the same as for a dilute suspension. However, specific requirements for the instrument setup and specification of test sample preparation are required for concentrated suspensions. At high concentrations, particle-particle interactions and multiple light scattering can become dominant and can result in apparent particle sizes that differ between concentrated and dilute suspensions.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 9276-1, *Representation of results of particle size analysis — Part 1: Graphical representation*

ISO 9276-2, *Representation of results of particle size analysis — Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
particle
minute piece of matter with defined physical boundaries

Note 1 to entry: A physical boundary can also be described as an interface.

Note 2 to entry: A particle can move as a unit.

[SOURCE: ISO 26824:2013, 1.1, modified]

3.2
average hydrodynamic diameter
$\overline{x}_{DLS}$
hydrodynamic diameter that reflects the central value of the underlying particle size distribution

Note 1 to entry: The average particle diameter is either directly determined without calculation of the particle size distribution, or calculated from the computed intensity-, volume- or number-weighted particle size distribution or from its fitted (transformed) density function. The exact nature of the average particle diameter depends on the evaluation algorithm.

Note 2 to entry: The cumulants method yields a scattered light intensity-weighted harmonic mean particle diameter, which is sometimes also referred to as the "z-average diameter."

Note 3 to entry: Arithmetic, geometric and harmonic mean values can be calculated from the particle size distribution according to ISO 9276-2.

Note 4 to entry: Mean values calculated from density functions (linear abscissa) and transformed density functions (logarithmic abscissa) may significantly differ (ISO 9276-1).

Note 5 to entry: $\overline{x}_{DLS}$ also depends on the particle shape and the scattering vector (and thus on the angle of observation, laser wavelength and refractive index of the suspension medium).

3.3
polydispersity index
PI
dimensionless measure of the broadness of the size distribution

Note 1 to entry: The PI typically has values less than 0,07 for a monodisperse test sample of spherical particles.

3.4
scattering volume
volume defined by the intersection of the incident laser beam and the scattered light intercepted by the detector

3.5
scattered intensity
intensity of the light scattered by the particles in the scattering volume

3.6
count rate
photocurrent
I_S
number of photon pulses per unit time

Note 1 to entry: It is also a photodetector current which is proportional to the scattered intensity as measured by a detector.

3.7
validation
proof with reference material that a measurement procedure is acceptable for all elements of its scope

Note 1 to entry: Evaluation of trueness requires a certified reference material.

3.8
reference material
RM
material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

[SOURCE: ISO Guide 30:2015, 2.1.1, modified]

3.9
certified reference material
CRM
reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

[SOURCE: ISO Guide 30:2015, 2.1.2, modified]

3.10
qualification
proof with reference material that an instrument is operating in agreement with its specifications

4 Symbols and units

$C(\Gamma)$	normalized distribution function of decay rates or characteristic frequencies	dimensionless	
D_T	translational diffusion coefficient	metres squared per second	m^2/s
D_c	collective diffusion coefficient	metres squared per second	m^2/s
D_s	self-diffusion coefficient	metres squared per second	m^2/s
f	frequency, $f = \omega/(2\,\pi)$	hertz	Hz
$g^{(1)}(\tau)$	normalized electric field correlation function	dimensionless	
$G^{(2)}(\tau)$	scattered intensity correlation function	arbitrary units	
$G(\Gamma_j)$	normalized distribution function of the individual decay rate Γ_j	arbitrary units	
I_s	scattered intensity, count rate, photocurrent	arbitrary units	
I_0	intensity of the incident light	arbitrary units	
M	number of steps in the histogram	dimensionless	
n	refractive index of the suspension medium	dimensionless	
$P(\omega)$	power spectrum	arbitrary units	
PI	polydispersity index	dimensionless	
$\Delta Q_{\text{int},i}$	scattered light intensity-weighted amount of particles in size fraction i, i.e. $x_{i-1} < x < = x_i$	dimensionless	
x	within this document: hydrodynamic diameter of a particle	nanometres	nm
$\bar{x}_{\text{DLS}}$	average hydrodynamic diameter	nanometres	nm

$\bar{\Gamma}$	scattered light intensity-weighted average value of the distribution function of the decay rate or characteristic frequency	reciprocal seconds	s^{-1}
Γ_{max}	maximum decay rate (histogram method)	reciprocal seconds	s^{-1}
Γ_{min}	minimum decay rate (histogram method)	reciprocal seconds	s^{-1}
η	viscosity of the suspension medium	millipascal seconds	mPa·s
θ	scattering angle	degrees	°
λ_0	wavelength of the laser light in vacuum	nanometres	nm
μ_2	second cumulant of the distribution function of decay rates or characteristic frequencies	reciprocal square seconds	s^{-2}
ρ	particle density	grams per cubic centimetre	g/cm^3
τ	correlation time	seconds	s
q	modulus of the scattering vector	reciprocal nanometres	nm^{-1}
φ	particle volume fraction	dimensionless	
ω	angular frequency	radian per seconds	rad/s

5 Principle

Particles suspended in a fluid are in constant Brownian motion as the result of the interaction with the molecules of the suspending fluid. In the Stokes-Einstein theory of Brownian motion[1], particle motion of smooth spheres at very low concentration is determined by the suspending fluid viscosity and temperature, as well as the size of the particles. Thus, from a measurement of the particle motion in a fluid of known temperature and viscosity, the particle size can be determined.

The DLS technique[2][3][4][5][6] probes the particle motion optically. The suspended particles are illuminated with a coherent monochromatic light source. The light scattered from the moving suspended particles has a time-dependent phase imparted to it from the time-dependent position. The time-dependent phase of the scattered light can be considered as either a time-dependent phase shift or as a spectral frequency shift from the central frequency of the light source. Measured over time, random particle motion forms a distribution of optical phase shifts or spectral frequency shifts. These shifts are determined by comparison either with all scattered light (homodyne or self-beating mode) or by using a portion of the incident light as reference (heterodyne mode). Regardless of the setup, the optical signals received from the particles are related to the scattering efficiency of the particles and are thus scattered intensity-weighted.

Sedimentation of particles, dependent on their density, sets an upper limit to the particle size that can be assessed by the technique; typically, the upper limit is much less than 10 µm.

DLS was developed for static suspensions. Provided that orthogonal flow and observation axes are adopted, flowing samples may, under some circumstances, be measured if the procedure is properly validated (see Annex C).

Different modes of diffusion, particle-particle interaction, multiple scattering and fluorescence can significantly influence the apparent particle diameter calculated from a DLS experiment. Annex B should be consulted.

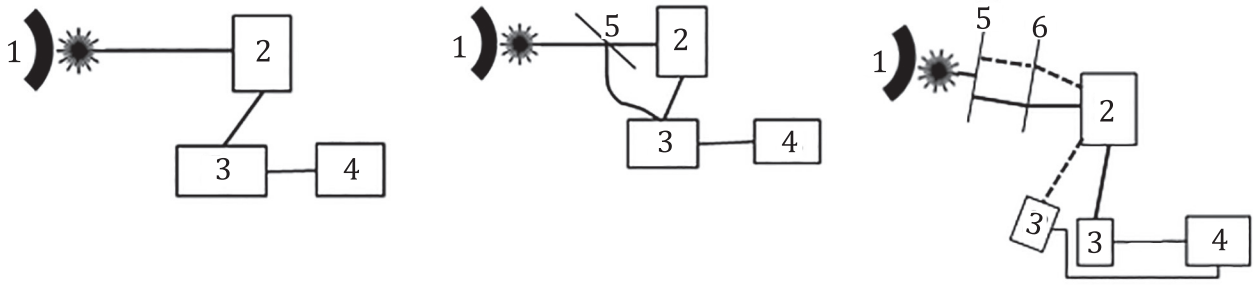
6 Apparatus

A typical apparatus consists of the following components:

6.1 Laser, emitting coherent monochromatic light, polarized with its electric field component perpendicular to the plane formed by the incident and detected rays (vertical polarization). Any kind of lasers may be used, e.g. gas lasers (He-Ne laser, Ar-ion laser), solid-state lasers, diode-pumped solid-state lasers and laser diodes.

6.2 Optics, lenses and equipment used to focus the incident laser light into a scattering volume and to detect scattered light. Optical fibres are often used as a part of the detection system and for light-delivering optics.

The use of a coherent optical reference allows using interference between the scattered light and the reference to measure the frequency shift of the scattered light. Two methods of referencing are commonly used and are illustrated in Figures 1 a) and b).



a) Homodyne **b) Heterodyne** **c) Cross-correlation (= two simultaneous homodyne experiments)**

Key

1 laser
2 sample
3 detector
4 correlator or spectrum analyser
5 beam splitter
6 lens

Figure 1 — Typical optical arrangement for DLS

— In homodyne detection (also referred to as "self-beating detection") [Figure 1 a)], the mixing at the optical detector of all of the collected scattered light provides the reference for frequency- or phase-difference measurement.

— In heterodyne detection [Figure 1 b)], the scattered light is mixed with a portion of the incident light. The unshifted incident light provides the reference for the frequency- or phase-difference measurement.

NOTE In DLS, "heterodyne" is understood as mixing of scattered light with unscattered light from the same source. This convention differs from, for example, the use in optical interferometry.

— In a cross-correlation setup [Figure 1 c)], two homodyne scattering measurements are performed simultaneously in such a way that the two scattering vectors and scattering volumes are the same, but the corresponding wave vectors are not coincident. These two laser beams produce two correlated fluctuation patterns. The correlation is not perfect, since on the one hand, both detectors collect

light from the other scattering experiment, and on the other hand, multiply scattered light of the incoming laser beams is totally uncorrelated. The two contributions of the multiply scattered light to the detector signal, however, do not contribute to the time-dependent signal but to an enhanced background.

6.3 Test sample holder, allowing fluctuations of the sample temperature to be controlled to within ±0,3 °C. While precise knowledge of the sample temperature is required for evaluation, it is not necessary to regulate the temperature to any defined value.

6.4 Photodetector, with an output that is proportionally related to the intensity of the collected scattered light. A photomultiplier tube or an (avalanche) photodiode is typically used. Detectors can be placed at any angle. Data collection can be performed in a linear or logarithmic manner.

6.5 Signal processing unit, capable of taking the time-dependent scattered light intensity signal and outputting the autocorrelation function, cross-correlation function or power spectrum of the input signal. This correlation can be performed by hardware and/or software correlators, operating linearly, logarithmically or in a mixed mode.

The resulting output from either mode contains a distribution of characteristic frequencies or time-dependent phases representative of the particle size of the suspended particles. Photon detection has a probability distribution of photon arrival times, which means that a fluctuating signal is obtained even if the intensity of the incident light is constant. The intensity of the photons arriving at varying time intervals is superimposed on this already fluctuating signal. In correlation analysis, the uncorrelated signal is constant, whereas the signal associated with the diffusing particles decays exponentially. In spectrum analysis, the uncorrelated signal is akin to a DC or zero frequency term which is not recorded. The time-dependent component is analysed to determine the particle-size distribution using the theory of DLS.

6.6 Computation unit, capable of signal processing to obtain the particle size and/or particle size distribution. Some computation units also function as the signal processing unit.

— Evaluation via the autocorrelation function allows determination of a mean diameter without determination of the particle size distribution, but determination of the distribution is also possible.

— Evaluation via the frequency distribution determines the particle size distribution using the power spectrum of the signal.

— Evaluation via photon cross-correlation allows quantification/minimization of the effects of multiple scattering, thus extending the useful concentration range towards higher concentrations (however, the effect of particle-particle interaction cannot be eliminated). The disadvantage of this method is that it requires a more complex optical setup.

6.7 Instrument location, placed in a clean environment, free from excessive electrical noise and mechanical vibration and out of direct sunlight. If organic liquids are used as the suspension medium, there shall be due regard to local health and safety requirements, and the area shall be well ventilated. The instrument shall be placed on a rigid table or bench to avoid the necessity for frequent realignment of the optical system.

WARNING — DLS instruments are equipped with a low- or medium-power laser whose radiation can cause permanent eye damage. Never look into the direct path of the laser beam or its reflections. Ensure highly reflecting surfaces are not in the path of the laser beam when the laser is on. Observe local regulations for laser radiation safety.

7 Test sample preparation

7.1 General

Test samples should consist of well-dispersed particles in a liquid medium. Dispersion procedures like sonication, filtration, etc. may influence the result and therefore have to be reported. The suspension liquid shall

a) be sufficiently transparent (non-absorbing) and non-fluorescent at the laser wavelength,

b) be free of particulate contamination,

c) not dissolve, swell or coagulate the particulate material,

d) have a known refractive index that is sufficiently different from that of the particulate materials,

e) have a known value of viscosity within ±2 % over the operational range of temperature to be used, and

NOTE As $\bar{x}_{\mathrm{DLS}}$ is directly proportional to η, the uncertainty of $\bar{x}_{\mathrm{DLS}}$ will always be larger than the uncertainty of η.

f) meet the guidelines of the instrument for low background scattering.

(This can be checked by measuring the count rate for the suspending medium alone and the dark count with no sample or solvent present. The former should be at least one order of magnitude lower than the sample, and the latter should be within the recommended range for the instrument.)

Inadequate suppression of the double layer can have a significant influence on the hydrodynamic diameter. A medium with ionic strength high enough to suppress the electric or diffuse double layer can improve agreement between results obtained by DLS and electron microscopy. A conductivity of 1 mS/cm is usually sufficient to achieve this between the hydrodynamic diameter and that obtained by microscopy techniques, especially for small particles.

Water is often used as a suspension medium. The use of freshly deionised and filtered (pore size 0,2 µm) water is recommended. A trace of ionic additive (e.g. NaCl at a concentration of 10 mmol/l = 0,6 g/l) may be added to such samples to reduce the double-layer thickness. However, precaution has to be made that such ionic strength adjustment will not make sample unstable or that the additive does not react with the sample (e.g. Cl with Ag ions).

7.2 Concentration limits

The lower concentration limit of the working range of DLS is determined, amongst other factors like particle size, detector sensitivity, etc., by the number of particles that are present in the scattering volume.

The scattered light intensity (e.g. expressed as count rate or I_S) of the sample containing the dispersed particles should ideally be ≥10 times the signal obtained by the suspension medium alone. Scattered intensity ratios below 10, either caused by low particle mass fractions or by very broad particle size distributions, will result in higher variation of results and poorer precision.

The maximum concentration of dispersed particles that can be measured without the concentration influencing the particle size reported is determined by particle-particle interaction and multiple scattering. This concentration limit should be determined empirically by dilution (see Annex B).

7.3 Checks for concentration suitability

Different instruments adopt differing optical observation angles and optical arrangements. The observations and checks given are for the general case, but the specific instrument operational advice should also be considered.

The following observations and checks are recommended.

a) Visually inspect the dispersed sample prior to placing it into the instrument. At low concentrations, the sample will look almost transparent. At higher concentrations, a milky or opaque appearance is seen.

b) Ensure that the sample is placed in the instrument prior to performing the measurement, allowing the sample to equalize its temperature. Check the count rate or signal level. The count rate or signal level (I_S) can be adjusted by changing an aperture in the receiver (which also changes the degree of coherence of the detector), by adjusting the gain of the receiver or by adjusting the laser power itself, either by adjusting the power applied to the light source (limited adjustment) or by employing a neutral density filter in front of the light source or in front of the detector.

c) For instruments using correlation analysis in autocorrelation mode, conduct a measurement using appropriate correlator settings and examine the intercept value which should be above the value specified by the vendor of the instrument. A low value of intercept may result from either a poorly aligned optical system, multiple scattering or from very weakly scattering samples requiring the detector aperture be increased, resulting in a multiple coherence area detection. For larger particles, the measurement volume may need to be increased to accommodate an adequate number of particles. This can also reduce the intercept value. A lower-than-expected intercept value may also be caused by sample absorbance or fluorescence. All of these factors may reduce the intercept value requiring further tests to establish the reason.

d) Measurements performed at different concentrations should give the same results within their measurement uncertainties. A decreasing particle size with increasing concentration indicates a significant amount of multiple scattering, a change of viscosity of the suspension caused by different viscosities of diluent and original suspension and/or collective motion of particles. Measurement at different concentrations is also used to extrapolate the particle size to infinite dilution [see Clause 8, list item k)].

In many applications, a volume fraction (ϕ) of dispersed particulate material in the range 10^{-5} to 10^{-4} fulfils the requirements for particle sizes below about 500 nm. For cross-correlation or backscatter techniques, higher concentrations can be achieved dependent on the sample. For polydisperse and/or larger particles, it may not be possible to find a concentration that satisfies all requirements without either increasing the coherence aperture of the receiver or increasing the diameter of the incident laser beam in order to increase the measurement volume. If this is the case, then the intercept values obtained may not meet the criterion set out in c). For particle sizes above 1 µm, the requirements c) and d) can only be fulfilled in exceptional cases.

All sample preparation steps (suspension medium, particulate concentration, dispersion procedure) should be recorded. (Recommendations for sample preparation are given in Annex D.)

8 Measurement procedure

a) Switch the instrument on and allow it to warm up. Typically about 15 min to 30 min is required to stabilize the laser intensity and to bring the sample holder to an equilibrium at the desired temperature.

NOTE 1 Monitoring the temperature rather than controlling it is sufficient for many applications provided a known value of viscosity is available for the reported monitored temperature.

b) A measurement cell filled only with suspension medium should be checked to ensure a low count rate or signal level, without radical fluctuations, which could indicate particle contamination. A high count rate or signal level might indicate cell flare or dirty cell walls. For instruments that provide background subtraction, measure and store the background signal for the dispersion medium being used.

c) Visually inspect the sample for the presence of optically visible particles, flocs, fibres and other possible contaminants. If these are present, repeat the sample preparation.

d) Transfer a required amount of sample to a suitable and clean measurement cell. The measurement cell may be disposable (e.g. plastic) or re-usable (e.g. optical-quality glass or quartz). Compared to disposable plastic cells, glass cells have as main advantage that there can be less refraction when light scatters across the cell and thus the actual observation angle will be the same as the geometrical angle between the detector and the incident light beam. The material of the measurement cell must be chemically compatible with the dispersion medium and the particles. Prior to sample loading, clean the cuvettes with filtered de-ionised water or solvent if non-aqueous dispersions are measured. In case of residual impurities, a mild surfactant or soap solution, specifically for optical cells, may be used. Rinse the cuvettes several times with filtered de-ionised water in order to remove residual surfactant. Let the water drain away by inverting the cuvette. Keep cleaned cuvettes capped until needed.

   The pore size of the filter used for filtering the water or solvent should be appropriate to the application. Ideally, a membrane with a pore size smaller than the smallest particle to be measured should be used.

   Alternatively, disposable plastic cuvettes (e.g. PMMA, polystyrene) may be used. The walls of plastic cuvettes are easily scratched and do not provide the optical quality of glass or quartz. Therefore, disposable cuvettes should not be used with weak scattering samples, and should be cleaned only by rinsing with suspending medium and/or use of a particle-free air stream to remove loose dust on the cell walls. Take care not to touch the cuvette windows with bare hands or to wipe cuvette surface with any potentially abrasive material (including optical paper or tissue).

   Place the test sample in the instrument or place the measurement probe into the sample. Allow temperature equilibrium to be established. The temperature value should be known to better than ±0,5 °C with minimal fluctuation of the value during the measurement.

   An alternative method for instruments without a temperature sensor in the cell is to measure the room temperature and then set the instrument to control the sample holder temperature to within ±0,3 °C of the room temperature. Samples can then equilibrate at room temperature and be measured immediately after insertion in the sample holder. Alternatively, samples can be equilibrated in a temperature-controlled bath whose temperature is controlled to ±0,3 °C of that of the instrument sample holder. In this case, dry the outside of the cell before inserting it into the DLS instrument.

   Uncertainties in particle size determined in aqueous suspensions will be approximately 2 % per degree Celsius at ambient temperature if the test sample has not reached thermal equilibrium.

   NOTE 2 For a temperature change of 3 °C, it can take about 10 min for the liquid in the measurement volume of a measuring cell holding a 1 ml of sample to thermally equilibrate.

e) Ensure that no air bubbles are entrapped in the test sample for non-air bubble samples. Ensure that no bubbles are attached to the walls of the cell.

f) Record test sample identification, date and time of the measurement and measurement duration, number of individual measurements, measurement temperature, temperature fluctuations during measurement, refractive index and viscosity of the suspension medium, particle concentration/dilution, wavelength of laser and scattering angle, as well as the particle concentration, if known.

g) Check the average scattered intensity of the sample.

   For homodyne optical arrangements, it is preferred that the average scattering intensity be controlled by adjusting the light output power, using neutral density filters, or by minimizing the detector aperture, to maintain detection coherence, while adjusting the receiver sensitivity within the limits specified by the manufacturer. The scattering signal from the test sample should be ≥10 times the signal from the dispersing medium alone.

   For heterodyne optical arrangements, the reference signal should be substantially greater than the test sample scattering signal (a ratio reference signal:scattering signal of 10:1 should be aimed for).

It is preferred that the reference signal can be blocked so that the scattering signal can be assessed as being greater than the suspension signal alone.

h) Measurements should not be continued if the light signal intensity contains isolated bursts of high count rates which may indicate contamination of the test sample.

i) Measurements should not be continued if the correlation function does not decline monotonically or if the power spectrum is not of Lorentz type.

j) Record the average particle diameter, $\overline{x}_{\mathrm{DLS}}$, and polydispersity index, PI, for each of the measurements performed using the cumulants method.

k) If a systematic concentration dependence of the average particle size is observed, the results of an extrapolation to infinite dilution (or the results obtained at the lowest acceptable concentration) shall be reported. The dilution shall be performed with particle-free suspension medium, containing the same concentration of salts, surfactants, pH, etc., in order to not alter the particle-solution interactions.

Although the checks described here will minimize biasing effects due to multiple scattering, particle interactions may, in particular for particles below 100 nm (diameter) at volume fractions above 0,01, bias the estimation of the average diameter. Therefore, for unknown dispersed systems, it is recommended that measurements are performed on at least two concentrations varying by a factor of at least two.

l) Check at the end of the measurement that no significant sedimentation has occurred in the test sample, either by visually checking for sediment or by inspecting the results of multiple, sequential measurements for trends. If sedimentation is found, then it should be decided whether it is small enough so that its effects on the precision of the measurement are acceptable or whether the sample is unsuitable for DLS measurements.

9 Evaluation of results

9.1 General

DLS is a low resolution method that is not capable of resolving narrowly spaced peaks in the particle size distribution. The low resolution also means that values away from mass-median-diameter, D_{50}, will have increasingly large uncertainties.

The original signal obtained by DLS is scattered intensity weighted. Many instruments allow calculation of volume or number-weighted results from the scattered intensity-weighted signal. For most methods, this involves smoothing of the scattered intensity-weighted particle size distribution, Q_{int}, which introduces uncertainties. A second issue affecting all methods is the highly nonlinear dependence of the scattering intensity on the particle size. Therefore, transformation of scattered intensity-weighted distributions to volume number distributions is not recommended and number distributions from DLS are specifically deprecated, especially for methods involving the smoothing of Q_{int}.

A particle size distribution determined by DLS is based on different physical properties than one determined by, for example, laser diffraction (diffraction of light) or microscopy (transmission of electrons), and therefore they can be different.

Note that, for a given size distribution, the distribution $C(\Gamma)$ of decay rates or characteristic frequencies is dependent on laser wavelength and state of polarization and on the scattering angle θ. Hence, x_{DLS} and PI are of a given sample depending on those quantities.

Different algorithms exist for the evaluation of results. These algorithms will, for the same sample, often give different results. Reporting of the algorithm and any internal settings or choices, together with the results, is therefore crucial. This clause summarizes those algorithms that are sufficiently standardized today. More information on the theoretical background is given in Annex A and a very brief overview is found in Reference [7].

9.2 Correlation analysis

9.2.1 Cumulants method

The cumulants analysis compresses the entire multi-exponential decay distribution into the exponent and then expands the exponent into a polynomial expression. Two parameters describing particle size distributions, i.e. the average particle diameter $\bar{x}_{\mathrm{DLS}}$ and the polydispersity index PI, are determined by a variant of the so-called "cumulants method"[4].

Obtaining $\bar{x}_{\mathrm{DLS}}$ and PI from the correlation function using the cumulants method can be accomplished by a nonlinear least-squares fitting of the correlation function by using the gradient method, the Gauss-Newton method or the Levenberg-Marquadt method. $\bar{x}_{\mathrm{DLS}}$ and PI can also be obtained by a linear least squares fit to the logarithm of the correlation function with proper statistical weighting for each data point, since the operation of taking the logarithm of the data affects the weighting of the data points. The data points now are not equally significant, although the error associated with counting statistics may not be significantly different between correlator channels. The average value and polydispersity obtained from the cumulants method are scattering intensity weighted.

9.2.2 Distribution calculation algorithms

In these algorithms, the distribution of diffusion coefficients for a particle suspension or molecular solution is calculated by applying a multi-exponential fitting algorithm to the correlation function. The output of these algorithms is a particle size distribution from which, if needed, an average particle size can be determined.

9.2.2.1 Regularized non-negative least squares (NNLS)

The non-negative least squares (NNLS) algorithm fits the exponential decay of the correlation function algebraically. There are a variety of parameters that can be altered in an NNLS algorithm, but the two principal ones are the "weighting scheme" and the "alpha parameter" or "regularizer." Data weighting is used in DLS algorithms to amplify subtle changes in the larger and more significant correlation coefficients over noise in the baseline. In the absence of data weighting, noise in the baseline can lead to the appearance of artefact peaks and erroneous data interpretation.

The regularizer or alpha parameter in NNLS-based dynamic light scattering algorithms controls the acceptable degree of "spikiness" in the resultant distribution. Deconvolution of the DLS-measured correlation function is accomplished using an inverse Laplace transform that is ultimately reduced to a linear combination of eigenfunctions. The caveat to this approach is that when the eigenvalues are small, a very small amount of noise can make the number of possible solutions extremely large, hence the labelling of the DLS method as an ill-posed problem. In order to overcome the problem, a stabilizing term, in the form of the "first derivative" of the distribution solution, is added to the set of eigenfunctions.

The alpha parameter is the multiplier applied to this stabilizing term and defines the emphasis placed upon the derivative of the solution. Large alpha values (0,1) limit the spikiness of the solution, leading to smooth distributions. Small alpha values (0,000 01) decrease the weighting or importance of the derivative, subsequently generating more spiky distributions. Therefore, the alpha parameter can be loosely described as an estimate of the expected level of noise in the measured correlation function. There is no ideal or best alpha parameter — the appropriate value depends on the sample being analysed. For mixtures of narrow mode (low polydispersity) and strongly scattering particles, decreasing the alpha parameter can sometimes enhance the resolution in the scattered intensity particle size distribution.

9.2.2.2 CONTIN

The CONTIN algorithm[8][9][10] uses a constrained regularized NNLS method combined with eigenfunction analysis to generate a smoothed solution and to reduce the number of degrees of freedom.

The algorithm generates several solutions, of which the least tailed distribution consistent with the data are selected. It can resolve decay rates as long as they differ by at least a factor of 2.

Prior knowledge of the expected distribution is needed, as users need to specify a number of constraints, e.g. the expected distribution range and the number of data points. The regularization parameters themselves can be automatically chosen based on an F-test and confidence levels.

9.2.2.3 Histogram method

The histogram method is an iterative method in which an initial Γ($\Gamma = D_r q^2$) is repeatedly processed to give a final answer. In the analysis program, the initial Γ is a histogram with all the steps being of equal height. The algorithm is repeated to change the histogram to fit the raw data[11].

The histogram approach has the advantage that the number of steps M is a variable. Thus, when the data become less precise, the M can reduce correspondingly. Initial values for $G(\Gamma_j)$ and for $\Gamma_{\min}$ and $\Gamma_{\max}$ signalling the start and stop of the range of the histogram are immaterial. The fitting procedure terminates when the limits of error are within the statistical error of measured data. Distributions obtained by this method tend to be broad and have connected peaks.

9.2.2.4 Other algorithms

There are a number of other algorithms and programs which include L-Curve[12][13] regularized positive exponential sum[14] and others under various names[15][16], which are also based on NNLS analysis. In general, the difference between these algorithms is the optimization of variables (e.g. the "regularizer" or alpha parameter and the weighting scheme implemented) within the NNLS analysis in order to optimize it for a given set of instrument and sample conditions.

9.3 Frequency analysis

In frequency analysis, the signal from the detector is sampled at regular intervals and at a frequency permitting the analysis of the highest frequency required. The sampled values are then subjected to Fast Fourier Transformation (FFT) to yield a frequency power spectrum.

The basis for the calculation is the iterative fitting of the experimentally obtained power spectrum using a number of predefined size and frequency channels to obtain the complete particle size distribution. For example, a size range of 1 nm to 6 500 nm requires a 16K linear power spectrum. The 16K linear channels are then converted into 80 channels with channel width in a logarithmic progression. Size channels are defined in the same progression. From this distribution, mean diameters, PI and other metrics can be calculated:

— Step 1: Size channels 1-N are defined in a logarithmic progression. Frequency channels 1-N are defined in the same logarithmic progression and the measured linear power spectrum is converted to logarithmic channels.

— Step 2: A response is calculated for each of the N frequency channels using Formula (A.25) yielding a first approximation of the particle size distribution, PSD.

— Step 3: The calculated response and the measured response are compared and a correction to the approximate PSD is made.

Steps 1 to 3 are repeated until the error between the calculated response and the measured response is minimized, yielding the best fit PSD. Typically, 80 size channels are used between 1 nm and 6 500 nm. The iterative deconvolution results in a full PSD in compliance with ISO 9276 (all parts) with all size channels having equal scattered intensity weighting.

10 System qualification and quality control

10.1 System qualification

Since particle size determination by DLS is an absolute method based upon first principles, calibration of the particle size scale with a particle size calibrant is not necessary. To ensure SI-traceable results, a number of the instrument or test parameters should be measured with traceably calibrated tools (such as test temperature by the user, or laser wavelength and scattering angle by the manufacturer during production of the instrument). In addition, verification of the instrument's performance should be performed with suspensions of particles of certified size after installation of the instrument and at time intervals thereafter or in case of doubt.

Instrument performance can be qualified using a suspension of particles of certified size after the first installation of the instrument and at regular time intervals thereafter.

Failure of the qualification indicates a problem with this particle suspension, the preparation of the test sample or the instrument.

Certified reference materials with values assigned for DLS using the same evaluation algorithm should be used. Ideally, the chemistry and morphology of the particles should match the test samples as closely as possible.

Alternatively, certified suspensions of polystyrene latex with narrow size distribution such as 5 % CV (= standard deviation/average diameter) with average particle diameter as measured by DLS or electron microscopy can be used.

To assess potential bias, the uncertainty for the mean value of the CRM should be combined with a tolerance for the measurement uncertainty and expanded with a factor of 2 to obtain an expanded uncertainty. The absolute difference between the certified value and the average of 5 results shall be smaller than this combined uncertainty.

For suspensions of particles of about 100 nm diameter, tolerance for the measurement uncertainty is 1,5 % , the standard deviation of 5 repeats shall be better than 2 %; the PI shall be smaller than 0,1.

EXAMPLE A polystyrene latex CRM with a certified mean value of 102 nm ± 3 nm (expanded uncertainty; k = 2) was measured five times. The average of these five measurements was 105,9 nm, the standard deviation was 1,4 nm. The check for acceptable bias and precision is performed as follows[26]:

1) Convert the expanded uncertainty of the CRM into a standard uncertainty by division by the k-factor stated on the certificate: u_{CRM}=3 nm/2 =1,5 nm.

2) Calculate the tolerance for the measurement uncertainty for the measured average. As the measured value is close to 100 nm, a tolerance of 1,5 % is used: u_{meas} = 1,5 % × 105,9 nm = 1,6 nm.

3) Combine the results of steps 1 and 2 quadratically and expand with a k-factor of 2 to obtain the expanded uncertainty U:

$$U = 2\sqrt{u_{CRM}^2 + u_{meas}^2} = 2 \cdot \sqrt{1,5^2 + 1,6^2} = 4,4\ \text{nm}$$

4) The absolute difference between the measured average and the certified value is 105,9 nm – 102 nm = 3,9 nm. As this difference is smaller than U, the measurement fulfils the test for bias.

5) The relative standard deviation of the 5 measurements is 1,3 %, hence smaller than the required 2 %.

10.2 Quality control of measurement results

10.2.1 A valid measurement result should fulfil the following conditions:

a) particle size distribution independent of the delay time range, as long as the smallest range covers the set of decay times;

b) peak positions of the particle size distribution independent of the chosen range of particle sizes;

c) no peaks at the extremes of the chosen size range;

d) plausible and consistent with the physical and chemical properties of the particles;

e) plausible when compared with other sizing techniques.

10.2.2 For measurements based on autocorrelation analysis, the maximal value of the intercept of the correlation function can be determined as follows.

a) If necessary and if possible, select a collecting aperture for which a value of B_{max} is available.

b) Determine the intercept B (see Annex A) with suspensions of polystyrene latex (diameter about 100 nm) with at least two different concentrations meeting requirements 7.3 c) and d);

If B depends on particle concentration in a systematic way, extrapolate the results to infinite dilution.

10.3 Method precision and measurement uncertainty

The achievable precision depends on the nature of the sample and the evaluation algorithm. In general, results obtained for homogeneous, monodisperse samples will exhibit less variability than results obtained for very polydisperse samples.

— **Repeatability**: For monodisperse materials with diameters between 50 nm and 200 nm, relative repeatability standard deviations of $\bar{x}_{DLS}$ should be below 2 %.

NOTE Even within one laboratory, there can be additional variations between runs, between operators, between instruments, between locations, etc.

— **Reproducibility**: For monodisperse materials with diameters between 50 nm and 200 nm, between-laboratory standard deviation of 5 % is achievable.

— **Trueness**: Results obtained by DLS depends on many factors, including the scattering angle and evaluation algorithm, so results on polydisperse materials are specific to the instrumentation and evaluation setup used. For monodisperse samples of spherical particles, lower limits of the trueness contribution to the measurement uncertainty can be estimated (see Reference [17]).

The measurement uncertainty of each result should include at least contributions from repeatability and reproducibility.

11 Test report

The test report shall contain at least the following information [points k) to r) are taken from ISO/IEC 17025]:

a) average particle size, $\bar{x}_{DLS}$, and its uncertainty;

b) an indication of the polydispersity of the sample (for example, the polydispersity index);

c) the number of individual measurements upon which reported average values are based;

d) if the mean values of $\bar{x}_{DLS}$ and PI are concentration dependent, their values extrapolated to infinite dilution or the value obtained at the lowest concentration;

e) all the information required for the complete identification of the sample, including details of particle shape and homogeneity;

f) reference to the sampling plan and procedures used by the laboratory or other bodies where these are relevant to the validity or application of the results;

g) the evaluation algorithm used, together with reference to this document if the algorithm is within the scope of this document. Also stated should be the type of mean (arithmetic, geometric or harmonic mean, median and modal value), the type of scale (linear or logarithmic) and the particle size range covered;

h) the suspension conditions:

— dispersing liquid and its filtering procedure (if applicable),

— concentration of particulate material (if known),

— dispersing agents and their concentration,

— dispersing procedure, including sonication conditions: time, frequency and applied power (if applicable), and

— viscosity and refractive index of the suspension liquid;

i) the measurement conditions:

— actual concentrations investigated, if known,

— duration of measurement, and

— temperature of the sample;

j) all operating details not specified in this document, or regarded as optional, together with details of experimental conditions that may have influenced the result(s);

k) a title (e.g. “Test Report”);

l) the name and address of the laboratory, and the location where the tests were carried out, if different from the address of the laboratory;

m) unique identification of the test report (such as the serial number), and an identification on each page in order to ensure that the page is recognized as a part of the test report, and a clear identification of the end of the test report;

n) the name and address of the customer;

o) a description of, the condition of, and unambiguous identification of the item(s) tested;

p) the date of receipt of the test item(s) where this is critical to the validity and application of the results, and the date(s) of performance of the test;

q) the name(s), function(s) and signature(s) or equivalent identification of person(s) authorizing the test report;

r) where relevant, a statement to the effect that the results relate only to the items tested or calibrated;

s) all relevant evaluation and measurement parameters — these, however, do not have to be added to the test report.

Annex A
(informative)

Theoretical background

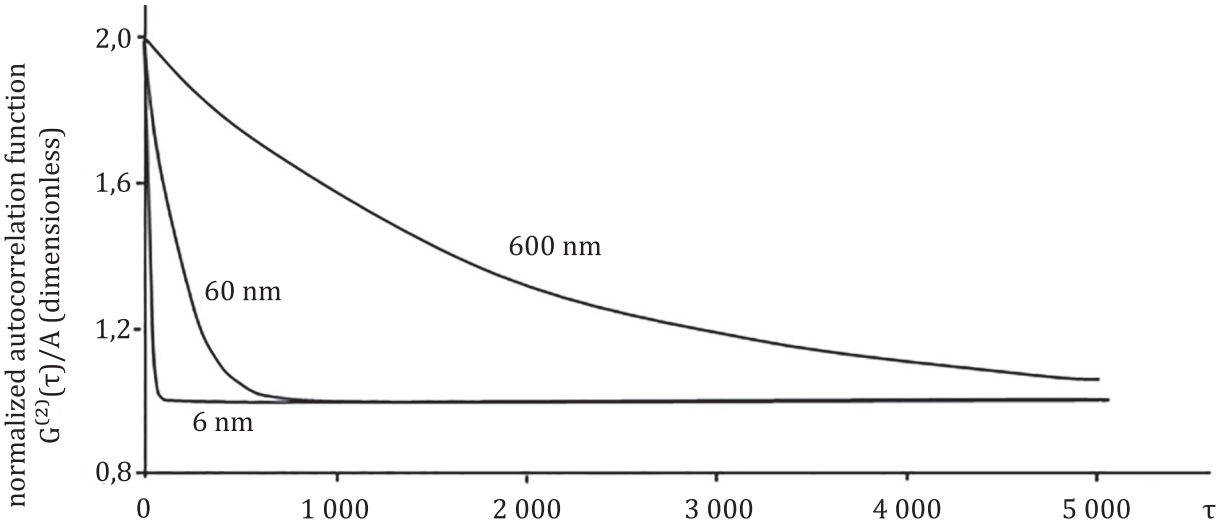
A.1 Correlation function analysis

A.1.1 Autocorrelation and cross-correlation

In a typical DLS experiment, the investigated suspension is illuminated by a narrow monochromatic and coherent source, i.e. a laser beam with one single wavelength, λ_0. The light scattered by the dispersed particles is coherently detected at an angle θ with respect to the incident radiation. Since the dispersed particles are in continuous Brownian motion, the observed scattered intensity $I(t)$ will fluctuate with time. Therefore, an analysis as a function of time of these intensity fluctuations provides information on the motion of the dispersed particles. In correlation analysis, the time analysis is carried out with a correlator which constructs the time autocorrelation function $G^{(2)}(\tau)$ of the scattered light intensity, as given in Formula (A.1):

$$G^{(2)}(\tau) = \left\langle I(t) \cdot I(t+\tau) \right\rangle \tag{A.1}$$

This correlation function depends only on the time difference τ and is independent of the arbitrary time t at which the evaluation of $G^{(2)}(\tau)$ is started. The symbol $\langle ... \rangle$ refers to an average value of the product $I(t) \cdot I(t+\tau)$ for various times t. In Figure A.1, an example of autocorrelation functions is shown.



Key

A	baseline
$G^{(2)}(\tau)$	autocorrelation function, arbitrary units
τ	correlation time, in microseconds

Figure A.1 — Autocorrelation functions (normalized)

In cross-correlation, two monochromatic and coherent laser light beams are focused in a test sample. The two light beams cross each other within the test sample. The overlap of the two laser beams forms the measurement volume. Light scattered by the particles is detected under the scattering angle by two

detectors. Thus, two independent scattering measurements are performed at the same measurement volume. This reduces the effect of multiple scattering on the measurement result.

There are several cross-correlation arrangements possible, e.g. using different scattering angles, using lasers of different wavelengths, etc. These can be evaluated in the same way as long as the scattering vector is the same. Measured cross-correlation function looks like the autocorrelation function shown in Figure A.1.

For a large number of monodisperse particles in Brownian motion in the measuring volume V, $G^{(2)}(\tau)$ is essentially an exponential decaying function of the time difference τ, as given in Formula (A.2):

$$G^{(2)}(\tau) = A[1 + B\exp(-2\Gamma\tau)] \tag{A.2}$$

where

A is, in principle, a time-independent constant proportional to the square of the time-averaged scattered intensity $\langle I \rangle$, called "baseline" in this document;

B can be considered as an instrument factor with $B \leq 1$. (In this document, it is designated as the intercept of the correlation function.)

The decay rate, Γ, is linked to the translational diffusion coefficient D_T of homogeneous spherical particles undergoing Brownian motion as given in Formula (A.3):

$$\Gamma = D_{\mathrm{T}} q^2 \tag{A.3}$$

where q is the modulus of the scattering vector, as given in Formula (A.4):

$$q = \frac{4\pi n}{\lambda_0} \sin\left(\theta/2\right) \tag{A.4}$$

The scattering vector $\vec{q}$ is defined as the vector difference between the incident and scattered wave vectors $\vec{k}_{\mathrm{i}}$ and $\vec{k}_{\mathrm{s}}$ respectively: $\vec{q} = \vec{k}_{\mathrm{s}} - \vec{k}_{\mathrm{i}}$. The modulus of both $\vec{k}_{\mathrm{i}}$ and $\vec{k}_{\mathrm{s}}$ are equal to $2\pi n/\lambda_0$, where n represents the real part of the refractive index of the suspension liquid.

Note that with DLS, the diffusion coefficient D is determined and not the particle size. The latter quantity can only be determined by relating the diffusion coefficient to the particle size. For non-interacting, spherically shaped particles dispersed in a medium with viscosity η, the diffusion coefficient D_{T} is related to the particle diameter x by the Stokes-Einstein equation, as given in Formula (A.5):

$$D_{\mathrm{T}} = \frac{kT}{3\pi\eta x} \tag{A.5}$$

where k is the Boltzmann constant and T is the absolute temperature.

The conditions imposed by this document on particle concentrations ensure that biasing effects of particle number fluctuations in the measuring volume, multiple scattering and particle interactions on particle size are avoided or at least minimized. In order to minimize bias by particle number fluctuations, this document requires that at least 1 000 particles are present in the measuring volume.

The criterion for the minimum of 1 000 particles relates to monodisperse materials only. For polydisperse samples, a much larger number of particles smaller than x_{DLS} may need to be present in the measuring volume.

Although the use of previous equations is, in principle, limited to homogeneous spherical particles, measurements on non-spherical and non-homogeneous particles, where both translational and rotational diffusion processes are present, can be made and analysed by these formulae. In this case, Formulae (A.1) to (A.3) and Formula (A.14) define an apparent and equivalent spherical radius.

For the practical cases of polydisperse samples, Formula (A.2) is written as Formula (A.6):

$$G^{(2)}(\tau) = A[1 + B\left|g^{(1)}(\tau)\right|^2] \tag{A.6}$$

where the modulus of the field autocorrelation function $g^{(1)}(\tau)$ is now related to the normalized distribution function of decay rates $C(\Gamma)$ as given in Formula (A.7):

$$g^{(1)}\left(\tau\right) = \int_0^\infty C\left(\Gamma\right)\exp\left(-\Gamma\tau\right)\mathrm{d}\Gamma \tag{A.7}$$

with Formula (A.8):

$$\int_0^\infty C\left(\Gamma\right)\mathrm{d}\Gamma = 1 \tag{A.8}$$

In Formula (A.8), $C(\Gamma)\mathrm{d}(\Gamma)$ is proportional to the fraction of the intensity scattered by particles with decay rates in the range Γ and $\Gamma + \mathrm{d}\Gamma$.

Several kinds of polydispersities may cause a distribution in decay rates:

1) polydispersity in size, i.e. all particles have the same shape and composition, e.g. homogeneous isotropic spheres, whereby only the linear dimensions of the individual particles differ;

2) polydispersity in shape, i.e. the particles differ in shape, e.g. mixtures of spheres, discs and rods, but are equal in volume and composition;

3) non-homogeneous and anisotropic materials, i.e. the material is distributed differently from particle to particle, e.g. mixtures of homogeneous spheres with layered spheres;

4) all possible combinations of the previous kinds of polydispersity.

In the application of particle sizing by DLS, only the first kind of polydispersity is assumed.

In this document, the distribution of decay rates is characterized by two parameters:

a) the average decay rate $\langle\Gamma\rangle$ defined in Formula (A.9):

$$\overline{\Gamma} = \int_0^\infty \Gamma C\left(\Gamma\right)\mathrm{d}\Gamma \tag{A.9}$$

b) a dimensionless polydispersity index PI, i.e. a measure of the broadness of the distribution, defined in Formula (A.10):

$$\mathrm{PI} = \frac{\mu_2}{\overline{\Gamma}^2} \tag{A.10}$$

where μ_2 is computed in Formula (A.11):

$$\mu_2 = \int_0^\infty \left(\Gamma - \overline{\Gamma}\right)^2 C\left(\Gamma\right) \mathrm{d}\Gamma \tag{A.11}$$

Note that for a Gaussian distribution of decay rates with mean decay rate $\overline{\Gamma}$ and standard deviation σ, i.e. Formula (A.12):

$$C\left(\Gamma\right) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{\left(\Gamma - \overline{\Gamma}\right)^2}{2\sigma^2}\right] \tag{A.12}$$

the PI is related to $\overline{\Gamma}$ and σ as given in Formula (A.13):

$$\mathrm{PI} = \frac{\mu_2}{\overline{\Gamma}^2} = \frac{\sigma^2}{2\overline{\Gamma}^2} \tag{A.13}$$

Since the Stokes-Einstein diameter is inversely proportional to the decay rate [see Formulae (A.3) and (A.5)], Formula (A.9) is also used to define the average DLS diameter $\overline{x}_{\mathrm{DLS}}$ in this document:

$$\frac{1}{\overline{x}_{\mathrm{DLS}}} = \int_0^\infty \frac{1}{x} C\left[\Gamma\left(x\right)\right] \mathrm{d}\left(\frac{1}{x}\right) \tag{A.14}$$

Since in Formula (A.14), $C[\Gamma(x)]\mathrm{d}(1/x)$ represents the fraction of the intensity scattered by particles with a diameter in the range x and $x + \mathrm{d}x$, the average DLS diameter $\overline{x}_{\mathrm{DLS}}$ defined through Formula (A.14) is a harmonic scattered intensity-averaged diameter.

Note that this average diameter is in general different and larger than a weight-averaged diameter. It is also different from the average diameters determined by, for example, (low-angle) laser light scattering or diffraction.

Note also that, for a given size distribution, $C(\Gamma)$ is dependent on laser wavelength and state of polarization and on the scattering angle θ [see Formulae (A.3) and (A.4)]. Hence, $\overline{x}_{\mathrm{DLS}}$ and PI are for a given sample dependent on those factors. For distributions with particle diameters below 30 nm, there is no significant dependence of $\overline{x}_{\mathrm{DLS}}$ and PI on laser wavelength (and on scattering angle).

A.1.2 Cumulants analysis

In the cumulants method, the factor $\exp(-\Gamma\tau)$ in Formula (A.7) is expanded around $\exp(-\overline{\Gamma}\,\tau)$, yielding a polynomial in delay time. Truncating this polynomial at the second-order term, Formula (A.6) can be approximated by Formula (A.15):

$$G^{(2)}(\tau) \approx A[1 + B\exp(-2\,\overline{\Gamma}\,\tau + \mu_2\tau^2)] \tag{A.15}$$

This formula is the basis of the determination of the average decay rate $\overline{\Gamma}$, μ_2 and hence of the average diameter $\overline{x}_{\mathrm{DLS}}$ and polydispersity index PI. In order to obtain a linear regression, Formula (A.15) is transformed as shown in Formula (A.16):

$$y(\tau) = \frac{1}{2}\ln\left[G_2(\tau) - A\right] \cong \frac{1}{2}\ln AB - \overline{\Gamma}\tau + \frac{\mu_2}{2}\tau^2 \tag{A.16}$$

or in Formula (A.17)

$$y(\tau_j) = a_0 - a_1\tau_j + a_2\tau_j^2 (j = 1,2,3\ldots m) \tag{A.17}$$

where j is the number of the delay channel of the correlator.

The baseline for point A can be determined in two ways: by the total number of photon counts in a total time duration of the considered experiment or from an estimate of $G_2(\tau)$ for delay times $\tau >> 25/\overline{\Gamma}$. It is recommended that both estimates of the baseline A are determined and that the largest of both is retained. However, for relative differences between the two estimates of the baseline larger than 10^{-3} times the smallest value, measurement shall be discarded for further analysis.

The range of values to be retained for $y_j = y(\tau_j)$ shall correspond to a range in $[G_2(\tau_j) - A]$ of $[G_2(\tau_1) - A] > [G_2(\tau_j) - A] > \{[G_2(\tau_1) - A]/100\}$ with at least one value smaller than $\{[G_2(\tau_1) - A]/50\}$. All values of $[G_2(\tau_j) - A]$ in this range must be positive; otherwise, the measurement shall be discarded for further analysis.

Finally, the number m of values of y_j in the acceptable range shall be at least 20.

The parameters a_0, a_1 and a_2 are determined by least-squares fitting of the experimental estimates of $y(\tau_j)$ to Formula (A.15) whereby the following function is minimized in Formula (A.18):

$$s(a_0, a_1, a_2) = \sum_{j=1}^{m} w_j \left(y_j - a_0 - a_1\tau + a_2\tau_j^2\right)^2 \tag{A.18}$$

In Formula (A.18), the normalized weighting factor w_j accounts for the nonlinear transformation of the raw data G_2 into the values for $y(\tau_j)$.

The average particle diameter $\overline{x}_{\mathrm{DLS}}$ is calculated from a_1 by Formula (A.19):

$$\overline{x}_{\mathrm{DLS}} = \frac{1}{a_1}\frac{kT}{3\pi\eta}\left[\frac{4\pi n \sin(\theta/2)}{\lambda_0}\right]^2 \tag{A.19}$$

where

k is the Boltzmann constant;

T is the absolute temperature;

η is the viscosity of the suspension medium;

n is the refractive index of the suspension medium;

θ is the scattering angle;

λ_0 is the laser wavelength *in vacuo.*

The polydispersity index PI is related to a_2 and a_1 by Formula (A.20):

$$\mathrm{PI} = 2a_2/a_1^2 \tag{A.20}$$

The actual value of the intercept B is computed from a_0 and A by Formula (A.21):

$$B = [\exp(2a_0)]/A \tag{A.21}$$

and is to be compared to the maximum value obtainable in the given experimental conditions, $B_{\max}$. Autocorrelation measurements for which the ratio $B/B_{\max} < 0{,}8$ shall be discarded. The variance, equal to Formula (A.22):

$$s/(m-4) \tag{A.22}$$

where

s is the target function of the fitting calculated by Formula (A.18);

m is the number of measurements.

can be used as a criterion for the goodness of the fit. For spherical, monodisperse particles, the variance should be ≥0,8. No general rule can be given for other particles, for which the variance should be fit for purpose.

A.2 Frequency analysis

In the frequency analysis mode, the output of the photodetector collecting the scattered light is first processed using the digital Fast Fourier Transform (FFT) technique. The FFT calculates the frequency power spectrum of the fluctuations detected by the photodetector. For a monodisperse system, the forms of the power spectrum, $P(\omega)$, are given by Formula (A.23) for heterodyne detection (see Reference [18], 5.4):

$$P(\omega) = I_{\mathrm{R}} \left\langle I_{\mathrm{s}} \right\rangle \frac{2\omega_0}{\omega^2 + \left(\omega_0\right)^2} \tag{A.23}$$

where

I_{R} is the intensity of the reference beam;

I_{s} is the scattered intensity;

ω_0 is the characteristic frequency;

ω is the angular frequency.

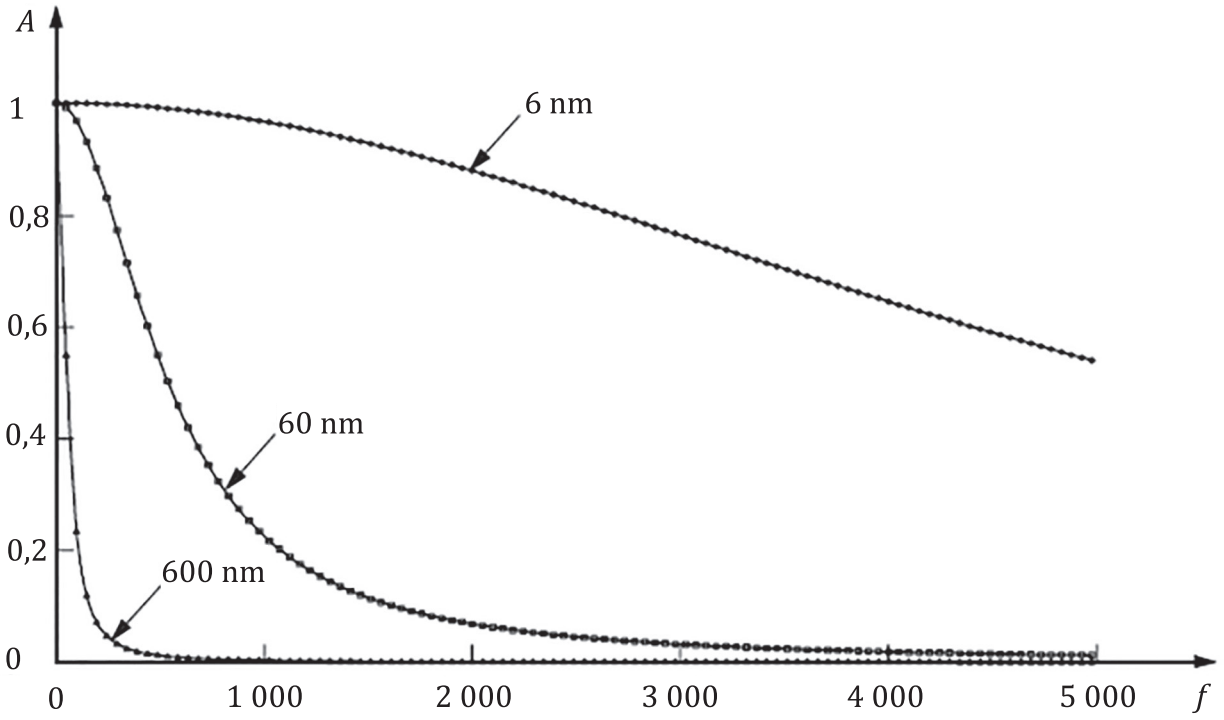
In the homodyne mode, the amplitude of the power spectrum is proportional to the square of the scattered intensity, $\langle I_s \rangle^2$, which is proportional to the sample concentration. In this mode, it is assumed that no unscattered primary beam is mixed with the scattered light. It is assumed that the condition $I_0 << \langle I_s \rangle$ at the detector is met. This ensures that the mode is purely homodyne with no mixture of heterodyne and no flare. It also assumes a noiseless detector and no flare or scattering from the test cell or optics.

In the heterodyne mode, a reference component of the laser input is mixed with the collected scattered light. The power spectrum in the heterodyne mode will always have a component of the homodyne mode. In order to treat the power spectrum as pure heterodyne, the homodyne component has to be arranged to be very small compared with the heterodyne component. This can be met if $I_0 >> \langle I_s \rangle$. At high concentrations, and thus high $\langle I_s \rangle$, be careful to ensure that $I_0 >> \langle I_s \rangle$ at the detector is met. Mixing of the modes combines power spectra with different characteristic frequencies, ω_0, in the heterodyne mode and 2 ω_0 in the homodyne mode.

The characteristic frequency, ω_0, is inversely proportional to the particle diameter x, the temperature T, the laser wavelength λ_0, the scattering angle θ and the viscosity of the medium η and represents the half power point of the spectrum as shown in Formula (A.24).

$$\omega_0 = \frac{1}{x}\frac{16\pi kT}{3\lambda^2\eta}\sin^2\left(\theta/2\right) \tag{A.24}$$

Figure A.2 plots the power spectrum for different particle sizes in the heterodyne mode. The inverse relationship of the characteristic frequency with particle size is evident.



Key

A amplitude, arbitrary units

f frequency, in Hz

Figure A.2 — Power spectra for different particle sizes in the heterodyne mode

The measured response vector $\vec{r}$ is the product of a Lorentzian matrix L with a vector consisting of the volume-weighted particle size distribution $\vec{v}$, [Formula (A.25)]. To a distribution of particle sizes is a weighted sum of individual particle size responses.

$$\vec{r} = L \cdot \vec{v} \tag{A.25}$$

Inversion of Formula (A.25) to determine the particle size distribution, $\vec{v}$, is difficult due to the ill-conditioned Lorentzian form of the power spectrum.

For a polydisperse system, the normalized power spectra are related to the distribution of characteristic frequencies for heterodyne detection, by Formula (A.26) (see Reference [6]):

$$P(\omega)\mathrm{d}\omega = I_0 \langle I_s \rangle \frac{2\omega_0}{\omega^2 + \omega_0^{\,2}} \mathrm{d}\omega \tag{A.26}$$

Transforming the frequency coordinates from a linear to a logarithmic basis [Formulae (A.27), (A.28)] results in Formula (A.29).

$$x = \ln(\omega) \tag{A.27}$$

$$x_0 = \ln(\omega_0) \tag{A.28}$$

$$P(x)\mathrm{d}x = I_0 \langle I_s \rangle \frac{1}{e^{(x-x_0)} + e^{-(x-x_0)}} \mathrm{d}x \tag{A.29}$$

In logarithmic coordinates, the response matrix is shift invariant. The single particle responses are equivalent and only shift along the log frequency axis. For particle size distributions, each log frequency channel will have differing amplitudes depending on the contribution to the power frequency spectrum by the size fractions present, complete with the effects of scattered intensity weighting proportional to the 6th power of the particle radius and noise. Figure A.3 shows the response functions for three particle sizes. The functions peak at the log of the characteristic frequency, ω_0. The matrix Formula (A.25) becomes a convolution with R, the response function as in Formula (A.30).

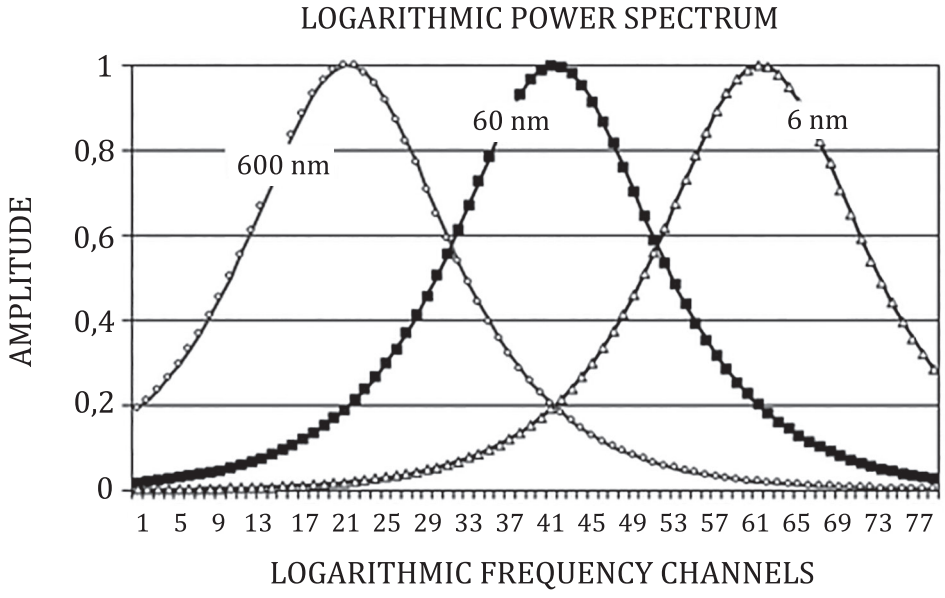


Figure A.3 — Power spectrum logarithmic coordinates

$$r_n = \sum R\left(x_m - x_n\right)v\left(x_n\right) \tag{A.30}$$

The inversion of the logarithmic coordinate frequency power spectrum [Formula (A.30)] is greatly simplified over the inversion of Formula (A.25) and can be accomplished with linear iteration techniques[19].

Annex B
(informative)

Guidance on potential measurement artefacts and on ways to minimize their influence

B.1 General

Various factors can influence a DLS result. This annex summarizes a number of influences and ways to minimize their effects to obtain valid results.

B.2 Measurements of high-concentration suspensions

B.2.1 General

For an unknown sample, it is not possible to predict at what concentration the limitations noted above will begin to affect the apparent size of the particles. Therefore, it is advised to perform a series of measurements at concentrations over several orders of magnitude. At higher concentrations, multiple scattering, particle-particle interaction and other effects (e.g. non-spherical geometry) may affect the measurement result. In this case, extrapolation of the apparent measured size to zero concentration is necessary in order to obtain an unbiased particle size. Quality procedures should determine the maximum concentration allowed for measurement of a specific material-dispersant combination. This means that a validation procedure has to be executed for measurement of a given type of sample to be analysed with a given instrument. Keep in mind that by diluting a sample, the particle size may change because of changes in the chemistry of the sample or the extent of the electrical double layer. However, the apparent particle size computed with the Stokes-Einstein formula for a fixed finite particle concentration can be used for quality control purposes even though this apparent size may not be the same as that measured by diluting the sample to a proper concentration.

In order to avoid biasing effects of particle interaction and multiple scattering, it is recommended to perform measurements at several concentrations and, if a systematic concentration dependence is observed, to extrapolate the obtained results to infinite dilution.

B.2.2 Multiple scattering

DLS analysis assumes that only single scattered light is collected. At high concentrations, light scattered from one particle may be scattered again from another particle before it reaches the detector. This means that additional phase factors add and broaden the spectrum of light intensity fluctuations. Particles appear to move faster, and hence, a smaller diameter is deduced.

Several strategies are used to minimize the effect of multiple scattering.

One technique utilizes an optical system which minimizes the optical path in and out of the test sample. There are a number of means of accomplishing a minimum optical path in the test sample, including the use of backscatter optics. Backscatter optics allow a short pathlength to be designed. Even if the test sample is optically opaque for long pathlengths, multiple scattering over short pathlengths may be negligible.

Another way is to use the cross-correlation method, as, in principle, this eliminates the contributions from multiple scattering. In practice, multiple scattering effects are minimized, not eliminated. The upper concentration limit for dispersed material is reached when single-scattered light can no longer be observed. Similar to the backscatter optics, higher concentrations can be achieved by reducing optical pathlengths.

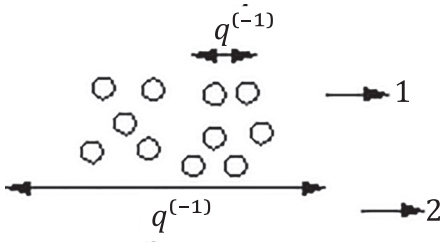
B.2.3 Particle-particle interactions

The formulae for converting the diffusion coefficient into a hydrodynamic diameter assume that the movement of each particle is not influenced by the movement of other particles. At low concentrations, this condition is usually met, but at higher concentrations, the particle diameter as determined by DLS will differ from the correct particle size. Indications of a significant particle-particle interaction include larger polydispersity, appearance of multiple modes and a larger apparent diameter.

For concentrated samples, DLS probes the diffusion of an ensemble of many particles rather than measuring a single particle diffusion coefficient. Depending on the particle size, concentration, scattering angle and laser wavelength, two different diffusion coefficients can be distinguished. The ratio of the average inter-particle distance, h, to the inverse of the modulus of the scattering vector q^{-1} will determine which of the following situations occurs (see Figure B.1).

If $q^{-1} < h/2\pi$, self-diffusion of single particles in the presence of many others is observed by DLS.

If $q^{-1} > h/2\pi$, the collective diffusion of an ensemble of particles is observed.



Key

1 self-diffusion

2 collective diffusion

Figure B.1 — Effect of the ratio of mean inter-particle distance and inverse scattering vector modulus on diffusion

Both modes can be described by a different diffusion coefficient, namely that of self-diffusion, D_S, and that of collective diffusion, D_C. Both diffusion coefficients are concentration dependent, but in different ways.

Therefore, at low concentrations, the self-diffusion of particles is observed. When the particle concentration is increased and/or for relatively larger particles, the mean inter-particle spacing between particle centres becomes smaller than the scattering vector, and collective diffusion instead of self-diffusion of particles is observed.

As a result, the apparent particle size computed with the Stokes-Einstein equation from the measured diffusion coefficient can change with particle concentration.

Note that the above discussion, and in particular the Stokes-Einstein equation for the translational diffusion coefficient, holds only for spherical particles. For a non-spherical particle, the measured diffusion coefficient is a superposition of translational and rotational diffusion coefficients.

Finally, inter-particle interactions have an important influence on the measured particle diffusion. There are several types of interactions, ranging from purely hydrodynamic hindering, to electrostatic repulsions caused by the existence of a surface potential of the particles, to van der Waals attraction. They can also all occur simultaneously. Due to the presence of particle interactions, the measured diffusion coefficients are no longer individual particle properties, but properties of the whole particle suspension.

Measuring the sample at different dilutions can also demonstrate presence or absence of multiple light scattering.

The onset of the effects of particle-particle interaction is system-specific: long-range interactions can introduce significant effects at low concentrations, whereas in rare cases, particles diffuse unhindered at higher concentrations. However, for each particle/fluid system, there is a limit where particle-particle interactions become significant and dilution is required for the determination of the correct particle size.

B.2.4 Restricted diffusion

Restricted diffusion describes the phenomenon where the presence of other particles hinders free particle diffusion. An example can be diffusion of particles in a polymer network. Symptoms include a shift in size, with no change to modality or polydispersity, to larger sizes when the solvent viscosity is used for size calculations at high sample concentrations or a concentration dependence of the mean diameter which parallels that of the bulk viscosity of the sample.

B.3 Sedimentation

For DLS to yield valid results, the rate of sedimentation should be much slower than the rate of diffusion. Whether this condition is met can be seen from the correlogram, which, for random Brownian motion, should show monotonous decay. Increases in correlation at high times indicate non-random movement, i.e. sedimentation.

The stability of the amplitude of the correlation function also gives an early indication of the dispersion stability of the sample.

B.4 Number fluctuations

The number of particles in the measurement volume should be constant. This may not be the case if large particles or samples with low particle concentrations are measured. Intercept values in the correlogram >1 and increases of the correlation at high times indicate number fluctuation for autocorrelation setup (this may differ with other measurement setups).

B.5 Large particles/dust

Large fluctuations in recorded scattered time-averaged signals (count rate) on short time scales (e.g. 0,1 s intervals) with bursts of high count rates may indicate the presence of large particles, either from potentially sedimenting coarse particle fractions in the sample or from contaminating dust. The appearance of sparkling centres in the beam also usually indicates the presence of large particles in the sample. If the large particles are due to contamination by dust, then the liquids shall be further cleaned (by filtration and/or distillation) before use. If it is concluded that these large particles are part of the original samples, users may either accept a poorer precision of the results due to the broad particle size distribution, filter the sedimenting sample or sample the supernatant suspension after sedimentation. Any sample treatment should be described in the test report.

B.6 Different modes of diffusion

Non-spherical particles contribute both rotational and translation diffusion components to the scattered light intensity fluctuations. The proportion of each depends on the angle of observation. The hydrodynamic diameter reported may depend on these factors[20].

B.7 Fluorescence

Fluorescent particles may absorb some of the incident light which is re-radiated at a longer wavelength. If this re-radiated light is within the pass band of the receiver, it can increase the non-correlated signal. Narrow band filters may be used to minimize this influence.

Annex C
(informative)

Online measurements

In principle, DLS requires stationary samples. Moving streams add extra movement of particles and hence may give an additional contribution to the apparent diffusion coefficient measured. Despite this fundamental issue, online DLS instruments are commercially available and practical experience shows that such devices can provide meaningful data. When using online systems, the following issues need to be taken into consideration.

— The effects of bulk flow will be most pronounced for large particles, as their correlation function decays slower and is hence most affected by the additional movement. Flow will therefore limit the maximum particle size that can be reliably measured.

— Turbulent flow, because of general conditions of either turbulence or near cell walls, may make it impossible to calculate the effect of the flow.

— In situations that deviate from steady state [e.g. varying particle numbers such as observed in field-flow-fractionation (FFF)], results obtained for a smaller number of particles (e.g. near the edges of the scattered intensity peaks in the FFF elution signals) are less reliable than those obtained for larger particle numbers (e.g. near the centre of the FFF peaks).

— In situations that deviate from steady state, changing particle concentration may also have an effect.

— Problems caused by moving particles are aggravated for polydisperse samples.

— As samples are often highly dilute, measurement of weak scatterers may be affected as well.

These potential effects require the user of flow systems to carefully validate the online system for the material, its size and concentration range to demonstrate the accuracy of the results. Comparison of the results of the online system (possibly after taking fractions) with static systems are highly recommended in this case.

Annex D
(informative)

Recommendations for sample preparation

D.1 General

Sample preparation consists of three parts: medium purification, cell cleaning and solution/suspension preparation. The first two parts are described in varying levels of detail in this annex. The last part depends on the particular type of sample. Some general guidelines are given at the end of this annex, and the preparation of a polystyrene latex suspension in water is described in detail.

One of the major problems in DLS measurements is dust. Dust is a general name given to any undesirable large scatterer that contributes to the signal. The presence of dust may seriously bias results for the average particle diameter.

Dust can be removed from liquids by filtration, distillation or centrifugation. Filtration is the easiest method, thanks to the availability of inexpensive, disposable filters. Distillation, however, may be necessary in order to deionize the liquid and to remove trace impurities. If filtration cannot be used, for example, due to interaction with the filter membrane, one can remove dust particles by centrifugation. In this case, the supernatant is transferred to the measurement cell following centrifugation.

The next subclauses describe several techniques for cleaning medium. Choose the one most suitable for your particular application. Try the simplest one first. Obviously, any other procedure that produces dust-free samples without altering the dispersed particles can be used.

D.2 Filtration

D.2.1 Water

To filter small quantities, use a 20 ml syringe in conjunction with a 25 mm diameter, 0,2 µm pore size disposable filter. Choose a syringe with a Luer-lock fitting. If a needle is required in your application, use a large diameter (18 gauge). Airborne dust is attracted to the increased surface area obtained by atomizing water through the tip of a narrow-gauge needle. Therefore, disperse gently; do not atomize.

Clean and flush the syringe and the needle several times to remove coarse particles which will clog a filter prematurely. If possible, use filters manufactured without adhesive residues (often polymeric coatings) which add particles to the solvent being filtered. Flush the filter several times before using to remove any such residues. Consider pre-filtering with coarser filters if liquids are heavily laden with particles. This will extend the life of the fine-pore filters.

To filter large quantities, use a 47 mm or larger-diameter filter housing with a large-area 0,2 µm pore size filter. Attach the inlet of the filter housing to a water tap. Run at least 20 ml water through the filter in order to remove possible particles present on the filter. This flushes the residue from the surface of the filter, though it also reduces the filter's life. During filtration, let the water drip into the cell or dilution bottle to avoid creating a large surface area.

D.2.2 Organic media

If the medium is compatible with the plastic filter housing and the filter material, then use the same procedures described in D.2.1 for water. lf not, use a stainless steel filter housing and a filter material suitable for use with the particular medium. Compatibility charts are available from filter manufacturers.

To filter small quantities, use a 13 mm or 25 mm diameter stainless steel filter holder and filters in conjunction with a glass syringe. Be sure to choose O-rings that will not dissolve. Handle filters with smooth forceps to avoid puncturing them.

D.3 Purification

D.3.1 Water

Residual ions may affect the chemistry and interaction of the scatterers. Ions may help to stabilize a sample, or they may cause flocculation. Changes in shape may affect the observed diffusion coefficient and, therefore, the results of DLS measurements.

Commercial water purification systems are available. They consist of replaceable cartridges that remove ions, adsorb trace organic materials, and filter to 0,2 µm particle size. They are convenient, compact, safe and easy to maintain.

An alternative approach is to connect an ion-exchange cartridge to a boiling flask/condenser, followed by a collecting bottle and a 0,2 µm pore size filter.

Use polytetrafluoroethylene (PTFE) connections throughout, except for PVC tubing on the input to the ion-exchange cartridge and on the cooling-water ports. Do not grease any of the joints.

Once assembled, run several litres of water through the system prior to produce purified water for use during the preparation of test samples.

D.3.2 Organic media

A deionizing column is usually not required for nonpolar liquids. Replace the plastics filter housing with a 47 mm PTFE one and appropriate filters. Replace with PTFE (second choice, fluoroplastic rubber) any PVC tubing that will come into contact with the solvent. Extreme care should be taken in distilling flammable solvents.

D.4 Cell cleaning

D.4.1 General

The degree of cell cleaning necessary depends on the application. The simplest case — for use with disposable, individually packed, clean cells — involves blowing off dust using filtered compressed air. Avoid fingerprints in the area where light enters or exits by holding the cell near its top.

D.4.2 Detergent and water

More rigorous cleaning procedures follow. Choose the one most suitable for your work. First, try the simplest ones. Rinse cells thoroughly with purified water. Use a non-abrasive detergent to clean the inside and the outside of the cell. Do not use a brush. Scratches of just a few micrometres can cause problems. Shake the cells containing the detergent and water vigorously instead.

To clean more thoroughly, place the cells in a small (40 W is sufficient) ultrasonic bath containing clean dilute detergent. Sonicate for several minutes. Use heat if available. To avoid scratches, prevent cells from touching each other or the walls of the container. Change the ultrasonic cleaning liquid frequently.

Rinse thoroughly with purified water. Let dry upside down or cover to prevent dust from collecting inside the cell or use particle-free HEPA filtered benches to clean cells and to maintain cleanliness.

D.4.3 Acid

Sometimes, samples adsorb onto walls of the cell. Sometimes, grease spots and biological materials are not completely removed by detergent and water. If the filtered water does not dry evenly and flow in even sheets from the cell, consider the following cleaning treatment.

Soak the cell for 1 h in concentrated sulfuric acid. Use PTFE-covered tweezers to remove the cell and for further treatment. Rinse with purified water; ultrasonicate in a detergent solution; rinse several times with filtered water. Cover and dry.

If left to dry, latex samples can form a tough film on glass and quartz cells. Concentrated sulfuric acid is very effective in eliminating this film.

D.5 Solution/suspension preparation

The most important rule is to clean everything (cells and caps, dilution bottles and caps, transfer pipettes or syringes, glassware for buffer solutions and surfactants, etc.) with purified liquid. Prepare buffer and surfactant solutions with purified and filtered liquid.

Exposure to compressed air is usually sufficient to remove dust from plastic disposable pipette tips, but not from glass ones which, if they have been left uncovered, collect grease and dust.

To reduce the chance of reintroducing dust, minimize contact between open air and liquids used in the final phase of sample preparation. Wherever possible, use direct connections, avoiding contact with open air. Do not store filtered water for long periods. Bacteria, which scatter light nicely, grow in stored water. Prepare small amounts of fresh solutions daily if possible. If not, filter prior to use.

Where possible, avoid spraying liquids into flasks, bottles or sample cells. Allow liquid to run down the side of a clean, smooth surface; less dust is reintroduced this way.

Never shake samples violently once prepared. This may entrap dust-laden air and dissolve air in the solvent. Bubbles, too small to see, scatter more light than most particles of interest. Gentle swirling is best. Adding diluent to a concentrated drop of sample promotes uniform distribution faster than adding the drop to the diluent.

When making true molecular solutions, follow advice given in the specialized literature. Beware of gels and other supramolecular species which can dominate the light scattering and lead to false conclusions.

If particles clump together and obviously do not go into suspension, try wetting the particles with a few drops of ethanol first, followed by water.

Do not overuse surfactants. Beyond the critical micelle concentration (typically a volume fraction of around 10^{-4} % to 10^{-5} %), a surfactant can actually promote flocculation.

D.6 Preparation of a latex suspension

For your first DLS measurement, choose a polystyrene latex of narrow particle size distribution with a diameter of about 100 nm as measured by electron microscopy or DLS. A dilute aqueous suspension of this sample will scatter very well and serve as one test of the instrument alignment. It can also be used to determine the maximum intercept B_{max} [see 7.3, point c)].

There are several sources of such polystyrene samples. Do not use samples which are more than one year old. Store these latex samples in closed vials at a temperature recommended by the manufacturer.

Prepare 200 ml of a solution of c(NaCl) = 10 mmol/l = 0,6 g/l. Use a disposable 0,2 µm pore size filter to clean the liquid. Rinse thoroughly a 25 ml dilution vial and its screw-on cap with the filtered solution. Rinse a suitable sample cell and its cap. Cover both until ready for use.

NOTE The electrolyte can interact with the sample. For example, NaCl will precipitate Ag ions.

The latex samples typically come in small, plastic squeeze-bottles and usually contain surfactant. The concentration usually varies from a mass fraction of 1 % to 10 % solids, in which case, add about four drops into the dilution vial. Add about 20 ml of the filtered 10 mmol/l NaCI solution. The result is a slightly turbid suspension suitable for measurement with a 5 mW, He-Ne laser. If the laser is considerably more powerful, use fewer drops, or dilute in two stages.

Ultrasonicate the sample in the dilution vial for a total of about 2 min using short bursts, perhaps 10 s, of sonic energy followed by a few seconds of no energy.

Clean a cell. Use the detergent-and-water technique described in D.4.2. Do not use anything abrasive. Rinse thoroughly with purified water followed by filtered water. Rinse the cell cap. Gently pour the sample from the dilution vial into the cell. Cap it.

If wet, dry the outside of the cell prior to placing it in a sample holder. Do not wipe the cell with an abrasive towel. Pat it dry, or roll it gently an adsorbent paper, preferably one designed for use with optical-quality glass.

Place the vial in the path of a laser beam, e.g. a laser pointer or the instrument itself, if the laser beam is visible. Follow the procedures given in Clause 7. A distinct scattering line should be visible. If the line is thick and fuzzy, the sample is too concentrated. Dilute it. If the line is extremely weak, i.e. not much more visible than in filtered water, the sample is too dilute. Add more latex. If random bursts of light are readily apparent, prepare a new sample, clean the dilution vial and water more carefully.

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