Particle size analysis by dynamic light scattering

The method is based on ISO standard 22412:2017 *Particle Size Analysis - Dynamic light scattering (DLS)*.

1 INTRODUCTION

Dynamic light scattering (DLS) can be used to measure the average particle size and the broadness of the size distribution of submicron particles dispersed in a liquid.

Average particle size and particle size distribution are important characteristics of dispersed systems such as emulsions, suspensions and liposome formulations.

DLS can be used to measure particles in the submicron range and is therefore particularly suitable for the particle size analysis of dispersed systems that are composed of randomly moving particles measuring up to approximately 1 µm.

2 PRINCIPLE

Submicron particles dispersed in a liquid, and that are free from sedimentation, are subject to a perpetual random movement, known as Brownian motion. When these particles are irradiated with a laser, scattered light intensity from the moving particles fluctuates depending on their diffusion coefficients. The intensity of the scattered light from larger particles fluctuates more slowly, because larger particles move more slowly and conversely the intensity of the scattered light from smaller particles fluctuates more rapidly.

In dynamic light scattering measurements the diffusion dependent fluctuations of the scattered light intensity are measured and analyzed. The diffusion coefficient and the particle diameter are related by the Stokes-Einstein equation.

$$x = \frac{kT}{3\pi\eta D} \times 10^{12}$$

x: hydrodynamic diameter of an equivalent spherical particle (nm)
k: Boltzmann constant (1.38×10^{-23} J·K^{-1})
T: absolute temperature (K)
η: viscosity of the dispersing medium (mPa·s)
D: translational diffusion coefficient (m^2·s^{-1})

The intensity of light scattered by the diffusing particles exhibits a time dependency which can be described as either a time-dependent phase shift or as a spectral frequency shift.

Based on these concepts, the time-dependent intensity of the scattered light is processed either by photon correlation spectroscopy (PCS) or by frequency analysis.

In PCS, the time-dependent intensity of the scattered light is correlated with a time-delayed copy of itself (autocorrelation function) or with the signal from a

second detector (cross-correlation function). Both the auto- and cross-correlation function of a monodisperse system decay exponentially with correlation time. The decay rate depends on the fluctuation of the scattered light as a function of particle size (slower for large particles and faster for small particles).

In frequency analysis, the frequency-based power spectrum of the scattered light is analysed. For a monodisperse system, the power spectrum is a Lorentzian type function.

These two methods are mathematically equivalent. The time-based autocorrelation function in PCS is equal to the Fourier transform of the frequency-based power spectrum in frequency analysis. Therefore, the average diameter ($\bar{x}_{DLS}$) and the polydispersity index (PI), which indicates the broadness of the particle size distribution, can be evaluated with each method.

Different mathematical approaches are applied for data evaluation, including a Laplace inversion for particle size distribution or the cumulants method to evaluate the time-based autocorrelation function.

Two types of optical detection are used with DLS instruments: homodyne detection, in which only the scattered light is measured and heterodyne detection, in which the scattered light and a portion of the incident light are combined for interference.

3 INSTRUMENT

The measuring system typically consists of:

(i) A laser: a monochromatic and coherent laser beam polarized with its electric field component perpendicular to the plane formed by the incident light beam and light-receiving optical axes (vertical polarization), illuminating the sampler in the measuring cell.

(ii) A sample holder: the sample holder must maintain the temperature of the sample to within ±0.3 ˚C.

(iii) Optics and a detector: a light detector positioned at a fixed angle relative to the incident laser beam measuring (usually at only one scattering angle) the apparent scattered light intensity (i.e. the sum of the scattered light from all the particles in the scattering volume) at appropriate intervals. When a polarization analyser is included, it is positioned so that the transmittance of the vertically polarized light is maximized.

(iv) A correlator (photon correlation spectroscopy) or spectrum analyser (frequency analysis).

(v) A computation unit and data processing software (some computation units also function as correlators or spectrum analysers).

4 INSTRUMENT PERFORMANCE

As the particle sizes obtained by DLS are not relative values calculated using standard particles but absolute values based on the first principle, calibration cannot be performed.

However, the performance of the instrument must be checked after it is first installed or if abnormal performance is suspected using particles with a certified diameter; it is recommended to repeat this check at least once a year thereafter. Certified reference materials with values assigned for DLS must be used.

Dispersions of polystyrene latex with narrow size distribution with certified particle diameter of about 100 nm or other suitable size can be used.

The measured average particle size must be within the stated range of the certified reference material expanded by 2% on each side. The polydispersity index must be smaller than 0.1 and the relative standard deviation of at least five repeated measurements on a sample must be equal or lower than 2 %.

5 PROCEDURE

5.1 SAMPLE PREPARATION

(i) Samples consist of the test substance well-dispersed in a liquid. To eliminate the influence of multiple light scattering, their concentration must be within an appropriate range. The dispersion medium must:
   a. be non-absorbing at the wavelength of the laser;
   b. be compatible with the materials used in the instrument;
   c. not induce particle dissolution, swelling or agglomeration/aggregation;
   d. have a known refractive index different from that of the test substance;
   e. have known viscosity to within ±2 per cent over the operational range of temperature to be used;
   f. be clean and free of dust for low background scattering.

(ii) The range of the particle concentration is determined so that the results of the measurements do not vary significantly. The range is determined beforehand based on measurements of systematically diluted samples.

It is also important to remove dust since it may affect the measurement, and to prevent its re-introduction during preparation. Typically, scattered light signals from the dispersion medium used for sample dilution must be undetectable or very weak. If large fluctuations in the scattered light signals accompanied by abnormally strong signals are recorded or if light spots appear in the path of the laser light in the sample, foreign particles are likely to have been incorporated into the sample. In such cases, further purification of the dispersion medium is necessary (by filtration, distillation, etc.) before use. The lower limit of the particle concentration range is determined mainly so that scattered light from the dispersion medium and foreign particles will not affect the measurement.

When water is chosen as the dispersion medium, use of fresh distilled water or desalted and filtered (nominal pore size 0.2 μm) water is recommended.

Long-range electrostatic interactions arising between highly charged particles may affect the measurement result. In such cases, a small amount of salt (for example, about 10^{-2} mol/L sodium chloride) may be added to the dispersion medium to reduce the effect. Air bubbles may also appear in the test sample, particularly when measuring a refrigerated sample at room temperature, and are to be avoided.

5.2 TEST PROCEDURE

Switch the instrument on and allow it to warm up.

Clean the measurement cell if necessary. The degree of cell washing required depends on the conditions of the measurement. When an individually packaged clean disposable cell is used, cleaning is not necessary. When a cell is intended to be washed, it is rinsed with water or an organic solvent. If required, a non-abrasive detergent may be used.

Place the measurement cell containing the sample in the sample holder, and wait until temperature equilibrium is reached between the sample and the

sample holder. It is recommended to measure and maintain the temperature to within ±0.3°C.

Perform a preliminary measurement of the sample, and set the particle concentration within the appropriate range (see Sample preparation).

Perform the measurement with the appropriate measuring time and number of acquisitions.

Record the average particle diameter and the PI for each measurement. If the measured values are dependent on the particle concentration, take the extrapolated infinite dilution values of the average particle diameter and the PI (or the measured values at the lowest particle concentration).

Confirm that no significant settling has occurred in the sample at the end of the measurement. The presence of a sediment indicates that the sample may have agglomerated/aggregated or precipitated, or that it may not be a suitable candidate for DLS.

5.3 REPEATABILITY

The achievable repeatability of the method mainly depends on the characteristics of the test substance (emulsion/suspension; robust/fragile; broadness of its size distribution; etc.), whereas the required repeatability depends on the purpose of the measurement. Mandatory limits cannot be specified in this chapter, as repeatability (different sample preparations) may vary appreciably from one substance to another. However, it is good practice to aim for repeatability at a relative standard deviation of not more than 10 per cent [n ≥3] for $\bar{x}_{\mathrm{DLS}}$.

6 RESULTS

The test report must include the average particle diameter and PI.

It must state the dispersion medium used, the refractive index, viscosity and temperature of the test sample, and give sufficient information about the measurement system, including the principle of measurement (PCS or frequency analysis), optical configuration (homodyne or heterodyne) and observation angle. The measuring time or number of acquisitions, the sample (nature, concentration and preparation method), the dispersion conditions, and the measurement cell type must also be described. As the results depend on the instrument, data analysis program and optical model used, these details must also be provided.

7 GLOSSARY

(i) Average particle diameter, $\bar{x}_{\mathrm{DLS}}$: Harmonic intensity-weighted averaged particle diameter expressed in nanometres.

(ii) Polydispersity index, PI: dimensionless measure of the broadness of the particle size distribution.

(iii) Scattering volume: section of the incident laser beam viewed by the detector optics. Its order of magnitude is typically 10^{-12} m^3.

(iv) Scattered intensity, count rate: intensity of the light scattered by the particles in the scattering volume as measured by a detector. In PCS, the number of photon pulses per unit time expressed in counts per second. In frequency analysis, the photodetector current which is proportional to the

189 scattered light intensity.
190 (v) Viscosity, η: viscosity of the dispersion medium in mPa・s.
191 (vi) Refractive index, n: dimensionless refractive index of the dispersion
192 medium at the wavelength of the laser.