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Add the following:

▲⟨430⟩ PARTICLE SIZE ANALYSIS BY DYNAMIC LIGHT SCATTERING

This method is based on ISO standard 22412:2017, *Particle size analysis—Dynamic light scattering (DLS)*. Portions of the chapter text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (♦♦) to specify this fact.

1. INTRODUCTION

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

Particle size distribution is an important characteristic of dispersed systems such as emulsions, suspensions, and liposome formulations.

DLS can be used to determine hydrodynamic size of 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 ♦2♦ μm. ♦For discussion of the theory and principles of measurements, see *Analytical Methodologies Based on Scattering Phenomena—Dynamic Light Scattering ⟨1430.3⟩*, which may be a helpful, but not mandatory, resource.♦

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 equivalent spherical diameter are related by the Stokes-Einstein equation.

$$\chi = \frac{kT}{3\pi\eta D}$$

χ = hydrodynamic diameter of an equivalent spherical particle (m)

k = Boltzmann constant (1.38×10^{-23} J·K^{-1})

T = absolute temperature (K)

η = viscosity of the dispersing medium (Pa·s)

D = translational diffusion coefficient (m^{2}·s^{-1})

The intensity fluctuations of the scattered light can be evaluated either as 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 functions of a disperse particle system decrease with increasing correlation time. This can be described by an exponential decay. 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 analyzed. For a disperse particle system, the power spectrum can be described by 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 ($\bar{\chi}_{DLS}$) 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.

Three types of optical detection are used with DLS instruments: homodyne detection, in which only the scattered light is measured; heterodyne detection, in which the scattered light and a portion of the incident light are combined for interference; and cross-correlation setup, which corresponds to two simultaneous homodyne experiments.

3. INSTRUMENT

The measuring system typically consists of a(n):

1. **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
2. **Sample holder**: the sample holder must maintain the temperature of the sample to within ±0.3°
3. **Optics** and **detector**: a beam splitter as used for heterodyne detection or cross-correlation setup, 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 analyzer is included, it is positioned so that the transmittance of the vertically polarized light is maximized.
4. **Correlator** (photon correlation spectroscopy) or **spectrum analyzer** (frequency analysis)
5. **Computation unit** and **data processing software** (some computation units also function as correlators or spectrum analyzers)

4. CONTROL OF INSTRUMENT PERFORMANCE/QUALIFICATION

As the particle sizes obtained by DLS are not relative values calculated using ♦particle standards of known sizes, but are calculated values based on the first principles,♦ 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. The use of certified reference materials with appropriate average particle size verified by DLS is recommended or electron microscopy if applicable.

Dispersions of polystyrene latex with a narrow size distribution with a 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. Using the cumulant analysis, the polydispersity index must be not more than 0.1, and the relative standard deviation of at least five repeated measurements on a sample must be not more than 2%.

5. PROCEDURE

5.1 Sample Preparation

1. Test samples consist of the article well dispersed in a liquid. The dispersion medium must:
   A. Be nonabsorbing 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% at the measuring temperature
   F. Be clean and free particle contamination (e.g., dust) for low background scattering

2. To eliminate the influence of multiple light scattering, the samples' concentration must be within an appropriate range. When appropriate, the particle concentration range is determined prior to the analysis based on the measurements of systematically diluted samples to ensure that the results of the measurements do not vary significantly. 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. Typically, scattered light signals from the dispersion medium used for sample dilution must be undetectable or very weak.

It is also important to remove dust as it may affect the measurement and to prevent its reintroduction during preparation. 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 or other intrinsic large particles are likely to be present in the sample. In such cases, further purification of the dispersion medium is necessary (by filtration, distillation, etc.) before use.

When water is chosen as the dispersion medium, use of fresh distilled water or desalted and filtered (nominal pore size of 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 (e.g., 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.

If measured values are dependent on the particle concentration, ensure that the concentration range is appropriate for the sample of interest.

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 nonabrasive 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°.

Perform a preliminary measurement of the sample, and set the particle concentration within the appropriate range (see *5.1 Sample Preparation*).

Perform the measurement with the appropriate measuring time, number of acquisitions, ♦number of replicates, and number of samples♦.

Record the average particle diameter and the PI for each measurement.

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 NMT 10% [n ≥ 3] for $\bar{x}_{DLS}$.

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), laser wavelength, and observation angle. The measuring time or number of acquisitions, the sample (nature, concentration, and preparation method), the dispersion conditions, the instrument settings, and the measurement cell type must also be described. As the results depend also on the data analysis program, these details must be provided as well.

GLOSSARY

Average particle diameter ($\bar{x}_{DLS}$)**:** Scattered light intensity-weighted harmonic mean particle diameter expressed in ▲▲ (ERR 1-May-2024) meters. $\bar{x}_{DLS}$ is also commonly referred to as the *z*-average diameter or "cumulants diameter".

Polydispersity index (PI)**:** Dimensionless measure of the broadness of the particle size distribution.

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

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 of time expressed in counts per second. In frequency analysis, the photodetector current, which is proportional to the scattered light intensity.

Viscosity (η)**:** Viscosity of the dispersion medium in ▲pascal-seconds (Pa·s).▲ (ERR 1-May-2024)

Refractive index (n)**:** Dimensionless refractive index of the dispersion medium at the wavelength of the laser.▲ (USP 1-May-2024)

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