
Good practice for dynamic light scattering (DLS) measurements

*Bonnes pratiques pour l'analyse de la dispersion lumineuse
dynamique (DLD)*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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Introduction

Dynamic light scattering (DLS) is a widely used technique for the characterization of particles with equivalent hydrodynamic diameters below a few micrometres. Modern instruments allow users with minimal training or background to use this technique. The downside is that not all users are familiar with the potential pitfalls, limitations and proper interpretation of results for DLS.

Therefore, this document has been developed as a guidance for good practice in DLS and complements ISO 22412:2017.

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Good practice for dynamic light scattering (DLS) measurements

1 Scope

This document provides practical guidance for performing and interpreting measurements using dynamic light scattering (DLS) that goes beyond the treatment of measurement artefacts in ISO 22412:2017.

This document is intended to help users with experiments planning, in particular with respect to obtaining the necessary information on the sample and deciding whether DLS is the most appropriate method. It provides information on how to prepare samples in an appropriate way, verify the proper functioning of the instrument and interpret the data correctly, including ways to assess data quality.

This document focuses on the practical steps required to obtain DLS results of good quality, rather than on theoretical considerations, and covers not only the measurement of solid particles, but also emulsions and bubbles.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

4 Instrument types

4.1 General

A discussion on what constitutes good practices requires knowledge of the instrument type being considered. Different optical configurations require different adjustment to control the optical layout: different signal processing techniques require different techniques to allow for background conditions; different analysis techniques require different conditioning parameters of the processed signal. Two commonly applied variants are homodyne detection with correlation function processing (see ISO 22412:2017, 9.2) and heterodyne detection with frequency spectrum processing (see ISO 22412:2017, 9.3).

Additionally, good practice, as it relates to instrument type, also depends on the scattering angle used for the measurement. For instance large spurious particles generally scatter more power into forward angles than higher angles, so that samples measured in forward-scatter typically require significantly more care regarding the cleanliness of the cuvette used, prior to filling with sample, the filtering of the sample between the particle size distribution (PSD) of interest and unwanted large size fractions and the dispensing to waste of the first few drops of sample from a syringe filter to remove filter spoil. Additionally, the single-scattering relaxation time is known to be well approximated by higher order scattering from concentrated samples as the scattering angle approaches 180 °, thereby allowing the characterization in backscatter of concentrated samples, so long as bulk scattering losses through

concentrated media can be avoided. Losses are mitigated in many commercial instruments by moving the optical detection point closer to the cuvette wall using opto-, mechanical or opto-mechanical means.

4.2 Information prior to analysis

4.2.1 Sample information

The customer or submitter of a sample for DLS analysis provides, as available, all information relevant to the measurement of the sample. Absence of information does not preclude analysis, but availability of information aids the analyst with respect to sample preparation, measurement design and interpretation of results. In general, the more information is available about a sample, the more likely the analysis will be successful and the results meaningful for the customer. Availability of this information can also reduce the uncertainty for the overall measurement result.

The following questions are answered where possible.

a) Questions related to the analysis step in DLS.

1) What is the primary composition of the sample?

The composition will determine the scattering properties and the complex refractive index, as well as colloidal stability.

2) What is the crystallographic phase (if known)?

3) What is the density of the sample? Has a Stokes' law calculation been carried out to show the settling rate for particles of different sizes?

4) Is the sample coated (e.g. is there a polymeric coating, ligand or surfactant that modifies the surface functionality and stability)?

5) What is the colloidal stability (sedimentation/agglomeration/dissolution...)?

If the colloid sediments or agglomerates, measurement results will change with time. This does not necessarily invalidate the results, but it is important to know whether such changes are expected and what information the user wants to obtain from the analysis (see [4.6.5.1](#), [4.6.5.2](#) and [4.7.3](#)).

6) What is the complex refractive index of the particles?

The complex refractive index consists of a real and imaginary part. The former defines the light scattering behaviour, the latter defines the light absorption behaviour. The real and the imaginary part of the refractive index of the particle are necessary for converting intensity-weighted to volume-weighted results.

b) Questions related to sample preparation.

1) What is the dispersing medium?

2) If the sample requires dilution, a diluent of similar chemical composition, ionic strength, pH and that contains the same other additives is chosen.

3) What is the refractive index of the medium?

The refractive index of the medium is required for analysis. If unknown, it can be looked up in tables or measured.

4) Does the medium contain surfactants necessary to maintain stability? If so, the surfactant and its concentration are identified.

5) What is the viscosity of the dispersion medium?

The viscosity of the dispersion medium is required for analysis. The viscosity of common fluids is available in tabulated forms. If this is not available, it can be measured.

6) Can the sample be filtered to remove large scatterers such as dust or residual aggregates?

This depends on the size of the principal component and desired information from analysis of sample.

7) If the sample is in suspension, is it clear? Does it contain sediment?

8) Are specific ingredients or procedures required for preparation of the sample?

9) Does the medium show non-Newtonian behaviour?

c) Questions related to the choice of the appropriate particle concentration.

1) What is the mass concentration of the sample (e.g. 0,01 mg/ml)?

d) Questions related to the selection of the appropriate evaluation algorithm.

Is the sample polydisperse (e.g. does it contain multiple size populations or a very broad size range, is it agglomerated) and what is the anticipated size distribution?

Providing any available information about degree and nature of polydispersity can help to set up the analysis.

The size distribution is in most cases the purpose of the measurement, but an expectation can help not only to set up the experiment, but also allows checking the plausibility of the result.

Many modern instruments are capable of characterising multi-modal samples with distribution-based analyses such as non-negative least squares. This is often the first step, prior to use of cumulants to provide a z-average size and polydispersity if and only if the sample is monodisperse. Laser diffraction may be considered for large aggregates; however, the user needs to be aware that laser diffraction reports the hard sphere size in comparison to dynamic light scattering which reports the hydrodynamic diameter.

e) Questions related to the identification of potential artefacts.

1) Are there other (non-principal) scattering components in the sample (e.g. proteins, a second solid phase, micelles)?

Providing the known sizes of secondary components helps in the interpretation of results.

2) Are the principal particles highly asymmetric (e.g. rod-like)?

f) Questions related to sample storage before analysis.

1) Are special conditions necessary for sample storage before analysis (e.g. refrigerated, in dark, exclusion of CO₂ uptake etc.)?

2) Is the sample material subject to dissolution?

4.2.2 Desired outcome of analysis

In addition to providing the analyst with basic information about the sample, it is equally important for the customer or submitter to stipulate the purpose and desired outcome of the analysis. This will

determine the level of effort expended and will aid the analyst in the experimental design. The following questions are most relevant.

a) In which context will the results be used?

- 1) Applications in quality control (QC) typically require less stringent analysis than applications in research and development or product characterization. Often the goal is detection of change rather than accurate determination of size/size distribution. In these cases, factors that lead to a constant bias (non-Newtonian media, inaccurate knowledge of the refraction indices etc.) will not affect the conclusion drawn from e.g. a time series.
- 2) Applications in research and development or product characterization may require higher levels of scrutiny depending on the application need.

b) Is DLS able to deliver results at the required uncertainty level?

On a material with a narrow size distribution and a median diameter of 100 nm, relative expanded uncertainties of the z-average of ≤ 3 % are achievable (see ISO 22412:2017, 10.1). For more polydisperse materials, higher uncertainties are expected. More data on uncertainties are given in e.g. References [4], [5], and [6]. Depending on how the result is used, DLS may not be able to deliver results with the required accuracy.

c) Should a mean size and polydispersity index value be reported?

- 1) This typically involves cumulants analysis, which delivers robust results for a monomodal Gaussian size distribution. It is not applicable to highly polydisperse systems or samples with a more complex distribution.

NOTE The scattering intensity into all scattering angles from particles of diameter $< 1/10$ th of the wavelength of the illuminating light beam is well-approximated, to within a few per cent, as proportional the 6th power of the particle radius.

- 2) The polydispersity index can be indicative of sample quality and hence for the suitability of DLS to measure the sample in question.

- 3) Cumulants may be useful for QC applications in particular, but see caveat in 1) above.

- 4) The use of software that generates Gaussian distributions from a mean value and a polydispersity index is deprecated, as the generated distributions may not correlate to the actual particle size distribution of the sample.

d) Is a size distribution rather than just a mean/modal size required?

- 1) The basic distribution analysis yields a scattered intensity-weighted hydrodynamic size distribution.
- 2) To convert the intensity-weighted distribution to volume or number basis, the complex refractive index for the sample material is required. However, it is deprecated to convert from intensity to volume and especially to number basis due to the inherent errors involved in this process, except for comparative purposes.
- 3) Due to low resolution of DLS, deciles (e.g. x_{10} , x_{20}) can carry high uncertainties, especially those away from the median diameter. Therefore, use of deciles is not recommended.

NOTE The mean size of the distribution can differ from that obtained by cumulants analysis.