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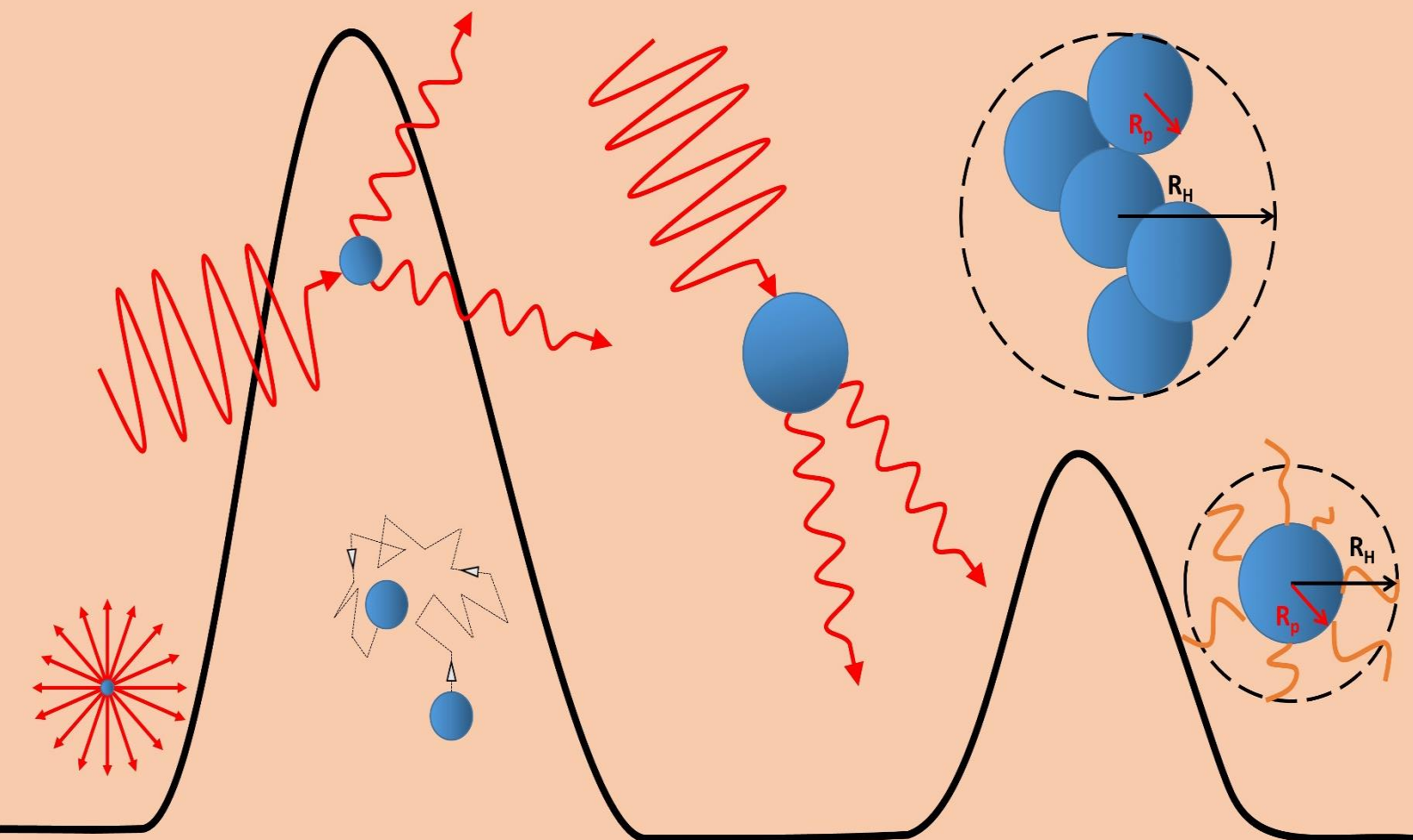
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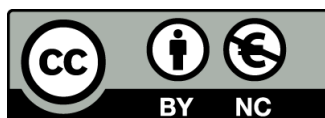


SIZE ANALYSIS OF DISPERSION IN LIQUID PHASE

First Edition - April 2022 - v1.0
by Anthony Hutin

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Preface

Why this guide?

I have often found myself, when starting a new project or a new study, having to delve into the theory and the methods in order to better understand the operating principles of the scientific tools that I would have to use to characterize my systems. The question often arose: “where to start?”

If you look at specialized books, you quickly get the feeling that you have lost the thread as soon as you get past the first two or three introductory chapters. It is a difficult task to differentiate small details from important information, and a time-consuming task to check all the references among the many citations. Very often, each chapter (which looks like an article) is written by a different researcher without the editor’s will to tell a common story, so we frequently end up with a lot of articles dealing with very detailed topics that only experts in the field will be able to appreciate.

If we now look at the articles, few of them take the time to lay the groundwork and explain how the various measurements were made. The introductions and the “material and methods” sections are often succinct and not very detailed, leaving the emphasis on the results. I quickly came to the conclusion that the articles are made for those who already know a lot about the subject. The solution is to find a good review that can afford to be more than 20 pages long and will try to give the current state of the art of a knowledge or a technique. This is often the solution if you are lucky enough that the subject has already been covered.

Another option is to open the equipment’s user manuals. This practice is far too rare because many researchers will stop at the basics of getting the equipment up and running or the quick training given on the day of installation (or by a colleague who received training from a colleague who was there on the day of installation...). Some instrument manuals introduce theory to better explain the operation and the principle of measurement. This is an essential thing in physicochemistry, a scientific instrument is neither a button press nor a black box. Moreover, today, many theoretical and application notes are written and made available free of charge by the instrument suppliers. If you have exhausted all these possibilities, there are still many scientific forums such as Researchgate where you can ask a question or read the many detailed answers that are available. It is just regrettable that the question is often being asked before the person even tried to find an answer by all the means described above.

As mentioned above, there are different ways to learn and/or refresh your memory. However, it is difficult to find one’s way through the large quantity of documents available and to quickly extract the desired information. I thought that instead of highlighting key paragraphs in articles and books, I could gather the key information in short articles on each topic of interest to save time for the next steps. I realized eventually that there was enough material to put together and share with the scientific community.

Content

This guide was made in order to help non-specialists to better understand the important concepts for size analysis of dispersions in liquid media. It is not meant to be exhaustive because I want it to be a simple tool that allows you to enter directly into the subject while finding practical advice. You will not find all the references but the main ones that are essential to go further. I have also added a “reading tips” section at the end of some chapters to help you know where to start your research. This guide will also be suitable for more experienced users who will use it as a support document or everyday memo.

I wanted to start this guide by laying out the basics of size analysis. It has often come to my ears the same questions

like “I have a size distribution, do I plot the curve, an average or the d_{50} ?”. I think it’s very important to know what parameters you want to analyze and how to compare them before getting interested in the technique you are going to use. In this book, I detail the most well-known techniques to measure the sizes of dispersions such as static and dynamic light scattering, or particle tracking analysis. I try to show the advantages and disadvantages of each technique by making comparisons. It is important to remember that there is no miracle technique and that it is necessary to take into account both the problems related to your systems and the various approximations made by the measurement techniques. One chapter also deals with the Z-average which is the most used number produced by dynamic light scattering.

This guide is not set in stone, it is intended to evolve over time. I will make corrections, improvements, clarifications, and new chapters. I therefore invite you to examine it and do not hesitate to send me your comments.

If you also wish to participate and bring your contribution to this project, do not hesitate to contact me to discuss.

I wish you a good reading.

Anthony Hutin

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Recurring abbreviations and notations

Abbreviations

PSD	Particle Size Distribution
PSA	Particle Size Analysis
SLS	Static Light Scattering
DLS	Dynamic Light Scattering
COV	Coefficient of variation
MALS	Multi-angle laser light scattering
PI	Polydispersity index
DI	Demineralized water
PTA	Particle Tracking Analysis
NTA	Nanoparticle Tracking Analysis
ZP	Zeta potential

Notations

d	Diameter
R_g	Radius of giration
T	Temperature
σ	Standard deviation
n_i	Refractive index of the material i
λ	Wavelength
k_B	Boltzmann constant
η	Dynamic viscosity
R_H	Hydrodynamic radius
Z_{av}	Intensity weighted mean diameter calculated from cumulant analysis
t	Time

Chapter I

Particle Size Analysis (PSA)

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Introduction

Particle size influences many properties of particulate materials and is a valuable indicator of quality and performance. It is then crucial to know the particle size distribution (PSD) in order to control or improve the qualities of a product. Particle size analysis (PSA) determines and reports the size range of the particles in a powder or a liquid sample. PSD is usually expressed based upon the technique by which it is determined.

All the concepts discussed in this part also apply to drop in emulsions.

1 Particle size definition

Particles are 3-dimensional objects, and unless they are perfect spheres (e.g. emulsions or bubbles), they cannot be fully described by a single dimension parameter such as a radius or a diameter.

Indeed, non-spherical particles can be described using multiple length and width measures (**Figure I.1**). These descriptions provide greater accuracy, but also greater complexity. In order to simplify the measurement, many techniques define the particle size using the concept of equivalent spheres. The reported value is the diameter of an equivalent sphere having the same property (e.g. volume or mass) as the actual particle (**Figure I.2**).

Although this approach is not accurate, the shapes of particles generated by most industrial processes are such that the spherical assumption does not cause serious deviations. However, if the particles have a very large aspect ratio (ratio of its width to its length), such as plates or needles, the size in at least one dimension can differ significantly from that of other dimensions. For example, a cylinder with a length of 100 nm and a width of 10 nm is volume

equivalent to a sphere of 24 nm diameter which is not a very accurate description of its true dimensions (**Figure I.3**).

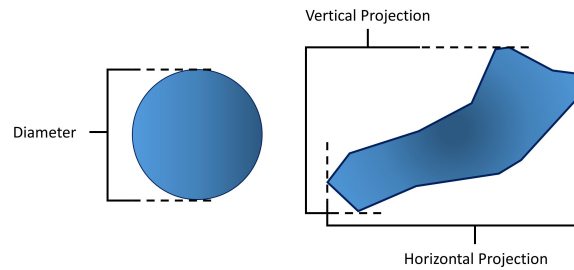


Figure I.1: Effect of shape factor on characteristic lengths of particles.

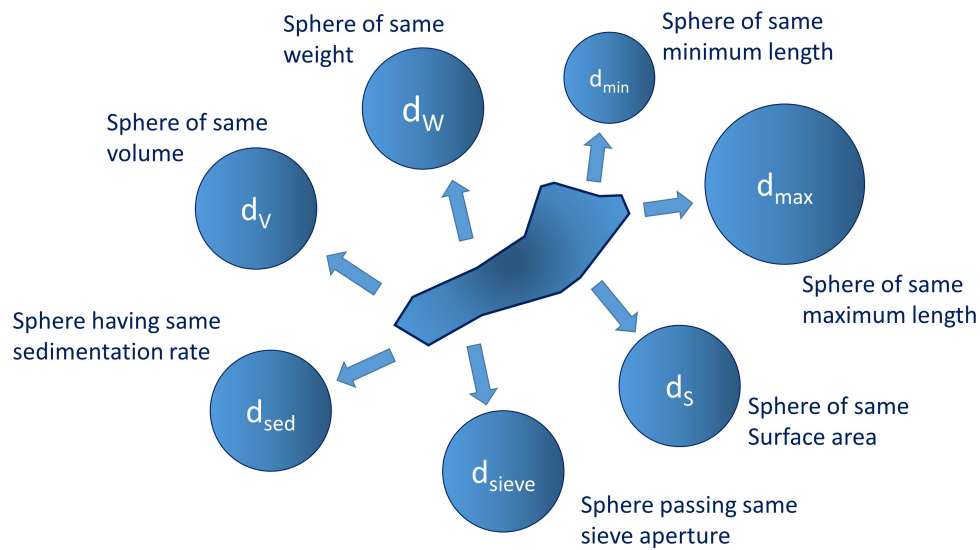


Figure I.2: Concept of equivalent spheres.

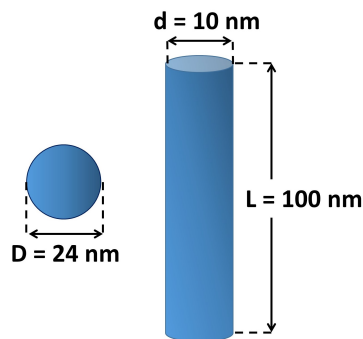


Figure I.3: Effect of aspect ratio on volume equivalent sphere of a needle shaped particle.

Shape factor causes disagreements when particles are measured with different particle size analyzers: each measurement technique detects size through the use of its own physical principle. For example, a light scattering device will average the various dimensions as the particles flow randomly through the light beam, producing a distribution of sizes from the smallest to the largest dimensions.

The only techniques that can describe particle size using multiple values are microscopy or automated image analysis. An image analysis system could describe the non-spherical particle seen in **Figure I.1** using the longest and shortest diameters, perimeter, projected area, or again by equivalent spherical diameter.

When reporting a PSD the most common format used even for image analysis systems is an equivalent spherical diameter on the x axis and a percent on the y axis. It is only for elongated or fibrous particles that the x axis is typically displayed as length rather than equivalent spherical diameter.

2 Particle size distributions (PSD)

Unless the sample is perfectly mono disperse, i.e. every single particle has exactly the same dimensions, it will consist of a statistical distribution of particles of different sizes. Size distribution can be monomodal (one population) or multimodal (several populations), and monodisperse (narrow distribution) or polydisperse (broad distribution) (**Figure I.4**). For instance, a batch with a mean size of 300 nm and described in the literature as "monodisperse" does not mean that all of the particles measure 300 nm but that the distribution is symmetric and centred around 300 nm.

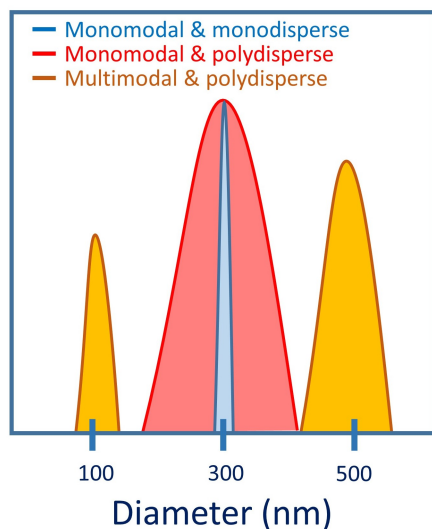


Figure I.4: Schematic representations of particle batches corresponding to a monodisperse or polydisperse monomodal population and a bimodal polydisperse population.

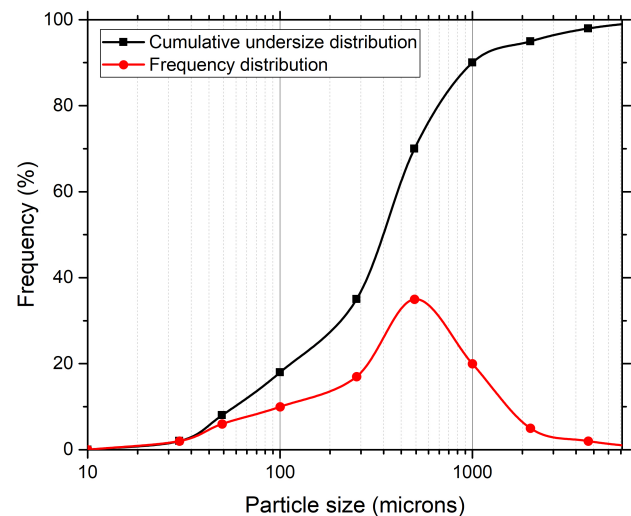


Figure I.5: Difference between a cumulative undersize distribution and frequency distribution.

It is common practice to represent this distribution in the form of either a frequency distribution curve or a cumulative (undersize or oversize) distribution curve (**Figure I.5**). PSD can be represented in different ways with respect to the weighting of individual particles. The weighting mechanism will depend upon the measuring principle being used.

Number weighted distributions A counting technique such as image analysis will give a number weighted distribution where each particle is given equal weighting irrespective of its size. This is most often useful when knowing the absolute number of particles is important or when high resolution (particle by particle) is required.

Volume weighted distributions Static Light Scattering (SLS) techniques such as laser diffraction will give a volume weighted distribution. The contribution of each particle in the distribution relates to the volume of that particle (equivalent to mass if the density is uniform), i.e. the relative contribution will be proportional to (size)³. This is useful from a commercial perspective as the distribution represents the composition of the sample in terms of its volume/mass.

Intensity weighted distributions Dynamic Light Scattering (DLS) techniques will give an intensity weighted distribution, where the contribution of each particle in the distribution relates to the intensity of light scattered by

the particle. For example, using the Rayleigh approximation, the relative contribution for very small particles will be proportional to (size)⁶.

When comparing particle size data for the same sample measured by different techniques, it is important to realize that the types of distribution being measured and reported can produce very different particle size results. This is clearly illustrated in the **Figure I.6**, for a sample consisting of equal numbers of particles with diameters of 10 nm and 100 nm. The number weighted distribution gives equal weighting to both types of particles, emphasizing the presence of the finer 10 nm particles, whereas the intensity weighted distribution has a signal one million times higher for the larger 100 nm particles. The volume weighted distribution is intermediate between the previous two.

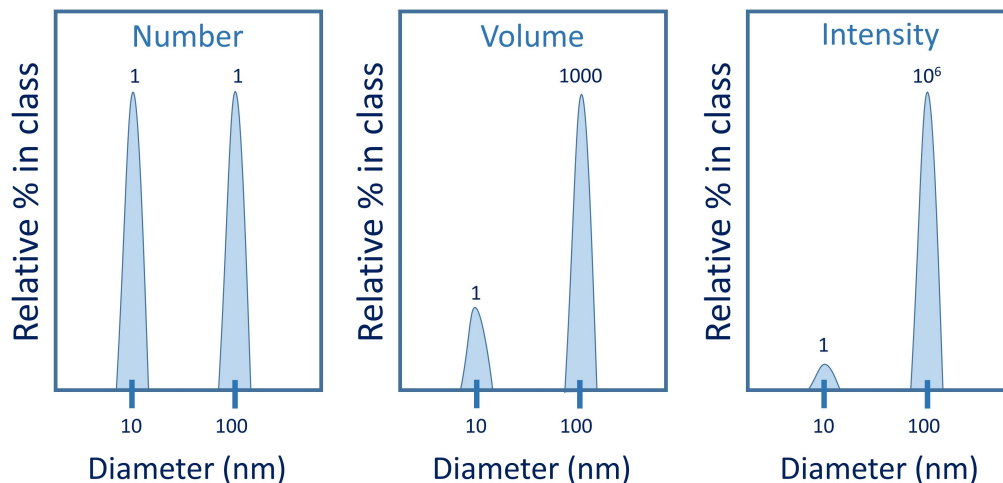


Figure I.6: Difference between number, volume, and intensity weighted particle size distributions.

3 Distribution statistics

In order to simplify the interpretation of PSD data, a range of statistical parameters can be calculated and reported. The choice of the most appropriate statistical parameter for any given sample will depend upon how that data will be used and what it will be compared with. For example, if you wanted to report the most common particle size in your sample you could choose between the following parameters:

- mean: 'average' size of a population,
- median: size where 50 % of the population is below/above,
- mode: size with highest frequency.

For symmetric distributions all central values are equivalent with $mean = median = mode$. If the shape of the PSD is asymmetric, as it is often the case in many samples, you would not expect these three values to be exactly equivalent, as illustrated in **Figure I.7**.

3.1 Means

There are multiple definitions for mean because the mean value is associated with the basis of the distribution calculation (number, surface, and volume).

The abbreviated notation $D[p,q]$ is used to define weighted mean values as :

$$D[p,q] = \frac{\sum d^p}{\sum d^q}$$

When $(p - q)$ is equal to 1, an arithmetic mean is represented and when the difference is above 1, the mean is geometric.

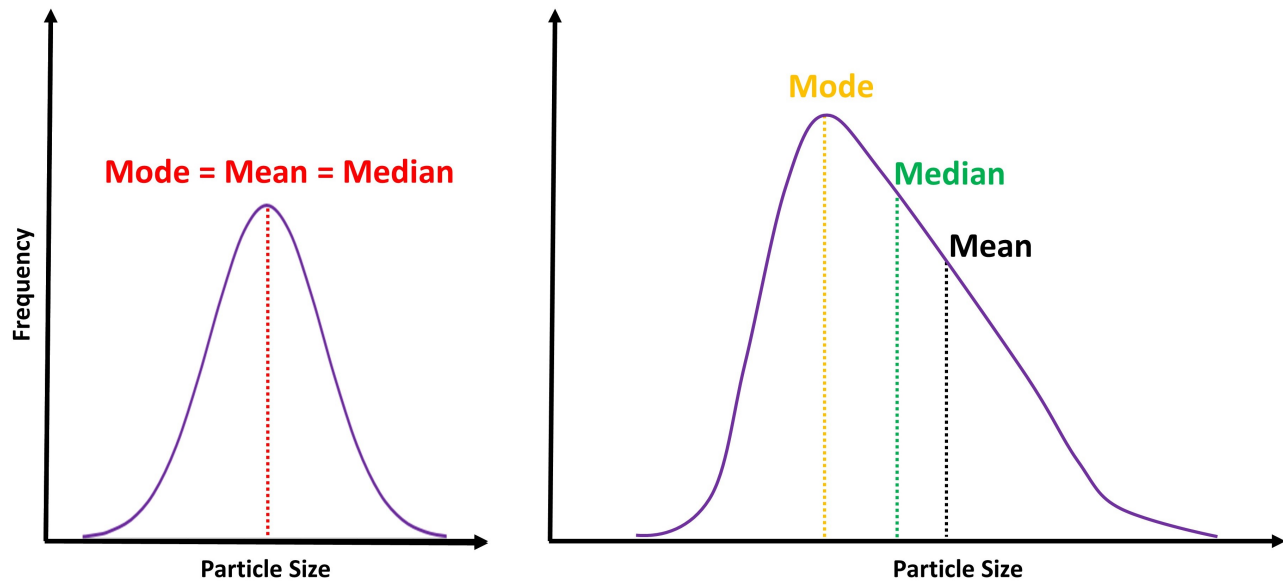


Figure I.7: Difference between a symmetric and a non-symmetric distribution for mode, mean, and median.

Number length mean $D[1,0]$: The number length mean, often referred to as the arithmetic mean is:

$$D[1,0] = \frac{\sum d^1}{\sum d^0} = \frac{\sum d}{n}$$

with $\sum d$ the sum of the diameters of all the particles and n the number of particles.

This number is most important when the number of particles is of interest, e.g. in particle counting applications). It can only be calculated with the total number of particles in the sample and is therefore limited to particle counting applications.

Surface area moment mean $D[3,2]$:

$$D[3,2] = \frac{\sum d^3}{\sum d^2}$$

The surface area mean (Sauter mean diameter) is most relevant when specific surface area is important, e.g. bioavailability, reactivity, dissolution. It is most sensitive to the presence of fine particulates in the size distribution.

Volume moment mean $D[4,3]$:

$$D[4,3] = \frac{\sum d^4}{\sum d^3}$$

The volume moment mean (De Brouckere mean diameter) is relevant for many samples as it reflects the size of those particles which constitute the bulk of the sample volume. It is most sensitive to the presence of large particulates in the size distribution.

These formulas indicate around which central point of the frequency the surface area or volume/mass distribution would rotate. The advantage of this method of calculation is obvious: the formulas do not contain the numbers of particles and therefore calculations of the means and distributions do not require knowledge of the number of particles involved. For example, laser diffraction initially calculates a distribution based around volume terms and this is why the $D[4,3]$ is reported in a prominent manner.

An example of the surface area and the volume moment means is shown in the **Figure I.8**. If the aim is to monitor the size of the coarse particulates that make up the bulk of this sample, then the $D[4,3]$ would be most appropriate. On the other hand, if it is more important to monitor the proportion of fines present, then it might be more appropriate to use the $D[3,2]$.

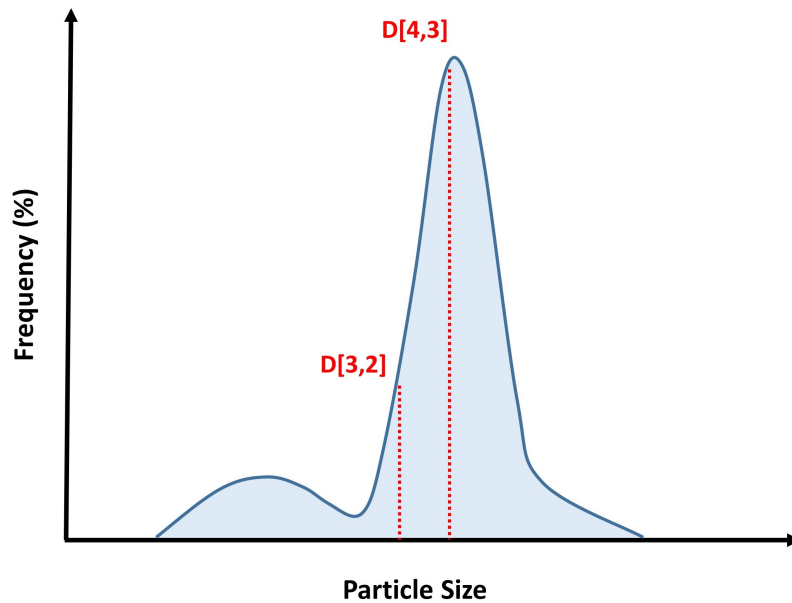


Figure I.8: Influence of fine presences on $D[4,3]$ and $D[3,2]$ for a polydisperse size distribution.

3.2 Mode

The mode is the peak of the frequency distribution, or it may be easier to visualize it as the highest peak seen in the distribution. The mode represents the particle size (or size range) most commonly found in the distribution. Less care is taken to denote whether the value is based on volume, surface or number; so either run the risk of assuming volume basis or check to assure the distribution basis. The mode is not as commonly used, but can be descriptive; in particular if there is more than one peak to the distribution, then the modes are helpful to describe the mid-point of the different peaks.

3.3 Median and Percentiles

For volume weighted particle size distributions, such as those measured by laser diffraction, it is often convenient to report parameters based upon the maximum particle size for a given percentage volume of the sample.

Percentiles are then defined as XaB where:

- X : usually D for diameter,
- a : distribution weighting, e.g. n for number, s for surface, v for volume, and i for intensity.
- B : percentage of sample below this particle size e.g. 50 % or 0.5.

For example, the $Dv50$ would be the maximum particle diameter below which 50 % of the sample volume exists, also known as the median particle size by volume. Since the primary result from laser diffraction is a volume distribution, $D50$ is often cited in reference to the $Dv50$ without including the v .

The most common percentiles reported are the $Dv10$, $Dv50$ and $Dv90$, as illustrated in the frequency and cumulative plots (**Figure I.9** and **I.10**). By monitoring these three parameters it is possible to see if there are significant changes in the main particle size, as well as changes at the extremes of the distribution, which could be due to the presence of fines, or oversized particles/agglomerates.

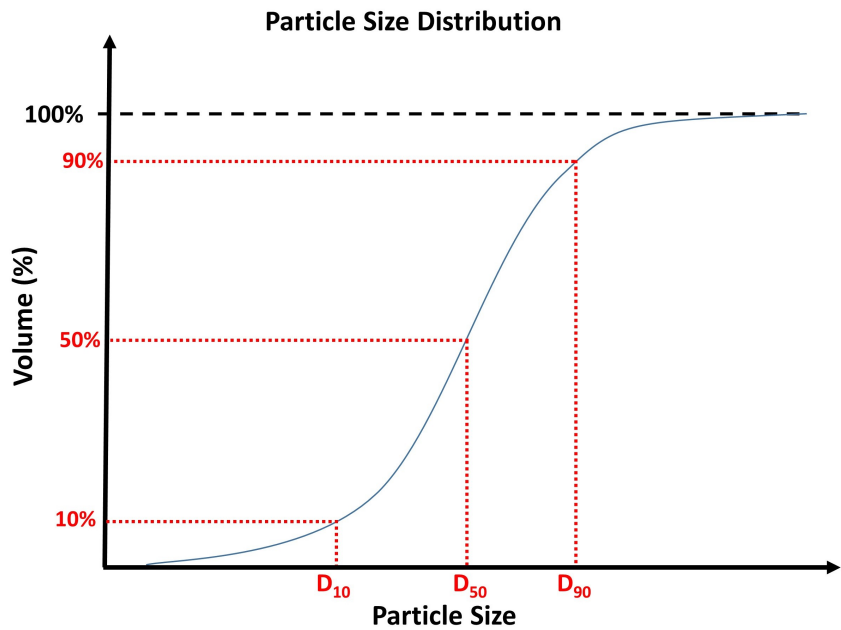


Figure I.9: Representation of Dv_{10} , Dv_{50} , and Dv_{90} for a cumulative undersize distribution.

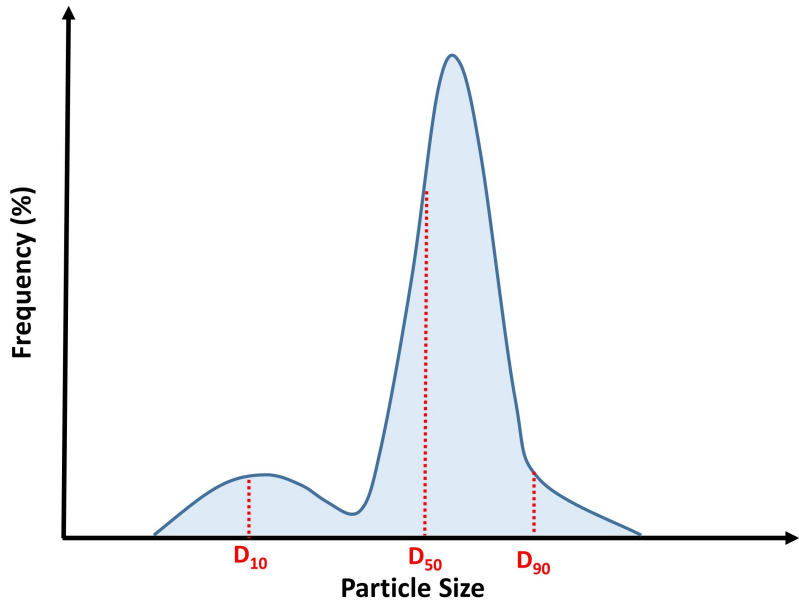


Figure I.10: Representation of Dv_{10} , Dv_{50} , and Dv_{90} for a polydisperse size distribution.

3.4 Distribution widths

The measurement of the particle sizes implies an interest in the width or breadth of the distribution. The field of statistics provides several calculations to describe the width of distributions:

3.4.1 Standard deviation

Standard deviation (σ) is an indication of the spread of the curve about the mean. As shown in **Figure I.11**, 68.27 % of the total population lies within $\pm\sigma$, and 95.45 % lies within $\pm2\sigma$.

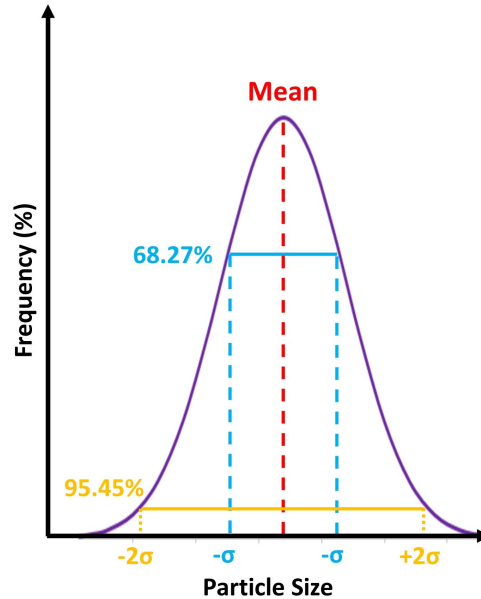


Figure I.11: Standard deviation for a normal distribution.

3.4.2 Variance

Variance (V) is the square of the standard deviation:

$$V = \sigma^2$$

It is also an indication of the spread of the data points. When two factors act together to cause variability in a distribution, their standard deviation is a square root function. Squaring it to obtain a value is therefore convenient in certain applications.

3.4.3 Span

Although occasionally cited, the use of standard deviation declined when hardware and software advanced beyond assuming normal or Rosin-Rammler distributions. One of the common values used for laser diffraction results is the *Span*, the distance between two points equally spaced from the median:

$$Span = \frac{Dv90 - Dv10}{Dv50}$$

In rare situations the *Span* equation may be defined using other values such as $Dv80$ and $Dv20$.

3.4.4 Coefficient of Variation

An additional approach to describe the distribution width is to normalize the standard deviation through division by the mean:

$$COV = \frac{\sigma}{Mean}$$

This is the Coefficient of Variation (COV), also called the Relative Standard Deviation (RSD) when reported as a percentage.

COV is both used and encouraged as a calculation to express measurement result reproducibility. *ISO 13320* encourages all users to measure any sample at least 3 times, calculate the mean, standard deviation, COV, and the standard sets pass/fail criteria based on the COV values.

4 Particle characterization techniques

There is a wide range of commercially available particle characterization techniques that can be used to measure particulate samples. Each has its relative strengths and limitations and there is no universally applicable technique for all samples and all situations.

A number of criteria must be considered when deciding which particle characterization techniques you need:

- Particle properties
- Particle size range
- Samples polydispersity (wide dynamic range)
- Quickness to make measurements
- Measurement resolution
- Wet or dry sample

The following tables are designed to provide some basic guidelines to help you decide which of some of the commonly used techniques could be most suitable for a particular application. The particle size ranges indicated in **Table I.1** are a guide only and exact specifications may vary from one instrument to another. **Table I.2** indicates which technique is the most (+++++) or least (+) efficient depending on the parameter to be measured.

Particle size range	1 nm	10 nm	100 nm	1 μ m	10 μ m	100 μ m	1 mm
Laser Diffraction							
Dynamic Light Scattering							
Automated Imaging							
Sedimentation							
Electrozone sensing							
Sieving							

Table I.1: Measured particle size range.

Techniques	Dynamic range	Rapid	Resolution	Sampling	Shape	Wet	Dry
Laser Diffraction	+++++	+++	++	+++		✓	✓
Dynamic Light Scattering	+++	+++	++	++		✓	
Automated Imaging	++	++	+++	++	✓	✓	✓
Sedimentation	++	+	++	++		✓	
Electrozone sensing	+	++	+++	+		✓	
Sieving	+	+	+	+		✓	✓

Table I.2: Effectiveness of the measure depending on the technique: from least (+) to most (+++++).

5 Selecting a particle size analyzer

The decision process may be different if the instrument is being purchased for a specific application as opposed to a general analytical technique for many possible samples. For specific application it makes sense to search the industry literature to determine if a particular technique is favored over others. If for example 90 % of all literature found in this field is DLS, then the decision is simple.

Sometimes the goal to buy a new instrument includes being able to correlate to existing data. Accomplishing this goal can range from easy to difficult. Just upgrading from an older to newer model diffraction analyzer could cause a change in results. The changes originate from many sources including differences in dynamic range, advances in algorithms, and mechanic improvements to samplers. Switching from an existing technique such as sieving to newer techniques like laser diffraction or dynamic image analysis could also lead to changes in results. Data from sieves are typically smaller than data from laser diffraction depending on the shape of the particles. The less spherical the particle, the greater the difference will likely be.

PSD is sufficient information for the majority of particle characterization applications. But some techniques have a higher resolution than others. Laser diffraction and dynamic light scattering are powerful techniques that are “resolution limited” compared to high resolution techniques which are based on particle counting. If the goal of the measurement is finding small populations of particles larger or smaller than the main distribution, then an investigation of the sensitivity to second distributions should be part of the selection process.

Particle shape information may be either desirable or critical depending on the degree to which shape affects product performance. Particle shape influences bulk properties of powders including flow and compaction behavior and the viscosity of suspensions. When particle shape information is required, microscopy and image analysis are the only techniques that delivery the desired data.

5.1 When to choose laser diffraction

Laser diffraction (Static Light Scattering, SLS) is the most popular particle size technique for reasons including speed, ease of use, and flexibility. The most basic laser diffraction system can measure size of dispersion in suspensions and emulsions, and also dry powders in air with the addition of a dry powder feeder. This is a low concentration technique, so dilution is often required. The complex refractive index of the sample and diluent must be known for optimum accuracy.

Since this is such a powerful and flexible technique, it is often the best option for companies buying their first analyzer, or hoping to satisfy multiple needs and applications.

5.2 When to choose laser dynamic light scattering

Dynamic Light Scattering (DLS) can measure suspensions and emulsions from 1 *nm* to 1 *μm*. Both the lower and upper limits are sample dependent.

The lower limit is influenced by concentration and how strongly the particles scatter light. A low concentration sample of weakly scattering particles near 1 *nm* can be difficult to reproduce.

The upper size limit is determined mainly by the density of the particles. DLS algorithms are based on all particle movement coming from Brownian motion. Motion due to settling is not interpreted correctly by DLS systems. In addition, particles settled on the bottom of the sample cuvette can not be inspected by the laser light source. The upper limit of DLS may be 8 *μm* for emulsion samples where the two phases have similar density. The upper limit of uranium particles may be as small as 300 *nm*. The upper limit of particles with a density of 1.7 may be around 1 *μm*.

Using DLS does not require any knowledge of the sample refractive index (it would only be required to convert from intensity to volume distribution), or concentration. What is required is viscosity, especially for higher concentration samples. Although most modern DLS systems claim the ability to work at higher concentrations, this is again sample dependent. Serious DLS work could involve a dilution study to determine the nature of the particle-particle interactions and presence of multiple scattering.

5.3 When to choose image analysis

Many laboratories are now replacing manual microscopy with automated image analysis. While microscopy provides qualitative accuracy and shape information, it requires automated image analysis to inspect the number of particles required to obtain statistically valid quantitative results. Choosing image analysis is often driven by the desire to generate results that are accurate, sensitive to second populations, contains shape information, and includes images of the particles.

Dynamic image analysis is generally used for particles ranging from 30 μm to 30 mm . Static image analysis is typically a research tool for measuring particles in the 0.5 to 1000 μm range. Deciding between dynamic or static image analysis is not difficult, as the applications are typically better served by one technique or the other.

Review and reading tips

- ✓ The language of particle size, R. Wolfrom, Journal of GXP Compliance, vol. 15, no. 2, spring 2011.
- ✓ A guide book to particle size analysis, Horiba, 2012.
- ✓ A basic guide to particle characterization, Malvern Whitepaper, 2015.
- ✓ Recommended Practice Guide: Particle Size Characterization, A. Jillavenkatesa et al., 960-1, NIST Pubs.

Chapter II

Static Light Scattering (SLS)

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Introduction

Laser diffraction, also called Static Light Scattering (SLS) is a widely used particle sizing technique, the main reasons for its success are:

- Wide dynamic range : from submicron to the millimetre size range.
- Rapid measurements : results generated in less than a minute.
- Repeatability : large numbers of particles are sampled in each measurement.
- Calibration not necessary : easily verified using standard reference materials.
- Well-established technique (*ISO 13320*).

The principle of SLS is presented here as well as the different measured parameters and how to optimize the measurement depending on the sample and its artifacts.

1 Principle

Laser diffraction measures the angular variation in intensity of light scattered as a laser beam passes through a dispersed particulate sample (see **Annex** for more details). SLS experiments consists in a laser illuminating a cuvette containing the sample to be analyzed (**Figure II.1**). One or many detectors are used to measure the scattering intensity in dependence of the scattering angle θ . This angular scattering intensity curve $I_s(\theta)$ is then analyzed to calculate the particle's size (volume equivalent sphere diameter), its shape, and molar mass.

A sample dispersion units, for dry and wet sample, is crucial to deliver the particles to the measurement area of the optical bench at the correct concentration and in a suitable state of dispersion. Wet sample dispersion units use a liquid dispersant, aqueous or solvent based, to disperse the sample. In order to keep the sample suspended and homogenized it is recirculated continuously through the measurement zone.

SLS is an in-situ technique where, as opposed to direct imaging techniques such as Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM), the sample can be measured in its natural state as long as the particle concentration is small enough to avoid multiple scattering effects. False measurements due to multiple scattering result in significant errors. They can only be avoided if special techniques such as cross-correlation are used to suppress the multiple scattering.

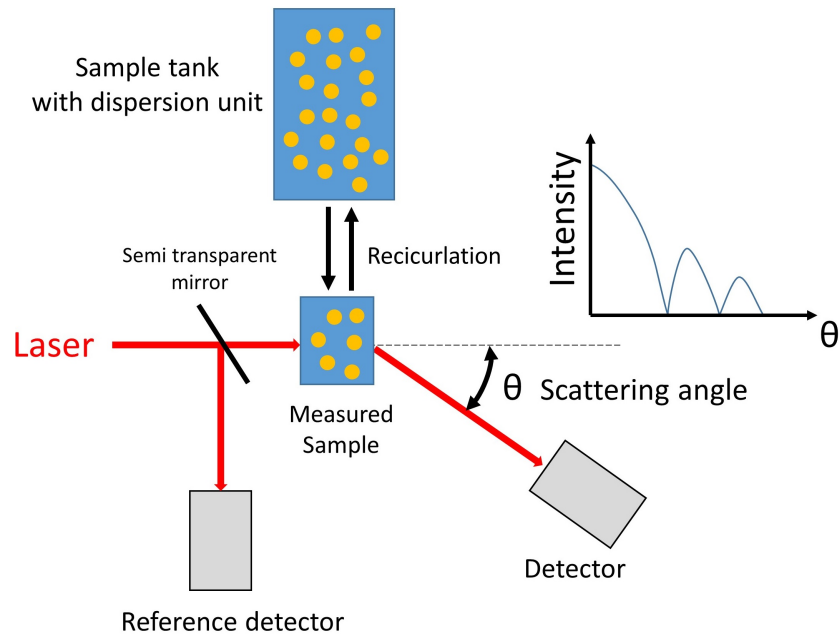


Figure II.1: Simplified diagram of measurement by SLS.

1.1 Size limit

Larger particles scatter light at small angles relative to the laser beam and small particles scatter light at large angles, as illustrated in **Figure II.2**. The limit between large and small particles is determined as a multiple (40x) of the wavelength of light used for the measurement and typically approximated at $50\ \mu\text{m}$.

For particles larger than $50\ \mu\text{m}$ the vast majority of light is scattered by diffraction and not by refraction. The scattered light is at relatively high intensity and low angle for these larger particles. This means the measurement will not benefit from the use of a refractive index to accurately interpret refracted light.

For particles smaller than $50\ \mu\text{m}$ refracted light becomes increasingly important to calculate an accurate particle size. The scattered light is at relatively low intensity and wide angle for these smaller particles. Laser diffraction uses Mie theory of light scattering to calculate the particle size distribution, assuming a volume equivalent sphere model. Mie theory requires knowledge of the optical properties (refractive index and imaginary component) of both the dispersant and the sample being measured. A simplified approach is to use the Fraunhofer approximation,

which does not require the optical properties of the sample. However it should be used with caution whenever working with samples which might have particles below $50\text{ }\mu\text{m}$ or where the particles are relatively transparent.

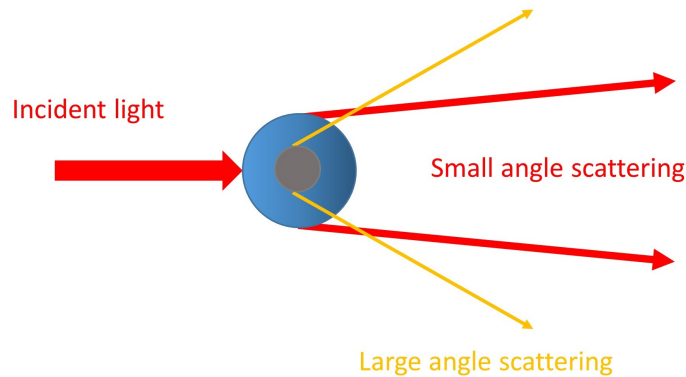


Figure II.2: Scattering of light from small and large particles.

1.2 Fraunhofer approximation

Fraunhofer approximation assumes that:

- the particle is much larger than the wavelength of light employed. *ISO 13320* [1] defines this as being greater than 40x wavelength,
- all sizes of particle scatter with equal efficiencies,
- the particle is opaque and transmits no light.

These assumptions are incorrect for many materials and for small particles they can give rise to errors approaching 30 %, especially when the relative refractive index of the material and medium is close to unity, or when the particles are transparent. When the particle size approaches the wavelength of light the scattering efficiency becomes a complex function with maxima and minima present.

1.3 Mie theory

Mie theory completely solves the equations for interaction of light with matter. This allows completely accurate results over a large size range and with all materials under all conditions.

This theory was developed to predict the way light is scattered by spherical particles and deals with the way light passes through, or is adsorbed by, the particle. It assumes the particle is spherical, as opposed to Fraunhofer which is a projected area prediction. The counterpart for such accuracy is that the refractive indices of the material and the medium must be known as well as the absorption part of the refractive index (imaginary part).

2 Mie or Fraunhofer?

The *ISO 13320* standard recommends the use of the Mie and Fraunhofer theories for spherical particles. It is also recommended to adjust the concentration of the measurement medium in order to avoid the phenomenon of multi-diffusion in order to separate the particles of at least 40 times the wavelength of the incident light.

The diagram shown in **Figure II.3** enables the most suitable theory to be chosen as a function of the parameters available to the operator. If the refractive index of the material is unknown or if the size of the particles is above $50\text{ }\mu\text{m}$, the user can use the Fraunhofer theory. In other cases, the Mie theory will give the best results. In the case of glass beads, the particle size shown in **Figure II.4** have a similar appearance whatever the theory applied.

However, when the Fraunhofer theory is used, we observe the presence of a population of particles between 1 and $5\text{ }\mu\text{m}$. This population is not present when the Mie theory is used with a complex refractive index of the material

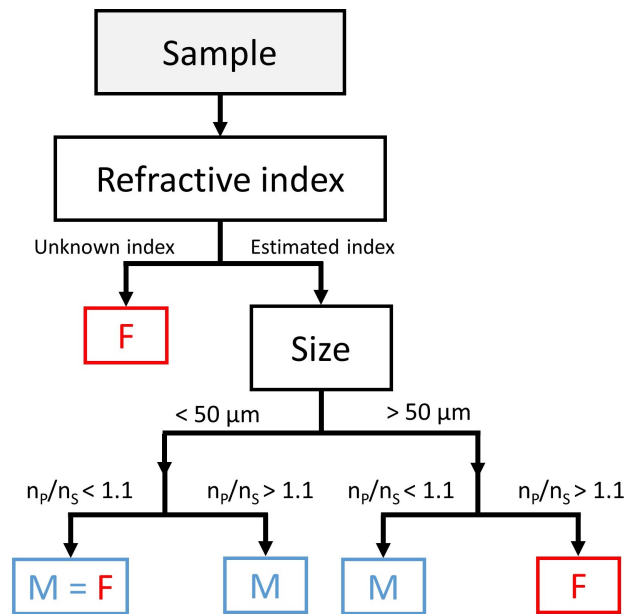


Figure II.3: Guide for choosing the most suitable theory as a function of the material. F and M correspond to the use of the Mie or Fraunhofer theories. n_p and n_s correspond to the refractive index of the particle and the solvent. (adapted from [2])

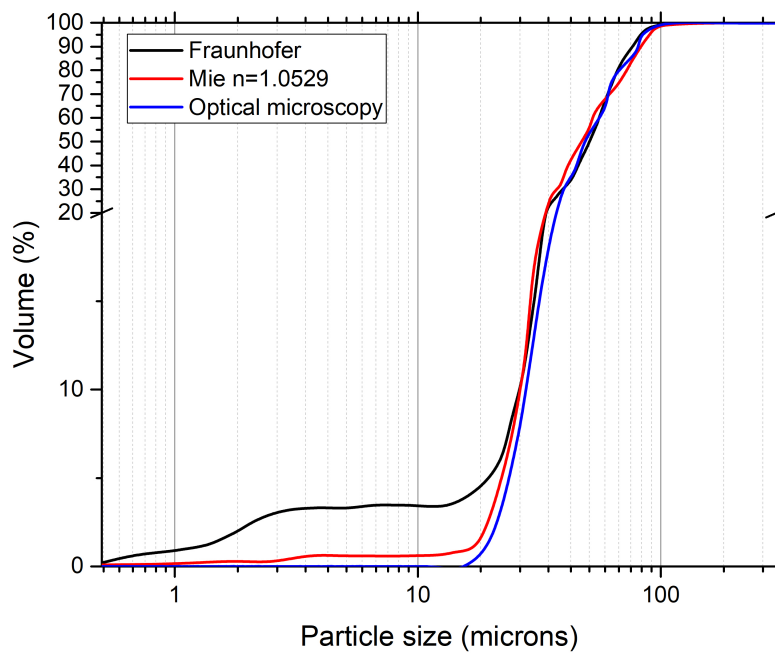


Figure II.4: Particle size distributions of glass beads using the Mie and Fraunhofer. models (adapted from [2])

equal to $n = 1.529$. Observations made by optical microscopy on the sample of glass beads enabled the corresponding particle size distribution to be drawn. It is similar to that obtained when the Mie theory is applied. The population between 1 and 5 μm when the Fraunhofer theory is used has therefore no physical reality. This population is uniquely generated by the limit of the model applied: glass beads are transparent material.

In conclusion, in the case where the complex refractive index is not known, the Fraunhofer theory is a good alternative for determining the particle size distribution over 50 μm . However, use Mie theory at all times if the complex reactive is known.

3 Multi-angle light scattering (MALS)

Multi-Angle Light Scattering (MALS), also called Multi-Angle Laser Light Scattering (MALLS), is a multi-angle SLS method. The term "multi-angle" refers to the detection of scattered light at different discrete angles, measured by a single detector moved over a range of selected angles or by an array of detectors fixed at specific angular locations. This technique allows to measure the absolute molecular weight (M_w) and the radius of gyration (R_g) of a sample.

3.1 Molar masses

MALS can determine M_w of polymers from below 10^3 to 10^7 g/mol (daltons). It measures the molar mass directly, no matter what the structure (whether they are rods, random coils, or spheres).

When laser light hits a macromolecule, the oscillating electric field of the light induces an oscillating dipole within it. This oscillating dipole will re-radiate light, much like the antenna for a radio station sends out radio waves. The intensity of the radiated light depends on the magnitude of the dipole induced in the macromolecule. The more polarizable the macromolecule, the larger the induced dipole, and hence, the greater the intensity of the scattered light.

Therefore, in order to analyze the scattering from a solution of such macromolecules, it is necessary to know their polarizability relative to the surrounding solvent. This may be determined from a measurement of the change of the solution's refractive index Δn with the molecular concentration change, Δc , by measuring the dn/dc ($=\Delta n/\Delta c$) value using an differential refractometer.

When there are many macromolecules in solution, each macromolecule scatters light via the induced dipole mechanism. Hence, the intensity of the scattered light is proportional to the concentration of the macromolecules in solution; twice as many molecules scatter twice as much light.

Consider now the important case where two monomers aggregate to form a dimer in solution. The scattered light from the dimer is twice as intense as the total light scattered from two independent monomers. Simply by doubling the molar mass, even while keeping the mass/volume concentration the same, the intensity of the scattered light doubles. The intensity of light scattered is directly proportional to the molar mass. Thus, light scattering represents a powerful technique for determining the molar mass of macromolecules in solution as well as monitoring the presence and formation of aggregates.

3.2 Molecule size

The size of a molecule affects the angular variation of the scattered light intensity. If a light scattering instrument makes measurements at a single angle only, the angular dependence of scattered light cannot be determined. Only MALS instruments can be used to determine molecular sizes directly.

Depending on the number of angles used, a MALS instrument can determine molecular radii from about 8–10 nm to larger than a micrometer. Small, compact molecules (< 10 nm) have little or no angular variation in scattered light. MALS instruments never require a priori conformational assumptions. Since the mean square radius is determined from the slope of the angular variation, a minimum of three angles is needed.

If the angular dependence of the scattered light is measured in the horizontal plane, it is possible to determine the size of the molecule. This size measurement is known as the root mean square radius, or sometimes the radius of gyration R_g . R_g is a measure of the molecule's size weighted by the mass distribution about its center of mass. If the molecule's conformation is determined to belong to a specific class (e.g., random coil, sphere, or rod), R_g can be related to its geometrical dimensions. A sample containing a broad molecular weight distribution can be separated, the light scattering data acquired at each elution volume can be used to determine M_w and R_g . The measured radius of gyration may be plotted against the correspondingly measured molar mass to determine the sample's conformation.

3.3 Others

Other benefits of MALS involve the ability to determine branching properties directly, since branching calculations depend on the measurement of the molecule's mean square radius. MALS instruments also can be used to detect

protein aggregation that UV and RI detectors often miss completely or to determine the oligomeric state of conjugated proteins like membrane proteins. In addition, MALS instruments can be used to study the homo- and hetero-association of proteins and other biological macromolecules.

4 Parameters affecting the measure

4.1 Sample preparation and concentration

Good sample preparation is critical. The sample should be correctly mixed before a measurement is taken. Wet samples also have to be correctly dispersed in a liquid dispersant. Using the wrong dispersant can cause the sample to stick together in clumps, float on the surface, or even dissolve.

The analysis of the sample is concentration dependant, usually the software of the device reports exactly what level of signal the sample generates and whether this is ideal or not. The sample concentration is controlled by monitoring the obscuration of the laser beam caused by the sample. The obscuration is simply the fraction of light “lost” from the main beam when the sample is introduced. For example, an obscuration of 10 % means that 10 % of the incident laser beam has been lost through scattering or absorption. Fortunately, the technique has a wide range of concentrations that are ideal so concentrations do not have to be precise.

4.2 Artifacts [3]

Also referred to as “ghost peaks”, artifact peaks are well recognized among laser diffraction instrument operators. The term “artifact peak” is meant as a general description to differentiate any outlier peak from the true particle size distribution that does not correspond to a tangible presence (such as a bubble, an unintended emulsion droplet, or agglomerated particles). Several common associated causes are discussed below.

4.2.1 Bubbles

When a dispersion of particles is subjected to liquid dispersion analysis, it can be generally assumed that any bubbles or gaseous occurrence in the dispersion are not meant to be taken as part of the intended sample for analysis. SLS method cannot distinguish between species present in a distribution and therefore, it will report the sample and the bubbles indiscriminately.

In laser light scattering instruments, air bubble peaks can occur at 40-500 μm in water. This size range can vary depending on the viscosity and surface tension of the dispersing medium. **Figure II.5** is a typical example of a bubble peak occurring alongside a particle distribution in an aqueous dispersion. In this example, the bubble peak occurs at the coarse end at approximately 200 μm . There is a clear demarcation between the particle peak and the bubble peak. Though the bubble peak does not overlap with the sample particle size distribution peak, it will clearly influence the cumulative size moments and will render any associated data erroneous.

It is important to be able to identify these peaks as distinct from the particulate to be measured, and to thereby invalidate them in a regulated environment. A simple check under the microscope is often sufficient to rule out the presence of particulate in the size range of the suspected bubble peak.

While certain sample types and dispersions, such as those containing surfactants or highly viscous carriers, might have a greater likelihood of bubble occurrence, in general, most bubble peaks occur inconsistently, which makes it easy to rule them out as part of the true particle size distribution.

4.2.2 Thermal

Like bubble peaks, thermal artifact peaks are inconsistent in their occurrence. These form when volatile dispersing media do not reach thermal equilibrium within the instrument. Equilibration is usually accomplished by allowing longer recirculation times prior to taking a background measurement.

This signal discrepancy is caused by the slight change in refractive index of the sample, because refractive index is temperature dependent. As this temperature difference between the initial measurement and final measurement increases in magnitude, so does the artifact peak at the coarse end of the distribution, which broadens over time until equilibrium is reached.

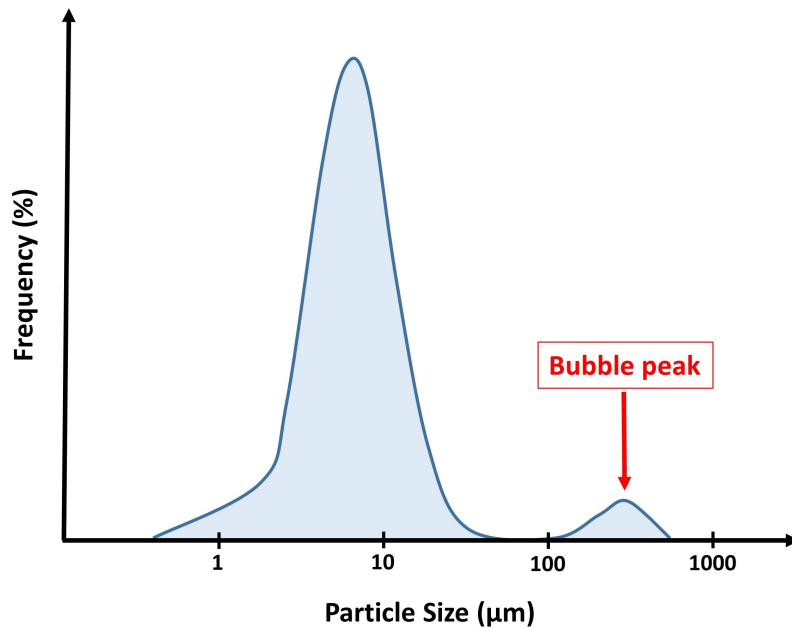


Figure II.5: Bubble peak in an aqueous dispersion. (adapted from [3])

Thermal peaks can be avoided in most cases when proper precautions are taken to ensure that the temperature of a volatile carrier medium has reached thermal equilibrium within the recirculator of an instrument prior to taking a background measurement. When they do occur, they correlate to a peak at the coarse end of the distribution. The thermal difference results in “beam steering,” where a larger signal reaches the inner or low angle detectors in some instruments. Depending on the size of the primary particulate, the thermal peak is often completely detached from the “true” particle size distribution.

4.2.3 Opalescent and reflective particles

In certain cases when a particle is particularly shiny, reflective, pearlescent, or otherwise opalescent, a coarse-end artifact peak may occur in a laser diffraction measurement.

This artifact peak is associated with the concentration of opalescent/reflective particulate within the recirculator of the instrument. Cutting back the concentration of the particulate is helpful in avoiding this occurrence, which can be sporadic over multiple measurements.

4.2.4 Optical model

Optical model artifact peaks can be the most insidious because they can occur repeatably and consistently. While they follow a distinct pattern, they can be more difficult to scientifically rule out because of their particle size orientation and the limits of optical microscopy.

Luckily, unlike the other problematic peaks discussed, an optical model-related artifact peak does not invariably necessitate a complete reanalysis. Often a simple recalculation of the data is sufficient to remedy the problem.

Optical model artifact peaks are associated with the complex refractive index assigned to a particle for laser diffraction analysis using Mie theory. *ISO 13320* defines the complex refractive index of a particle as consisting of both a real and an imaginary (absorption) component [1]. The particle’s refractive index (real part) can be directly measured using a Becke line test, for example. The imaginary or absorption component represents the reduction of intensity of a light beam that is not due to scattering. Particle properties, such as surface roughness, texture, and reflectivity; internal reflection or refraction; or color and opacity (i.e. actual absorption) can affect the beam intensity, thereby influencing the absorption value. Approximations can be made through volume concentration experiments in many instruments to diminish this effect. Beekman et. al. [4] provides an excellent discussion on the

importance and impact of the imaginary and absorption component on laser diffraction particle size distribution data.

An inappropriate choice of optical model may result in significant bias of the resulting particle size distribution. **Figure II.6** is an extreme example of an inappropriate choice of optical model. Generally, when the value selected differs greatly from the actual optical properties of the particle type, a detached or partially-detached peak may occur in the distribution, usually at the fine end (typically resulting in a sub-micron mode). While the particle's real refractive index value can contribute to this problem, the absorption value often poses a more significant influence. Simply changing the optical properties used for the particle drastically changes the resulting recalculated distribution, see red and black distributions in **Figure II.6**.

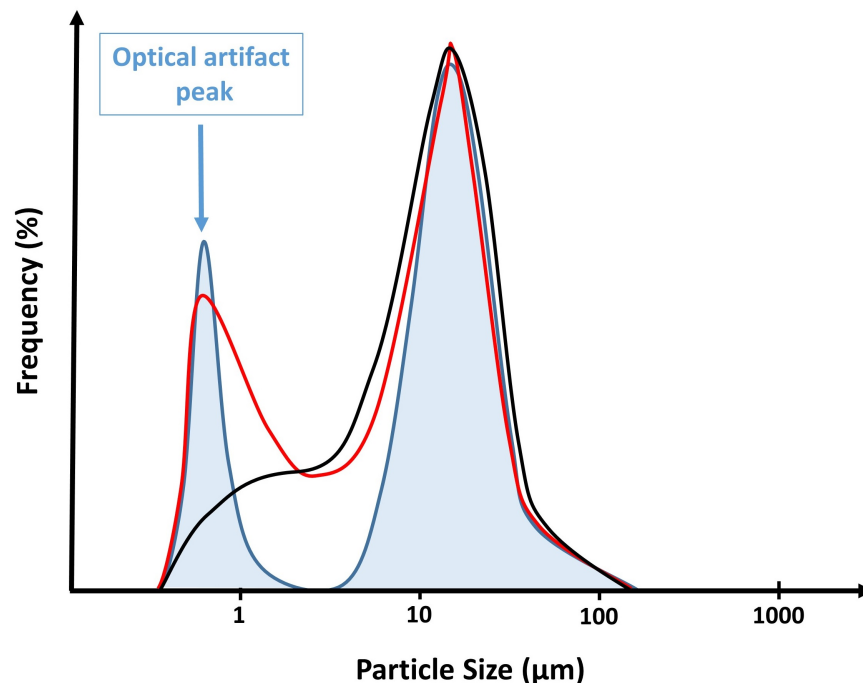


Figure II.6: Optical artifact peak (blue distribution) and influence of changing adsorption values (red and black distributions). (adapted from [3])

5 Recommendations

5.1 Wet samples

5.1.1 Dispersant

The majority of samples used water as the dispersant. An alternative must be used if the particles are soluble in water or react chemically. For example, flour added to water will float on the surface but it disperses well when added to propan-2-ol. Solubility in the dispersant can be screened with the observation of the obscuration figure: if the obscuration decreases the sample is probably dissolving. The dispersant may itself contain impurities or particles that could be significant. Filtering the dispersant before use at $1\ \mu\text{m}$ is generally adequate.

If the dispersant is stored under pressure or at low temperature it may be necessary to consider degassing before use. A pressure release or a temperature rise will reduce the solubility of gasses, resulting in the formation of bubbles in pipes and cells. As we discussed before (see 4.2.1), bubbles are a problem as they are measured with the sample and are counted as particles, biasing the results. The solution is to store sufficient dispersant at room temperature and pressure for several hours before use to allow the degassing to occur.

Cold dispersant in a warmer environment can also give rise to condensation on the outside surfaces of the cell

windows leading to an increase of the obscuration. A quick remedy is the addition of warm water to the sample tank, the increase in obscuration caused by the condensation will quickly disappear. Another solution is to warm the dispersant (for water typically to 60-80 °C) and then allow it to cool before use.

When analysing particles suspended in a liquid dispersant, one of the most important decisions is which liquid to use. The dispersant can be any clear (at 633 nm wavelength), optically uniform liquid that does not interact with the sample causing it not to change its size.

5.1.2 Surfactants and admixtures

If experiencing problems such as the sample floating on the surface of the dispersant, addition of a surfactant or admixture may help.

i) Surfactants

Adding a surfactant may help in preparation of the sample, it will lower the surface tension, which promotes wetting of the particles. Surfactants have to be added in trace amounts, usually one drop per litre of dispersant. If too much surfactant is added the action of stirring and pumping the sample may cause it to foam, causing bubbles to be introduced into the system. Antifoaming agents may be added to prevent the formation of bubbles but, owing to the fact that these may contain particulates, they should be added to the dispersant before the background is measured.

Try adding a drop of surfactant to a quantity of sample and dispersant mixed in a small beaker. If the sample sinks to the bottom of the beaker in large clumps, dispose of the sample and start again. This time add the sample to a dry beaker, add a drop of surfactant and mix thoroughly to a thick paste. Add the dispersant and mix well. This usually avoids the agglomeration caused by adding the dispersant first.

A list of recommended surfactants in order of common use is given below:

- Non-ionics : Igepal, Teepol L, and Synperonic N
- Anionic : Aerosol OT and Sodium dodecyl sulphate
- Cationic : Hyamine 2389

ii) Admixtures

Admixtures work by causing the particle surface to become charged, causing particles to repel each other (i.e. providing a zeta-potential to help dispersion). Admixtures are added in larger quantities, typically 1 g/L. A list of commonly used admixtures is given below:

- Sodium Hexametaphosphate (e.g. Calgon)
- Sodium Pyrophosphate
- Trisodium Phosphate
- Ammonia Oxalate
- Calcium Chloride

As many of these are solid materials dissolved into the dispersant, the solution should be filtered after preparation to remove impurities.

5.1.3 Ultrasonics

In addition to the processes described above, ultrasonics can be applied to help the dispersion, whether or not it contains a surfactant. When mixing the sample in the suspension medium, a visual inspection often shows whether ultrasonics are necessary. If large agglomerates of particles sink to the bottom of the beaker, try applying two minutes of ultrasonics by placing the sample in an ultrasonic bath. It will be immediately apparent if this has been effective.

Further ultrasonics can be applied when the sample is added to the tank, if necessary. This often prevents re-agglomeration, but is not always needed. Beware of ultrasound with fragile particles; the action of ultrasound can break up the particles themselves.

5.1.4 Samples with unstable concentrations

Obscuration may change during the dispersion period: most samples disperse very quickly so users will not notice the obscuration value rising. With some samples the slow dispersion of the sample can be clearly seen. Do not make a measurement until the obscuration has stabilised, indicating that the sample has properly dispersed.

The obscuration and its behaviour during the dispersion of the sample can also warn of other potential problems. If the obscuration decreases, the size of the particles within the sample may be increasing; either the sample is sticking together or the particles are actually swelling due to the dispersant.

Other causes could be the larger particles settling out due to inadequate pumping and stirring or even the particles dissolving. If obscuration increases rapidly, particles may be attaching themselves to the cell windows due to surface charges. This means material is in the laser beam continuously and the obscuration appears to increase. To solve this, use an admixture.

5.2 Dry samples

If the product is to be used and stored in a dry form, a dry analysis may be preferred. Some samples can only be measured in a dry state as they react with all wet dispersants, for example they may dissolve or the particles may swell when in contact with a liquid.

Another consideration is whether the material in its dry state is free flowing. Good pouring characteristics indicate a non-cohesive powder which will usually disperse well in a dry powder feeder without any difficulties, whereas a highly cohesive material tends to stick and clump together, giving biased measurements. Sample clumping together can often be overcome by drying the sample in an oven. Obviously care should be taken with delicate materials where drying in the oven may damage the sample. To drive the moisture from the sample, set the oven to the highest temperature possible below its melting point. If it is obvious that using an oven is going to damage the sample, use a desiccator. A fresh sample that has not had time to absorb moisture from the atmosphere is always preferable and usually gives better results.

Similar to volatile liquid artifact peaks, dry dispersion artifact peaks occur at the coarse end of a dry dispersion distribution analysis due to the non-equilibrated state of the air current. These occurrences may be minimized when steps are taken to stabilize and equilibrate the dispersion environment prior to analysis. Care must be taken to ensure that agglomerated particulate is not part of the problem and microscopic examination is necessary to verify the suspect peak is distinct from the primary particle peak.

5.3 Important questions before buying a SLS instrument

5.3.1 How many scattering angles?

The importance of the number of angles cannot be overemphasized and is critical since it determines the precision (reproducibility or absence of fluctuations) of the measurement, as well as the accuracy of the molar mass and size determination.

More angles mean more data are collected, and more data mean greater accuracy. Indeed, the precision of a light scattering measurement is roughly proportional to the square root of the number of detectors. Obviously, at least two angles are necessary in order to get any sense of the variation of scattering intensity. Yet in the presence of noise, a weighted linear fit of the data requires a minimum of three angles.

5.3.2 Is my light scattering detector really absolute?

Absolute means that the instruments measure molar mass directly from the scattered light intensity, without reference to a standard of “known” molar mass. Usually, instrument calibration is done by measuring the scattered light from a pure solvent, such as toluene.

Once calibrated, the solvents can be changed without the need for recalibration. This absolute calibration has a tremendous practical advantage over pseudo-light scattering instruments. Such units require recalibration for every solvent and then compare the light scattering to the scattering from reference standards in each solvent used. These other instruments then adjust their results to make them agree with the standard, with no direct proof that the standard is actually correct. There are many problems that may result from trusting calibration standards. Some of these standards were made in large batches years ago and have aged. There are many solvent systems for which there are no standards.

5.3.3 How stable is the laser source?

This may seem a trivial question since most commercially available solid state lasers contain power output monitors that may be used to ensure beam power stability. Unfortunately with age and/or temperature fluctuation, such lasers are prone to so-called “mode-hopping”, which degrade their stability.

5.3.4 Are there a lot of publications that used the same equipment?

Procuring an instrument that has been used to collect publication-quality data is critical, since its performance will have been questioned rigorously by peer review. Look carefully at the publications that have been generated using different light scattering equipment and in what disciplines.

Review and reading tips

- ✓ Static Light Scattering (SLS)/Laser Diffraction Particle Size Distribution Analysis, Technical Notes, Horiba.
- ✓ Light Scattering Demystified : Theory and Practice, L. Øgendal, 2019.

Annex

Light scattering

When light strikes a surface it is either : diffracted, refracted, reflected and absorbed (**Figure II.7**). Diffraction is also known as "edge diffraction" as that is where it occurs. Refraction occurs as light changes angle traveling through the particle. Diffracted and refracted light is useful for this purpose. Absorbed and reflected light works against this purpose and must be taken into account during measurement and size calculation.

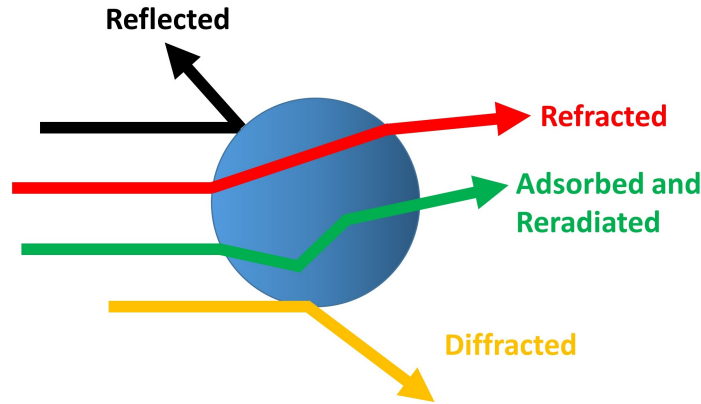


Figure II.7: Simplified scheme of when a light beam strikes a particle.

Angle of scatter is inversely proportional to particle size (see **Figure II.2**), then small particles scatter at larger angles than large particles. The amount of light scattered directly proportional to particle size, large particles scatter more light than small particles.

Light scattering intensity is expressed as:

$$I_{\theta} = \frac{I_0}{2k^2a^2} \{[S_1(\theta)]^2 + [S_2(\theta)]^2\}$$

with I_{θ} the total scattered intensity as a function of angle θ , I_0 the intensity of the incident light, k the wavenumber, and a the distance from scattered to detector.

$S_1(\theta)$ and $S_2(\theta)$ are dimensionless, complex functions defined in general scattering theory, describing the change of amplitude in respectively the perpendicular and the parallel polarized light as a function of angle θ with respect to the forward direction. Computer algorithms have been developed in order to allow computation of these functions and, thus, of $I(\theta)$.

In Fraunhofer approximation, if the following assumptions are correct:

- all particles are much larger than the light wavelength (only scattering at the contour of the particle is considered; this also means that the same scattering pattern is obtained as for thin two-dimensional circular disks),
- only scattering in the near-forward direction is considered,

then $S_1(\theta)$ and $S_2(\theta)$ are equal as:

$$S_1(\theta)^2 = S_2(\theta)^2 = \alpha^4 \left[\frac{J_1(\alpha \sin(\theta))}{\alpha \sin(\theta)} \right]^2$$

with α a dimensionless size parameter and J_1 is the Bessel function of the first kind of order unity.

Scattering can be subdivided into elastic and inelastic scattering. If the scattered light has exactly the same wavelength as the incident light meaning that the scattered photons have exactly the same energy as the incident photons, the scattering is called elastic. If the photons come out of the scattering process with a changed energy

the scattering process is termed inelastic. This means that the scattered light will have either a longer or a shorter wavelength than that of the incident light.

Both of the above mentioned main interaction phenomena have as a result that a light beam is being attenuated when it passes through a solution. The intensity of the light beam is weakened by either absorption or scattering (or both). In both cases the transmitted intensity decreases exponentially with the thickness x of the layer of material through which the light has to pass. In the context of scattering the frequency is written as:

$$I = I_0 \cdot \exp^{-\tau x}$$

with τ the turbidity coefficient of the medium.

While absorption of light is most easily discussed in terms of the photon concept, scattering of light is most easily understood in terms of a wave description of light, i.e. considering light as electromagnetic radiation.

SLS measures the average intensity of the scattered light at many different scattering angles and uses this to derive information about molecular weight, particle size, particle shape and particle interactions.

In order to be useful for quantitative light scattering the light itself has to fulfil two conditions:

- The light has to be monochromatic, i.e. to have a well-defined wavelength.
- The light has to be in a collimated beam, i.e. the rays have to be parallel

In order to obtain a measure which is independent of apparatus characteristics but only depends on the properties of the system one defines the Rayleigh ratio, R_θ , for the solution under investigation:

$$R_\theta = \frac{I_s(\theta)r^2}{I_0V_s(\theta)}$$

where the subscript θ indicates that the Rayleigh ratio is a function of the scattering angle, $V_s(\theta)$ is the scattering volume (i.e. the volume which is illuminated by the laser while at the same time being visible for the detector), and r is the distance from the scattering volume to the detector.

R_θ depends on:

- the molecular weight of the suspended particles (molecules),
- the concentration of the suspended particles (molecules),
- the refractive index of the pure solvent,
- the refractive index of the suspended particles (molecules),
- the size of the suspended particles (molecules),
- interaction forces between the suspended particles.

More details on the theory can be found in [5].

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Chapter III

Dynamic Light Scattering (DLS)

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Introduction

Dynamic Light Scattering (DLS) is a simple and fast method to investigate the hydrodynamic size of dispersions (droplets and particles): mean size, size distribution, and polydispersity.

This technique is non-invasive and requires a low amount of sample (up to few milliliters). However, the results should be interpreted cautiously because several parameters such as viscosity, temperature, concentration, and particle sedimentation may influence the data. Moreover, certain limits must be observed to guarantee the accuracy of the measurement.

1 Principle

Dispersed particles scatter incident light proportional to the 6th power of their diameter (d^6) [1]. When the particles are smaller than $\lambda/10$, with λ the wavelength of the incident light, they will scatter the light in all directions with the same energy (elastic scattering) which means that this phenomenon is not angle-dependent (Rayleigh scattering)[2].

However, when the size of the particles exceeds this threshold of $\lambda/10$ then Rayleigh scattering is replaced by anisotropic Mie scattering where the scattered light is unequal in energy (inelastic scattering) to the incident light and angle-dependent (**Figure III.1**). The scattered light is then most intense towards the direction of the incident light [3, 4]. This size threshold ($\lambda/10$) is due to the way electromagnetic waves (e.g., light) interact with a particle.

If we consider thousands of stationary particles: the screen would now show a speckle pattern consisting of bright and dark areas. **Figure III.2** shows the propagated waves from the light scattered by the particles. The bright areas of light are where the light scattered by the particles arrives at the screen with the same phase and interferes constructively to form a bright patch. The dark areas are where the phase additions are mutually destructive and cancel each other out.

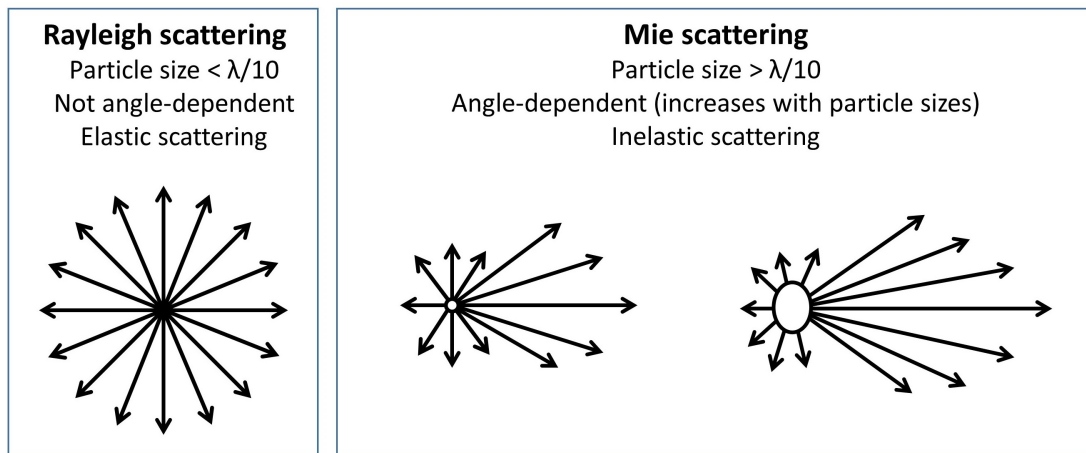


Figure III.1: Difference between Rayleigh and Mie scattering. (adapted from [5])

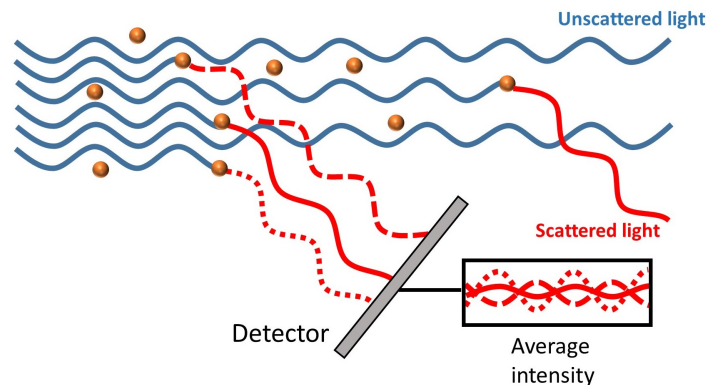


Figure III.2: Scattered light falling on the detector.

In practice, particles suspended in a liquid are never stationary. The particles are constantly moving due to Brownian motion (movement of particles due to the random collision with the molecules of the liquid that surrounds the particle). An important feature of Brownian motion for DLS is that small particles move quickly and large particles move more slowly. The relationship between the size of a particle and its speed due to Brownian motion (diffusion constant D_t) is defined in the Stokes-Einstein equation:

$$D_t = \frac{k_B T}{6\pi r \eta}$$

where k_B is the Boltzmann constant, T the temperature, r the radius of the spherical particle, and η the dynamic viscosity of the fluid. This law is valid for the macromolecules of solute. It is empirically modified to better show the experimental results relating to the smaller molecules.

Because of this movement, the intensity at any particular point appears to fluctuate. The system measures the rate of the intensity fluctuation due to Brownian motion and uses it to calculate the size of the particles. The particle size measured in a DLS instrument is the radius of the sphere that diffuses at the same speed as the particle being measured (hydrodynamic radius R_H). The system determines the size by first measuring the Brownian motion of the particles in a sample using DLS and then interpreting a size depending on the different theories.

R_H is a hypothetical measurement as such hard spheres rarely exist in colloidal dispersions (**Figure III.3.a**). In reality the dispersed particles could be agglomerated (**Figure III.3.b**) and hydrated/solvated which along with its corona (**Figure III.3.c**) are often not spherical. The composition of the corona, especially the soft corona, is dynamic and fluctuates over time depending on the ionic strength, types of smaller and bigger molecules present in the environment, and nature of solvents [6, 7]. Therefore, DLS provides only an indicative size of the colloid.

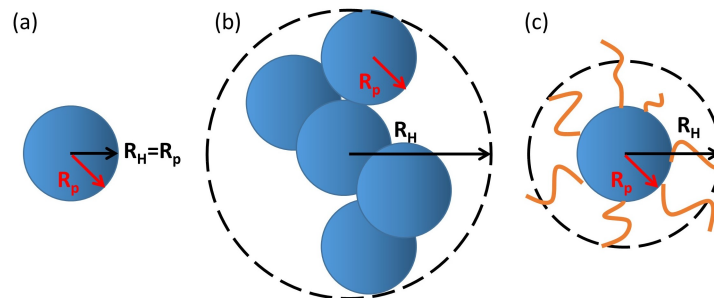


Figure III.3: Difference between Hydrodynamic radius R_H and Particle radius R_P for different cases: (a) an isolated particle, (b) an aggregate of particles, and (c) a corona formation.

DLS is designed for colloidal particles, and is therefore not suitable for particles larger than $1\ \mu m$, except when a monomodal population with a narrow size distribution and slow sedimentation rate is analysed. Particles larger than $1\ \mu m$ may be characterized by laser light diffraction (e.g. see **Chapter II**), which presents two main limitations: the large amount of sample required and the over-estimation of small size population (smaller than $500\ nm$).

2 Measured parameters

2.1 Intensity, volume, and number distributions

A very simple way of describing the difference is to consider a sample that contains only two sizes of particles ($10\ nm$ and $100\ nm$) but with equal numbers of each size particle (**Figure III.4**).

The first graph shows the result as a number distribution. As expected the two peaks are of the same size (1:1) as there are equal number of particles.

The second graph shows the result as a volume distribution. The area of the peak for the $50\ nm$ particles is 1000 times larger the peak for the $10\ nm$ (1:1000 ratio). This is because the volume of a $100\ nm$ particle is 1000 times larger than the $10\ nm$ particle (volume of a sphere is proportional to d^3).

The third graph shows the result as an intensity distribution. The area of the peak for the $100\ nm$ particles is now 1 million times larger than the peak for the $10\ nm$ ($1:10^6$ ratio). This is because large particles scatter much more light than small particles, the intensity of scattering of a particle is proportional to the sixth power of its diameter d^6 (from Rayleigh's approximation).

Although the fundamental size distribution generated by DLS is an intensity distribution, this can be converted, using Mie theory, to a volume distribution. This volume distribution can also be further converted to a number distribution. However, number distributions are of limited use as small errors in gathering data for the correlation function will lead to huge errors in distribution by number.

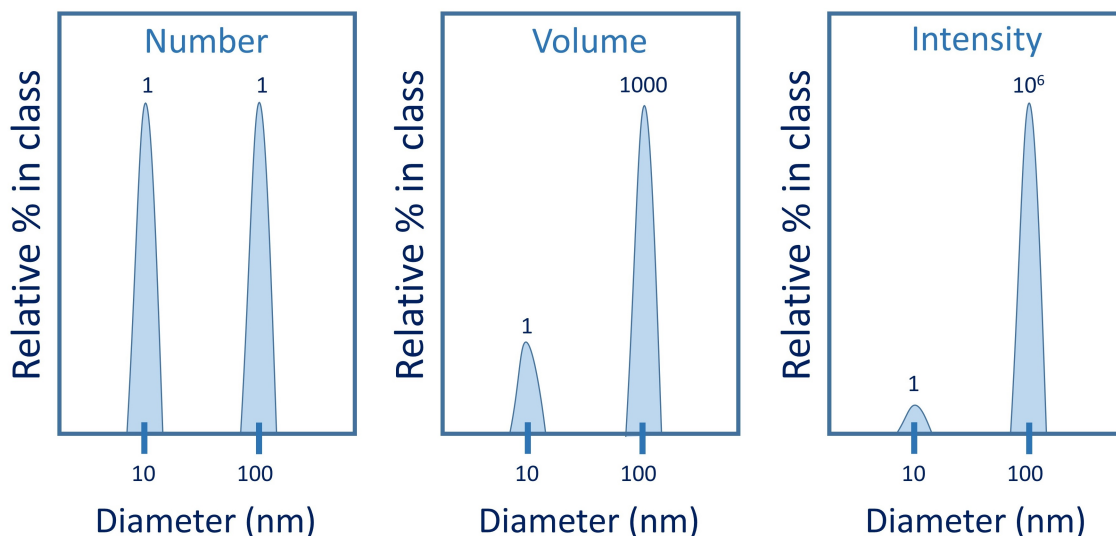


Figure III.4: Difference between size representation in number, volume, and intensity.

2.2 Standard deviation

The size, calculated from a single data set, is usually presented along with standard deviation (σ). σ is a statistical calculation that indicates the distribution of the size around the mean (see **Chapter 1.3.4.1**). Typically, size measurements are repeated several times on the same sample. Most of the results given by the data processors of DLS equipment give the standard deviation on the means obtained at each run. Therefore, the data do not represent the variation on the whole particle population but the variation of the median on the same sample. This may be quite misleading and a typical result such as $126 \pm 2 \text{ nm}$ found in the literature does not mean that all the particles are between 124 and 128 nm but that 2 nm represents the error made on three measurements and not the distribution of the particle population.

2.3 Z-average and polydispersity index

Z-average (Z_{av}), intensity weighted mean diameter calculated from the cumulants analysis, is the most important and stable number produced by DLS technique. The polydispersity index (PI) is associated to the Z_{av} calculation and estimates the width of the distribution.

The calculation is defined in the *ISO* standards 22412 [8], so all systems that use this calculation as recommended should give comparable results if the same scattering angle is used. It is important to note here, that the cumulants analysis algorithm does not yield a distribution: it gives only the intensity weighted Z_{av} and the PI . This method applies for monomodal distributions with polydispersity lower than 0.25. In case of higher polydispersity, Z_{av} and PI are not sufficient to describe precisely the size distribution of the sample and a multimodal analysis is necessary.

The cumulants analysis and the use of Z_{av} and PI are discussed in more detail in **Chapter IV**.

3 Parameters affecting the measure

3.1 Effect of aggregation/agglomeration

Nanoparticles tend to agglomerate and while some of these agglomerations are reversible, often it is not the case. Note that the difference between agglomerate and aggregate comes essentially from the forces holding the particles together. In the case of aggregates, these forces will be of covalent type and will be very difficult, even impossible most of the time to break.

It is difficult to obtain high quality data from dispersions with agglomerated particles as the bigger agglomerates scatter too much light which masks low intensity scattered light from smaller particles. Hence, broadened peaks emerge while confidence in the data is reduced. Agglomeration is enhanced by increasing concentrations and

thus, DLS is reliable only at dilute concentrations (typically 50–100 $\mu\text{g}/\text{mL}$) which makes it unsuitable for many formulations.

From the interaction with light, particle size is determined, generally at an observation angle of 90° . The calculation model is based on the equivalent sphere principle, in which each particle is viewed as a sphere. Thus, the presence of a few aggregates will tremendously increase the mean size. Moreover, the method may not be reliable in analysing samples with a mixed population of size. To improve the accuracy in size determination, working with variable angle systems is recommended.

3.2 Shape of particles

Usually, particles are not spherical and for such particles the DLS provide a R_H which by definition is the radius of a hypothetical hard sphere moving at the same speed to that of the aspherical particles within dispersion (**Figure III.5**).

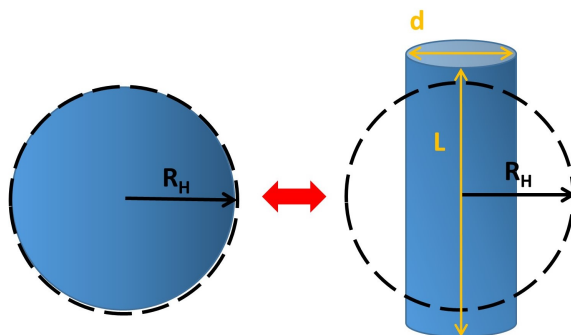


Figure III.5: Difference in R_H between particles of the same volume but different shape (hard sphere and cylindrical nanotube).

Nair et al. have modified the Stokes-Einstein to fit the data obtained from cylindrical structures (e.g., nanotubes):

$$D_t = \frac{k_B T}{3\pi\eta L} \left[\ln\left(\frac{L}{d}\right) + 0.32 \right]$$

where k_B is the Boltzmann constant, T the temperature, and η the viscosity of the dispersant, L the length and d the diameter of the cylinder. The aspect ratio (L/d) is known for nanotubes and hence, D_t for such cylindrical nanomaterials can also be determined by DLS.

3.3 Effect of sample concentration

Higher concentration of particles results in multi-scattering where the scattered light from one particle interacts with other particles and loses intensity before reaching detector [9]. As a result the obtained size is artificially smaller. On the contrary, using too dilute samples may not generate enough scattered light to analyze. Therefore, finding optimal sample concentration is essential.

Whenever possible, the sample concentration should be selected such that the sample develops a slightly milky appearance (slightly turbid). In practice, it may be necessary to run DLS experiments on serial dilutions to determine the optimal concentration: a concentration should be chosen such that the result is independent of the concentration chosen.

3.4 Colored and fluorescent samples

Ideally, colored and fluorescent samples should be avoided in DLS [10]. It is essential to run control experiments to exclude the possibility of absorption of light by the fluorophores at the wavelength of laser. In case of absorption, the intensity of the scattered light will be lower and hence, artificial smaller size for the particles will be estimated [11]. Fluorescent light is non-coherent and recorded as noise.

3.5 Issues related to cuvette

Plastic cuvettes should be avoided for samples with organic solvents or experiments requiring temperature over 50 °C. Especially for glass cuvettes, when the area of illumination is close to its wall, a part of the reflected laser from the wall may be recorded by the detector as high intensity light. This is known as flaring and typically shows up as sharp peak at 1–10 μm . To avoid flaring the area of illumination should be moved towards the middle of the cuvette using the focusing lens.

3.6 Solvents

Using deionized (DI) water is usually not recommended as the absence of ions fails to shield the long-distance interactions between particles. Hence, the size obtained in DI water is always 2–10 nm larger than actual size. In few circumstances (e.g., Poly(ethylene carbonate) PEC) using dilute saline water gives better data as the ions shield the particles from long-distance interactions. Using 10 mM KNO_3 is better compared to NaCl as chloride ions are highly reactive.

Some solvents (e.g., toluene) scatter light [12] which interferes as background noise while some like dimethyl sulfoxide (DMSO) shows considerable changes in viscosity with variation in temperature.

4 Recommendations

4.1 Sample preparation

The samples for DLS measurements should be clear, homogeneous and without haze. Checking the bottom of the cuvette for precipitation is useful. Any precipitation confirms the presence of bigger particles which can be due to poor dispersion, wrong pH, or inadequate sonication which compromises the experiment.

Filtering samples to exclude dust particles or aggregates is helpful although can produce artificial narrow size distribution. Using properly washed filters of pore sizes three times bigger (e.g., 5 μm) than the expected largest particles within samples can be done. Large particles of low density may float on top of the solvent layer (creaming) and can render DLS ineffective.

For powder formulations stirring vigorously can disperse the nanoparticles. Sonication deserves caution especially when proteins are involved. For polymeric nanoparticles it can take up to 24 hours by sonication to obtain a stable and homogeneous dispersion. Moreover, direct sonication generates particles from the probe which distorts the results or prevents the measurements due to possible sedimentation [13].

It is difficult to provide a general guideline on the ideal concentration for DLS as it varies and depends upon factors related to both the instrument (e.g., scattering volume, angle of scattering, laser power, detector sensitivity) [14] and material properties (e.g., molecular weight, compactness) of the particles [15]. For example, nanoparticles that are spherical and more compact scatter more light than particles that are less compact [16].

4.2 Resolution of DLS

An inherent weakness of DLS is its low resolution [17]. For example, DLS is unable to distinguish between particles of 90 and 110 nm and a broad peak with high PI will appear. In order to offer peak resolution, DLS requires particles different in size by at least a factor of 3 (e.g., 10 and 30 nm , 50 and 150 nm) [18]. This is a limitation of DLS especially for polydisperse samples. If a scale from 0 to 1 is considered, a PI lower than 0.1 might be associated with a high homogeneity in the particle population, whereas high PI values suggest a broad size distribution or even several populations. Therefore, a good polydispersity is expected to be as close as possible to 0.1. Usually results with a PI above 0.5 should be avoided and considered with more care especially if the sizes are close.

To increase resolution prior size-separation of particles can be done [19]. Latest DLS instruments can be coupled with analytical instruments which enable DLS to determine size of every fraction precisely. DLS resolution is discussed in more detail in **Chapter VI**.

4.3 Expressing R_H based on intensity, volume or number?

A common dilemma faced by researchers is how to express the particle size as most of the current DLS softwares offer options to express particle size distribution based on intensity, number and volume. Expressing the data on these three parameters often produce three different R_H and size distributions. The principles of DLS are based on measuring intensity of scattered light and hence, the representative R_H value should always be deduced on intensity measurements while the other two parameters (volume and number) should be used as supporting information only.

4.4 Data from DLS measurements

Softwares needs to be fed with data regarding both the dispersant (refractive index, viscosity) and dispersed (refractive index, absorption) phases. For Rayleigh scatterers ($\leq 100\text{ nm}$) this information is often not required. However, for particles $> 100\text{ nm}$ this information becomes crucial. Knowing the accurate RI and viscosity of the sample is essential in such cases and can be obtained by use of refractometer and rheometer, respectively. It is difficult to predict how nanoparticles alter the viscosity of the sample. As a working guideline, it can be stated that if the number of particles per unit volume of dispersant increases with increasing concentration then it usually also increases the viscosity of the dispersion [20]. It is crucial to know these parameters about the dispersant and instruct the software accordingly to avoid erroneous data.

Review and reading tips

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Chapter IV

Z-average

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Introduction

When measuring the sizes of a dispersion with Dynamic Light Scattering (DLS), the question often arises as to whether a single number can describe the size distribution of the sample or whether it is always necessary each time to report the entire distribution with the average size of the different peaks.

Z-average (Z_{av}), intensity weighted mean diameter calculated from the cumulants analysis, is the most important and stable number produced by DLS technique. The polydispersity index (PI) is associated to the Z_{av} calculation and estimates the width of the distribution.

We will discuss in this part how Z_{av} is obtained and how to use it to best describe the particle size distribution of a dispersion.

1 Principle

DLS measures the speed at which the particles are diffusing due to Brownian motion. This is done by measuring the rate at which the intensity of the scattered light fluctuates when detected using a suitable optical arrangement (**Figure IV.1**). Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship. It is possible to directly measure the spectrum of frequencies contained in the intensity fluctuations arising from the Brownian motion of particles, but there is no point in doing it. The best way is to use a device called a digital autocorrelator.

1.1 Correlator and Correlation function

A correlator is a signal comparator designed to measure the degree of similarity between two signals, or one signal at varying time intervals. For a signal derived from a random process such as Brownian motion, the comparison of the intensity of the signal at time t to the intensity a very small time later $t + \tau$ shows a strong correlation. As the particles move, the correlation with subsequent τ signals decreases with time until a complete decorrelation at

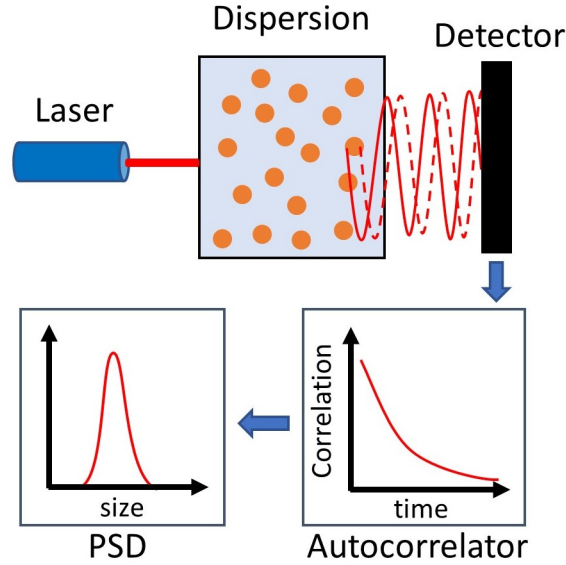
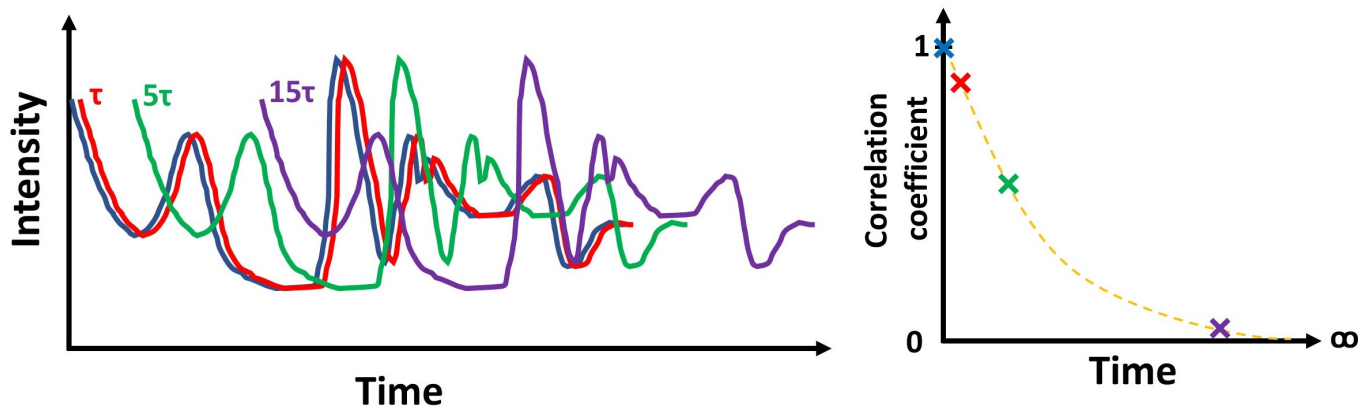


Figure IV.1: DLS principle: from laser to size.

$t = \infty$ (Figure IV.2). Perfect correlation is indicated by unity and no correlation is indicated by zero. The period of time τ is usually very small, maybe nanoseconds or microseconds and is called the sample time of the correlator.

Figure IV.2: Correlation function obtained from one signal compared at varying time intervals τ .

The correlator constructs the correlation function $G(\tau)$ of the scattered intensity:

$$G(\tau) = \int_0^{\infty} I(t)I(t + \tau)dt$$

where I is the scattering intensity and τ is the sample time of the correlator.

For monodisperse samples containing one group of particle size, the correlation function is an exponential decaying function of the correlator time delay τ :

$$G(\tau) = B + Ae^{-2\Gamma\tau}$$

where A is the amplitude of the correlation function and B the baseline.

The decay rate Γ is :

$$\Gamma = Dq^2$$

where D is the diffusion coefficient of the particles and q is the magnitude of the scattering wave vector. The scattering wave vector is defined as the difference between the incident and the scattered wave vectors, and its magnitude q is given by as:

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

where n is the refractive index of the dispersant, λ_0 the wavelength of the laser in vacuum, and θ the scattering angle.

For polydisperse samples, the equation can be written as:

$$G(\tau) = B + Ag_1(\tau)^2$$

where $g_1(\tau)$ is the sum of all the exponential decays contained in the correlation function:

$$g_1(\tau) = \int_0^\infty G(\Gamma) e^{-\Gamma\tau} d\Gamma$$

For large particles, the signal will be changing slowly and the correlation will persist for a long time, whereas small particles move rapidly then correlation will reduce more quickly (**Figure IV.3**). The time at which the correlation starts to significantly decay is an indication of the mean size of the sample. The steeper the line, the more monodisperse the sample is. Conversely, the more extended the decay becomes, the greater the sample polydispersity. The amplitude of the correlation function at time 0 (called intercept) can be used to evaluate the signal-to-noise ratio from a measured sample and thus is often used to judge data quality. For a good measurement it will be between 0.85 and 0.95. Lower values will be obtained for measurements on weaker scattering sample, or in turbid sample where background scattering is significant. Values >1 will only occur for samples with dust that makes the sample count rate fluctuate wildly.

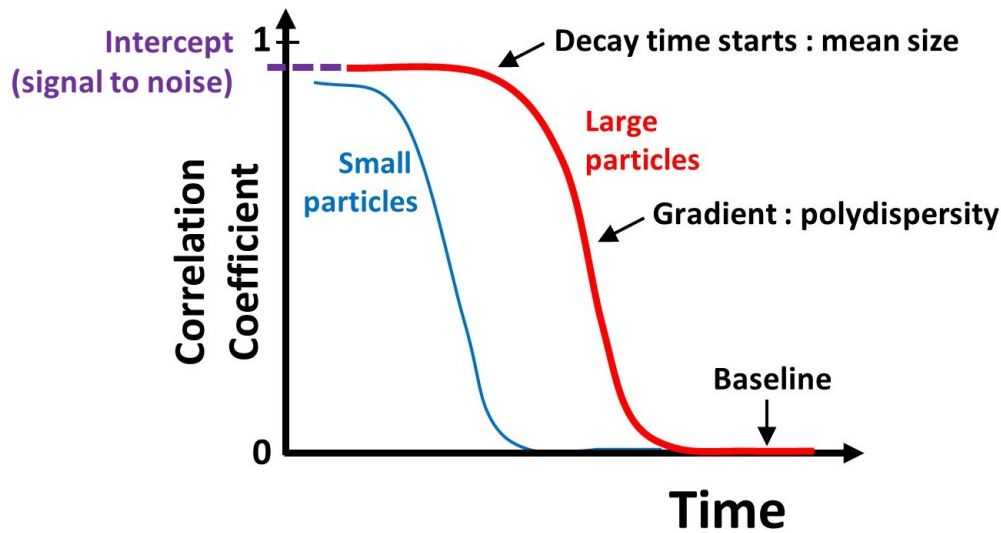


Figure IV.3: Correlation function as a function of particle size.

1.2 Size from correlation function

Size is obtained from the correlation function by using various algorithms. There are two main approaches:

- **Cumulants:** fit a single exponential to the correlation function to obtain the mean size (Z_{av} diameter) and an estimate of the width of the distribution (polydispersity index, PI);
- **Non-negative least squares (NNLS) or CONTIN:** fit a multiple exponential to the correlation function to obtain the distribution of particle sizes. The size distribution obtained is a plot of the relative intensity of light scattered by particles in various size classes and is therefore known as an intensity size distribution.

1.2.1 Cumulants analysis

It is based on the assumption that the correlation curve should be a smooth, single exponential decay function for a mono-size particle dispersion. This monomodal model enables the determination of the average particle size and width of the particle size distribution of a dispersion. The cumulants analysis fits the data by approximating the single exponential decay by a polynomial to the log of the correlation function:

$$\ln[G_1] = a + bt + ct^2 + dt^3 + et^4 + \dots$$

Only the first three terms a , b , and c are used in the standard analysis to avoid over-resolving the data, it is thus just a question of adjusting a polynomial of the third degree. Generally, the fit is done only with the initial part of the correlation function up to 10 % of the intercept. The decay rate Γ is obtained from the first order cumulant, then the hydrodynamic diameter can be calculated from the diffusion coefficient by using a variation of the Stokes-Einstein equation.

Figure IV.4 shows a graphical representation of the procedures employed within the cumulants analysis to calculate the Z-average (Z_{av}) and polydispersity index (PI). The initial slope (red) of the correlation function is related to the Z_{av} and the curvature (blue) indicates the non-linearity due to the width of the polydispersity. The value of b is known as the second order cumulant, or the Z_{av} diffusion coefficient. It is converted to a size using the dispersant viscosity and some instrumental constants. Z_{av} is an intensity-based calculated value and should never be confused with or directly compared to a mass or number mean value produced by other methods.

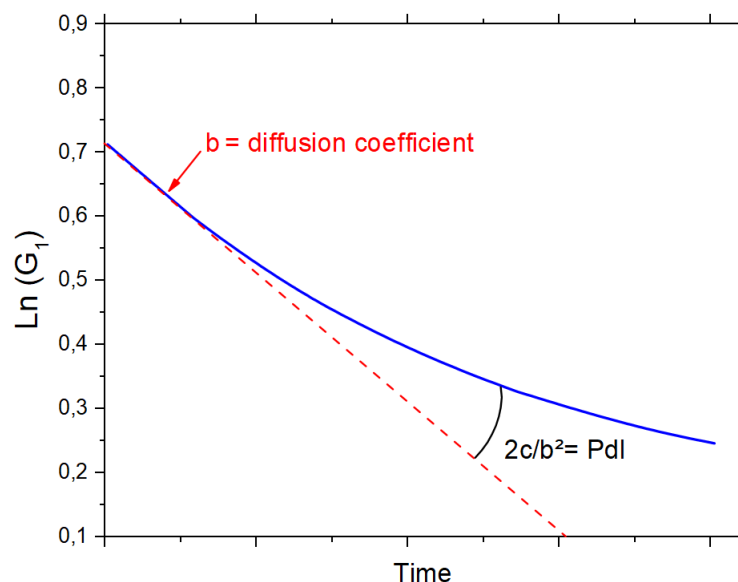


Figure IV.4: Determination of Z_{av} and PI from log of the correlation function.

The coefficient of the squared term c when scaled as $2c/b^2$ is known as the PI . PI is dimensionless and scaled such that values smaller than 0.05 are rarely seen other than with highly monodisperse standards. Values greater than 0.4–0.5 indicate that the sample has a very broad size distribution and is probably not suitable for the DLS technique.

The calculation is defined in the *ISO* standards 22412 [1], so all systems that use this calculation as recommended should give comparable results if the same scattering angle is used. It is important to note here, that the cumulants analysis algorithm does not yield a distribution: it gives only the intensity weighted Z_{av} and the PI . This method applies for monomodal distributions with polydispersity lower than 0.25. In case of higher polydispersity, Z_{av} and PI are not sufficient to describe precisely the size distribution of the sample and a multimodal analysis is necessary.

1.2.2 CONTIN

For multimodal samples, the CONTIN method consists in fitting the correlation data with a multi-exponential algorithm [2, 3]. In DLS software, this fit is denominated as “distribution fit”, “data fit” or “size fit”. It gives a better understanding of particle size distribution than Z_{av} and PI as it can be seen in **Figure IV.5**.

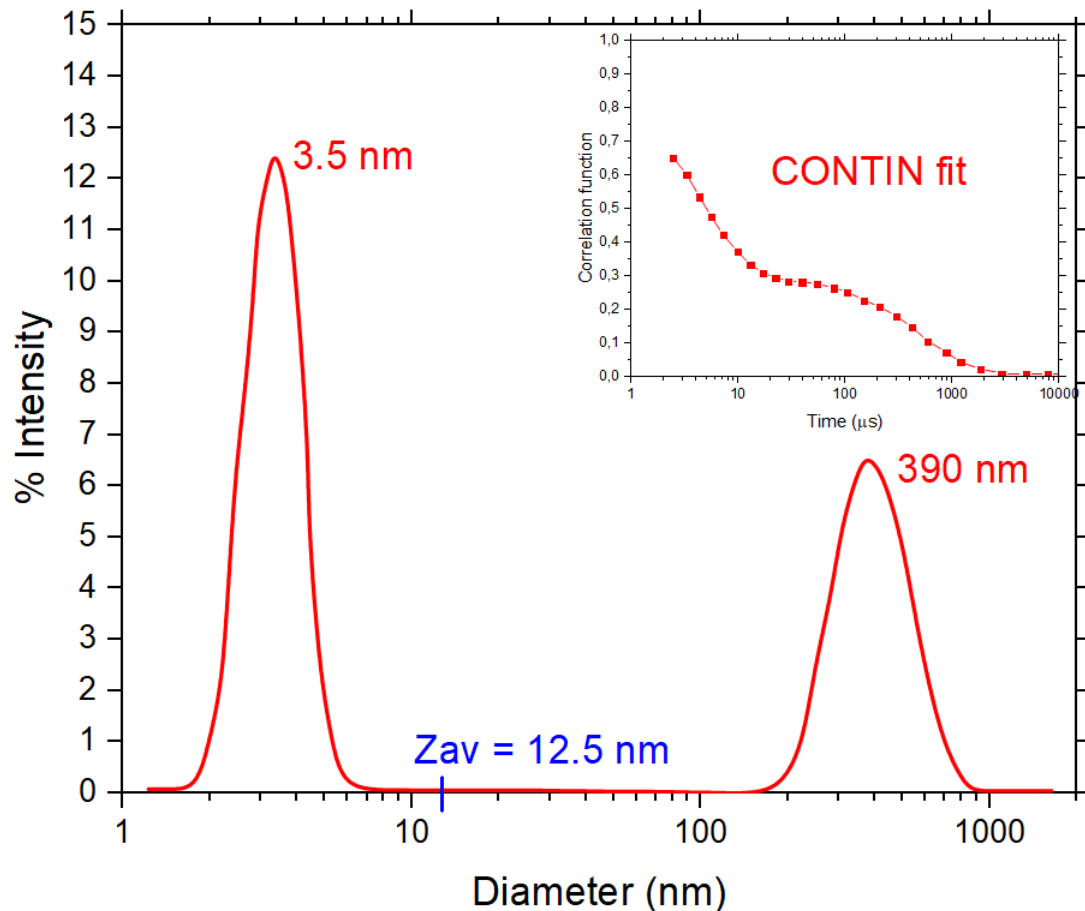


Figure IV.5: PSD from CONTIN fit of the correlation function.

A Z_{av} of 12.5 nm is specified when one exponential cumulant algorithm is used. Although Z_{av} is useful for citing an average value, it cannot suitably describe the distribution results. Here, the correlation function is fitted against longer periods of time and provides size distribution analysis with average size and width for every peak.

Although CONTIN is one of the best choice if a bi-modal particle distribution is present in the sample, however it is very sensitive to any noise present in the data. Often the only way to obtain reliable results is by making additional assumptions. This however means that there different versions of CONTIN. Typically the assumption of a Gaussian distribution for the individual particle populations is required. But other more severe restrictions are frequently applied. Due to the limitation of CONTIN, a recent algorithm called CORENN (COstrain weighted, Regularized Non-linNear) has been developed and implemented by LS Instruments [4, 5].

2 Is the size distribution or the overall mean size better?

On the one hand, Z_{av} is a preferred DLS size parameter as it is insensitive to experimental noise. However, it can be sensitive to even small changes in the sample, e.g. the presence of a small proportion of aggregates. On the other hand, PSD allows to display the size and percentage by either intensity, volume or number and to calculate peak means. Therefore, it is important to understand in which cases is it more advisable to use peak means rather than Z_{av} .

2.1 Monomodal

For a perfectly monodisperse sample, Z_{av} should be equal to the mean of the single peak of the distribution. In real applications, even for monodisperse samples with a fairly smooth peak, this is likely not the case and there will be small differences because all the distributions are not perfectly Gaussian as shown in **Figure IV.6**.

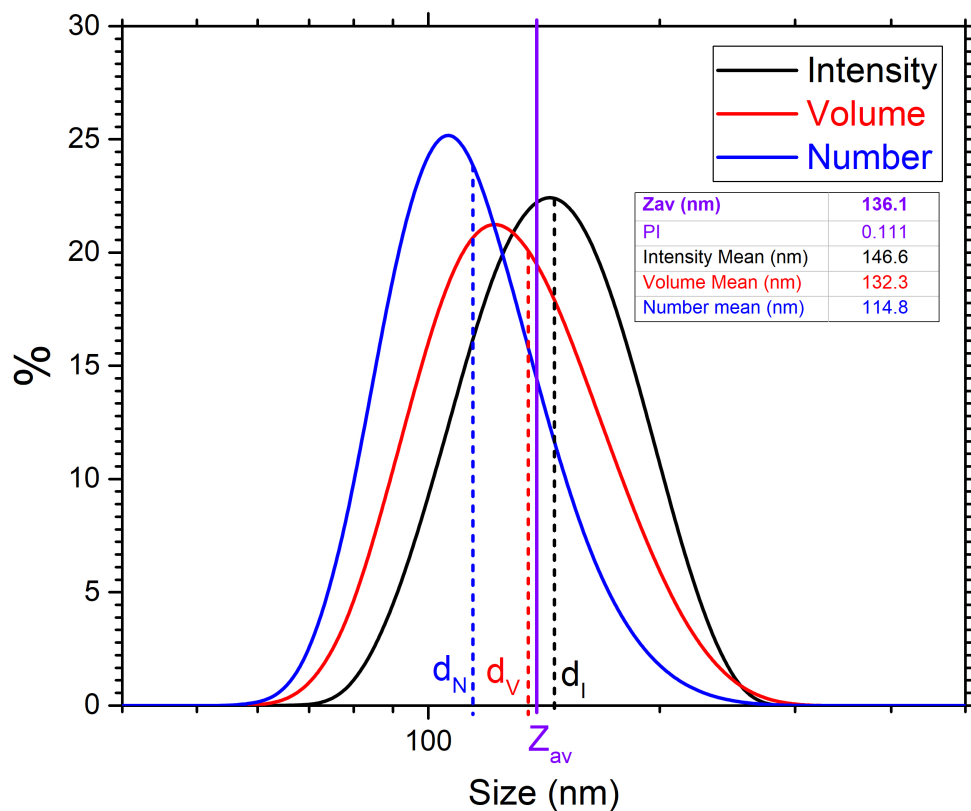


Figure IV.6: Difference between Z_{av} and mean size in intensity (d_I), volume (d_V), and number (d_N) for a monodisperse distribution.

As PI is less than 0.25, the cumulants analysis is correct and allows to consider the size distribution as a monomodal Gaussian with the mean diameter size $Z_{av} = 136 \pm 45 \text{ nm}$. The intensity peak mean (d_I) is slightly greater than Z_{av} confirming that the size distribution is not perfectly Gaussian. The distribution by intensity is a single fairly smooth peak, then there is little point in doing the conversion to a volume (d_V) and/or number (d_N) distribution using the Mie theory. If the optical parameters are correct, this will just provide a slightly different shaped peak as it can be seen in **Figure IV.6**.

2.2 Multimodal

For polydisperse samples, Z_{av} can not be equal to d_I as Z_{av} will still be only a single number, whereas the intensity distribution will show two or more peaks, each peak having its own mean and width.

For bimodal distribution, Z_{av} can be larger or smaller than the main peak size when the distribution shows a small

and a large component. It depends mainly on the size difference between the two peaks and on the intensity of the main peak compared to the second peak (**Figure IV.7**). It is even possible that Z_{av} is the same as the main peak size when one of the peaks happens to match the overall average size.

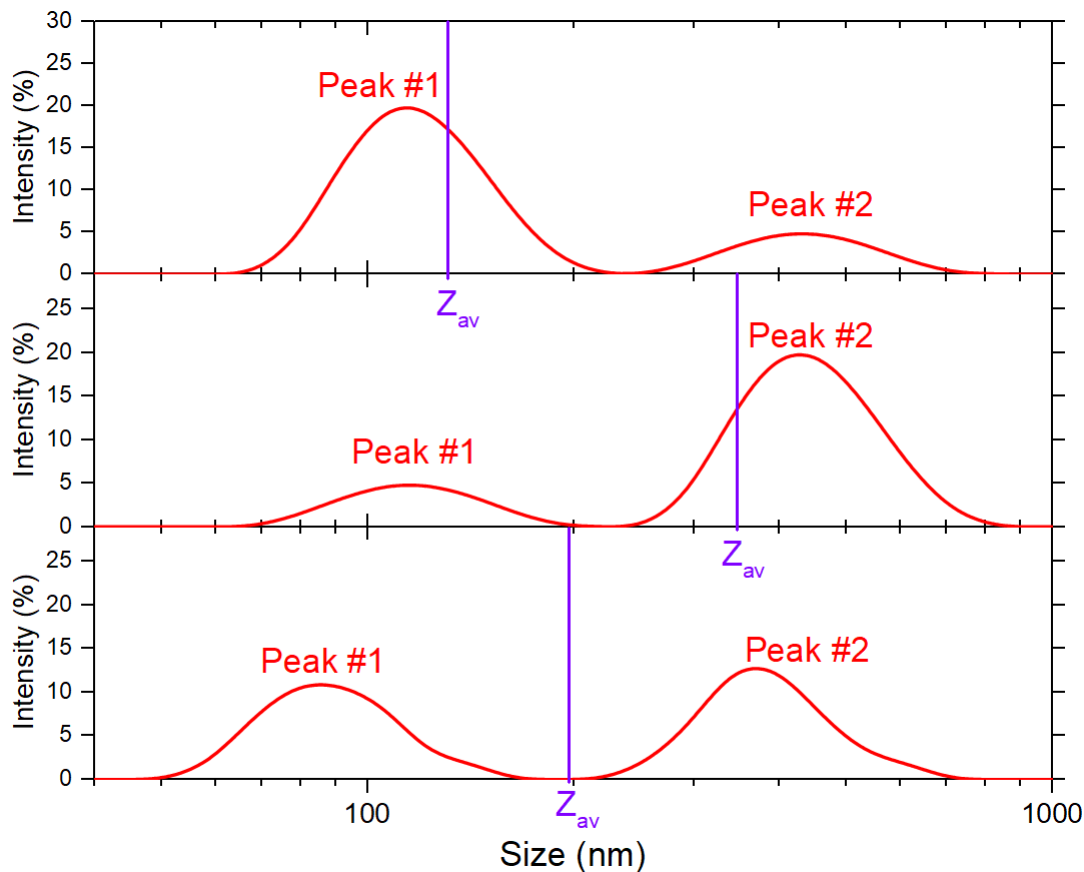


Figure IV.7: Evolution of Z_{av} as a function of the bimodal distributions.

In the previous cases, the intensity distribution can be converted into a volume or number distribution for a better interpretation. This will then give a more realistic view of the importance of the different peaks.

Z_{av} represents the mean size contributing to the major part of the signal in intensity. For polydisperse samples, this value mostly gives a hint on the aggregation state of the particles but does not reflect the hydrodynamic size of most of the dispersed particles.

Conclusion

Z_{av} defined as the harmonic intensity averaged particle diameter or the cumulants mean is the most important and stable (insensitive to experimental noise) number produced by the DLS technique. Z_{av} is independent of the calculations and models used in instruments of different brands, as well as input parameters such as refractive index.

However, Z_{av} is a number and cannot be representative of a multimodal size distribution; moreover, it is sensitive to even small proportion of aggregates in the sample. It gives a good description of the size that is comparable with other methods of analysis for spherical, reasonably narrow monomodal samples, i.e. with polydispersity below a value of 0.25. For samples with a slightly increased width, Z_{av} and PI will give values that can be used for comparative purposes. For broader distributions, where PI is over 0.5, it is unwise to rely on the Z_{av} , and a distribution analysis should be used to determine the peak positions.

The polydispersity index PI is just as important as Z_{av} because it the criterion to identify the conditions for which a size distribution may or may not be summarized by Z_{av} .

Review and reading tips

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Chapter V

Particle Tracking Analysis (PTA)

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Introduction

Particle Tracking Analysis (PTA), also known as Nanoparticle Tracking Analysis (NTA), is a method to visualize, count, and measure particles in liquid suspension in the size range from 10 to 1000 *nm*. PTA tracks the particles by means of laser illumination and measures the diffusion movement of the particles, the hydrodynamic diameter is then related to Brownian motion via the Stokes-Einstein equation. This technique allows the measurement at comparably low concentration levels, down to 10^6 particles per *mL*.

It can also be used to measure the zeta potential of particles and identify fluorescent particles.

1 Set-up and theory

PTA consists in illuminating the dispersion with a laser beam of wavelength in the visible region (**Figure V.1**). The angle of incidence and refractive index of the glass is designed to be such that the laser emerges in a compressed beam with a reduced profile and a high power density. The scattered light of the particles can be easily visualized

via a conventional optical microscope and recorded with a CCD (charge coupled device) or CMOS (complementary metal oxide semiconductor) camera arranged at a 90° angle to the irradiation plane. The instrument's software records a series of sequential images, and thus tracks the position of each particle as a function of time moving within a field of view of approximately 100x100x10 μm . The size of each tracked particle is calculated, allowing the size distribution and concentration to be determined.

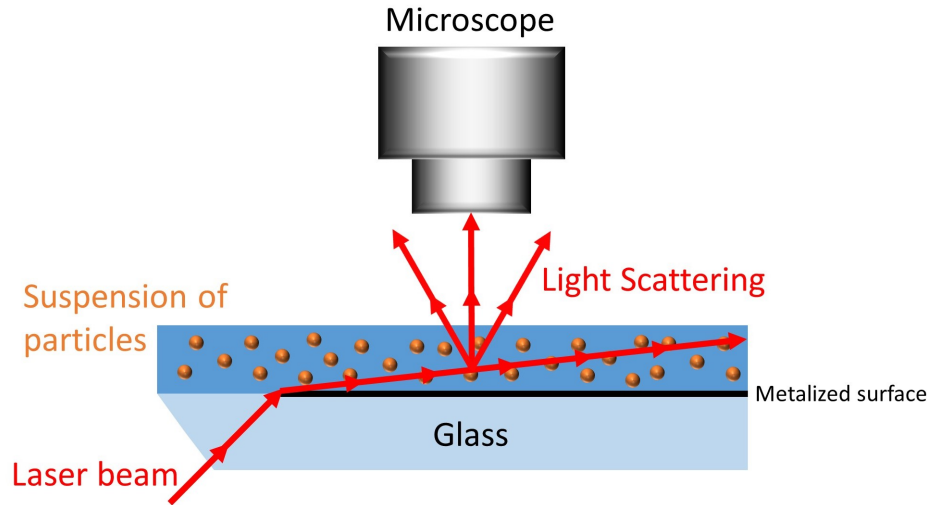


Figure V.1: Schematic of the PTA configuration.

Small particles dispersed in a liquid move randomly in all directions, this phenomenon is called diffusion and characterized by the diffusion coefficient (D). For a given time interval, diffusing particles move inside certain volume elements. In PTA, the time t between two observation points is quite short ($\sim 30 \text{ ms}$) and the movement of the particles per time is recorded in two dimensions as the mean square displacement $\overline{(x, y)^2}$. The diffusion coefficient D is then calculated as:

$$D = \frac{\overline{(x, y)^2}}{4t}$$

where t is the time period between the start of adjacent frames. The hydrodynamic particle diameter d can be calculated with the Stokes-Einstein relationship, as:

$$d = \frac{k_B T t}{3\pi\eta D}$$

It depends on the diffusion coefficient D , the temperature T and the viscosity η of the liquid, and the Boltzmann's constant k_B . After combining the Stokes-Einstein relationship and the two dimensional mean square displacement, the equation can be solved for the particle diameter d with:

$$d = \frac{4k_B T}{3\pi\eta \overline{(x, y)^2}}$$

The temperature T and the viscosity η are the only input parameters needed to estimate d .

2 Operating principles

The first setting is the adjustment of the observation field in the (x, y) direction by movement of the optics or of the sample chamber and in the z -direction by adjustment of the focus. The observed position shall be such that illumination of visible particles is maximised, background interference is minimised, and as large a proportion of particles as possible can be seen in sharp focus (**Figure V.2.a**). Once the correct region of the sample is in focus, suitable image capture settings should be selected. These can include camera gain and shutter, gamma correction, greyscale conversion and analysis duration.

After thermal stabilization and drift settling of the sample, one or more videos of the illuminated sample may be recorded. After selection of the analysis settings, the centre of each particle in every frame of the video is located by the software, and for each particle the distance moved between every frame of video in which it is visible is measured. Over the course of a single video, hundreds or thousands of particles can be identified and tracked (**Figure V.2.b**), and the mean squared displacement between frames is calculated for each particle individually. This mean squared displacement per time is used in conjunction with the known temperature of the sample and the viscosity to calculate the hydrodynamic diameter of the observed particles on a particle-by-particle basis.

Finally, particle size data are combined to produce a 2D particle size distribution in either histogram or frequency per size bin format or a 3D representation with the intensity (**Figure V.3**). Visual inspection of the particle tracks on the screen can give a good estimate for the data quality obtained.

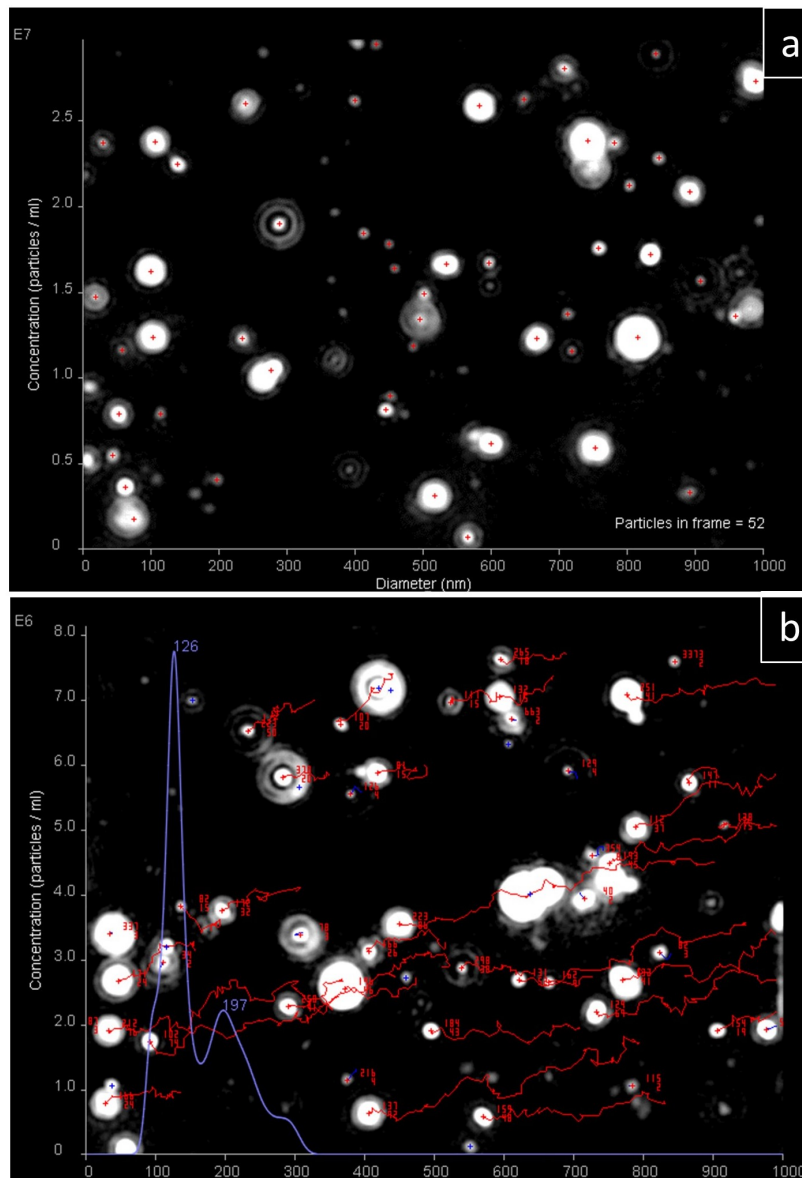


Figure V.2: a) Video capture of particles. b) Track of the center of each particle and measure of the average distance moves per frame.

A detailed schematic of the process flow can be found in *ISO 19430* [1].

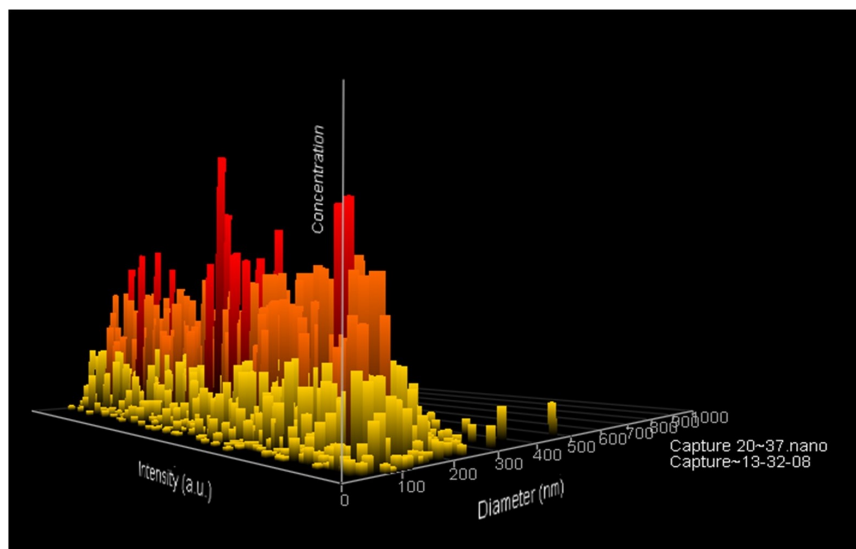


Figure V.3: 3D plot representing the size of the particles vs. concentration vs. intensity.

3 Limits of measurement

PTA has detection limits in terms of the particle size and the particle number concentration. These limits depend mainly on the particle material, solvent, and polydispersity of the dispersion. The typical working diameter range is from 10 *nm* to about 1 μm .

3.1 Size

3.1.1 Lower limit

The amount of light scattered by the particle and the efficiency with which that light can be detected acts to limit the smallest size at which nanoparticles can be analyzed by PTA.

The amount of light scattered by a particle in any given direction is a function of many variables fixed by the equipment (incident illumination power, wavelength, angle and polarization) and the sample: particle size, refractive index (real and imaginary) and shape, as well as solvent refractive index. Therefore, the two main variables affecting the lower limit of detection are the particle size and the refractive index difference between the particle and its dispersion solvent. A large refractive index difference results in higher scattering and therefore lower detection limit. For example, nanoparticles of gold and silver are strong scatterers due to the comparably large refractive indices of 2-4 and can be detected down to sizes of ~ 10 *nm*. Biological material have refractive indices of around 1.37-1.45 resulting in a limit of detection of 35-60 *nm* [2]. **Table V.1** show examples of lower hydrodynamic diameter limit of detection for monodisperse suspensions.

Particle material	Lower detection limit
Gold and silver	10–20 <i>nm</i>
Silica	75 <i>nm</i>
Other metals or metal oxides	25 <i>nm</i>
Polystyrene	45 <i>nm</i>
Biologic materials	35–60 <i>nm</i>

Table V.1: Lower hydrodynamic diameter limit of detection for monodisperse suspensions of nanoparticles.

In a polydisperse sample, large particles scatter a lot more than small particles making it difficult to detect or track small size particles. All the values in **Table V.1** are higher for polydisperse samples.

3.1.2 Upper limit

The upper particle size limit is approached when the rate of Brownian motion becomes so low that long observation periods may be required. This limit is typically around 1-2 μm for particles in aqueous suspension.

In the limit of very large particles the sample may separate with particles sedimenting. This effect should be checked and avoided at all times for PTA measurement.

3.2 Volume concentration

Usually PTA equipments require approximately 1 mL of sample to be used for measurement. Since the optical field of view is fixed (about $100 \times 100 \mu m$) and the depth of the illumination beam is about 10 μm , the scattering volume is about 0.1 nL . Larger sampling volumes may be obtained for optical systems with larger field of view or lower magnification.

Particle number concentration limits should always be considered for analysis with the PTA method. PTA requires highly diluted samples and the optimal particle concentration is sample-dependent. The optimal concentration of particles must be evaluated by preliminary tests on a series of dilutions.

3.2.1 Minimum

It is important that a sufficient number of particles are tracked to reach a specified level of sampling repeatability and statistical viability. Thus, the minimum particle number concentration is determined by the sampling level chose by the result that the user is trying to obtain. Moreover, if the particle size distribution (PSD) is over a wide range of sizes, the minimum particle number concentration required is larger as there are more size bins over which the tracking data should be spread. A concentration in the region of 10^7 to 10^{10} particles per mL provides a good PSD within a timescale of typically 30-60 seconds. For low particle number concentrations ($< 10^7$ per mL), the analysis times must be increased (e.g. 2-3 minutes) to increase the number of detected particles and thus obtain results statistically reproducible.

3.2.2 Maximum

The more particles tracked, the more representative the obtained particle distribution is. However, a too large number of particles affects the ability to independently track particles in the field of view. In highly concentrated samples, many particle paths intersect and tracks are discarded, resulting in less tracked data. A typical value of maximum particle number concentration measurable with PTA is 10^{10} particles per mL .

4 Precision and uncertainties

4.1 Precision

The number of tracks of different particles determines the level of representative sampling. Tracking enough particles for an appropriate statistical representation of the sample is important [3]. To record representative size distribution profiles it is recommended to analyze a range of 1,000–10,000 single particles. An example of an approximate relationship between the number of tracks, number of frames, video length, and the PTA measurement precision expressed in coefficient of variation (COV) of modal particle size for a monodisperse 100 nm sample is reported in **Table V.2**.

Number of tracks & frames	Video length	COV
400 / 130	5 s	< 10 %
700 / 230	8 s	< 8 %
1000 / 300	10 s	< 5 %
2000 / 600	20 s	< 3 %

Table V.2: Accuracy of PTA results as a function of acquisition parameters (number of tracks and images, video length).[?]

The values in **Table V.2** represent a relatively ideal case as the dispersion is monodisperse and the particles are spherical. This means that, to achieve a desired precision, a higher number of tracks should be counted for more polydisperse samples. More information about calculation of the number of tracks required for a given precision can be found in *ISO 19430*.

4.2 Counting efficiency

It is important to remember that PTA systems give an indication of the number of particles in a sub-volume ($0.1 nL$) of the sample.

Measurement of particles critically depends on the ability of the PTA system to detect them in this sub-volume. Smaller particles move greater distances than larger ones between the frames, thus they can exit the field of view and in that case may be disregarded from the calculation. Conversely, larger particles at the same edge of the field of view are more likely to be tracked for longer thus contributing more significantly to the overall count. However, a small particle outside the field of view is more likely to enter the field of view faster than a large particle at that same position. Therefore, provided the number of particles of a given size per frame is reported, this does not lead to a bias in the measurement.

Because larger particles can be detected more easily than smaller ones, samples that contain particles of very different sizes may therefore overcount larger particles. Tracks that are too short or intersecting tracks are rejected by the processing software.

4.3 Sizing accuracy

The size estimated by the PTA method depends on temperature and viscosity of the sample (see **section 1**) .

ISO 19430 evaluated that an uncertainty of $\pm 3 K$ in temperature results in approximately 1 % additional error in diameter evaluation. The temperature of the sample must therefore be controlled and kept within this uncertainty. It is also important to bring both sample and instrument into thermal equilibrium before starting any measurement.

As viscosity varies with temperature, so such variation shall be less than ± 2 % over the operational range of temperatures used in an experiment. In the case of water at room temperature, the ± 2 % requirement for the accuracy of the viscosity implies a more stringent requirement on knowledge of the absolute temperature (than $\pm 3 K$).

Any vibration or motion other than the Brownian motion of the particles should be avoided as it will lead to a decrease in the reported particle size. Therefore, measures should be taken to prevent vibration from affecting the measurement. Tracking software must be able to detect non-Brownian motion and either correct for it or at least alert the operator to its presence.

4.4 Size resolution

Size resolution refers to the ability to distinguish two closely size-related particle populations. This parameter is determined by the uncertainty in measurement accuracy of a single particle, the reproducibility of the data in each size bin as well as the counting efficiency in each size class of the particle distribution.

4.5 Quality and repeatability

Quality standards of PTA results can only be achieved by enlarging statistical numbering and following standardised shared guidelines [1, 4]. Indeed, using best practice in-house developed protocol does not always guarantee consistency with other laboratories. An interlaboratory study on the comparison of size measurements using PTA confirmed that in the absence of shared guidelines on how to use a system and prepare samples, variability across laboratories can be large even for relatively monodispersed samples [5]. Standardisation of the PTA technique is difficult to achieve due to the number of software options that a user is required to choose in order to make a measurement.

5 Additional parameters

The main application when analyzing particles is size and concentration determination. However, some PTA manufacturers implement their device with zeta potential measurement and fluorescence detection to enable understanding of underlying processes or to quantify and discriminate particles.

5.1 Particle identification

Comparing the brightness of particles of the same size allows them to be differentiated. They can be expected to be brighter as their index of refraction is high. **Figure V.4** shows differentiation of populations of gold and polystyrene particles with the same size but different scattering intensities. The higher intensity gold population (higher refraction index) and the lower intensity polystyrene population are only visible due to the third dimension of the graph.

However, absolute intensity measurements are difficult because, depending on the profile of the laser beam, a particle may scatter less light simply because it is in a less intense part of the laser beam and not due to of the actual material difference. Therefore, these measurements are more qualitative than quantitative.

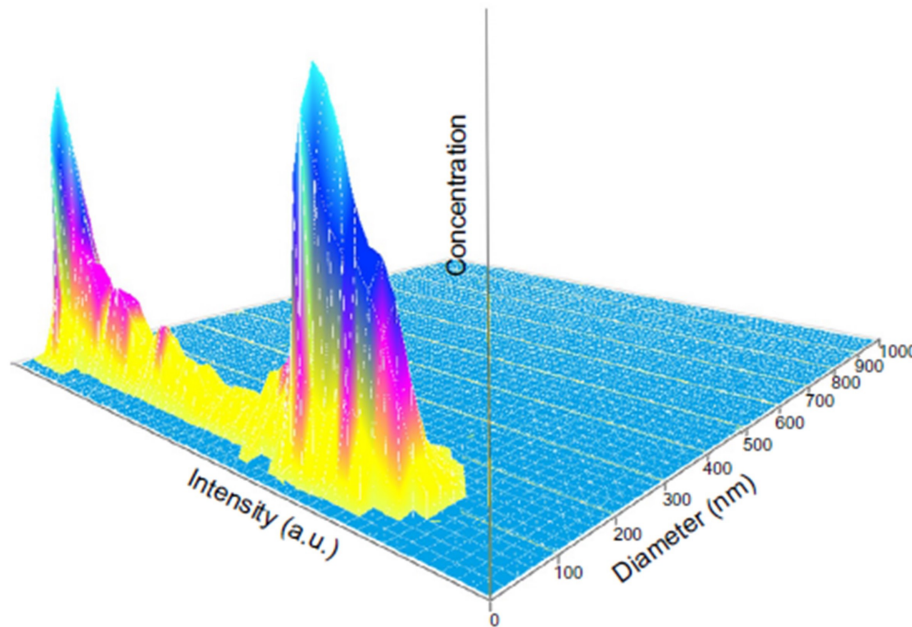


Figure V.4: 3D plot of the size of particles vs. concentration vs. intensity for a mix of 100 nm polystyrene and 100 nm gold particles. [6]

5.2 Fluorescence

Shorter wavelength lasers, such as blue (405 and 488 nm) and green (532 nm), can be used to enable selective tracking of fluorescent particles through the use of appropriate optical filters. It is therefore possible to selectively identify the size and count only certain sub-populations of particles in a mixture thanks to fluorescent labeling.

The fluorescent signal needs to be sufficiently intense for the particle to be seen and its dynamic behaviour tracked. This method also requires stable fluorophores which do not decay within the tracking time period to determine their diffusion coefficient (e.g. 0.5 seconds). For faster bleaching of fluorophores, the sample can be slowly circulated through the laser beam so that there is a continuously refreshed population of fluorescently labeled particles to be analyzed.

5.3 Zeta potential

Zeta potential (ZP) can be determined in PTA cell by applying an electric field through the sample by use of immersed electrodes. ZP is obtained from the movement of the charged particles due to the potential difference between the electrodes, called electrophoretic motion. ZP reflects the surface charge of given particles, which is related to their stability by electrostatic forces.

However, the application of a constant electric field through an aqueous liquid causes electroosmotic flow of the solvent. This flow is independent of the motion of charged particles, but the total motion of the particles will be the superposition of electrophoresis and electroosmosis motions at any position. There are two possibilities to obtain the electrophoretic velocity:

- measure the electrophoretic flow directly by measuring the particle motion at the point of zero electroosmotic flow;
- make several measurements through the sample chamber to map the electroosmotic profile (based on the assumption that the sample chamber is closed and that the electroosmotic profile will sum to zero) and subtract it from the observed velocities made on the charged nanoparticles.

Although not as fast as conventional zeta analysis methods (which generate a weighted average based on the intensity of the zeta potential of the population), the unique advantage of PTA is that it can create true three-dimensional plots of high-resolution particles mixtures. It is important to note that mixtures of charged particles of different polarity can also be discriminated.

6 Advantages and disadvantages

6.1 Advantages

Even though the PTA technology is relatively new on the market, it is considered a mainstream technique with an intermediate capital costs (typically in the 50–100 k\$), and a negligible running costs. It has many advantages:

- measurement technique is quick to learn and run;
- low sample volumes and little sample preparation;
- non-destructive;
- significantly higher resolution than DLS;
- less impacted by polydispersity than other techniques;
- measurement of concentration;
- materials can be distinguished through their different refractive indices;
- ability to measure fluorescence, intensity, and zeta potential on particle-by-particle;
- a direct visualization of the suspension.

6.2 Disadvantages

- fast computer systems capable of coping with computationally intensive video analysis in a reasonable time frames;
- limited size range 10-1000 nm;
- operates within a relatively narrow concentration range (~ 1 – 2 orders of magnitude and $\sim 10^8$ particles/mL), thus most samples require a dilution;
- requires some level of subjective input (capture, focus, grayscale to differentiate a particle from camera noise), which impacts the final results.

Reviews and Readings

- ✓ Particle size analysis - Particle tracking analysis (PTA) method. ISO 19430:2016.
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Chapter VI

DLS vs. PTA

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Introduction

DLS and PTA are two techniques that use light scattering to determine the particle size distribution of colloidal dispersions. DLS is the fastest and most user-friendly technique but it is also known to have several disadvantages, which are mainly related to the principles of the technique. Unlike DLS, PTA can visualize and track individual nanoparticles to both evaluate size and concentration. **Table VI.1** summarizes the main differences between the two techniques.

	DLS	PTA
Size range	1-1,000 <i>nm</i>	30-1,000 <i>nm</i>
Time consumption	2 to 5 <i>min</i>	5 <i>min</i> to 1 h
Concentration range	Broad (10^8 - 10^{12})	Limited (10^7-10^9)
Sample vizualisation	No	Yes
Particular output	Intensity distribution, Z_{av} , PI	Number distribution
Size accuracy	Monodisperse	Monodisperse & Polydisperse
Peak resolution	Low	High
Population ratio	Narrow range	Very broad range
Reproducibility	More reproducible	Less reproducible
Contaminations	Large influence	Little influence

Table VI.1: Comparison of DLS and PTA (red: disadvantages, blue: advantages). (Adapted from [1])

1 DLS vs. PTA

1.1 Intensity vs. number

DLS reads the intensity change of scattered light to find the diffusion coefficient of particles, whereas PTA calculates the diffusion coefficient based on the movements of individual particles. Therefore, the difference in the detection principles results in a difference in the way size is reported: weighted by intensity for DLS and by number for PTA. In comparing the two quantities, one must be converted to match the weighting of the other.

Although the fundamental size distribution generated by DLS is an intensity distribution, it can be converted, using Mie theory, to a volume distribution. This volume distribution can also be further converted to a number distribution. However, these conversions rely on several assumptions and the number distributions are of limited use because small errors in the data collection for the correlation function will result in huge errors in the number distribution. Thus, the number distributions as calculated by DLS are generally considered inaccurate and cannot be compared to the weighted by number results obtained from PTA.

The number-weighted size distribution from PTA can be converted into an intensity weighted distribution. Kim et al.[2] converted size distribution of vesicle samples from PTA into intensity and noticed that while the mean size given by PTA after conversion is very close to the value before conversion, the relative variance after conversion is much lower than that from DLS and PTA. Given the nature of the vesicle samples, it is questionable whether the low relative variance after reconstruction is reliable. Therefore, converting a number-weighted size distribution from PTA to an intensity-weighted distribution does not seem to be trustworthy, and the size information from DLS and PTA is better compared in their original weightings.

The intensity weighted mean hydrodynamic size (Z_{av}) and the polydispersity index (PI) are obtained from the autocorrelation function of DLS, these 2 numbers cannot be compared to particle size distribution (PSD) but to mean size and standard deviation values obtained by PTA. However, the cummulants analysis only applies for monomodal distributions with polydispersity lower than 0.4. Therefore, in case of higher polydispersity and/or multimodal distribution, Z_{av} and PI are not sufficient to describe precisely the intensity size distribution of the sample and a multimodal analysis is necessary.

As a conclusion, Z_{av} from DLS can only be compared to mean size from PTA for monodisperse samples. Otherwise DLS intensity distribution should always be directly compare to PTA number distribution. This difference in the detection principles of PTA results makes it an excellent technique for verifying DLS results.

1.2 Concentration

PTA requires concentrations of 10^7 – 10^9 particle per mL , while DLS concentration range is about 10^8 – 10^{12} particles per mL . DLS concentration is less critical and depends upon a number of instrumental and sample properties.

1.3 Time

PTA requires several optimization steps (setting for the video capture and the tracking of particles) and is therefore more time consuming than DLS. Moreover, DLS can automatically adjust the attenuator to accommodate a wide range of sample concentrations, while finding the right sample concentration for a successful PTA measurement may require various dilution steps.

1.4 Monodisperse samples

In general, both techniques show good sizing accuracy and relatively narrow distributions for monodisperse samples. Hence, Z_{av} and mean size are close for monodisperse samples. However, the error bars of the size distribution are smaller with DLS, which is consistent with the larger amount of statistical data collected compared to PTA. These higher errors bars in the PTA results come from differences in detection by the software which sometimes detects slightly more or slightly less particles. The overall shape of the PTA particle size distribution is not affected by this counting difference but the measured concentrations will be quite different, which brings attention to the imprecision of the particle concentration given by PTA.

1.5 Polydisperse samples

It is well-known that one of the major drawbacks of DLS is its low peak resolution, i.e. it can only resolve particle populations that differ in size at least by a factor of 3. Filipe et al. [1] confirmed the difficulty of DLS in separating peaks from polydisperse samples of close sizes (e.g. 60 nm and 100 nm; 100 nm and 200 nm). Whereas, PTA was able to differentiate the two size populations in all mixtures and gave accurate estimates of the bead sizes in the mixtures.

The ratio number between the two populations is also an important parameter. Indeed, it is important to be able to detect and characterize small amounts of a certain particle size population (smaller or bigger), different from the main population. Filipe et al. [1] mixed 100 and 400 nm polystyrene beads at different number ratios of 3:1, 6:1, 15:1, 150:1 and 300:1; DLS was not able to distinguish the two bead sizes for the lower bead number ratios of 3:1 and 6:1, for which mainly the larger beads were detected. DLS started to better detect the smaller population when the ratio is 150:1. When the number ratio reached 300:1, the larger particles were no longer shown by DLS. On the other hand, analysis with PTA enabled accurate sizing and a clear distinction of the two size populations for all the ratios analyzed.

Another concern of DLS is the influence of small amount of large particles (dust, aggregates) on PSD measurement. Filipe et al. [1] confirmed that PTA is less sensitive than DLS to small amount of large particles but it made the PTA measurements considerably more complex and it resulted in the underestimation of the number of smaller beads.

In addition, van der Pol et al. have recently shown how differences in refractive index may help to differentiate single nanoparticle populations not only by size, but also by their scattering intensity [3]. Additional discrimination parameters that can be used in PTA make it a preferred tool for polydisperse samples.

2 How to choose?

In summary, each technique has its advantages and disadvantages, as it can be seen in **Table VI.1**. The final choice really depends on what you want to do, if you:

- have perfectly monodisperse samples : DLS & PTA
- have polydisperse distributions and want higher resolution of peaks : PTA
- want concentration of nanoparticles : PTA
- need to show the smallest nanoparticles : PTA
- need to show the aggregates : DLS
- want a tool for quality control of nanoparticle production : DLS
- want to detect aggregated batches at the earliest time possible : DLS
- want to detect nanoparticles of different material : PTA

In an ideal scenario, you might not choose but consider a combination of the two systems to take advantage of the complementary information the two techniques can provide.

References

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