

# Particle Light Scattering Methods and Applications

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## Abbreviations

<b>DIAL</b>	differential absorption LIDAR
<b>DLS</b>	dynamic light scattering
<b>DWS</b>	diffusing wave spectroscopy
<b>FBRM</b>	focused beam reflectance measurement technology
<b>FNU</b>	formazin nephelometric units
<b>HMW</b>	high molecular weight
<b>LIDAR</b>	light detection and ranging
<b>MALS</b>	multiangle light scattering
<b>NTU</b>	nephelometric turbidity units
<b>Pdl</b>	polydispersity index
<b>R<sub>H</sub></b>	hydrodynamic radius
<b>RI</b>	refractive index
<b>SEC</b>	size exclusion chromatography
<b>SLS</b>	static light scattering

## Introduction

The detection and measurement of scattering from particles is a key analysis tool across many different industries. Different types of scattering methods, which rely on the use of many different types of incident radiation including visible/UV light, microwaves, near-IR, X-rays and neutrons, allow the analysis of material defects and particles across a wide size range (angstroms to microns). Of the types of incident radiation listed above, the scattering of visible light is the most widely used as a non-invasive analysis tool. For the purposes of this article, only laser light scattering will be discussed along with its applications. This form of electromagnetic scattering can be either elastic, where there is no change in energy between the incident and scattered light, or inelastic, where a change in energy occurs. Rayleigh scattering and the more general Mie solution are the two best known theories for elastic scattering. These scattering theories form the basis of a number of light scattering methods and applications that will be discussed further in this article. Inelastic scattering, such as Raman scattering, is discussed elsewhere in this encyclopedia and will not be covered here.

The advent of the laser enabled rapid advances to be made in the field of light scattering as the use of a coherent, monochromatic beam of light greatly improved the quality and sensitivity of the measurements. The use of light scattering for the characterization of polymers was initially the most prevalent application area.

However, the expansion of methodologies and applications has continued, and forms of light scattering, extending from light detection and ranging (LIDAR) to dynamic light scattering (DLS), are now used in many different industrial sectors including atmospheric analysis in meteorology, powder analysis in cosmetics, and drug substance analysis in the biopharmaceutical industry.

This article describes a number of applications of light scattering, where the technique plays an important analytical role. In particular, the focus will be on the application of DLS and Doppler velocimetry light scattering (electrophoretic light scattering) to the characterization of nanoparticles and biotherapeutic drugs. Emphasis will be placed on the analysis of light scattering data and the importance of certain measurement parameters as well as sample preparation methods and their impact on the data quality.

## Elastic Light Scattering Theory

Electromagnetic scattering, and in particular the scattering of electromagnetic waves in the visible range (light scattering), is an understood phenomenon with well-established theories. The two most widely used theories for the explanation of elastic light scattering are Rayleigh scattering and Mie scattering.

### Rayleigh Scattering

Lord Rayleigh provided the first quantitative theoretical treatment of light scattering for small, spherical, homogeneous particles, which is now known as Rayleigh theory. The theory applies to spherical particles whose size is much smaller than the wavelength of light being used. Typically this means that the particle diameter has to be less than one-tenth of the wavelength of the incident light. When light waves hit a particle, they cause a distortion in the molecular electronic distribution inducing an oscillating dipole, which will irradiate (scatter) in all directions. The size of this dipole governs the intensity of the scattered light and is dependent on the polarizability of the particle, while the angular dependence of the intensity provides information on the size and shape of the particle. The polarizability of a particle is linked to its molecular weight. For this reason, Rayleigh scattering, or classical light scattering, can be used to obtain the molecular weight of particles so long as the particle

concentration and specific refractive index (RI) increment ( $dn/dC$ ) are known.

### Mie Scattering

In 1908 Gustav Mie developed a theory to explain the scattering of radiation by homogeneous, spherical particles at all particle diameter-to-wavelength ratios. Mie's complete solution to Maxwell's electromagnetic field equations has become important to a number of application areas, notably in the field of meteorology where the sizes of the scattering particles found in clouds are similar or larger than the wavelength of the incident light. The theory has also found use in the imaging of substances such as milk and biological tissue. The theory uses a number of assumptions, namely that the particles being measured are spherical, the scattered light is measured from a single scattering event, the optical properties (absorbance and RI) of both the particle and dispersant are known, and the particles are homogeneous. Despite Mie theory being developed at the turn of the twentieth century, and updated formulations being developed in the 1940s, only recently has modern computing power allowed the theory to be applied to light scattering experiments such as laser diffraction measurements over a large dynamic range of particles sizes. Earlier instruments used the Fraunhofer approximation to estimate particle sizes. However, while simpler to use than the Mie theory, the Fraunhofer approximation model can lead to significant errors in the estimation of the particle size fraction for particles less than 50  $\mu\text{m}$  in diameter. These errors become particularly critical when the particles are transparent. The Fraunhofer approximation makes a number of assumptions above and beyond those used in Mie theory that only hold for the scattering of light by large particles. This is why modern laser diffraction instruments, which are required to measure particles ranging in size from 2000 to 0.02  $\mu\text{m}$ , use Mie theory to inversely model the observed scattering intensities.

## Overview of Methods and Applications

### Environmental Applications

#### *Atmospheric measurements*

The light scattering technique known as LIDAR is well established as a means of measuring atmospheric composition, cloud formations, and aerosols for both meteorological and military reasons. The method works in a similar way to radar but uses much shorter wavelength radiation, such as visible light, which scatters more strongly from rain droplets and aerosols. By using high-powered pulse lasers, remote sensing of the backscatter signal from aerosols can be carried out to determine distance profiles. An adaptation of LIDAR, where two

lasers with different wavelengths known as the online and offline wavelengths are used, is known as differential absorption LIDAR (DIAL). The online wavelength is tuned so that it is absorbed by a gas of interest, while the offline wavelength is not absorbed. Measurement of the difference in absorbance between the two wavelengths allows range-resolved concentration measurements to be made of a number of atmospheric constituents, such as water vapor and ozone. All of these measurements rely on modeling using Mie theory and a good knowledge of the optical properties of these atmospheric components.

Laser Doppler velocimetry, which will be discussed below in more detail in the context of zeta potential measurements of proteins and nanoparticles, has been used alongside LIDAR to measure the speed of global winds. Doppler LIDAR, as the technique is known, uses the frequency shift of the backscattered light to estimate the speed at which atmospheric particles are travelling and, hence, the velocity of the air. This application of light scattering is currently being used to help the renewable energy sector with the positioning of wind farms by analyzing certain characteristics of the wind patterns in locations of interest.

#### *Water analysis*

The water industry uses light scattering techniques for a number of important measurements of water effluent and sewage sludge flocculation. Traditionally, water treatment plants monitor the quality of water that is released into the public distribution system by either using turbidimetry (nephelometry) or more recently particle counters. The former technique is more established and relies on the measurement of scattered light from the water. This is a simple, qualitative measurement that requires the user to calibrate the instrument using standards that are traceable to the primary standard formazin. Samples are usually illuminated using a broad-spectrum tungsten lamp. The scattered light, in the wavelength range 400–600 nm, is detected at a 90° angle, while the intensity of the remaining light is detected in the forward direction. The ratio of the light intensities at the two detectors allows the turbidity to be calculated. Measurements are reported as either nephelometric turbidity units (NTU) or more correctly as formazin nephelometric units (FNU).

Focused beam reflectance measurement technology (FBRM) is another light scattering technique that is commonly used in the sewage treatment industry. The technique allows real-time, in-process measurement of highly turbid samples. The technique uses an inline probe containing a laser that is focused to a point at the surface of the probe window, which is in contact with the process sample. The focused beam is rotated in a circular path at a controlled scan speed, and pulses of backscattered light are detected by the probe as the focused beam passes over particles and

agglomerates. The length of these pulses correlate to the distance across each particle and, by multiplying the length of each pulse by the scan speed, the distance across each particle (chord length) can be calculated. High numbers of chord lengths are measured each second, which enable changes in the particle size distribution and particle count to be monitored. An example where this technique has been used is the analysis of cationic polyelectrolyte-induced flocculation of sewage sludge. The technique proved very useful for the choice and concentration optimization of the polyelectrolyte flocculant.

### **Industrial Applications**

Light scattering is used in a number of industrial processes to analyze particle size distribution and ensure that quality control measures are met. A number of different light scattering techniques and methods are used across these application areas, such as laser diffraction, diffusing wave spectroscopy (DWS), static light scattering (SLS), nephelometry, DLS, and FBRM technology.

#### ***Cosmetics industry***

The cosmetics industry uses light scattering to analyse the size distribution of particles and emulsions in many of its products such as facial powders, moisturizers, and lipsticks.

The selective absorptive and reflective properties of the pigments used in lipsticks, for instance, are intrinsically linked to the size of the pigments. The ability to absorb light, known as the tinctorial strength, increases with decreasing particle size, while gloss is governed by the fraction of material greater than 1  $\mu\text{m}$  in size. The use of laser diffraction, at the milling stage of production, enables rapid analysis of the milling process across a wide size range of both powders and dispersions. Moisturizers are essentially oil-in-water emulsions and their stability depends, critically, on the size distribution of the oil droplets and their zeta potential. Depending on the size of these droplets, DLS can be used to measure the size distribution, while electrophoretic light scattering can be used to measure the zeta potential.

#### ***Cement industry***

By far and away the most popular technique within the cement industry to measure particle size distributions of dispersed cement powders is laser diffraction (low-angle laser light scattering). The cement industry uses light scattering to ensure consistency and predict performance of its products. Computational modeling of the hydration process requires accurate knowledge of the particle size distributions. If the particles are too fine, then cracking can occur during the curing process, while, if they are too coarse, long curing times are encountered. As with most applications of light scattering, sample preparation

(which in the case of cement means dispersion of the particles in a liquid (wet method) or aerosol form (dry method)), knowledge of the complex RI of the material and which data analysis method to use are all critical to obtaining the correct particle size distribution. The two data analysis methods typically used are Mie theory and Fraunhofer approximation. As discussed earlier in this chapter, both of these approaches have disadvantages. Mie theory, however, provides a more robust data analysis method when an accurate value of the complex RI is used and the particle sizes are close to the wavelength of the incident light. Over the last few years, the industry has participated in studies to try and develop a reference material and standard method in order to improve the accuracy and precision of particle size distribution analysis. These have led to best practice guides and the development of standard sample preparation procedures for both wet and dry dispersion methods.

#### ***Food and beverage industry***

Numerous products within the food and beverage industry require particle size analysis in order to ensure the consistency of the final product. Particle size is a critical characteristic as it influences stability (in the case of emulsions such as high-concentration flavor additives), mouth-feel, and flavor. A number of different techniques are used for the analysis of food colloids, ranging from rheology to ultrasonic spectroscopy and DLS. All of these techniques provide valuable information about the system they measure; however, they all require some kind of sample manipulation prior to measurement. In the late 1980s, a new light scattering method was proposed called diffusing wave spectroscopy. This new technique is similar to DLS in that it measures the time-dependent fluctuations in the intensity of scattered light and inversely relates this to the diffusion coefficient of the dispersed particles. Where this method differs from DLS is in the capability to gain information on the size of sub-micrometer particles at very high concentrations, where the sample is in the multiple scattering regime. DWS exploits the fact that, in the multiple scattering regime, the transport of light through the sample can be treated as diffusive. While treating the scattered light in this way, it is important to be aware that the functions that describe the correlation of the time-dependent fluctuations in the scattered intensities are dependent on the geometry of the experimental setup.

The technique is still not fully established in the food industry; however, numerous studies, of undiluted milk, have been made using DWS to measure the size of casein micelles and to investigate the process of gelation. DWS has also been used to study the effect of various polysaccharides on the properties of oil-in-water emulsions and detect structural differences at the micrometer level during destabilization experiments.

### Pharmaceutical industry

Light scattering is used to monitor many processes within the pharmaceutical industry, for product development and quality control purposes, and provide non-invasive information on mean particle size, particle size distribution, molecular weight, and product stability and uniformity. These processes can include biopolymer degradation monitoring, determination of the second virial coefficient ( $A_2$ ) and percentage polydispersity for crystallization screening studies, determination of protein molecular weight (native state and aggregated forms), and detection of trace amounts (<0.01% by weight) of high molecular weight (HMW) components in drug formulations.

The most widely used light scattering techniques are SLS, multi-angle light scattering (MALS), and DLS. Another technique, electrophoretic light scattering, is used for the measurement of zeta potential, which is a key physical property of any particle dispersed in a liquid environment. By measuring the zeta potential, colloid and drug product formulations can be optimized, with respect to the colloidal stability. When coupled with thermodynamic information, product stability predictions can be made.

MALS is most commonly used to obtain the molecular weight of particles such as biopolymers and proteins. As discussed in a previous section, Rayleigh theory indicates that the scattering intensity is proportional to the product of the scattering particle's molecular weight and concentration. If the particle is small (hydrodynamic diameter  $\leq \lambda/10$ ), then Rayleigh scattering predominates and the angular dependence of the scattering intensity is minimized. When the particles are large or have extended nonspherical structures, then Mie scattering predominates and the observed scattering intensity is highly dependent on the detection angle. There is much debate, within industry and the literature, about whether single-angle versus multiple-angle detectors should be used, especially when studying small particles that can aggregate into larger species, for example, proteins. MALS is required for applications such as the measurement of HMW polymers; however, for globular proteins smaller than 500 kDa, the scattering profile is isotropic and so only a single angle measurement is required. Sizing errors can become significant when single-angle instruments are used and large aggregates, or nonspherical proteins, are studied as Mie scattering dominates. MALS has been used very successfully in conjunction with another sizing technique, size exclusion chromatography (SEC), for the absolute assignment of molecular masses. The combined technique is unsurprisingly called SEC-MALS, and the use of the light scattering detector allows correct molecular masses to be assigned to proteins that are hard to assign with SEC alone due to, for example, unwanted column-matrix interactions. SEC-MALS has also been used to characterize PEGylated

versions of therapeutic proteins to provide information on the degree of PEGylation and to detect oligomeric species that can lead to loss in product efficacy or immunogenic responses in patients.

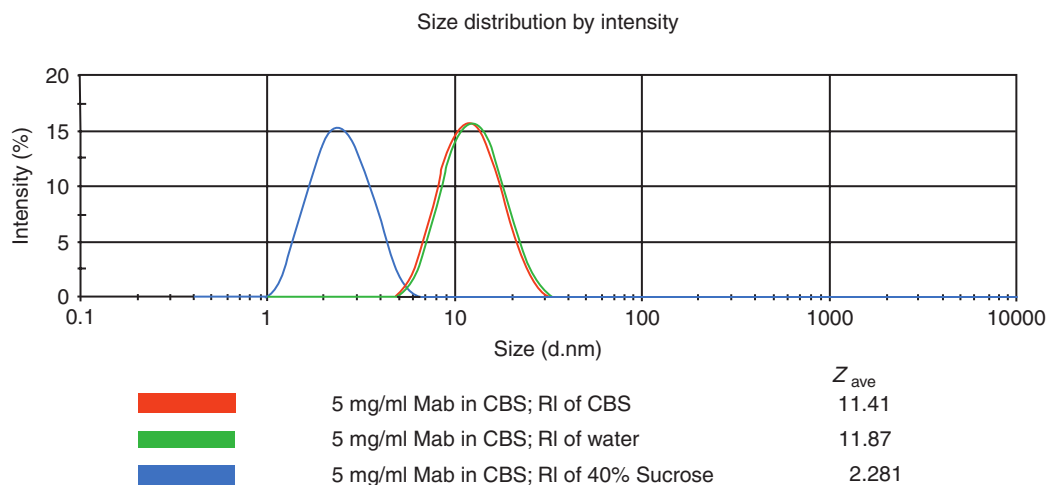
The following sections will explore DLS and electrophoretic light scattering more thoroughly, focussing on their use for protein and nanoparticle characterization. The importance of sample preparation will be discussed alongside data analysis strategies. A description will be given of the key measurement parameters obtained from these light scattering techniques with examples of how best to use and interpret them.

### Dynamic Light Scattering

The light scattered from an induced oscillating dipole will have the same frequency as the incident light but a different phase. Because the light is irradiated from different sources (particles), this difference in phases will result in constructive and destructive interferences leading to variations in the intensity. These variations, in turn, are not static due to the Brownian motion of particles. A DLS experiment measures the time-dependent fluctuations in the scattering intensity due to the particles' Brownian motion and, by analysing these fluctuations, it determines the particles' translational diffusion coefficient. Therefore, DLS is an indirect method where the hydrodynamic radius is not measured directly but rather determined from the measurement of the diffusion coefficient.

To get reliable and robust results from a DLS experiment, it is necessary to know the RI of both the dispersant and of the material, to control the temperature, and to know the viscosity of the solution. The knowledge of the RI is important because the excess of scattered light depends on the difference between the RIs of the dispersant and the material. The viscosity and the temperature, instead, affect the Brownian motion: the diffusion coefficient depends both on the temperature and on the frictional coefficient, which is related to the shape and size of the particles and to the viscosity of the solution. Therefore, errors can be introduced in the measurement by an incorrect estimation of the viscosity or by a short equilibration time. In [Figure 1](#) is shown the difference in hydrodynamic diameter obtained from the intensity distribution due to an incorrect estimation of the sample RI and viscosity. All three distributions refer to a single acquisition, the only difference being the values for the dispersant (RI and viscosity) fed into the program for the analysis.

The kind of solution (Rayleigh or Mie) used to analyse the scattering data depends on the size of the particles, as explained in the previous sections. For the approximation of spherical and homogeneous particles, the hydrodynamic radius ( $R_H$ ) can be obtained from the Stokes–Einstein



**Figure 1** Example of the effect of viscosity on the calculated hydrodynamic diameter ( $D_H$ ). The distributions were obtained from the same raw data, feeding different values of RI and viscosity into the analysis program. CBS, citrate buffered saline.

equation, and it represents the radius of the hypothetical sphere that diffuses with the same speed as the particle. This assumption has to be taken into consideration when analyzing a material because the scattering signal is isotropic only when the wavelength of the incident light is much larger than the particle's dimension. With a light source such as the He–Ne laser (633 nm), the upper limit for isotropic scattering is at around 100 nm. DLS doesn't require calibration, because it is a first principle technique, but the instrument performance should be verified using latex particles according to relevant standards such as ISO13321 part 8 1996.

### The Correlogram and Data Analysis Approaches (Cumulant vs. Multimodal)

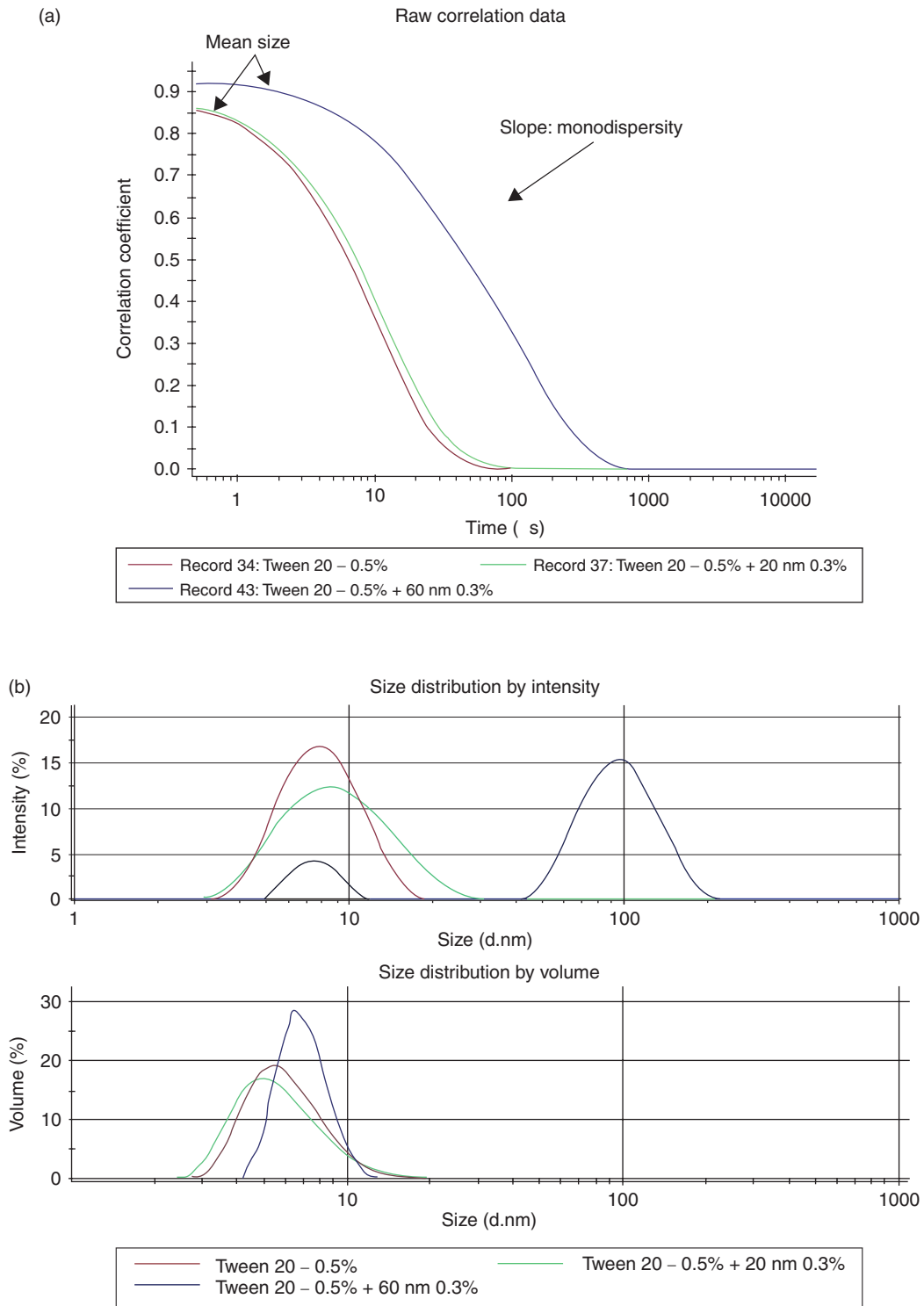
The correlogram is a measure of the loss in similarity (loss of correlation) between two signals (scattered intensities) over time due to the Brownian motion of the particles. The correlation is seen to decay exponentially as a function of time, and the cumulant analysis, which is recommended by the ISO guide, fits the correlogram to a monoexponential function giving a value for the hydrodynamic radius ( $Z_{ave}$ ) and a polydispersity index (PDI). The PDI is related to the width of the distribution and, therefore, is indicative of the monodispersity of the sample. In general, PDI values of 0.05 or below are from very monodisperse particles, values between 0.05 and 0.08 are indicative of samples that are nearly monodisperse, values between 0.08 and 0.7 indicate midrange polydispersity, while values above this indicate a highly polydisperse sample. It is suggested that when the PDI value is above 0.25, the  $Z_{ave}$  value should not be considered appropriate. In the cumulant analysis, the exponential function is only fitted to the first part of the correlogram, with the assumption that the sample comprises of a single family of

spherical, homogeneous scatterers, and there is no effect due to multiple scattering.

The shape of the correlogram gives an idea of the size and monodispersity of the samples and of the 'goodness' of the data. The intercept of the correlation function corresponds to the signal-to-noise ratio for the measurement; this value should lie between 0.2 and 0.8 to get good quality data. The point where the correlation function starts to decay gives an indication of the mean size of the particles in solution, while the slope is related to the polydispersity of the sample.

As an example in **Figure 2(a)** are reported three correlograms: the first one is of Tween micelles ( $\sim 9$  nm), the second and the third are of the same micelles doped with, respectively, 20 and 60 nm latex standards. From a visual inspection of the correlogram it is clear that the three samples are different, though the difference between the solution of pure micelles and the one spiked with the 20 nm particles is small. The  $Z_{ave}$  and PDI values (**Table 1**), which are obtained from the cumulant analysis, clearly indicate that only the first sample is monodisperse. It has to be noted though that when the micelles are doped with the 20 nm particles (**Table 1**), the PDI is still below 0.25 but the intensity distribution (multiexponential) is broader (**Figure 2(b)**). Therefore, though a PDI limit of 0.25 is often suggested, it is clear that PDI above 0.1 is indicative of more than one species present in solution. This data demonstrates one of the limitations of DLS: though it is very sensitive to the presence of large particles, it does not have sufficient resolution to separate the contribution of different sized particles when they differ in size below a limit of 2 to 3 times the diameter or 8 times the molecular weight.

The intensity distribution is determined using a multiexponential function (non-negative least square or CONTIN) to fit the correlation data. In this kind of



**Figure 2** Correlogram (a) and size distribution by intensity and by volume (b). (a) The point where the correlation function starts to decay gives an indication of the mean size of the particles in solution. The slope is related to the polydispersity of the sample. (b) The presence of 20 nm nanoparticles in a solution of Tween micelles (~9 nm) determine an increase in the width of the distribution (which is related to the polydispersity of the sample), but the two peaks (micelles and 20 nm particles) cannot be resolved because the limit of DLS resolution has been reached. On the other side, the 60 nm nanoparticles peak can be resolved from the 9 nm peak of the micelles, though the values for  $D_H$  are higher than expected. From the volume distribution it is evident that the major species in solution are the Tween micelles (~9 nm).

**Table 1** Comparison of the hydrodynamic diameters obtained for a monodisperse sample (Tween micelles) and for non-monodisperse samples (micelles spiked with 20 and 60 nm particles) using both cumulant analysis ( $Z_{\text{ave}}$  and Pdl) and multiexponential analysis (intensity and volume distributions)

Sample name	$Z_{\text{average}}$ (d.nm)	(Pdl)	Size peak 1 intensity (d.nm)	Size peak 2 intensity (d.nm)	Size peak volume (d.nm)
Tween 20 – 0.5%	7.428	(0.074)	7.531	0	5.615
Tween 20 – 0.5% + 20 nm	8.355	(0.179)	8.721	4801	4.849
Tween 20 – 0.5% + 60 nm	47.43	(0.684)	91.28	7.531	68.06

analysis, the presence of more than one family of particle size is taken into consideration. These multiexponential algorithms, for which there is no standard and which generally vary from manufacturer to manufacturer, will generate an intensity distribution and, using the Mie theory, a distribution normalized for the volume of the scattering particles (Figure 2(b) – intensity and volume distribution). Going back to our example, we can notice, in the case of the micelles doped with the 60 nm particles, that the high polydispersity index (Pdl) indicates that the  $Z_{\text{ave}}$  is not a reliable size estimation of what is present in solution. In this case, the hydrodynamic diameter of the first peak from the intensity distribution gives a value closer to that of the nonspiked micelles (Table 1). Because the intensity of the scattered light is proportional to the square of the molecular weight, and to the sixth power of the diameter, the presence of large aggregates/particles cause a bias in the cumulant analysis: in these cases the intensity distribution will account for the presence of these other species. Moreover, if multiple peaks are present in the intensity distribution, the volume distribution will give an idea of the weight of these peaks (e.g., if the corresponding species are present only in trace amounts, the peaks will not be present in the volume distribution – see Figure 2(b)). When Mie theory is utilized to carry out the transformation from the intensity distribution to the volume distribution, two main assumptions are made that the particles are both spherical and homogeneous. Therefore, the volume (and number) distribution should be considered only when comparing results from measurements of the same system. The value for the hydrodynamic diameter of the main species present in solution should be taken from the intensity distribution, whenever it is not possible to use the  $Z_{\text{ave}}$  from the cumulant analysis.

### Sample Preparation

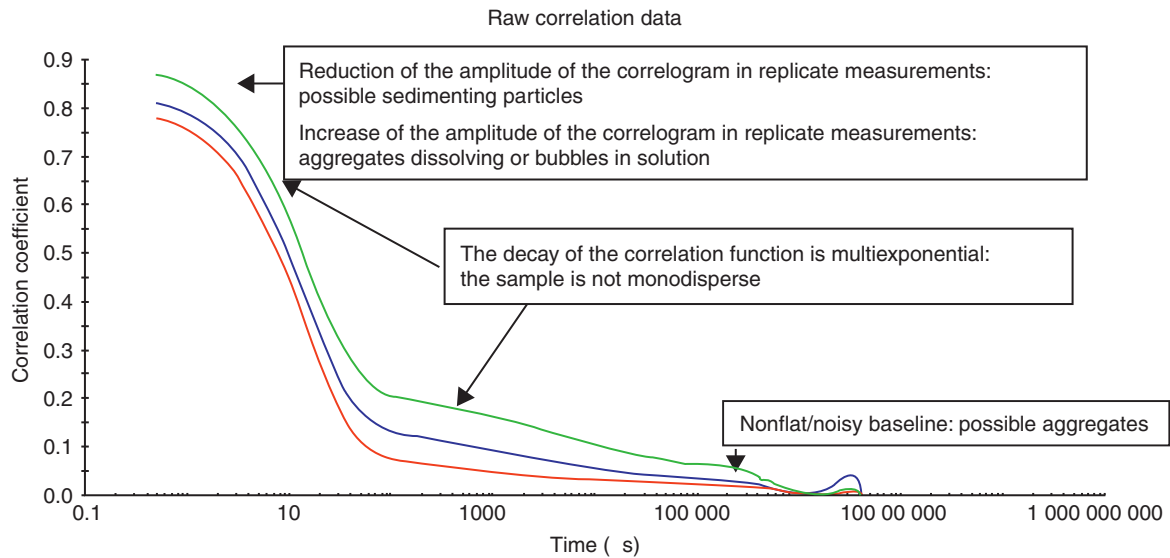
In a DLS experiment it is very important to have a sample free from very large contaminants, such as dust, because their presence can affect the measured hydrodynamic radius, especially when the cumulant analysis is used ( $Z_{\text{ave}}$ ). Moreover, it is advisable to acquire a minimum of three measurements for each sample to check for the solution stability. The quality of the data can be

inferred from the correlogram, and Figure 3 shows examples of some ‘signatures’ indicative of poor sample quality. The presence of dust/large aggregates will result in a nonflat baseline in the correlogram (Figure 3); in these cases the sample should be filtered, whenever possible. Filtration will eliminate the dust, but care must be taken to avoid altering the equilibrium population of the oligomers/aggregates present in solution. For certain applications this filtration step is not advisable. For example, when testing the stability of protein formulations, the interest is in analysing the equilibrium population of the sample. Therefore, the measurement must be made without any pretreatment, and care should be taken in recognizing any signs of the presence of aggregates

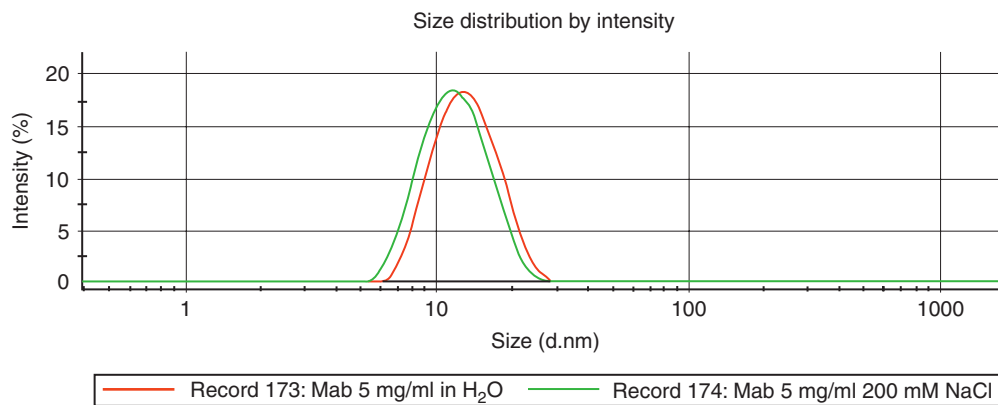
and, subsequently, in interpreting the results. In these cases, the sensitivity of DLS for large aggregates can be an advantage because these can be detected even when present at very low concentrations (<0.01% by weight).

DLS experiments need a relatively diluted solution to avoid multiple scattering events. These events occur when photons, scattered by one particle, are rescattered by other particles before reaching the detector. The rescattering increases the randomness of the signal, leading to a faster loss in signal correlation, which results in a smaller apparent size. Certain instruments detect the backscattered signal and use automatic positioning of the laser to optimize the intensity of scattered light, thereby avoiding multiple scattering events. The advantage of this optical configuration is that it is possible to measure a wider range of concentrations. In general, if a size versus concentration trend is observed, it is suggested to perform measurements at different concentrations and then extrapolate the results to infinite dilutions (i.e., zero concentration).

One of the factors affecting the diffusion of particles, and therefore the hydrodynamic radius, is the ionic strength. This physical property of the dispersant needs to be taken into consideration when comparing data from the same sample in different buffers. Figure 4 shows the size distribution by intensity of a monoclonal antibody in H<sub>2</sub>O and in the presence of NaCl. The presence of salt will suppress the electrical double layer, thereby



**Figure 3** The correlogram and the quality of the data.



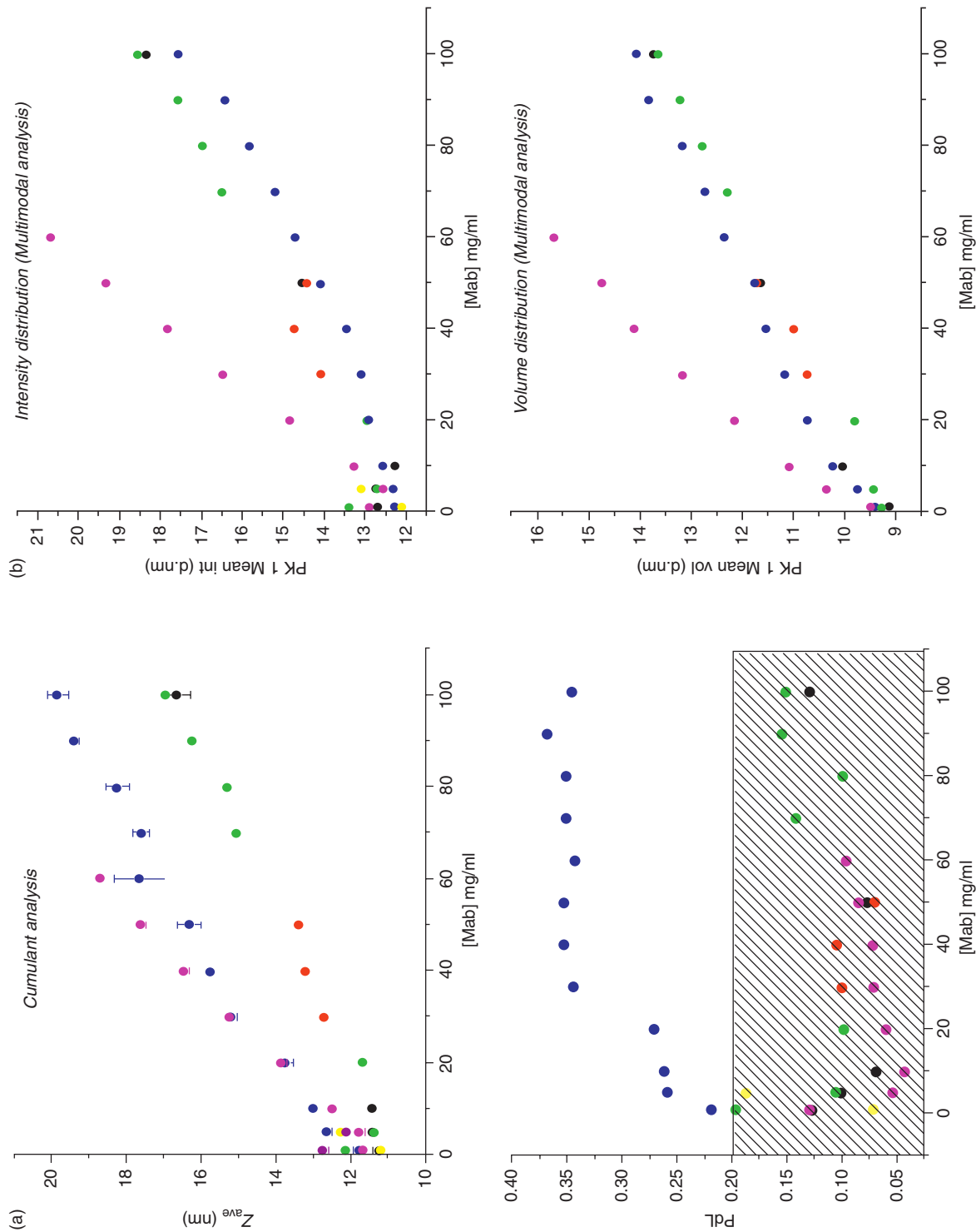
**Figure 4** Effect of the ionic strength on the apparent size distribution. The red data refers to a protein diluted in water; the green data refers to the same protein diluted in 200 mM NaCl.

increasing its translational diffusion coefficient and, as a net result, the particle will appear smaller.

**Figure 5** reports an example of how a superficial evaluation of the data could have led to the wrong conclusion. The data relates to a protein solution, formulated at high concentration, and measured at different dilutions in its formulation buffer. The measurements were performed on different days, and by different operators (different colors in **Figure 5**). Moreover, a protein preparation was buffer-exchanged in a dispersant different from its formulation buffer. A dilution series of this preparation (pink data set in **Figure 5**) was measured to check if the buffer exchange process had caused any aggregation/oligomerization. The increase in  $Z_{ave}$  with increasing concentration, for both of the preparations, is probably due to the increase in the viscosity of the sample. If only

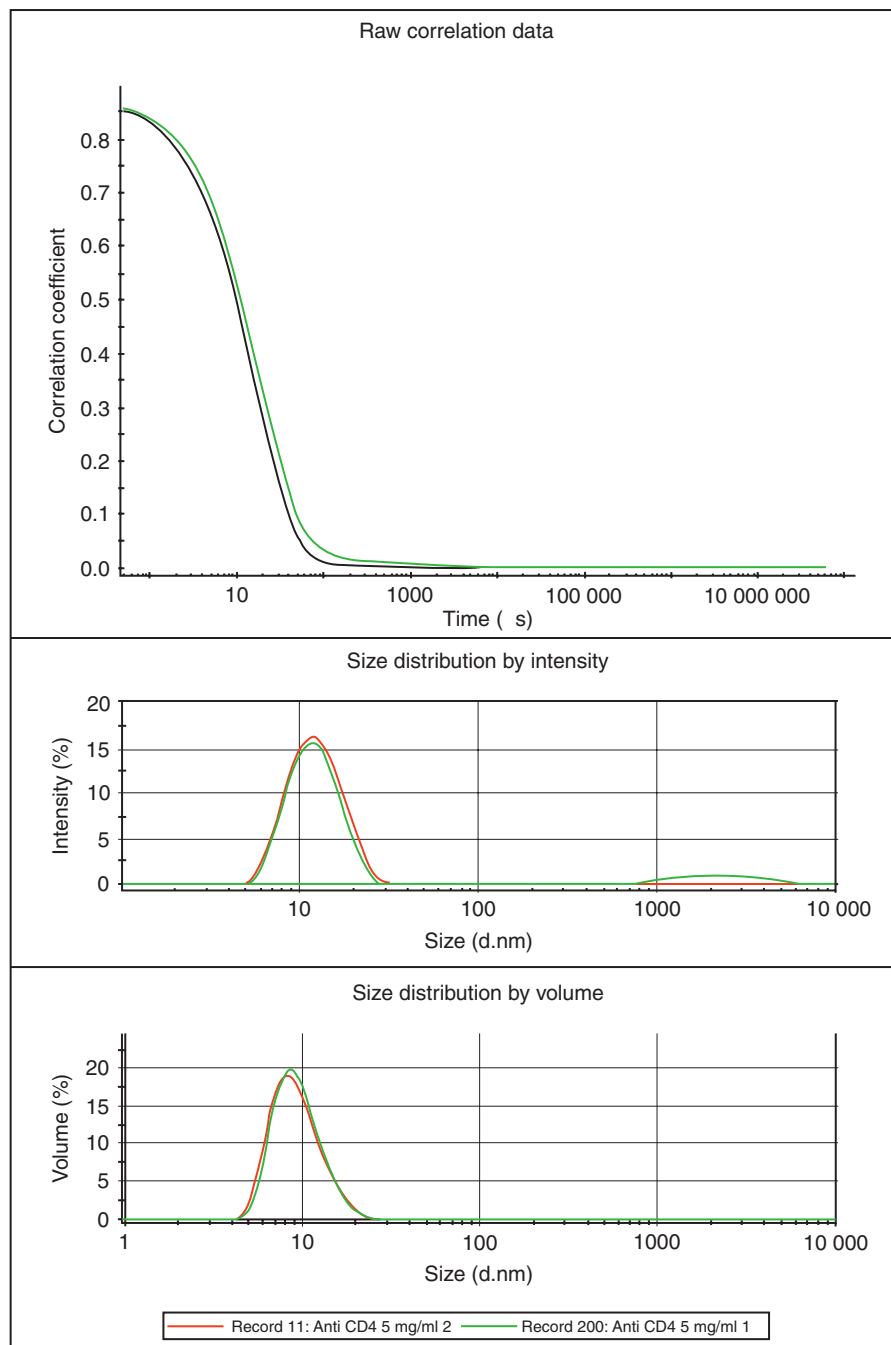
the cumulant analysis is taken into consideration, both the buffer-exchanged sample (pink data set) and one of the dilution series in formulation buffer (blue data set) could be considered outside specification. Comparing, instead, the hydrodynamic diameters, for each of the dilution points obtained from the intensity and volume distributions (**Figure 5**: trend in  $D_H$  obtained from the intensity and volume distributions), it is evident that the differences observed in the case of the blue data set are due to the presence of large aggregates, probably dust. The change in hydrodynamic diameter in the second buffer (pink curve) is, instead, a real effect. The presence of dust in the blue dilution series is also suggested by the higher PDI values and the shape of the correlograms. **Figure 6** shows, for simplicity, only the comparison between the correlograms and distributions obtained at 5 mg/ml for two dilution





**Figure 5** DLS measurements of a protein at different concentrations in two different buffers performed by two operators. Yellow, black, orange, and green data sets (formulation buffer and operator 1); and pink data set corresponds to measurements performed after nonideal sample preparation (formulation buffer and operator 2).

[Protein] = 5 mg/ml in its formulation buffer  
Two different days and operators



**Figure 6** Correlograms, intensity, and volume distributions for one dilution point in [Figure 5](#) (5 mg/ml). The green data refer to the 5 mg/ml data point of the blue series in [Figure 5](#). The red data refer to the 5 mg/ml data point of the black series in [Figure 5](#).

series in formulation buffer, of which one is the blue data set (the green data set in [Figure 6](#) corresponds to the data point at 5 mg/ml of the blue data set in [Figure 5](#)). From this data it is evident that the higher values of  $Z_{ave}$  for the blue set are due to the presence of a broad peak at HMW,

which introduces an error in the cumulant analysis. The HMW peak can be seen in the intensity distribution, but is not observed in the volume distribution indicating that it is present only in trace amounts.

In summary, the correlogram, the  $Z_{\text{aver}}$  PDI, and intensity and volume distributions should all be compared to produce a meaningful interpretation of the data.

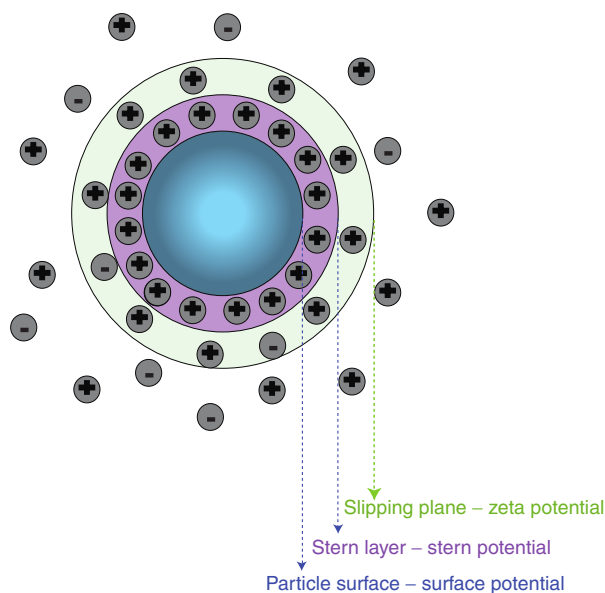
## Electrophoretic Light Scattering (Laser Doppler Velocimetry)

The total potential energy of a colloidal system can be considered as the combination of two main potential energy terms: one attractive ( $V_a$ ), due to van der Waals interactions, and one repulsive ( $V_r$ ), due to the repulsion between the electrical double layers formed at the surface (DLVO theory). According to this theory, a colloidal system is stable when the repulsive forces overcome the attractive forces. Therefore, approaches to stabilize a colloidal system include increasing the steric repulsions (e.g., by adding a polymer layer), or increasing the repulsive forces due to the electrical double layer (e.g., using different buffer systems or counter ions). An increase in the repulsive forces can be an easy way to achieve stabilization; therefore, parameters related to the extent of surface charge can be used to predict colloidal stability.

The zeta potential is defined as the potential at the slipping plane, which is the layer of resident counter ions in the double electrical diffuse layer (Figure 7), and is used as an indication for the colloidal stability. The most important factor affecting the zeta potential is the pH, because it affects the surface charge and therefore the extent of the repulsion between particles. Other factors include the ionic strength, which affects the thickness of the double layer and contributes to the reduction of the particle's effective charge. Finally, the presence of additives can have similar effects on the reduction of the effective charge. In general a particulate solution is considered colloidally stable if the value of the zeta potential is above  $+30\text{mV}$  or below  $-30\text{mV}$ , with values in between indicating a colloidally unstable system.

The zeta potential can be evaluated by using light scattering measurements of the Doppler shift in the frequencies of particles undergoing Brownian motion. The Doppler effect arises when an object moves relative to a source of energy. In this particular case the charged particles, moving in an electric field, will scatter the incident light with different frequency (higher) than that of the incident light (or reference beam). The magnitude of the Doppler shift, observed for the particles in an applied electric field, is related to their speed and, therefore, to their charge.

Avoiding or measuring the contribution of electro-osmosis is one of the main problems in determining the electrophoretic mobility and various manufacturers achieve this in different ways.



**Figure 7** Zeta potential – definition.

Zeta potential measurements should be optimized by measuring different sample concentrations and checking which one provides the best quality data. The dilutions should be carried out in the same buffer as the original material to preserve the surface charge, and attention should be paid to the conductivity of the sample because it can affect the results. Because the technique works on first principles, instruments do not need to be calibrated but, as for the DLS, they require verification through the use of a standard.

## Conclusions

Light scattering techniques allow rapid, often real-time, noninvasive analysis of many types of materials in environments ranging from manufacturing plants to field-based measurements from aircraft. For this reason a large number of methods and applications have been developed. Robust theories exist, which enable data to be analyzed and interpreted. However, these theories rely on key assumptions, which must be understood in the context of the measurement and material being analyzed, otherwise the quality of the results will be questionable. When analyzed correctly, data from light scattering measurements can provide information on the average particle size, particle size distribution, polydispersity, molecular weight, and sometimes information on the particle shape. These physical characteristics govern many of the properties of particles and their colloidal suspensions. Critically, this information can be gained noninvasively, meaning sensitive or complex systems can be analyzed without unnecessary manipulation. That

said, for certain light scattering techniques such as DLS, MALS, and electrophoretic light scattering, sample preparation is extremely important and the material's and dispersant's optical properties must be known while other key physical properties of the sample must be controlled during the measurement.

See *also*: Laser Spectroscopy Theory, Light Sources and Optics, Scattering and Particle Sizing Applications, Scattering Theory.

### Further Reading

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