Measurement of nanoparticles by light-scattering techniques

Satinder K. Brar, M. Verma

Nanoparticles (NPs), due to their unique physical and chemical properties, especially their minute particle size (⩽100 nm), find applications in numerous industrial, commercial and consumer products. After their end-user applications, these NPs find their way into the environment and food products. The NPs so discharged need to be quantified accurately to determine their toxicity and exposure levels.

At this time, there is a need to develop a unified method for their determination. There are plenty of techniques available in the market that were initially used for colloidal particles (e.g., microscopy, spectroscopy and the recent addition of magnetic resonance), but each of these techniques has a certain degree of uncertainty.

Further, sample homogeneity, sample preparation, instrument-operating procedures, and statistical practices are likely to add to the complexity of the problem. In this context, this review attempts to understand the widely-used light-scattering techniques, including their theory, practice and real-world use in determination of NPs in environmental and food applications.

Keywords: Determination; Environment; Exposure; Food; Light scattering; Nanoparticle; Particle size; Sample homogeneity; Sample preparation; Toxicity

Abbreviations: DLS, Dynamic light scattering; NP, Nanoparticle; PSD, Particle-size distribution; SLS, Static light scattering

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1. Introduction

The extensive use of nanotechnology in various spheres of science and technology has led to the release of nanoparticles (NPs) from various applications (e.g., dyes, cosmetics, catalysts, print materials, food, and lacquers). The most important physical property of NPs is particle size, which needs to be estimated accurately, as it may also correspond to a particle-size distribution. Since the advent of interest in nanotechnology, NP measurements have come a long way. Light scattering is one of the most prevalent, commonly-used techniques.

Elaborate laboratory studies have shown the potential of nanomaterials (e.g., carbon nanotubes, metal NPs, and quantum dots) to be transported in aquatic systems, to interfere with biological systems and to lead to toxicity [1–3]. Further, with the increased interest in studying the fate of NPs in the environment and the food sector, there is a need to evolve analytical methods that can measure these NPs. This will lead to a clear understanding of their behavior and their effects, two key factors to ascertain their impacts. An appropriate analytical method to measure NPs is the largest gap in NP research, more so at environmentally relevant concentrations. In this regard, this review examines the possibility of using light-scattering techniques as valuable methods for measurement and analysis of NPs.

Particle-size distribution (PSD) plays a fundamental role in controlling the properties of different nanomaterials. For example, chemical reactivity of nanomaterials, which mostly differs from that of macroscale or microscale materials, is usually affected by NP surface/volume ratio. Particle size also determines NP diffusivity, and the ability of NPs to permeate cell membranes. PSD analysis thus allows monitoring of NP aggregation or release of NP-surface modifiers. These modifications may be attributed to preservation conditions, concentration effects or chemical properties of the dispersing medium (pH, ionic strength), and they alter NP properties. Size characterization is a key step in not only the quality control of NP synthesis, but also proliferating application of NPs and their ultimate release into the environment.

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NPs are characterized by a variety of techniques {e.g., electron microscopy (transmission, TEM, or scanning, SEM), atomic force microscopy (AFM), Fourier-transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), nuclear magnetic resonance (NMR) and dynamic light scattering (DLS) [4–13]}. Evaluation of the particle size of NPs in numerous fields involving environmental and food samples has mostly been carried out by DLS, which is now considered an established, traditional technique. However, the variations in the types of DLS instrument and the analytical procedures adopted may often limit accurate information on the apparent sizes of particles, making it difficult to understand the size dependence of the NPs.

DLS measures the Brownian motion of NPs and relates this movement to an equivalent hydrodynamic diameter, with the motion of smaller particles being overestimated. In reality, DLS measures the time-dependent fluctuations in scattering intensity caused by constructive and destructive interference resulting from the relative Brownian movements of the NPs within a sample. Through application of the autocorrelation function and subsequent calculation of the exponential decay, average particle size can be calculated from time-dependent fluctuations in light intensity (see ISO 13321 for further details). The challenge with most of the nanomaterials in natural environments is difficult because they exhibit a duality of physical and chemical characteristics as they switch from hydrophobic to polar forms upon exposure to water. In aqueous environments, this is expressed as their initial tendency to:

(1) self-assemble into aggregates of substantial size and hydrophobicity; and, subsequently,
(2) interact with the surrounding water molecules and other chemical compounds in natural environment, thereby acquiring negative surface charge.

Thus, measurement of nanomaterials by one single method is a daunting, irreproducible task. Table 1 sets out the different barriers to environmental analysis of nanomaterials. However, light scattering has been employed on a large scale, as it is the most important, key technique in many environmental applications [14–16].

This review discusses the different light-scattering techniques, the logic behind their widespread use compared to other techniques as well as their pitfalls. To begin, it is necessary to understand the theory of light scattering.

2. Principle of light scattering

Light scattering can be divided into three domains based on a dimensionless size parameter, α, which is defined as:

$$\alpha = \frac{\pi D_p}{\lambda} \quad (1)$$

where πDp = circumference of a particle, and λ = wavelength of incident radiation. Based on the value of α, these domains are:

$\alpha \ll 1$: Rayleigh scattering (small particle compared to wavelength of light);

$\alpha \approx 1$: Mie scattering (particle about the same size as wavelength of light); and,

$\alpha \gg 1$: Geometric scattering (particle much larger than wavelength of light).

Particles in suspension are in constant random motion (Brownian motion) due to interaction with the molecules of the suspending fluid. According to the Stokes-Einstein theory of Brownian motion [17], particle motion is determined by the suspending fluid viscosity, the temperature, electrical charge, electrical mobility and the size of the particle, as seen in Equations (2) and (3):

$$D = \frac{k_B T}{6\pi\eta r} \quad (2)$$

$$D = \frac{\mu_q k_B T}{q} \quad (3)$$

where D = diffusion constant, q = electrical charge of a particle, μ_q = electrical mobility of the charged particle

Table 1. Environmental barriers for analysis of nanomaterials

Environmental barriers	Responses
Trace levels	Often difficult to measure with good reproducibility by single method; often requires several methods in tandem
Other particles not of interest also present	Segregation difficult leading to ambiguous results
Particle-size changes (aggregation, agglomeration, condensation)	Often leads to composite data making characterization difficult
Volatilization of organics during sample preparation	Loss of sample in conjunction with lower quantities leads to erroneous measuring
Static charges	-
Extraction efficiency weak	Leads to underestimated concentration levels
Aquatic stability due to formation of colloids	Nanoparticles lose their individual characteristics
Absence of quality-control materials (references/surrogates)	Non-comparable results across global laboratories
Laboratory background levels	Contamination of samples demanding several replicates; often precious samples are lost

(i.e. the ratio of the particle's terminal drift velocity to an applied electric field), k_B = Boltzmann's constant, T = absolute temperature, η = viscosity, and r = radius of the spherical particle. Equation (3) is also known as the electrical mobility equation, which defines the diffusion of charged particles in a given medium.

The particle motion in a fluid of known temperature and viscosity can finally lead to the determination of particle size. The DLS technique measures optical motion [18,19]. The suspended particles are illuminated with a coherent light source. The time-dependent position or velocity of the suspended particles imparts frequency shifts due to the light scattered from the suspended particles.

Theoretically, the scattering pattern produced by a single spherical particle comprises a series of light and dark concentric bands that decrease in intensity with increasing radial position. The principal optical model applied in laser-light scattering (LLS) is Mie scattering. The Mie model takes into account both diffraction and scattering (absorption, refraction and reflection) of the light around the particle in its medium. The dimensionless size parameter is estimated as a function of particle diameter and refractive index.

Ultimately, random particle motion forms a complete distribution of optical frequency shifts measured over time. There is an effect of NP interaction and gravity on NP measurement by light-scattering techniques, as shown in Fig. 1. Light-scattering techniques are of two principal types: classical and dynamic.

2.1. Classical

Classical light scattering (also known as static, Rayleigh or multi-angle light scattering) provides a direct measure of particle size and can be often used for the measurement of molecular mass, which is an important application in food with respect to proteins. It is therefore very useful for determining whether the native state of a particle is simple or complex, and for measuring the masses of aggregates or other non-native species. It also can be used for measuring the stoichiometry of complexes between different particles (e.g., colloidal, larger particle complexes or particle-organic matter complexes). Like-

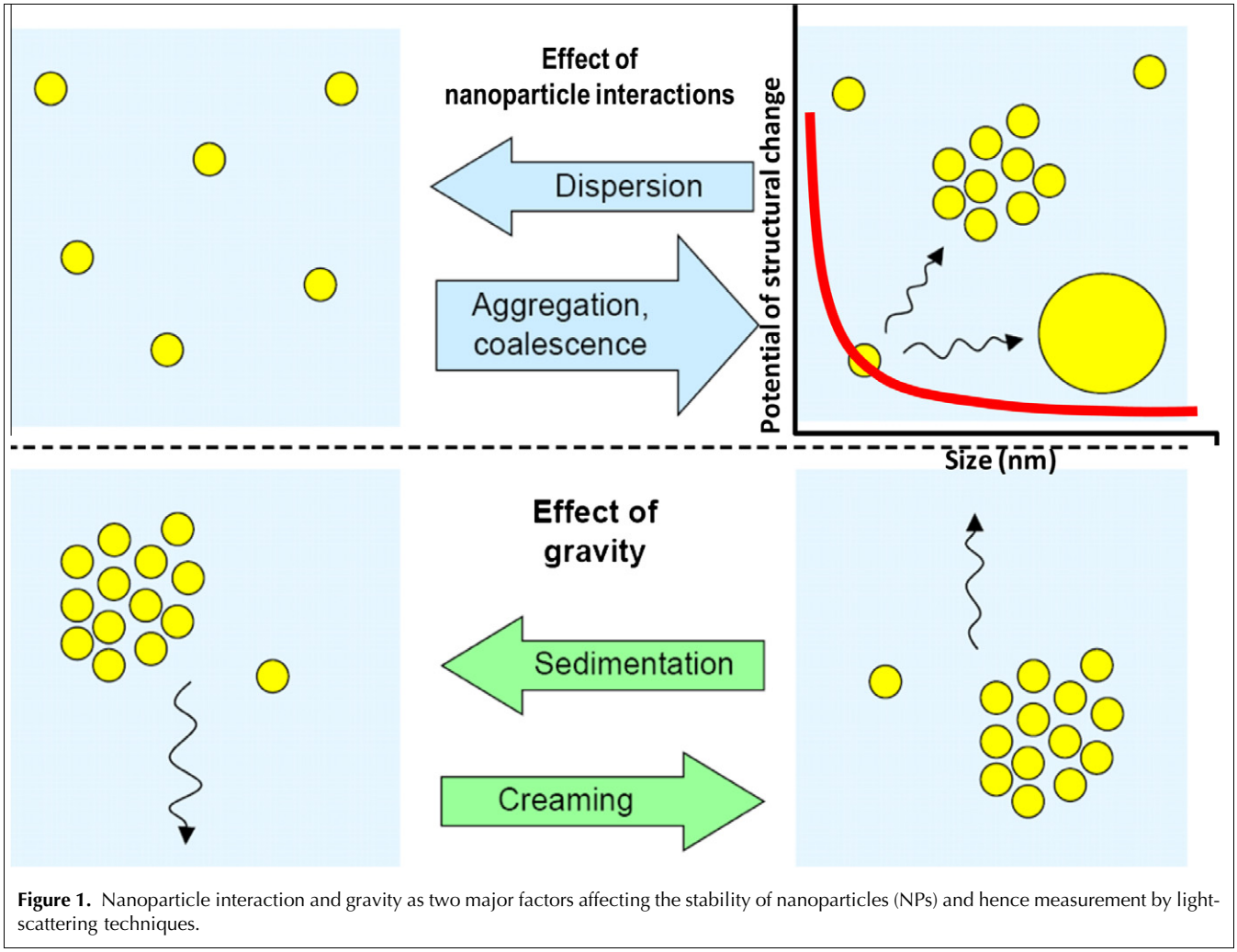

Figure 1. Nanoparticle interaction and gravity as two major factors affecting the stability of nanoparticles (NPs) and hence measurement by light-scattering techniques.

wise, it can give information on particle structure and, in combination with DLS or field-flow fractionation (FFF), particle shape can be also determined.

The most common equation used for determination of average molecular weight, M_w, by static light scattering (SLS) is the Zimm Equation as explained below in Equations (4)–(8):

$$\frac{K_c}{\Delta R(\theta,c)} = \frac{1}{M_W P(\theta)} + 2A_2 c = \frac{1}{M_w\{1 + q^z(\frac{R_g^Z}{\Xi})\}} + 2A_2 C \quad (4)$$

where:

$$K = 4\pi^2 n_0^2 (dn/dc)^2 / N_A \lambda^4 \quad (5)$$

and:

$$\Delta R(\theta,c) = R_A(\theta) - R_0(\theta) \quad (6)$$

with:

$$R(\theta) = \frac{I_A(\theta) n_0^2}{I_T(\theta) n_T^2} \frac{R_T}{N(\theta)} \quad (7)$$

and the scattering vector for vertically polarized light is:

$$q = 4\pi n_0 \sin\left(\frac{\theta}{2}\right)/\lambda \quad (8)$$

where n_O = refractive index of the solvent, λ = wavelength of the light source, dn/dc = refractive index increment of the solution, N_A = Avogadro's number (6.023×10^{23}), and c = solution concentration. The intensity of the analyte, A, is measured at an angle $I_A(\theta)$. The subscript, T, is for toluene with the Rayleigh Ratio of toluene, R_T, being 1.35×10^{-5}/cm for an HeNe laser. As described above, the radius of gyration, R_g, and the second virial coefficient, A_2, are also calculated from the Zimm Equation. A simple SLS experiment entails the average intensity of the sample that is corrected for the scattering of the solvent and yields the Rayleigh ratio, R, as a function of the angle or the wave vector, q, as mentioned in Equation (7). Thus, by using the Zimm Equation, molecular mass of particles, such as proteins, can be determined.

2.2. Dynamic

DLS [also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS)] uses scattered light to measure the rate of diffusion of the particles. This motion data is simply processed to derive a size distribution for the sample, where the size is given by the Stokes radius or hydrodynamic radius of the particle. This hydrodynamic size depends on both mass and shape (conformation). For food applications, DLS is particularly good at sensing the presence of very small amounts of

Table 2. Dynamic light-scattering technique – advantages and disadvantages

Characteristics	Advantages	Disadvantages	Remarks
Size range (nm)		2–3000	Other methods (e.g., tracking analysis) can have a larger size-range analysis
Size resolution	1:3 in theory, 1:4 in practice*	-	Relatively higher than other known methods
Measurement of NPs in polydispersions		Average particle size, which is intensity biased towards the larger or contaminant particles within a sample	Other approaches can have particle-by-particle approach enhancing resolution
Measurement of NPs in monodispersions	Slightly more reproducible than other methods due to average particle size from many more particles		Other methods look at fewer NPs, hence decreasing reproducibility
Refractive index		Requires solvent refractive index. In samples with NP mixture, analysis is weighted towards the more refractile particles	Other methods may not need this information, causing no interference
Size distribution	Intensity distribution which can be converted into a volume distribution. No accurate information about particle concentration can be calculated.		It is equivalent to other methods where particle distribution is possible and volume distribution is not. Often in liquid environmental and food samples, volume is more important
Dilution	Lower dilution		Less compromise on particle aggregation, a major factor in NP measurement

Advantages correspond to the positive points of light scattering versus negative points correspond to disadvantages, as per the analysis; DLS is a win-win situation on many accounts.
*1:3 means 100-nm particles can be resolved from 300-nm particles; and, 1:4 means 100-nm particles can be resolved from 400-nm particles.

aggregated protein (<0.01% by weight) and studying samples containing a very large range of masses. It can be quite valuable for comparing stability of different formulations, including real-time monitoring of changes at elevated temperatures.

The three key strengths of DLS are:

(1) analysis of samples containing very broad distributions of species of widely differing molecular masses (e.g., a native particle and various sizes of large aggregates) can also be carried out;
(2) it can detect very small amounts of the higher mass species (<0.01% in many cases); and,
(3) as it is a batch-mode measurement, there will be no chromatographic separation or dilution involved; moreover, there is no precaution to be taken on the molecule aggregates, as they are not affected within a chromatographic column or dissociated by dilution.

Table 2 sets out the major advantages and disadvantages of DLS technique. The primary limitation of DLS is that it is often difficult to quantify accurately the amounts of any aggregates that may be present, so the size fractions cannot be correlated with a specific composition [20]. For example, interferences can be caused by a range of possible artifacts (e.g., dust particles), which influence the scattering intensity compared to smaller particles, so that they influence the sizing result. Moreover, the particles are considered spherical by DLS techniques, so that the actual size of the particles in the dispersion can be underestimated. Also, data obtained from samples containing particles with heterogeneous size distributions are difficult to interpret, as is often the case in environmental applications. Fig. 2 is a representation of a typical set-up for measurement of NPs by light-scattering techniques.

Nonetheless, DLS can be a very good technique for relative comparisons, such as indicating which formulation, sample treatment, or purification process produces more aggregates, especially in food applications. The application of DLS and its sensitivity are also dictated by the protocol used for sample preparation, as this entire procedure can lead to underestimation or overestimation of results.

3. Sample-preparation techniques

Experimental conditions (e.g., light scattering detected off-line or in combination with fractionation or any other technique) and sample parameters (e.g., amount, temperature, pressure, and viscosity) determine the choice of sample cells. When light-scattering detectors are to be used in combination with chromatography or FFF, special, commercially-available flow cells have to be used. For most off-line measurements of aqueous or non-aqueous solutions of the scattering NPs or polymers, cylindrical quartz-glass cuvettes are used. Table 3 represents the key sample parameters and characteristics that need to be measured to analyze the samples.

As light-scattering techniques usually involve single-particle measurements, they entail dilution of the sample as far as possible to suppress interparticle interactions, which may lead to interparticle interferences. If the dilution factor is not respected, the Stokes–Einstein equation (Equation (1)) is no longer valid, and diffusion coefficients determined by DLS yield wrong hydrodynamic radii and lead to erratic measurements. However, the concentration of scattering particles within the solution and/or suspension has to be large enough to create sufficient scattered intensity at all scattering an-

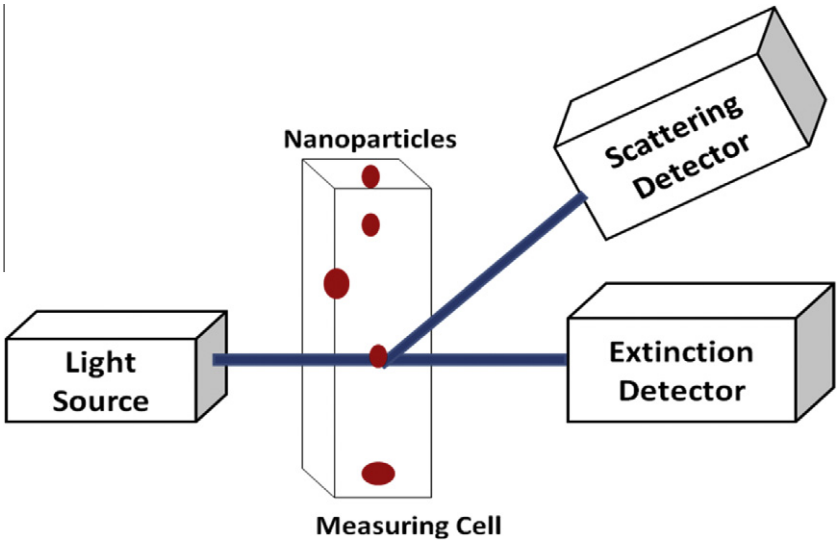

Figure 2. Typical light-scattering technique set-up for measuring size of nanoparticles (NPs).

Table 3. Sample characteristics required for data analysis using light-scattering techniques

Parameter	Static light scattering	Dynamic light scattering	Remarks
Solvent viscosity, η	NO	YES	Determines the magnitude of scattering vector q according to Equation (6) (which can be obtained from physical chemistry handbook)
Solvent refractive index, n_D	YES	YES	Can be obtained from physical chemistry handbook
Sample concentration in g/L	YES	NO	Known for the specific sample
Sample temperature	NO	YES	Known for the specific sample
Refractive index increment, dn_D/dc	YES	NO	Has to be measured using standard interferometers, especially for the environmental samples, which are mixtures of different components

gles of interest. The scattered intensity depends on the following parameters:

(1) mass or size of the scattering particles, which cannot be adjusted at all to experimental needs;
(2) solute-particle concentration, which should be as small as possible; and,
(3) refractive-index difference of solvent and solute particles or, more accurately, refractive-index increment dn_D/dc, which can be adjusted by choosing an appropriate solvent.

Often, certain samples containing polyelectrolytes cannot be handled in water and organic solvents, due to the long-range Coulombic interactions between the scattering solute particles. In environmental applications, this can be a big hurdle and a challenge, due to the presence of certain polyelectrolyte-modified NPs for potential application. Salts can help overcome the Coulombic interactions, but their concentration has to be limited to reduce the possibility of affecting the refractive index. A comprehensive review on polyelectrolytes has been already published [21].

The changes in refractive index have to be determined under Donnan equilibrium conditions, which correspond to chemical potential and not constant salt concentration. The theory of Donnan equilibrium can be elaborated by adopting the partitioned chamber concept. Consider two chambers partitioned by a semi-permeable membrane. In the initial phase, the left chamber contains an aqueous solution of negatively-charged NPs and positive counterions only, whereas the right chamber contains an aqueous salt solution (e.g., NaCl). The membrane is permeable to the small ions (Na^+, Cl^-) only, and impermeable to the larger, negatively-charged NPs. To establish the electrochemical equilibrium, Cl^- ions start to flow through the membrane from the right chamber into the left chamber, leading to an electric membrane potential also attracting the Na^+ ions from right to left. Thus, an equilibrium is established, as seen in Fig. 3, referred to as the ''Donnan equilibrium'', when the electrochemical potential of the left chamber matches that of the right chamber. Both refractive-index increments and average scattered intensities have to be determined under these membrane-equilibrium conditions to determine accurately the true particle molar mass, M_w. Thus, various artifacts encountered during light-scattering techniques (e.g., aggregate formation, presence of dust, and absorption of the incident laser light) must be taken into consideration while measuring the particle size of NPs. Samples with heterogeneous size distribution and of diverse composition are hard to interpret, and, unless the sample content is known, size fractions cannot be related to particles of a specific composition [22,23].

Despite the artifacts, careful sample preparation makes the light-scattering techniques simple and robust methods, which find wide application in the environmental and food sectors.

4. Environmental applications

NPs entering the environment may not initially be toxic to the living organisms in the environment, but, during the course of their life cycle, they may become toxic. NPs could react with other substances in the environment, break down in the environment, provide a catalyst (speeding up) for the reactions already taking place, or inhibit essential reactions taking place and even mimic certain metabolic pathways resulting in identical natural products.

In order to understand the mechanism of NPs in the environment and their various interactions, an effective measuring tool is key. For example, waste NPs from a manufacturing plant could alter the pH of the river, which lead to dissolution of metals that normally do not dissolve (e.g., aluminum). In turn, aluminum in the water supply would be toxic to the inhabiting living organisms. In order to carry out the complete analysis of the NPs, consideration needs to be given to the size, the shape, the surface and the bulk of the particles [24–28]. All these parameters could influence the properties of NPs, and, in turn, their behavior and eventual risks in the environment. Thus, unintended (e.g., waste, wastewater, and sludge) and intended (e.g., groundwater remediation) release of NPs into the environment may

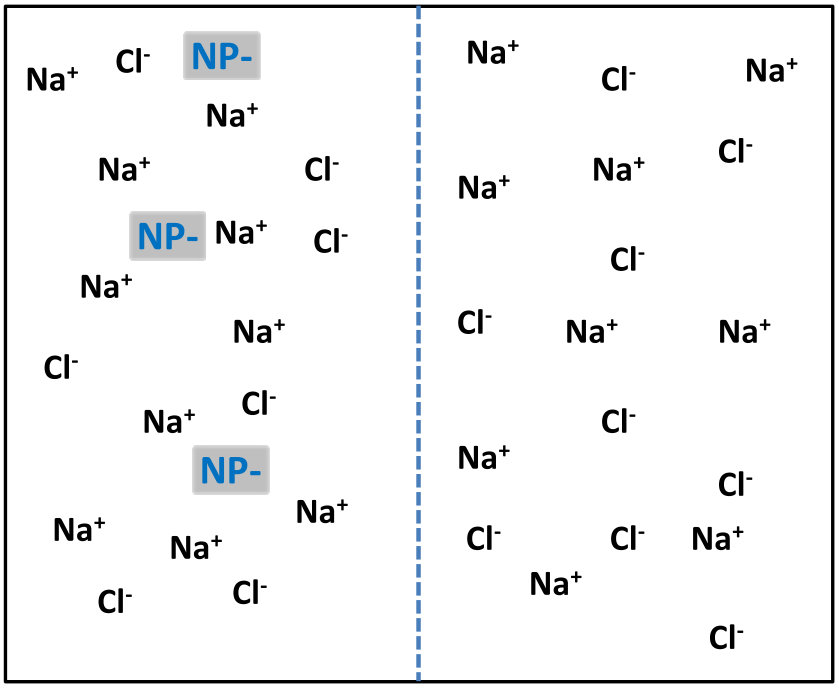

Figure 3. Donnan equilibrium with charged nanoparticles (NPs) and salt solution.

lead to indirect human exposure (e.g., via drinking water, and the food chain), which needs to be quantified.

While considering environmental samples for detection and analysis of NPs, different environments and ecosystems need to be considered. The different ecosystems comprise different media, mainly, solid, liquid or gas with constituent variations (e.g., plant tissue and cells, and soil types), which provide challenges to methods of detection and analysis.

NPs could also be present within consumer products and nanocomposites, which also provide challenges to detection and analysis. Light-scattering techniques are mostly applicable to NPs in the liquid phase. Drawbacks of these techniques are that, often, frequent sampling is required, and samples need to be treated before their analysis. These not only increase error in quantitative analysis but also prevent *in situ* analysis of the NPs in the environment resulting in unrealistic analysis of NPs.

A recent study by Domingos et al. [29] used a multimethod for characterizing manufactured NPs in suspension. The authors used different techniques [e.g., transmission electron microscopy (TEM), atomic force microscopy (AFM), DLS, fluorescence correlation spectroscopy, NP-tracking analysis, and FFF]. Their study demonstrated that none of the methods was ideal and that each method had inherent advantages and limitations, so characterization of NPs involves a combination of methods rather than a single method.

There are two aspects to measuring NPs in the environment:

(1) use of NPs to measure other contaminants precisely – which also incorporates their determination; and,
(2) measurement of engineered NPs released into the environment.

Both these aspects are discussed in this section. Localized surface-plasmon resonance (LSPR) applies surface-enhanced spectroscopic processes of metallic NPs, so LSPR spectroscopy has become a powerful technique for chemical and biological analysis. In the environment, a recent homogeneous of Hg^{2+} assay was performed by measuring enhanced LSPR scattering signals resulted from Hg^{2+}-DNA complex-induced aggregation of gold NPs (AuNPs). These light-scattering signals can therefore be observed by dark-field microscopy and measured by spectrofluorometer [30]. Compared to colorimetry, this technique was highly sensitive.

DLS has been used to determine the amount of arsenic in Bangladeshi well water, bottled drinking water and Mississippi tap water. Label-free AuNPs are used in a selective colorimetric assay and a highly-sensitive DLS assay for the detection of arsenic in concentrations as low as 3 ppt [31].

Another aquatic environment study confirmed that DLS offered good advantages as a technique to analyze NPs. Miglietta et al. [32] studied the dispersion of carbon black and carbon nanotubes in the aquatic environment. The study also considered the influence of mechanical agitation (e.g., sonication and stirring) on the agglomeration state and particle-size distribution. The authors reported that such processes seemed

to have little or no effect as far as agglomeration was concerned. But, it depended on the sonication frequency and stirring speed, as a high rate of stirring along with high sonication frequency can cause dispersion as well as agglomeration by increasing particle density and hence enhancing inter-particle interactions.

The toxicity of copper NPs against *Escherichia coli* was studied using a centroid mixture design of experiment that adopted DLS so that a correlation was obtained between toxicity and particle size [33]. DLS techniques require information on viscosity and refractive index, which is often not available or difficult to find in environmental samples that are highly complex, thus making the overall estimate quite difficult, unless the sample is spiked. This necessitates further development of these simple methods in order to adapt them to the real environmental situations.

C60 fullerenes comprise a class of nanomaterials that are gaining immense popularity due to their applications, so that novel analytical methods are required for their quantification with high sensitivity. Although the fullerenes are insoluble in water, colloidal suspensions of stable C60 NPs can be formed in high concentrations and transported in water between biological networks [34]. The high chemical stability of C60, even in the presence of acids or bases [35], indicated that its biodegradation is difficult in biological and environmental systems, though their functional groups, in fullerene derivatives, may be lost. Most methods of fullerene analysis in the literature involve extraction of fullerenes by organic solvents combined with ultraviolet-visible (UV-vis) spectrophotometry [36] and other expensive techniques {e.g., high-performance liquid chromatography (HPLC) [37–41] and high-performance thin layer chromatography (HPTLC) [42]}, both using MS or UV-vis for detection. However, direct quantification of fullerenes in water by spectrophotometry is limited for higher concentrations of NPs in suspension. A simple alternative for direct quantification of C60 in water would be scattering techniques (e.g., nephelometry or turbidimetry), extensively used for quantification of solids in suspension [43], or spectrofluorimetry, which is often limited by the lack of strong fluorescence bands, as is the case in most C60 molecules and C60 NPs [44]. For nephelometry, the intensity of the scattered radiation is greatest if the particles are small enough so that Rayleigh scattering occurs. For larger particles, the scattering intensity decreases at 90° so that turbidimetry is more suitable [43]. A study by Sene et al. [45], who employed nephelometry to measure n-C60 concentrations, found that the detection limit of the proposed method was about 0.0090 ± 0.0008 mg/L, in a linear concentration range of 0.007–0.360 mg/L. A comparison of this nephelometric technique with spectrophotometry showed that, for nephelometry, the threshold concentrations detected were about 20 times lower than for spectrophotometry studied at the second-order lines.

Likewise, a recent study investigated the possibility of adding guar gum to stabilize sterically zero-valent iron NPs, which are widely used in remediation of contaminated groundwater. Tiraferri et al. [46] found that the sedimentation profiles of the different iron-NP suspensions confirmed the improved stability of the iron NPs in the presence of guar gum. Thus, this strategy can also be used to measure these NPs by DLS techniques as, often, their aggregation limits precise measurement.

Titanium dioxide (TiO_2) NP suspensions were recently determined for their particle size using DLS techniques to establish a reliable method for in vitro toxicology assessment of these NPs [47]. It was established that the NP sizes were comparable with values determined using other analytical procedures and other instruments, thus confirming the accuracy of these techniques.

In recent years, the resonance light-scattering (RLS) technique has also gained importance as a valid technique for analysis of NPs in the environment [48–51]. For species that aggregate, enhancements in light scattering of several orders of magnitude, efficiently carried out by RLS techniques, can be observed at wavelengths characteristic of these species. RLS is characterized by high sensitivity [52,53], ease of performance and simplicity in equipment (usually a common spectrofluorometer).

The RLS technique has been widely used in determination of proteins and some metal ions, due to the strongly-enhanced RLS signals of the bindings of dye-proteins or dye-metal-ion complexes [54–57].

Recently, a study reported the use of RLS where the signal of CdS NPs was strongly enhanced by the silver ion, and the response signal was linearly proportional to the concentration of the silver ion [58]. This technique can therefore be adopted for determination of environmental pollutants, most commonly in surface waters.

Most of the research studies pertaining to the use of light-scattering techniques in literature are linked to waters with none conducted on soils. Soils pose a different set of problems, due to their complexity and the possibility of NP-organic-matter speciation and other interactive effects.

Even wastewater is a domain that is yet to be investigated, due to the complexity of the systems and myriad possible interactions in this milieu. We found in the literature one reported study on the presence and the measurement of NPs in wastewater, which we discuss below, as it is the principal sink for the NPs and hence the major route of exposure.

The most important aspect is that nano-structured components are intrinsically less stable than their microstructured counterparts, so that there is loss of structural stability in nano-structured components, which is a difficult parameter to analyze by existing light-scattering techniques.

4.1. Case of wastewater-treatment plant

An increasing number of consumer products, including cosmetics, medicines, cleaners and even food, use various types of NPs as active or inert ingredients, so wastewater-treatment plants (WWTPs) (the treated effluent as well as the wastewater sludge) have become a major pathway for NPs entering the environment. Although there is no evidence that such NPs pose an environmental threat, their ultimate fate is an enigma, as determination of their presence in WWTPs has not so far been successful.

A recently reported study by Jarvie et al. [59] conducted a study using neutron-scattering techniques and stated that the bare NPs remained suspended in simulated study, and coated NPs tended to aggregate in sediment, which was explained by interactions of NPs with organic matter and surfactant molecules. By contrast, long-term electrolyte effects were not so prominent. Apparently, polyelectrolyte effects may be predominant, due to the possibility of Coulombic interactions, as discussed earlier.

Nanotechnology can certainly be used in a positive manner in the environment (e.g., for remediation of contaminated groundwater and soils). However, the risks of NPs throughout their life cycles in the environment must be established, and that needs an integrated platform of precise measurement techniques. When considering environmental samples, they can be distinguished as simple (monodispersions) or complex (polydispersions). Hence, for less complex environmental media (e.g., water-only ecotoxicity studies and studies on aggregation in water), it may be possible to use some of the simpler, faster analytical approaches (e.g., DLS) along with centrifugation and/or microscopy to cover the entire range of space and time for manufactured NPs. However, in complex media (e.g., sorption and persistence studies in sludge, soils and sediment/water systems, water-sediment ecotoxicity studies, or bioavailability studies), analysis becomes significantly more challenging, and some techniques (e.g., DLS and electron microscopy) apparently fail to distinguish between the engineered NPs to be tested and naturally-occurring nanomaterials as well as between dissolved and particulate forms. Ideally, under these conditions, more sophisticated methods of separation, further coupled with MS, can take over. To date, no single protocol exists that can precisely characterize NPs *in situ*, or in general in complex media (e.g., water, sediment and soil). Separating a wide range of particle sizes (~1–1000 nm) in physico-chemically diverse, polydispersed samples at low concentrations is a major bottleneck.

5. Food applications

The uses of nanotechnology-derived food ingredients, additives, supplements and contact materials are expected to grow rapidly in future. Food safety will also potentially benefit from the introduction of nano-based detectors, sensors and labeling [48]. In some countries, nanomaterials are already used in food supplements and food packaging, with nanoclays as diffusion barriers and nano-silver as antimicrobial agents [60–62]. The principal NPs in food applications comprise TiO_2, silicates and aluminosilicates. TiO_2 (designated E171 in Europe) is used for whitening and brightening foods, especially for confectionary, white sauces and dressings, and certain powdered foods [63,64]. Particulate silicates and aluminosilicates (E554, E556 and E559 in Europe) are used in the food industry as anti-caking agents and to allow the flow of powders, and some are present in cheeses, sugars and powdered milks [63]. The major five food sources of particulate silicates are salt, drinking powders, chewing gum, instant pot savory snacks and icing sugar. Pharmaceuticals or nutraceuticals and toothpaste are also major sources of particulate silicate and aluminosilicate intake [63].

Limited work has been reported to date on detection and characterization of engineered NPs in food, based on searching the databases of Science Direct and ISI Web of Science, so we have drawn a simile from natural NPs. Moreover, with existing techniques, it is difficult to distinguish between natural and engineered NPs. Thus, isotopic labeling techniques must be designed to perform the same with both natural and engineered NPs. Further, analysis of engineered NPs in food has mostly been performed by microscopy techniques, rather than light-scattering techniques, and a review has been already published on this aspect [65].

As discussed in the case of environmental applications, determination of NPs in food samples requires information on not only concentration, but also size distribution and other properties that influence their presence and distribution in the food samples. The physico-chemical properties of NPs that would play an important role in their overall assessment for potential risks include: particle size; particle distribution; surface area; surface charge and topography; composition and purity; hydrophobicity and solubility; chemical reactivity and bioactivity; and, dispersion or aggregation state.

Moreover, on one side, application of NPs in food is thought to ensure food safety, and, on the other side, the NPs themselves incur toxicity. Given the huge diversity of engineered NPs for use in the food sector (e.g., chemical composition, size, size distribution, and surface activity or modification) and potential interaction with food-matrix components (e.g., proteins), measurement of NPs is a challenging task requiring tailored solutions [66–70]. The processes affecting two or more particles in food will depend heavily on environmental factors (e.g., concentration, pH, ionic strength, and temperature). As a result, when investigating the physico-chemical properties of a food product, it is very important to maintain the study system in an environment as close as possible

to the original conditions. In a food product, the major constituents (e.g., proteins, polysaccharides, lipids and flavors) are in dynamic, and often weak, equilibrium with themselves as well as the environment, making the analysis further challenging.

Conventional light-scattering techniques often require the sample to be diluted, so that that they cannot maintain the original conditions of the sample. This restricts their application in the industrial environment [71]. However, often the backscattering benefits of recent advances in DLS techniques can help overcome this limitation, as shown in Fig. 4, as recent advancements in DLS techniques can improve performance by enhancing sensitivity and covering a larger NP-size range for dilute, low-molecular-weight samples. Likewise, the technique is able to measure higher concentrations with better reproducibility for concentrated high-molecular-weight samples.

The SLS technique has been generally described as a rapid, reproducible technique that covers a broad particle size range (0.05–2000 μm). SLS has been used to measure the size of dairy components in suspension – individually fat globules [72], lactose crystals [73], or casein micelles [74] – and in composite media {e.g., skimmed milk [75] or whole milk [76,77]}. DLS techniques have been mostly restricted to milk and to simple emulsions, together with model systems. Many factors contribute to this:

(1) milk is a liquid containing colloidal particles;
(2) these particles scatter light well, and they may be diluted with proper care; and,
(3) dilution does not disrupt the texture that is natural to the food, as would be the case for preparations (e.g., sauces and mayonnaises).

It is evident that DLS experiments can provide some information on the surface structures of the casein micelle NPs by relatively simple, rapid experiments [78–80]. The major concern in these studies is whether or not the micelles preserve their original state when they are diluted into buffer, so methods need to be devised to take the utmost care to avoid premature dissociation of these micelle molecules. Although DLS is very sensitive to changes in shape and size of the particles observed, detection of the structural changes taking place in small globular proteins prior to denaturation is very difficult. However, the processes of aggregation or nucleation or transitions to the denatured form can easily be detected [81–83].

A technique that is also catching on in the field of food colloids, and hence NPs, is phase-analysis light scattering (PALS), which was developed to measure the electrophoretic mobility of charged particles, and it offers enhanced sensitivity at the low mobilities. In PALS, laser light is split, a frequency modulation is applied to one portion of the light, which is then used to generate a scattering pattern from a suspension of particles. If the particle moves in the applied voltage gradient, the relative phase will shift, and can be detected by a phase comparator. This method was principally used in the determination of isolelectric point and elctrophoretic mobility of whey-protein-isolate solution, and provided better reproducibility [84]. The electrical properties of globular proteins in solution cannot easily be characterized by light scattering, as they are relatively weakly scattered, and have, at certain pH values, very low charge. In this context, PALS has certainly given a more sensitive, reproducible analysis of such proteins.

Recently, diffusing wave spectroscopy (DWS) has been used to overcome the disadvantages of conventional light-scattering techniques, as it can operate in a very turbid medium (or "multiple scattering" regime). DWS has proved to measure coagulation times accurately and it is also possible to correlate qualitatively the intensity of the scattered light to the elasticity of the structures formed in the gelation of milk via rennet [85–87]. The combination of back and transmission DWS was used to monitor the stability of sodium caseinate-stabilized emulsions in the presence of excess sodium caseinate [88]. By studying the oil-droplet dynamics, flocculation and/or creaming was correlated as a function of oil-volume fraction when sodium caseinate was present in excess in solution. Other authors [89–91] monitored the early stages of droplet kinematics to detect the differences between aggregation leading to creaming or to gel formation by presence of extracellular polysaccharide in emulsions stabilized with whey-protein isolate. Although these studies did not supply precise quantitative information, their interesting point was the clear indication of the mechanism of interactions of proteins and polysaccharides with different food systems, and that too under realistic concentrations. The DWS system is still in its formative stage with regard to its use in food, particularly in emulsion systems. Nevertheless, it will prove a valuable tool, more particularly in the case of complex interactions in food systems, where more than one scattering element is always present. For toxicity tests of NPs in food samples, a separate dispersion experiment can be conducted under optimum conditions to compare with the dispersion behavior in the affecting media and during the course of the affecting experiment. This comparison experiment with maximum dispersion may include surfactants, co-solvents, pH changes, certain ionic strength and sonication. Comparing the results in the realistic effect/exposure experiments with those from this comparative experiment can give valuable information on the degree of aggregation.

Further, analysis requires knowledge of the type of medium (i.e. whether it is a pure medium or a complex mixture comprising heterogeneous systems). Another aspect to be considered is the characterization of the NPs under a range of relevant biological conditions (e.g., complex food products, where they can interact with

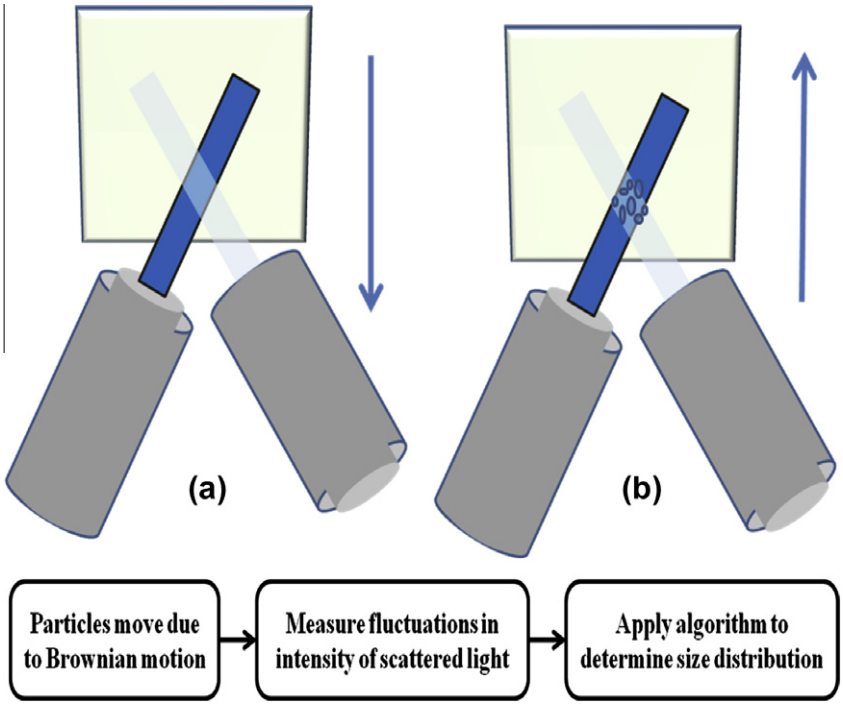

Figure 4. Backscattering benefits of recent development in light dynamic scattering techniques: (a) for dilute low-molecular-weight samples (enhanced sensitivity and larger size range); and, (b) concentrated high-molecular-weight samples (higher concentrations and better reproducibility).

proteins, lipids, sugars or other biomolecules). This may have consequences for the surface composition of the NPs and their aggregation behavior, as the adsorbed proteins and biomolecules may result in totally different hydrophobicity, charge and charge distribution than synthesized NPs. The adsorbed proteins and other biomolecules thus confer what is called a ''biological identity'' (the biomolecule corona) [92,93] to the NPs. NPs can also alter the functioning of the adsorbed biological molecules (e.g., enzyme activity, degradation and other properties), making them more or less active than the unbound form [94].

While the light-scattering techniques may be applied to carry out measurements of NPs in the pristine form, the measurement and the quantification of NPs in food, biological tissues and other biological matrices presents considerable challenges, since suitable equipment and measurement strategies are not yet available [95,96]. Many food products also contain considerable amounts of naturally-occurring NPs (e.g., proteins, silica or traces of TiO_2), which makes detection of added NPs difficult, as it rules out techniques (e.g., elemental mapping), where there are already significant background levels. Further, light-scattering techniques are mostly destructive, and often the samples are precious, so this is another aspect that needs to be investigated and improved, especially for environmental and food applications.

6. Future research and challenges

Particle-analysis techniques can generally be classified as ensemble or single-particle techniques. With ensemble techniques, individual particles cannot be isolated. Instead, ensemble techniques measure the response from statistically significant numbers of particles simultaneously. Laser-light diffraction is a commonly employed ensemble technique. Obviously, there is not one single ''best technique'' for all situations. Determining the best technique for a particular situation requires knowledge of the particles being analyzed, the ultimate application of the particles, and the limitations of techniques being considered. There are plenty of techniques available in the literature covering specific ranges of NP-size distribution, as shown in Fig. 5.

Depending on the application of interest, a number of techniques can be used to analyze and to characterize NPs. In industries where aerosols play an important role, some tools [e.g., the Differential Mobility Analyzer (DMA)] are commonplace. With fine powders, light-scattering techniques are common. A prerequisite for toxicological, toxicokinetic, migration and exposure assessment is development of analytical tools for the detection and the characterization of NPs in complex matrices (e.g., food and environment). We can therefore conclude that nanotechnology research in the environment will principally be governed by newer, simpler,

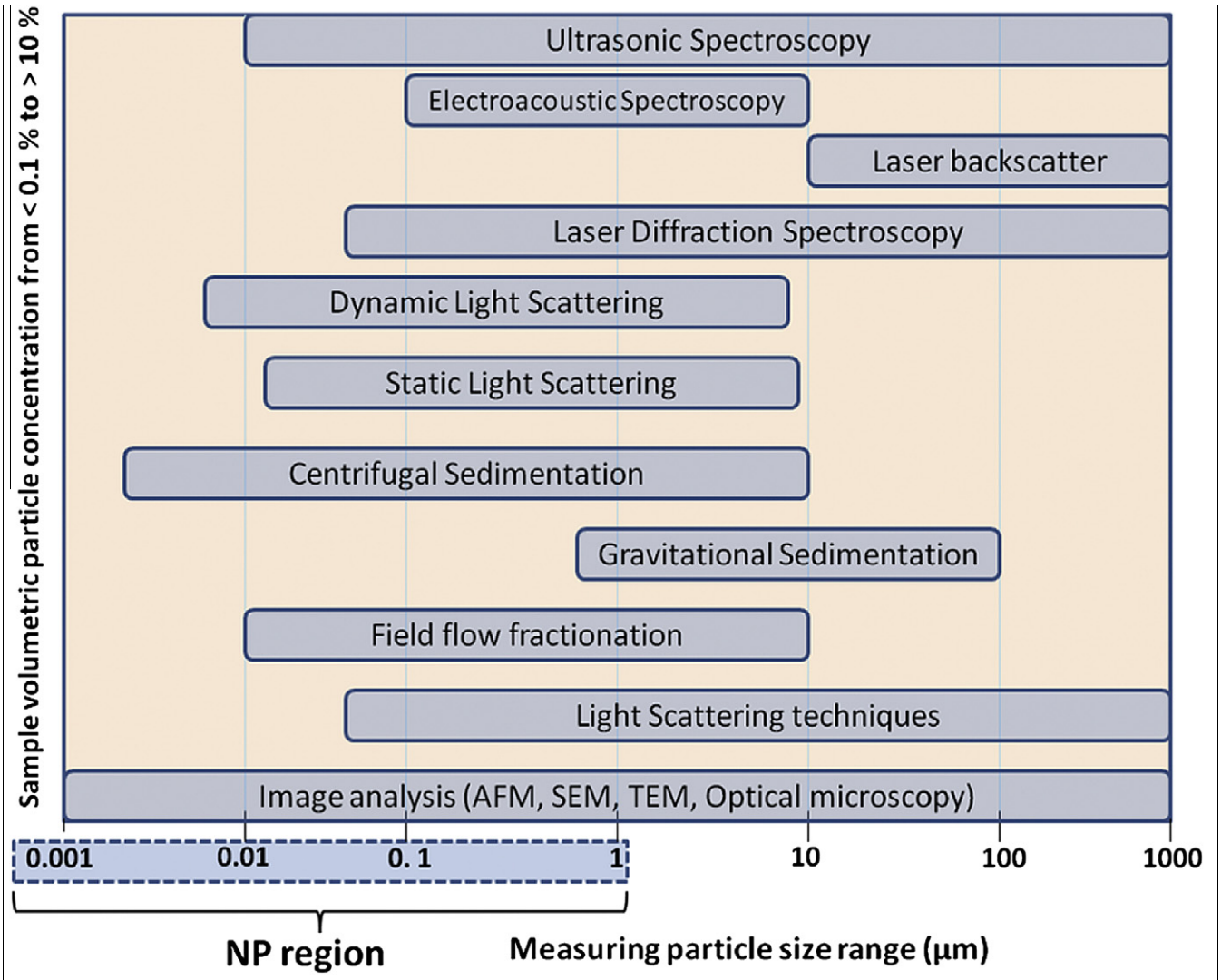

Figure 5. Performance of different particle-size-measurement techniques (Note: only a few techniques can measure particles in the nanoscale region).

faster and more efficient characterization techniques for nanomaterials. This particle-by-particle approach avoids the ensemble assumptions of DLS and provides a unique approach, going beyond light scattering in assessing polydisperse systems and providing insight into aggregation. Moreover, when the same analysis has been carried out using different methods, we need to adopt a caveat, as particle-size distributions reported in terms of volume, number or scattering intensity usually produce vastly differing results, despite the data coming for the same physical material. Another fundamental particle characteristic, zeta potential, must also be measured rapidly using light-scattering techniques. This effectively quantifies the parameter controlling electrostatic stabilization, and can be used in precise NP analysis to avoid the instability that can result from particle-particle attraction, further leading to aggregation (which is a major pitfall of conventional light-scattering techniques).

The use of the light-scattering techniques is a further aid to NP-size determination and characterization, which have obviously become important aspects in future food applications, more so with respect to exposure and toxicity. In 2007, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) affirmed that specifications and acceptable daily intakes for food additives that have been evaluated in other forms are not intended to apply to NP materials [97]. These considerations have led food companies to focus attention on pre-market approval, traceability and other regulatory aspects related to the risk management of these materials. In the case of food and environmental samples, characterizations of the parameters of NPs are made at different times, and can also be additional uncontrollable variables of the physical properties. All these limitations make the development of a characterization tool desirable that can accomplish its functions in the same ambient conditions, in real-time,

and in a non-destructive way. From this perspective, light-scattering techniques outperform structural techniques (e.g., TEM and AFM) and hold the advantages in terms of being non-destructive, real-time and in situ methods. In future, this factor will be crucial in the development of the nanosciences, their technological applications and finally monitoring in food and environmental samples for risk analysis and safety. The very different nature of nanotechnology and the complexity and dynamism of environmental and food samples means that conventional standards, measurement methods and instrumentation are increasingly unable to provide the necessary measurement parameters, range, uncertainty and accuracy.

7. Conclusions

The advantages of DLS are rapidity of analysis, no requirement for calibration, and sensitivity to NPs. By contrast, SLS requires cleaner samples than DLS, and advance knowledge of the optical qualities of particles. Light scattering offers simple, sensitive and fairly selective analytical techniques that do not use expensive or complicated test equipment for the determination of environmental and food-based NPs, with the increasing interest in environmental and food contamination. Many published data have not characterized or have only poorly characterized environmental and food systems. Typically, this means that uptake and effects of true NPs have not been studied, but only for agglomerates that behave as microparticles *per se* due to interactions with the surrounding environment. Food and environmental samples have to be prepared carefully to avoid interferences (i.e. induction of aggregation, agglomeration, dissolution or even dispersion) that are not representative of the real environment. Proper sample preparation strengthens NP-size analysis using light-scattering techniques.

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