

Overcoming the Challenges of Nanoparticle Characterization with a Light Scattering Toolkit

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Introduction

Engineered Nanoparticles (ENPs) are of increasing importance to a diverse range of industrial and commercial products including pharmaceuticals, cosmetics and food. Whereas the physical properties of bulk materials remain constant regardless of their size, the size of nanoparticles often dictates their physical and chemical properties. Accurate and comprehensive characterization of nanoparticle size is therefore essential to controlling their properties and understanding their potential for new applications.

Additional parameters that are fundamental to nanoparticle development, optimization, formulation, toxicology and quality control include shape, zeta potential and aggregation. A variety of analytical techniques are available to address this parameter set, each with their own distinct advantages and disadvantages.

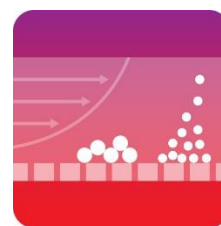
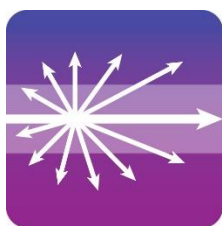
Perhaps one of the most versatile sets of nanoparticle characterization tools is that based on light scattering, utilizing the three basic flavors of analytical light scattering: multi-angle, dynamic and electrophoretic (MALS, DLS and ELS, respectively). These offer determination of size and size distributions, shape or conformation, and propensity for aggregation or flocculation. Auxiliary techniques that enhance and complement light scattering include Field-Flow Fractionation (FFF), which separates nanoparticles

according to size prior to analysis, and Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) for analysis of elemental composition.

The Nanoparticle Characterization Challenge

One significant challenge encountered in nanoparticle engineering is the sensitivity of their properties to small differences between seemingly similar particles. Detailed characterization is therefore required to know if ENPs made by nominally equivalent processes are actually identical. In addition, nanoparticles may be out of equilibrium with their environment so properties can change as a function of time. Slight variations in surrounding media can cause particles to aggregate, change size, and react with their environment to oxidize or absorb contaminants. Hence understanding the time dependence of these properties is important for storage, environmental and health impacts as well as for manufacturing and product stability.

The regulations governing the use of ENPs are not fully defined. Current guidance by the US Environmental Protection Agency (EPA) requires "...manufacturers of new (nanoparticle) chemical substances to provide specific information to the Agency for review prior to manufacturing chemicals or introducing them into commerce" [1]. A comprehensive approach to analysis is therefore recommended, particularly in regard to particle size, which impacts critical aspects of ENP behavior such as rate of diffusion or uptake by an organism.



A Light Scattering Toolkit

Light scattering comes in several flavors, each of which provides different insight into nanoparticle properties. The most common form of light scattering for nanoparticle analysis is dynamic light scattering (DLS). More advanced forms include multi-angle light scattering (MALS) and electrophoretic light scattering (ELS).

Dynamic Light Scattering



DLS is among the most commonly used ENP characterization tools. It is a fast, low resolution technique for measuring the diffusion coefficient and hence **hydrodynamic size** r_h of nanoparticles in solution or suspension.

Particle size and coarse size distributions from a radius of less than 0.2 nm and up to thousands of nm, such as that shown in Figure 1, may be estimated from the diffusion coefficients in a matter of seconds to minutes, often without dilution or additional sample handling beyond pipetting into a cuvette or microwell plate. DLS is suitable for all types of ENPs, regardless of the material and (usually) the optical properties, including metallic, semiconducting, dielectric, organic, etc. Fluorescing nanoparticles do present a challenge; if the ENPs fluoresce under excitation by the DLS laser potential solutions include changing the laser wavelength or installing narrow-band fluorescence-blocking filters.

While traditional DLS is carried out in single-sample cuvettes in instruments such as the **DynaPro® NanoStar®**, a more advanced system measures dozens or hundreds of samples automatically, in microwell plates, for dramatically increased throughput and productivity when characterizing ENPs. Wyatt's **DynaPro Plate Reader II** performs DLS measurements *in situ* in standard microwell plates containing 96, 384 and even 1536 samples, completing hundreds or thousands of samples per day in order to screen many solution conditions for optimal stability and other properties. It has also been used to optimize the process conditions of drug nanoparticles prepared by acoustic milling [2].

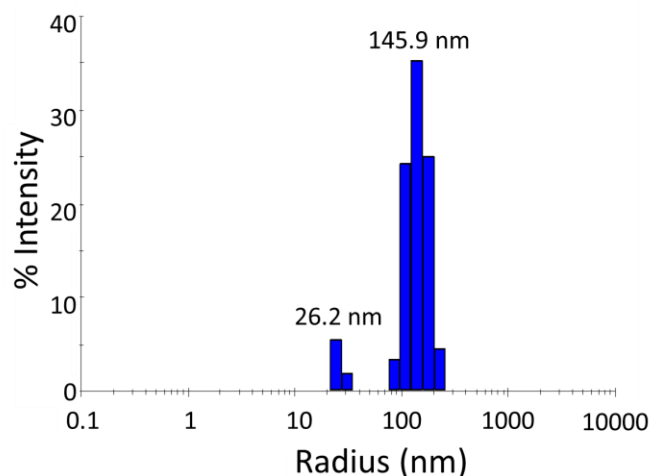


Figure 1. Size distribution from batch (unfractionated) dynamic light scattering (DLS) measurement of semiconducting nanoparticles, exhibiting a bimodal population. Scattering intensity is approximately proportional to the 6th power of the radius, so while the peak at 145.9 nm appears much larger in the scattering intensity than the 26 nm peak, in terms of mass content it is actually much smaller.

Multi-angle light scattering



MALS utilizes the anisotropic (i.e., angle-dependent) scattering behavior of ENPs to determine a different measure of size, the root mean square radius r_g , from 10 nm to ~ 500 nm. Unlike DLS, unfractionated MALS measurements do not establish a size distribution, just a z-averaged size of the overall population of particles in solution.

However, MALS is particularly well suited to making measurements in flowing systems such as the field-flow-fractionation (FFF) system described below, and therefore is ideal for obtaining high-resolution size distributions with the aid of FFF.

The combination of r_h and r_g yields the shape factor $\rho = r_g/r_h$, which is a good indicator of **shape and conformation**, e.g. the ellipticity of an ENP or discriminating between a shell vs. filled sphere. For larger particles such as carbon nanotubes, the angular intensity distribution indicates both the rod-like shape and the length, as shown in Figure 2. The **DAWN® HELEOS II®** from Wyatt Technology, which can incorporate an embedded **WyattQELS™** DLS detection module for enhanced capabilities such as ρ analysis, is the most widely used and versatile MALS detector to characterize ENPs with FFF.

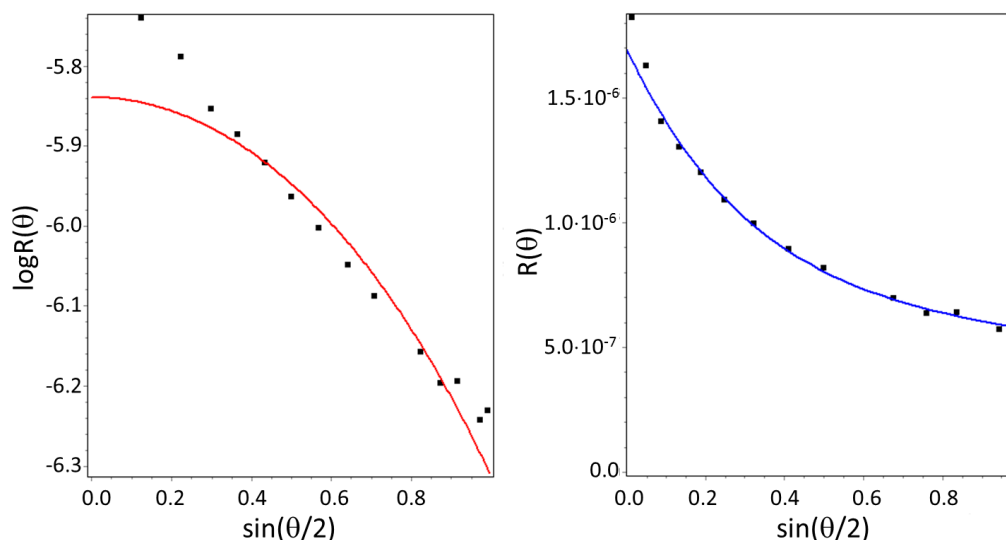
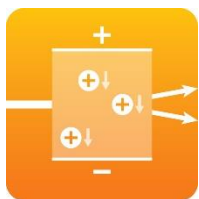


Figure 2. Angular distribution of scattered intensity for carbon nanotubes as determined by multi-angle light scattering (MALS), and fits to two shape models. Left: best fit to sphere model. Right: best fit to rod model. While the sphere model does not fit the data, the rod model does, indicating a half-length of 330 nm.

Electrophoretic light scattering



ELS is a means for determining the charge on an ENP suspended in a fluid, usually presented as the **zeta potential**. This method operates by measuring the drift, and hence electrophoretic mobility, of a nanoparticle under an applied electric field using the laser Doppler technique.

Most ELS instruments incorporate a DLS detector for measuring size as well as mobility, calculating zeta potential from mobility, size and solution conductivity. Similarly

to most colloidal systems, the zeta potential of ENPs is a good predictor of stability against aggregation or flocculation as well as an indicator of surface modifications that might affect charge. When automation of zeta potential and size measurement is desired, Wyatt's **Möbius®** instrument allows for connection to an autosampler in order to carry out dozens of measurements, unattended, carrying out ELS and DLS simultaneously in order to monitor possible sample degradation due to application of the electric field.

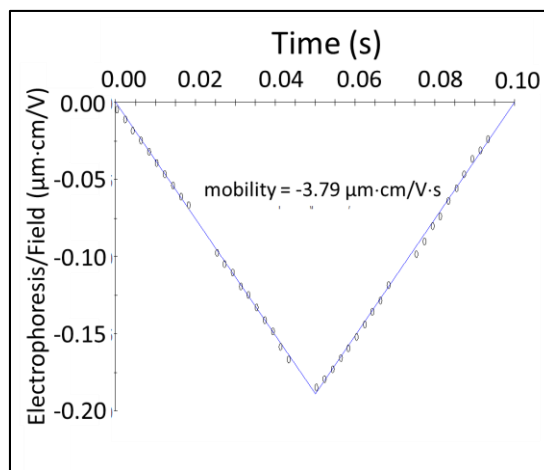
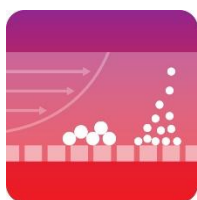


Figure 3. Left: electrophoretic light scattering (ELS) is usually carried out in cuvette-like cells, but when automation of many samples is desired a flow-cell-based instrument with simultaneous ELS and dynamic light scattering capability may be connected to an autosampler. Bottom: graph shows drift vs time under a field of alternating polarity, and the fitted mobility result.

By monitoring changes in particle size distribution with DLS, formulators are able to assess gross protein aggregation across a dynamic temperature range, pH, or following essential aspects of the formulation process. For example, Figure 1 shows the particle size distribution of two monoclonal antibody (mAb) formulations with and without sucrose, before and after lyophilization, measured using the DynaPro Plate Reader II. Lyophilized samples were reconstituted to the same concentration as pre-lyophilized solutions.

The key to high resolution: field-flow fractionation



Batch (unfractionated) measurements such as those described above can at best yield low-resolution size distributions. In order to obtain high-resolution size-based separation upstream of the light scattering detector is required. There are several different types of **field-flow-fractionation** (FFF) methodology that have been developed to fractionate simple to complex colloidal sample mixtures. The most commercially-successful form of FFF technology to date is asymmetric-flow field-flow fractionation (AF4), where samples in solution or suspension, with sizes ranging from 1-1000 nm, are separated within an open channel by liquid forces alone. Fractionation is only one part of the story; the eluting material then can be characterized post-fractionation

for size, elemental composition and other properties by a variety of online detectors.

In AF4, fractionation of the sample is based purely on hydrodynamic size, and occurs as a result of the competing effects of drift under an applied field, and diffusion, within a flowing channel. The AF4 'field' is a cross-flow field: the bottom surface of the channel is a semi-permeable membrane, and solvent is forced to flow through it, drawing particles to accumulate near the membrane. However, diffusion transports particles away from the membrane, back up into the channel – the smaller the particle, the farther it diffuses. Separation is achieved when 'channel' flow is applied parallel to the membrane, since the laminar flow profile is subject to higher velocity the farther it is from the membrane, as illustrated in Figure 4. The ratio of cross-flow to channel flow is adjustable to balance the overall size range and resolution of the fractionation process.

While in principle it is possible to calibrate size vs AF4 elution time, this is not as reliable a measurement as is obtained with the addition of MALS and/or DLS detectors downstream. Whereas unfractionated DLS at best distinguishes particles that differ in size by 3-5x, **AF4-MALS** and **AF4-DLS** can characterize accurately particles that differ in size by just a few percent, as well as help determine their shape. Compared with imaging techniques such as electron microscopy, AF4-MALS/DLS does not provide highly detailed shape and morphology, but it does provide a statically valid sample for determining reliable size distributions. Figure 5 illustrates a chromatogram for a poly

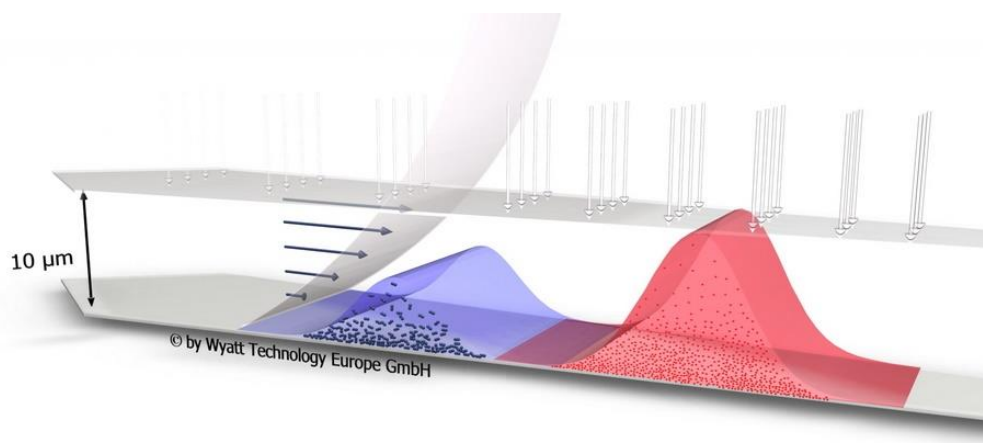


Figure 4. Process of fractionation in an AF4 flat channel during sample elution with cross-flow and channel flow.

ethylene glycol suspension of gold nanoparticles (AuNP). Individual AuNPs, nominally 10, 20, 30, 40, and 60 nm, were fractionated with Wyatt's AF4 system, the Eclipse DualTec™, and characterized using online DLS (DAWN HELEOS II with WyattQELS). Each fraction is successfully separated and clearly resolved. Separation and characterization of AuNPs by AF4, MALS and DLS has been studied exhaustively [3].

Light scattering is an essential element of nanoparticle characterization. However, its reliability may be limited at ultra-low concentrations of smaller particles, where validation with an additional particle sizing technique may be in order. Single-particle inductively-coupled mass spectroscopy (SPICP-MS) is another means of measuring the size of metallic nanoparticles such as gold or silver, as well as their composition. AF4 + SPICP-MS has been used to examine the size and chemical composition of nanoparticles found in foods as well as the type of metals associated with nanoparticulate fractions recovered from natural sources such as lakes and streams.

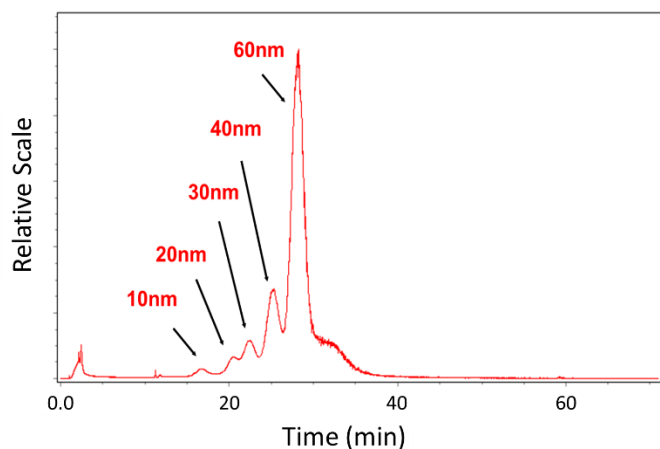


Figure 5. Fractionation and characterization of gold nanoparticles in aqueous solution with FFF and DLS.

The combined utility of AF4-LS-ICP-MS has been particularly powerful in assessing nanoparticle size and composition in the area of food packaging applications. Silver nanoparticles have been shown to be effective antimicrobial

additives, and some food packaging containers are now being manufactured with these particles incorporated into liners with the intention of preventing food spoilage. Food scientists can sample the food product using AF4-LS-ICP-MS to determine whether the nanoparticles remain in the packaging liners or have migrated into the food.

Combining AF4 with ELS

A novel characterization scheme has been demonstrated recently in our lab, combining AF4 separation and electrophoretic light scattering to determine nanoparticle electrophoretic mobility by size with high resolution. The AF4-ELS apparatus included an Eclipse Dualtec AF4 system, Möbius ELS/DLS instrument and an Optilab T-REX refractive index detector (Wyatt Technology) to measure concentration. Figure 6 presents the results of AF4 separation + flowing ELS and DLS measurements of polystyrene latex nanoparticle standards, along with particle concentrations. The heterogeneity of the 50 nm diameter standard is evident, along with the inverse relationship between mobility and size. AF4-ELS promises to be an important method for better characterization of heterogeneous nanoparticle samples.

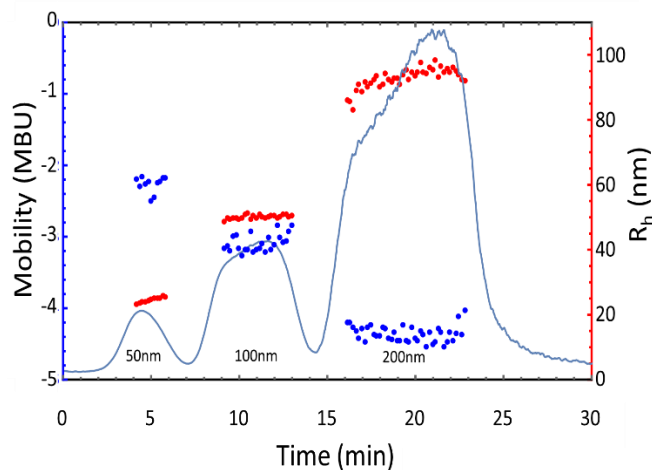


Figure 6. Electrophoretic mobility and size measurements of polystyrene latex nanoparticle standards separated by AF4.

Conclusion

The light scattering toolkit provides essential analytical instrumentation for the characterization of nanoparticles combined with unparalleled levels of automation, sensitivity and resolution. In unfractionated mode, DLS, MALS and ELS. The real advantage to FFF technology comes from joining the fractionation with online detection, including multi-angle and dynamic light scattering analysis for size and conformation determination. Adding additional downstream detection such as ICP-MS or ELS, or spectroscopic detectors such as UV/Vis/NIR absorption or fluorescence, increases the amount of information about the sample. This versatile suite of techniques is essential to scientists and engineers working in nanoparticle characterization.

References

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- [2] D. H. Leung, D. J. Lamberto, L. Liu, E. Kwong, T. Nelson, T. Rhodes, A. Bak. *A new and improved method for the preparation of drug nanosuspension formulations using acoustic mixing technology.* **Int. J. Pharm.** (2014) 473(1-2):10-19.
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