Is Multi-Angle Instrumentation Essential For MW Measurements?

The dogma that multi angle instrumentation is essential for absolute molecular weight measurements is a common misconception, left over from the days when light scattering instruments were not sensitive enough to measure small particles. Modern static light scattering instruments, such as the Zetasizer Nano system, are quite capable of measuring small particles well below the upper size limit for Rayleigh scattering, wherein the scattering profile is independent of the detection angle.

When light (of a non-absorbing frequency) interacts with matter, an oscillating dipole is induced. Inherent to this oscillating dipole is acceleration of charge, which necessitates the release of energy. For visible wavelength light, this energy release is in the form of scattered light. Particles much smaller than the wavelength of the incident light are defined as Rayleigh scatterers. As shown in the Figure 1, the scattering profile for a small Rayleigh scattering particle is independent of the scattering angle (θ).

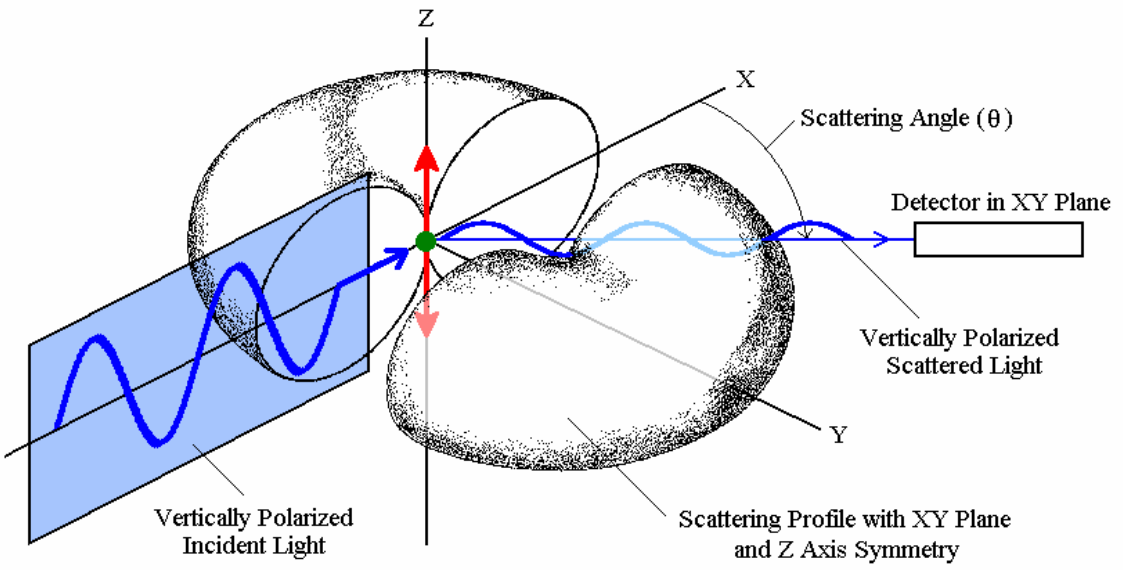


Figure 1: Schematic describing the absence of an angular dependence in the scattering profile for a small Rayleigh scattering particle.

As the particle size increases, so does the probability that multiple photons will interact with the particle simultaneously, thereby inducing multiple dipoles. Each of these dipoles will "scatter", and as the waves are combined at the detector, an angular dependence is observed, due to constructive and destructive interference effects. Figure 2 shows a schematic describing the angular dependence of the scattering of large "non-Rayleigh" scattering particles.

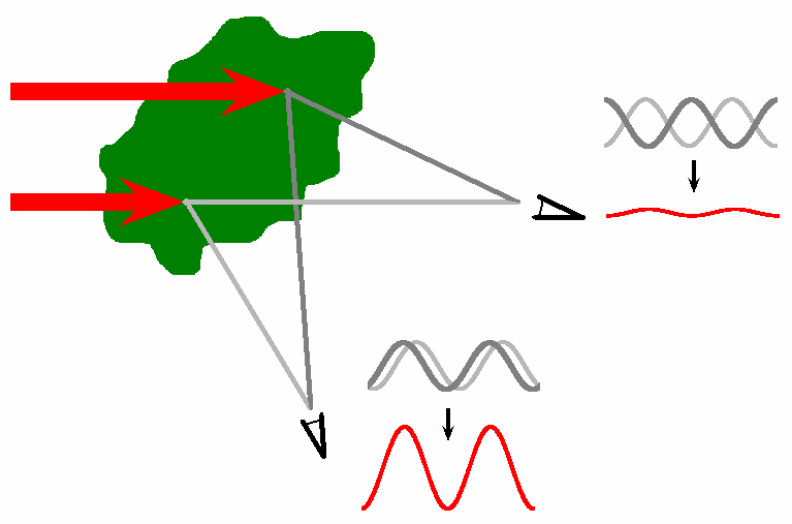


Figure 2: Schematic (top view) showing the constructive and destructive interference effects of multiple dipoles on the measured scattering intensity of a large particle.

The question then of whether or not multi-angle light scattering instrumentation is essential for molecular weight measurements can be reduced to the following:

If the particle is large, Mie scattering should be assumed and multi angle instrumentation should be used for molecular weight measurements. If the particle is small however, Rayleigh scattering can be assumed and multi-angle instrumentation is redundant.

Defining the boundary between "large" and "small" however, is a bit more complex, and is best approached by examining the error in the molecular weight introduced by a single angle limit. The Rayleigh expression describing the intensity of light scattered from a particle in solution is given below, where K is an optical constant, C is the particle concentration, R_θ is the Rayleigh ratio of scattered to incident light intensity, M is the weight average molecular weight, A_2 is the 2nd virial coefficient, $1/P(\theta)$ is the angle dependent term, R_g is the radius of gyration, λ_o is the vacuum wavelength of the incident light, θ is the scattering angle, N_A is Avogadro's number, $\tilde{n}_o$ is the solvent refractive index, and $d\tilde{n}/dC$ is the solvent and analyte dependent refractive index increment.

$$\frac{KC}{R_\theta} = \left(\frac{1}{M} + 2A_2C\right)\frac{1}{P(\theta)}$$

$$K = \frac{2\pi^2}{\lambda_o^4 N_A}\left(\tilde{n}_o \frac{d\tilde{n}}{dC}\right)^2$$

$$\frac{1}{P(\theta)} = 1 + \frac{16\pi^2 \tilde{n}_o^2 R_g^2}{3\lambda_o^2}\sin^2\left(\frac{\theta}{2}\right)$$

In static light scattering measurements, the molecular weight is determined using a Debye plot of KC/R_θ vs. C. For large particles, a Debye plot will consist of a group of parallel lines, one for each angle, with the molecular weight being determined from the Y intercept in the limit of $\theta = 0$, where $1/P(\theta) = 1$. For small particles, where $R_g << \lambda$, the collection of parallel lines

will condense to a single line, since 1/P(θ) = 0 for all θ. For single angle measurements, the error in the measured molecular weight can be calculated using the expression shown below, where $M_{Apparent}$ is the inverse of the intercept of a single angle Rayleigh plot, and M_{True} is $M_{Apparent}$ * 1/P(θ).

$$\%E_M = \frac{|M_{Measured} - M_{True}|}{M_{True}} \times 100 = \frac{\left|\frac{1}{b} - \left(\frac{1}{b}\right)\left(1 + \frac{16\pi^2 \tilde{n}_o^2 R_g^2}{3\lambda_o^2}\sin^2\frac{\theta}{2}\right)\right|}{\left(\frac{1}{b}\right)\left(1 + \frac{16\pi^2 \tilde{n}_o^2 R_g^2}{3\lambda_o^2}\sin^2\frac{\theta}{2}\right)} \times 100$$

$$\%E_M = |P(\theta) - 1| * 100$$

For a given optical configuration and set of experimental conditions, all of the parameters in the above %Error expression are known. As such, a plot of the %Error in a single angle MW measurement vs. particle size can be generated. Figure 3 shows an example of such a plot, generated for aqueous globular protein samples, using the NIBS optical configuration in the Zetasizer Nano ZS system.

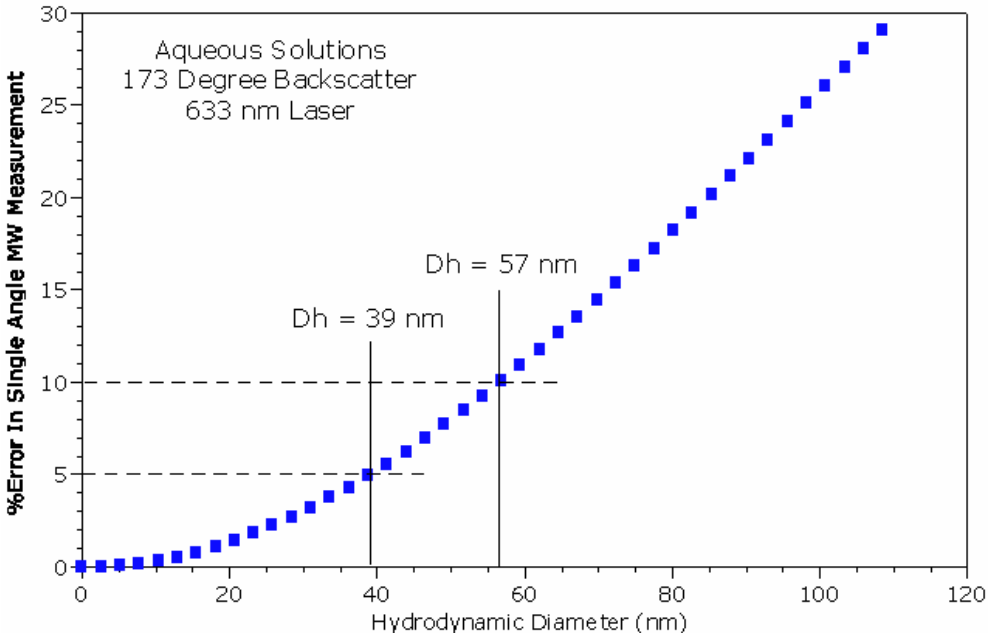
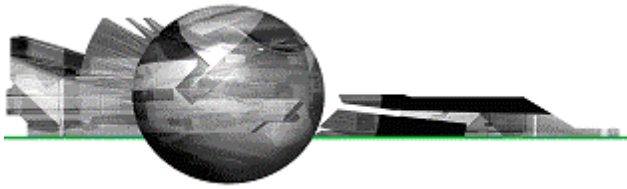


Figure 3: The %Error in single angle molecular weight measurements as a function of the hydrodynamic diameter for aqueous globular protein samples measured with a Zetasizer Nano ZS system.

As seen in the above figure, the error in a single angle molecular weight measurement is < 5% for globular proteins up to 39 nm in hydrodynamic diameter. This size corresponds to a molecular weight on the order of 3.5 million Daltons (3,500 kDa), which would included the vast majority of protein samples. If an error of 10% is acceptable, then the upper limit for assumed Rayleigh scattering is 57 nm on the hydrodynamic diameter.



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