Why are DLS measurements in high concentration solutions difficult?

Brookhaven Instruments • Holtsville, New York

Dynamic Light Scattering (DLS) is an effective measurement technique used for measuring the hydrodynamic size of common nanomaterials including colloids, nanoparticles, proteins, and polymers. Despite the versatility of this technique, there are several important considerations that cannot be ignored when using light scattering to characterize high-concentration solutions. While it is possible to make measurements on high volume-fraction samples without dilution, it raises additional questions about the meaning of the hydrodynamic size. To understand why this is the case we need to discuss two effects encountered in concentrated solutions: multiple scattering and mutual diffusion.

Equivalent Hydrodynamic Size

The size obtained from DLS is a hydrodynamic size, which is derived from measurements of particle diffusion. All freely diffusing particles are constantly undergoing Brownian Motion in proportion to thermal energy expressed as the product of the Boltzmann constant by the temperature (k_bT). A single particle would experience Brownian Diffusion in inverse proportion to its size, thus the diffusion coefficient, D_T, of this particle can be used to calculate its hydrodynamic diameter, d_H, as shown in the Stokes-Einstein equation:

$$D_T = k_b\, T\, /\, 3\pi\eta d_H$$

d_H can be obtained if the temperature, T, and viscosity, η, are also known. This single particle diffusion coefficient is the result of self-diffusion; therefore, in the limit of infinite dilution, this calculated size is fully equivalent to its hydrodynamic size. In the simplest case a single decay rate, Γ, is extracted from the

DLS autocorrelation function (ACF). This Γ is the reciprocal of the characteristic relaxation time, τ_t, such that:
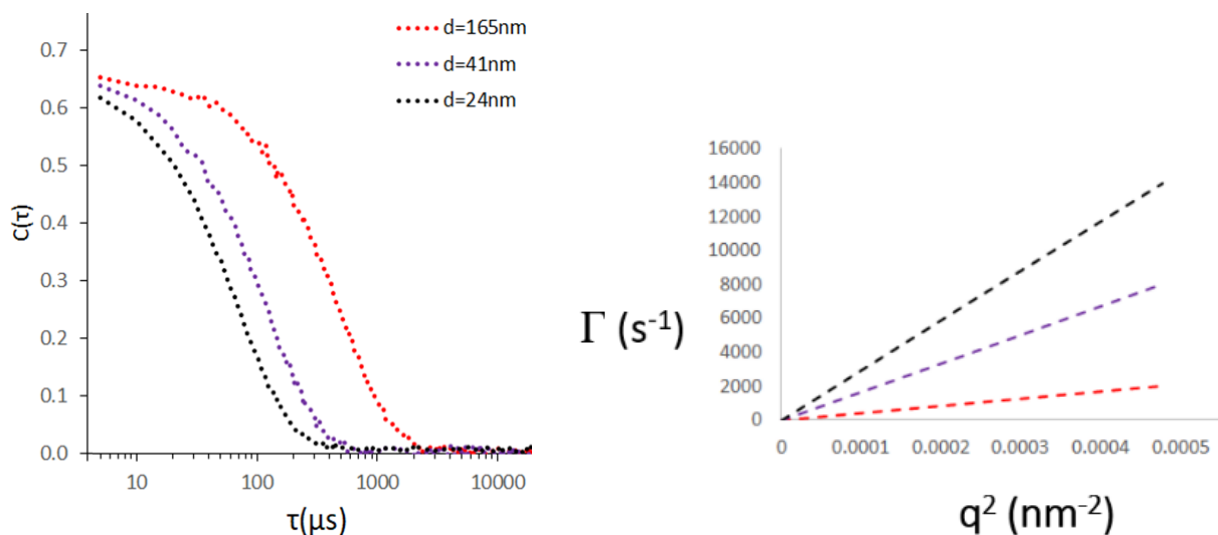
$$g^{(1)}(\tau) = \exp(-\Gamma\tau)$$

Figure 1.0 *Simulated ACF's for decay rates of Γ = 7000, 4000, and 1000 s^{-1} (corresponding to 24, 41, and 165 nm diameter spherical particles). The relationship between Γ, and hydrodynamic size will be addressed in the following sections.*

Note the most rapid decay corresponds to the smallest effective particle size, which in the dilute case corresponds to the diffusion coefficient of a single particle. The diffusion coefficient would come from the slope of the above plot, although it is extremely common to measure at a single q, as is the case with fixed-angle DLS measurements. For the vast majority of compact globular particles, a single scattering angle can still be used to obtain hydrodynamic size.
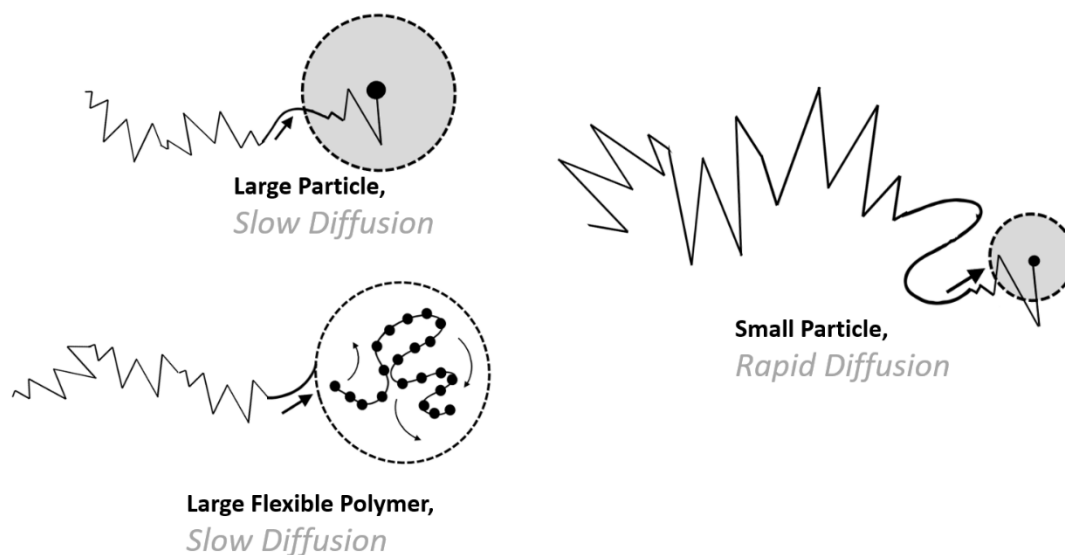
Brookhaven Instruments
750 Blue Point Road
Holtsville, NY 11742
Phone: +1 (631) 758-3200
Fax: +1 (631) 758-3255
E-mail: info@brookhaveninstruments.com
Web: brookhaveninstruments.com

Brookhaven Instruments, a Nova Instruments Company

NOVA INSTRUMENTS

Figure 1.1 *Given two spherical particles, the larger particle will diffuse more slowly. Also of note, that flexible polymers frequently have multiple modes of relaxation corresponding to diffusion and also of internal motion of the polymer chain.*

The rate of diffusion of the particle factors directly into the computed size, where rapid decay rates correspond to smaller particles, and lower decay rates correspond to larger particles. For a given q value, Γ is related to the translational diffusion coefficient, D_T, via the following expression:

$$D_T = \Gamma / q^2$$

For the sake of this calculation, all decays are treated as resulting purely from diffusion, and all diffusion coefficients are treated as though they result from the *thermal motion of a single particle*. This is often not the case, and important exceptions are noted below. *This becomes more complicated when interactions with other particles are considered.*

Self-Diffusion vs. Mutual Diffusion

The concept of infinite dilution is an abstraction; you cannot measure a real sample at a theoretical limit. By making serial dilutions of a concentrated solution or suspension, you incrementally dilute away

interactions between particles. By reducing these interparticle interactions the effect of mutual diffusion is minimized, and thus the mutual diffusion coefficient approaches the value expected for self-diffusion as concentration is lowered. At excessively high concentrations, the measured diffusion coefficient will not result from self-diffusion; thus, while it can be measured, the size computed would not be the true particle size. *This size would not appear to be constant with respect to concentration.*   Artificially lower (or higher) sizes would result, depending on the type of interaction.

Multiple Scattering

If sample concentration is high enough, a single photon of laser light will scatter off multiple particles sequentially before reaching the detector. The longer the pathlength, the more secondary scattering events occur, and the less correlated the detected light is to the original signal. In contrast to dilute solution conditions, this rapid loss of correlation is not the result of particle motion, and thus not relatable to particle size. See figure 1.2.
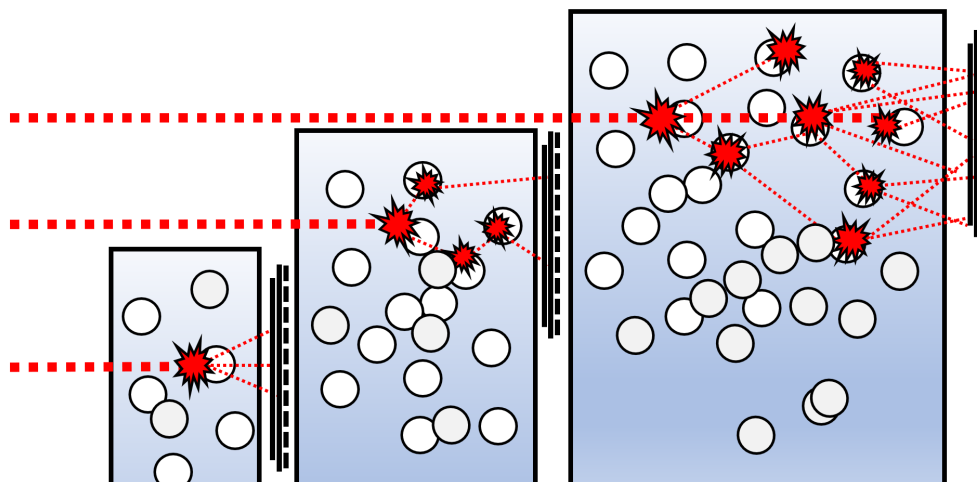

Figure 1.2 Multiple scattering is more pronounced as pathlength is increased

Thus, in the case of multiple scattering, the signal appears to decay faster, therefore this larger decay rate would appear to be rapid diffusion. In reality, this is a *non-diffusive* decay; thus, not only is the computed size not a size, the diffusion coefficient it is being calculated from is not a real diffusion coefficient. Samples showing multiple scattering will skew towards smaller sizes since $d_H \propto k_b T/ D_T$. The extent of this skew will also depend heavily on concentration, such that the apparent size will decrease systematically as the signal becomes dominated by multiple scattering.

Particle-Particle Interactions:

One option for reducing multiple scattering is to minimize the effective pathlength, and thus the amount of sample in the beam path. However, eliminating multiple scattering by using a shorter pathlength fixes one problem, but not the other. While it becomes possible to make a measurement at very high concentrations, this does not eliminate the effect of particle-particle interactions.

The decay, or loss of correlation, is no longer artificially rapid, but direct interactions between particles still prevent measurement of a true single-particle property: particle size. There are several significant types of inter-particle interactions: repulsion, attraction, clustering, or any other type of forces that can produce coupled motion. You are once again not measuring true size, even though you are now able to obtain a valid diffusion coefficient.
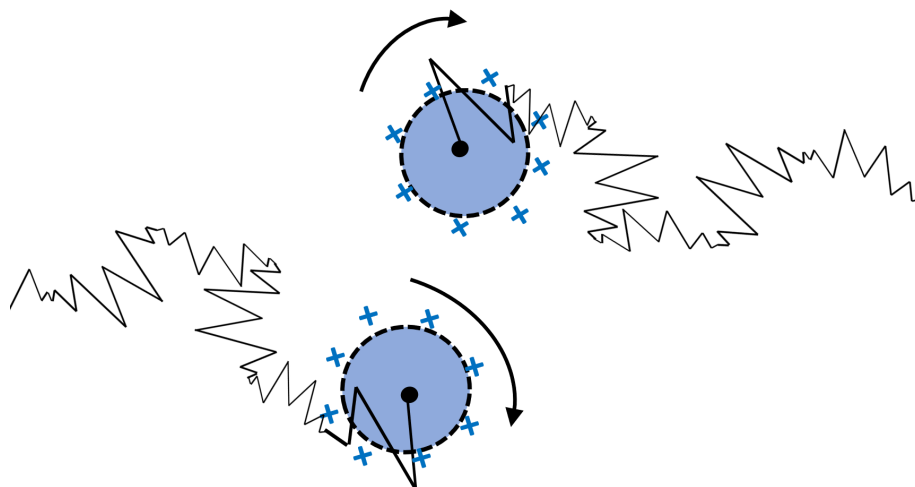

Figure 1.3 *One significant and common interaction is charge at the particle surface as shown for positively charged colloidal particles (above) where like-charges cause repulsion between particles.*

At high concentrations, it is no longer possible to ignore the interaction potentials between particles; whether the potential is attractive or repulsive, it still results in a form of mutual diffusion as the motions of multiple particles can no longer be decoupled. The initial relationship between diffusion coefficient and concentration, c, can be expressed as $D_T(c) = D_0(1+kc)$ where k is a concentration coefficient that is related to the strength of the interaction. Higher order terms in c may occur, but to obtain D_o, the true self-diffusion coefficient and the true size, extrapolate from the linear region in c.

True Size

What is dilute enough? Working in The Dilute Limit.

Serial dilution is an effective way to determine if a sample is sufficiently dilute to make a reasonable DLS measurement. As you approach the dilute limit, the apparent particle size converges on a limiting, or invariant, hydrodynamic size. As shown for the following flexible polymers: Sodium Polystyrene Sulfonate (NaPSS) and Polyvinylpyrrolidone (PVP).

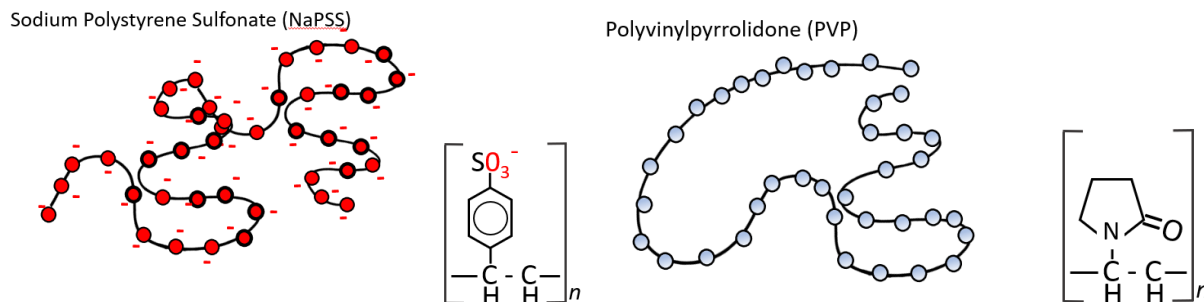Sodium Polystyrene Sulfonate (NaPSS)

Polyvinylpyrrolidone (PVP)

Figure 2.0 The two flexible polymers being compared are NaPSS and PVP. NaPSS has a high linear charge density, due to the presence of permanent negative charges. In contrast, PVP is uncharged. Both samples are of similar Mw and chain length.
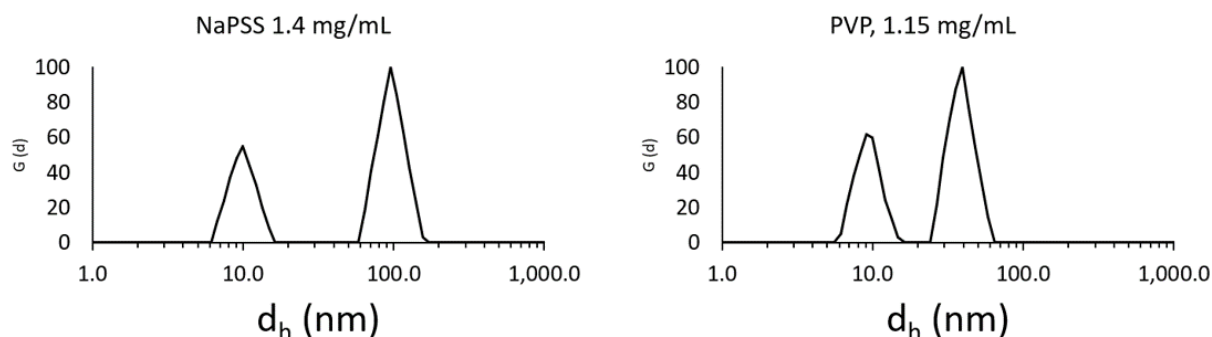

Figure 2.1 Even at high dilution a second mode is observed in the size distribution, with hydrodynamic sizes on the order of 10nm.

Given its high charge density NaPSS has extremely strong particle-particle interactions, resulting from electrostatic repulsion between sidechains. Plotting diffusion coefficient as a function of concentration for the two polymers gives us the following plots:
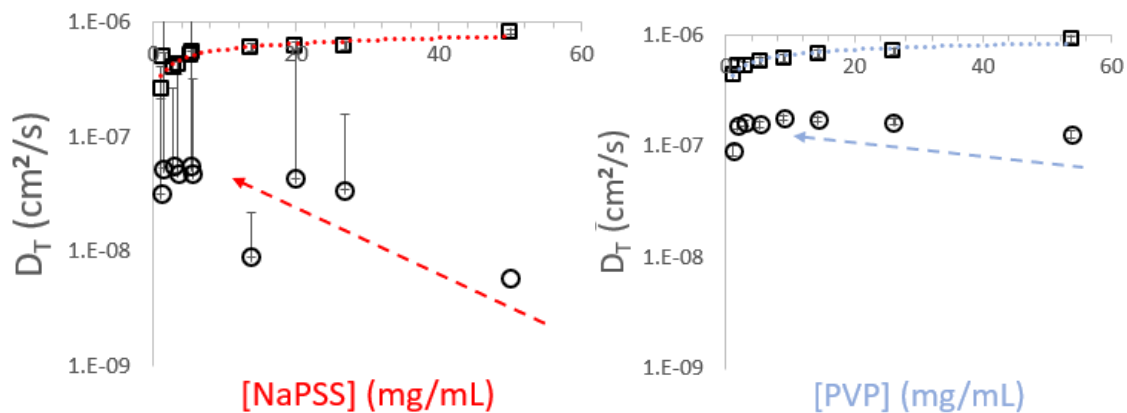
Figure 2.2 *Dilution of NaPSS and PVP from starting concentrations of 50 mg/mL continued until the measured diffusion coefficients began to approach constant values. The fast modes, $D_T > 1x10^{-7}$ cm^2/sec, corresponds to the size of an individual polymer chain.*

Highest concentrations measured for both are on the order of 50 mg/mL, both solutions are fully optically transparent, and yet both exhibit signs of multiple scattering and intense particle-particle interactions. Starting at the highest concentrations, both polymer solutions show bimodal size distributions. The mode corresponding to the larger diffusion coefficient gradually approaches a limiting value as concentration is lowered. The mode corresponding to the lower diffusion coefficient falls off rapidly between 50 mg/mL and around 15 mg/mL. This effect is more pronounced for NaPSS, unsurprising considering the larger interaction potential, where high negative linear charge density gives rise to strong repulsive interactions enhancing inter- and intra- molecular interactions, especially in comparison to PVP which is an uncharged linear polymer of similar molecular weight.

Extrapolation of the fast mode to zero concentration gives hydrodynamic diameters approaching 10nm for PVP and between 10-13nm for NaPSS, values expected from their respective molecular weights. At the highest concentrations, both polymers start out above their critical overlap concentrations, C*, the volume fraction above which polymer chains are no longer spatially separated from one another in solution. This critical concentration depends on the molecular weight, chain length, and radius of gyration of the polymer chain. Only as the lower end of the semi-dilute region is approached, does the calculated hydrodynamic size actually correspond to the dimensions of the chains.

Summary

Two major factors impact our ability to measure particle size at high concentrations: <u>multiple scattering</u> and <u>interparticle interactions</u>. Measurements made in dilute solution or suspension can be used to reliably calculate particle size directly. Even in the absence of multiple scattering, measurements made in the semi-dilute region do not necessarily reveal particle size, and thus great care must be used to interpret such results. It is also demonstrated that serial dilution can be used to identify the region in which the sample is sufficiently dilute to measure.

Be sure to stay tuned for the next entry in our **Learning Corner** series. Contact us at learningcorner@brookhaveninstruments.com for more info.