Whitepaper

An Introduction to DLS Microrheology

Microrheology techniques involve tracking the motion of dispersed probe (or tracer) particles in a complex fluid, to extract local and bulk rheological properties of the matrix. Analogous to mechanical rheometry techniques, a stress is applied to the system by motion of the probe particle, and the deformation (or strain) is measured through changes in the probe particle position.

Dynamic Light Scattering (DLS) Microrheology is classified as a passive technique, whereby the colloidal probe particles undergo thermal fluctuations in a system at thermodynamic equilibrium. The Mean Square Displacement (MSD) of the probe particles with time is followed by DLS, to enable linear viscoelastic parameters for the complex fluid matrix to be extracted.

DLS Microrheology offers significant measurement advantages for low-viscosity, weakly structured complex fluids since it offers a much wider frequency range than conventional mechanical rheometry (fundamentally limited by inertia), and can access the very high frequencies required to measure the critical (short timescale) dynamics of such low-viscosity materials. DLS Microrheology also requires very small sample volumes—microliter-scale volumes are possible—and enables rheological characterization of material types not available in larger volumes, e.g., protein-based formulations.

This paper introduces Microrheology techniques for the rheological characterization of soft complex fluids, with subsequent emphasis on DLS Microrheology and the underlying theory. It goes on to review the applicability of DLS Microrheology measurements, and some important practical aspects of method development to ensure robust data. Finally, some example measurements are shown, including the high-frequency viscoelastic characterization of a polymer solution, and the use of the technique for protein solutions, both as a method for making viscosity measurements over an industrially relevant concentration range, and for evaluating the onset of aggregation and structure development in denaturing systems.

Microrheology overview

Rheological characterization of complex fluids

Rheology is the study of flow and deformation of materials under applied stress. The limits of material behavior are the ideal (Newtonian) fluid, which is characterized by a viscosity and has negligible elasticity, and the ideal (Hookean) solid, which is characterized by an elastic modulus and does not flow (in the limits of experimentally accessible timescales).

Between these extremes are a vast array of soft material types that exhibit a combination of viscous and elastic responses, or viscoelasticity (and have a non-Newtonian shear rate-dependent viscosity). Depending on the timescale of the applied deformation, these materials can both store energy (an elastic response) and dissipate energy (a viscous response).

Consider the class of materials termed "complex fluids"—including synthetic and biopolymer or protein solutions, surfactant systems (with their wide variety of self-assembled phases), and dispersions (colloidal and non-colloidal suspensions and emulsions). While these complex fluids have a liquid-base, they also encompass supramolecular structures formed by the constituent polymer molecules or dispersed-phase particulates, and it is these microstructures that convey viscoelastic properties. Not only do such fluids have a range of rich dynamics (over multiple length scales and timescales) that continue to drive academic studies—they are also ubiquitous in many everyday products including foodstuffs, personal care, household, and industrial chemicals, and their rheological properties determine processing behavior and end-use product performance.

Conventional rheological techniques, such as a rotational rheometer, measure the bulk shear flow or deformation properties of materials by using a mechanical system to impose a controlled force or displacement onto a sample, of typically milliliter-scale volume. The sample response to the applied deformation is recorded, enabling properties such as shear rate-dependent viscosity and frequency-dependent linear viscoelastic moduli to be measured (i.e., G'—the elastic [or storage] modulus and G''—the viscous [or loss] modulus). A modern rotational rheometer is a highly sophisticated instrument that is capable of measuring material properties over multiple decades, but there are inherent limitations to mechanical techniques that inhibit certain types of material characterization.

Consider low-viscosity materials—of the order of a few mPas, e.g., synthetic polymer or protein solutions—where macromolecular relaxation times are of the order of milliseconds, and therefore high frequencies are required to characterize their viscoelastic response. Accessing the dynamics of such materials is impossible with a rotational rheometer, where oscillation measurements become dominated by instrument inertia from around 100 rads⁻¹, which is orders of magnitude below the frequencies required to probe timescales relevant to molecular relaxation times.

The relatively weak modulus and often highly strain-sensitive structures that are developed in many complex fluids require very low stresses for linear viscoelastic characterization—typically only a tiny fraction of the lowest torque range available from a rotational rheometer, which is optimized for testing over multiple decades of torque. Now include sample volume restrictions down to microliter-scale—as can occur with high-value, early-stage protein-based formulations, for example—and the

demands on ultralow torque and deformation control are simply outside the capabilities of a traditional mechanical rheometer system.

Microrheology techniques

The term Microrheology is used to describe a range of techniques that extract local and bulk rheological properties of soft materials by measuring and analyzing the motion of embedded colloidal probe (or tracer) particles in the sample.

While the "micro" in Microrheology refers to the size of the (typically) submicrometer probe particle used to investigate microscale dynamics, it also points to the microliter scale sample volumes that these techniques make accessible. In this latter respect, the term Microrheology can also be used in relation to microfluidic-based measurements, where complex fluids are subjected to controlled flow and deformation regimes in sub-millimeter scale channels to extract rheological properties. While microfluidic techniques are another area of microscale rheological characterization with significant research activity, this paper addresses embedded probe particle-type Microrheology techniques only.

Passive and active probe-based Microrheology

From the description of embedded probe-based Microrheology techniques, two types of measurements can be defined:

- Passive Microrheology—linear rheological properties are extracted from the motion of colloidal probe particles undergoing thermal fluctuations in a system at thermodynamic equilibrium, i.e., no external forces are exerted on the probe particles. (Passive Microrheology can also be described as thermal diffusion Microrheology.) Relationships have been derived that enable quantitative rheological data over a wide range of frequencies, and which have been shown to hold, in general, across a variety of complex fluid types.

- Active Microrheology—rheological properties are extracted from the forced motion of colloidal probe particles in the system, which can extend measurements from the linear into the non-linear regime.

Note that moving into the non-linear regime poses challenges for theory and interpretation, as the non-equilibrium state of the sample microstructure has to be understood to relate probe motion to the underlying rheological properties. External drive forces on the probe particles have included the use of laser tweezers to exert optical forces, or magnetic tweezers. Active Microrheology techniques can also extend into the use of multiple particles (two-particle correlation).

Generally, passive techniques are advantageous for measuring weakly structured materials which have low values of predominantly viscous modulus, whereas active techniques better facilitate measurements on materials which have significant elastic properties.

Measuring probe particle behavior

An additional classification for Microrheology measurements can be made based on the method used to measure the behavior of the embedded probe particles in the sample under investigation:

- Light scattering Microrheology describes the case where the average motion of an ensemble of probe particles is determined by a scattering technique. Dynamic Light Scattering (DLS) is applicable for samples with optical characteristics ranging from transparent to slightly turbid, such that the condition of single scattering is maintained from the dispersed probe particles. Measurements can be extended into opaque materials by the use of Diffusing Wave Spectroscopy (DWS), which is applicable in the opposite limit of multiple scattering.

- Particle tracking Microrheology describes the case where the motion of individual probe particles is followed using a video-microscope, and the probe particle tracks are subsequently analyzed using image processing software. The image processing aspects can be the most challenging practically, although using Microrheology techniques where the imaging and tracking of a single probe particle only is required can be much more efficient, and offer the benefit of very good spatial resolution.

Literature reviews

The above brief overview of Microrheology fits with the scope of this paper, but in an active and developing field of research, several review papers have been published in the scientific literature that provide extensive background and references to all Microrheology methods and the applications that have been targeted.[1-4]

Theory of DLS Microrheology

Dynamic Light Scattering (DLS), sometimes referred to as Photon Correlation Spectroscopy (PCS) or Quasi-Elastic Light Scattering (QELS), is a commonly used technique for measuring the size of particles suspended in a liquid, typically in the sub-micrometer regime.[5] DLS actually measures the Brownian motion of the particles in the liquid, and the technique is the founding basis of embedded probe particle Microrheology techniques.

The Generalized Stokes-Einstein Relation

For a particle moving freely due to thermal fluctuations in an ideal viscous (Newtonian) fluid, the Mean Square Displacement (MSD) increases linearly with time, with the slope of this increase given by the diffusion coefficient of the particle[6]:

$$\left\langle \Delta r^2(t) \right\rangle = 2dDt \tag{1}$$

where $\left\langle \Delta r^2(t) \right\rangle$ is the MSD, d is the dimensionality of the motion (2 for motion in a plane, 3 for motion in space), and D is the diffusion coefficient of the particle.

In a purely viscous (Newtonian) system, the diffusion coefficient can be written as a function of the size of the particle, the viscosity of the fluid medium, and the temperature using the Stokes-Einstein Relation:

$$D = \frac{k_B T}{3\pi \eta a} \tag{2}$$

where a is the diameter of the particle, η is the viscosity of the fluid medium, T is the temperature, and k_B is the Boltzmann constant. The Stokes-Einstein Relation therefore links the MSD of the particle to the fluid's viscosity.

However, for viscoelastic (non-Newtonian) complex fluids, where the medium exhibits both dissipation of energy (viscosity) and storage of energy (elasticity), Eq. (2) does not describe the full behavior of the system. As the elasticity of the suspending medium becomes significant, particle motion becomes sub-diffusive and deviations from linearity in the slope of the MSD are evident. The validation of a more general approach to extend the strategy of linking MSD to measure the linear viscoelastic moduli of complex fluids using DLS formed the basis of modern Microrheology.[7]

A generalized Langevin equation is used to describe the thermally driven motion of a particle dispersed in a complex fluid[8,9]:

$$m\dot{v}(t) = f(t) - \int_0^t \zeta(t-\tau)v(\tau)d\tau \qquad (3)$$

where m is the mass of the particle, $\dot{v}$ is the particle acceleration, f is the total force acting on the particle, and ζ is a time-dependent memory function that accounts for the elasticity in the system. Solving the equation of motion for the case where the only forces on the particle are due to thermal fluctuations in the system yields the Generalized Stokes-Einstein Relation[7,8] that relates the Mean Square Displacement (MSD) of the tracer to the viscoelastic modulus of the complex fluid medium surrounding the particle:

$$\widetilde{G}(s) = \frac{k_B T}{\pi a s \langle \Delta \tilde{r}^2(s) \rangle} \qquad (4)$$

where the tilde symbol represents the Laplace transformation, and s is the Laplace frequency; the rest of the variables are as in Eq. (2). $G*$ is the viscoelastic modulus of the system as experienced by the probe particle. Eq. (4) can be recast in Fourier space by using the identity $s = i\omega$ to obtain:

$$G*(\omega) = \frac{k_B T}{\pi a i \omega \langle \Delta r^2(i\omega) \rangle} \qquad (5)$$

where the angular frequency ω is given by $\omega = 2\pi/t$.

The Generalized Stokes-Einstein Relation is based on the assumption that the complex fluid medium is a continuum around the particle, i.e., the length scales of the microstructures in the complex fluid are small in comparison to the size of the particle.

Extracting rheological data from DLS measurements

In a Dynamic Light Scattering experiment, the autocorrelation function (ACF) of the light scattered by particles undergoing thermally driven motion within a material under study is measured. It can be shown that the ACF, $g_1(\tau)$, can be written as a function of the Mean Square Displacement (MSD) of the scattering particles thus[9]:

$$g_1(\tau) = g_1(0)\exp\left[-q^2\langle \Delta r^2(\tau) \rangle / 6\right] \qquad (6)$$

where $g_1(0)$ is the value of the autocorrelation function at zero time (or intercept), and q, the scattering vector, is given by:

$$q = \frac{4\pi n}{\lambda}\sin(\theta/2) \qquad (7)$$

where n is the refractive index of the medium, λ is the wavelength of the light, and θ is the scattering angle. The correlation time τ is related to the time t by $\tau = 2\pi t$, so that $\omega = 1/\tau$.

A DLS Microrheology experiment therefore requires the addition of probe (tracer) particles of a known size into the medium of interest, in order to measure the ACF of the light scattered by the probe particles. Then, from Eq. (6), the following relation between the MSD of the probe particles and the ACF of the scattered light is obtained:

$$\langle \Delta r^2(\tau) \rangle = \frac{6}{q^2}\left[\log(g_1(0)) - \log(g_1(\tau))\right] \qquad (8)$$

where $g_1(0)$ is the estimated intercept of the value of the autocorrelation function, and q is the scattering vector as defined above. In general, a large value of the intercept (above 0.8) indicates the absence of multiple scattering (scattering from the probe particles is expected to be high in comparison to background scattering from the suspending medium).

The viscoelastic modulus can be obtained by calculating the Fourier transform of the MSD and substituting it in Eq. (5). However, since the correlation data is usually logarithmically spaced in time, and doesn't lend itself to the usual methods of obtaining the Fourier transform, a method based on a power law expansion of the MSD can be used instead.[8] This method estimates the complex shear modulus algebraically by expanding $\langle \Delta r^2(t) \rangle$ locally around $t = 1/\omega$ to give:

$$\langle \Delta r^2(t) \rangle \approx \langle \Delta r^2(1/\omega) \rangle (\omega t)^{\alpha(\omega)} \qquad (9)$$

where $\langle \Delta r^2(1/\omega) \rangle$ is the magnitude of $\langle \Delta r^2(t) \rangle$ at t = 1/ω and:

$$\alpha(\omega) = \frac{d\ln\langle \Delta r^2(t) \rangle}{d\ln t}\bigg|_{t=1/\omega} \qquad (10)$$

is the power law exponent describing the logarithmic slope of $\langle \Delta r^2(t) \rangle$ at $t = 1/\omega$.

For thermally driven motion of the probe particles, the slope of the logarithmic time derivative of the MSD will be one in a purely viscous medium (diffusive motion), zero in an elastic medium (completed arrested motion), and will lie between these extremes in a complex viscoelastic fluid medium.

Evaluating the Fourier transform of the power law behavior of the MSD, substituting into Eq. (5) and using Euler's equation results in expressions for the frequency-dependent viscoelastic moduli G', elastic (storage) modulus, and G'', viscous (loss) modulus[8]:

$$G'(\omega) = G*(\omega)\cos[\pi\alpha(\omega)/2]$$
$$G''(\omega) = G*(\omega)\sin[\pi\alpha(\omega)/2] \qquad (11)$$

where

$$G*(\omega) = \frac{k_B T}{\pi a \langle \Delta r^2(1/\omega) \rangle \Gamma[1+\alpha(\omega)]} \qquad (12)$$

and where Γ denotes the gamma function which is a result of the Fourier transform of the power law behavior of the MSD.

Complex viscosity, **η***, can then be calculated by using the following relation:

$$\eta^*(\omega) = \sqrt{\frac{[G''(\omega)]^2 + [G'(\omega)]^2}{\omega^2}}$$ (13)

Applicability of DLS Microrheology

Consideration of the conditions where either DLS measurements, or the assumptions within the Generalized Stokes-Einstein Relation become unreliable, is necessary to ensure robust and consistent DLS Microrheology data.

From a DLS measurement perspective, it is important to ensure that the dispersed probe particles are the dominant scatterers in the system, and that the condition of single scattering is maintained. As mentioned above, there are certain quality criteria that can be applied, for example, to the autocorrelation function, to highlight appropriate data quality requirements. The use of defined method development procedures is also central to performing valid DLS Microrheology measurements, to evaluate satisfactory dispersion of probe particles, for example.

The Generalized Stokes-Einstein Relation is the underpinning basis of Microrheology techniques, and its general applicability has been shown to hold across a wide range of complex fluid types, enabling quantitative rheological data to be measured across a wide range of frequencies. However, particular cases have been identified where assumptions in the Generalized Stokes-Einstein Relation can break down, and where agreement between Microrheology data and bulk rheological measurements cannot generally be expected.[4]

For DLS Microrheology measurements, the following are some notable cases where assumptions within the Generalized Stokes-Einstein Relation may be infringed, and where agreement with bulk (macro) rheological measurements may not occur:

Influence of probe particle-matrix interactions

A key requirement of a Microrheology experiment is to minimize particle matrix interactions, since the existence of significant physical (e.g., depletion or electrostatic) or chemical interactions of the embedded probe with the surrounding material can alter the local material environment and affect diffusivity in a measureable way. Ensuring an appropriate choice of particle surface chemistry is an essential element of method development for DLS Microrheology measurements for a particular complex fluid.

Influence of probe particle size and material heterogeneity

The probe size used in DLS Microrheology measurements can have a significant impact on the extracted rheological parameters.[10] The probe size should be larger than the relevant microstructural length scale, e.g., mesh size in a polymer network, such that it probes the bulk response, i.e., the assumption of continuum viscoelasticity holds. (Note also that the particle size must not be so large that sedimentation becomes significant on the timescale of the experiment.) Ensuring an appropriate choice of probe particle size to enable bulk material properties to be extracted is another central element of method development for DLS Microrheology measurements for a particular complex fluid.

It is important to note that the ability to probe local material heterogeneity on a wide variety of microstructural length scales with targeted Microrheology techniques enables new information (not available from bulk measurements) to be obtained, for further novel insight into material microstructures and characterization.

Materials with evolving or aging microstructure

A material can be considered as quasi-steady state if the timescale of the material structural evolution is slow compared to (experimental) data sampling times—in which case DLS Microrheology can be used to monitor material structure evolution.

Aggregating or gelling systems close to the gel point

DLS Microrheology is a valuable technique for tracking the onset of aggregation or gelation, due to its sensitivity to probe very soft structures, and discern very low values of viscoelastic modulus.[11] Beyond the gel point, the system becomes nonergodic, and the contribution of the aggregated structures to the overall scattering increases significantly to the point of making single scattering DLS measurements unreliable.

Benefits of DLS Microrheology measurements

For the rheological characterization of weakly structured complex fluids, which have optical characteristics ranging from transparent to slightly turbid—the technique of DLS Microrheology, allied to advances in instrument design, provides the following benefits:

- Measurements require very small sample volumes—typically microliter-scale volumes—and therefore enable rheological characterization of material types not available in larger volumes, e.g., protein-based formulations.

- Laser-based DLS Microrheology probes a much wider frequency range than conventional mechanical rheometry (fundamentally limited by inertia), which enables rheological studies into dilute, weakly structured macromolecular solutions where critical material dynamics occur on very short timescales (or at very high frequencies).

- A DLS Microrheology measurement effectively probes a complete range of measurement frequencies simultaneously by measuring ensemble statistics arising from thermal (Brownian) motion of probe particles, which allows a much faster frequency-dependent rheological characterization method, e.g., for rapid formulation screening.

- By utilizing the Brownian motion of tracer particles, a DLS Microrheology measurement probes the linear dynamics of materials under conditions of very low applied stress (the probe energy is of the order of $k_B T$, where k_B is the Boltzmann constant and T is the temperature), which enables measurements in the linear regime of highly strain-sensitive systems.

- The use of a DLS technique with improved single scattering detection[12] allows the use of a lower concentration of probe particles, which can be an important factor for some biological systems to minimize probe-sample interactions.

- The use of improved single scattering detection[11,12] for DLS Microrheology also allows measurements of more concentrated and slightly turbid solutions, which can be advantageous for characterizing high

concentration protein solutions, for example. DLS Microrheology enables a quick measurement technique for viscosity in protein solutions over an industrially relevant concentration range, and can also detect the onset of aggregation by following the evolution of elasticity in dilute protein solutions.[11]

Practical aspects of DLS Microrheology measurements

The use of a defined experimental procedure is recommended in order to ensure a reliable method is generated for DLS Microrheology characterization of a particular complex fluid type, which avoids the possible measurement pitfalls that have been highlighted as part of the above review.[11]

Method development

The following sequential steps are advised for characterization of a new sample type:

- Assess suitability of probe particle chemistry to minimize particle-matrix interactions—measure and compare zeta potential of probe particles in the base solvent only, and then in the sample.

The zeta potential of a colloidal particle is the electrostatic potential between the dispersant matrix and the layer of fluid attached to the dispersed particle.[13] Zeta potential can be calculated by measuring the electrophoretic mobility of the colloidal particles.

The zeta potential of a colloidal particle will change if the surface properties of the particle change, e.g., if components of the dispersant matrix, such polymer molecules adsorb onto the particle surface.

For a DLS Microrheology measurement—if the zeta potential of the probes in the presence of the sample is significantly different from the value obtained without the sample, this may be indicative of particle-matrix interactions, and different probe chemistry should be investigated.

- Assess suitable concentration of probe particles for measurement in single scattering regime and that probe particles are dispersed properly—add amount of probe particles to sample (e.g., 5 µL of known probe particle dispersion into 500 µL of sample, for example), and measure autocorrelation function and intensity Particle Size Distribution (PSD) of resulting sample.

The intercept of the autocorrelation function with a value >0.8 is indicative of a single scattering regime. The intensity PSD should show a narrow peak in a position corresponding to the size of the probe particles, which indicates the probe particles are dispersed properly, and not aggregated. The scattering signal should be dominated by the probe particle scattering, as compared to any scattering from a matrix network, which can be seen by comparing intensity peaks in the PSD. An appropriate amount of probe particles should be added to the sample to meet the above conditions.

- Measure autocorrelation function (ACF) and Mean Square Displacement (MSD) of probe particles to extract Microrheology data for sample under test.

- Check that the Microrheology data obtained are independent of probe particle concentration and probe particle size—repeat measurements at different probe concentrations and with different probe size (if possible).

Perform Microrheology measurement at two concentrations of probe particles higher than the concentration determined above, to check the MSD obtained is independent of concentration.

Perform Microrheology measurement with a larger probe size, of the same surface chemistry, to check the MSD obtained is independent of probe particle size.

The above DLS Microrheology methodology is relatively straightforward to implement, and, of course, once a particular sample type has been assessed as above for a suitable probe particle type, size, and concentration, then only an ACF measurement needs to be run to extract Microrheology data. It is at this point that the possibility of rheological testing of samples quickly and easily, with minimal volumes (below 20 µL is possible) really opens up.

Example DLS Microrheology measurements
Water
Measurements on an ideal (Newtonian) liquid
- Using DLS to obtain the viscosity of a Newtonian liquid, with dispersed probe (tracer) particles of known size, is straightforward by simply applying the Stokes-Einstein Relation (Eq. [2]) to obtain the viscosity from the diffusion coefficient.

- The measurements on water detailed below utilize the full DLS Microrheology approach to show that there is no frequency dependence of the viscosity.

- Two probe (tracer) particle sizes —60 nm and 200 nm latex—are dispersed in water, and the ACF of the light scattered by both tracer sizes is measured, to enable determination of the MSD.
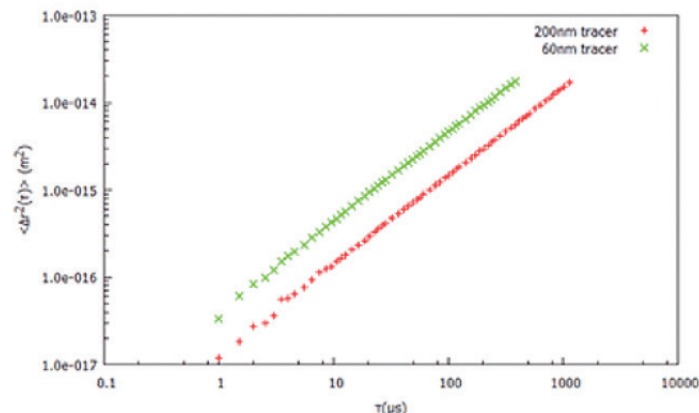

Figure 1 – Mean Square Displacement $\langle \Delta r^2(t) \rangle$ with time for two different probe sizes in water.

- Linear response on log-log scale indicating purely diffusive motion for an ideal Newtonian liquid.

- From the MSD, the procedure described above (using the approach in Ref. 8) is applied to calculate to viscoelastic moduli.
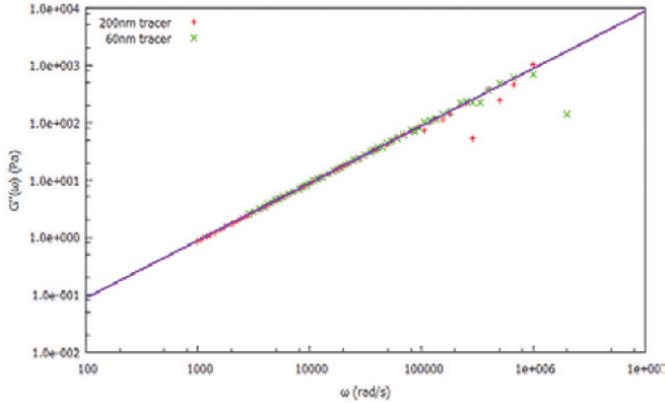

*Figure 2 – Viscous (loss) modulus, **G′ ′**, of water measured using 60-nm and 20-nm tracer particles.*

- Both tracer particles produce essentially the same results—the 60-nm latex extends the data to higher frequencies, whereas the 200-nm latex gives access to lower frequencies.
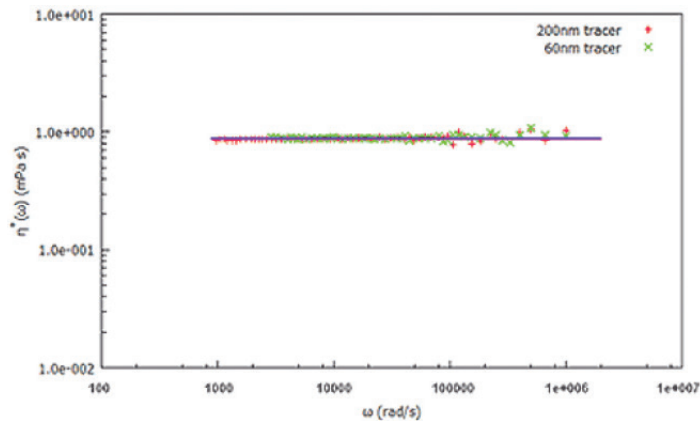

*Figure 3 – Complex viscosity, **η***, for water for the two probe sizes at 25 °C.*

- Frequency-independent behavior as expected from an ideal (Newtonian) liquid.

- Viscosity data is accessible for frequencies in excess of 10^5 rads^{-1}.

Polyethylene oxide (PEO) solution

Viscoelastic polymer solution—PEO can be used as a model system for comparison of DLS Microrheology data and mechanical (rotational) rheology data.

- Measurement data shown here was obtained with 700-nm latex tracer particles in a 2% by weight solution of PEO in water.

- The PEO solutions in water were rolled gently until fully dispersed.

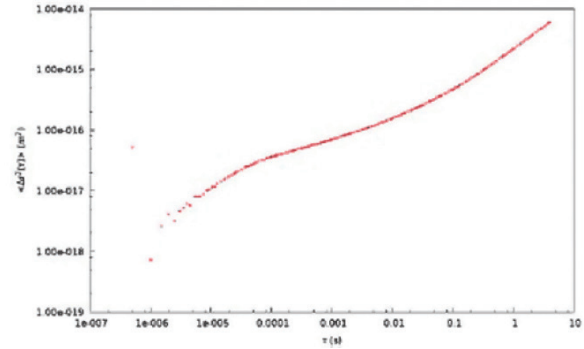- The tracers were added to the fully dispersed PEO solution and again rolled until fully dispersed.

Figure 4 – Mean Square Displacement $\langle \Delta r^2(t) \rangle$ with time for 700-nm tracer probes in 2% PEO solution.

- The MSD of the tracer particles in PEO is not linear with time, due to the viscoelasticity of the sample.

- At short timescales, the MSD behavior is sub-diffusive, indicative of an elastic modulus. At the shortest timescales, the probe particles experience "hindered" motion, and at longer timescales (where the MSD curve trends to a plateau) the probe particles are effectively "trapped" by the elasticity of the system. On the longest timescales, the probe particles are able to "escape" as the material relaxes, i.e., the escape time is the timescale beyond which their motion becomes diffusive in nature, ~ t.

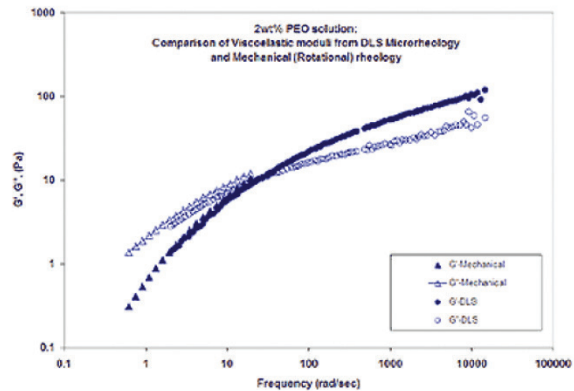*Figure 5 – Viscoelastic moduli (elastic (storage) modulus, **G′,** and viscous (loss) modulus, **G′ ′**) as a function of frequency for a 2wt% PEO solution in water.*

- Data is shown from mechanical (rotational) rheology and DLS Microrheology measurements (using 700-nm latex probe particles).

- Good agreement is shown for data in the region of overlap for the two techniques.

- DLS Microrheology extends the viscoelastic data into the high-frequency regime (not accessible by mechanical rheology due to inertia limitations), and therefore permits a more complete characterization of rheological properties and dynamics of polymer solutions—from dilute through semi-dilute and into concentrated regimes.
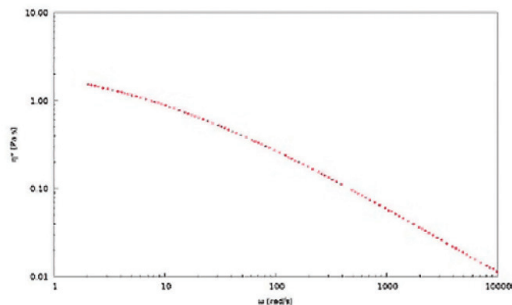

Figure 6 – Complex viscosity, η*, for a 2wt% PEO solution in water measured with 700-nm latex tracer particles.

- Frequency-dependent viscosity showing non-Newtonian, shear-thinning behavior.

Protein solutions: Bovine Serum Albumin (BSA)

The use of DLS Microrheology to extend the measurement range for polymer solutions is, of course, directly applicable to characterizing protein solutions, which also exhibit weak viscoelastic behavior on short timescale (high-frequency) deformations. For protein solutions, in particular, the benefits of small sample volume and the passive (low stress) nature of the measurement technique can be particularly advantageous.

(a) Viscosity measurements of BSA solutions of varying concentration:
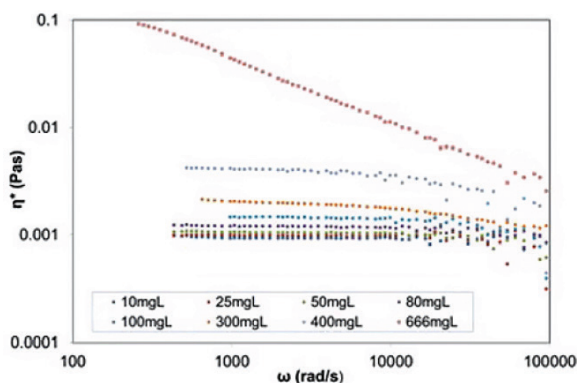

Figure 7 – Plot of complex viscosity against frequency for varying concentration BSA in PBS solutions (from 10 mg/mL up to 666 mg/mL), from DLS Microrheology measurements (using 615-nm carboxylated melamine probe particles).

- At concentrations above 80 mg/mL, the viscosity is no longer constant with frequency, and the BSA solutions exhibit (non-Newtonian) shear thinning behavior.

- Zero Shear Viscosity can be obtained by extrapolating the complex viscosity data to zero frequency.

- The Relative Viscosity can then be obtained from a ratio of the Zero Shear Viscosity (for each BSA concentration) to the viscosity of the PBS (phosphate buffered saline).

- The DSV uses a capillary flow measurement technique, and the data shows excellent agreement with the DLS Microrheology measurements.
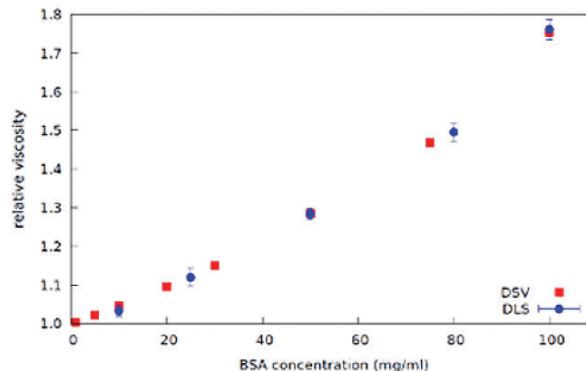

Figure 8 – Plot of relative viscosity against concentration for BSA in PBS solutions—data from DLS Microrheology (ref. Figure 4) and Dilute Solution Viscometer (DSV), at 25 °C.

- Once the choice of probe chemistry and probe size has been optimized, DLS Microrheology can be effectively utilized to make quick measurements on an industrially relevant protein concentration range, with much smaller sample volumes than other available techniques.

(b) Viscoelastic measurements to assess onset of aggregation in BSA solutions following thermal denaturation:
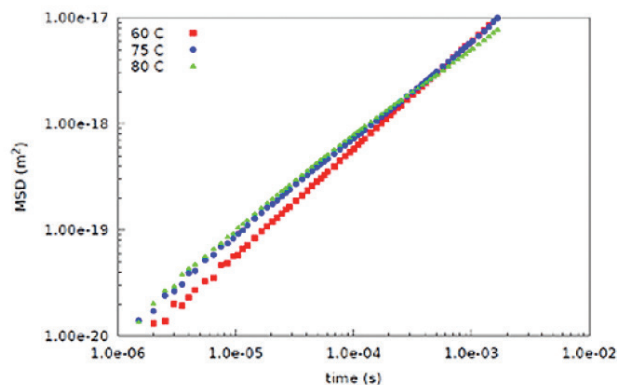

Figure 9 – Variation of reduced Mean Square Displacement (MSD) with time for 10 mg/mL BSA solution at 60 °C, 75 °C, and 80 °C (using 615-nm carboxylated melamine probe particles).

- At 60 °C, the MSD has a slope of 1—the probe particles are showing purely diffusive behavior, i.e., in a viscous medium.

- At higher temperatures, BSA starts to denature, and a process of aggregation ensues,[11] which leads to structure development within the fluid.

- Both of the MSDs at 75 °C and 80 °C show curvature away from a slope of 1—this short time sub-diffusive behavior is an indication of the presence of elasticity in the system.

- As aggregation progresses, the viscoelastic moduli accessible from DLS Microrheology can provide insight into the possible interconnection of aggregates and subsequent system gelation.

- The evolution of the elastic (storage) modulus, **G′**, in particular is strongly indicative of the development of an interconnected microstructure.
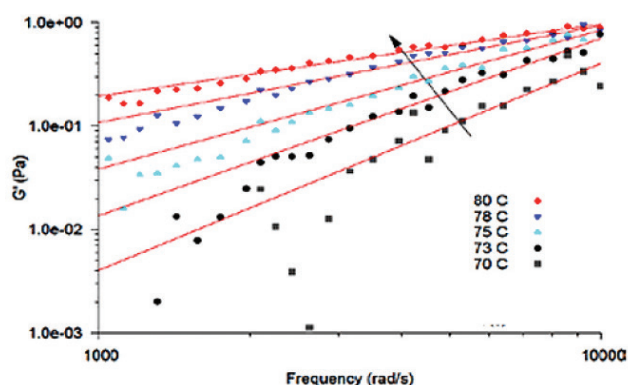

*Figure 10 – Evolution of elastic (storage) modulus, **G′**, with temperature for 10 mg/mL BSA solution over a range of temperatures (the arrow points toward increasing temperature). Lines on the graph are power law fits to each data set (**G′** ωP).*

- A weak elastic response from this system can be detected at 70 °C—although the data are noisy due to presence of very weak **G′** at this temperature.

- Data quality for **G′** improves with temperature as protein denaturation and subsequent aggregation progresses, and a microstructure with a stronger elastic structure evolves.

- **G′** increases by more than an order of magnitude going from 70 °C to 80 °C.

- The values and trend of power law exponent, P, for both **G′** and **G′′** (viscous modulus—data not shown in Figure 6), offers further insight into structure development in the denaturing protein solution. The **G′** exponent shows a relatively fast monotonic decrease from 2 (at 70 °C) to around 0.68 (at 80 °C), whereas the **G′′** exponent decreases from 1 to 0.8 over the same range. The overall decrease of the exponents, and the faster decrease of the **G′** exponent, is indicative of a sol-gel transition, with the gel point occurring when the exponent reaches a value of 0.5.[14]

- The observation of exponent behavior and the rapid decrease of the **G′** exponent are strong indications that network connectivity is evolving for this protein solution in this temperature range.

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