WYATT TECHNOLOGY

6300 Hollister Avenue
Santa Barbara, CA 93117
Tel: +1 (805) 681-9009
Fax: +1 (805) 681-0123
Web: www.wyatt.com

Live Webinar Q&A Sheet:

Characterizing Nanostructures by Light Scattering

The recorded webinar may be viewed from the Polymers webinars page. These questions were submitted by live viewers. Additional information on MALS, DLS, and FFF may be found on the Wyatt web Library under webinars, application notes, featured publications and bibliography, as well as on the corresponding Product page and Theory page of our web site.

Please contact info@wyatt.com with any additional questions.

Q: *How does one determine the swelling ratio during the adsorption process of VOCs on the hyper-crosslinked polymer?*

A: The swelling ratio can easily be measured with dynamic light scattering by measuring the polymer size in the presence and absence of VOC's. A DLS measurement takes only a few seconds, so it may also be possible to monitor the rate of swelling in the presence of VOC's depending on the speed of this process. If something is known about the conformation of the particle, then a total volume change can also be calculated (in the case of spherical or globular polymers, this is simply the volume of a sphere).

Of course, the degree of branching and crosslinking within a polymer will have a large influence on the degree of swelling. More highly crosslinked polymers will swell less since there is less freedom of movement within the polymer.

Q: *Can we use this technique to determine morphology of a polyelectrolyte complex coacervate with varying salt ion content and confirm salt content in the complex?*

A: As long as the overall size and molecular weight of the coacervate is within the limits of light scattering, you should be able to get information such as Mw, Rg, Rh. Coacervates can get very large – maybe as big as 100 microns – which means many complexes are above the size limits of light scattering techniques.

It's important to distinguish that the values we get from light scattering are representative of the whole complex and cannot tell us how the various parts of the coacervate are interacting. We cannot distinguish if salt ions are part of the structure or where they are located – you could certainly try the experiment at different salt concentrations and get valuable information indirectly.

Q: *Must the refractive index (or relative refractive index) of the nanoparticle or gel be known to accurately use DLS?*

A: The refractive index of the sample does not have to be known to accurately use DLS. However, we do need to enter details about the solvent such as refractive index, viscosity, and how these parameters respond to temperature changes.

Q: *Could you talk about how to select sovlent for micorgel characterization using DLS? Would it affect the results, pH, ionic strength, size, and charges...?*

A: DLS is a great screening tool for solvent conditions since factors like pH and ionic strength can make a big difference in how a microgel behaves. The way to do this is simply suspend microgels in different buffers and take size measurements over a range of temperatures. With a cuvette-based instrument, this can take a significant amount of time since only 1 sample can be analyzed at a time. However, the DLS plate reader allows for screening of many conditions all at once and greatly improves the speed at which different buffer conditions can be analyzed. Solvent screening is very commonly done for development of drug formulations.

Many researchers will do a similar type of experiment to study the effect of sample concentration on the size and potential aggregation of the sample. DLS is ideal for this kind of experiment since it is particularly sensitive to aggregates and size changes over a wide range of concentrations.

Q: *How do you differentiate between highly-branched polymer and microgel - where is the demarcation line?*

A: It's tough to draw a line – microgels are highly branched polymers, they just exist at such large dimensions that the normal terminology gets stretched. We also tend to think of them more as continuous meshes of polymeric material rather than an individual polymer with many branch points.

Q: *I have a mixture of linear polymer and microgel – how do I analyze it? Is it possible separate the microgel with SEC?*

A: It may be possible to separate the linear polymer and microgel using an SEC method, but this will depend on the size and molecular weight of each species. Most microgels are above the size limitations of SEC columns, so the separation will not be effective. This is why an alternative fractionation technique like field flow fractionation (FFF) must be used for large nanoparticles. FFF would likely be very effective at separating a linear polymer from a microgel particle as long as they have a different hydrodynamic radius.

Q: *What prevents fouling of the membrane with the larger particles? Is it controlled by concentration and flow rate? Do these requirements impose limits on the dynamic range?*

A: Typically, membrane fouling is not a problem with larger nanoparticles. However, if fouling becomes an issue, there are several ways around the problem such as using a different membrane material, a different membrane pore size, or changing the flow rates within the channel. The dynamic range for FFF is dependent on flow rates and the channel geometry, but not necessarily on the identity of the membrane.

Q: *What features of the light scattering data tell you that a polymer is branched vs. linear?*

A: To analyze branching, we plot either RMS radius or intrinsic viscosity vs. molar mass. The slope of the resulting graph is then compared to the plot for a linear standard of the same material and branching characteristics can be calculated.

Q: *Is it possible to measure particle count for a specific size? What is the smallest size range that particle count can be assigned?*

A: The DLS directly measures the number of photons hitting the detector/second, which is called the count rate. To get a measure of particle count, you would need to make a standard curve of count rate vs. concentration, then compare your measured count rate to the curve. This type of experiment can be done for the smallest size that the DLS can measure, which is 0.2 nm.

Q: *How do these techniques apply to hydrogels that are composed of individual molecules bound by non-covalent forces? What's the best way to characterize such gels?*

A: Individual molecules bound by non-covalent forces will scatter light as a single larger entity. For example, aggregates held together by hydrophobic forces will have a larger size in DLS measurements and a higher molecular weight in MALS measurement when compared to the monomer. One should consider the overall dimensions of the interacting molecules and the strength of the non-covalent interaction when choosing the best way to analyze the samples. DLS is a very gentle and non-destructuve technique that will work well for aggregates. Liquid chromatography combined with MALS may be appropriate in many cases, but sometimes the shear forces in a column will disrupt aggregates or loosely bound polymer particles. If shear forces are a concern, consider FFF-MALS to fractionate the samples since shear forces are extremely small in this technique. If fractionation is good, MALS will determine molecular weights for both the unbound individual molecules and the larger complexes.

Q: *Is there a way to apply charge to the membrane further segregating particles not only based on size but also based on surface charge?*

A: Separation based on charge is possible with the FFF. Instead of charging the membrane, we apply an electric field across the channel to create a gradient for particle separation. Although not yet commercialized, this technique is not only possible but has been used successfully by many groups. For more information, please visit our FFF bibliography at http://www.wyatt.com/library/fff-bibliography.html.

Q: *How is rms measured in the degradable microgels in MALLS? Is it related to the rg measurement?*

A: RMS radius and Rg are actually just different terms for the same number. Like most scientific fields, light scattering has an abundance of terms for the same measurement!

RMS radius is measured by looking at how the intensity of light scattering off a particle varies with angle. Depending on where we put a detector around the flow cell, it will "see" different amounts of light when a particle is larger than 10 nm. This angular variation is directly related to the particle size.

Q: *Are significant errors introduced when particle properties (such as refractive index) change over test conditions (temp, pH, structure changes due to degradation etc.)?*

A: Not really – there may be minimal changes in refractive index upon pH changes or particle degradation. However these changes are small enough to not affect the measurement. Temperature changes, however, can have a big impact. We correct for the refractive index change of the solvent as temperature is increased or decreased.

Q: *Have you looked at size at different salt concentrations?*

A: Yes, microgel size decreases with increasing salt concentration due to more ions being available for charge screening. This allows the polymer chains in the particle to be closer together and changes the overall particle dimensions.

Q: *How was density (rho) obtained for two different nanoparticles from Rh, Rg? Or was there another detector used?*

A: The density was determined by Rg/Rh.

Q: *Polymers that will not separate on a column - are you talking about solubility?*

A: Microgels are well solubilized in water or buffer, but they are simply too big to separate with an SEC/GPC column – they will be filtered out by the stationary phase. Thus, we have to use an alternative separation technique like FFF.

Q: *Is it possible to do SLS with charged polymers?*

A: Absolutely! The polymer charge does not negatively affect the SLS measurement, and this kind of sample is very common.

Q: *In your model, you used a solid sphere. Can I use protein cage nanoparticles for encapsulation of microgel polymers?*

A: This may be possible although microgels are quite large compared to typical material that is encapsulated in this way.

Q: *If the particles is in the same ionic strength buffer but different monovalent ion (for example Na or K), would the size be the same?*

A: We did not explicitly test this, although during the course of various experiments, many buffers were tried. There were no obvious trends that jumped out; most likely the change is so minimal that it's not observable by current techniques.

Q: *Many swollen gels are optically clear. Does that interfere with the ability to perform scattering measurements?*

A: If a particle has an identical refractive index to its surrounding solvent, then light scattering measurements cannot be performed. However, most optically clear gels are clear simply because they are so swollen in solvent, but they will still scatter light effectively. Dilute solutions of microgels are completely clear at room temperature, but the large nanoparticles scatter lots of light and often must be diluted further to avoid oversaturating the detectors.

Q: *What does mean if the Rg/Rh is >>>> 2?*

A: A ratio of 2 or more indicates a *very* extended conformation. Most likely, the sample is a linear chain that is not folded or a rod-shape with high aspect ratio.

Q: *Can DLS or SLS give information about degree of crosslinking of a polymer?*

A: DLS is not very useful for this experiment, but SLS is very useful! Either the RMS radius or the intrinsic viscosity can be plotted vs. the molecular weight for branching

analysis. It is possible to measure the branching ratio, branch units per molecule, and long-chain branching.

Having the Wyatt viscometer inline has several advantages. First, you can measure the hydrodynamic radius. Second, you can get branching information for very small samples (samples must be larger than 10 nm to use the RMS radius method). Third, you can measure the Mark-Houwink-Sakurada Parameters *K* and *a* for unknown polymers.

Q: *Could you mention the name of the analysis software? Is it the same software that comes with all DLS machine from Wyatt?*

A: The DLS analysis software is called *Dynamics* and comes with all Wyatt dynamic light scattering instruments.

Q: *Do you have any experience with adding salt to a microgel-suspension (to reduce electrostatic repulsion of the different polymer-chains) and then increasing the temperature? That would reduce the particle size permanently, wouldn't it?*

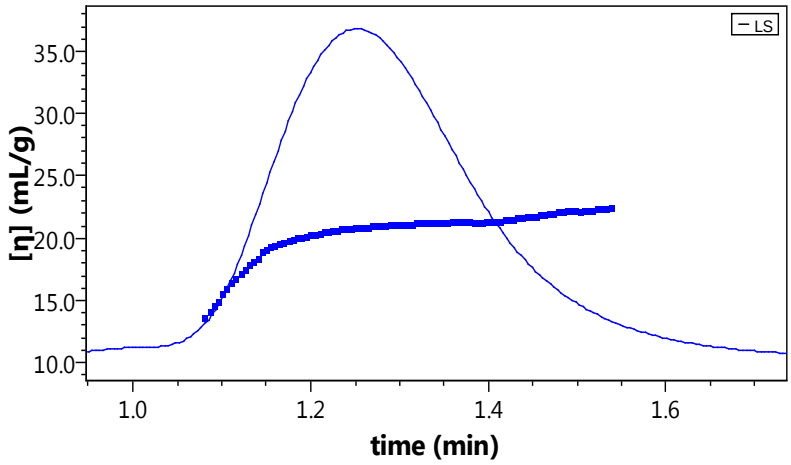
A: Adding salt will increase the amount of charge screening inside the microgel structure and therefore cause the particle to deswell slightly. As long as the salt concentration is not changed, the microgel will stay at the reduced size indefinitely. Example: 300 nm in water, then 200 nm in 5% sodium chloride.

If a microgel in a high salt environment is heated up, it will deswell further. But upon returning to room temperature, it will re-swell to the starting dimensions. Example: 200 nm at room temperature, then 100 nm at 40 degrees C, then 200 nm when cooled to room temperature.

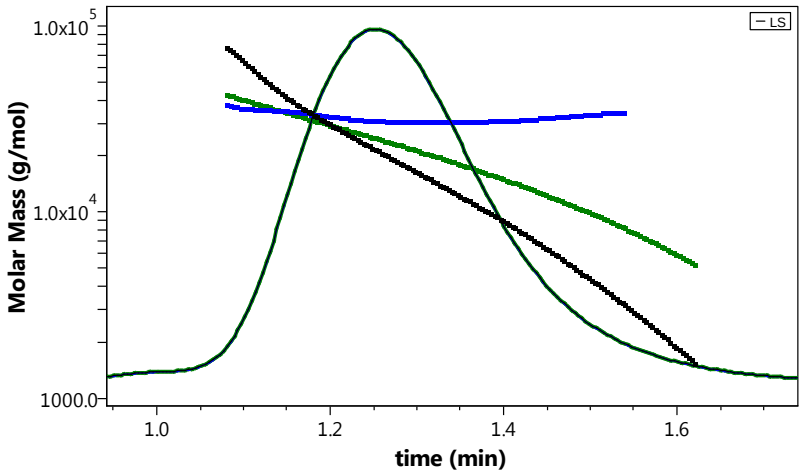
Q: *If we do the mw analysis by intrinsic viscosity and MALS, is there any difference in the result and why?*

A: Most likely the two ways of calculating Mw will result in different values. Measuring Mw by intrinsic viscosity is often referred to as "universal calibration" since it is thought to apply to almost all samples. However, this type of calibration has some limitations.

Both column calibration and universal calibration dictate that species eluting at a later time and later volume *must* be of lower molecular weight due to the negative slope of the calibration curve; this axiom is inherent in the experimental method. However, peaks in a chromatogram often have a fixed molecular weight across the entire peak width and time. For samples such as a narrow 30 kDa standard, the molecular weight is constant, which also means that the intrinsic viscosity is basically consistent across a peak (see image below).



However, since the product of intrinsic viscosity and molar mass must be decreasing (the universal calibration curve has a negative slope), then molecular weight *must* decrease with elution time. In other words, the inescapable mathematical framework of column calibration is a cause of error in the measurement. The figure below overlays calibration and MALS measurements for a narrow 30 kDa polystyrene standard and shows that the molar mass measured by MALS (blue) is consistent across the peak. In contrast, the molar mass results from column calibration (green) and universal calibration (black) slope sharply downward and results in significant error, particularly on the right side of the peak. Both the polydispersity and average molecular weight are affected by using calibration vs. MALS.



For more information on this topic, see a webinar on our website:
http://www.wyatt.com/library/webinars/essential-polymer-characterization-with-mals-and-fff.html

Q: *Do you have any tricks for sample preparation? I notice that this technique is dust sensitive.*

A: Yes – cleanliness is crucial! For dynamic light scattering in particular, we recommend filtering your solvents and possibly also the samples. All solvents should be filtered to 0.1 µm or smaller. If working with proteins, the filter should be 0.02 – 0.1 µm. If working with nanoparticles, the filter must be large enough to allow the sample to pass through.

Q: *Can the DLS plate reader tell if the polymer is linear or spherical?*

A: DLS in general is quite good at detecting small changes in the sample hydrodynamic radius. For example, you can observe the change in size during molecular unfolding. The instrument cannot explicitly tell you whether the polymer is linear or spherical, but you can compare the estimated molecular weight from the radius to predictive models in the software for each type of conformation. Alternatively, you can measure the rms radius with MALS and compare to the hydrodynamic radius from DLS for a true measure of conformation.

Q: *Can this technique be used for protein (MW, predict protein structure, folded or unfolded forms, ...)?*

A: Absolutely! DLS is very commonly used to monitor protein folding and unfolding under various experimental conditions. For example, chemical denaturation can be observed while holding temperature consta nt, or unfolding and/or aggregation can be observed as the temperature is increased. Some of our DLS instruments can also measure molecular weight in addition to size.

Static light scattering is a powerful tool for measuring the molecular weight of proteins, and when combined with chromatography, MALS can measure oligomeric states.

Q: *What is the maximum molecular weight/radius gyration that can be measured by SLS? and also maximum hydrodynamic radius that can be measured by DLS?*

A: The maximum molecular weight that can be measured with SLS is 1 GDa. The maximum size is 1 µm. The maximum size that can be measured with DLS is 10 µm, but this number may decrease depending on the optical configuration.

Q: *What limits the maximum size of the range for DLS measurements?*

A: There are 2 limiting factors on the upper end of the size range. First, large particles will settle out of solution, which means that their diffusion characteristics are affected by gravity. Thus, their diffusion is no longer random, but is directed which will

distort the measurement. Second, large particles diffuse so slowly that it's difficult for the instrument optics to detect the change in scattered light based on particle position. These two factors combined set an upper limit around 10 µm.

Q: *Is there a limit on the polydispersity to obtain an accurate average particle size?*

A: The limit for accurate average particle size measurement is about 30% polydispersity.