



## Live Webinar Q&A Sheet:

### Investigating Hydrogel Microparticles with Light Scattering and Microscopy Tools

The recorded webinar may be viewed from the [ELS](#) webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS, DLS, CG-MALS, 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](mailto:info@wyatt.com) with any additional questions.

#### DLS/ELS general questions

*Q: Can the measurement of DLS or ELS change the sample itself? How would I check if it does?*

A: 1) In general, DLS is a non-invasive measurement. The exception would be a sample that was sensitive to the laser wavelength of 532 nm. ELS measurements apply an electrical field which may induce changes in particle size and/or surface charge for sensitive samples. 2) Software provides full control of collection settings. Test DLS: Continuously measure samples over time to confirm that the sample size does not change. Test ELS: Measure samples with DLS only first and then with DLS and ELS. Confirm that the size and mobility is consistent across both measurements.

*Q: How long do you need to equilibrate the system at each temperature before making the size and mobility measurements? Is this dictated by the instrument or the sample?*

A: The software can be set to continuously measure the size and mobility as the temperature is ramped. The instrument is calibrated so that the reported temperature at a given size and mobility measurement is accurate even during a temperature ramp. Alternatively, the software can be set to ramp the temperature in steps and to only measure the size and mobility when the temperature has stabilized at each temperature, which would typically take about a minute.

*Q: 1) Is there a calibration process necessary for both these instruments? 2) If so, what is that process? 3) If so, what is the time required and frequency?*

A: 1) No calibration needed. But Wyatt does recommend validating the system with a mobility standard. 2) Load mobility standard into PALS cell and measure DLS & PALS with an applied voltage of 3V. 3) Once a quarter or shorter.

*Q: Is the cell used for zeta potential reusable?*

A: Yes, the dip cell and flow cell are both reusable. This is because they are constructed of highly robust materials: platinum electrodes, fused silica windows, Kalrez o-rings, and a PEEK housing.



Q: *Is the zeta potential instrument commercially available?*

A: Yes, the instrument mentioned in this webinar is the Wyatt [Mobius™](http://www.wyatt.com/Mobius). To learn more, go to [www.wyatt.com/Mobius](http://www.wyatt.com/Mobius).

Q: *How are the light scattering measurements impacted when particles have high refractive index, e.g. TiO<sub>2</sub>?*

A: A higher refractive index will enhance the scattering efficiency and will therefore improve the actual size and mobility measurements.

Q: *Is it possible to use DLS for the characterization of magnetic nanoparticles that tend to quickly aggregate?*

A: Yes. You may load the sample cell, place it in the instrument, and begin collection in seconds. Feel free to reach out to Wyatt if you would like to see a demonstration with your samples.

#### **Hydrogel-specific DLS/ELS measurements**

Q: *Is it possible to study the swelling and de-swelling efficiency over multiple cycles?*

A: Yes, the software can be set to simultaneously measure size and mobility as the temperature is cycled between 20 and 60° C multiple times.

Q: *How can you check that the change in size is a swelling/de-swelling process rather than the formation/de-formation of aggregates?*

A: This can be investigated by observing the 'Normalize Intensity' value which represents static light scattering and is related to the product of concentration and molar mass. A change in size that is associated with aggregation would also cause an increase in the normalized intensity since the molar mass increases, while swelling/deswelling does not change the molar mass and the normalized intensity would remain constant.

Q: *The scale of the punctate nanostructures appears to be tens of nanometers, or perhaps a hundred. Would this be evident in light scattering data other than the overall particle size?*

A: One might observe changes in overall scattering intensity due to the formation of those nanostructures, but such changes are not clearly evident in our data. A multi-angle light scattering detector such as a [DAWN®](#) would be more sensitive to structural details such as these.

Q: *Once formed, how stable are microgels with high temperature exposure?*

A: In aqueous media, the particles are quite stable at neutral pH. However, under acidic or basic conditions, one might observe amide hydrolysis.



Q: *DLS shows a sharp decrease of particle sizes from room temperature to 32°C at pH 4.5, but from the AFM image, the sizes look similar. Why?*

A: These may be particle pinning/drying artifacts associated with the strong adhesion of the particles to the surface.

Q: *For linear chain structure, can the structural heterogeneity be also proved by combination of DLS and SLS?*

A: This is theoretically possible, but we have not performed those studies at this time.

Q: *What information can DLS/ELS give regarding gels that form via a 3D networks of fibers?*

A: The size from DLS and mobility from PALS would be that of the whole 3D network.

Q: *Since these hydrogels have a very high level of water, why are the particles easily seen by dynamic light scattering?*

A: Mobius has excellent sensitivity due to its high-power, 50 mW laser and the high-quality detectors. In addition, the software can be set to integrate the signal longer for low-scattering samples.

#### **AFM measurements**

Q: *How do you dry your particles for AFM analysis so that they retain their wet state structures (i.e. without seeing significant folding of particle walls on itself)?*

A: When deposited on positively charged substrates, the negatively charged microgels tend to get “pinned” in their swollen conformation and then dehydrate anisotropically during drying.

Q: *How was the AFM performed? Was tapping mode employed? Was contrast achieved via height or phase?*

A: Non-contact, AC mode was used, and the data presented were in height mode.

Q: *What substrate was used for the AFM work?*

A: Glass coverslips modified with amino-silanes.

#### **Hydrogel miscellaneous questions**

Q: *If used in vivo, how will the particles leave the body? Renal or liver other?*

A: Typically, one would design the particles to be biodegradable, at which point the decomposition products would be excreted renally. We have not yet developed those degradation chemistries in our lab.



- Q: *In the analysis of the origins of the pH effect, it looks like you are assuming that the pKa of the acrylic acid is the same in the hydrogel environment as it would be for monomers in solution. Is justified, or might incorporation into the hydrogel cause some dispersion for molecules incorporated into different microenvironments?*
- A: Indeed, shifts in pKa and dispersion in their values is typically observed in microgels. Those effects tend to be quite modest in loosely crosslinked systems where the acidic comonomer makes up a small fraction of the composition, such as those studied here. However, when the acidic comonomer concentration is increased or the particle is more densely crosslinked, one must be more cautious with assumptions regarding the use of solution pKa values.
- Q: *How do you keep your particles separate (not aggregated) from each other above LCST for size measurement?*
- A: When charged (due to comonomers and/or initiator fragments) the particles are stabilized coulombically against aggregation.
- Q: *How do you purify your particles after synthesis?*
- A: Extensive dialysis using high-molecular-weight-cutoff tubing.
- Q: *What conjugation chemistry do you use for fibrin coupling?*
- A: The fibrin-binding proteins are coupled via EDC/NHS coupling.
- Q: *In which case was the use of SDS (surfactant) was necessary? In which % was it used in your synthesis?*
- A: SDS was not used in the syntheses reported in this webinar, but the SDS concentration can be varied to produce particles of various sizes, with larger SDS concentrations resulting in smaller particles. In all cases, concentrations lie below the CMC for SDS.