



**Live Webinar Q&A Sheet:
A quick guide to properly measuring zeta potential
and particle size for challenging samples**

The recorded webinar may be viewed from the [ELS](#) 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: In DLS analysis is the cumulants vs regularization analysis an option or automatically selected?

A: DYNAMICS automatically calculates and outputs the radius by both cumulants and regularization analysis in the Datalog Grid. Further information can be found in Technical Note TN2005 - Cumulants versus Regularization Analysis Rev B. Let us know if you have any further questions about how DYNAMICS performs this analysis.

Q: Is the 20Hz frequency unique to Wyatt's Mobius, or is that frequency also available in other zeta potential instruments as well?

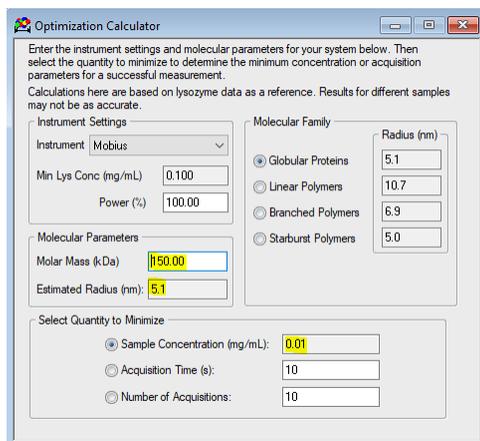
A: To the best of my knowledge only Wyatt's Mobius can go up to 20Hz. Let us know if you would like to discuss this feature further.

Q: While measuring the Zeta Potential of the samples we normally dilute with MilliQ water. Is there any effect of water on the Zeta potential of samples?

A: MilliQ water vs. standard water should give the same result. However, dilution in general can impact the resulting Zeta Potential if the ionic strength changes significantly. For example, if your sample has 150 mM NaCl and you dilute with water 10 fold then the ionic strength will only be 15 mM. In the webinar I discuss how this can change the model that should be used. It would be best to use Henry model and type in the final ionic strength.

Q: What is the ideal dilution (10 X, 100 X) to measure the size and Zeta potential?

A: This is sample dependent. If you have large particles (>100nm) then you should make sure that the solution is dilute enough so it isn't cloudy. If you have small particles (<10nm) you should make sure you have a high enough concentration to yield a good correlation curve. DYNAMICS has a nice calculator that tells you what the minimum concentration needed depending on your samples molar mass (size). In the example below the calculator shows that 0.1 mg/mL is needed to measure a 150 kDa (~5nm) particle. Let us know if you would like to see a virtual demo of this feature.



Q: How is Z-potential valuable parameter for native proteins? Protein community is not much acquainted with the notion of Z-potential. Electrophoretic mobility is more common. Are there any databases of protein properties including Z-potential values?

A: Both are similar and can tell you about the interactions. But Zeta also accounts for the particle size and ionic strength. I do not know of any such database myself. You may be interested in the ability to pair Mobius with an autosampler which will allow you to screen dozens of samples automatically. Below are some publications that used Mobius to study protein applications.

- Effects of Ionic Strength and Sugars on the Aggregation Propensity of Monoclonal Antibodies: Influence of Colloidal and Conformational Stabilities. *Pharmaceutical Research* 2013 30(5) , 1263-1280. DOI: [10.1007/s11095-012-0965-4]
- The Role of Electrostatics in Protein-Protein Interactions of a Monoclonal Antibody. *Molecular Pharmaceutics* 2014 11(7) , 2475-2489. DOI: [10.1021/mp5002334]
- Whey proteinpectin soluble complexes for beverage applications. *Food Hydrocolloids* 2017 63() , 130-138. DOI: [10.1016/j.foodhyd.2016.08.027]



Q: I wanted to know whether nanoparticles in microemulsions (oil and surfactant) can be added to get zeta potential and DLS or nanoparticles require to filter and wash before measurement?

A: Measurements of microemulsions and should be fine. Measuring the sample filtered and unfiltered is useful. If you are worried about the unfiltered solution you can use the disposable cuvettes with Mobius. Your application sounds interesting. Let us know If you would like to speak to one of our Ph.D. Application Scientist to discuss the best method to analyze your samples.

Q: Are the analyses performed in static conditions? Can stirring conditions be used too?

A: Yes, in general the analysis is performed in static conditions. However, because of the flow cell design it is possible to measure the sample while it passes thru the flow cell. In addition, the Atlas accessory can pressurize the flow cell. I have seen that this pressurization step “kicks” the solution a bit. Are you worried about your particles settling out? Your application sounds interesting. Let us know If you would like to speak to one of our Ph.D. Application Scientist to discuss the best method to analyze your samples.

Q. Is it possible to measuring the Zeta potential variation with pH on-line?

A. This would be possible if you have an instrument upstream of the Mobius that is feeding in samples with different pH values. The Mobius software can be set to continually measure the Zeta Potential on-line. Your application sounds interesting. Let us know If you would like to speak to one of our Ph.D. Application Scientist to discuss the best method to analyze your samples.

Q: How do you differentiate change in size as a function of concentration due to self-association vs multiple scattering phenomena?

A: You do if you work at high concentrations. It would be ideal to measure at a few dilutions (10X, 100X) and keep diluting till the size does not change.

Q: What is the concentration range for zeta potential measurement? Is there a concentration limit for measuring the zeta-potential?

A: Because we are measure a frequency shift the size and concentration limit is likely as high as you would ever measure for you samples. But it would be good idea to know more about your samples. Let us know If you would like to speak to one of our Ph.D. Application Scientist to discuss the best method to analyze your samples.



- Q. *How to consider effect of PO4 buffer in viscosity (any Correction factor like Glycerol ??)*
- A. Most likely it will not impact the viscosity at standard concentrations (~150 mM). Maybe if you work at high concentrations (>1M).
- Q. *Is there a feature to measure the isoelectric point of a protein by Mobius?*
- A. To do this you would inject your sample at different pH values into the Mobius. The Mobius can then create a plot of Zeta vs pH which will allow you to trace the pH at which Zeta is zero. Let us know if you would like to discuss this application further.
- Q. *The DLS system I use has a "dust cutoff" variable. Can you explain what this means and how I might utilize it best during analyses?*
- A. My guess is that this is a type of filter that removes data that the system thinks is "dust". Usually if you have a sample with a lot of aggregates/dust the "dust cutoff" filtering feature will clean up the correlation curve thereby allowing you to get a better fit to the data for the smaller species. But I would say that you should always show the filtered and unfiltered data. Mobius also has some filtering options. Let us know if you would like a virtual demo of this feature.
- Q. *What would you suggest as a general approach to measuring a protein adjuvanted to Alhydrogel when there are populations of both protein (fibrous) and adjuvant (granular) aggregates?*
- A. Measure sample without filter and with aggregates filtered out. If you have Mobius look at the PALS amplitude for both samples. By comparing the PALS Amplitudes, you can determine the Zeta contribution of each species to the mixture.
- Q. *Have you developed method for protein based nanoparticle Zeta potential?*
- A. You may follow the method described in the webinar for all types of samples. Let us know if you would like to speak to one of our Ph.D. Application Scientist to discuss the best method to analyze your samples.



- Q. *Is there a university of Contract lab in California using Mobius that you can refer me to? Previously I have been led to believe that Zeta Potential could not be detected by any instrument it could only be calculated manually. Your mobius has software that can make the determination.*
- A. Yes. We have many in California. What city do you live in?