Protein Solution Microviscometry with the NanoLab 3D

Webinar Q&A Transcript

1 What is the viscosity range available with this technique?

DLS microviscometry is very sensitive in the low viscosity end and is, in fact, the most precise way of measuring viscosities close to that of the solvent, in the case of protein solutions. The lowest viscosity we can measure is in the order of 10^{-2} mPa.s. On the upper end, 10^3 to 10^4 mPa·s can be measured, depending on the tracer particles chosen.

2 What is the main advantage of light scattering compared to microfluidic techniques that can also measure small samples?

In DLS microviscometry, we always get the ideal zero-shear viscosity. Another advantage is the possibility to probe the sample over long times, or under different temperatures with the sealed cuvettes. At last, the measurements are also faster than with microfluidic devices.

3 What is the ideal size for the tracer particles?

A good compromise is in the order of 50 to 200 nm. The particles should be large enough to scatter more than the protein sample. At the same time, they shouldn't be too large, as they would move too slowly and exhibit angular-dependent scattering intensity.

4 Are tracers added before or after the SLS/DLS measurement?

DLS sizing and SLS measurements should be conducted without tracer particles. DLS microviscometry mandates the addition of tracers prior to the measurement.

5 In the NanoLab 3D, are we working under the Rayleigh scattering regime?

In DLS microviscometry, the scattering regime does not matter since we are not considering angular-dependent behavior. Having said that, with tracers particles in

the size range of 50 to 150 nm range, one is indeed in the RGD regime.

6 Is there a filtration step of the samples before measurements?

DLS microviscometry experiments are straightforward to conduct and dust is rarely a problem. If aggregates are present, they may be removed by centrifugation (we do not recommend using filters, as proteins may bind to them). For high precision measurements, it is also advisable to ensure that the buffer and particle stock solution do not contain contaminants. This may require filtration.

7 Do you take into account the second viral coefficient in the measurements using concentrated protein solutions?

While proteins may be present at high concentrations and therefore strongly interact with each other they only constitute the background media. The tracer particles, used to probe this media, are added at concentrations sufficiently low so that they do not interact with each other, but sufficiently high to shadow the original sample signal. Therefore, we do not need to take such corrections into account.

8 Can we perform measurements using fluorescent proteins?

This is possible with a proper choice for the fluorescent probe, so that it does not limit the wavelength of the laser used. It may also require optical filters to remove the fluorescent signal if present: for such a custom solution, please contact info@lsinstruments.ch

9 How to confidently ensure there is no protein particle interaction?

Most proteins are surface-active, and this is a key problematic, which can be addressed through several approaches.
First of all, at very low tracer particle concentrations, even if the proteins cover the surface of the tracer particles, only a tiny fraction will be lost to these monolayers and the measurement will not be affected.
Then, one can use tracer particles whose surface charge is compatible with that of the proteins. For this, one needs to compare the pH of the buffer to the isoelectric point (pI) of the proteins.
Finally, one can also use particles that are functionalized using PEG.
For more information, read: T. Garting, and A. Stradner, Colloids and Surfaces B: Biointerfaces 181

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10 Can you measure the viscosity locally in the sample, for example close to the container surface?

Due to the unique geometry of the 3D instrument, one indeed has a very well defined small scattering volume that can in principle be used to investigate local heterogeneities. The NanoLab 3D allows the user to displace the cuvette via a software tool. The scattering volume can thus be moved close to the wall of the cuvette.

11 What is the possible q-range?

The Nanolab is a fixed angle instrument, meaning that there are no variations in q. However, the LS Spectrometer from LS Instruments allows conducting measurements in the angular range from 8° to 155° with a precision of 0.01°. DLS microviscometry is also possible in this instrument using high precision NMR tubes with volumes as low as 50 uL.

12 In your example, we see the viscosity of BSA decreasing with time. Can you explain?

Most likely this is a random occurrence, and the variation observed is very small (1% for the Modulated 3D measurement) However, there can also be an effect arising from small temperature variations in the sample, if one launches the measurement directly after sample insertion.

13 Could I measure a protein coacervate that settles at the bottom of the tube (similar to a centrifuge pellet) upon phase separation with water? Can the laser measure at the bottom of the tube?

This is possible to some extent in the NanoLab 3D by adjusting the height of the cuvette. It is also possible with the LS Spectrometer. We suggest contacting LS Instruments at info@lsinstruments.ch for more details.

14 Can you measure the viscosity inside a porous material, as could be done with nuclear magnetic resonance?

This would be difficult since porous materials typically scatter strongly given the heterogeneity in the index of refraction. A solution would be to have the porous material index-matched with the solvent.

15 Do you sell TiO2 particles or coated TiO2 particles? And do you have experience with them in the context of protein solutions?

For DLS microviscometry, we tend to use organic tracer particles such as polystyrene, so as to avoid excessive scattering, and because their density is usually similar to that of the solvent. TiO2 particles are best for DWS microrheology. For additional information, please contact us at info@lsinstruments.ch.