



Live Webinar Q&A Sheet:

Development of a New Online Micro-Viscometer for APC and UHPLC Applications

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

Q: Can the μ DAWN- μ Viscometer- μ RI detector set be used with standard GPC?

A: Yes, subject the constraint that the microViscoStar applies a higher shear than the V3. This could be an issue for large molar mass or highly branched samples. Even so, one can measure the sample at two different flow rates (to change the shear). If the results are equivalent, this demonstrates that the measurement is being made sufficiently close to zero shear that the approximation that the intrinsic viscosity can be calculated as the ratio of the specific viscosity to the concentration.

Q: How does the sensitivity of the μ Viscometer compare with the standard viscometer?

A: The microViscometer has 2x higher sensitivity for the same concentration than the V3.

Q: *Are the delay columns matched to APC columns volumes?*

A: The microViscoStar will ship with one delay column that is appropriate for UHCLP/APC. If one wants to use it for standard chromatography, you can reconfigure the system with either the 3 or 6 column stack.

Q: *What are the overall range and sensitivity limitations of the UHPLC-MALS-IV-RI system?*

A: The UHPLC/APC systems are intended for molecules under 2MDa. For molar mass, we can measure down to around 100 Da. For the Rh derived from viscometry, the system can measure below 1nm.

Q: *Is MALS preferable over LALLS/RALLS detectors for molar mass determination?*

A: LALLS suffers from excessive noise because any contaminants in the mobile phase or dirt on the optics shows up in the low angle data and compromises the accuracy of the results. RALLS data is typically much less noisy, but if the molecules are above around 10nm, then one has to correct for the angular asymmetry of the light scattering data. With only two detectors, one of which is contaminated with stray light, this is difficult to do reliably. This is why the minimum number of angles should be three. With two points, you can fit a line. With three points, you can tell if the curve IS a line.

Q: *Does your software support universal calibration?*

A. The software supports traditional and universal calibration. It also supports determine molar mass from intrinsic viscosity if the Mark-Houwink-Sakurada coefficients are known *a priori*. However calibration methods are inaccurate when there is column interactions or other non-ideal chromatography. Since Molar mass from MALS does not depend on the elution time it is unaffected by non-ideal chromatography, which makes it the preferred technique.

Q. *Why do you recommend using the μ Viscometer alone for QC and process monitoring applications instead of including light scattering?*

A. We are not claiming Viscometry alone is preferred for QC! Some feedback I have heard is that since viscometry is insensitive to dust, it can often be used for samples that are not clean enough to get good MALS data.

Q: *Is the ASTRA software running the Waters APC when in use with a microDAWN and the Waters DRI?*

A: The Waters APC is controlled by their Empower software. ASTRA is responsible for data collection and analysis.

Q: *Have you sped up the run time for the IV with better quality of results?*

A: Yes, an analysis with the microViscoStar will be 3-5x faster with increased sensitivity.

Q: *Does this detector work for aqueous polymers, polysaccharides?*

A: Yes, the microViscoStar will work with aqueous samples.

Q: *How many columns can be connected in series before running into issues with the delay columns?*

A: The microViscoStar is can be configured to match the analytical columns. For the APC data presented in the talk, we used 4 Waters APC XT columns. The microViscoStar was configured with a delay of 5.4ml. The instrument can accommodate a maximum delay of 32ml, which will work with 4 standard chromatography columns.

Q: *Is the microViscometer able to handle aggressive solvents like HFIP on a continuous basis?*

A: Yes, the microViscoStar can handle HFIP in continuous operation.

Q: *Applications for proteins and antibodies?*

A: The microViscoStar can characterize the hydrodynamic radius and conformation of biomolecules. Thus, one can study how these parameters change when the biomolecules are exposed to different stimuli e.g. heat, buffer composition, intermolecular interactions.

Q: *Is there a reason to measure intrinsic viscosity for formulation development?*

A: Yes, measuring intrinsic viscosity can give you information on the conformation and size of your component molecules, so you can see how they behave in different formulation solutions.

Q: *How many Liters of HFIP does UHPLC uses in regards to using HPLC?*

A: A UHPLC setup will typically use 1/3 of the solvent compared to HPLC.

Q: *How do you ensure reproducibility even with additional backpressure due to the RI connected behind the Viscometer?*

A: The microViscoStar was designed with high pressure components so that back-pressure of downstream detectors do not affect its performance. It can operate with an inlet pressure as high as 30bar (420psi).

Q: *If we run a sample and get MH parameters from the instrument, and then fill in the exact parameters and our g' is 1.05 (5% off). Can this be due to heating problem in the viscosity meter? We run polyesters.*

A: Temperature does have a big impact on the solution specific viscosity, so incorrectly regulated and/or reported temperature from an online viscometer could ultimately result in some error in the calculation of intrinsic viscosity, and from which the branching ratio g' is calculated. Most measurements involving Wyatt Technology-brand light scattering detectors for molar mass determination, whether used in conjunction with viscometers, have an overall accuracy of 2-4% of a known molar mass. Branching ratios require measurement of molar mass and intrinsic viscosity of a linear reference standard in addition to the unknown sample, and an overall error of these combined measurements of 5% is not outside the range of overall accuracy for this measurement. A 5% error in measured branching ratio compared to some standard value is within the expected accuracy range of this technique. The temperature in the Wyatt ViscoStar is regulated to 0.01 °C and the current model of ViscoStar, the model III, has 3 Peltier units to add or remove heat as needed to make sure the

temperature inside the bench is well regulated throughout the whole interior section containing all the fluid handling tubing. The temperature inside the ViscoStar bench is recorded so that it can be checked after the measurement to see if it has changed from what it was set for.

Q: You have your detectors in series. Traditionally there is split flow between the RI and Viscometer. Have you tried this configuration or is it not necessary?

A: We have done extensive testing of detectors running in parallel and have found that it is very difficult to insure that the split ratio remains constant between the detectors. Moreover parallel configuration causes a lower flow rate through the viscometer, which results in reduced sensitivity. For these reasons we support series operation for the best sensitivity and reproducibility.

Q: What's the level of back pressure added by the ViscoStar. What do you suggest: should it be connected after LS or RI as an end detector?

A: The microViscoStar produces 3.2 bar in THF at 1.0ml/min (7.1 bar in water). The ViscoStar 3, which is tuned for standard chromatography produces 1.3 bar and 2.9 bar for THF and water respectively. The RI detector produces much lower noise when run as the last instrument in the chain. The preferred plumbing configuration is UV + MALS + microViscoStar + RI

Q: Why do you recommend using the Micro-Viscometer alone for QC and process monitoring applications instead of including light scattering?

A: We are not specifically advocating for using the microViscoStar alone for process monitoring/QC as LS can give valuable information as well. However, using the microViscoStar alone can offer certain advantages such as decreased sensitivity towards dust/particulates from a dirty sample.

Q: *Can we use the ViscoStar without LC separation? direct injection of amle to detector to determine Rh?*

A: Yes, you can inject an unfractionated sample into the microViscoStar to determine Rh.

Q: *Can we measure viscosity of all edible oils by these instruments?*

A: Yes, the intrinsic viscosity can be measured as long as the oils are soluble in a compatible solvent.