



## Live Webinar Q&A Sheet:

### Characterizing PLGA for FDA Generic Drug Approval Using SEC-MALS-IV

The recorded webinar may be viewed from the [MALS](#) 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.

#### General SEC-MALS-IV

*Q: How does PDI from analytical SEC compare to SEC-MALS?*

A: Typically, analytical SEC overestimates PDI. Consider a perfectly monodisperse sample: it has a finite peak width, which by definition gives  $PDI > 1$  in analytical SEC. Any tailing of the monodisperse sample due to non-ideal column interactions results in further broadening of the peak, and further apparent polydispersity. In both these cases, SEC-MALS would give the true polydispersity of 1.

*Q: Could this technique be used in conjunction with UPLC/UHPLC?*

A: Yes, Wyatt has UHPLC-compatible instruments: the [microDAWN™](#) multi-angle light scattering detector, [microOptilab™](#) differential refractometer, and [microViscoStar™](#) differential viscometer. These instruments feature increased pressure tolerances and reduced internal volumes compared to the standard [DAWN® MALS detector](#), [Optilab® RI detector](#) and [ViscoStar® differential viscometer](#) HPLC-compatible instruments.

*Q: Are there specific brands of HPLC that you have to use with Wyatt instruments?*

A: Wyatt instruments are compatible with a wide variety of HPLC manufacturers. Please contact a Wyatt sales representative for further information.

*Q: What is the typical run time of SEC-MALS and SEC-MALS-IV experiments?*

A: The run time of the SEC-MALS experiment will depend on the column configuration and the HPLC flow rate. A typical run time is around 45-60 min at 1 mL/min flow rate in a 3- or 4-column configuration. The inclusion of a ViscoStar for SEC-MALS-IV roughly doubles the run time.



## PLGA-specific

*Q: Is there any limitation of PLGA concentration on particle size detectability?*

A: The angular variation of a sample's light scattering signal gives the rms radius  $R_g$ . The light scattering signal is proportional to molar mass and concentration and  $dn/dc$ . Thus, the minimum detectable concentration will depend on both the molar mass of the particle and the solvent. Typically, such particles will be large enough that there is no issue with the light scattering signal unless the solution is extremely dilute.

*Q: Which type of column do you suggest for PLGA analysis? Is it silica based or polymer based?*

A: For generic SEC-MALS analyses of PLGA, a standard mixed bed column with divinylbenzene crosslinked polystyrene beads works well.

*Q: What are some other techniques that may be used to complement SEC-MALS-IV for PLGA characterization?*

A: The primary complementary technique would be nuclear magnetic resonance (NMR) spectroscopy. NMR produces the lactide:glycolide ratio which is pivotal in determining key properties of the polymer such as solubility and degradability.

Other standard polymer characterization techniques, such as TGA, DSC, and DMA, are also useful.

## Dn/dc

*Q: How is  $dn/dc$  determined?*

A: The  $dn/dc$  property of a sample can be determined in two ways. The batch, or offline, method uses a series of sample injections directly into the Optilab RI detector at different concentrations to create a plot of differential refractive index over concentration. The slope of this plot gives  $dn/dc$ .

There is also an online method in our chromatography software, [ASTRA®](#). Here,  $dn/dc$  is determined from the sample dRI peak from a SEC-MALS experiment. Here you need to know the flow rate of the HPLC, the sample concentration in the loop, the injected volume, and that the sample does not interact with the column. Essentially, you assume that 100% of the mass injected into the column, elutes out of the column and reaches the detector. ASTRA integrates the dRI peak area and compares to the injected mass to yield  $dn/dc$ .

*Q: Does  $dn/dc$  change with the degree of branching?*

A: No, the  $dn/dc$  value of a sample is independent of branching. It depends only on the sample's chemical composition and the solvent.



*Q: Are there any solvents that PLGA can be dissolved in to give a higher  $dn/dc$  for better light scattering and refractive index signals?*

A: Solvents with lower refractive indices like acetone and acetonitrile will work. However, be aware that higher ratios of glycolide in the PLGA may reduce the solubility of the polymer in these solvents.

### Dynamic light scattering

*Q: Does DAWN measure dynamic light scattering (DLS)? How exactly does that work and would it be useful for PLGA characterization?*

A: DLS stands for [dynamic light scattering](#). In short, this technique measures the short-term light scattering fluctuations due to a sample's Brownian motion. These are converted into a diffusion coefficient which is then used in the Stokes-Einstein equation to calculate hydrodynamic radius.

The DAWN MALS instrument is used for [multi-angle light scattering](#), which is a different technique from dynamic light scattering. However, the DAWN can be outfitted with an online DLS module called [WyattQELS™](#) to enable DLS measurements, so that the hydrodynamic radius of each eluting slice from the SEC column can be measured.

*Q: Size parameters were mentioned, but weren't discussed much. Are there any other uses for rms radius and  $R_h$  for characterization?*

A: The ratio of rms radius over hydrodynamic radius, called the 'shape factor'  $\rho$ , gives the conformation of a sample. For a spherical particle, this ratio will be 0.77 while a hollow sphere with a thin shell will be around 1.0. Extended conformations such as rods or ellipsoids have a shape factor greater than 1.0. As expected, this is useful for the characterization of nanoparticles including empty and full drug nanocarriers, gold nanorods and more.

### Intrinsic viscosity and Mark-Houwink-Sakurada plots

*Q: Is it possible to make a Mark-Houwink-Sakurada (MHS) plot with QELS data?*

A: No, a MHS plot graphs intrinsic viscosity versus molar mass, so a differential viscometer is required in addition to a MALS detector. However, you can create a conformation plot of size vs. molar mass with QELS and MALS data. The conformation plot is not identical to a MHS plot, but conveys much of the same information regarding conformation (compact, random coil, linear, etc.). Of course, it will not provide the MHS coefficients needed to interpret intrinsic viscosity data.



Q: *Is there any value in determining hydrodynamic radius  $R_h$  for PLGA from the Mark-Houwink equation in a solvent like THF, using a viscometer?*

A: The combination of differential viscometer and multi-angle light scattering detector *directly* measures the hydrodynamic radius of the sample. Therefore, the *indirect* determination of  $R_h$  via the Mark-Houwink equation, which requires that you know the correct MHS coefficients, should be of relatively little benefit.

### **Branching analysis**

Q: *Can you just use rms radius to do branching analysis for PLGA?*

A: Yes, rms radius from multi-angle light scattering can be (and often is) used to conduct branching analysis. This method is better than combining intrinsic viscosity with MALS: the radius and molar mass data are perfectly aligned, coming from the same detector, and also the drainage factor does not need to be calculated. The drawback is that the sample needs to be larger than 10 nm in rms radius.

Q: *Can you explain more about the drainage factor?*

A: The drainage factor is a measure of the solvent interaction with the sample. It is influenced by the extent of sample solvation along with the sample architecture. The drainage factor is used to convert the viscosity branching ratio ( $g'$ ) to the rms radius branching ratio ( $g$ ).