WHITE PAPER

AN1004: Absolute characterization of polymers with light scattering and UHP-SEC

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Summary

UHP-SEC offers multiple benefits for synthetic polymer characterization. Using short, narrow-bore columns, sub-3 µm beads and ultra-high pressures, size-exclusion separations may be completed in less than 90 seconds, potentially realizing throughputs of hundreds of analyses per day. Sample and mobile phase consumption are greatly reduced, benefiting the environment as well as the costs of analytics.

However, despite the clear advantages of UHP-SEC, its rapid, low-volume separation is more sensitive to column calibration errors and drift than traditional HP-SEC. Additionally, only a small selection of column chemistries is available for eliminating non-ideal sample-column interactions; when these non-idealities do occur, they invalidate column calibration by standards that are not chemically identical to the samples of interest. Therefore, it is essential to combine UHP-SEC with online, low-volume multi-angle light scattering instrumentation (SEC-MALS). SEC-MALS constitutes an absolute technique for determining the molecular weight and size of polymers, independently of retention time. SEC-MALS is necessary for UHP-SEC characterization of branched polymers, rod-like polymers and co-polymers, all of which have no appropriate column calibration standards.

Introduction

UHP-SEC is growing in popularity as the technique of choice for analysis of synthetic polymers. With greatly reduced run times and consumption of both sample and solvent, the time- and cost-saving benefits are compelling. Factoring in better resolution in separation and the consequential improvement in quantification of monomers and oligomers, as well as the flexibility to revert to standard GPC as necessary, the case for upgrading to a highly-productive UHP-SEC system is clear.

Pitfalls of analytical UHP-SEC

With all the advantages of UHP-SEC, several drawbacks need to be addressed. The first is the implicit assumption of a column calibration curve: that different elution times necessarily imply different molar masses, with decreasing molar mass corresponding to increasing elution time. By this logic, a narrow standard would elute in a very narrow peak, in fact narrower than the inherent broadening due to the finite column volume. In reality, even a narrow standard elutes with a width that—theoretically, by column calibration—corresponds to at least a two- to four-fold span in molar mass.

The second drawback is surface chemistry: when optimizing a separation method, it is important to find conditions wherein both the reference markers and sample to be tested do not interact non-ideally (e.g. via hydrophobic or charge interactions) with the column packing. This is because column calibration depends on the assumption that the reference molecules and sample undergo only ideal (steric) interaction with the column, and any additional 'stickiness' will render erroneous results. Compared with HPLC-SEC columns, there are relatively few surface chemistries available to UHP-SEC for method optimization, hence a higher probability that the method will not be fully optimized.

The third is column creep and drift in chromatography conditions: with such a fast separation, even a minor difference in elution time due to column aging, sample loading or changes to the pump operation conditions will lead to a relatively large error in estimated molecular weight.

The solution: light scattering

Online multi-angle light scattering (MALS)[1,2] measures polymer molar mass at each elution volume, absolutely, without reference to retention time. It does so by a first-principles physical relationship between the scattered intensity ($I_{scatter}$), molar mass (M), concentration (c) and scattering angle θ:

$$I_{\text{scatter}} = K \cdot M \cdot c \cdot \left(\frac{dn}{dc}\right)^2 P(\theta) \qquad \text{Eq. 1}$$

where dn/dc is usually known or readily measurable for a polymer in any given solvent, $P(\theta)$ is an angular function that is depends on the molecule's rms radius R_g, and the constant of proportionality K may be calculated from the measurement system properties such as laser wavelength. Hence measurement of $I_{scatter}$ and c directly yields the values of M and R_g, contiguously, as the solution passes through the MALS detector's flow cell. The determination of molecular weight is independent of conformation or shape as well as elution properties. For these reasons, SEC-MALS , utilizing a DAWN® MALS detector and Optilab® differential refractometer, has long been the *de facto* standard for rigorous analysis of proteins and polymers in solutions separated by standard HPLC-SEC.

With the addition of an embedded WyattQELS™ dynamic light scattering (DLS) module, a SEC-MALS system adds a second, independent determination of molecular size (hydrodynamic radius). Online measurement of intrinsic viscosity with a ViscoStar® is another method of determining hydrodynamic radius commonly used with polymers. The relationship between molar mass and molecular size may be further analyzed to determine polymer conformation such as random coil, branched or elongated.

The microDAWN® MALS detector and microOptilab® refractive index detector for UHP-SEC[3] confer the benefits of MALS and embedded DLS on the newer separation technology. UHP-SEC-MALS maintains the central benefits of UHP-SEC while preserving chromatographic resolution and providing absolute molecular weight and size of the eluting species.

The importance of UHP-SEC-MALS is exemplified in Figure 1, where the chromatograms of a 30 kDa polystyrene standard with two injection volumes are presented.

A naïve interpretation of the chromatograms based on elution time would suggest that the 50 µL injection contains primarily smaller species than those in the 4 µL injection. It would also indicate a broad molar mass range covering 10-40 kDa or 20-40 kDa in the respective peaks; yet MALS analysis shows that both injections consisted of narrow distributions around 30 kDa.

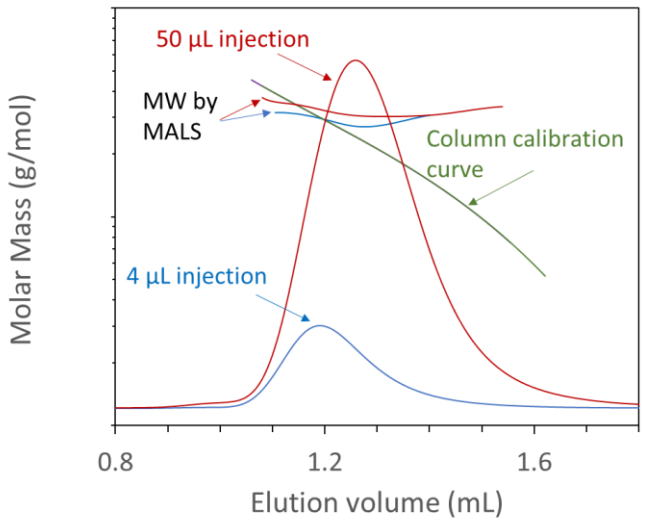


Figure 1. Elution of two injections of 30 kDa polystyrene (4 and 50 µL) by UHP-SEC overlaid with molar masses derived from light scattering. In contrast to the broad and disparate molar mass ranges implied column calibration, MALS proves that both contain narrow distributions around 30 kDa.

Knowns and unknowns

It is always helpful and even essential to validate an unfamiliar technique against commonly accepted standards. For UHP-SEC, such standards are linear polystyrenes, and SEC-MALS analyses of a series of polystyrene standards is quite informative. As shown in Figure 2a, the molar masses derived by light scattering align perfectly with the column calibration curve. However, the flat MALS results across most of the peaks provide absolute evidence that these polystyrene standards are quite homogeneous, something that could not be proven with column calibration alone.

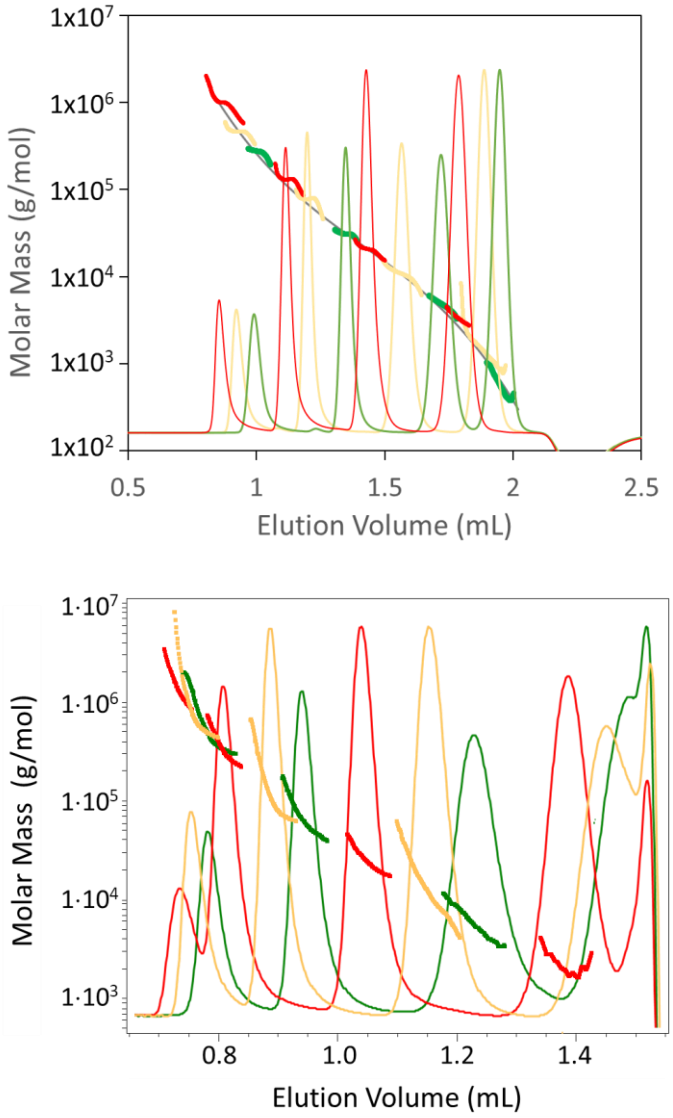


Figure 2. (A) Overlay chromatogram of a series of linear polystyrene standards. MALS analysis indicates high correspondence of calculated molecular weights with the calibration curve and in addition provides the true homogeneity within each peak. (B) Overlay chromatogram of a series of PMMA samples. MALS analysis shows the deviations from column calibration which result from branched conformations as well as internal heterogeneity for each peak.

PMMA, on the other hand, elutes quite differently from linear polystyrene due to branching. As shown in Figure 2B, the molar masses determined by MALS for these nominally 'unknown' samples do not align nicely with each other as for the polystyrene standards, and each peak is quite polydisperse. Nevertheless, MALS provides the full picture in terms of correct, accurate molar masses and distributions across the entire range from hundreds to millions of g/mol.

SEC-MALS vs. UHP-SEC-MALS

Not only is it essential to validate a new technique against standards, it is also important to compare it against the old technique. For decades, SEC-MALS has been the gold standard for evaluating polymer molar mass and size. While current UHP-SEC-MALS instrumentation does not cover the entire size range of standard SEC-MALS (MW: 300 Da – 10^9 Da, rms radius: 10 nm – 500 nm), it does cover the entire range of UHP-SEC columns (MW: 300 Da – 10^6 Da for linear polymers and higher for branched polymers, rms radius: 10 nm – 50 nm). Cross-validation has consistently shown excellent agreement between SEC-MALS and UHP-SEC-MALS.

The comparison is particularly illuminating in light of the concerns that have been raised about the potential for shearing polymers under high pressure on the tightly packed UHP-SEC column. Figure 3 present the analysis of a 1.5 MDa polystyrene sample, characterized by SEC-MALS (green, late-eluting chromatogram) and UHP-SEC-MALS (red, early-eluting chromatogram), both at flow rates of 0.5 mL/min. In both analyses the weight-average molar masses came out to precisely (1.56 ± 0.01) x 10^6 g/mol, and the z-average rms radii were nearly identical at 63.0 and 61.5 nm, respectively. Hence we may conclude that no shearing occurs, at least under these conditions. Potential for shear at higher flow rates remains to be tested.

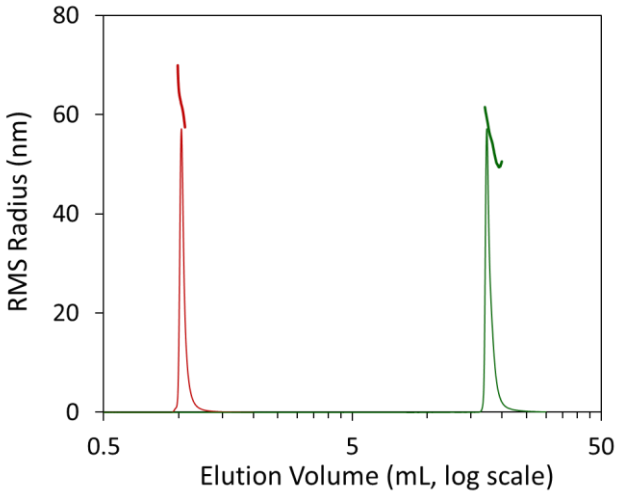


Figure 3. SEC-MALS (green, late-eluting) and UHP-SEC-MALS (red, early-eluting) chromatograms of a 1.5 MDa MW polymer, both at 0.5 mL/min. MALS analyses of weight-average molar mass and z-average rms radii indicate no degradation on the UHP-SEC columns compared to standard GPC columns.

Nonlinear conformation – not an obstacle

Polymers may take on a variety of conformations, including random coil, branched, and rod-like. Each conformation presents a different relationship between molar mass and hydrodynamic volume, and therefore a different relationship between molar mass and elution volume. Unlike PMMA or polystyrene, some epoxy resins are stiff and elongated, similar to rigid rods. Their elongated shape produces a comparatively large hydrodynamic volume and hence they elute much earlier, for a given molar mass, than either of these macromolecules.

Figure 4A presents three UHP-SEC chromatograms corresponding to these three polymer types along with the molar masses determined by UHP-SEC-MALS. While this particular PMMA sample is not heavily branched and therefore its molar mass/elution volume relationship diverges only slightly from that of polystyrene, the epoxy resin (EP) is clearly distinguished from the other two. At each elution volume, EP exhibits a molar mass that is about 40-50% lower than the corresponding polystyrene species. The discrepancy in elution behavior is a direct consequence of the differing conformations. While standard analytical SEC would not recognize the discrepancy, it is highlighted and revealed immediately by MALS and the true molar mass values are determined.

Figure 4B presents the chromatograms and overlaid molar mass values determined for two EP samples. The nearly perfect matchup of molar masses along the chromatogram indicate that these resins possess the same conformation, even though they span different ranges and exhibit quite different distribution shapes. The moments and polydispersity values presented in Table 1 indicate very good repeatability for each sample.

One of the most challenging tasks for polymer characterization is the analysis of copolymers. Since no well-characterized, narrow reference standards exist for these quite heterogeneous complexes, analysis by SEC with column calibration is impossible.

Triple detection combining MALS, UV and dRI is the most common method for analyzing copolymers[4]. The combination of two distinct concentration signals is sufficient to determine the copolymer ratio and weight-average *dn/dc* value, which are then plugged into the light scattering equation (Eq. 1) in order to determine the molar mass of each component in the complex as well as the overall molar mass. All the key components of a triple-detection system for copolymer analysis are readily available and integrate well in UHP-SEC format: UHPLC pumps and columns plus online UV, MALS and RI detectors. Hence the standard techniques developed for SEC-MALS are readily extendible to UHP-SEC-MALS.

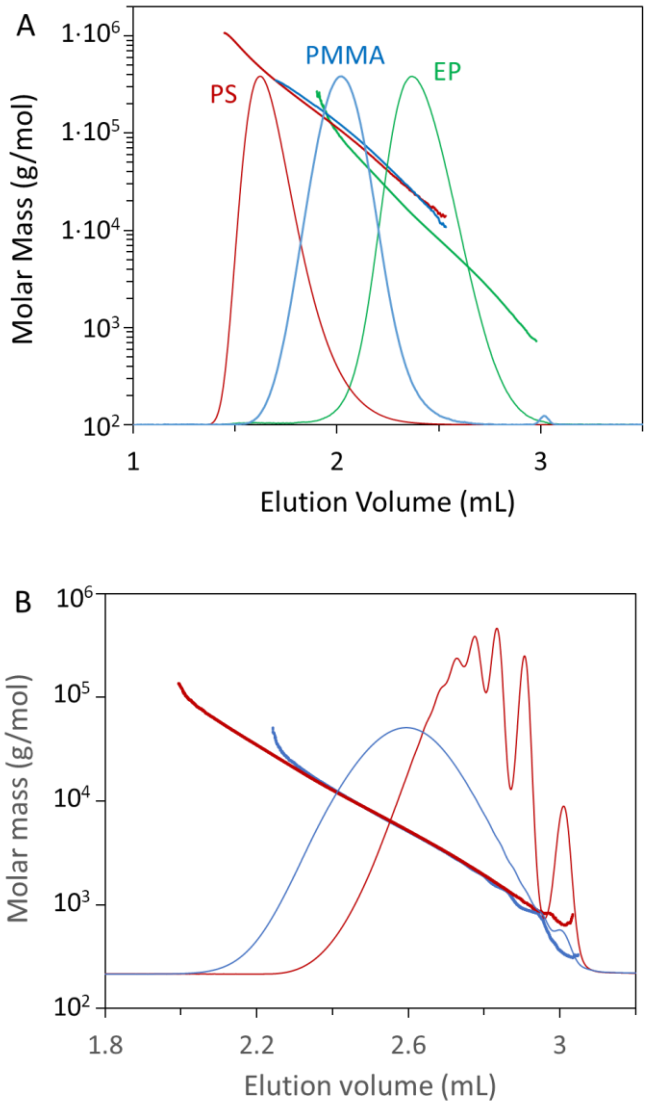


Figure 4. A) The elution behavior of stiff, rod-like epoxy (EP) vs. linear polystyrene (PS) or slightly branched PMMA by UHP-SEC-MALS. At each elution volume, the molar masses of PS and PMMA are quite similar while that of EP is significantly lower, a consequence of its stiff, rod-like conformation. B) UHP-SEC-MALS analysis of two epoxy resins samples. While one sample exhibits quite distinct low-molar-mass peaks, the molar masses overlay perfectly along the chromatogram, indicating that the two samples possess the same conformation.

Experimental

The data presented here were acquired using an Acquity® UPLC® system with APC 125 and 450 Angström pore SEC columns (Waters Corp., Milford, MA), a microDAWN MALS detector (Wyatt Technology Corp., Santa Barbara, CA) and a microOptilab refractive index detector for UHPLC (Wyatt Technology), and were analyzed with the ASTRA® software (Wyatt Technology). Typical conditions included 4- 20 µL injections and 0.5 – 1.0 mL mL/min flow rate. Additional tests have shown that the elution volume of any given species may vary with flow rate, but molar mass and size as determined by light scattering do not, over a range of flow rates from 0.05 µL/min to 2 mL/min.

	M_n (kDa)	M_w (kDa)	PD (M_w/M_n)
EP1(001)	1.55 ±0.02	3.26 ±0.01	2.10 ±0.03
EP1(002)	1.55 ±0.02	3.25 ±0.01	2.10 ±0.03
EP2(001)	3.58 ±0.07	7.90 ±0.02	2.21 ±0.04
EP2(002)	3.47 ±0.06	7.96 ±0.02	2.30 ±0.04

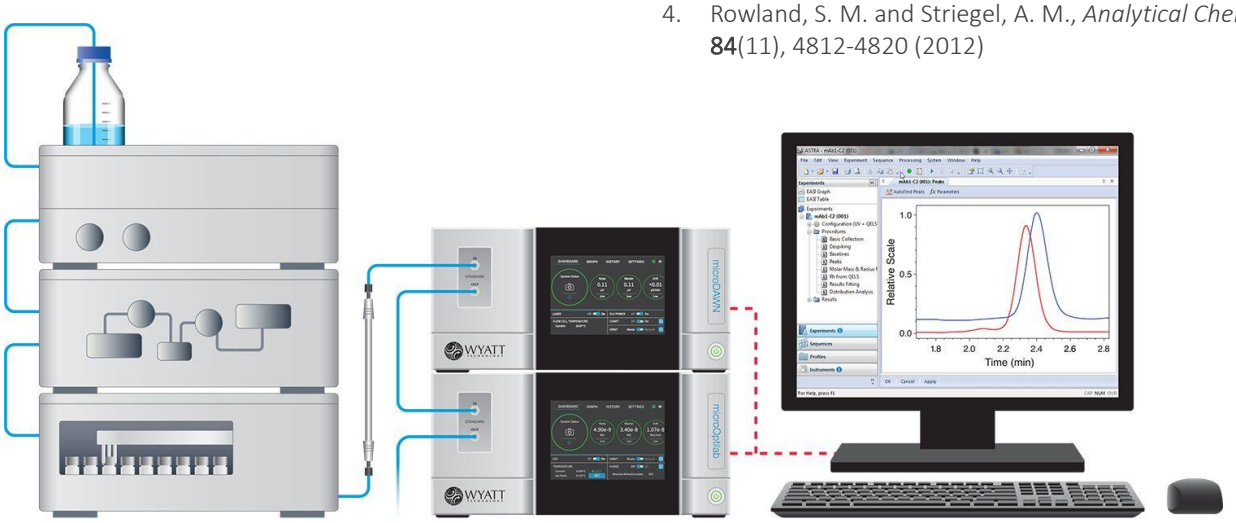
Table 1. Molar mass moments and polydispersity values along with experimental uncertainties (precision) for two epoxy resins, each with two replicates. The agreement between subsequent runs is quite good.

Conclusions

UHP-SEC provides many benefits for the characterization of synthetic polymers and other macromolecules ranging in size from hundreds to millions of g/mol. However, these can only be fully realized with the addition of a µDAWN online light scattering detector for absolute determination of molar mass and size, in order to overcome the inherent limitations of size exclusion chromatography which may be exacerbated in the context of UHPLC. In addition, UHP-SEC-MALS characterizes complex molecules such as copolymers, branched or elongated polymers which do not possess available reference markers. Therefore, light scattering is an essential tool for analytical, process development and QC labs implementing UHP-SEC. To learn more about these instruments and their uses for polymer characterization, contact info@wyatt.com.

References

1. Wyatt, P.J., *Analytica Chimica Acta* **272**, 1-40 (1993)
2. For brief overview of light scattering as applied to macromolecules and size-exclusion chromatography, see http://www.wyatt.com/Theory
3. Hsieh, V.H. and Wyatt, P.J., *Scientific Reports* **7**, article number: 10030 (2017)
4. Rowland, S. M. and Striegel, A. M., *Analytical Chemistry* **84**(11), 4812-4820 (2012)



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