APPLICATION NOTE

AN1312: Analysis of PLGA using APC-MALS

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Introduction

Poly(lactic-co-glycolic acid) (PLGA), is a copolymer formed from lactic acid and glycolic acid. Due to its biocompatibility and biodegradability, PLGA is used extensively in the biomedical field as medical devices or for drug delivery. Additionally, the component monomers themselves have very little toxicity. As they are naturally occurring, lactic acid and glycolic acid may be derived from renewable resources, making PLGA more attractive for use.

Determining the properties of PLGA is crucial in product formulation. For example, the lactic acid to glycolic acid ratio (L:G ratio) affects both the rate of hydrolytic degradation and solubility in organic solvents. Of particular importance is molecular weight (MW), which influences polymer strength and ease of processing into products. Traditionally, SEC with standard calibration has been used to analyze PLGA MW. However, there are several drawbacks to this method, including property disparities between standard and samples, and dependence on good chromatography.

Size-exclusion chromatography with multi-angle light scattering and intrinsic viscosity detection (SEC-MALS-IV) is an ideal technique to characterize PLGA: size and conformation are calculated along with absolute molecular weight. This ability to capture multiple properties of PLGA takes on greater importance as companies attempt to make generic versions of drugs containing PLGA. These companies must prove to the FDA that excipients in their generic product are the same as in the reference listed drug. In a recent paper by Hadar *et al.*, Wyatt detectors were used to characterize PLGA extracted from a commercial drug product.[1] The product-derived PLGA was then compared to PLGA standards using several parameters including, but not limited to, MW, molecular weight distribution, polydispersity, and number of branches.

The analysis can be enhanced further by replacing the high-performance liquid chromatography (HPLC) component with ultra-high-performance liquid chromatography (UHPLC). UHPLC provides faster experiment times without sacrificing resolution, and meets sustainability goals through reduced solvent consumption. This application note details the use of SEC-MALS-IV in conjunction with UHPLC for rapid and precise quantification of PLGA.

Materials and Methods

Samples consisted of linear and branched PLGA samples from PolySciTech dissolved in THF. The UHPLC setup consisted of the Waters™ ACQUITY™ Advanced Polymer Chromatography™ (APC™) System with Waters ACQUITY APC XT 45 Å, 4.6×150 mm; 125 Å, 4.6×150 mm; and 450 , 4.6×75 mm columns. The detector suite included the Wyatt microDAWN®, microViscoStar®, and microOptilab®.

Results and Discussion

Speed, sensitivity and resolution

Unlike homopolymers of lactic acid and glycolic acid, PLGA is soluble in a wide range of solvents, including tetrahydrofuran (THF) and dichloromethane (DCM). Even though the refractive index increment, d*n*/d*c*, of PLGA in these commonly used HPLC solvents is very low, around 0.040 to 0.050 mL/g—resulting in weak MALS and refractive index (RI) signals—the Wyatt detector suite possesses excellent sensitivity to ensure good data collection.

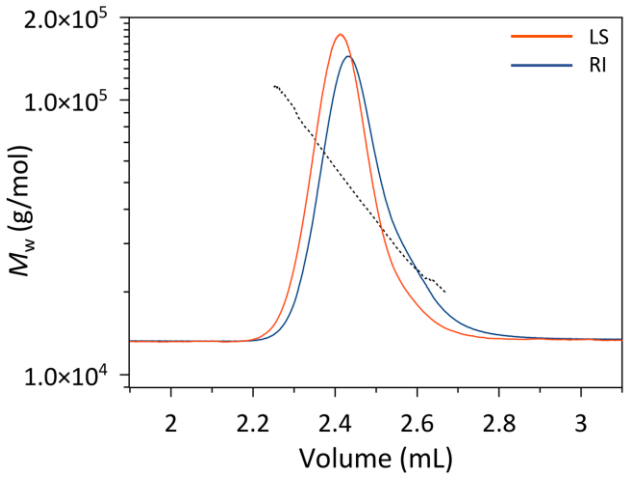


Figure 1. Molar mass vs elution of a branched PLGA sample in THF (2.6 mg/mL)

Liquid chromatography separations often sacrifice resolution for speed. Yet, the low-dispersion flow path of the Waters ACQUITY APC System, including the robust ACQUITY APC XT separation column, yields high resolution, short analysis times, and quick mobile phase changes. This high speed/high resolution separation technique works best with low-dispersion detectors, and Wyatt offers a low-dispersion triple-detection system that maintains the resolution of the APC separation while determining accurate molecular weights.

In this particular experiment, PLGA analysis with the APC was 2× faster and consumed 3× less solvent compared to the HPLC setup. This is useful for sustainability and reducing overhead costs, especially for expensive solvents like hexafluoroisopropanol (HFIP). As seen in Figure 1, the LS and RI signal peaks possess superb signal-to-noise ratio (SNR), permitting robust molar mass determination across the peak.

Conformation analysis

Branched vs. linear

Molecular weight is not the only parameter measured by SEC-MALS-IV. With the inclusion of the microViscoStar, PLGA size and conformation can also be determined. In Figure 4, Mark-Houwink-Sakurada (MHS) plots of linear and branched PLGA are shown.

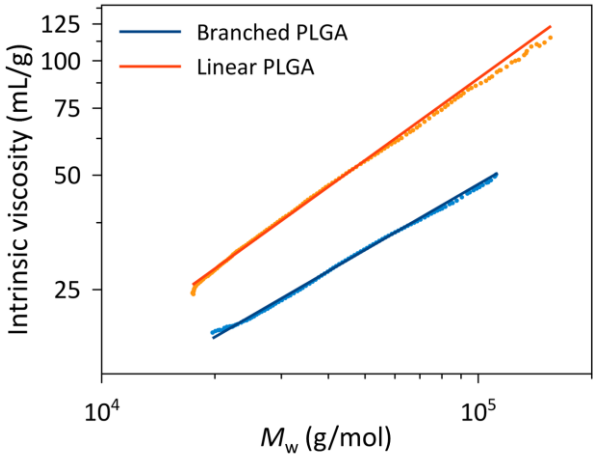


Figure 4. Mark-Houwink-Sakurada plot of a branched PLGA vs a linear PLGA. The branched PLGA has a lower slope indicative of a more compact conformation.

The branched PLGA exhibits a lower slope than the linear PLGA, indicating a more compact conformation for the branched PLGA. The two PLGA samples are readily distinguished from each other in the MHS plot. The weight-average molecular weights and hydrodynamic radii (R_h) of both samples are displayed in Table 1. The branched architecture is clearly indicated by a similar R_h value at a higher molar mass.

	M_w (g/mol)	R_h (nm)
Linear PLGA	27.2×10^3	6.0
Branched PLGA	42.3×10^3	6.1

Table 1. Molar mass and size of the linear and branched PLGA samples.

The effect of L:G ratio

The MHS plot can also differentiate PLGA polymers with different L:G ratios. Figure 5 shows the MHS plot of two linear PLGA samples.

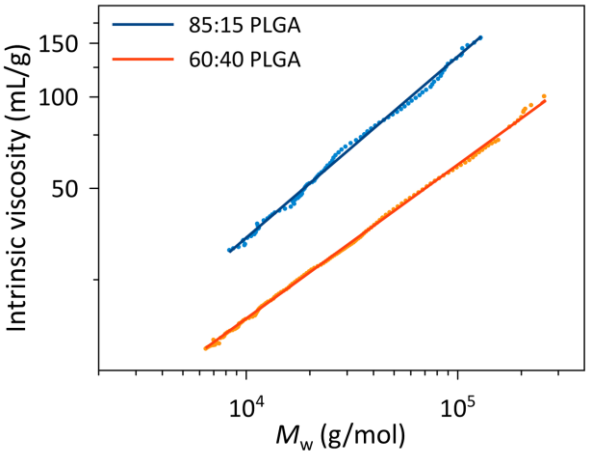


Figure 5. Mark-Houwink-Sakurada plot of an 85:15 PLGA sample versus a 60:40 PLGA sample. The 85:15 PLGA has a MHS *a* value of 0.60 compared to 0.51 for the 60:40 PLGA.

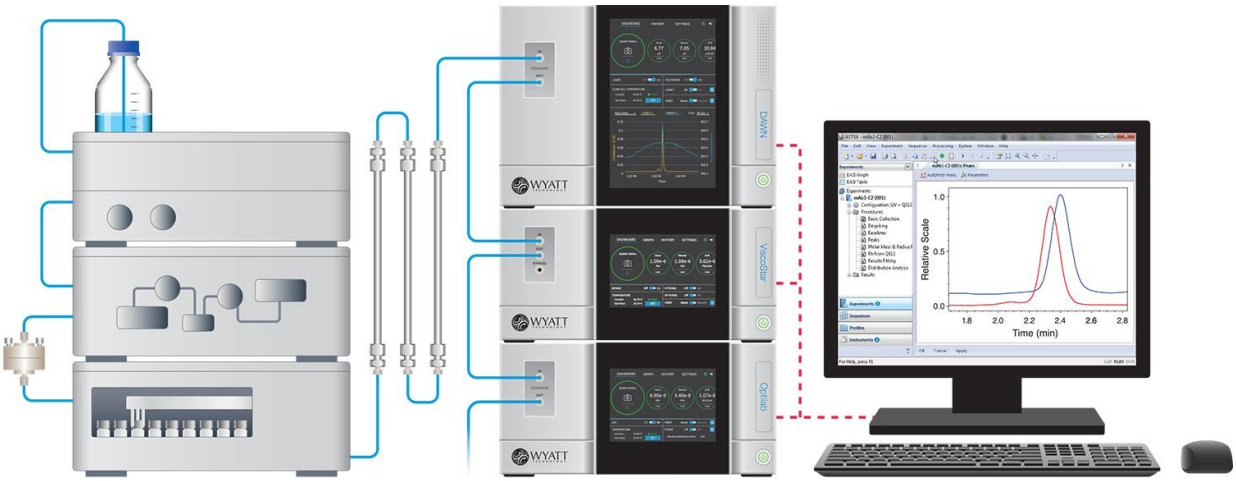
One sample has a L:G ratio of 85:15 while the other sample has a L:G ratio of 60:40. With a higher glycolic acid content, the 60:40 PLGA has reduced solubility in THF, resulting in a more compact conformation. This difference is reflected by the lower MHS slope of the 60:40 PLGA (0.51) versus the 85:15 PLGA (0.60). Both values are above 0.5, reflecting the linear nature of the PLGA samples.

Conclusions

The Wyatt complement of UHPLC-compatible detectors possess excellent resolution and sensitivity, allowing for facile analysis of a difficult sample like PLGA in THF. With the inclusion of an online microviscometer, the conformation and size of the samples are determined in addition to molar mass. This capability reveals the effect of varying PLGA composition on architecture. Finally, the powerful combination of Wyatt instruments and the Waters APC system produces faster experiments with improved resolution that consume less solvent, allowing for higher throughput, lower operating costs and overall enhanced productivity.

References

1. Hadar, J., Skidmore, S., Garner, J., Park, H., Park, K., Wang, Y., Qin, B., Jiang, X. Characterization of branched poly(lactide-co-glycolide) polymers used in injectable, long-acting formulations. *J. Control. Release.* **304**, 75-89 (2019).



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