White Paper

Wyatt Technology Corporation

Branching Revealed: Characterizing Molecular Structure in Synthetic and Natural Polymers by Multi-Angle Light Scattering

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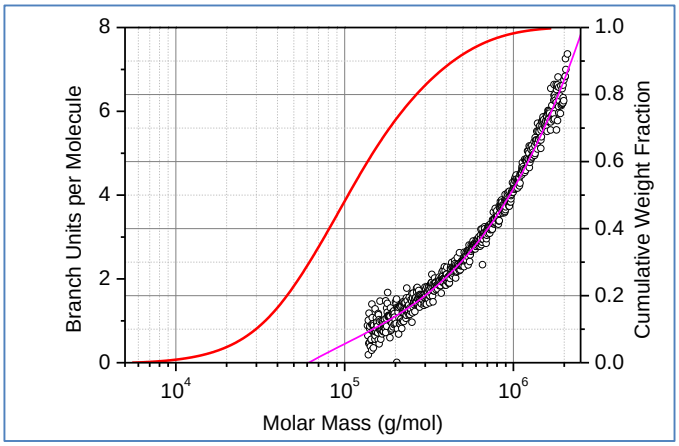
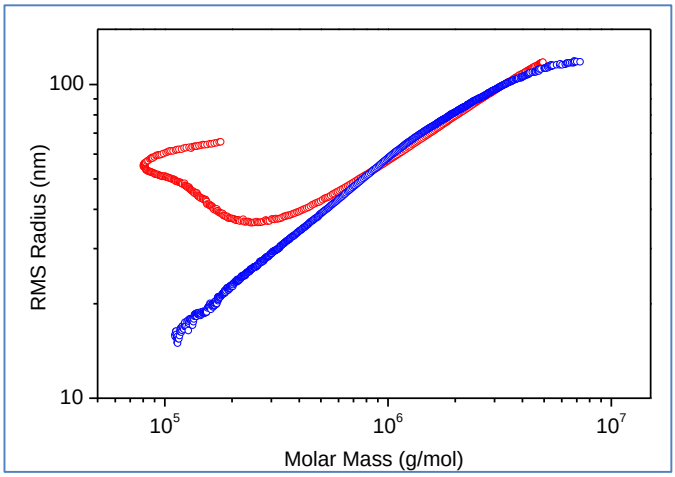
Summary

This note provides a brief overview of basic principles of the detection and characterization of branching by means of multi-angle light scattering (MALS) detector. The most common methods that can provide branching information are presented and elucidated by means of real experimental results obtained with Wyatt Technology instruments. The abnormal elution behavior of branched macromolecules in size exclusion chromatography (SEC) is explained and the data acquired by SEC-MALS are compared with those obtained by asymmetric flow field flow fractionation (A4F). The comparison of SEC-MALS and A4F-MALS results proves superior A4F separation of large and highly branched macromolecules compared to their separation by SEC. For those who are more interested in branching topic the Application Note offers several recent literature references.



Number of branch units per molecule plotted against molar mass, determined from SEC-MALS-RI analysis. The cumulative molar mass distribution (red) and the 3rd order fit to experimental data points (magenta) are overlaid.



Conformation plots of polymer containing branched macromolecules as determined by SEC-MALS (red) and A4F-MALS (blue). A4F separation is purely hydrodynamic hence the monotonic relationship between rms radius and molar mass.

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WYATT TECHNOLOGY

Introduction

Branching represents an important structural parameter of many synthetic and natural polymers, with significant impact on their properties. The properties affected by branching include various mechanical properties; viscosity and rheological behavior of polymer solutions and melts; thermodynamic properties; and the ability to crystallize or dissolve. Quantitative information about branching topology is vital for the development of novel polymer-based materials with enhanced properties and for understanding polymerization processes.

The characterization of branching by means of size exclusion chromatography (SEC) or asymmetric flow field flow fractionation (A4F) would not be possible without a multi-angle light scattering (MALS) detector. The importance of branching is obvious from numerous scientific papers devoted to branching topic. The fact that Wyatt instruments play essential roles in branching studies is evident from the Wyatt Bibliography where the key words 'polymer branching' result in 218 references. Selected recent papers on branching in synthetic polymers can be found in References.[1,2,3,4,5,6,7,8,9]

Although branching is mostly related to synthetic polymers, it is also an important parameter for many biopolymers. For example, Wyatt instruments were used to detect branching in hyaluronic acid, an important biopolymer with numerous medical and pharmaceutical applications, which was until recently believed to have a linear structure.[10] Several other examples of branching in biopolymers are cited in the References section.[11,12,13,14]

This Application Note shows the most common methods for characterizing branching, which use a MALS detector. More details and other methods can be found in reference 15.

Theoretical Background

The history of branching characterization began with a famous article by Zimm and Stockmayer who introduced a theoretically derived parameter called the 'branching ratio', g:[16]

$$g = \left(\frac{R^2_{branched}}{R^2_{linear}} \right)_M \qquad (1)$$

where R^2 is the mean square radius of branched and linear macromolecules having the same molar mass (M). As shown in reference 16, the branching ratio g is directly related to the number of branch units in randomly branched polymers or to the number of arms in star-branched polymers. The branching ratio g is ≤ 1, where the equality sign is valid for linear polymers. The lower the value of g, the higher the degree of branching, for example, a value of $g \approx 0.1$ indicates a highly branched structure.

Ten years after the definition of g by Zimm and Stockmayer, Zimm and Kilb introduced an alternative branching ratio based on the intrinsic viscosity:[17]

$$g' = \left(\frac{[\eta]_{branched}}{[\eta]_{linear}} \right)_M \qquad (2)$$

where $[\eta]$ is the intrinsic viscosity of branched and linear polymer molecules having the same molar mass. The relationship between g' and g is described via the so-called 'draining parameter' e:

$$g' = g^e \qquad (3)$$

The parameter e is expected to vary in the range of 0.5 – 1.5, while a value of $e \approx 0.7$ may be used reliably for the recalculation of g' to g.

Equations 1 and 2 show that in order to identify and characterize branching one needs information about the molar mass and the molecular size. That is exactly the power of MALS: it provides both pieces of information simultaneously and independently. The MALS detector is usually connected to an analytical separation technique - most commonly SEC - to determine branching parameters as a function of molar mass. Typically the parameters of interest are the branching ratio and the number of branch units per molecule. A certain limitation of MALS is given by the inability to determine RMS radius for relatively small polymers with $R < \approx 10$ nm, which corresponds to a molar mass of $\approx 10^5$ g/mol. In the case of such polymers an alternative size parameter can be used instead of RMS radius. The two most effective alternatives are intrinsic viscosity and SEC elution volume. The former can be used for the calculation of g' using Equation 2, whereas the latter can provide g using the approach of Yu and Rollings:[18]

$$g = \left(\frac{M_{linear}}{M_{branched}} \right)_V^{\frac{1+a}{e}} \qquad (4)$$

where M is the molar mass of linear and branched molecules eluting at the same elution volume V and a is the exponent of the Mark-Houwink equation for a linear polymer.

Experimental

The data presented here were acquired with the follow-ing Wyatt Technology Corporation instruments: a MALS photometer DAWN® HELEOS®, an online vis-cometer ViscoStar™, a refractive index detector Optilab® T-rEX™ and an A4F system Eclipse™ AF4. The acquired data were processed with ASTRA® 6 software. Tetrahydrofuran was used as a solvent for both SEC and A4F.

Methods for Characterizing Branching

Branching ratio–versus–molar mass plots can be obtained by one of the following methods:

1. *Radius method:* calculates g from the conformation plot (log-log plot of R–versus–M) using Equation 1.
2. *Viscosity method*: calculates g' from the Mark-Houwink plot (log-log plot of $[\eta]$–versus–M) using Equation 2.
3. *Mass method:* calculates g from the molar mass–versus–elution volume plot using Equation 4.

The advantage of methods 1 and 2 is given by the possibility of using literature constants of conformation or Mark-Houwink parameters. In contrast, the mass method requires measurement of a linear counterpart under the same SEC conditions as those used for branched sample. However, the mass method is suitable for small polymers for which the RMS radius cannot be measured. The pros of viscosity method include high sensitivity with respect to branching and possibility to measure over a broad range of molar masses down to about 1000 g/mol.

Results and Discussion

An example of the conformation plots of linear and branched polymer is shown in Figure 1. Branching can be easily identified from the slope of the plot: the slopes of the conformation plots of all linear polymers in thermodynamically good solvents are ≈ 0.58, whereas lower values are indicative of branching (0.33 for compact spheres). The conformation data allow calculation of

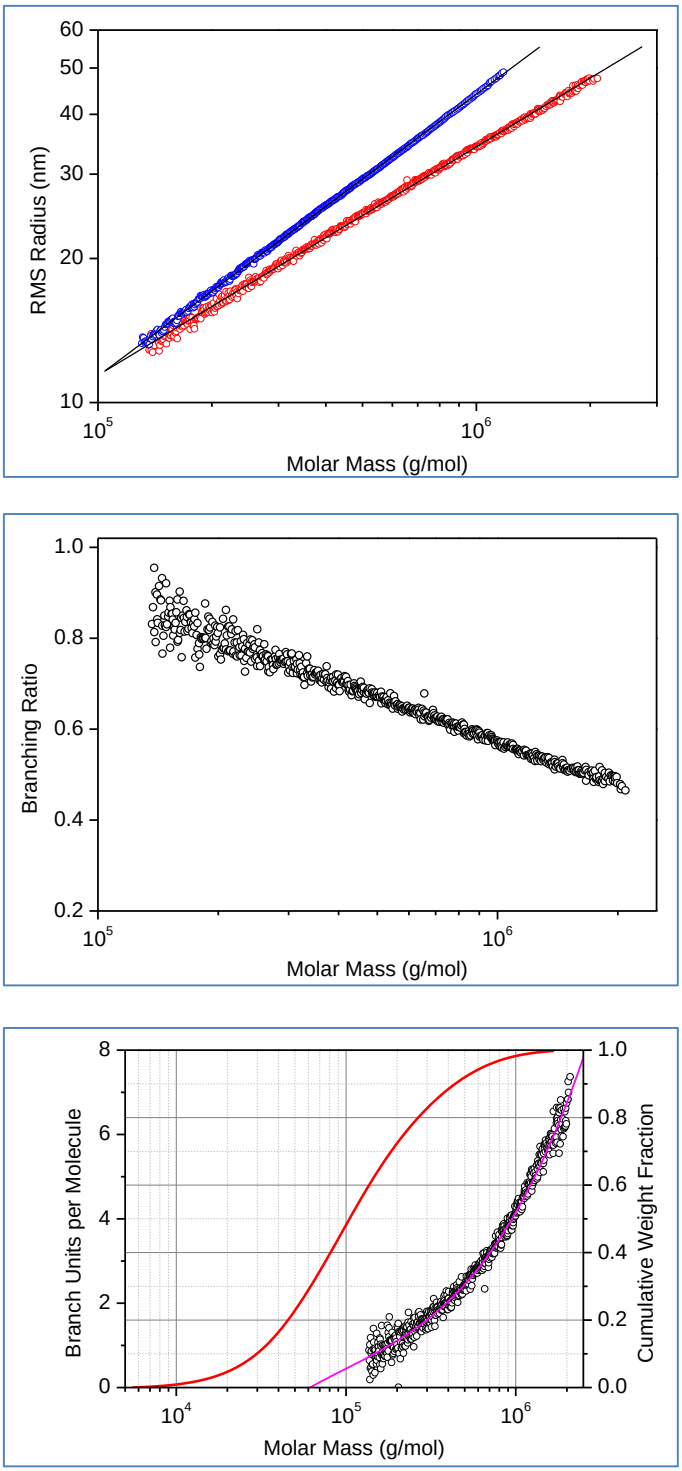


Figure 1. Top: Conformation plots of linear (blue) and branched (red) polystyrene. Center: The corresponding plot of branching ratio versus molar mass. Bottom: The number of branch units per molecule plotted versus molar mass. The plot of branch units per molecule versus molar mass is overlaid with the cumulative molar mass distribution (red), and the 3rd order fit to experimental data points (magenta). The slopes of the conformation plots of linear and branched polymer are 0.59 and 0.48, respectively.

plots of branching ratio–versus–molar mass and number of branch units per molecule–versus–molar mass as shown in Figure 1. To facilitate quantitative evaluation of branching it is beneficial to overlay the plot of branching parameter with the cumulative distribution of molar mass. Glancing at Figure 1 one can see that ≈ 28 % of molecules with molar masses below ≈ 60,000 g/mol do not contain branch units. Noteably, SEC-MALS is capable of detecting just a single branch unit in polymer chains.

The characterization of branching for small polymer molecules is depicted in Figure 2 which compares Mark-Houwink and molar mass–versus–elution volume plots of linear and branched polyesters based on lactic acid. This bio-compatible and bio-degradable polymer can find utilization as a drug delivery material whose ability to swell, degrade and release an active compound can be controlled by the degree of branching. Both plots can detect the presence of branched molecules and be used to calculate g by means of Equations 2–4.

The SEC-MALS radius method is unsatisfactory in two extreme cases: on the one hand very small polymers, and on the other certain large, highly branched polymers. For polymers with RMS radius below about 10 nm, the radius method runs up against the limitations of MALS measurements. The method may also fail for some large, highly branched polymers due to limitations of SEC, wherein the branches may be temporarily anchored in the pores of SEC column packing and elute abnormally – at a time which corresponds to a much smaller hydrodynamic volume than actually presented by the molecule.[19]

The abnormal SEC elution results in upswings on the conformation plots and consequently incorrect values of g. For polymers showing abnormal conformation plots A4F proved to be a superior separation technique providing excellent conformation plots.[15] The comparison of conformation plots obtained by SEC-MALS and A4F-MALS is depicted in Figure 3. The upswing on the conformation plot from SEC-MALS is caused by increased polydispersity of molecules eluting in the region of higher elution volumes. For polydisperse fractions the MALS detector measures the z-average RMS radii (R_z) and the weight-average molar masses (M_w). As the R_z is more sensitive to polydispersity than M_w, the conformation plot becomes upturned. The separation by A4F is not affected by the anchoring of branched molecules and the upswing is completely eliminated.

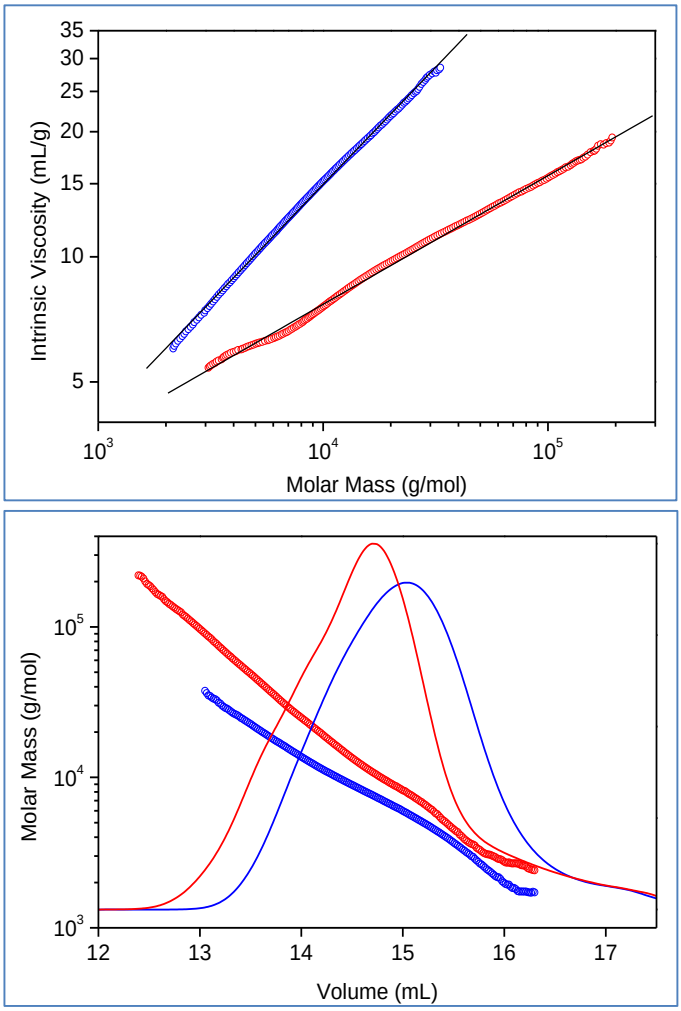


Figure 2. Top: Mark-Houwink plots of linear (blue) and branched (red) poly(lactic acid). Bottom: Molar mass–versus–elution volume plots, same colors. The slopes of Mark-Houwink plots for linear and branched samples are 0.56 and 0.31, respectively. RI chromatograms are overlaid in the molar mass–versus–elution volume plots.

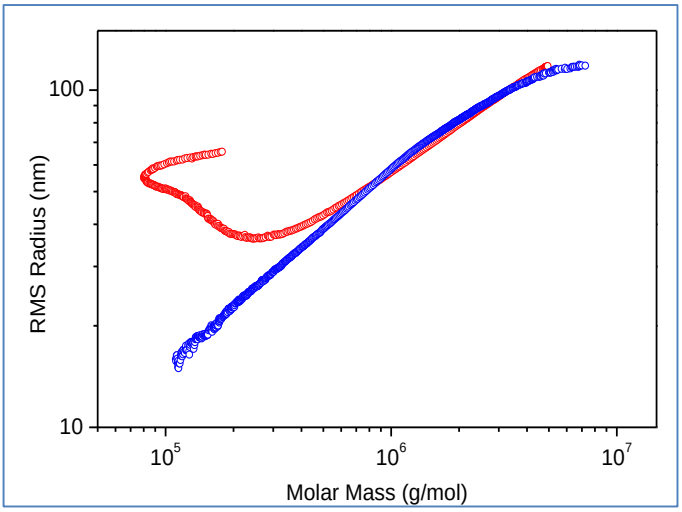


Figure 3. Conformation plots of polymer containing branched macromolecules determined by SEC-MALS (red) and A4F-MALS (blue).

Conclusions

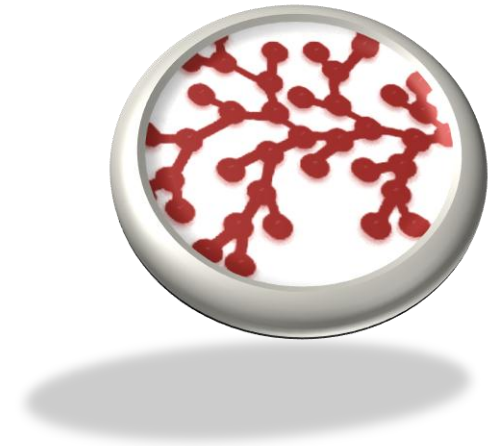
Wyatt MALS photometers allow direct determination of branching ratio *g* and number of branch units per molecule.

The radius method represents the most direct and fundamentally correct method of branching characterization, but it is limited to polymer molecules with RMS radii > 10 nm.

SEC-MALS set-up can be completed with an online viscometer to determine Mark-Houwink plot.

Smaller branched polymers can be characterized by means of Mark-Houwink plot or relation between the molar mass and elution volume.

Separation of some branched polymers is affected by the anchoring of branched macromolecules in the pores of SEC column packing. For such polymers A4F offers superior separation and yields correct conformation plots and values of *g*.



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