

WP1003: Branching revealed: Characterizing molecular structure in synthetic polymers by multi-angle light scattering

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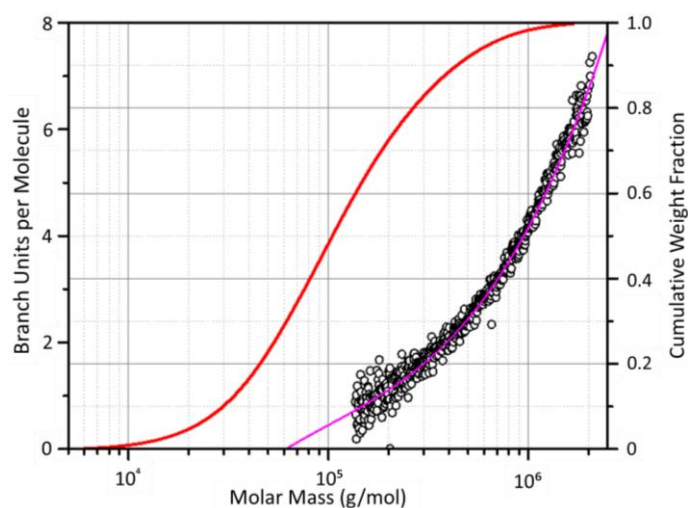
Summary

This white paper provides a brief overview of the basic principles of the detection and characterization of polymer branching by means of multi-angle light scattering (MALS). The most common methods for determining branching parameters are presented and elucidated by means of pertinent experimental results.

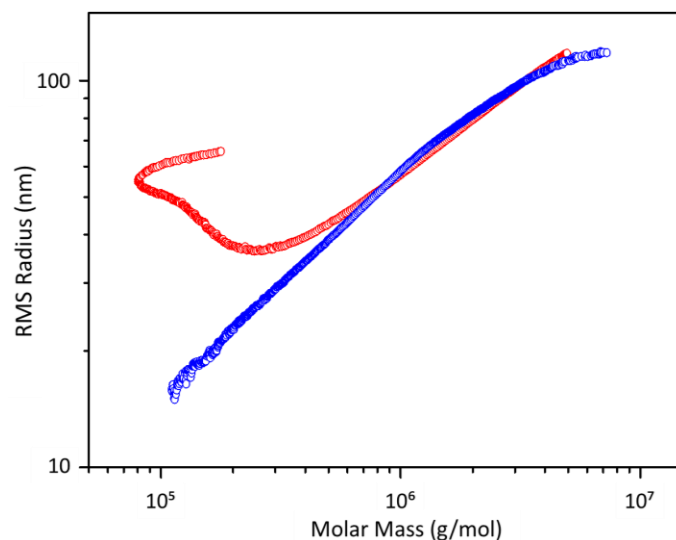
The abnormal elution behavior of certain branched macromolecules in size exclusion chromatography (SEC) is explained and the data acquired by SEC-MALS are compared with those obtained by asymmetric-flow field flow fractionation (AF4). The comparison of SEC-MALS and AF4-MALS proves superior separation of large and highly branched macromolecules by AF4 compared to SEC. The addition of MALS capabilities is invaluable for analyzing branched polymers.



Diagram of an AF4 system comprising HPLC degasser, pump, UV detector and autosampler; Eclipse AF4 control system and separation channel; and MALS and refractive index detectors.



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 AF4-MALS (blue). AF4 separation is purely hydrodynamic hence the monotonic relationship between RMS radius and molar mass.

Introduction

Branching represents an important structural parameter of many [synthetic polymers](#) 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; thermo-dynamic 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](#) (AF4) 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 over 200 references. Selected recent papers on branching in synthetic polymers can be found in [1-9].

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. Several other examples of branching in biopolymers are cited in the References section¹¹⁻¹⁴.

This white paper shows the most common methods for characterizing branching, which use a [MALS detector](#). More details and other methods can be found in [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]:

$$g = \left(\frac{R_{\text{branched}}^2}{R_{\text{linear}}^2} \right)_{\text{M}} \quad (1)$$

where R_{x}^2 is the mean square radius of branched and linear macromolecules having the same molar mass M .

As shown in [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 viscosity¹⁷:

$$g' = \left(\frac{[\eta]_{\text{branched}}}{[\eta]_{\text{linear}}} \right)_{\text{M}} \quad (2)$$

where $[\eta]_{\text{x}}$ 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 :

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

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. The parameters of interest are typically the branching ratio and the number of branch units per molecule. A certain limitation of MALS is its inability to determine RMS radius for relatively small polymers with $R_{\text{g}} < \approx 10$ 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 Rollings¹⁸:

$$g = \left(\frac{M_{\text{linear}}}{M_{\text{branched}}} \right)_{\text{V}}^{\frac{1+a}{e}} \quad (4)$$

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-Sakurada equation for a linear polymer.

Materials and Methods

The data presented here were acquired with the following instruments, all from Wyatt Technology Corporation: a DAWN® MALS photometer, a ViscoStar® online differential viscometer, an Optilab® differential refractive index detector and an Eclipse® AF4 field-flow fractionation system. The acquired data were processed with ASTRA® software. Tetrahydrofuran was used as a solvent for both SEC and AF4.

Methods for characterizing branching

Plots of branching ratio versus molar mass 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-Sakurada plot (log-log plot of $[\eta]$ versus M) using Equation 2
3. *Mass method*: calculates g from the plot of molar mass versus elution volume using Equation 3

The advantage of methods 1 and 2 is inherent in 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 the potential 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 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 $\approx 28\%$ of molecules with molar masses below $\approx 60,000$ g/mol do not contain branch units. Notably, 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 plots and plots of molar mass versus elution volume for linear and branched polyesters based on lactic acid. This bio-compatible and bio-degradable polymer is a potential 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 molecule¹⁹.

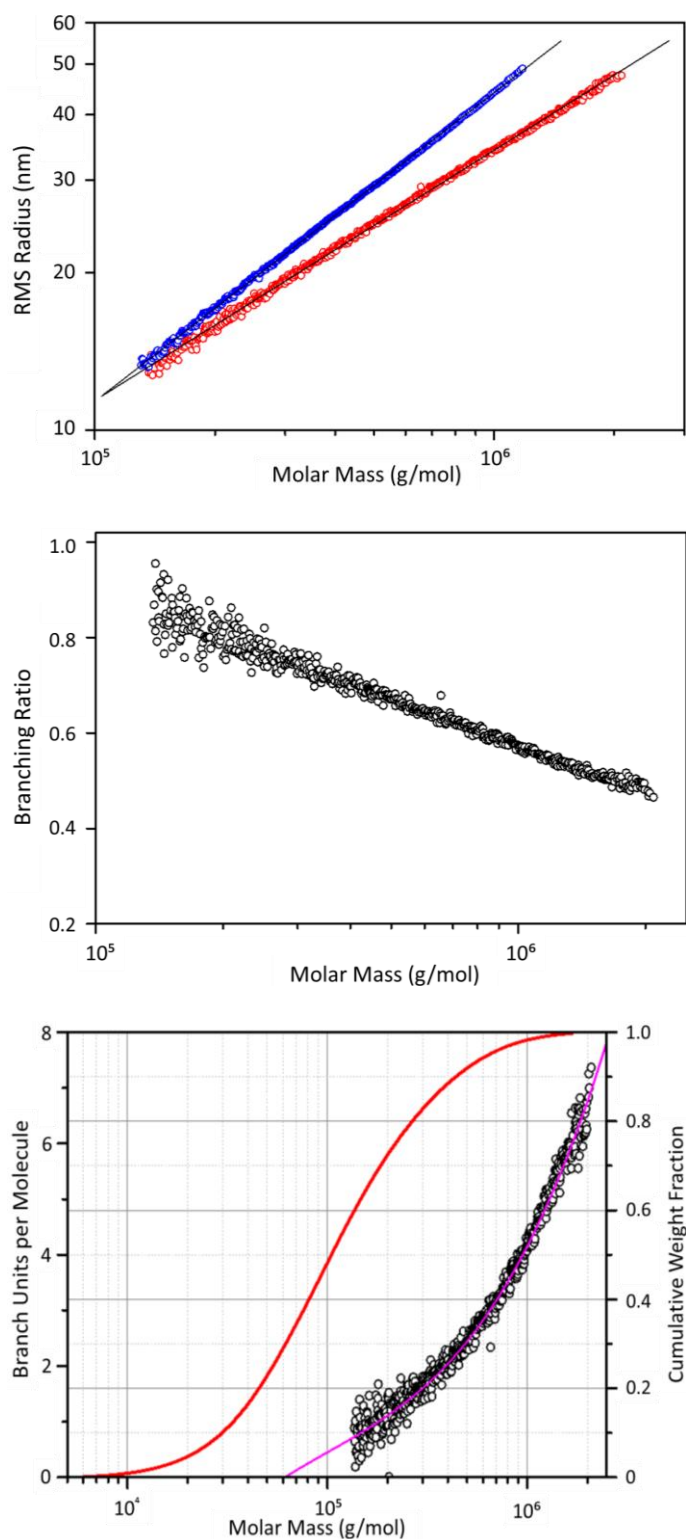


Figure 1. **Top:** Conformation plots of linear (blue) and branched (red) polystyrene. **Center:** The corresponding plot of branching ratio versus molar mass. **Bottom:** The number of branch units per molecule plotted versus molar mass. The plot of branch units per molecule versus molar mass is overlaid 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.

The abnormal SEC elution results in upswings on the conformation plots and consequently incorrect values of g . For polymers showing abnormal conformation plots AF4 proved to be a superior separation technique providing excellent conformation plots¹⁵. The comparison of conformation plots obtained by SEC-MALS and AF4-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 AF4 is not affected by the anchoring of branched molecules and the upswing is completely eliminated.

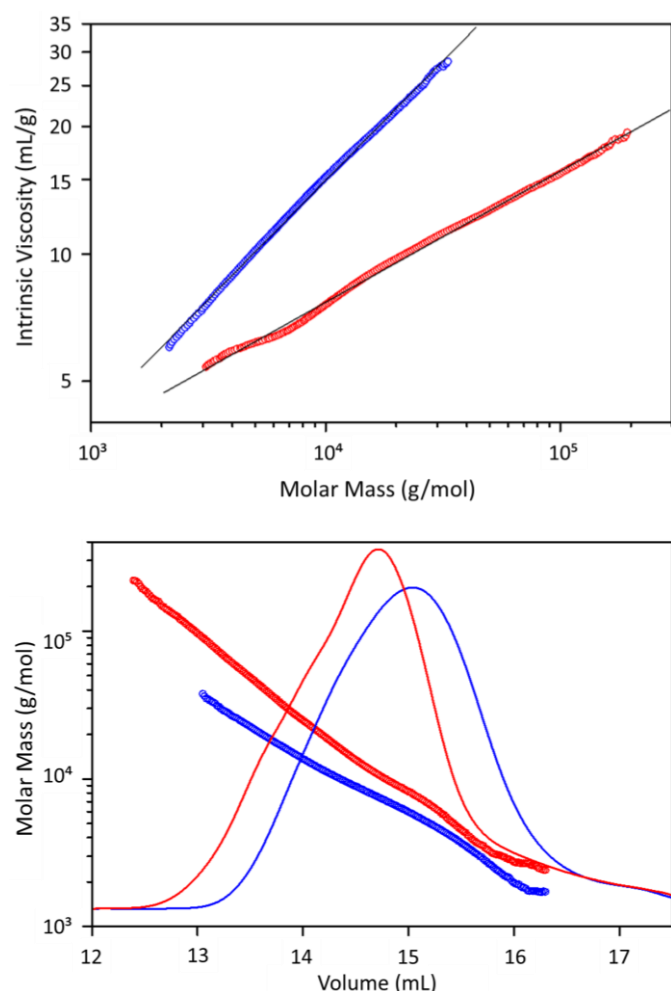


Figure 2. **Top:** Mark-Houwink-Sakurada plots of linear (blue) and branched (red) poly(lactic acid). **Bottom:** Plots of molar mass versus elution volume, same colors. The slopes of Mark-Houwink-Sakurada plots for linear and branched samples are 0.56 and 0.31, respectively. RI chromatograms are overlaid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

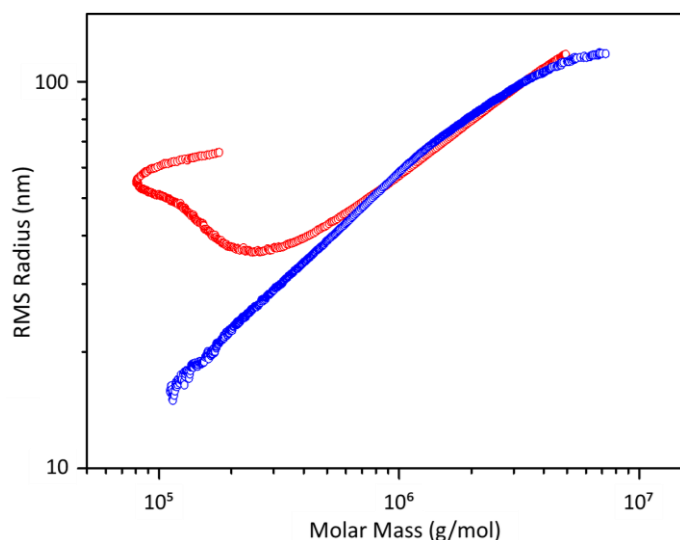


Figure 3. Conformation plots of polymer containing branched macromolecules determined by SEC-MALS (red) and AF4-MALS (blue).

Conclusions

SEC-MALS determines directly the polymer branching ratio g and the 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 $R_g > 10$ nm.

A SEC-MALS set-up can be enhanced with an online viscometer to determine Mark-Houwink-Sakurada parameters. Smaller branched polymers can be characterized by means of Mark-Houwink-Sakurada 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 AF4 offers superior separation and yields correct conformation plots and values of g .

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