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BEYOND GPC: USING LIGHT SCATTERING FOR ABSOLUTE POLYMER CHARACTERIZATION

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TOC
Table of contents

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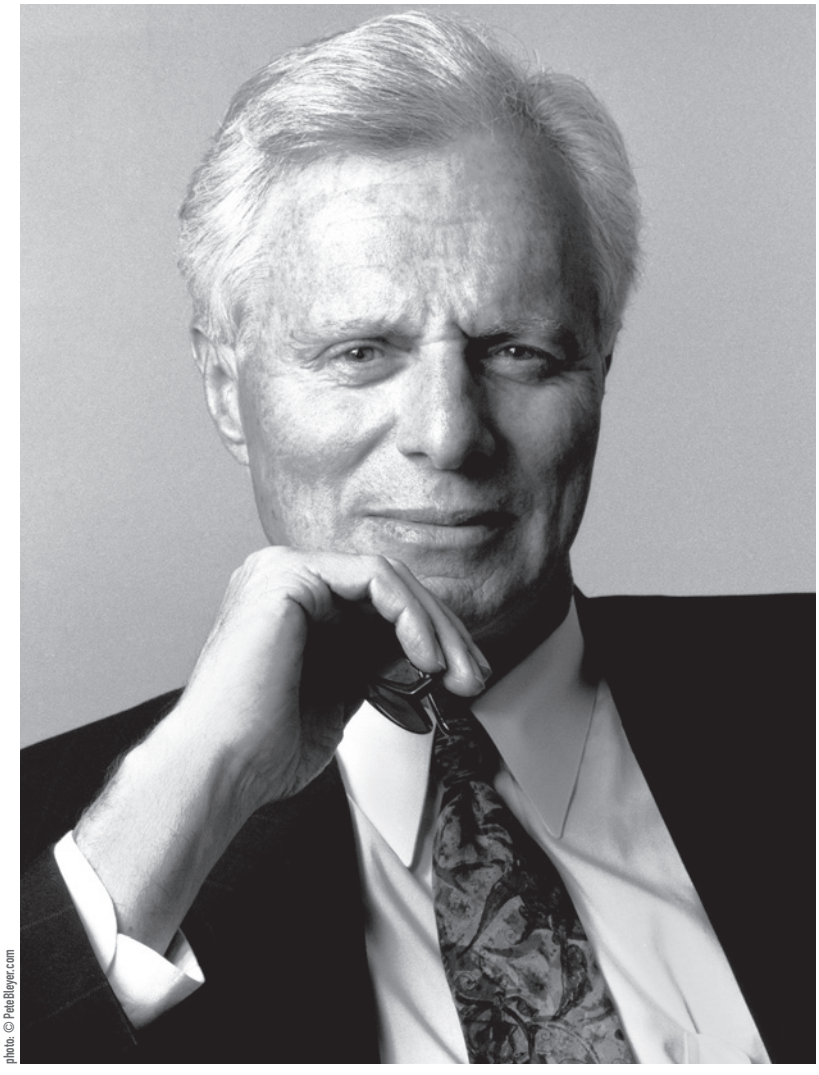
The Most Interesting Man in Light Scattering.



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We Call Him Dad.

Dr. Philip Wyatt is the father of Multi-Angle Light Scattering (MALS) detection. Together with his sons, Geof and Cliff, he leads his company to produce the industry's most advanced instruments by upholding two core premises: First, build top quality instruments to serve scientists. Check. Then delight them with unexpectedly attentive customer service. Check. After all, we don't just want to sell our instruments, we want to help you do great work with them. Because at Wyatt Technology, our family extends beyond our last name to everyone who uses our products.

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OVERCOMING FEAR, UNCERTAINTY, AND DOUBT IN GPC: THE NEED FOR AN ABSOLUTE MEASUREMENT OF MOLAR MASS

Mark W. Spears, Jr.

While conventional calibration for gel permeation (GPC) or size-exclusion chromatography (SEC) is useful, there are inherent disadvantages in this type of analysis that introduce experimental error. This uncertainty may cast serious aspersions on the rigour and utility of the results. Multi-angle light scattering (MALS) detection is quite simple to add to an existing chromatography system and can help overcome the challenges faced with single detector chromatography and conventional calibration-based methods. An alternative separation technique called *asymmetric flow field-flow fractionation (AF4)* offers tunable, column-free fractionation.

Gel permeation chromatography (GPC) and size-exclusion chromatography (SEC) are widely used techniques for the analysis of polymer molecular weight. In this technique, a sample is passed through a separation column and fractionated into differing molecular weight species. After exiting the column, the sample then travels through one or more detectors where some characteristic is measured. Traditionally, the downstream detector is either a refractive index (RI) detector or an ultra-violet (UV) absorption detector.

In order to be useful for calculating molecular weights, a column or column set must be calibrated using well-characterized standards where each species ideally has a narrow molecular weight distribution and the chosen standards span a broad range of molecular weights. Because a typical calibration curve has 10–12 points and can be time-consuming to collect, standards are often injected as mixtures with 3–6 species in a cocktail. In column calibration, the apex of each eluting peak is selected from the concentration detector, and the known molecular weight is plotted versus retention time. In universal calibration, a viscometer is added to the chromatography setup, and the intrinsic viscosity of the standard is now plotted along with known molecular weight to generate a calibration curve that may be

more generally applicable. There are at least two fundamental assumptions for GPC/SEC methods: 1) The polydispersity within each elution volume slice is negligible and 2) the elution time for a species is an accurate predictor of molecular weight when compared to a calibration curve. However, are these assumptions always true? If not, under what conditions do the assumptions fail?

Described below are several points at which column and universal calibration are challenged, and they illustrate that a method of absolute measurement is required. A multi-angle light scattering detector (MALS) can be plumbed in-line with a high performance liquid chromatography (HPLC) system and concentration detector (typically UV or RI) to provide this type of measurement.

Light scattering is an absolute technique, meaning that it does not depend on any calibration standards or calibration curves. The fundamental light scattering equation is:

$$I(\theta) \propto M \times c \times \left(\frac{dn}{dc}\right)^2 \times P(\theta) \quad [1]$$

where the intensity of scattered light at an angle θ is directly proportional to the product of the molar mass M, the concentration c, the square of the specific refractive index increment dn/dc (a constant for each sample), and an angular factor $P(\theta)$, which equals 1 at $\theta = 0$. The absolute intensity of scattered light extrapolated to $\theta = 0$ is used to calculate molecular weight, and the variance of this intensity with angle is used to calculate the root mean square (rms) radius of the sample. Other information can be gleaned from the data such as analysis of copolymers and polymer branching ratio.

The most recent major advance in polymer characterization is the applicability of ultrahigh-pressure liquid chromatography (UHPLC) to polymer separations. As pointed out by Bouvier and Koza (1), UHPLC offers greater resolution and throughput than traditional HPLC methods. Because of the short run times, the volume between peaks is greatly decreased, and the effects described below may be exaggerated, emphasizing the need for a MALS detector that makes an absolute measurement.

Potential problems with a column or universal calibration include:

- Poor fractionation, resulting from inappropriate column conditions or interaction with the stationary phase, will result in overlap and co-elution of different molecular weight species that will be assigned incorrectly by calibration curves. MALS will report a weight-average molar mass and size for each time point as sample passes through the flow cell, so high-quality data are dependent on good separation. However, an increase in polydispersity can be obtained from MALS data as evidence of co-elution.

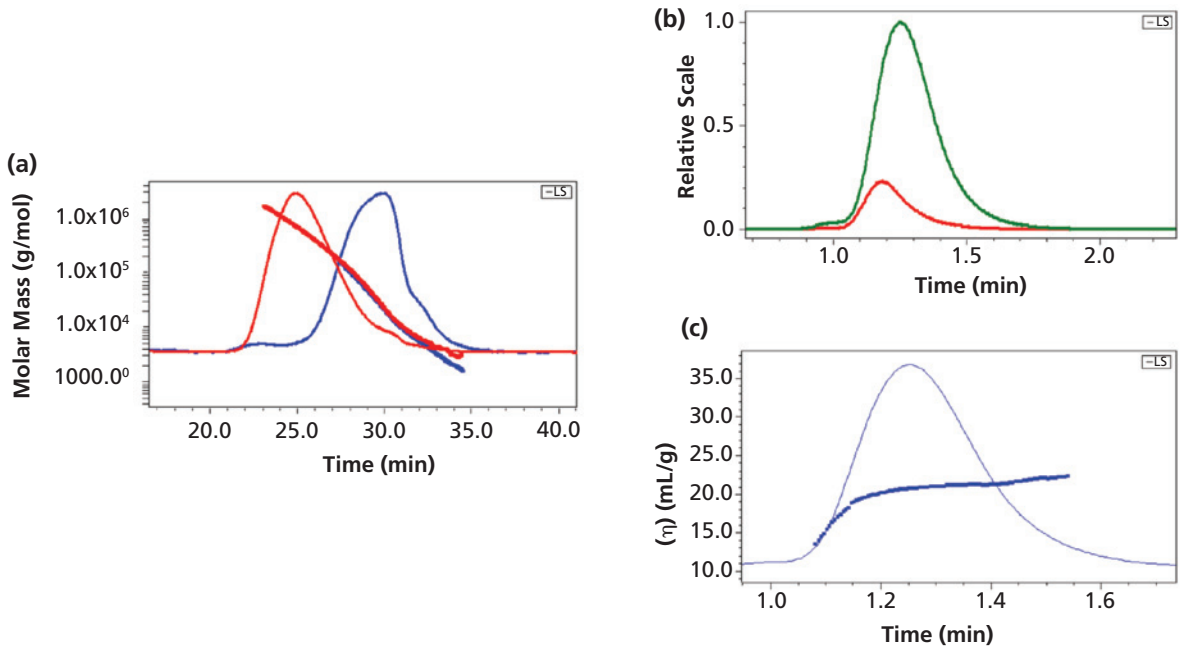
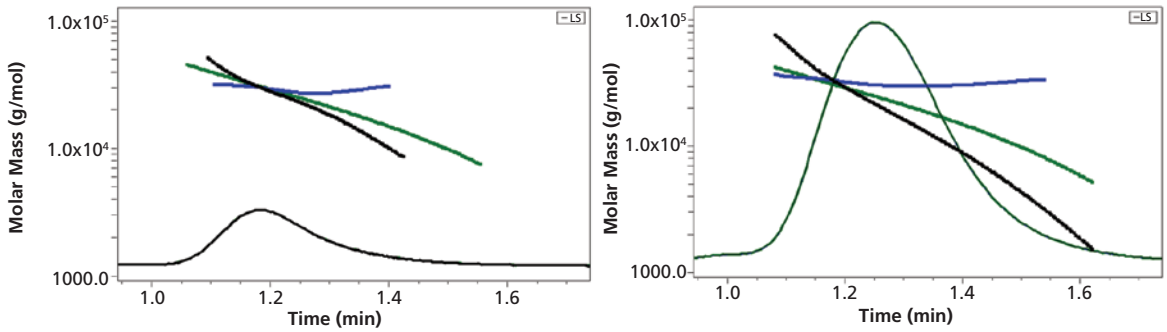


Figure 1: (a) Chromatograms of branched polymer 1 (red) and branched polymer 2 (blue) with overlaid molar mass. The elution time difference is a result of the conformational differences between the samples, which have the same molar mass. (b) Chromatograms for a 4 µL injection (red) and 50 µL injection (green) of an identical 30 kDa polystyrene standard. The peak apex shifts depending on injection volume. (c) Chromatogram for a 30 kDa polystyrene standard with intrinsic viscosity overlaid. The intrinsic viscosity is relatively constant across the peak.

- A well-characterized standard may not be available that matches the sample of interest; this mismatch will result in molecular weight error as a result of density or conformation differences (2). For example, two species of the same molar mass but different sizes will elute at different times (Figure 1[a]). Calibration will assign different molecular weights, but MALS will correctly measure molar mass regardless of conformation or retention time. Since MALS measures rms radius, it may also give insights into the cause of different elution times.
- Changing the injected mass of the sample can change the apex of eluting species, which will change the reported molecular weight according to a calibration curve. In Figure 1(b), an injection of 4 µL is compared to an injection of 50 µL with a clear shift towards longer time ($\Delta t \sim 0.7$ min.). MALS is insensitive to elution time and thus reports the same molecular weight for both peaks.
- Both column calibration and universal calibration dictate that species eluting at a later time and later volume must be of lower molecular weight because of the negative slope of



4 µl Injection	Column Calibration (Da)	Universal Calibration (Da)	MALS (Da)
Half max, left side	3.83E+04	4.44E+04	3.17E+04
Apex	3.07E+04	3.02E+04	3.01E+04
Half max, right side	2.23E+04	1.97E+04	2.71E+04
Average	3.04E+04	3.14E+04	2.96E+04
Std. Dev.	7.97E+03	1.24E+04	2.34E+03
Polydispersity	1.105	1.143	1.003

50 µl Injection	Column Calibration (Da)	Universal Calibration (Da)	MALS (Da)
Half max, left side	3.43E+04	4.15E+04	3.46E+04
Apex	2.47E+04	2.14E+04	3.07E+04
Half max, right side	1.57E+04	9.72E+03	3.06E+04
Average	2.49E+04	2.42E+04	3.19E+04
Std. Dev.	9.27E+03	1.61E+04	2.28E+03
Polydispersity	1.152	1.598	1.002

Figure 2: (Top) Chromatograms of 4 µL and 50 µL injections of a 30 kDa polystyrene standard in THF with molar mass results overlaid from MALS (blue), column calibration (green), and universal calibration (black). (Bottom) Molar masses resulting from the apex and half maximum of the peak in both the 4 and 50 µL injections. Standard deviation and polydispersity is much lower for the MALS measurement in both cases.

the calibration curve; this axiom is inherent in the experimental method. However, peaks in a chromatogram often have a fixed molecular weight across the entire peak width and time. For samples such as a narrow 30 kDa standard, the molecular weight is constant, which also means that the intrinsic viscosity is consistent across a peak (Figure 1[c]). However, because the product of intrinsic viscosity and molar mass must be decreasing (the universal calibration curve has a negative slope), then molecular weight must decrease with elution time. In other words, the inescapable mathematical framework of column calibration is a cause of error in the measurement. Figure 2 overlays calibration and MALS measurements for a narrow 30 kDa polystyrene standard and shows that the molar mass measured by MALS is consistent across the peak. In contrast, the molar mass result from calibration slopes sharply downwards and results in significant error, particularly on the right side of the peak.

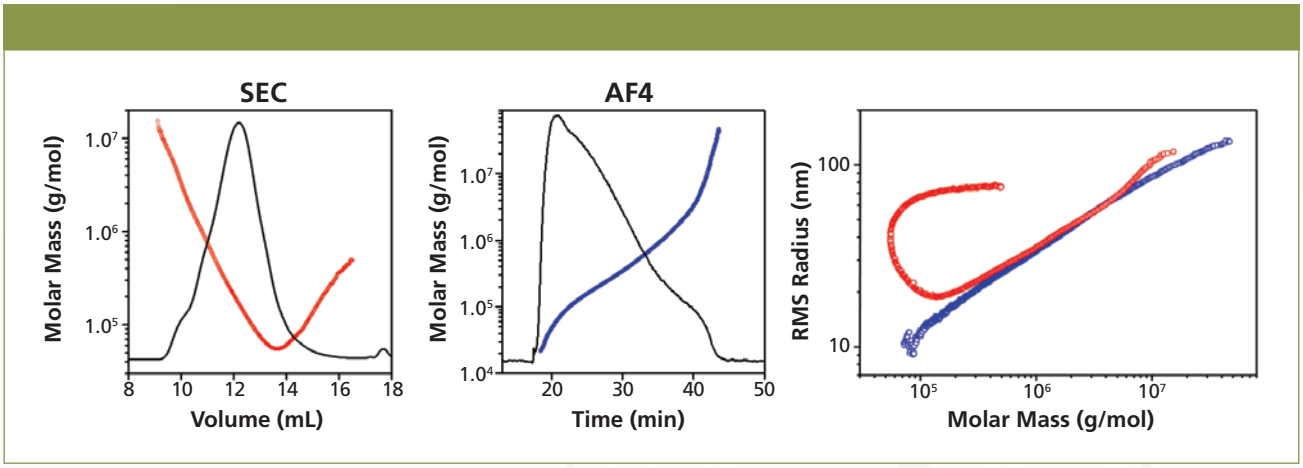


Figure 3: (Left) For a high molecular weight, branched polymer separated by SEC, MALS provides evidence that a fraction of the larger species elute abnormally late because of anchoring. (Middle) MALS analysis proves that AF4 properly fractionates the same sample. (Right) The conformation plot for the two runs comparing SEC (red) to AF4 (blue).

- Copolymers have a different elution time than any of the corresponding pure polymers (3). Copolymer standards are usually not available, and the copolymer composition will vary across a peak so that no standard would be adequate for comparison.

With so many possible ways to go wrong in column or universal calibration, why is the technique still widely used in so many laboratories? Firstly, it has been the accepted analytical technique for a long time and many laboratories are hesitant to change. Historical data may have been collected with single-detector calibration methods, so modern data must be compared to historical results. Secondly, standard operating procedures (SOPs) and protocols may have been written with this method, and they are difficult to change, especially if errors in molecular weight results are not easily detectable by the analytical equipment already installed in a laboratory. Lastly, it may be assumed that the technique works fine if the chosen standards are very similar or identical to the analyte of interest. However, the results above clearly indicate that an absolute measurement of molecular weight is needed to overcome the uncertainty of a previously established curve.

Fractionation by AF4

One common source of error in GPC/SEC not yet mentioned is interaction with the column packing material. In some cases, it is not possible to find a suitable column either because of the specific chemistry of the sample or because the molecular weight range is challenging. Another situation that arises frequently is that polymer chains "anchor" or entangle in the pores of the column packing material causing high-molecular-weight large species to elute at an unexpectedly late time in the chromatogram. This effect may mean large and small species will co-

elute, causing the researcher to assign an incorrect molecular weight to a significant percentage of the sample if depending on calibration curves. Equally concerning, entanglement will make proper polymer branching analysis impossible. These issues can be particularly pronounced for high molecular weight, highly branched polymers and show up most obviously in the conformation plot of rms radius vs. molecular weight as a characteristic upswing. The common, emblematic shape in the far right graph in Figure 3 is evidence of a branched sample and is an artifact from the poor SEC separation (4).

Asymmetric flow field-flow fractionation (AF4) is a technique that, similar to GPC/SEC, fractionates samples according to hydrodynamic diameter; however it does not have a stationary phase. Instead, the sample is introduced into a flow channel consisting of two parallel plates, where one facet comprises a porous membrane without any packed stationary phase. The sample is forced against the membrane by a cross-flow but then allowed to diffuse away from the membrane to a different height within the channel. The flow profile along the channel's length is parabolic, meaning that particles higher up in the channel will be influenced more by the faster flow. Smaller species diffuse more quickly than large particles and so end up farther from the membrane where the flow velocity is greater; in AF4, small particles elute first followed by large particles. Because there is no stationary phase, the possibility of polymer anchoring is eliminated. Thus, the conformation plot for AF4 (Figure 3, right) shows a straight line, which not only indicates good separation, but will also allow proper branching analysis.

Experimental

SEC–MALS data were acquired using an HPLC system (1100 series, Agilent Technologies), with a Dawn Heleos MALS detector, Optilab T-rEX differential refractive index detector, and ViscoStar differential viscometer, and analyzed in the Astra software package (all detectors plus analysis software from Wyatt Technology). FFF–MALS data were acquired using the same components as SEC–MALS with the addition of an Eclipse FFF system (Wyatt Technology).

Conclusions

In conclusion, while single-detector calibration experiments have proven useful, there are significant errors associated with the analysis whether column calibration or universal calibration is used. An absolute measurement with MALS is a more accurate and data-rich analysis, and it allows flexibility to change run conditions without having to re-generate a calibration curve. MALS detection also increases experimental throughput by eliminating the calibration steps. Adding MALS detection to an existing GPC setup is straightforward and helps eliminate the fear of uncertainty and doubt in experimental results. Even greater benefits may be achieved for certain problematic polymers by replacing the GPC column with AF4.

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PRINCIPLES OF DETECTION AND CHARACTERIZATION OF BRANCHING IN SYNTHETIC AND NATURAL POLYMERS BY MALS

Stepan Podzimek

Branching is an important structural parameter of many synthetic and natural polymers. It can influence the mechanical and thermodynamic properties of polymers, and also affect the viscosity and rheological behaviour of polymer solutions and melts. Quantitative data about branching topology is therefore vital to understanding polymerization processes and the development of novel polymer-based materials with enhanced properties. Multi-angle light scattering (MALS) is one analytical technique that can be performed to identify branching in macromolecules. This article provides insight into the basic principles of this technique, and how it can be applied to the detection and characterization of branching.

Branching is widely recognized as relevant to synthetic polymers, but has more recently become relevant to natural polymers. For example, hyaluronic acid, an important biopolymer with numerous medical and pharmaceutical applications, was believed to have a linear structure until multi-angle light scattering (MALS) analysis proved otherwise (1).

Full characterization of branching requires the coupling of a separation device to separate molecules of varying size over a period of time; and an analytical detector to determine molecular properties such as molar mass, size, or branching ratio. This coupling allows the detector to characterize each size fraction individually to obtain a complete and accurate distribution.

The most common method of separating polymers in solution is gel permeation or size-exclusion chromatography (GPC/SEC). SEC–MALS is a well-established technique for the absolute characterization of typical polymers; however, large and highly branched polymers can exhibit abnormal conformation plots in SEC (5). An alternative method is asymmetrical flow field-flow fractionation (AF4) coupled with MALS. AF4 does not require the diffusion of molecules in and out of a porous solid

phase, and is therefore not subject to the "anchoring" mechanism that leads to abnormal elution behavior. AF4–MALS is therefore ideal for the separation of large and highly branched macromolecules. MALS provides the required quantitative information about branching topology.

The Theory Behind Branching

The development of quantitative branching analysis began in 1949 when Zimm and Stockmayer[2] introduced the theoretically derived "branching ratio" (*g*):

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

R^2 is the mean square radius of branched and linear macromolecules having the same molar mass (*M*). *R* and *M* are both determined independently of MALS. A differential refractive index (dRI) detector is used for measuring concentration. 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 (2). In general, $g \leq 1$ where the equality sign stands for linear polymers. Lower values of *g* tend to correspond to higher degrees of branching. For example: $g \approx 0.1–0.2$ indicates a highly branched structure.

Ten years after the definition of *g* by Zimm and Stockmayer, Zimm and Kilb (3) introduced an alternative branching ratio based on intrinsic viscosity:

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

where [η] is the intrinsic viscosity of branched and linear polymer molecules having the same molar mass. [η] and *M* are determined using a MALS, dRI, and a differential viscometry (dVI) detector for intrinsic viscosity. 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, but a typical value is $e \approx 0.7$.

MALS can measure molar masses from below 1 kDa up to ~1 GDa, but is limited to determining root mean square (RMS) radii above ~10 nm (corresponding to a molar mass of $\approx 10^5$ g/mol for typical polymers). Alternatively, either intrinsic viscosity or SEC elution volume can be used as a size parameter. The former is used in equation 2, whereas the latter appears in the approach of Yu and Rollings: [4]

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

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. *M* is determined by MALS + dRI. In summary, the branching ratio may be obtained by performing the following methods:

- Radius method: Calculate *g* from the conformation plot (log–log plot of *R* versus *M*) using equation 1, MALS, and dRI detectors.
- Viscosity method: Calculate *g*′ from the Mark-Houwink plot (log–log plot of [η] versus *M*) using equation 2, MALS, dRI, and dVI.
- Mass method: Calculate *g* from the plot of molar mass versus elution volume using equation 4, MALS, and dRI, plus measurement of a linear counterpart under the same SEC conditions as those used for branched sample.

The radius method is the simplest to implement, but for polymers smaller than ~10 nm in radius, the viscosity or mass method is required.

Case Studies

Polyester based on lactic acid (PLA) is a biocompatible and biodegradable polymer that can be used as a drug delivery material. Its ability to swell, degrade, and release an active compound can be controlled by the degree of branching. The release of an active compound can be controlled by altering the degree of branching to alter the rate of swelling and degradation of the polymer.

Method: The data presented in this study were obtained with a Dawn Heleos MALS photometer, a ViscoStar on-line viscometer, an Optilab T-rEX refractive index detector, and an Eclipse A4F system, and processed with Astra 6 software, all from Wyatt Technology. SEC was performed with an Agilent 1100 HPLC instrument (Agilent Technologies). Tetrahydrofuran was the solvent for both the SEC and AF4 analysis.

The characterization of branching for small PLA molecules is depicted in Figure 1, which compares Mark-Houwink plots and plots of molar mass versus elution volume of linear and branched molecules. Both indicate the presence of branched molecules and may be used to calculate *g* by means of equations 2–4. Figure 2 shows conformation plots of linear and branched polystyrene. Branching is shown by the measured slopes of 0.59 and 0.48. These can be compared with the two limiting theoretical values: 0.58 for linear polymers in thermodynamically good solvents, and 0.33 for compact spheres that can be considered "infinitely branched".

The conformation data transform to plots of molar mass dependency of the branching ratio, and the number of branch units per molecule, shown in Figure 2. Overlaying the branching units plot with the cumulative distribution of molar mass facilitates quantitative evaluation of branching. Figure 2 shows that ≈ 28% of molecules with molar

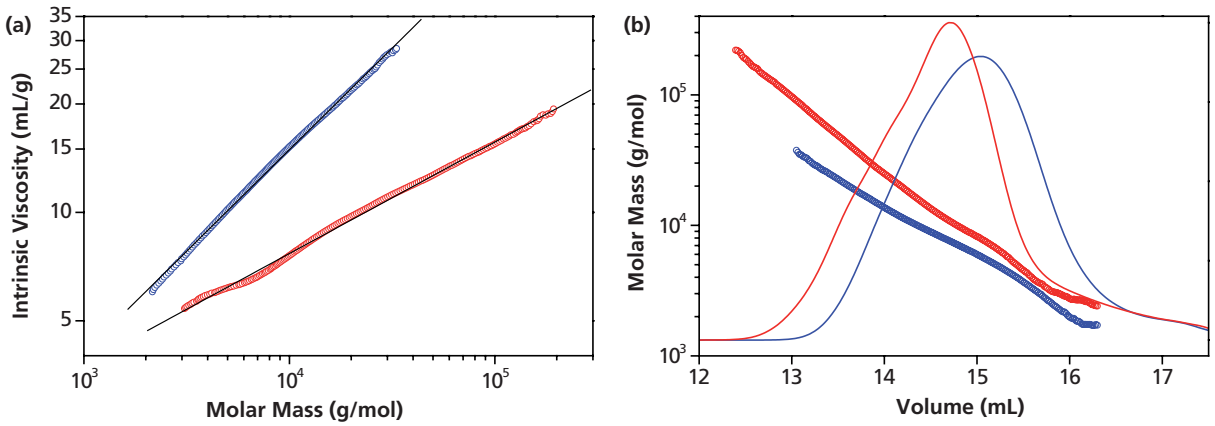

Figure 1: Analysis of linear (blue) and branched (red) poly(lactic acid). (a): Mark-Houwink plots, exhibiting slopes of 0.56 and 0.31 for linear and branched molecules, respectively. (b): Molar mass versus elution volume overlaid with RI chromatograms.

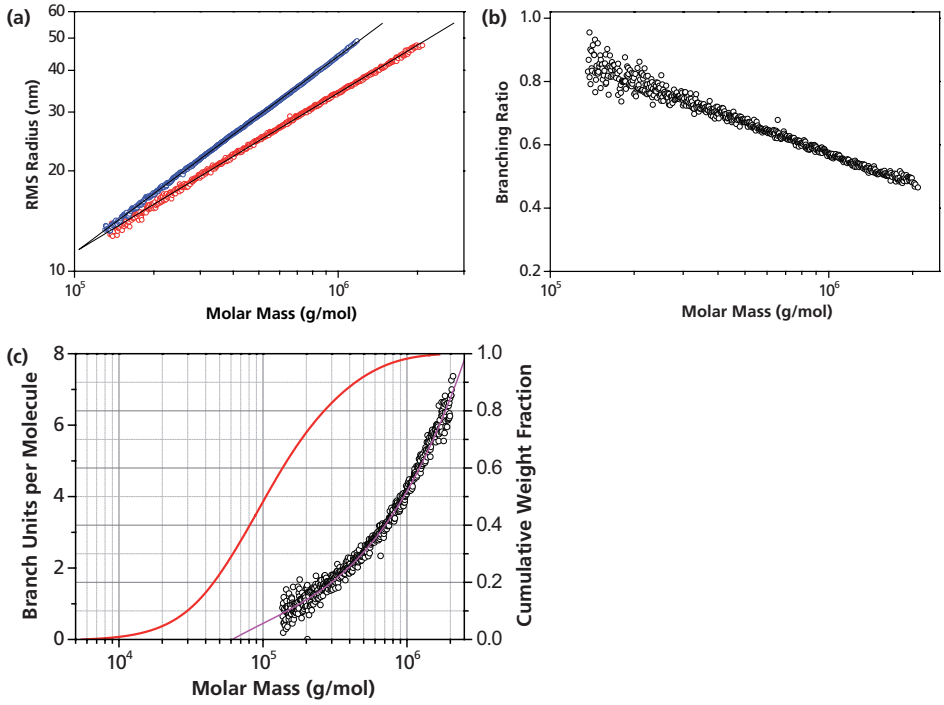

Figure 2: (a): Conformation plots of linear (blue) and branched (red) polystyrene. (b): The corresponding plot of branching ratio versus molar mass. (c): The number of branch units per molecule plotted versus molar mass. The plot of branch units per molecule versus molar mass is overlaid with the cumulative 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.

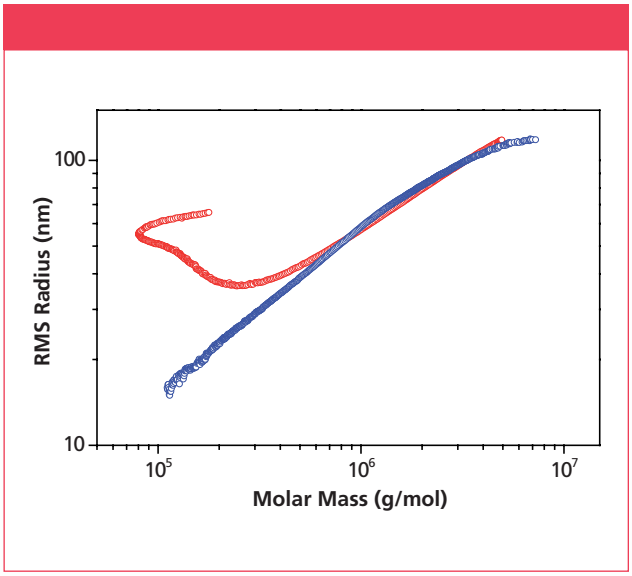

Figure 3: Conformation plots of cellulose tricarbanilate, a branched polymer exhibiting "anchoring", determined by SEC–MALS (red) and AF4–MALS (blue).

masses below ≈ 60,000 g/mol do not contain branch units. Notably, SEC–MALS is capable of detecting just a single branch until in polymer chains.

The SEC–MALS radius method may fail for some large, highly branched polymers because of limitations of the SEC separation mechanism, where the branches are temporarily "anchored" in the pores of SEC column packing (5). These polymers then elute abnormally at a retention time that corresponds to a much smaller hydrodynamic volume than actually presented by the molecule (5). As a result, fractions at large elution volumes become highly polydisperse containing both very small and very large branched species. MALS measures the weight-average molar mass (M_W) and the *z*-average RMS radius (R_Z). As long as elution fractions are reasonably monodisperse, the weightings of R_Z and M_W are nearly identical, but with increased polydispersity they diverge. The combination of polydisperse, abnormal SEC elution with disparate weightings of R and M by MALS results in upswings on the conformation plots at the low end of the molar mass axis (horizontal) and consequently incorrect values of g (5).

For such polymers, AF4 has proven to provide better results (6).

A comparison of conformation plots obtained by SEC–MALS and AF4–MALS for cellulose tricarbanilate is depicted in Figure 3. The separation by AF4 is not affected by the anchoring of branched molecules and the upswing is completely eliminated.

Conclusion

The demand for an absolute technique that can provide robust and reliable polymer characterization has led to recognition of the powerful capabilities of MALS. This study shows that the most direct and fundamentally correct technique for characterizing branching in polymers is the MALS-based radius method, though it is limited to molecules with RMS radii > 10 nm. Smaller branched polymers can be characterized by adding a differential viscometer to a SEC–MALS system for Mark-Houwink plots, or MALS-based determination of the relation between the molar mass and elution volume. When separation is adversely impacted by the anchoring of branched macromolecules in the pores

of SEC column packing, AF4–MALS offers great separation and yields correct conformation plots and branching ratios. AF4–MALS is suitable for all types of polymers.

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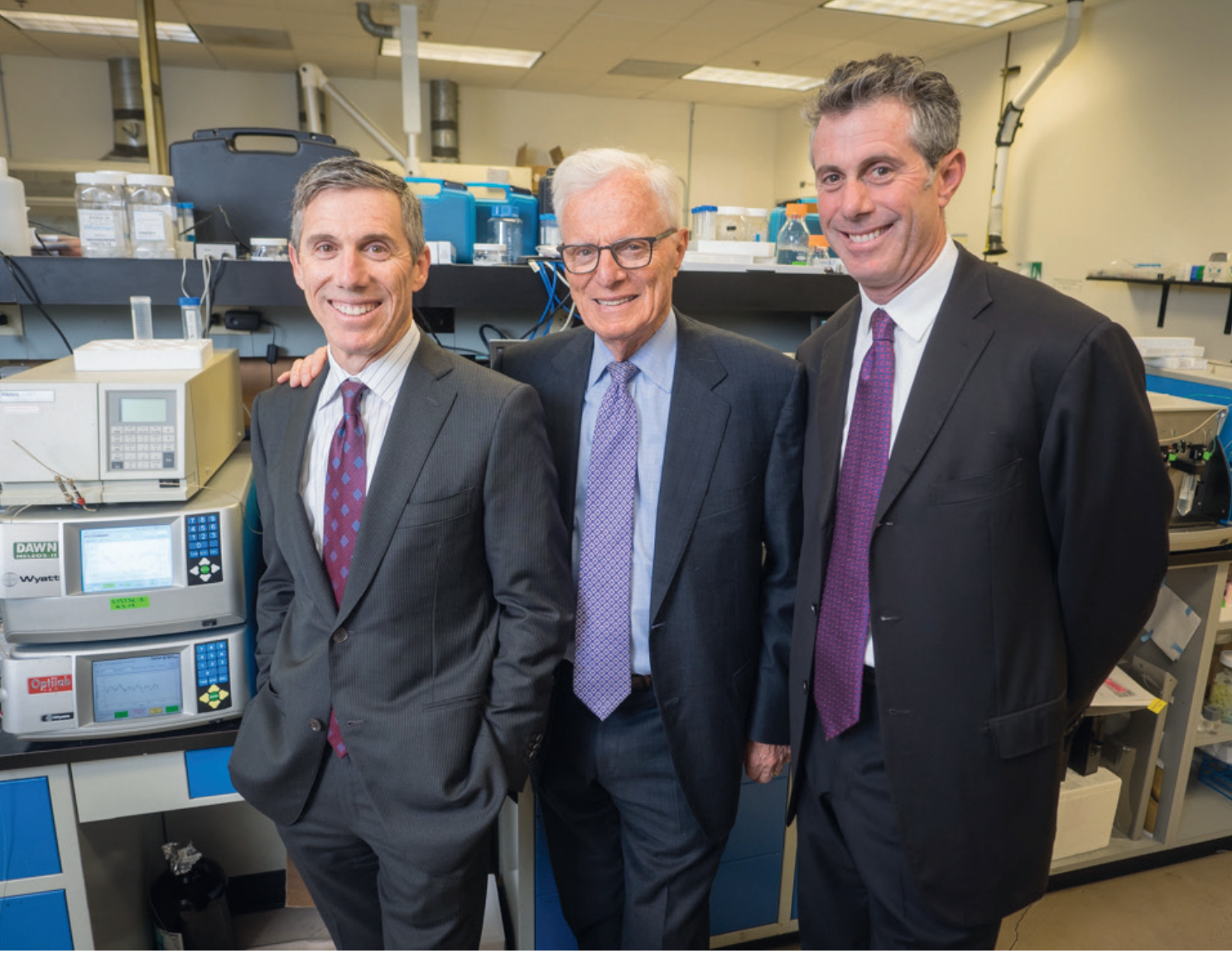

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LIGHT-SCATTERING TECHNIQUES FOR ANALYZING POLYMERIZATION PROCESSES

An interview with Dr. Judit Puskas of the University of Akron

Synthetic and natural polymers are extremely versatile and powerful materials with an extremely wide range of uses, from industrial applications to everyday consumer products to biomedical implants. Judit E. Puskas and her group at the University of Akron, in Akron, Ohio, strive to make polymer chemistry greener by developing more sustainable and environmentally friendly synthesis and functionalization methods and processes. Puskas recently spoke to us about some of her recent work to better understand and improve polymerization processes, and about how she used light-scattering techniques as an analytical method in those studies.

Your group investigated poly(α-lipoic acid) structures produced by thermal polymerization under reduced pressure (1) and found that the process produced branched structures instead of the interlocked ring structures proposed in an earlier study (2). What technique was used in your study to determine the molecular weights and molecular weight distributions of the polymer structures? What was the role of light-scattering detection in the analysis?

Light scattering gives us absolute weight average molecular weight data. The molecular weight distribution data depend on the quality of separation. We have six columns (100, 500, 1000, 10,000, 100,000, and 1,000,000 Å) that provide excellent resolution. We also took advantage of radii measurements that are very precise in the case of high-molecular-weight structures. The amplified structures had very high molecular weights.

How does the light-scattering approach compare with other methods for determining polymer molecular weights?

Light scattering gives us absolute weight average molecular weight data and is the best method for high molecular weights. It also gives us radii and conformation information. However, it is less sensitive to low molecular weights (oligomers).

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In a study (3) to evaluate the biocompatibility of a thermoplastic rubber—for its potential use as a protective coating on a cranial implant—you measured the rubber before and after implantation in rats using size-exclusion chromatography (SEC) with a interferometric refractometer (RI), a multiangle light scattering (MALS) detector, a viscometer, and a quasi-elastic light scattering (QELS) instrument. First, what exactly did you need to measure to determine its biocompatibility, and why?

We evaluated biostability—how stable the polymer is in vivo. The molecular weights did not decrease, verifying the stability of the polymer after implantation.

What was the specific role or contribution of the light-scattering techniques you used—MALS and QELS?

We use QELS for obtaining hydrodynamic radius (R_h) data, and comparing it to R_h values obtained from viscometry. We found excellent agreement. Comparison of radius of gyration (R_g) and R_h data gives us critical information about polymer architectures (4).

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CHARACTERIZATION OF STYRENE-BUTADIENE RUBBERS BY SEC–MALS AND AF4–MALS

Stepan Podzimek

Two samples of styrene-butadiene rubbers (SBR) were analyzed by size-exclusion chromatography (SEC) and asymmetric flow field-flow fractionation (AF4) coupled with a multi-angle light scattering (MALS) detector. The results were compared from the viewpoint of the molar mass distribution and the separation performance of SEC and AF4.

Styrene-butadiene rubbers (SBR) represent an important group of synthetic elastomers that are used in a variety of applications, generally as an abrasion-resistant replacement for natural rubber. The viscoelastic and mechanical properties of this material are affected by the molar mass distribution and by the topology of macromolecular chains. Traditionally, the molar mass distribution is characterized by conventional size-exclusion chromatography (SEC) with column calibration based on polystyrene standards. Polystyrene calibration results in incorrect molar mass distribution because of different hydrodynamic volumes of polystyrene and the polymer under analysis. Although various procedures for transforming the polystyrene calibration to the calibration valid for the polymers undergoing analysis have been developed (1–3) some of them specifically for SBR rubbers (4), the most effective way of solving the calibration problem is using a multi-angle light scattering (MALS) detector with SEC. The theory of light scattering and MALS detection has been described in detail in several papers and books (5–8).

Although a MALS detector converts a relative and calibration dependent SEC method into an absolute method of molar mass determination, there are still several potential issues when polymers of very high molar mass are characterized by SEC–MALS. These include possible shearing degradation and incomplete separation as a result of various non-SEC separation mechanisms (9). Branched macromolecules in particular show non-

SEC separation behaviour that may strongly affect the results obtained by SEC (10). Asymmetric flow field-flow fractionation (AF4) represents a powerful alternative to traditional SEC, with several advantages compared to SEC (5) These include the possibility to separate molecules with ultra-high molar mass with a significantly reduced possibility of shearing degradation, elimination of entlapic interactions with SEC column packing, and elimination of specific elution behaviour of branched macromolecules in SEC.

SBR are a good example of polymers that can benefit from AF4 separation as they typically contain high molar mass fractions with a molar mass over 10^6 g/mol and branched macromolecules. Since the introduction of AF4 by K.G. Wahlund,[11] the AF4 method has undergone a substantial development that has established it as a reliable analytical technique suitable for routine applications.

Experimental

SEC and AF4 set-ups consisted of an Agilent 1100 Series HPLC pump and a Waters 717 autosampler. The SEC separation was achieved using two 300 mm × 7.5 mm, PLgel Mixed-C columns (Agilent). The solvent was tetrahydrofuran (THF) at a flow rate of 1 mL/min (SEC) or detector flow rate of 1.8 mL/min (AF4). An AF4 system Eclipse 3+ (Wyatt Technology Europe) was used for AF4–MALS. A cross flow gradient from 2.4 mL/min to 0 mL/min was used for AF4 separation using a 350 µm spacer and a 5 kDa regenerated cellulose membrane. The detectors used were MALS photometer DAWN HELEOS and refractive index (RI) detector Optilab T-rEX (Wyatt Technology Corporation). The samples were prepared as solutions in THF at a concentration of approximately 2 mg/mL, the injected volume was 100 µL. The data were acquired and processed using light scattering software Astra 6 (Wyatt Technology Corporation).

Results and Discussion

Molar mass versus retention time plots obtained for an SBR sample by both SEC–MALS and AF4–MALS are contrasted in Figure 1. Corresponding plots of the root mean square (rms) radius are depicted in Figure 2. The upswings on the molar mass and rms radius plots at the end of the SEC chromatogram (see Figure 1[a] and Figure 2[a]) are typical for branched polymers and are caused by the specific elution behaviour of branched macromolecules in the pores of SEC column packing (5). The anchoring effect of SEC packing results in the increased polydispersity of the elution volume slices at the end of the SEC chromatogram. For polydisperse slices the MALS detector measures the weight-average molar masses and the *z*-average rms radii, which count mainly the high molar mass fractions. As a consequence, both plots show the curve-up trend. As the *z*-average is more sensitive to

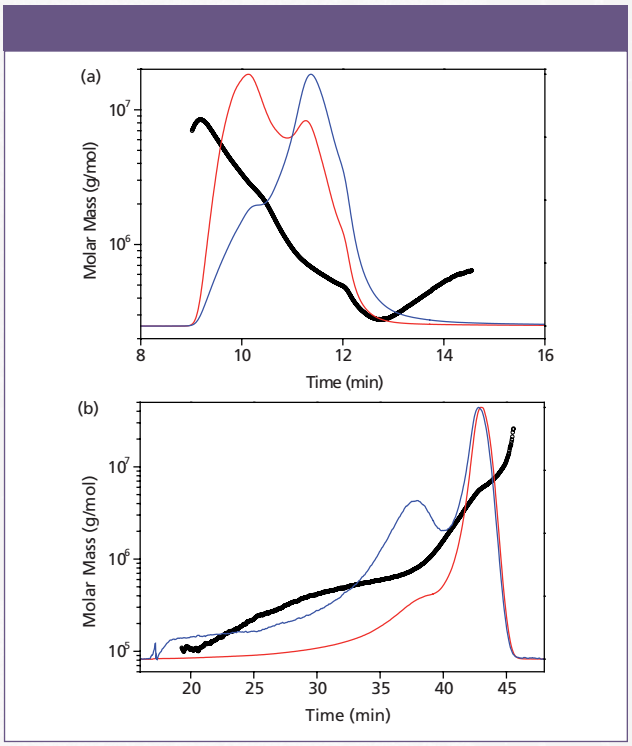

Figure 1: Molar mass versus retention time plots from (a) SEC–MALS and (b) AF4–MALS analysis of styrene-butadiene (SBR). Signals from MALS at 90° (red) and RI (blue) detectors are overlaid here.

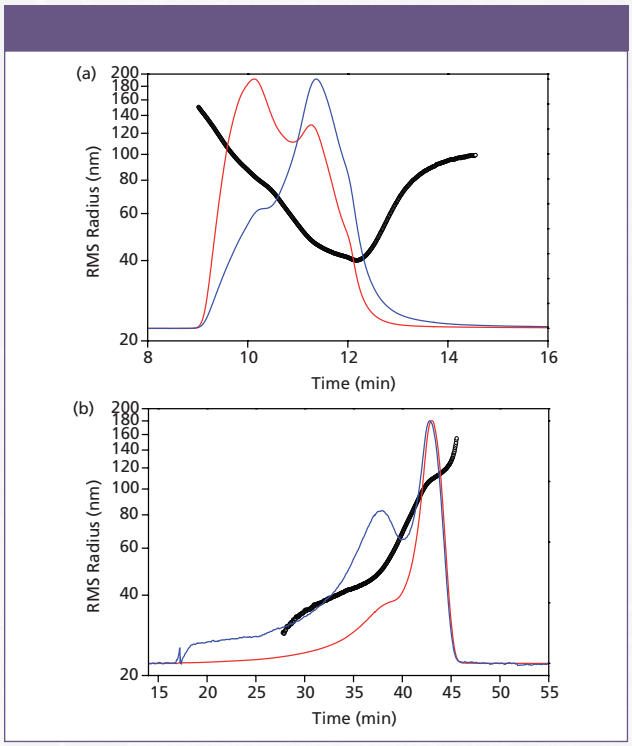

Figure 2: RMS radius versus retention time plots from (a) SEC–MALS and (b) AF4–MALS analysis of SBR. Signals from MALS at 90° (red) and RI (blue) detectors are overlaid here.

polydispersity than the weight-average, the corresponding conformation plot (log-log relation between the rms radius and molar mass) becomes up-turned (Figure 3).

In AF4, the separation takes place in an empty channel filled in solely by the mobile phase and the anchoring effect is completely missing. This is evident from Figures 1–3 which show no upswings on the plots yielded by AF4–MALS.

Information about branching can be obtained from the conformation plot. Unfortunately, the curved conformation plots obtained by SEC–MALS make the characterization of branching difficult or even impossible. The plots obtained by AF4–MALS are not curved and such accurate branching characterization can be achieved over the entire molar mass range. For example, the conformation plot from AF4–MALS shown in Figure 3 has a decreasing slope with an increasing molar mass — this is a typical pattern for polymer materials consisting of a mixture of linear macromolecules and branched macromolecules with a branching degree increasing towards high molar masses. The slope at the region of molar masses up to $\approx 800 \times 10^3$ g/mol is 0.57 (a typical value for linear random coils in thermodynamically

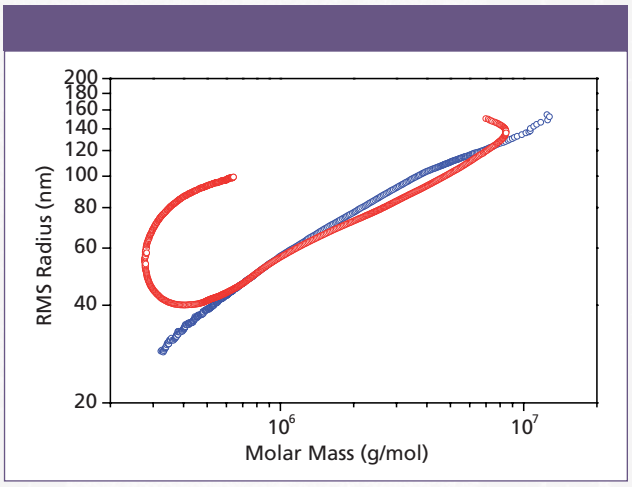

Figure 3: Conformation plots of SBR acquired by SEC–MALS (red) and AF4–MALS (blue).

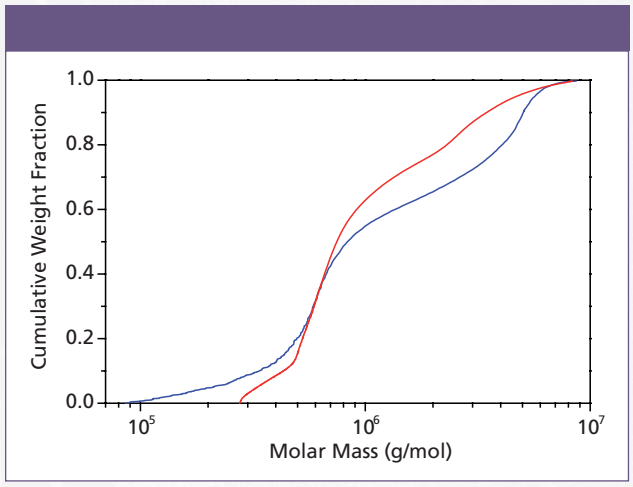

Figure 4: Cumulative molar mass distribution plots from SEC–MALS (red) and AF4–MALS (blue).

Table 1: Molar mass averages determined by SEC–MALS and AF4–MALS.

Sample	M_n (10^3 g/mol)		M_w (10^3 g/mol)		M_z (10^3 g/mol)	
	SEC–MALS	AF4–MALS	SEC–MALS	AF4–MALS	SEC–MALS	AF4–MALS
1	780	650	1610	2310	3960	5310
2	660	620	1270	2010	3280	6750

good solvents); the slope from ≈ 800×10^3 g/mol – 3×10^6 g/mol is 0.46 (a value typical for branched macromolecules); and the slope over ≈ 3×10^6 g/mol is 0.30 (a value typical for highly compact structures).

The anchoring of the large branched macromolecules in the column packing also affects the determination of the molar mass distribution at the region of lower molar masses, which results in the overestimation of the number-average molar mass (M_n) (**Table 1**) and the shift of the molar mass distribution curve towards higher molar masses as seen from Figure 4. In addition, shearing degradation in SEC columns may affect the high molar mass part of the distribution and the weight-average molar mass (M_W) and in particular the *z*-average molar mass (M_Z). Comparison of the data in Table 1 reveals not only the overestimation of M_n as a result of the anchoring effect, but also the underestimation of M_W and M_Z because of shearing degradation in SEC packing. Both anchoring and shearing degradation effects make the molar mass distribution narrower, as evidenced in Figure 4.

Conclusions

AF4–MALS provides better separation than SEC–MALS for high molar mass branched

SBR and other similar polymers. As a result of better separation not only can more accurate information about branching be obtained, but also correct molar mass averages are obtained. The results from AF4–MALS are unaffected by the anchoring of the branched molecules in the column packing and/or by shearing degradation of molecules with very high molar mass.

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