

# WP2304: Analysis of polymers in organic solvents by FFF-MALS

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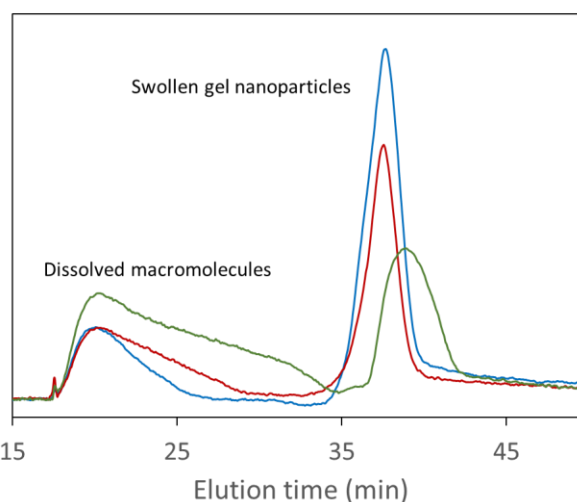
## Summary

Numerous properties of technically important polymers utilized in the paint, rubber and adhesive industries are dependent on details of the molar mass distribution and molecular structure. These include mechanical strength and elasticity, viscosity of solutions and melts, rheological behavior, glass transition temperature, ability to create films and fibers, solubility, chemical resistance, degradability and ability to crystallize. Polymer chain branching, in particular, is a key determinant of many of these properties. Hence, accurate characterization of molar mass distributions and branching parameters is essential for R&D and process development of such materials.

**Field-flow fractionation with multi-angle light scattering (FFF-MALS)** is an invaluable technique for characterizing polymers, especially those that are not analyzed well by other techniques, such as high-molecular-weight polymers. While FFF is most often used with aqueous solvents, it is also applicable to many polymers soluble in organic solvents.

In this article, FFF-MALS and its applications in [characterization of technical polymers](#) are reviewed. Several examples, relevant to the most important application areas, are demonstrated via measurements performed in the author's laboratory. The results acquired by FFF-MALS are contrasted with those produced [by size-exclusion chromatography with light scattering \(SEC-MALS\)](#) which, despite being markedly better known and more widely utilized, often fails to describe the true molecular structure of many technically important polymers such as elastomers, acrylic emulsion copolymers, branched polymers or macromolecular materials containing nanogels or nanoparticles.

The FFF technique is shown to be applicable not only to polymers that are difficult to separate properly by SEC, but also to "SEC-easy" polymers. Even for such samples, FFF-MALS can be helpful in confirming the presence of high-molar mass species that may be adsorbed and/or degraded by SEC columns, and thus remain undetected.



FFF allows separation and quantification of solutions comprising both swollen latex particles and fully dissolved macromolecules.

## Introduction

Asymmetric-flow field-flow fractionation (AF4), by far the most commonly used type of FFF and the only type implemented in Wyatt Technology's product line, belongs to a family of FFF separation techniques that were described in the 1980's.<sup>1</sup> The method advanced from prototype, lab-built apparatus to sophisticated instrumentation that is commercially available and highly reliable.

The principle of AF4 separation is illustrated in Figure 1. Following is a brief summary of the fractionation process.

1. Particles are separated in an open channel containing a semi-permeable membrane supported by a frit.
2. Fluid enters from the left and obtains a parabolic flow profile in the lateral direction as a result of laminar flow.
3. Constriction of the channel outlet forces a portion of the fluid, known as the cross flow, through the membrane, exiting via the cross-flow outlet. Cross flow is regulated by a flow controller.
4. Particles injected into the channel obtain an equilibrium height above the membrane that arises from a balance between cross flow and diffusion. The equilibrium height is directly related to the diffusion coefficient and hence the hydrodynamic radius.
5. Separation is achieved as a result of the height-dependent differential flow velocity. Smaller particles diffuse higher, experience a higher lateral velocity, and therefore are swept out of the channel more quickly. Conversely, larger particles remain close to the membrane and experience a low flow velocity.

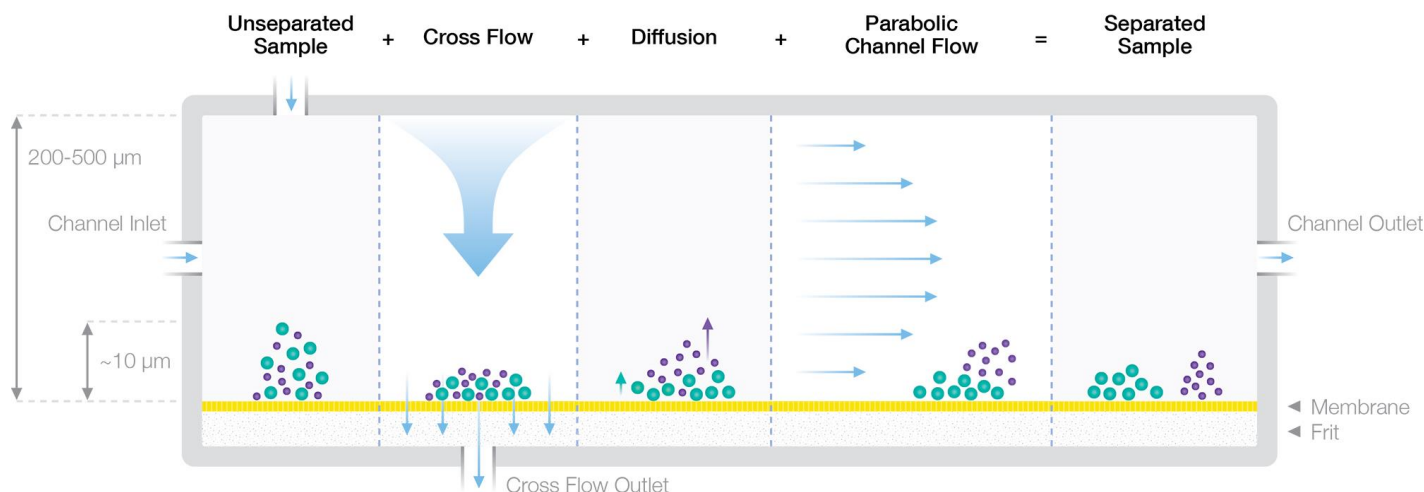


Figure 1. Schematic of size-dependent particle separation by asymmetric-flow field-flow fractionation.

As a size-based analytical separation technique, FFF is similar to the better-known size-exclusion chromatography (SEC), which is in extensive use. In contrast to SEC, FFF separation is achieved using only mobile phase with no stationary phase. Strictly spoken, it is not a chromatographic method, despite having many chromatographic attributes. This lack of stationary phase affords FFF several significant advantages over SEC:

- Reduced shearing degradation of ultra-high-molar-mass polymers
- Superior separation of branched macromolecules
- Separation and quantification of nanogels

- Elimination of enthalpic interactions between polymer molecules and stationary phase
- Detection and characterization of nanoparticles in technical polymers.

Additional advantages over SEC include (i) the ability to tune and optimize separation performance solely by changing flow rates, without changing the separation device; (ii) substantial decrease in unit cost per analysis, as the channel is practically indestructible and the price of membranes is a small fraction of that of SEC columns; and (iii) higher signal-to-noise ratio for light scattering due to the absence of column shedding, a phenomenon that is

prevalent in SEC and detrimental to light scattering analysis.

FFF theory enables calculation of hydrodynamic radius from the retention time, which can be determined with a standard online differential refractive index, or dRI, detector. However, far more information can be obtained by FFF coupled to a multi-angle light scattering (MALS) detector (in addition to dRI). MALS provides online determination of the absolute molar mass and the root-mean-square radius  $R_g$  (radius of gyration) of each eluting polymer fraction.<sup>2</sup> In MALS analysis, a dRI detector is included to determine the concentration of molecules eluting from the channel at each elution fraction.



**Figure 2.** Illustration of an FFF-MALS instrumental set-up consisting of degasser, HPLC pump, autosampler, UV detector, Eclipse separation module and channel, and MALS and differential refractive index detectors.

A UV/Vis concentration detector can also be valuable for characterizing copolymers containing a UV-absorbing monomer. Analysis of combined MALS-RI-UV signals determines the fraction of UV-absorbing monomer, as well as total copolymer molar mass, for each eluting fraction. Hence the copolymer composition can be determined as a function of molar mass. Styrene-butadiene rubbers and styrene-acrylic copolymers are typical examples of copolymeric macromolecules that may be subjected to this analysis.

FFF is implemented in Wyatt Technology's Eclipse® FFF system which comprises a sophisticated flow controller and a range of separation channels of different capacities. A complete FFF-MALS system is illustrated in Figure 2. The setup includes a degasser, HPLC pump, autosampler and UV detector together with the Eclipse controller and channel, a DAWN® MALS instrument and an Optilab® dRI detector.

## Experimental

The results shown in this paper were acquired using an Agilent HPLC system composed of isocratic pump, autosampler and variable wavelength detector, followed by an Eclipse FFF controller and channel, DAWN and Optilab. Data acquisition and processing were performed by ASTRA® software. Two Agilent PLgel Mixed-C or Mixed-B 300 × 7.5 mm columns were used for SEC-MALS analyses in place of the Eclipse controller and channel.

The solvent used as a carrier was tetrahydrofuran (THF). The membranes used with the Eclipse are compatible with THF and toluene. However, they are not compatible with more polar or aggressive solvents, which does place a certain limit on the range of synthetic polymers that may be analyzed by FFF.

## Results and Discussion

The following examples show characteristic applications of FFF-MALS in the area of THF-soluble synthetic polymers.

### “SEC-easy” polymer

Figure 3 depicts molar mass versus retention time plots of linear poly(methyl methacrylate) (PMMA) measured by SEC-MALS and FFF-MALS, along with the cumulative distribution curves and the number-average ( $M_n$ ), weight-average ( $M_w$ ) and z-average ( $M_z$ ) molar masses. The polymer is well-known as plexiglass, but it is also used as an essential material in medicine and dentistry and many other applications. Figure 3 reveals that both SEC-MALS and FFF-MALS yield similar results, evident from the nearly identical molar mass distribution curves and molar mass averages. This allows us to conclude that FFF is as efficient as common SEC and may be used without loss of information.

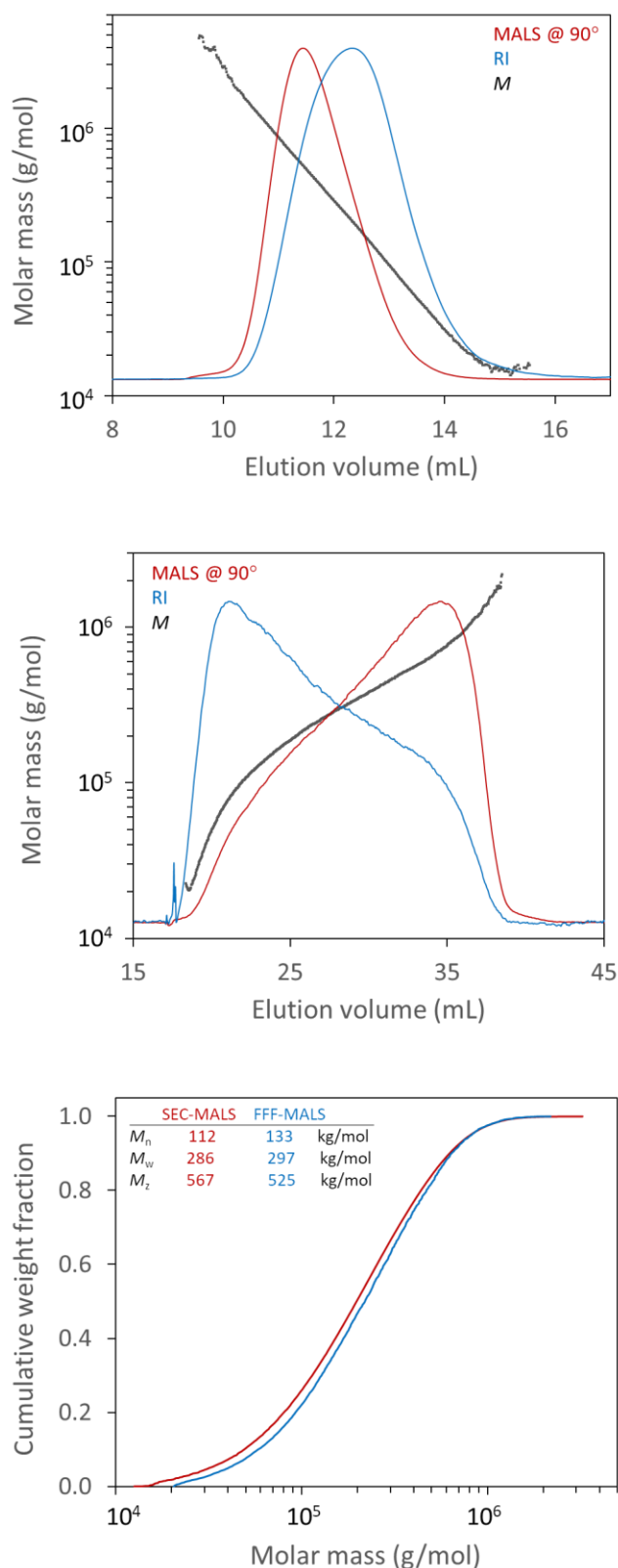


Figure 3. Linear PMMA: molar mass versus retention time overlaid with MALS and dRI signals acquired by SEC-MALS (top) and FFF-MALS (center), and corresponding molar mass distributions and moments (bottom).

## Branched and ultra-high molar mass polymer

Figure 4 shows data equivalent to those in Figure 3 for an acrylic emulsion copolymer. Acrylate-containing polymers prepared by emulsion polymerization usually contain branched species with very high molar mass as a result of chain transfer to acrylate monomer units.

The upswing in molar mass on the trailing edge of the SEC-MALS peak was first explained as the specific elution behavior of branched polymers.<sup>4</sup> In contrast to linear macromolecules, individual branches of large branched macromolecules can behave as independent molecules, permeating into the pores of SEC stationary phase and anchoring the entire molecule while the other parts remain in the mobile phase. This process is dynamic in the sense of diffusion of anchored branches out of the pores alongside permeation of other branches of the same macromolecule into the pores. Consequently, large branched macromolecules elute late, together with smaller molecules, at the trailing edge of the chromatogram. In the case of highly branched polymers one can even observe excessive tailing of MALS chromatograms far behind the limit of total permeation.

Analysis of SEC-MALS data is based on the assumption of nearly monodisperse fractions. Although this assumption is never completely fulfilled due to band broadening in the SEC columns or FFF channel, in the case of most linear polymers the polydispersity of the eluting fractions may be neglected and the data are treated as if the molar masses and RMS radii measured by the MALS detector were monodisperse molar masses  $M_i$  and RMS radii  $R_i$ . When anchoring occurs, the polydispersity at the end of SEC elution curve is no longer negligible and the MALS detector in actuality measures weight-average molar masses  $M_{w,i}$  and z-average radii  $R_{z,i}$ .

The z-average is, by definition, more sensitive to high molar masses than the weight average. The upturn in the RMS radius plot therefore appears at lower elution volumes than for molar mass plot and it is also more pronounced. Consequently, by plotting  $R_{z,i}$  versus  $M_{w,i}$  the conformation plot attains an abnormal U-shape pattern shown in the lower graph of Figure 5. The U-shaped conformation plot does unambiguously indicate the presence of branched macromolecules and anchoring. However, it is generally undesirable as detailed branching analysis over the entire molar mass range becomes impossible.

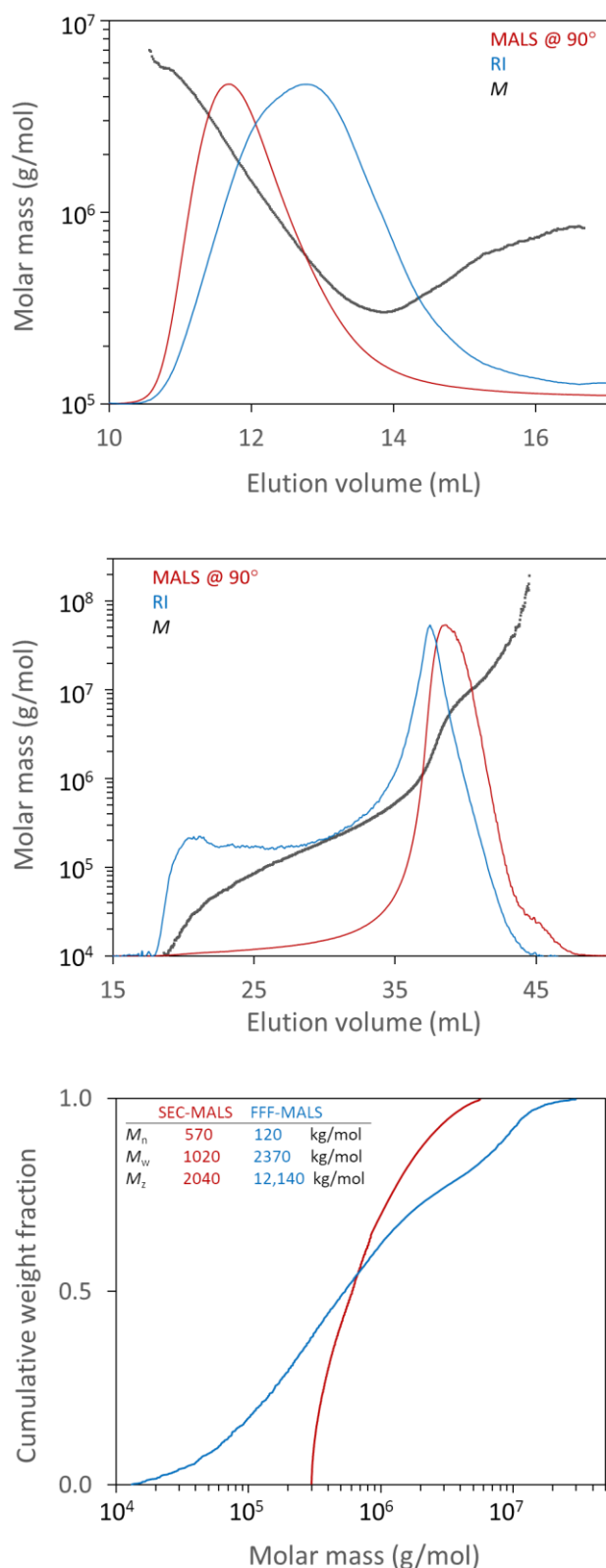


Figure 4. Emulsion copolymer methyl methacrylate-butyl acrylate: molar mass versus retention time and MALS and dRI signals, acquired by SEC-MALS (top) and FFF-MALS (center), and corresponding molar mass distributions and moments (bottom).

Note that the branching ratio  $g$  calculated in the region of lower molar masses would be markedly over unity, which is theoretically impossible. In an FFF channel containing no stationary phase, anchoring cannot occur, the fractions eluting from the channel are almost monodisperse and the resulting conformation plot is straight without any artefacts. This is seen in the bottom graph of Figure 5 which compares the conformation plots of a branched polymer obtained by SEC-MALS and FFF-MALS. Consequently, FFF-MALS makes possible accurate calculations of  $g$  and the number of branch units as a function of molar mass.

Another consequence of anchoring is that the values of  $M_n$  measured by SEC-MALS are significantly higher than those from FFF-MALS. The difference is explained by the fact that the number average  $M_n$  counts mainly the fractions with lower molar mass. When anchoring occurs, the lower molar mass fractions are masked by small amounts of delayed large molecules, and so become virtually invisible to MALS. Elimination of anchoring by FFF improves accuracy of the lower molar mass region of the distribution curve as well as of the number average  $M_n$ .

One more difference between SEC and FFF arises from shear degradation of high-molar-mass molecules, readily noted by comparing the two distribution plots depicted in the bottom graph of Figure 4. The SEC-derived distribution is shifted towards lower molar masses, and the weight- and z-averages  $M_w$  and  $M_z$  are lower than their FFF-derived counterparts, due to the shearing degradation of very large macromolecules by the SEC packing. FFF separation is noticeably gentler, a consequence of the absence of stationary phase which eliminates eddy forces and mechanical disruption.

A vivid example of how misleading certain SEC-derived results can be is presented in Figure 6 which compares two samples of emulsion core-shell acrylic copolymer, characterized by SEC-MALS and FFF-MALS. The SEC separation (top) not only provides false values of  $M_n$  due to the anchoring phenomena, but the shearing degradation makes the two samples almost identical with nearly overlapping distribution curves and very close molar mass moments. These are resolved in the FFF-MALS-derived distributions (bottom) which preserves the high-molar-mass fractions.



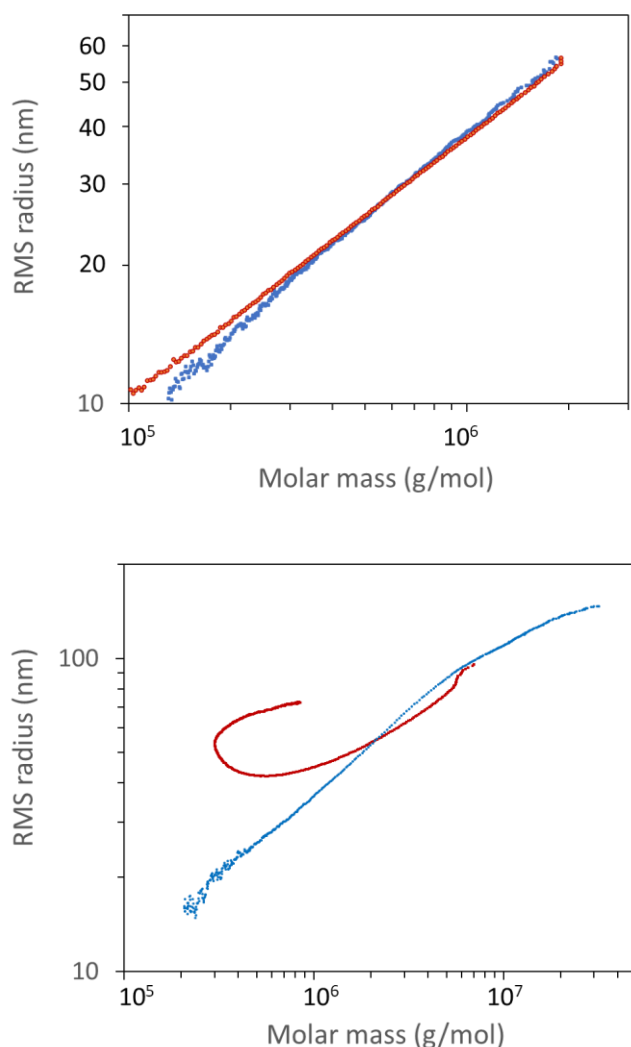


Figure 5. Conformation plots of linear PMMA (top) and branched emulsion copolymer methyl methacrylate-butyl acrylate (bottom) obtained by SEC-MALS (red) and FFF-MALS (blue).

### Polymers containing nanogels

Some technical polymers can contain crosslinked structures created by the intentional addition of multifunctional monomers into the polymerization mixture, excessive chain transfer to the polymer or, in the case of diene monomers, polymerization of the branch double bond after the 1,2-addition. Solutions of such polymers contain both individual macromolecules dissolved to the molecular level, and swollen cross-linked species which can be called nanogels. In SEC separation, these species are often partly or completely removed by SEC columns that, in effect, operate as filters. This leads not only to the loss of information about the samples under analysis, but to rapid deterioration of SEC column performance as well.

An example of a nanogel-containing sample is shown in Figure 7. The sample is a core-shell acrylic latex for paint applications. Crosslinking the latex core results in enhanced mechanical properties of the paint binders. It can be seen from Figure 7 that FFF provides baseline separation of soluble macromolecules from the crosslinked core. FFF-MALS quantifies the two species (based on the dRI peak areas), determines molar mass distributions and estimates the core compactness by simultaneously determining the molar mass and the RMS radius.

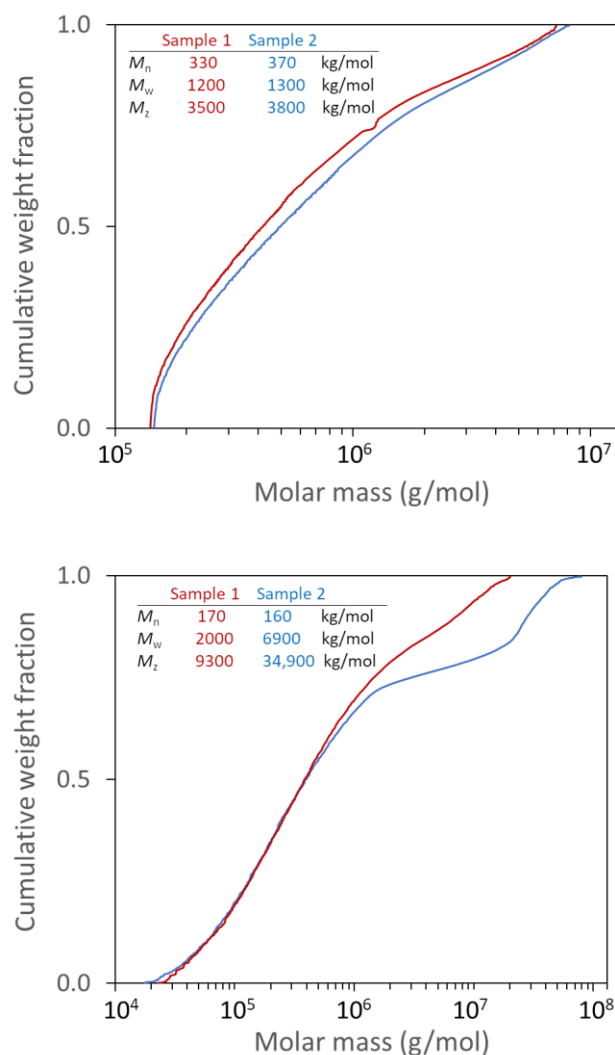


Figure 6. Emulsion acrylic core-shell copolymers: cumulative molar mass distribution curves and molar mass averages of two samples determined by SEC-MALS (top) and FFF-MALS (bottom).

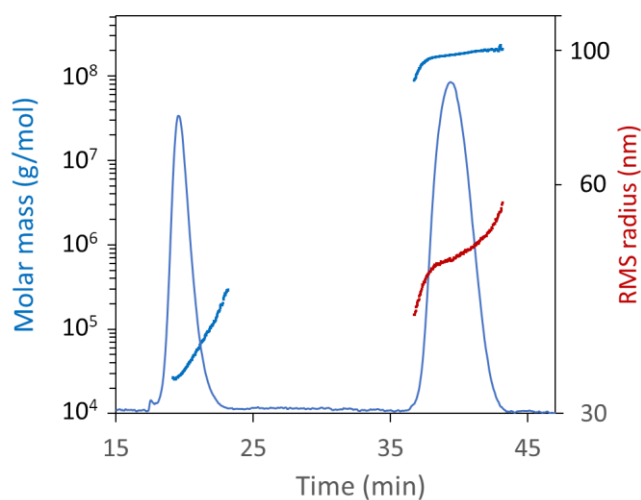


Figure 7. Core-shell acrylic copolymer prepared by emulsion polymerization: dRI fractogram, molar mass and RMS radius plots. The two peaks belong to dissolved macromolecules and swollen nanogels.

Note that the acrylic latex sample consists of macromolecules with molar mass in the range of tens to hundreds of kg/mol eluting around  $\approx 20$  min, and another species with extremely high molar mass of  $\approx 10^8$  g/mol, which is associated with RMS radii of only several tens of nm. For linear polymer chains in thermodynamically good solvents an RMS radius of  $\approx 50$  nm typically corresponds to a molar mass of  $\approx 1.2 \times 10^6$  g/mol, i.e., two orders of magnitude below the molar mass shown in Figure 5. This proves the expected highly compact, crosslinked structure of the core-shell copolymer.

### Styrene-butadiene rubbers

Styrene-butadiene rubbers (SBR) are materials from which a significant portion of car tires are made. Varying the ratio of soft (butadiene) and hard (styrene) blocks tunes the rubber's essential properties including adhesion, wet grip, rolling resistance, and abrasion resistance.

The fraction of styrene can be determined as a function of molar mass by incorporating a UV detector into the FFF-MALS system. The analysis, included in ASTRA software, is based on the different responses of the dRI and UV detectors to the two components: the dRI detector monitors both UV-absorbing (styrene) and non-UV-absorbing (butadiene) blocks, whereas the UV detector is only sensitive to the styrene blocks. With the knowledge of the specific refractive index increments ( $dn/dc$ ) of both parent homopolymers and the UV extinction coefficient

of polystyrene, ASTRA calculates the weight percentage of styrene along the molar mass axis.

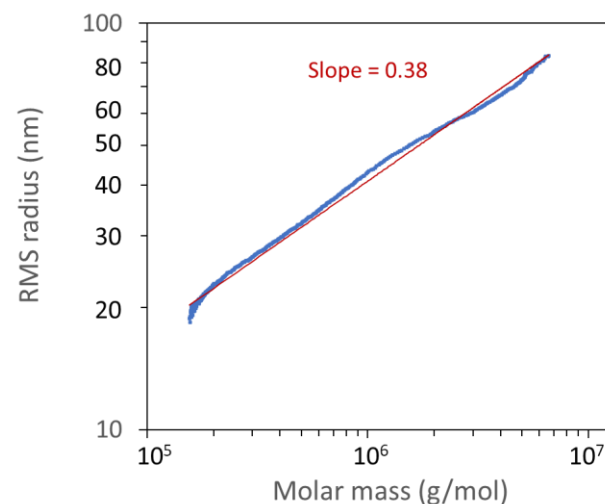
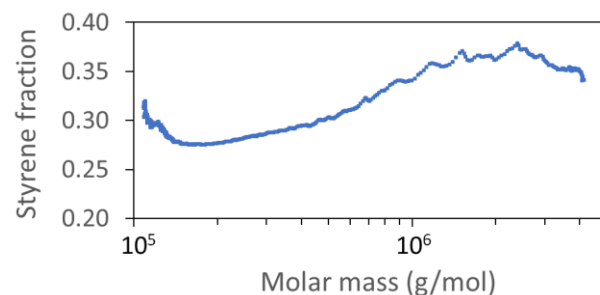
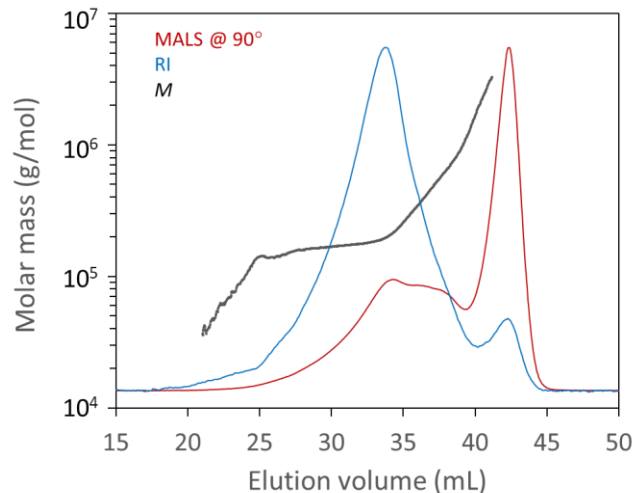


Figure 8. Styrene-butadiene rubber: molar mass versus retention time overlaid on MALS and dRI FFF fractograms (top), weight fraction of styrene as a function of molar mass (center), and conformation plot (bottom).

Such a calculation is demonstrated in Figure 8 which plots the molar mass versus retention time (top) of an SBR sample and the corresponding plot of styrene fraction

versus molar mass (middle). Figure 8 reveals a bimodal molar mass distribution with  $\approx 8\%$  high molar mass fractions, likely stars with multiple arms. The branched structure is evident from the slope of the conformation plot, 0.38 (Figure 8, bottom), that falls well below the typical value of linear polymer chains ( $\approx 0.58$ ). Figure 8 is an excellent example of the very detailed characterization of molecular structure of a technically very important polymer enabled by means of FFF coupled to MALS, dRI and UV detectors.

### Natural rubber

Although natural rubber (chemically, polyisoprene) has been partly replaced by synthetic alternatives, it still plays an important role in manufacturing vehicle tires and other elastic items such as protective gloves, shoes or conveyor belts.

As for other polymers containing fractions with molar mass above several millions of g/mol, FFF offers gentle separation and a corresponding reduction of shearing degradation. FFF-MALS analysis results in the determination of true molar mass distributions needed for understanding the mechanical properties and for differentiating samples from different geographical regions.

An example of molar mass, MALS and dRI signals versus retention time of a natural rubber is depicted in Figure 9. Were this sample to be analyzed by SEC, the fractions with molar mass of roughly  $10^8$  g/mol would be severely degraded, resulting in completely misleading conclusions regarding the molar mass distribution. The slope of the conformation plot drops from  $\approx 0.53$ , indicating moderate branching, to virtually zero, (surprisingly) well below the value of  $\approx 0.33$  which corresponds to compact spheres. Although this may sound implausible, one can imagine structures where additional mass is absorbed into the particles without increase of the RMS radius.

### Cellulose

At first glance, cellulose does not appear to be a good candidate for separation by FFF in THF. Cellulose in its native form is difficult to dissolve. Dissolution requires highly polar organic solvents such as dimethyl sulfoxide, often with complicated pre-treatment procedures. Derivatization to cellulose tricarbanilate, which is well-soluble in THF, is an efficient solution to the limited solubility problem.

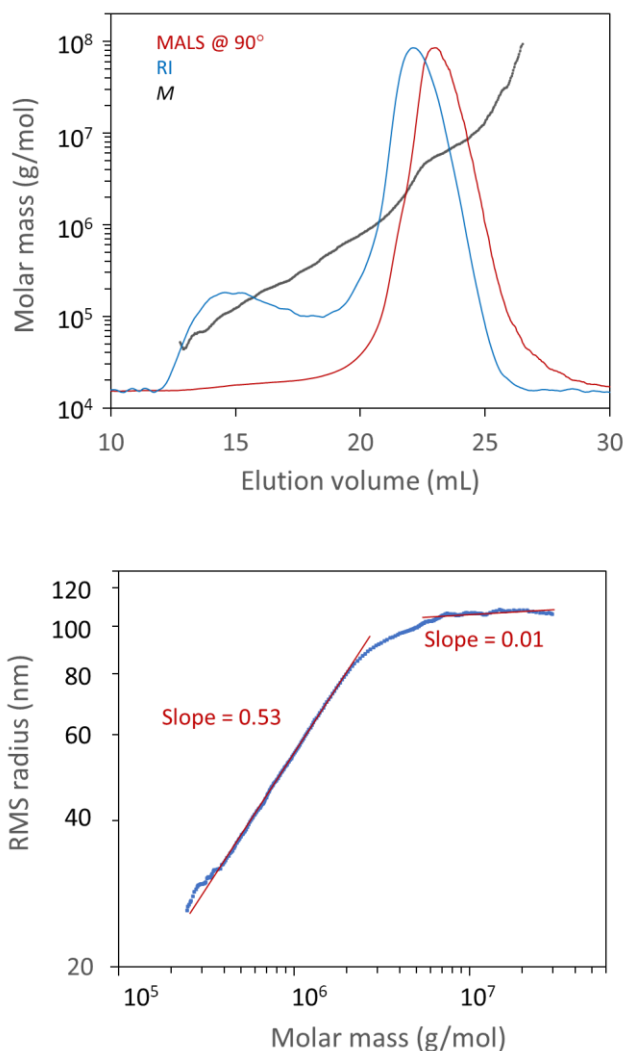


Figure 9. Natural rubber: molar mass versus retention time overlaid on MALS and dRI fractograms (top), and conformation plot with two different slopes indicating different molecular structures (bottom).

An example of FFF-MALS analysis of cellulose after derivatization to tricarbanilate is depicted in Figure 10. Comparative SEC-MALS analysis revealed shearing degradation, obvious from lower values of  $M_w$  and  $M_z$ . FFF also offers markedly better resolution of hemicellulose that appears as a well-separated peak on the FFF fractogram at  $\approx 19$  min, while there is only a shoulder on the SEC chromatogram at larger elution volumes. As in the previous examples of polymers containing branched molecules, one can see that FFF-MALS provides a more accurate description of the molar mass distribution in the region of lower molar masses.



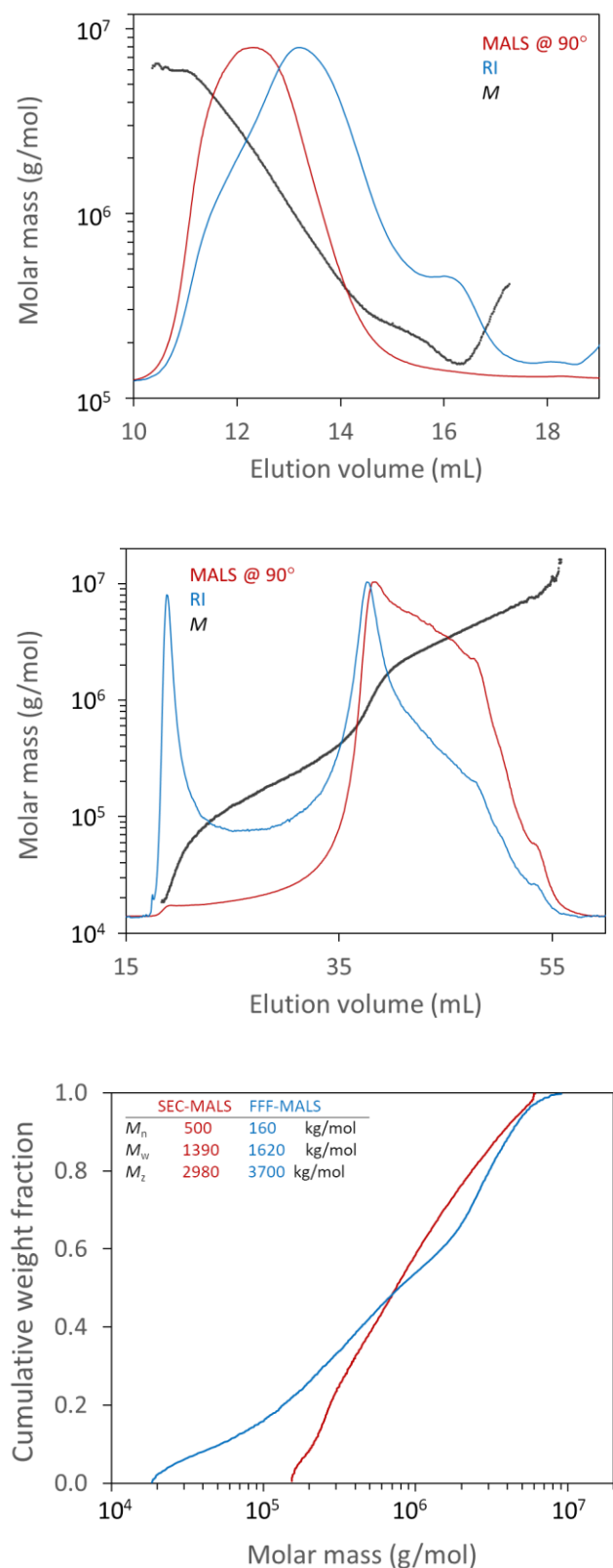


Figure 10. Cellulose tricarbanilate: molar mass versus retention time overlaid on MALS and dRI signals from SEC-MALS (top) and FFF-MALS (center), and corresponding molar mass distribution curves and molar mass averages (bottom).

## Conclusions

The combination of FFF separation with MALS detection brings to polymer characterization many capabilities unachievable by other techniques. Compared to SEC, which widely used, FFF provides superior separation of branched polymers, polymers with ultra-high molar mass fractions, and supramolecular structures. Although FFF is particularly powerful beyond the effective range of SEC separations, it is also quite efficient for polymers that are easily analyzed by SEC. FFF-MALS offers benefits such as very low MALS noise, simple tunability of the resolution and low unit analysis cost.

A great advantage of Wyatt's Eclipse FFF system is its foundation on chromatography modules from a leading HPLC manufacturer, making the entire setup highly reliable. Introduction of FFF-MALS into a polymer R&D or QC laboratory does not mean abandoning SEC; quite the contrary. Both techniques share the same chromatograph and detectors, so the entire system can easily be rearranged from FFF-MALS mode to SEC-MALS and vice versa in a few minutes.

## References and Further Reading

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