Comparison of Polymer Separation by Size Exclusion Chromatography and Asymmetric Flow Field Flow Fractionation

Stepan Podzimek,[1] Christoph Johann[2]

[1]SYNPO / University of Pardubice, Czech Republic,

stepan.podzimek@synpo.cz

[2]Wyatt Technology Europe, Dernbach, Germany

Overview

- Size exclusion chromatography (SEC/GPC) versus asymmetric flow field flow fractionation (A4F/AF4)
  - ✓ Multi-angle light scattering (MALS) detection for both SEC and AF4 (No calibration by polymer standards)
- Data to compare
  - ✓ SEC and AF4 separation of narrow polymers
  - ✓ Molar mass averages by SEC-MALS and AF4-MALS
  - ✓ Mass recovery in AF4
  - ✓ Molar mass distribution by SEC-MALS and AF4-MALS
  - ✓ Conformation plots (branching) by SEC-MALS and AF4-MALS
- Examples of application
- Conclusions

AF4-MALS and SEC-MALS Instrumentation

The presented data were acquired using instrumentation from

Wyatt Technology Corporation

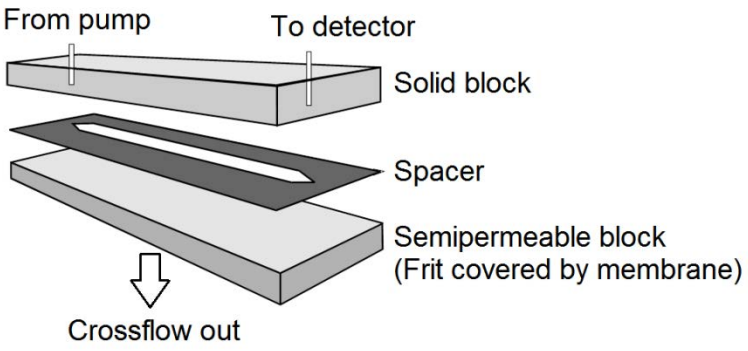
- AF4 system Eclipse AF4
- MALS detector HELEOS
- Refractive index detector Optilab T-rEX
- Software ASTRA 6

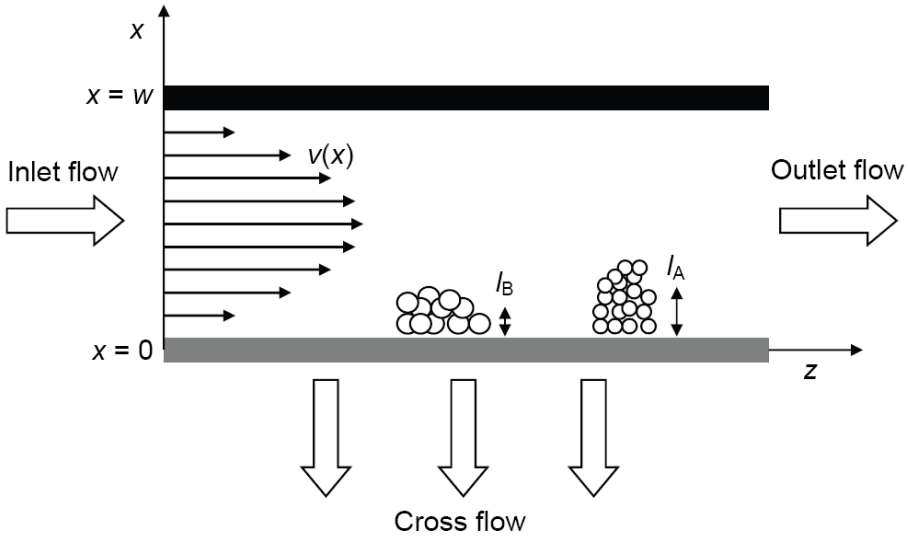
Waters and Agilent

- Agilent 1100 pump
- Waters 717 autosampler
- Agilent 2 × PLgel Mixed-C or Mixed-B columns 300 × 7.5 mm

What Is AF4?

- Alternative separation technique to SEC/GPC
- Separation according to hydrodynamic volume
- No stationary phase – only solvent
- Separation by flow in a thin ribbon like channel created by sandwiching a thin spacer between two blocks
- One block is solid, one is semipermeable – frit covered by semipermeable membrane





AF4-MALS and SEC-MALS Experiments

- Polymers of different molar mass, linear and branched, narrow and broad; polystyrene, poly(methyl methacrylate), polycarbonate, acrylic emulsion copolymers, …
- Mass recovery and molar mass averages
  - Stock solutions dispensed into well-sealed autosampler vials, each vial used just for a single injection, 100 µL 0.2 – 0.5 % solutions in THF, identical injected mass in SEC and AF4
  - Mass recovery in AF4 was compared relatively to SEC to avoid systematic errors given by RI calibration or *dn*/*dc*
  - The same injected mass in SEC-MALS and AF4-MALS
  - Four or five injections for each sample, presented data are averages
- THF as solvent for SEC and AF4
- Cross flow gradient in AF4, 5 kDa regenerate cellulose membrane

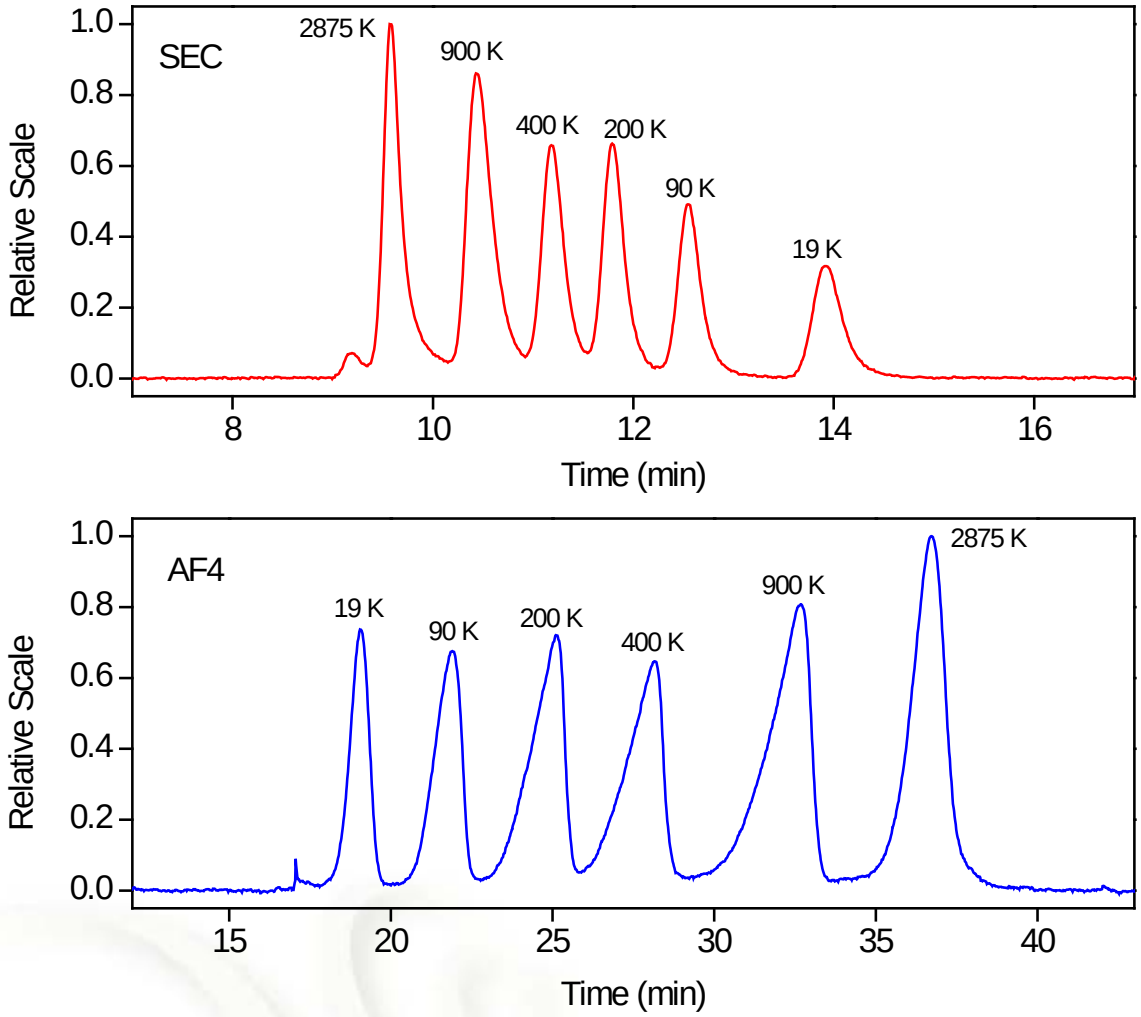
Processing AF4-MALS and SEC-MALS Data

- MALS of non-fractionated polydisperse polymers provides **weight-average molar mass** (M_w) and ***z*-average root mean square** (RMS) **radius** (R_z). The M_w and R_z count more the fractions with high molar mass, R_z counts more than M_w.

- **Assumption:**
  Slice polydispersity in SEC and AF4 is negligible, i.e., MALS detector measures molar masses M_i and RMS radii R_i and not averages
  - This assumption is never completely fulfilled due to limited resolution of SEC columns or AF4 channel
  - Typically the polydispersity can be neglected without introducing significant errors
    (good agreement of M_n measured by SEC-MALS and MO; and M_z measured by SEC-MALS and calculated from R_z)
  - **Simplification od data processing**

Narrow Polymers
(Polystyrene Standards)

Narrow Polystyrene Standards



MALS signals for a mixture of PS standards separated by SEC and AF4.
AF4 is less efficient (peak width) and more selective (retention time difference).

Average Molar Masses
Mass Recovery

Average Molar Masses and Mass Recovery

Polymer	M_n (10^3 g/mol)	
	SEC-MALS	AF4-MALS
Polycarbonate (38)	17 ± 1	27 ± 1
PMMA (J)	46 ± 2	62 ± 5
PS (NIST 706)	105 ± 4	156 ± 5
PS (AN)	113 ± 2	137 ± 6
PS (2)	131 ± 1	148 ± 4
PMMA (Y)	193 ± 2	233 ± 16
PMMA (dent)	621 ± 1	676 ± 3
PS branched (B2)	151 ± 1	121 ± 4
PS branched (43)	217 ± 4	110 ± 14

Polymer	M_w (10^3 g/mol)		AF4 recovery (% of SEC)
	SEC-MALS	AF4-MALS	
Polycarbonate (38)	34 ± 1	39 ± 1	91.0 ± 0.4
PMMA (J)	84 ± 1	91 ± 2	94.2 ± 0.6
PS (NIST 706)	271 ± 1	290 ± 2	97.0 ± 0.4
PS (AN)	279 ± 1	296 ± 3	98.0 ± 0.9
PS (2)	367 ± 1	387 ± 2	98.8 ± 0.2
PMMA (Y)	516 ± 1	555 ± 19	98.3 ± 0.2
PMMA (dent)	1448 ± 6	1572 ± 20	99.8 ± 0.9
PS branched (B2)	482 ± 6	648 ± 14	98.7 ± 0.3
PS branched (43)	1265 ± 19	2159 ± 149	99.7 ± 1.0

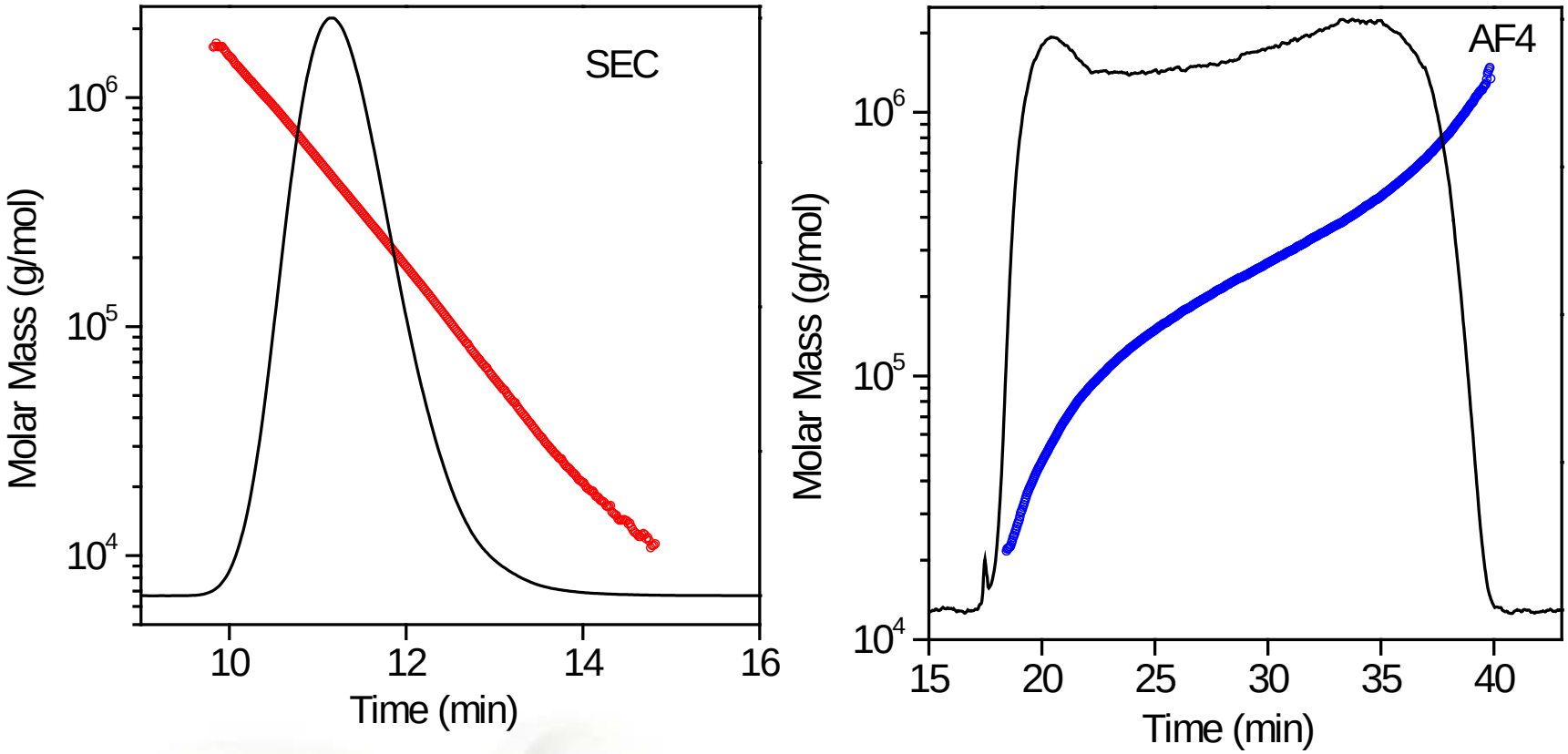
Polymer	M_z (10^3 g/mol)	
	SEC-MALS	AF4-MALS
Polycarbonate (38)	52 ± 1	54 ± 1
PMMA (J)	131 ± 1	132 ± 1
PS (NIST 706)	426 ± 1	426 ± 3
PS (AN)	468 ± 1	471 ± 6
PS (2)	858 ± 1	848 ± 11
PMMA (Y)	982 ± 5	968 ± 10
PMMA (dent)	2520 ± 20	2340 ± 20
PS branched (B2)	2190 ± 60	7240 ± 440
PS branched (43)	7720 ± 190	15510 ± 630

Average Molar Masses and Mass Recovery

- **Number-Average molar mass**
  - AF4 yields slightly higher M_n for linear polymers due to better resolution of SEC in lower molar mass region and/or permeation of oligomeric fractions through semipermeable membrane
  - Difference of M_n is larger for samples containing more oligomers
  - AF4 yields lower M_n for branched polymers due to more efficient separation of branched macromolecules
- **Weight-average molar mass**
  - AF4 yields slightly higher M_w as the concentration in A4F channel is typically lower compared to SEC column and thus lower error from neglecting the A_2
  - AF4 yields markedly higher M_w for polymers containing ultra-high molar mass fractions due to the absence of stationary phase and significantly lower pressure in AF4 channel, which reduces shearing degradation
- **Z-average molar mass**
  - Typically well-comparable M_z
  - AF4 yields markedly higher M_z for polymers containing ultra-high molar mass fractions due to reduced shearing degradation
- **Mass recovery**
  - No systematic deviation of AF4 from SEC
  - Mass recovery depends on the content of oligomeric fractions
  - Mass recovery in AF4 for samples not containing oligomers is close to 100 %

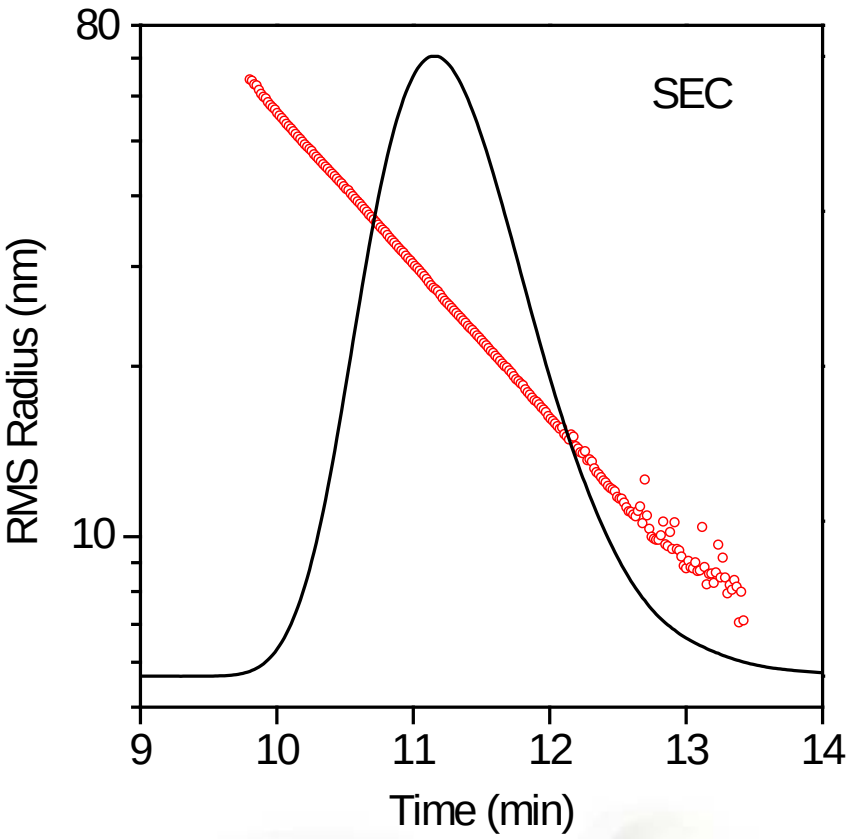
Molar Mass and Size Distribution

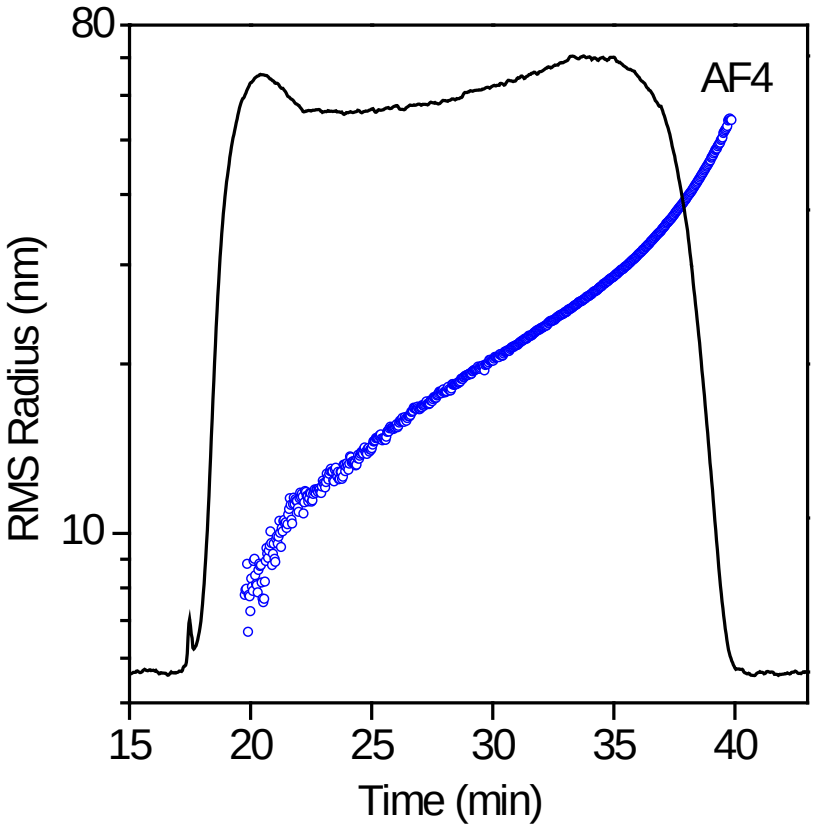
Linear Polymers: Molar Mass



Molar mass versus retention time plots of linear polystyrene determined by SEC-MALS and AF4-MALS. RI signals are overlaid here.

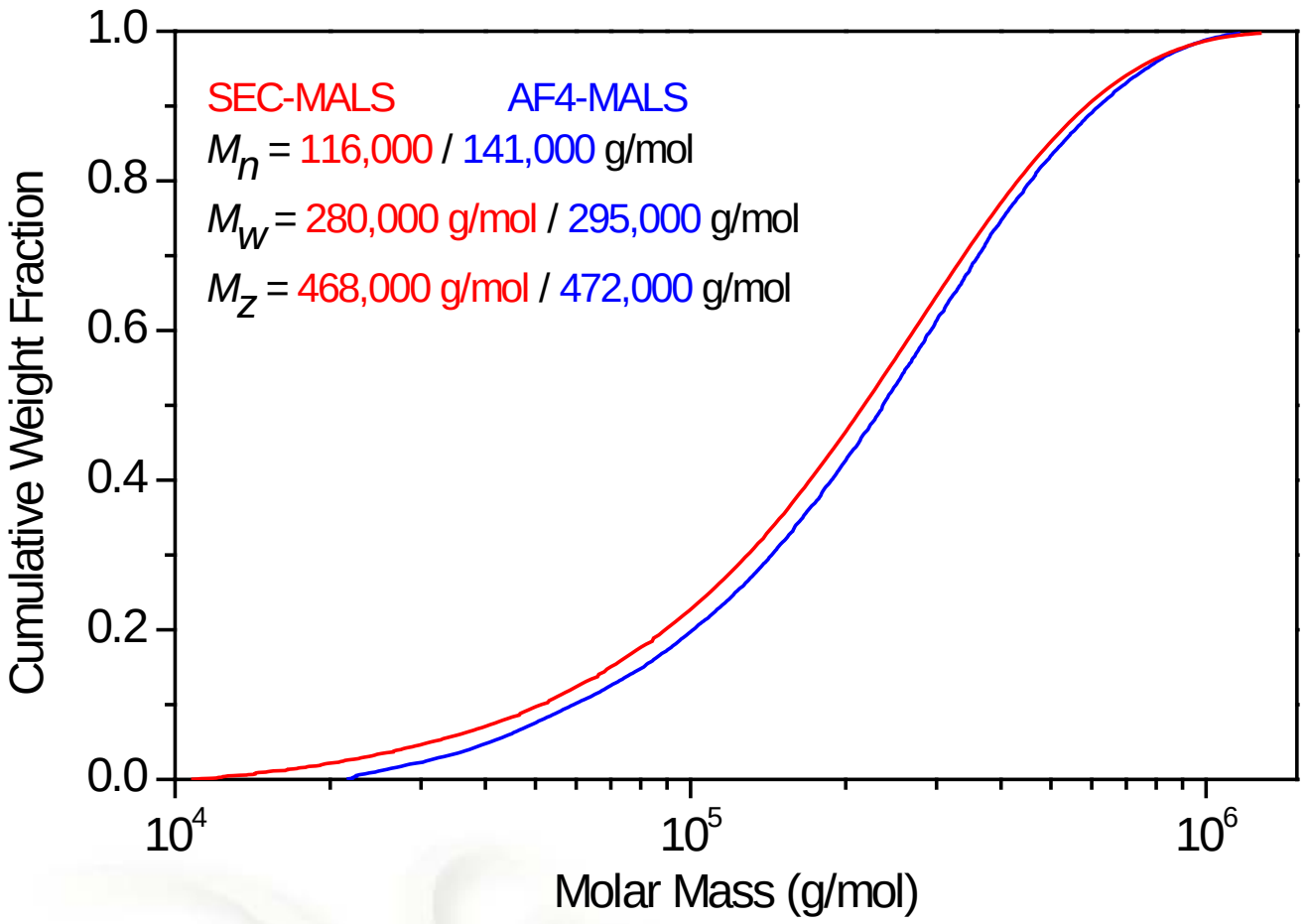
Linear Polymers: RMS Radius





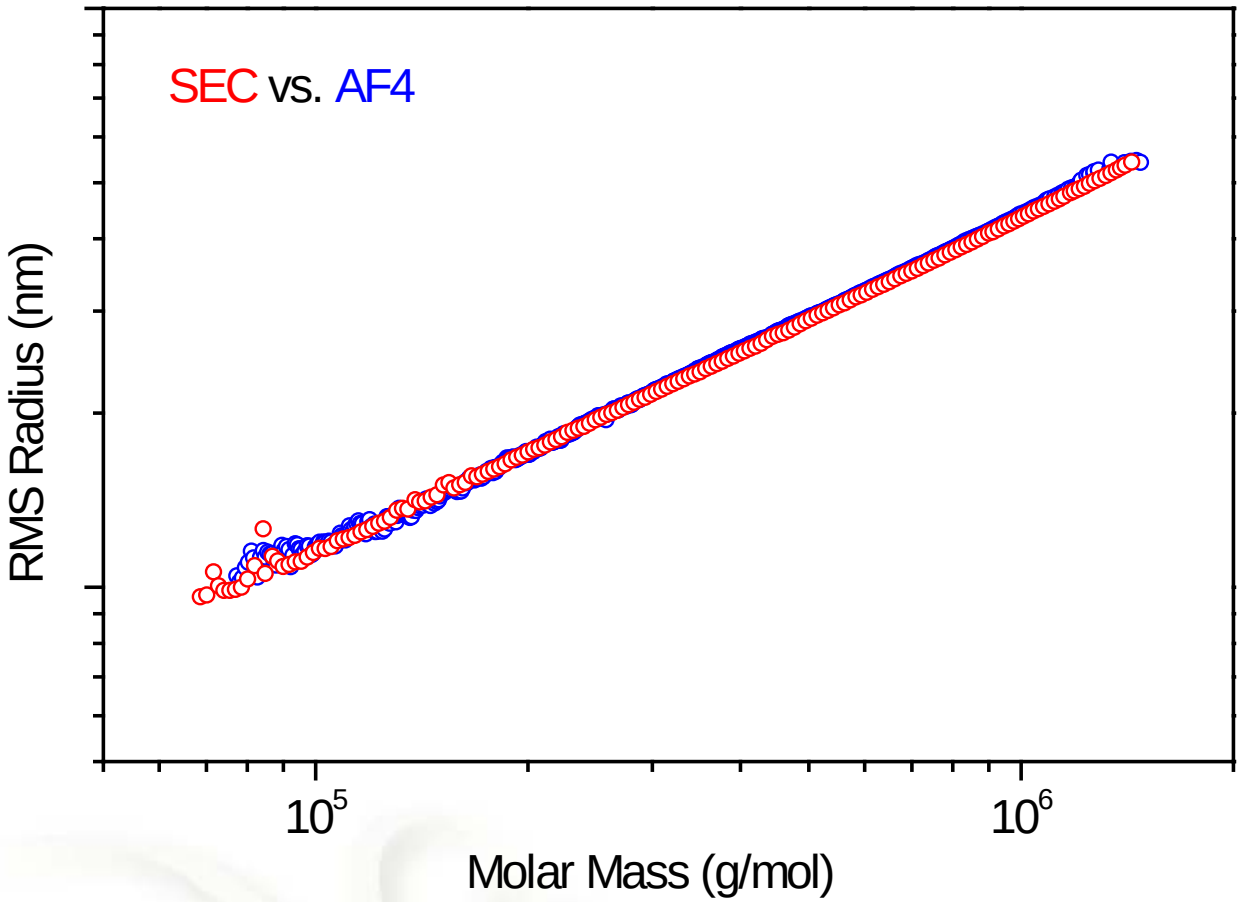
Root mean square (RMS) radius versus retention time plots of linear polystyrene determined by SEC-MALS and AF4-MALS. RI signals are overlaid here.

Linear Polymers: Molar Mass Distribution



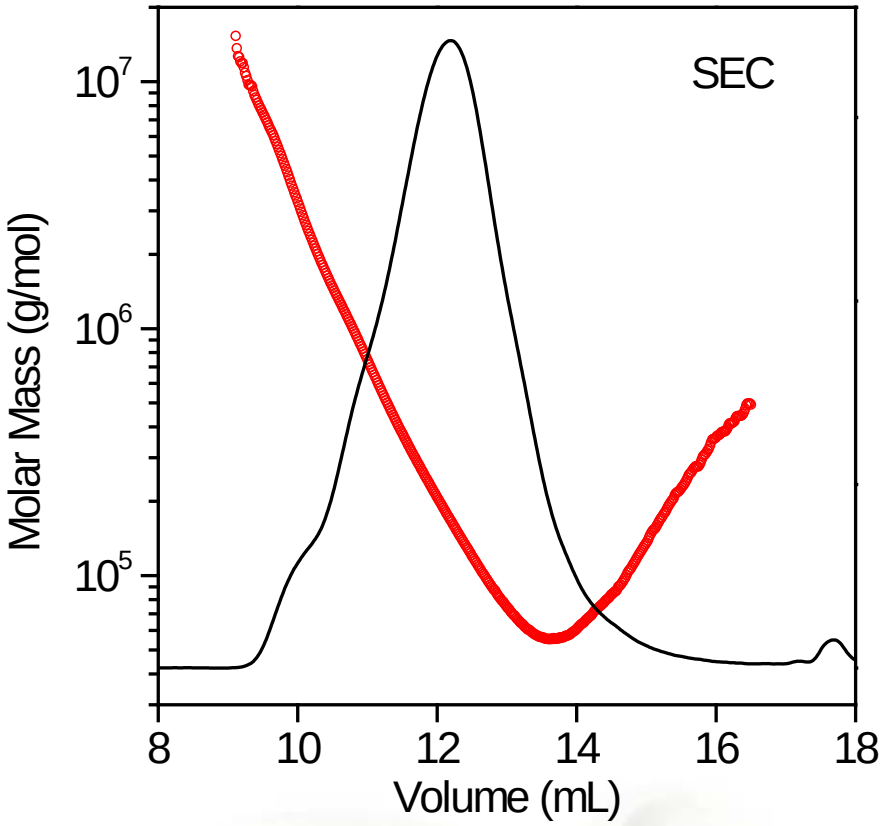
Cumulative molar mass distribution of linear polystyrene determined by SEC-MALS and AF4-MALS.

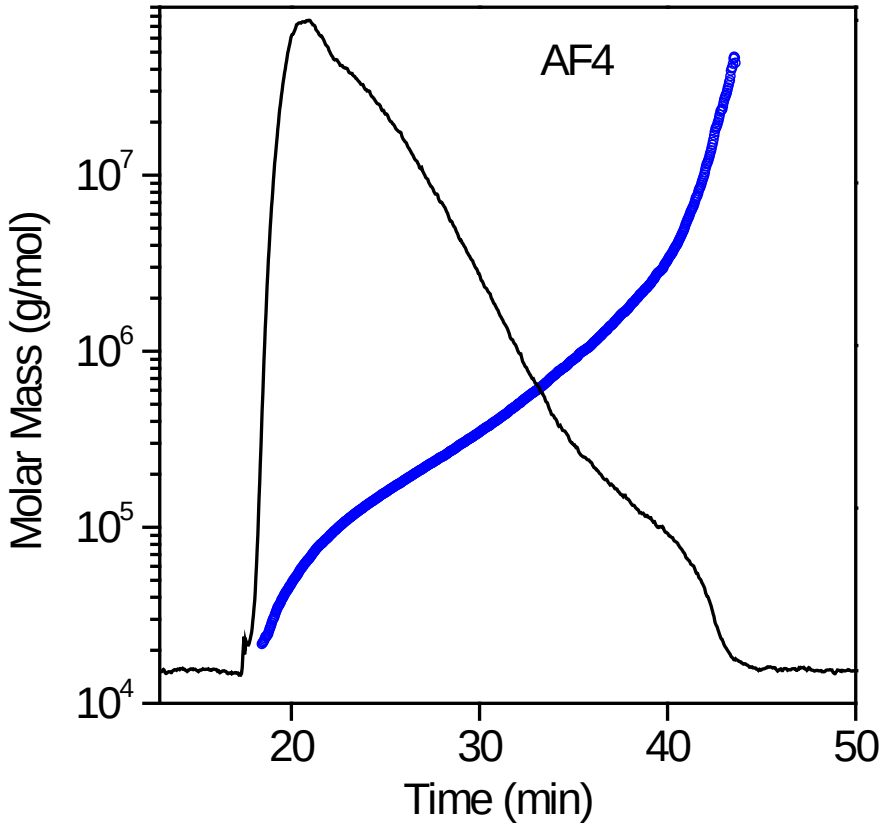
Linear Polymers: Conformation Plot



Root mean square (RMS) radius (radius of gyration) versus molar mass plots of linear polystyrene determined by SEC-MALS and AF4-MALS.

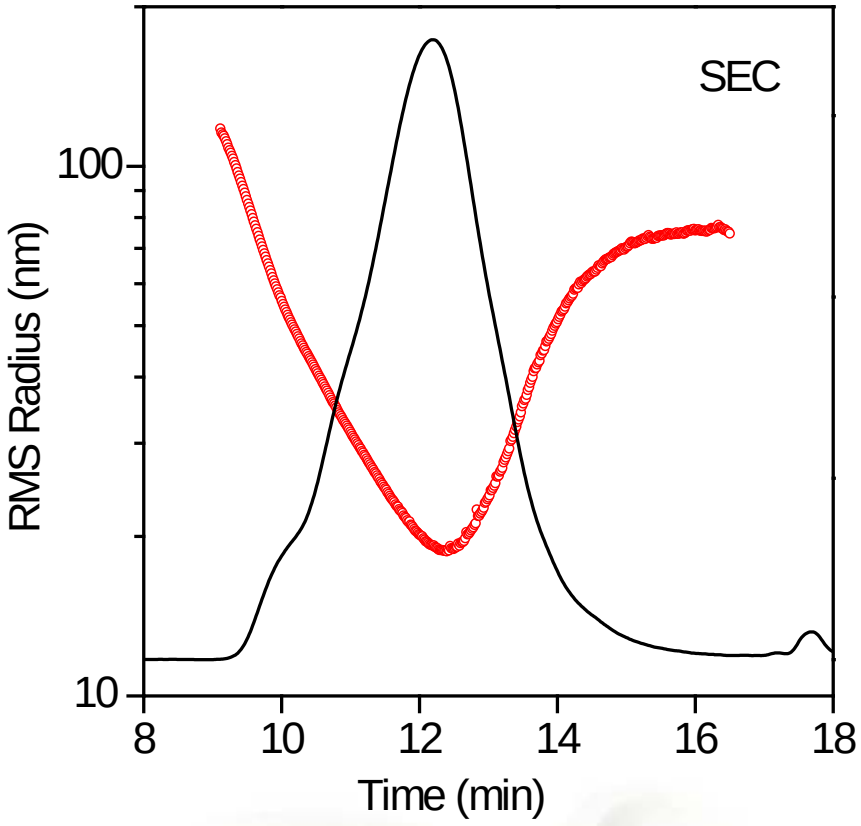
Branched Polymers: Molar Mass

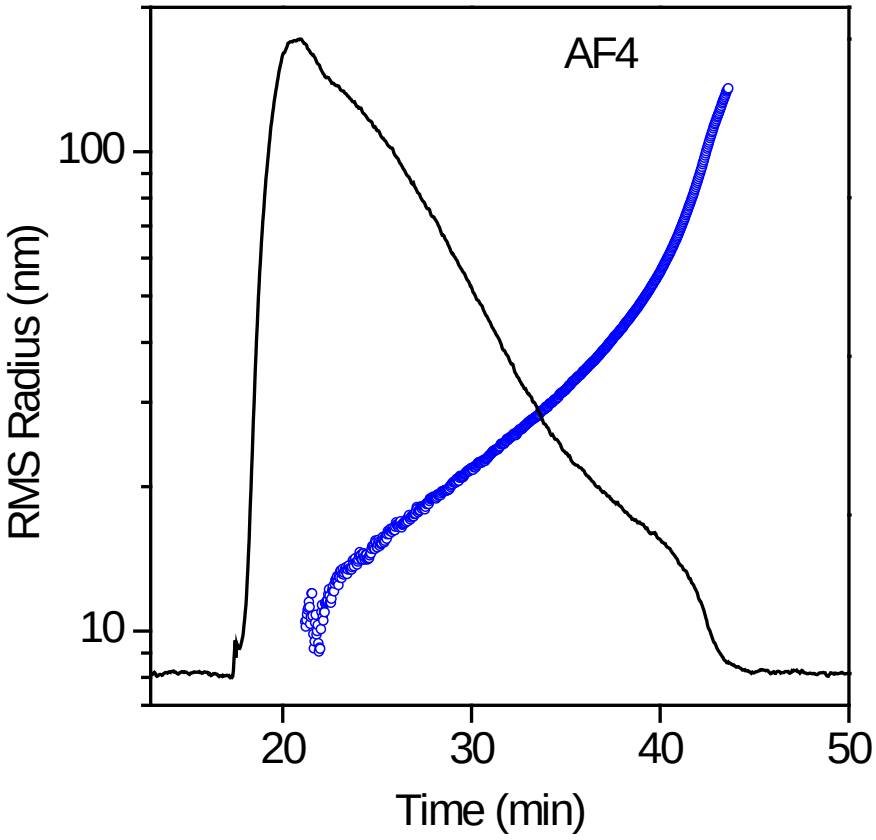




Molar mass versus retention time plots of branched polystyrene determined by SEC-MALS and AF4-MALS. RI signals are overlaid here.

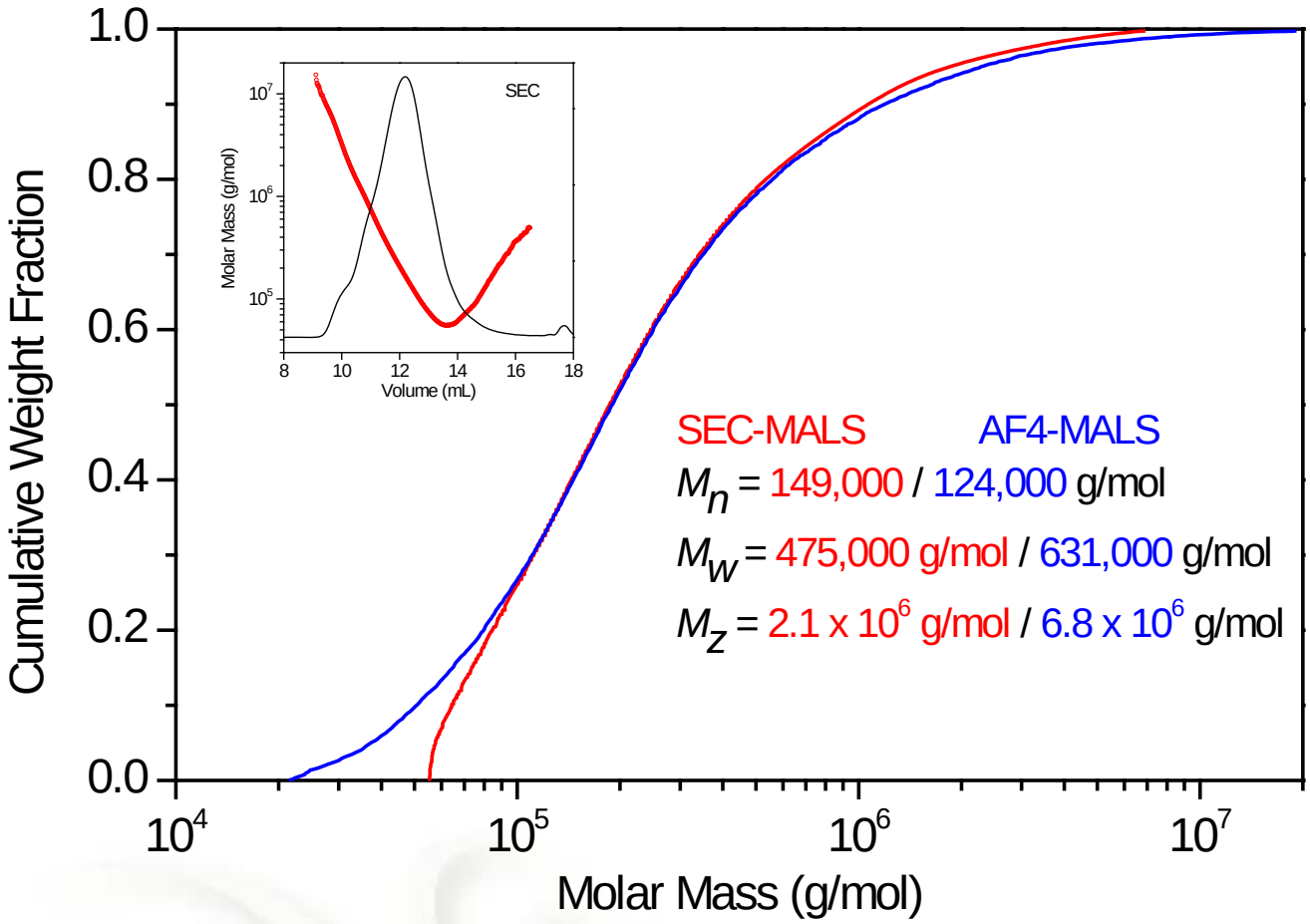
Branched Polymers: RMS Radius





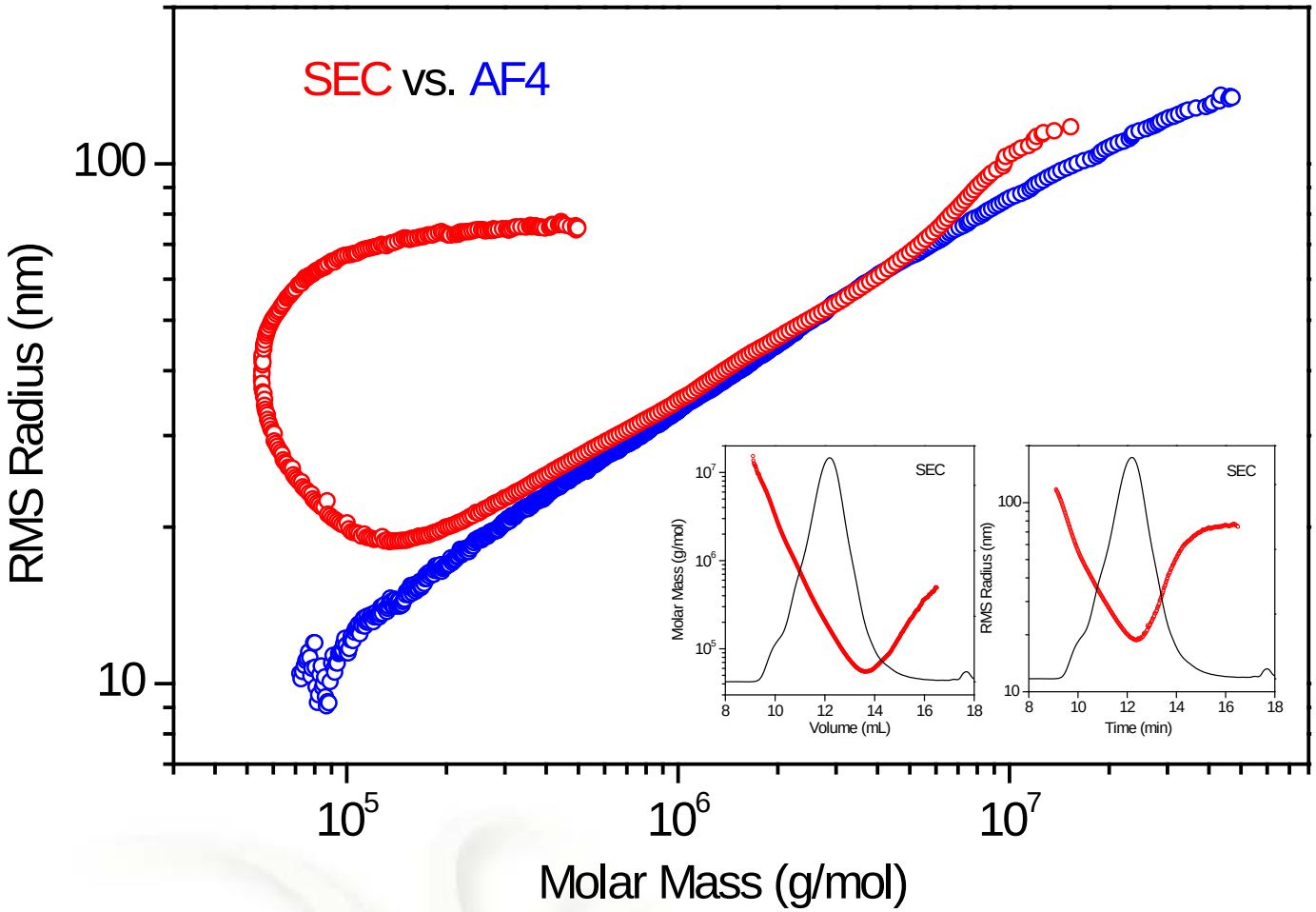
Root mean square (RMS) radius versus retention time plots of branched polystyrene determined by SEC-MALS and AF4-MALS. RI signals are overlaid here.

Branched Polymers: Molar Mass Distribution



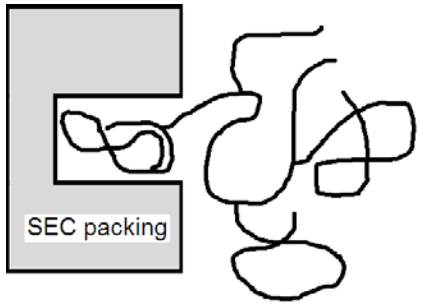
Cumulative molar mass distribution of branched polystyrene determined by SEC-MALS and AF4-MALS.

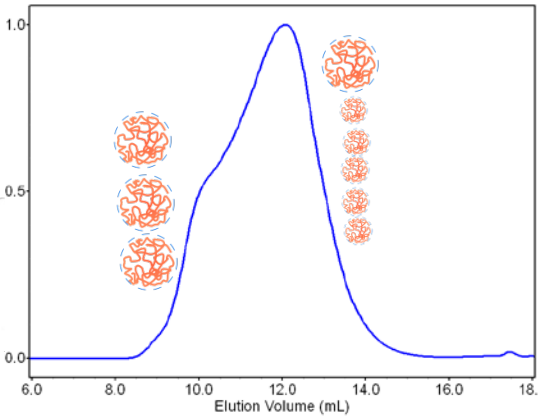
Branched Polymers: Conformation Plot

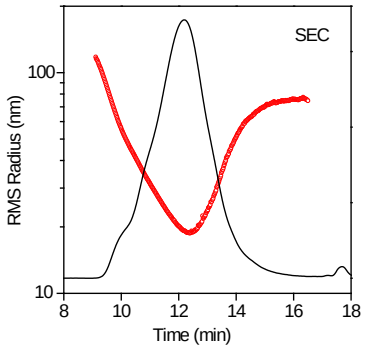


Root mean square (RMS) radius versus molar mass plots of branched polystyrene determined by SEC-MALS and AF4-MALS.
Basic assumption is not valid!

Delayed SEC Elution of Branched Polymers



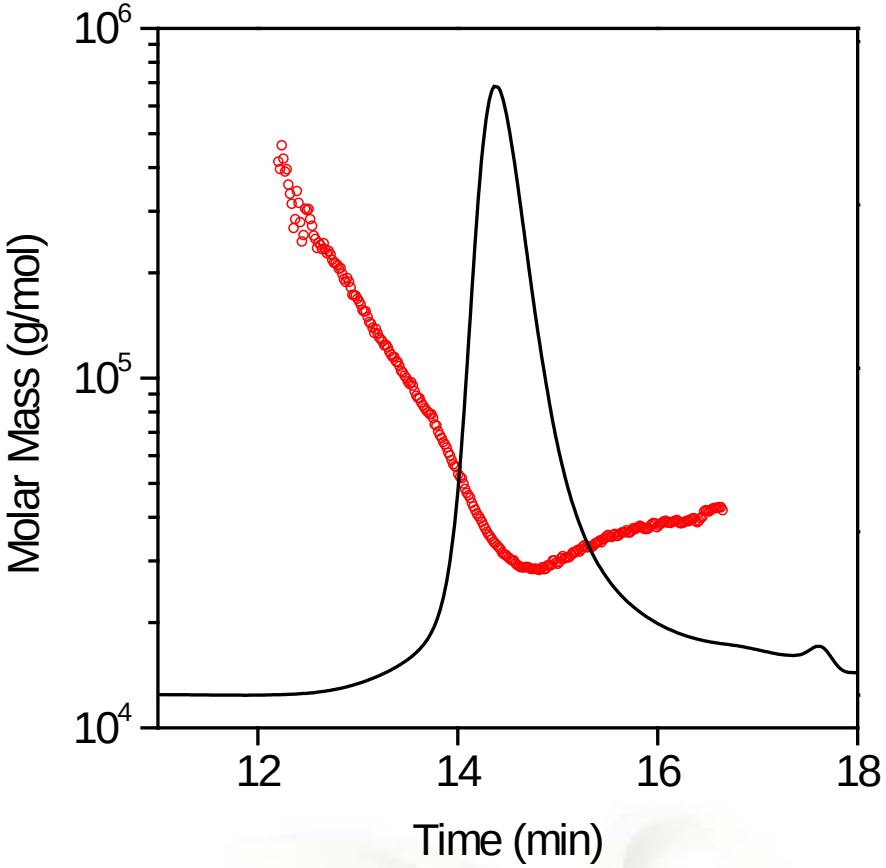


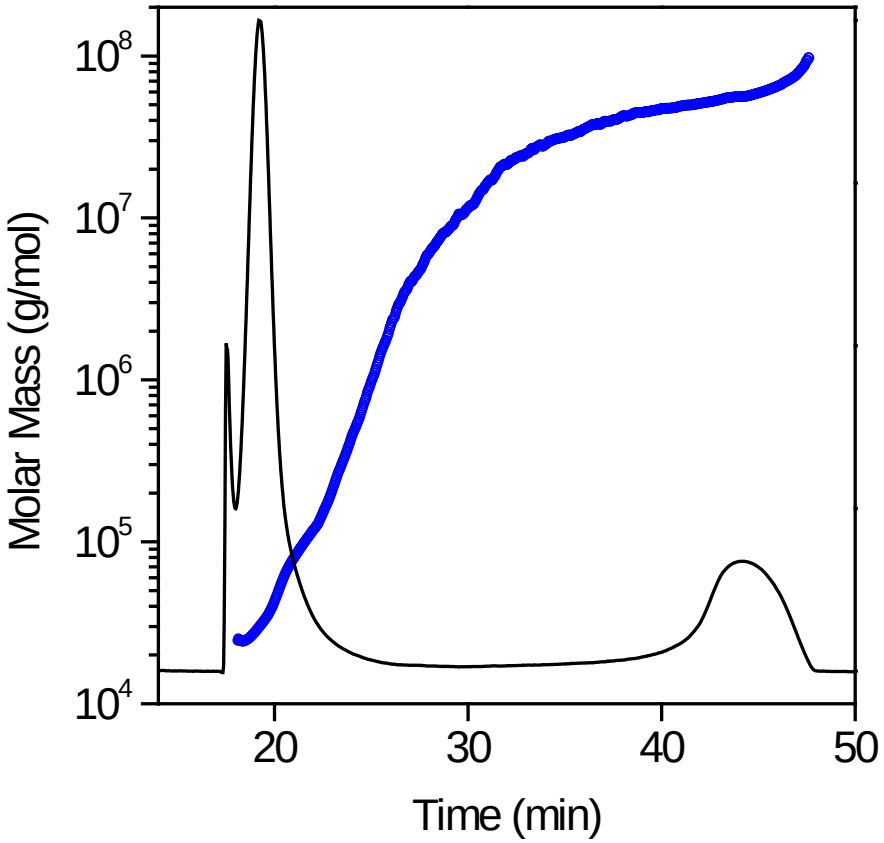


- Large branched molecules are anchored in pores and elute later than it would correspond to their hydrodynamic size
- Slices at high elution volumes are contaminated by delayed large molecules and MALS detector measures M_w and R_z
  The fundamental assumption od SEC-MALS and AF4-MALS is not fulfilled
- The delayed elution increases polydispersity of slices at the end of SEC chromatogram, which makes the conformation plots upturned

Polymers Interacting with Column Packing

Polymers Interacting with Column Packing





Molar mass versus retention time plots of functional polymer determined by SEC-MALS and AF4-MALS overlaid on RI signals.

Polymers Containing Insoluble Nanogels

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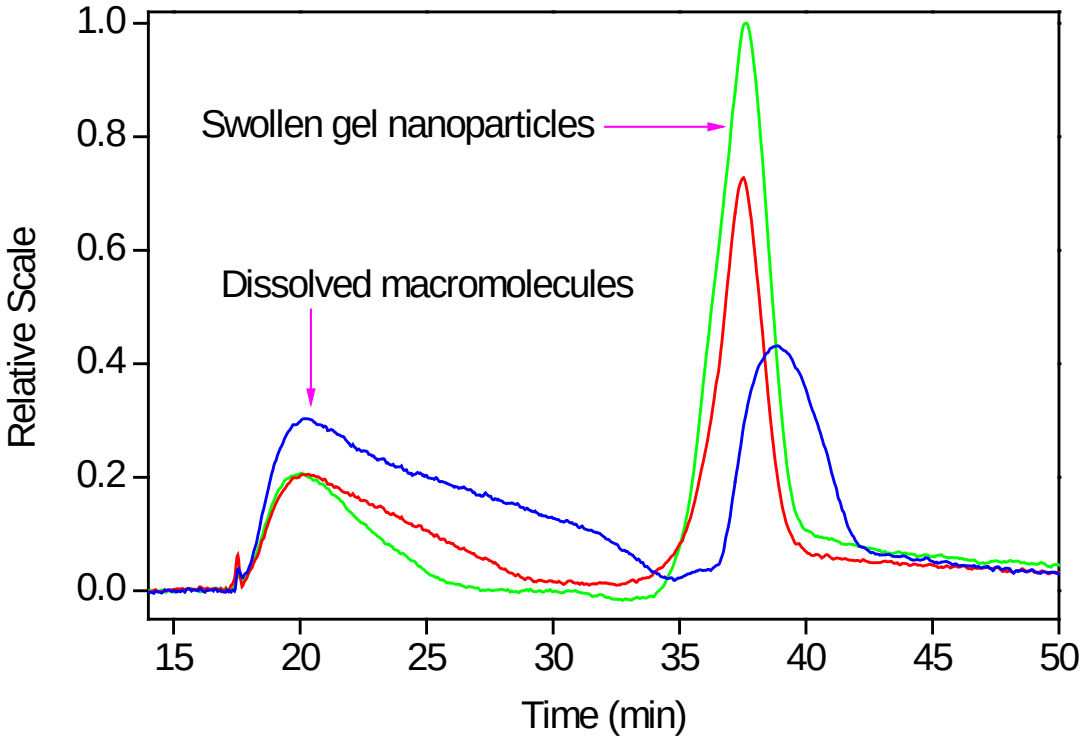
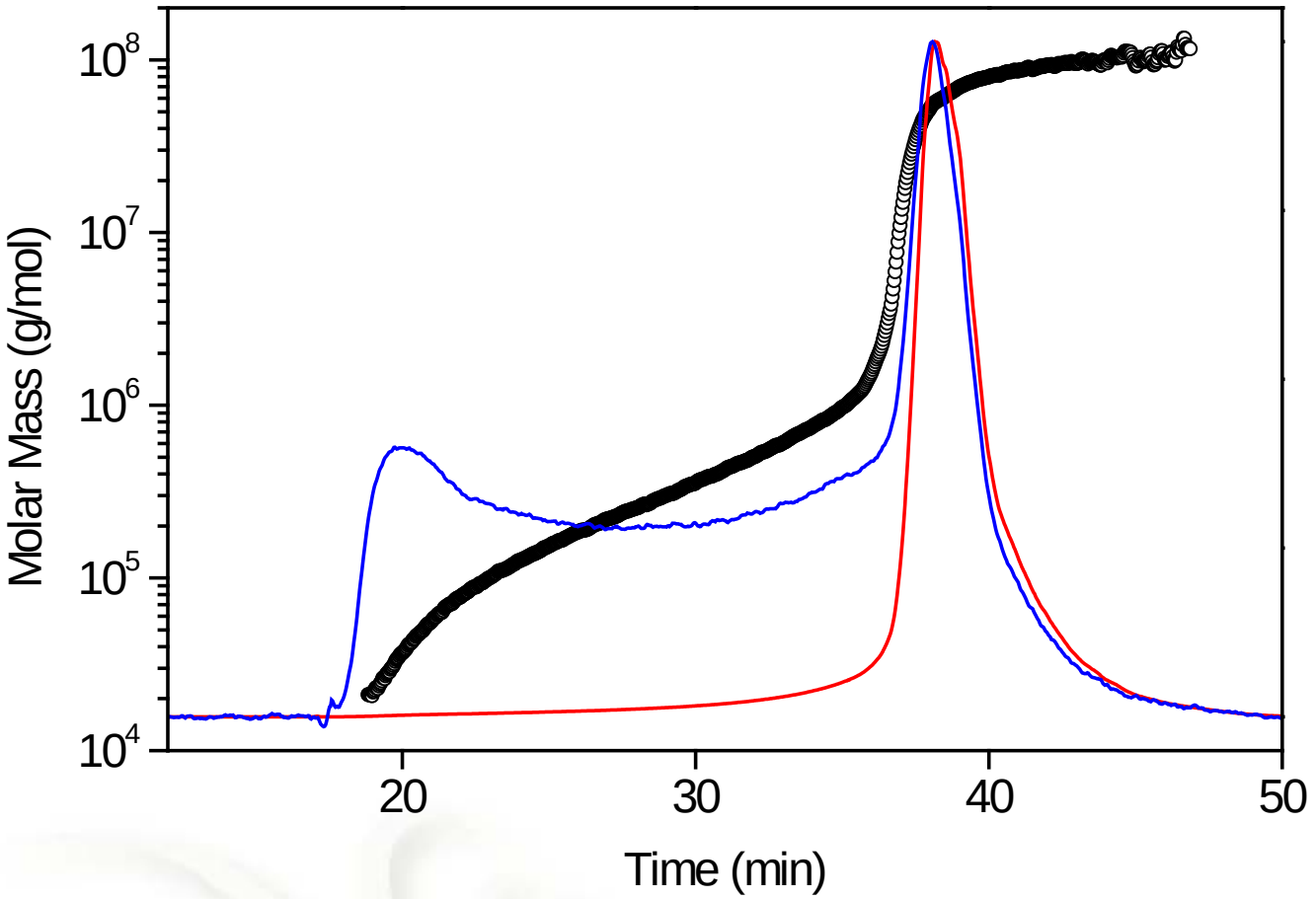


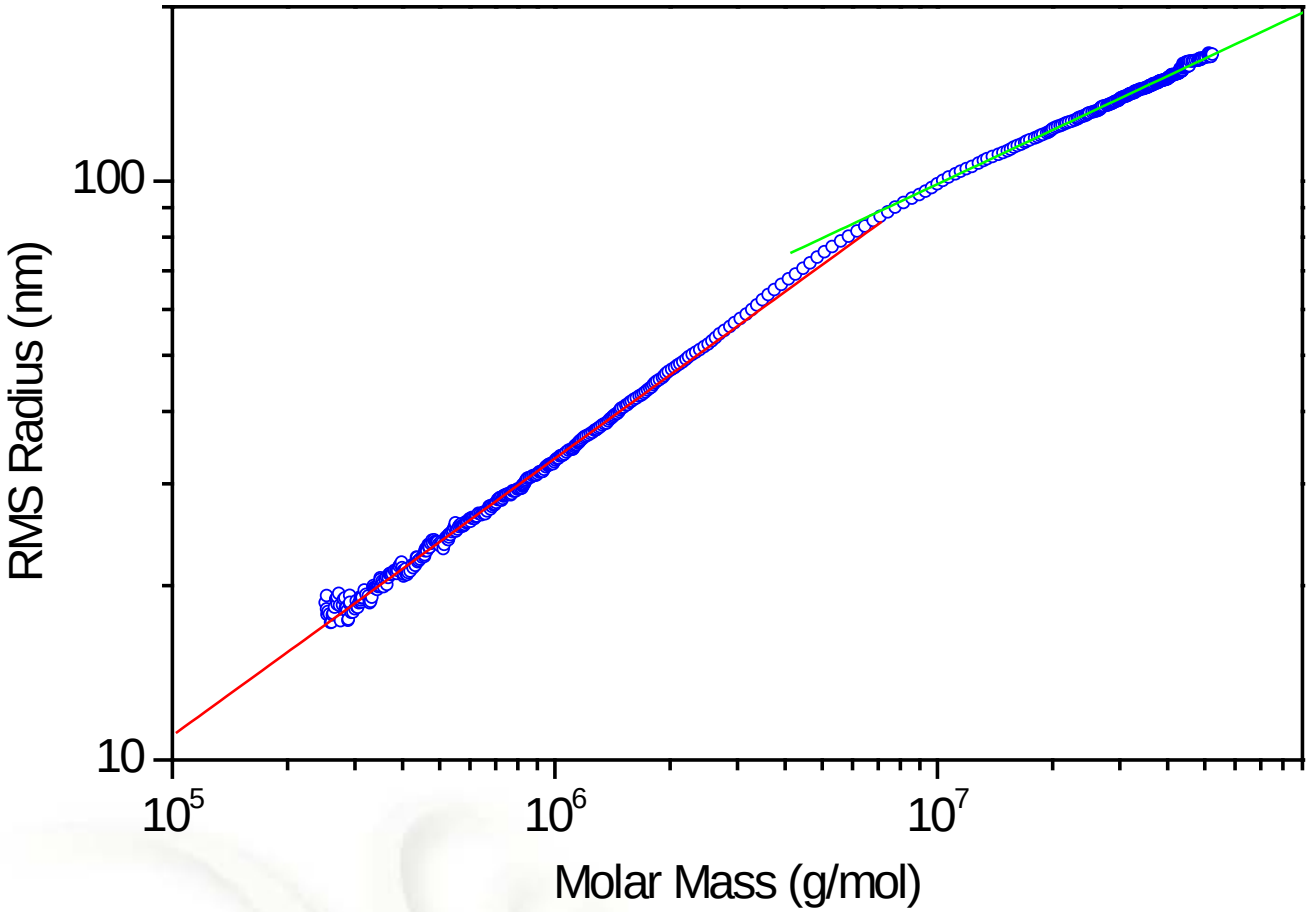
THF solutions of methyl methacrylate/butyl acrylate copolymers containing (from left to right) 0, 1, 2, 4, 6, and 8 % ethylene glycol dimethacrylate; and RI fractograms of copolymers containing 2 % (blue), 4 % (red) and 8 % (green) ethylene glycol dimethacrylate.

Polymers with Ultra-High Molar Mass Species



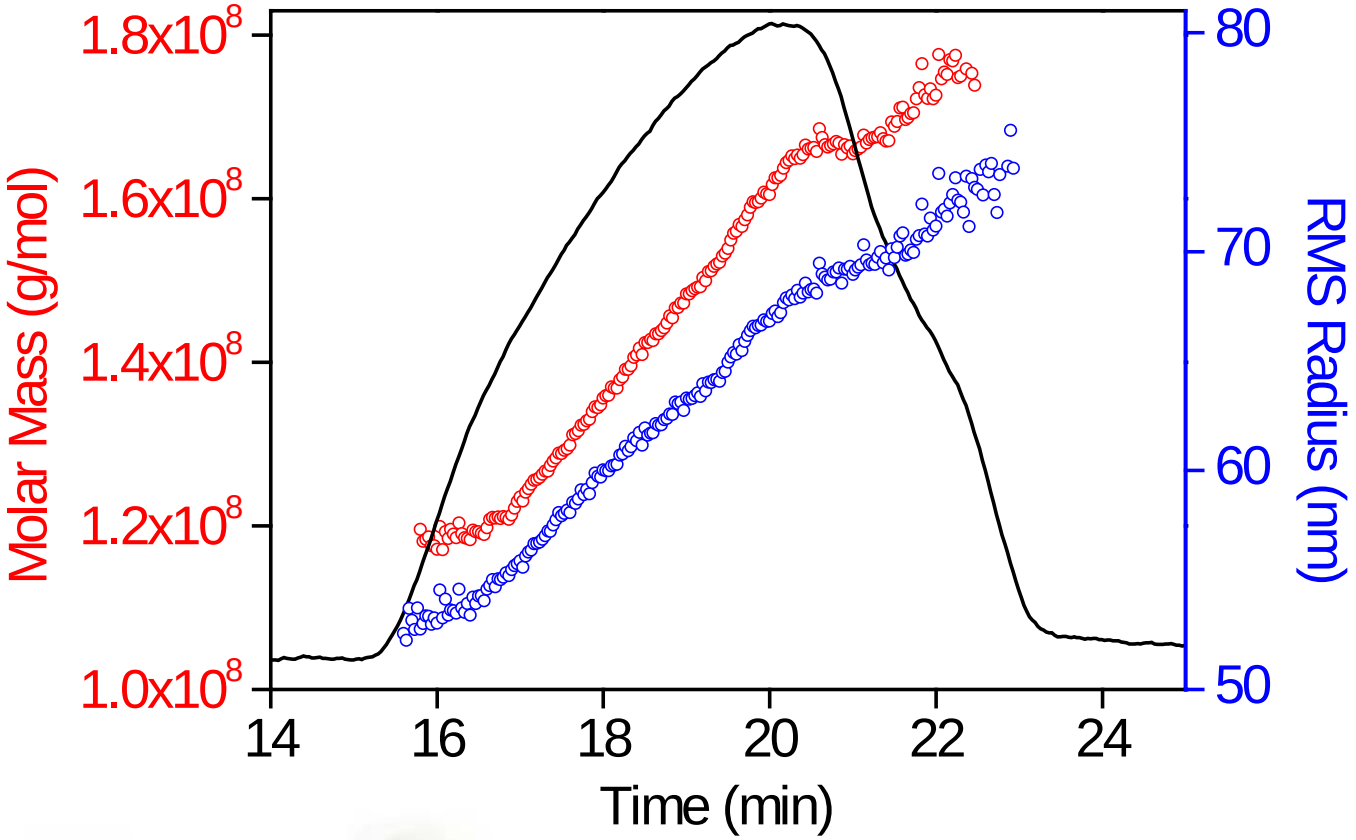
Molar mass versus retention time plot (o) and signals of MALS (red) and RI (blue) detectors of methyl methacrylate/butyl acrylate copolymer.

Polymers with Ultra-High Molar Mass Species



Conformation plot of methyl methacrylate/butyl acrylate copolymer showing two different slopes of ≈ 0.48 and 0.31.

Organic Nanoparticles in Epoxy Resin



RI fractogram and molar mass vs. retention time and RMS radius vs. retention time plots for organic nanoparticles added to epoxy resin.

For linear random coils RMS radius ≈ 65 nm corresponds to molar mass ≈ 1.9 x 10^6 g/mol → compact structure of nanoparticles.

Conclusions

AF4 compared to SEC

- Absence of stationary phase reduces shearing degradation, and eliminates anchoring effect and interactions of polymer with stationary phase
  1. Polymers containing ultra-high molar mass fractions
  2. Polymers containing highly branched species
  3. Polymers containing nano-gels and nanoparticles
  - Functional polymers interacting with SEC column packing
- The same HPLC pump, autosampler and detectors for SEC and AF4