Seznámení s přístroji Wyatt Technology

Štěpán Podzimek

SYNPO Pardubice
Univerzita Pardubice
Waters | Wyatt Technology

About Wyatt Technology

- Founded in 1982 by Dr. Philip J. Wyatt to commercialize multi-angle light scattering (MALS)
- Leading provider of light scattering instruments for solution-based characterization of macromolecules and nanoparticles
- Wyatt instruments have become standard analytical tools in protein, biopharma, drug delivery and polymer labs

Instruments and software have been cited and validated by thousands of peer-reviewed publications

Wyatt Technology is now a part of Waters

One company – One focus!

Cutting edge technologies and scientific expertise to support research, development and delivery of novel therapeutics and next-generation materials.

Wyatt Technology Instruments

Waters™ | WYATT TECHNOLOGY

Solutions for Macromolecular and Nanoparticle Characterization

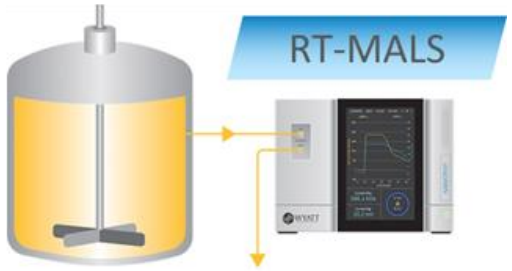
Batch systems | **Chromatography** | **Process**

DLS | HTP-DLS

SEC-MALS | IEX-MALS

AF4-MALS

RT-MALS

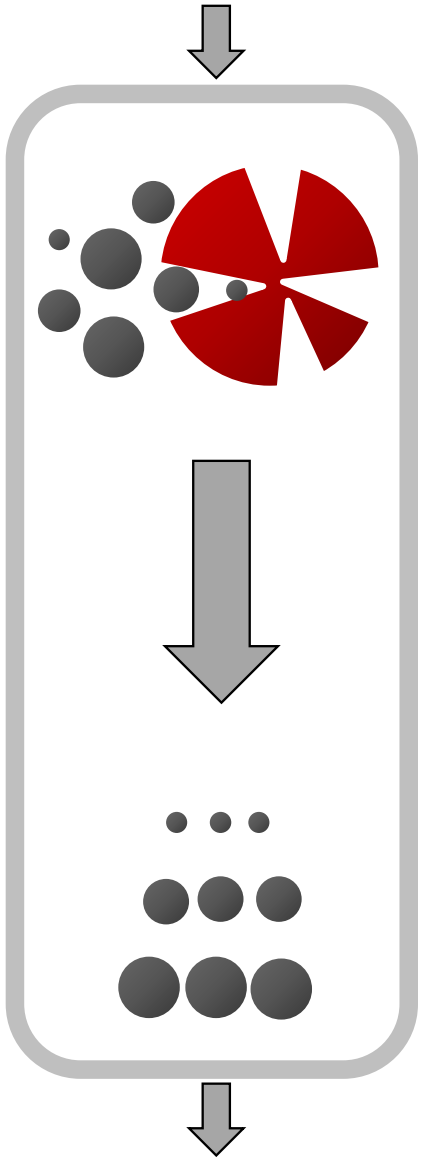


Dynamic Light Scattering (DLS)

DynaPro NanoStar II
DynaPro Plate Reader III

Multi-Angle Light Scattering (SEC MALS, FFF-MALS)

DAWN, miniDAWN, microDAWN, ultraDAWN,
WyattQELS, Optilab dRI, ViscoStar, Eclipse AF4



Detectors for SEC/GPC

Size Exclusion Chromatography (SEC)
Gel Permeation Chromatography (GPC)

Inception of GPC in Synthetic Polymer Science

Waters GPC-200 chromatograph;
SYNPO seventies – eighties.

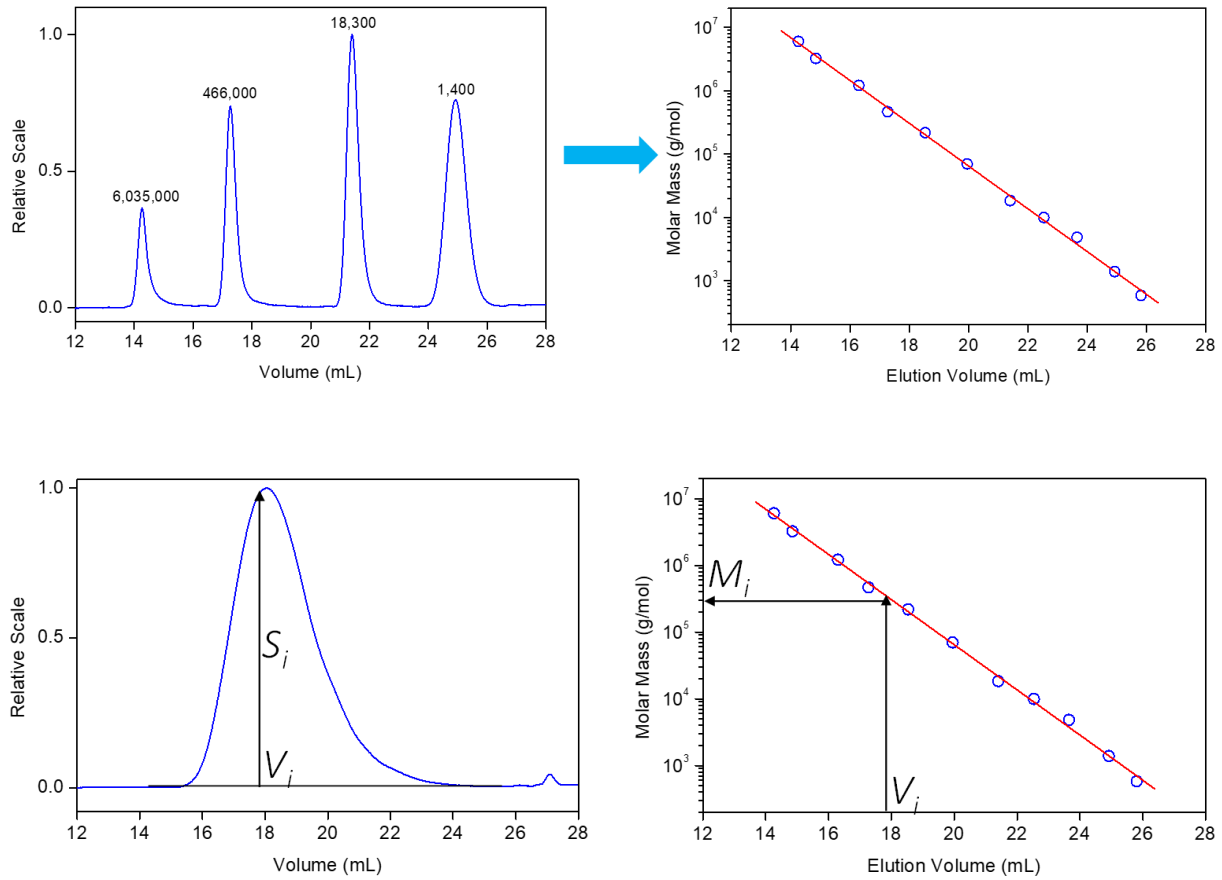
JOURNAL OF POLYMER SCIENCE: PART A VOL. 2, PP. 835–843 (1964)

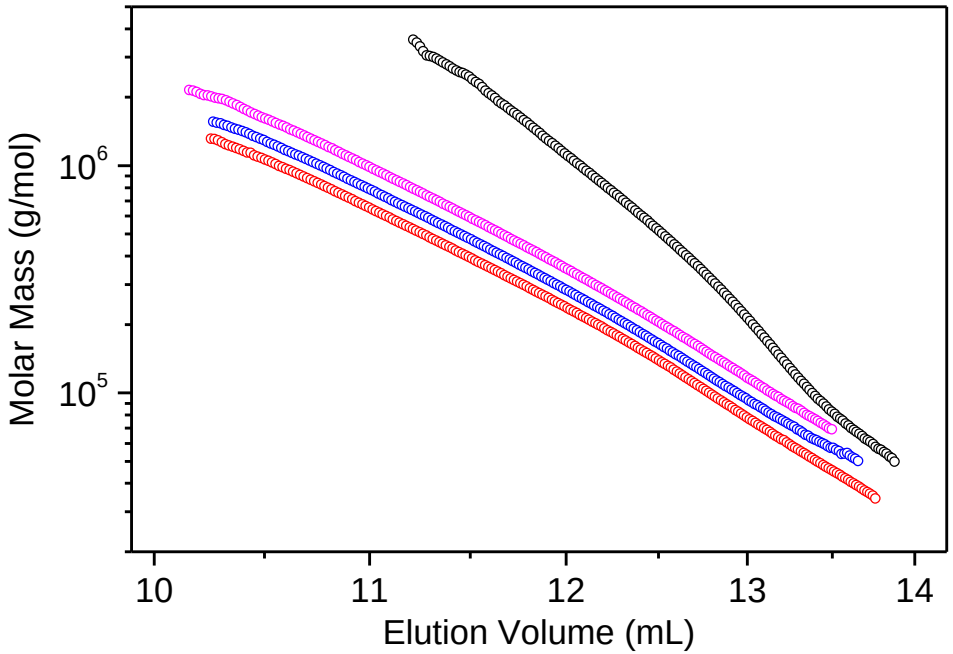
Gel Permeation Chromatography. I. A New Method for Molecular Weight Distribution of High Polymers

J. C. MOORE, *Texas Basic Research Department, The Dow Chemical Company, Freeport, Texas*

Waters 600 pump, 717 autosampler, 410 RI detector;
Wyatt DAWN MALS; SYNPO nineties od last century.

Molar Mass by SEC with Column Calibration





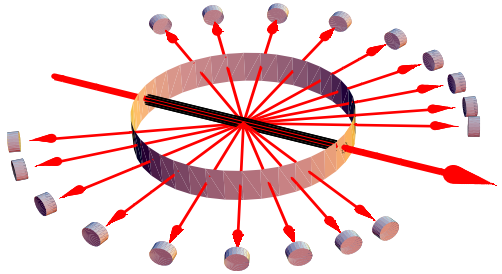
Polystyrene

Poly(butyl methacrylate)

Poly(2-ethyl hexyl methacrylate)

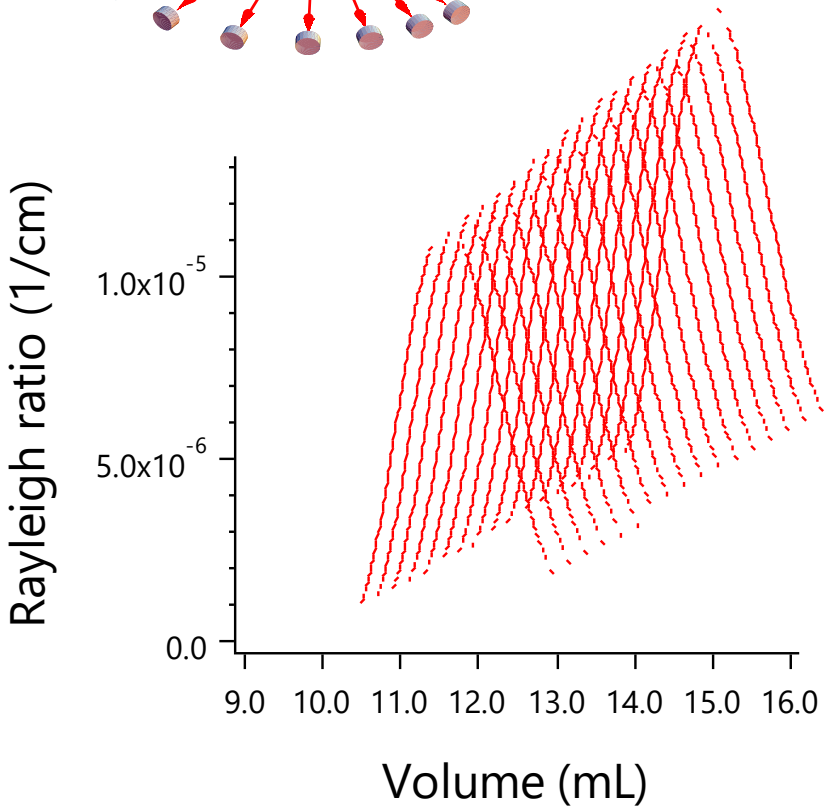
Star-like poly(butyl methacrylate)

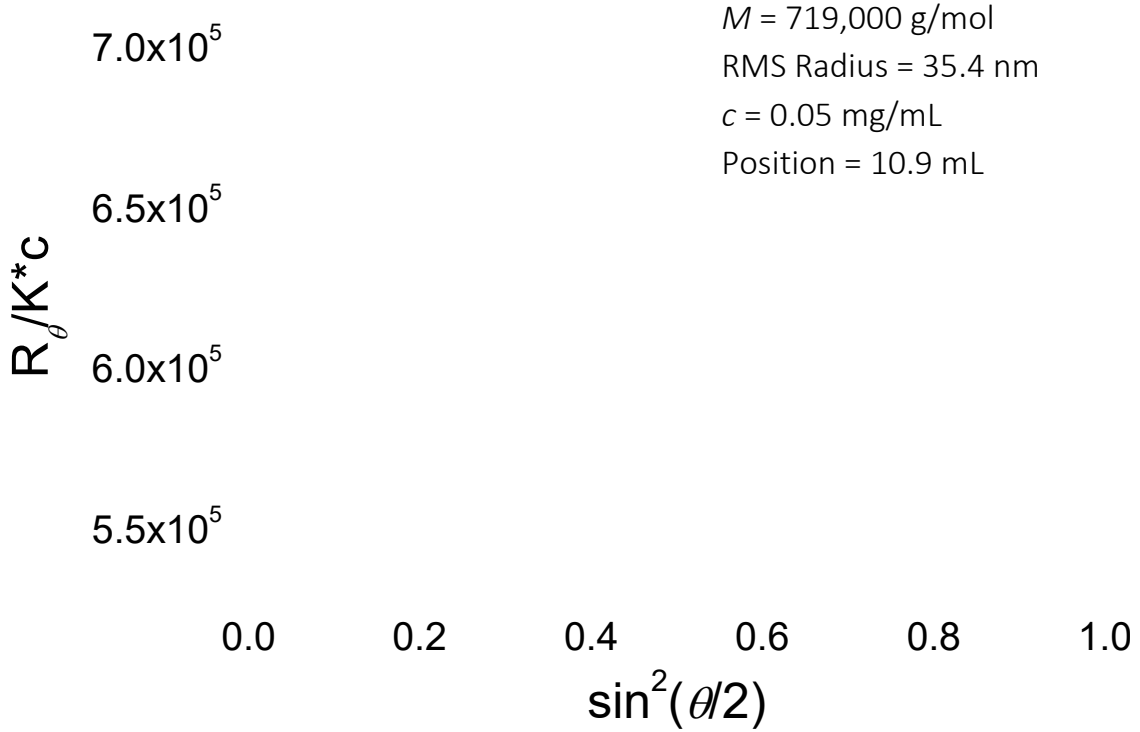
Molar Mass and Size Distribution by SEC-MALS



$$Scattered\ light\ intensity\ at\ zero\ \theta \approx K^{*}cM$$

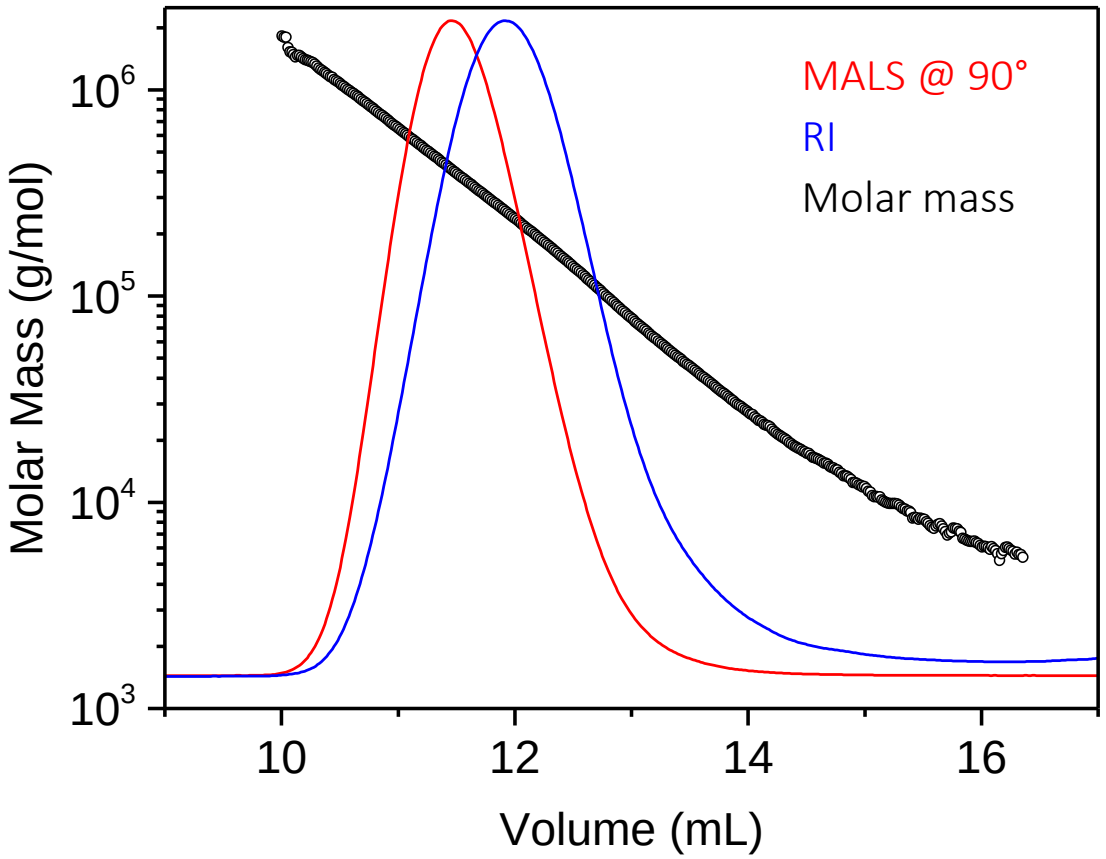
$$Slope\ of\ angular\ variation \approx size$$

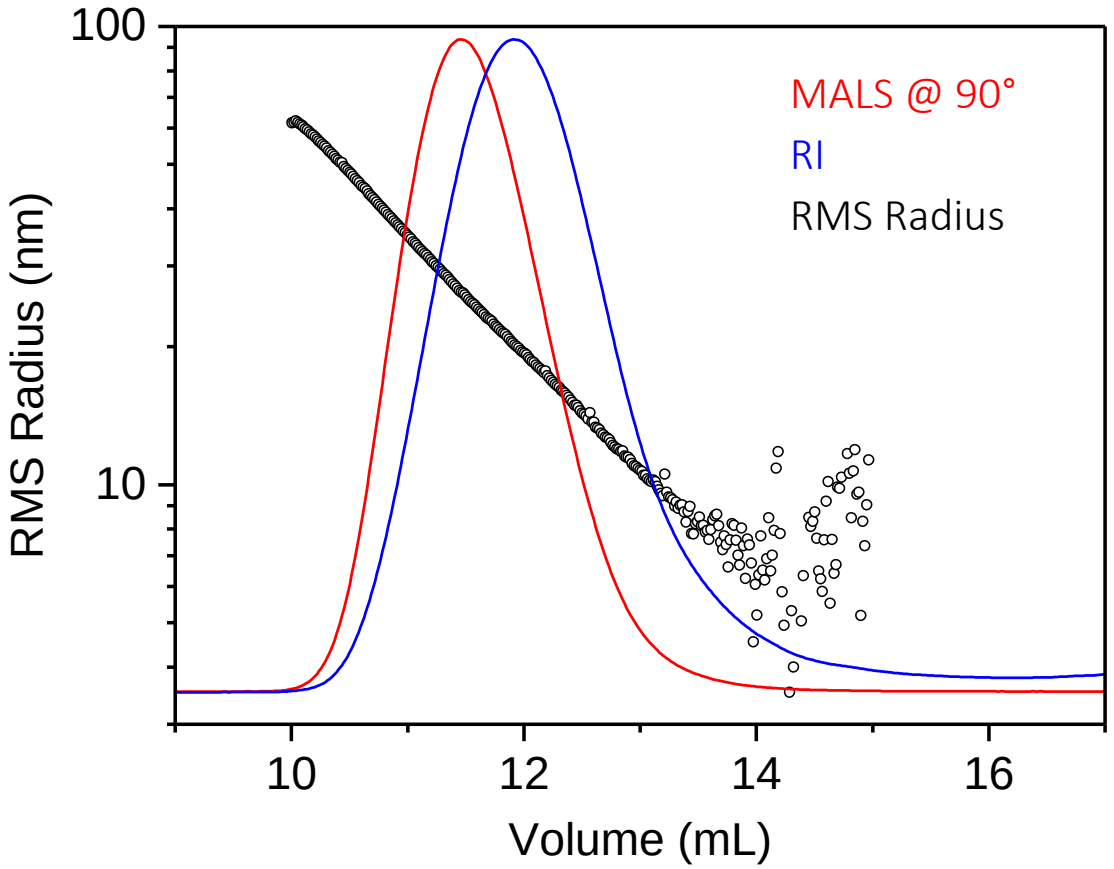




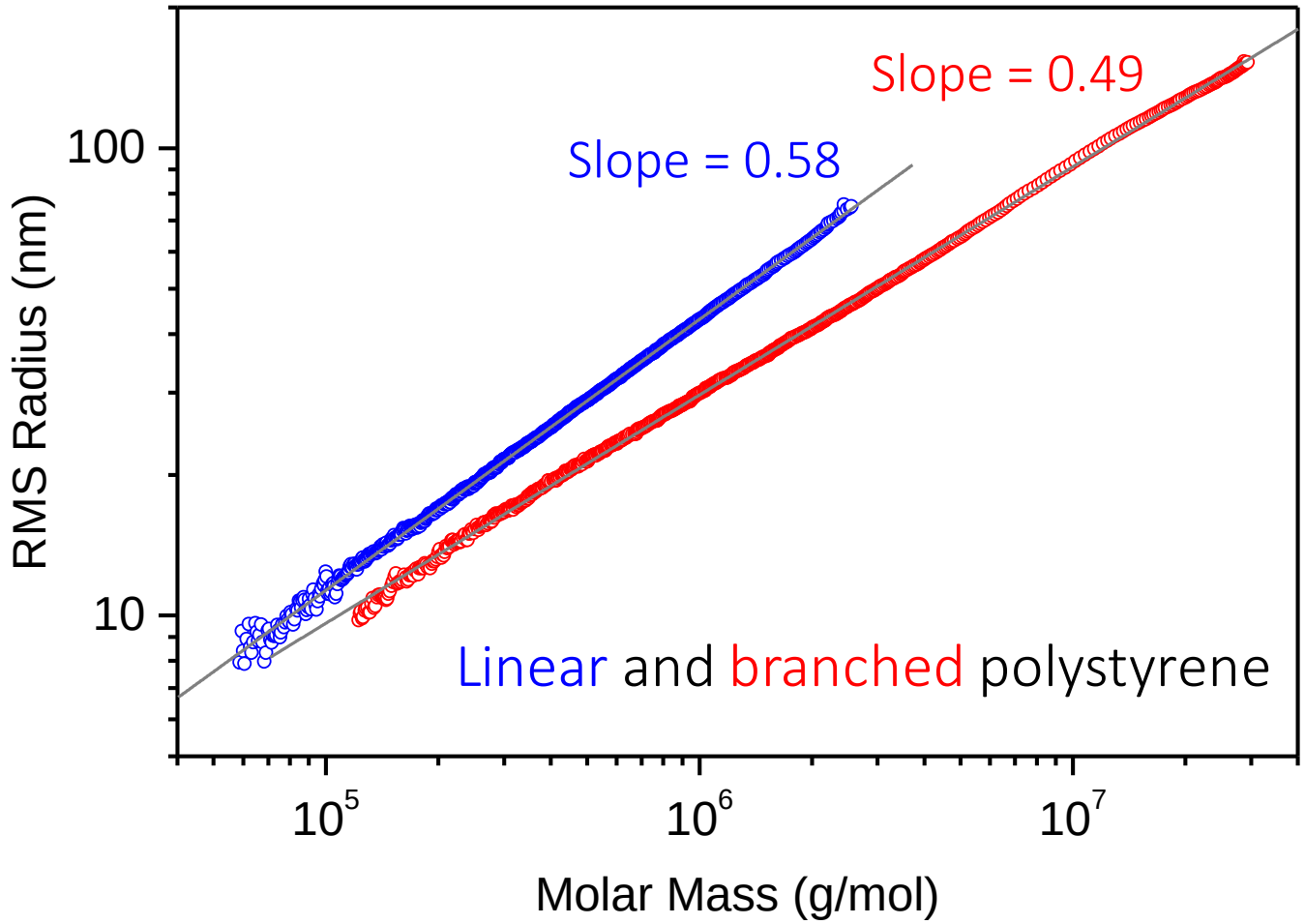
M = 719,000 g/mol
RMS Radius = 35.4 nm
c = 0.05 mg/mL
Position = 10.9 mL

MM and RMS Radius Distribution by SEC-MALS





Structure from SEC-MALS



- Slope
  - Sphere ≈ 0.33
  - Random coil ≈ 0.58
  - Branched polymer ≈ 0.3 – 0.55
  - Extended structure ≈ 0.65 – 1
  - Rod ≈ 1
- Branching calculation
  - Number of branch units
  - Number of arms in stars

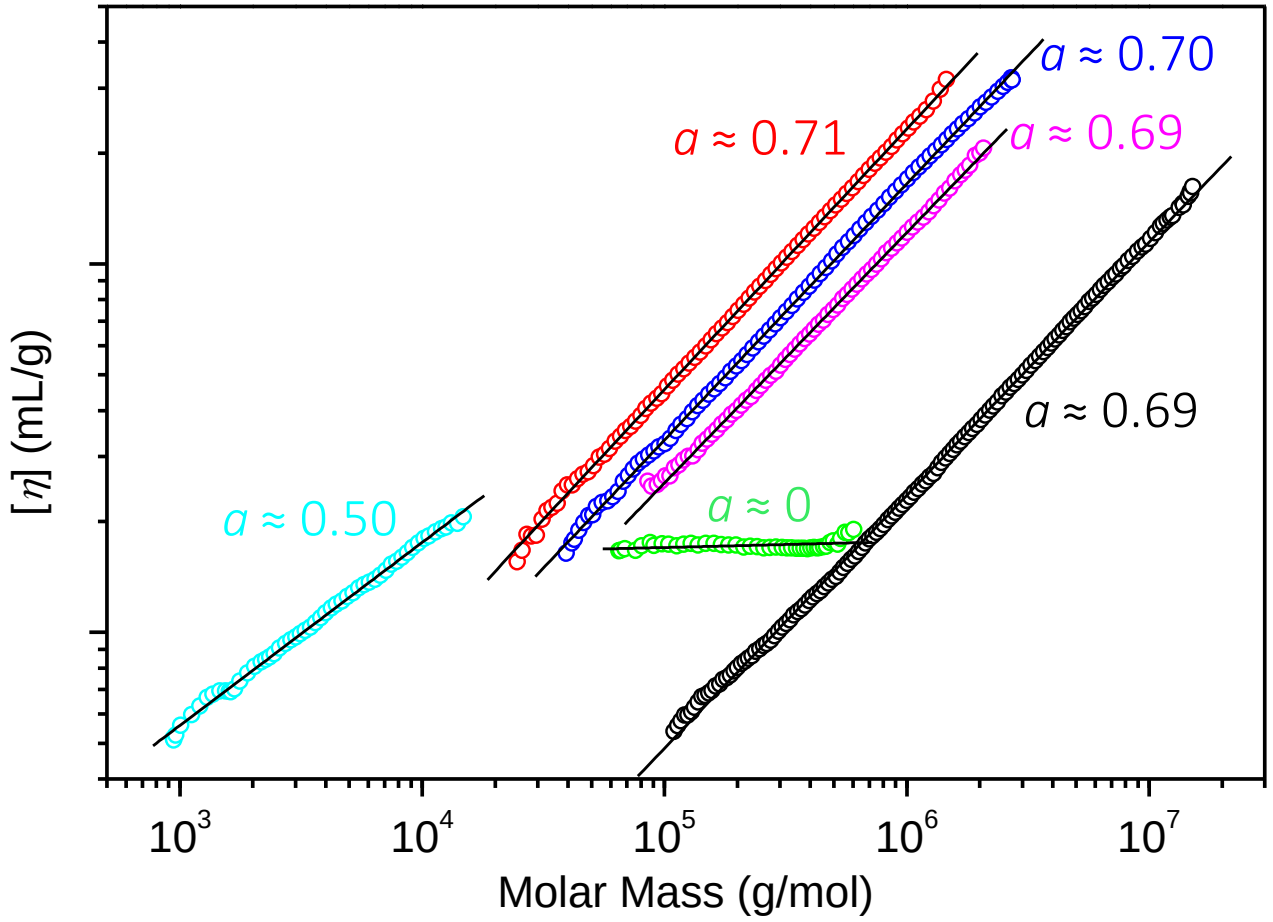
Wyatt Detectors for SEC/GPC

← Multi-angle light scattering detector (MALS)
DAWN; molar mass, RMS radius (radius of gyration)

← Online viscometer ViscoStar; intrinsic viscosity, hydrodynamic radius, radius of gyration, …

← Refractive index detector Optilab; concentration

Detailed Molecular Structure by SEC-MALS-Visco



Slope

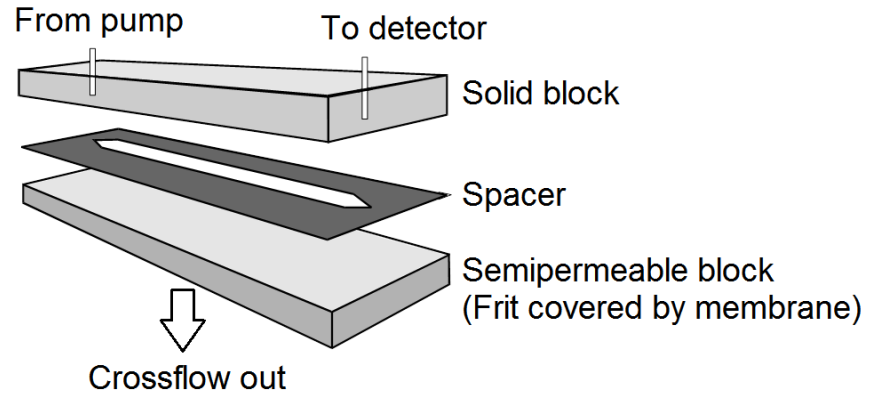
- Sphere ≈ 0
- Random coil ≈ 0.7
- Oligomer ≈ 0.5
- Rod ≈ 2
- Branched polymer ≈ 0 – 0.6
- Extended structure ≈ 0.8 – 1.5

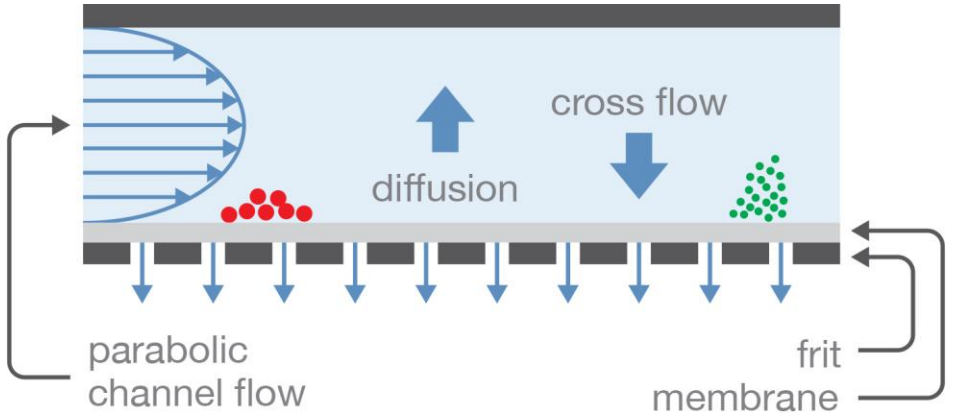
Epoxy resin, linear polystyrene, linear poly(methyl methacrylate), linear poly(benzyl methacrylate), linear poly(iBuPOSSMA), star-branched poly(isobutyl methacrylate) in THF.

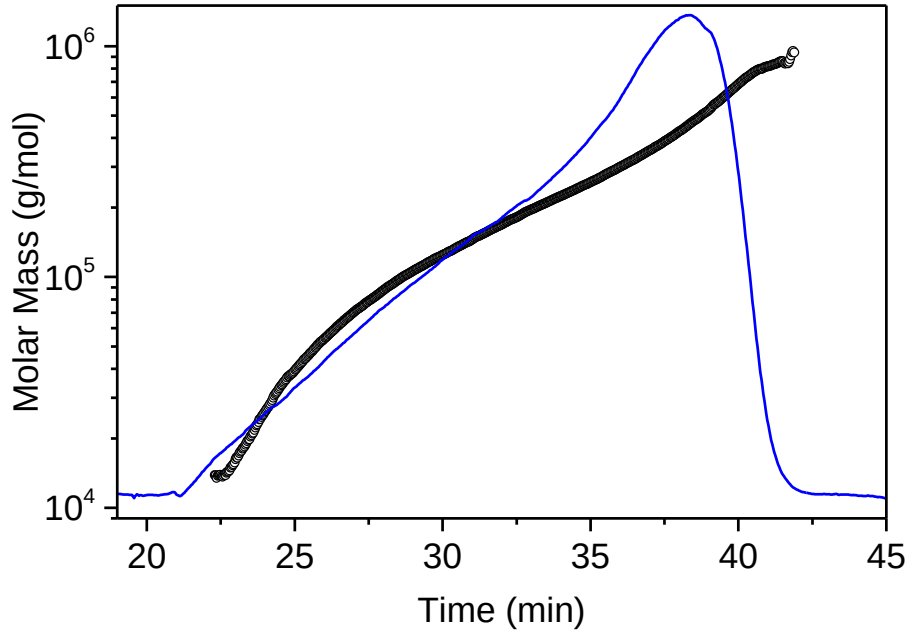
Asymmetric Flow Field Flow Fractionation (AF4)

Alternative Separation Technique to SEC/GPC

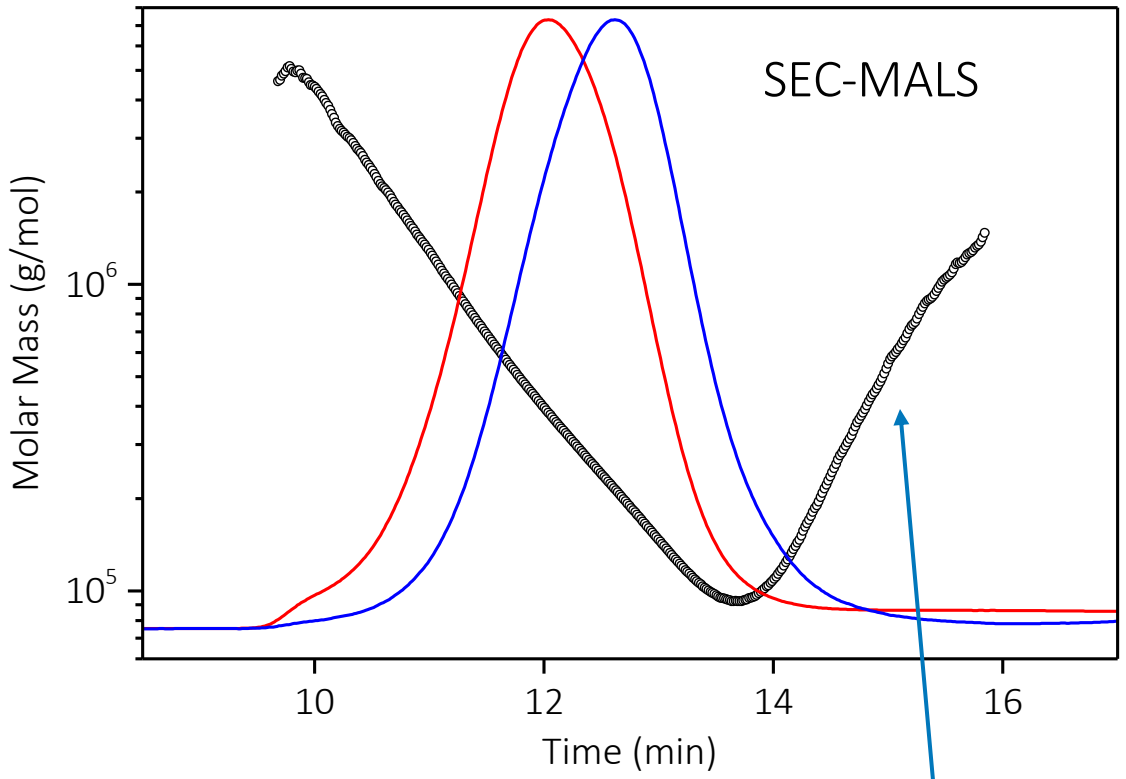
Asymmetric Flow Field Flow Fractionation (AF4/FFF)

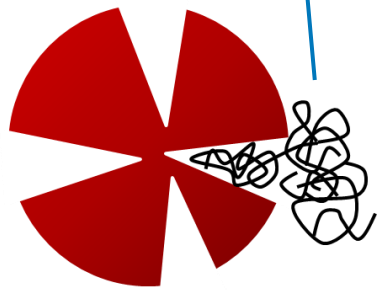


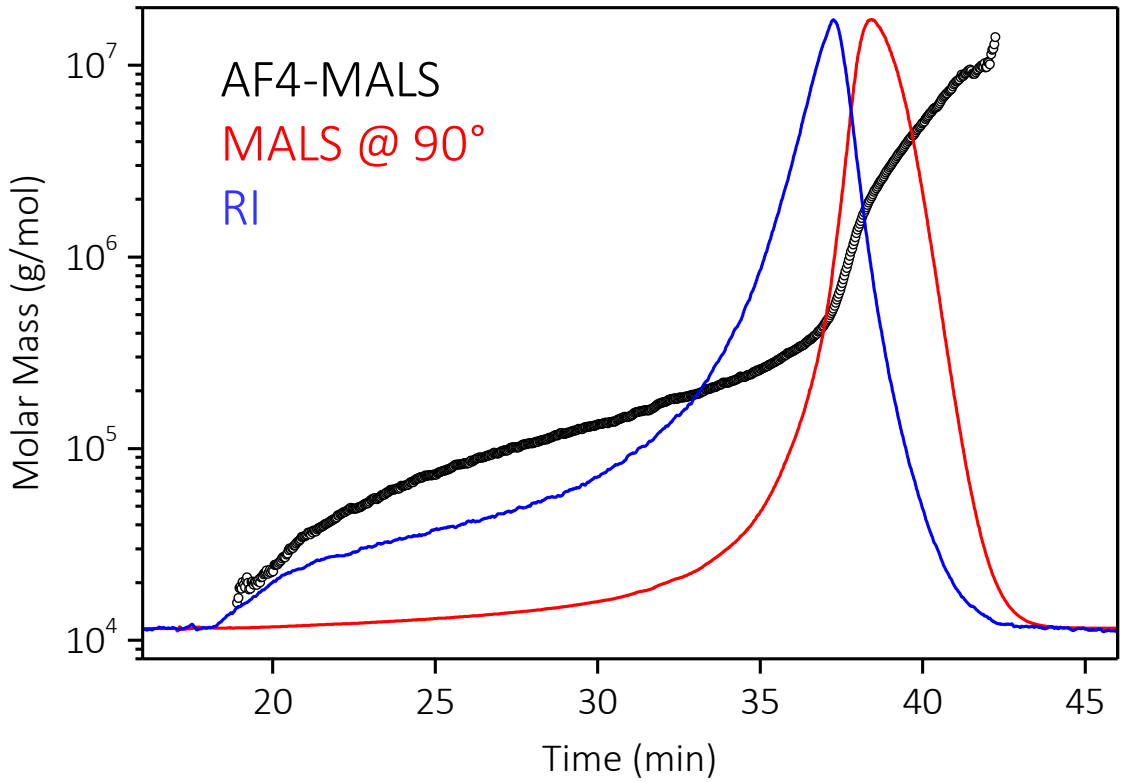




AF4 Solving Anchoring and Shear Degradation

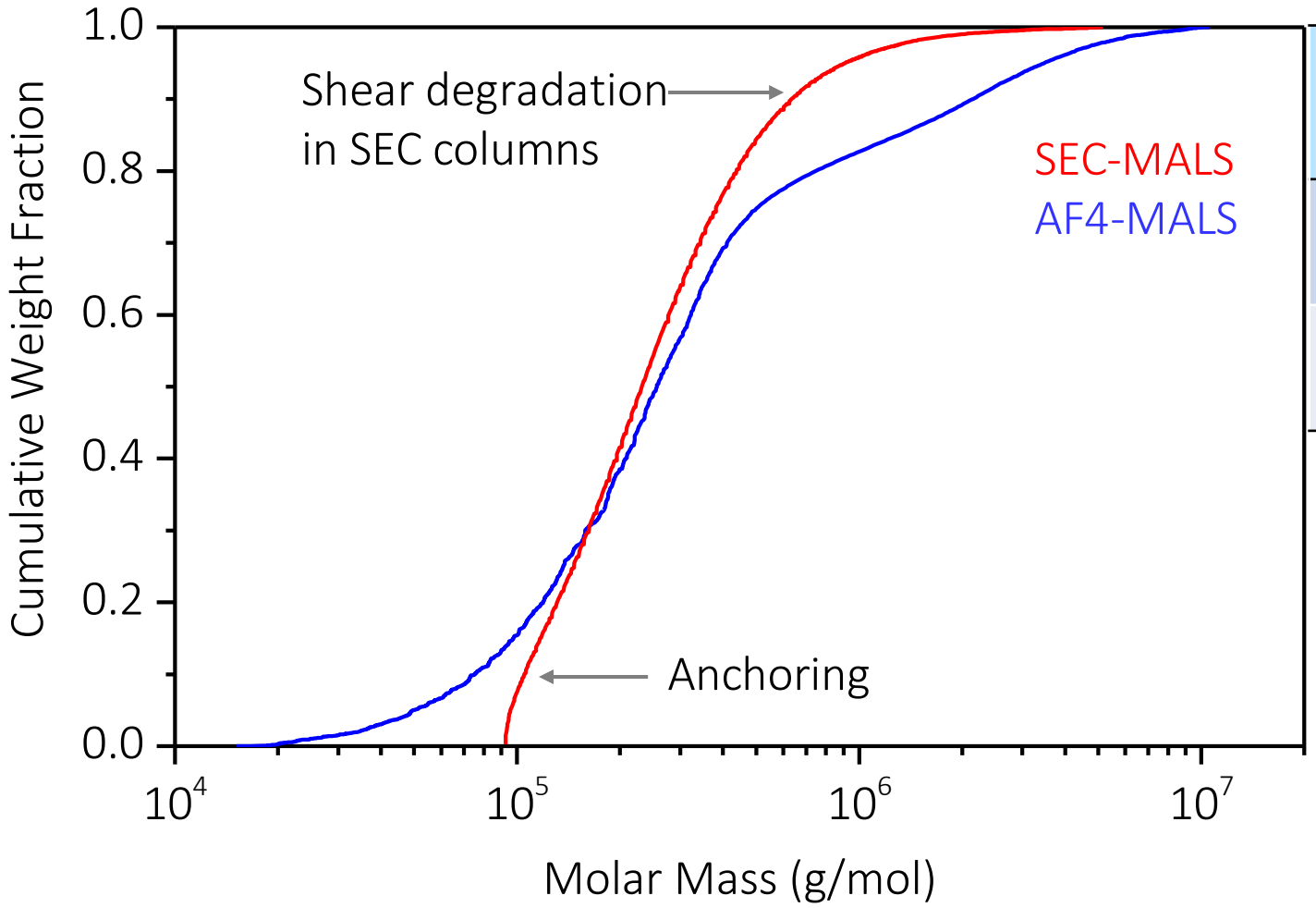






S. Podzimek, T. Vlcek, C. Johann: J. Appl. Polym. Sci. 81, 1588 (2001)

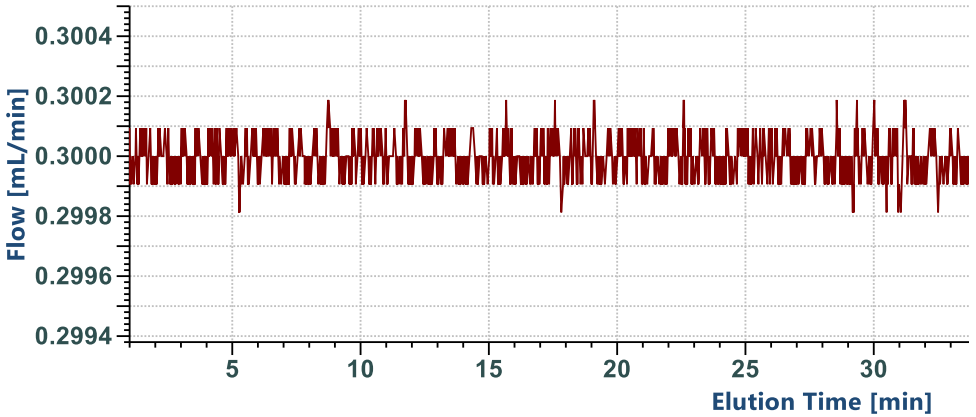
MMD: SEC-MALS versus AF4-MALS

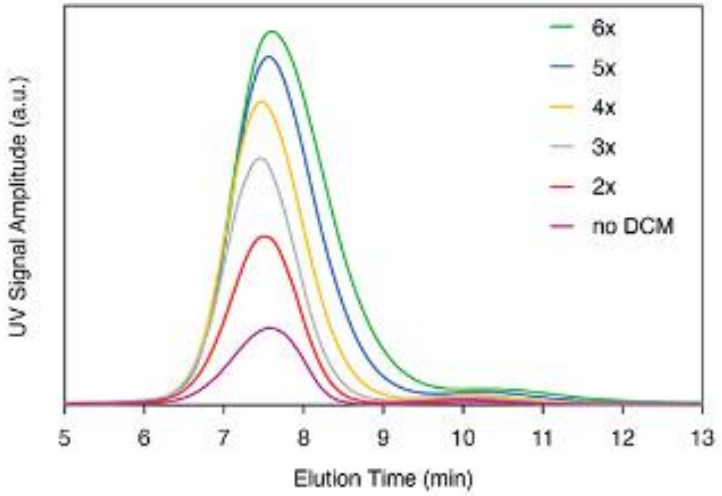


M_n (10^3 g/mol)	M_w (10^3 g/mol)	M_z (10^3 g/mol)
210	340	790
170	730	3080

Eclipse – Wyatt Instrument for AF4

- Dilution control module (DCM)
  - Major technology advancement in AF4
  - Increases the detector signal without loading more sample
  - Splitting away the upper fraction of the channel flow and thus concentrating the sample layer
- Precise control of flows

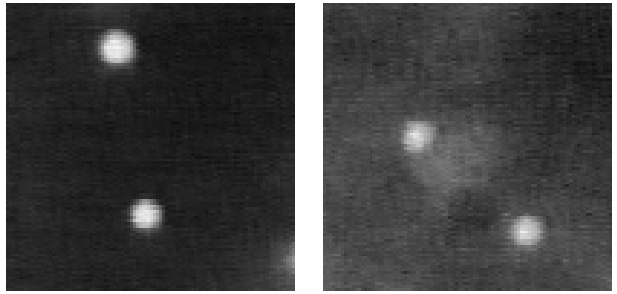


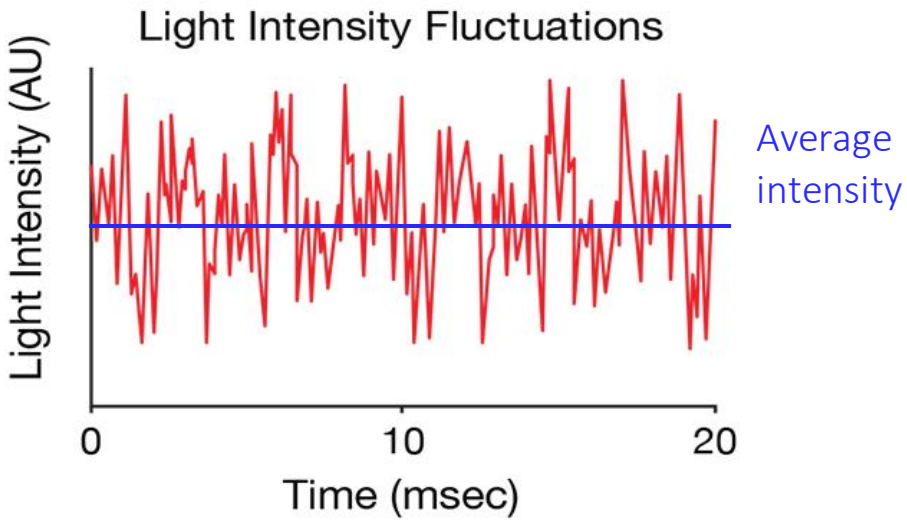


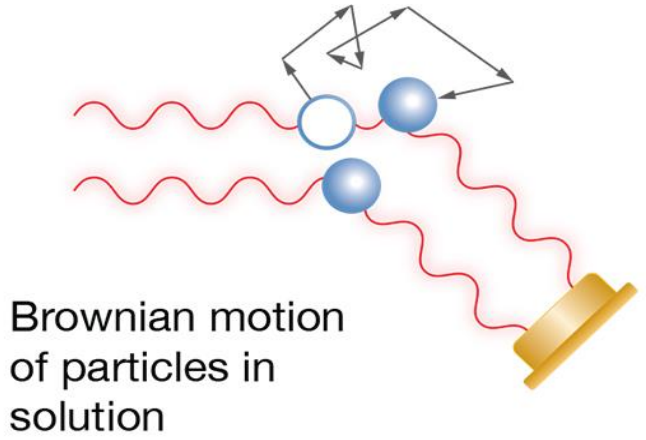
Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS)

- Measurement of the scattered light intensity oscillation
- Analytical technique for the determination of
  - Diffusion coefficient
  - Hydrodynamic radius

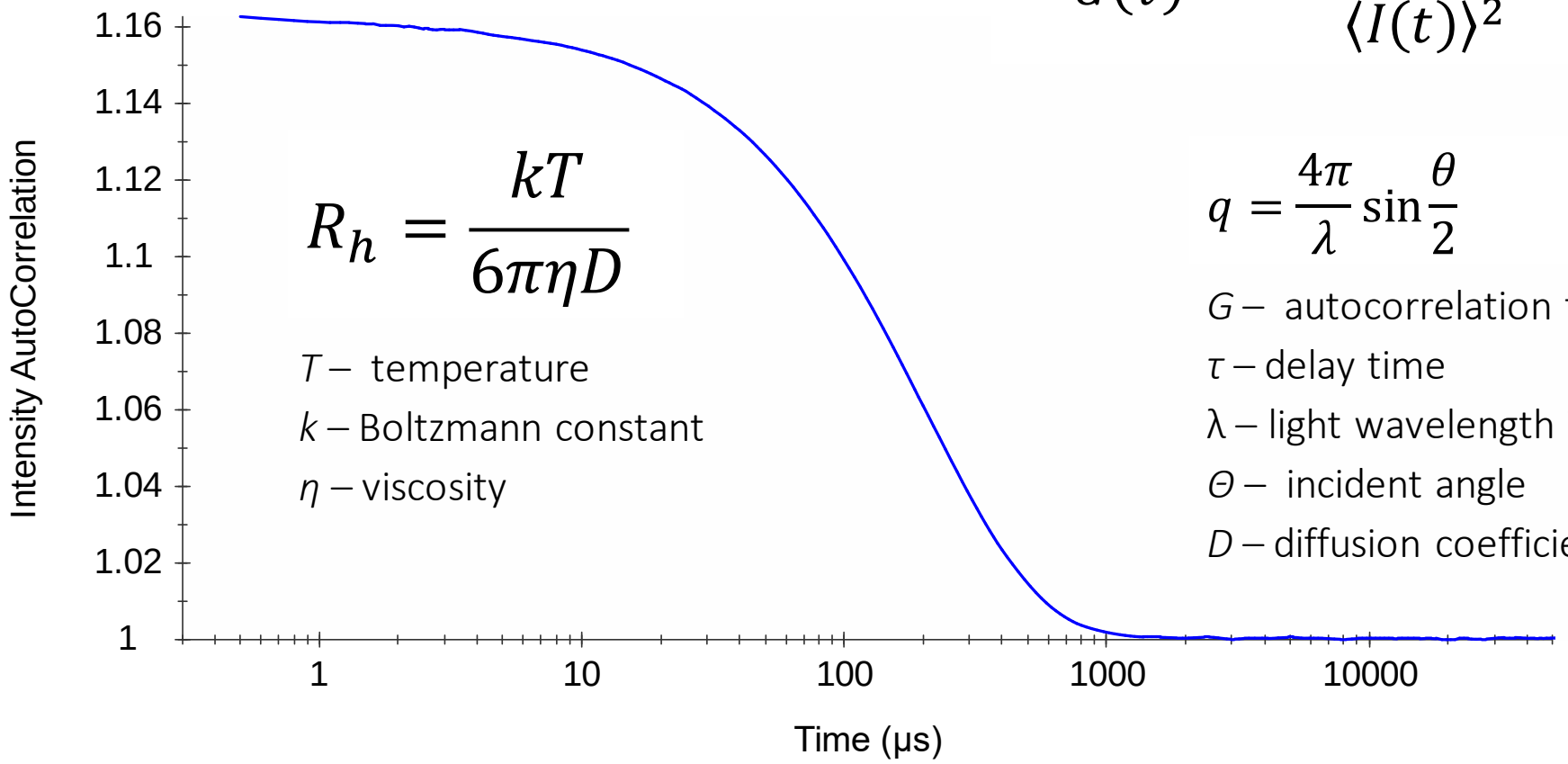






Dynamic Light Scattering (DLS)

$$G(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2} = 1 + \alpha e^{-2Dq^2\tau}$$



$$R_h = \frac{kT}{6\pi\eta D}$$

T – temperature

k – Boltzmann constant

η – viscosity

$$q = \frac{4\pi}{\lambda}\sin\frac{\theta}{2}$$

G – autocorrelation function

τ – delay time

λ – light wavelength in solvent

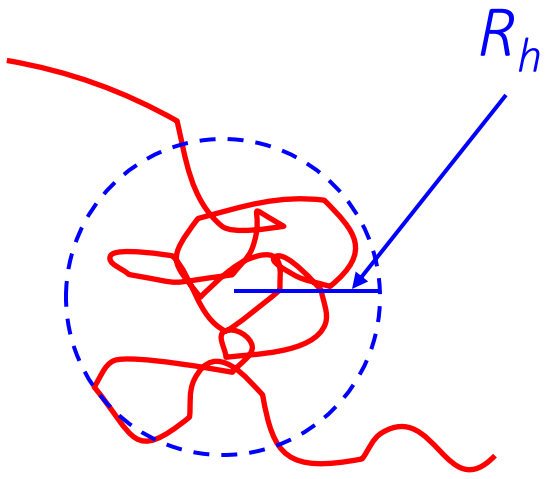
Θ – incident angle

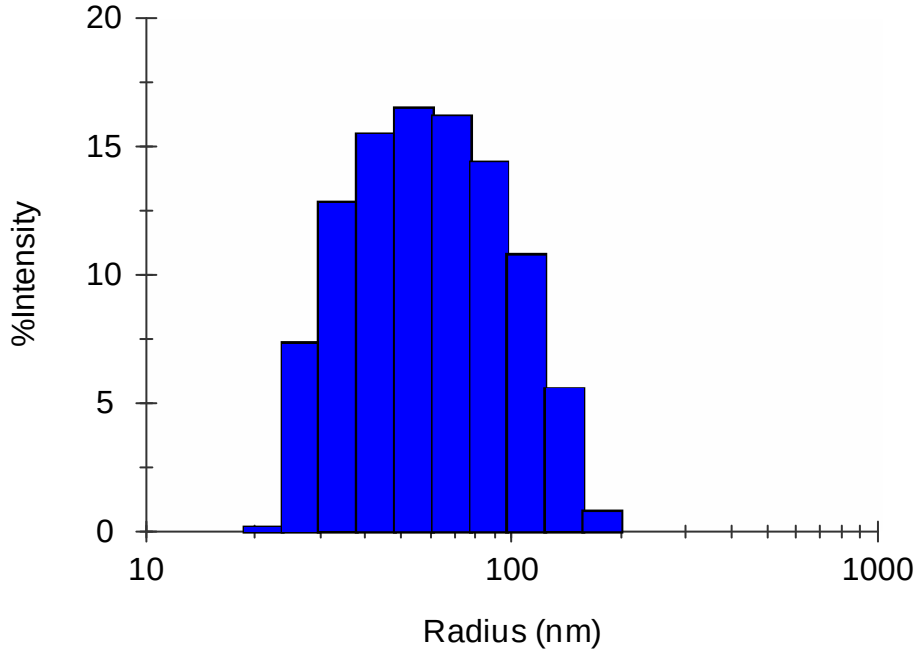
D – diffusion coefficient

Hydrodynamic Radius R_h

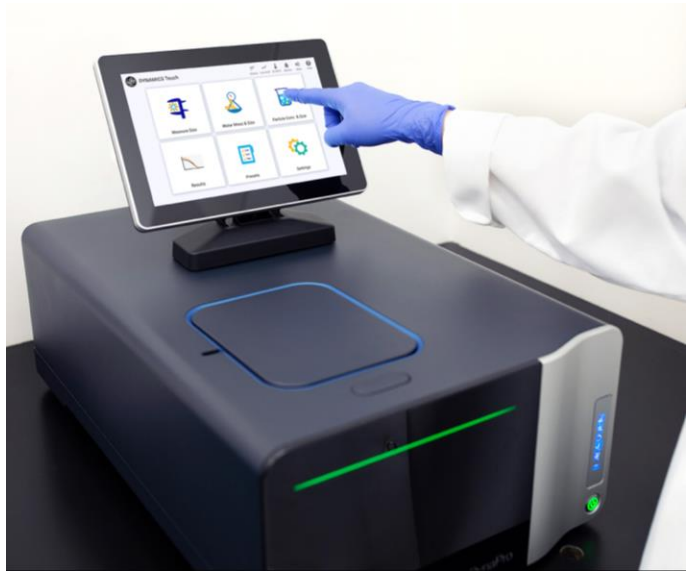
- Hydrodynamic radius is radius of sphere that would have the same diffusion coefficient as the molecules or particles under investigation.
- Hydrodynamic radius is not directly measured, but calculated via Stokes-Einstein equation.

$$R_h = \frac{kT}{6\pi\eta D}$$





DLS Instruments: Batch or Online



DLS instrument NanoStar

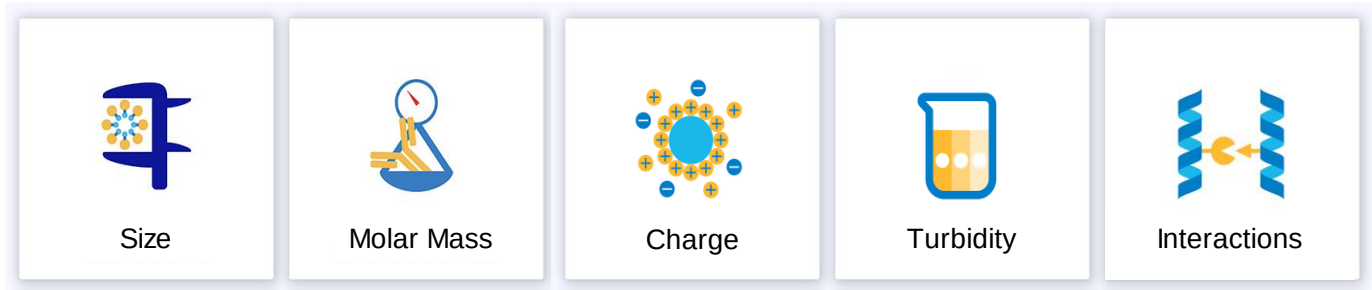
PlateReader

Online in DAWN

ZetaStar with DYNAMICS Touch

Quick and easy measurement just from a few µL

- Size *(R_h)* and size distribution
- Zeta-potential
- Concentration
- Molar mass
- Stability
- Turbidity



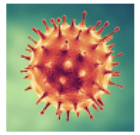
Wyatt Instruments: Overview

- Multi-angle light scattering (MALS): DAWN, miniDAWN
  - Online DLS optional
  - Usually coupled with SEC or AF4, concentration measured by RI detector Optilab
  - Molar mass, radius of gyration, molecular architecture (conformation and branching)
  - For detailed structural studies MALS can be completed with online viscometer ViscoStar
- Separation: Eclipse
  - Asymmetric flow field flow fractionation
  - Electrical AF4
- Dynamic Light Scattering (DLS): NanoStar, PlateReader, ZetaStar
  - Translational diffusion coefficient
  - Hydrodynamic Radius via Stokes-Einstein equation
  - Batch or online
  - Possible combination with the measurement of charge

Wyatt Instrument´ Applications: wyatt.com

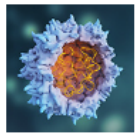
Wyatt Instrument´ Applications

Vaccines



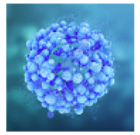
Wyatt's suite of solutions are used across vaccine discovery, development, production and quality control and analyze critical vaccine attributes, including: molar mass and size, viral physical titer and nucleic acid content, aggregation, and thermal and colloidal stability.

Gene Therapy



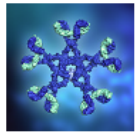
Dynamic light scattering (DLS) and multi-angle light scattering (MALS) coupled to separation technologies (SEC-MALS, FFF-MALS) reveal molar mass and size, aggregation, physical titer, empty:full ratio and stability of gene vectors.

Biotherapeutics



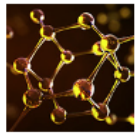
Light scattering technologies assist at each stage of the biotherapeutic R&D pipeline, with uniquely versatile technologies for biophysical screening and characterization, from target and candidate discovery to selection, optimization, purification, and formulation.

Proteins



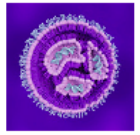
Proteins conjugates, aggregation, colloidal stability and protein-protein interactions can be analyzed with MALS, high-throughput Dynamic Light Scattering (DLS), and Composition-Gradient, Multi-Angle static Light Scattering (CG-MALS).

Polymers



A DAWN® MALS detector and Optilab® dRI detector work together to determine distributions of absolute molar mass and size of natural or synthetic polymers *independently of column calibration*.

Nanoparticles and Lipid Nanoparticles



Size, composition, mass and zeta potential of nanoparticles and lipid nanoparticles can be analyzed via MALS, Dynamic Light Scattering (DLS), Field-Flow Fractionation (FFF) and Electrophoretic Light Scattering (ELS).

Light Scattering University Training

- Light Scattering University (LSU)
  - MALS, DLS, FFF and Viscosity courses
  - Included with instruments purchase (2 credits/instrument)
  - Combined basic training, application support, and one-to-one discussions
  - Meets the needs of both novices and advanced users
  - Get to the Wyatt team and other users

Phone and E-Mail Support

support@wyatt.eu
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Support

- Hardware
- Software
- Applications


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