

Polymers in Solution

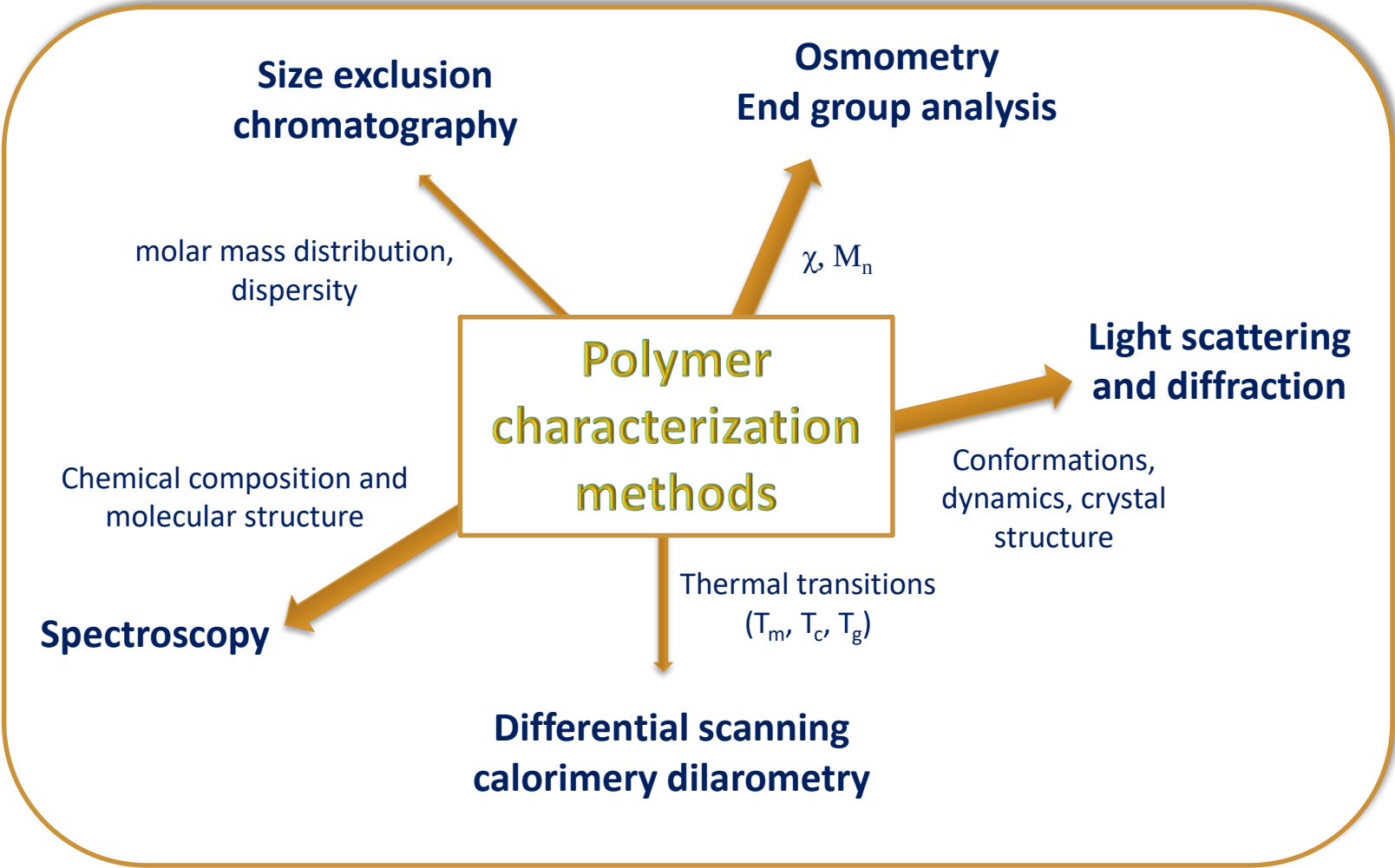
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5. Molar mass determination

1. Overview



1. Overview

Molar Mass in Polymers

Number average molar weight

$$M_n = \frac{\sum_i c_i}{\sum_i (c_i/M_i)} = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

Weight average molar weight

$$M_w = \frac{\sum_i (c_i M_i)}{\sum_i c_i}$$

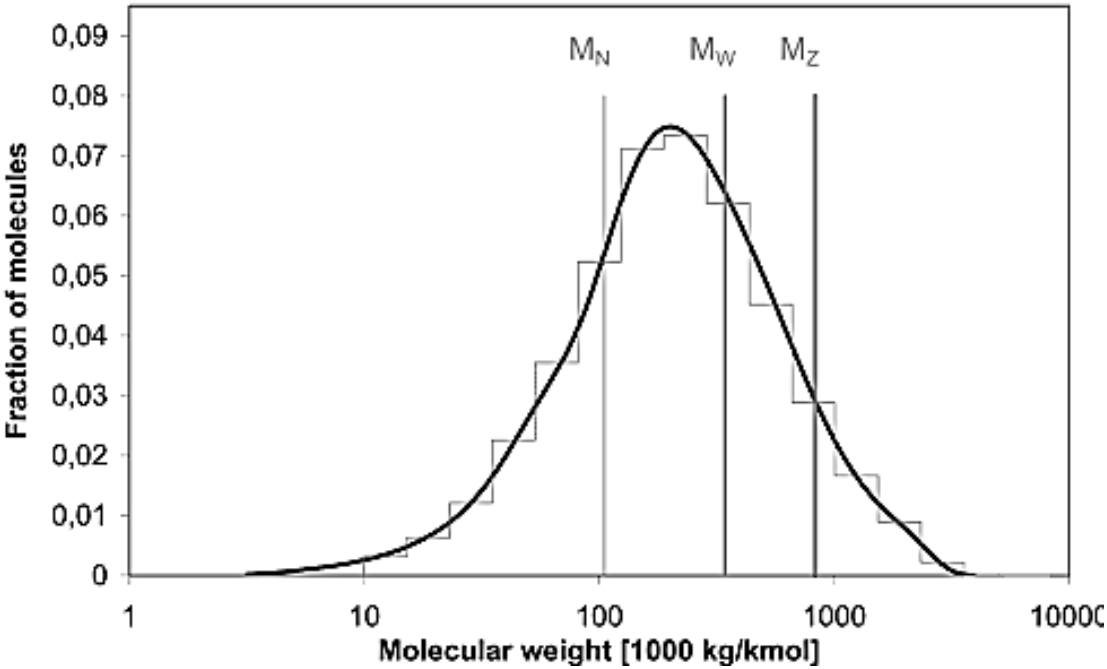
z-average molecular weight

$$M_z = \frac{\sum_i (c_i M_i^2)}{\sum_i (c_i M_i)} = \frac{\sum_i z_i M_i}{\sum_i z_i}$$

MMD (polydispersity) is with M_w/M_n

For monodisperse samples $M_w/M_n = 1$

Polydisperse polymers have $M_w/M_n > 1$



2. Methods for determining the molar mass of macromolecules

Method	Molar mass average	Molar mass range
Absolut method		
Ebulliometry, cryoscopy	M_n	$M < 5 \times 10^3$
Membrane osmometry	M_n	$10^4 < M < 10^6$
Isothermal distillation	M_n	$M > 5 \times 10^4$
Sedimentation velocity	M_n, M_w, M_z	$M > 10^2$
Equilibrium sedimentation	M_w, M_z	$M > 10^2$
Vapor pressure osmosis	M_n	$M < 2 \times 10^4$
Static Light Scattering	M_w	$M > 5 \times 10^2$
Turbidity measurements	M_w	$M > 5 \times 10^2$
Small-angle X-ray scattering	M_w	$M > 5 \times 10^2$
Small-angle neutron scattering	M_w	$M > 5 \times 10^2$
Dynamic Light Scattering	M_w	$M > 5 \times 10^2$
Mass spectroscopy- MALDI-TOF	M_n, M_w, M_z	$M > 5 \times 10$
Equivalent method		
End-group analysis- (titration, NMR, IR)	M_n	$M < 5 \times 10^4$
Relative method		
Dilute solution viscometry	M_n	$M > 10^2$
Gel Permeation chromatography	M_n, M_w, M_z	$M < 10^7$
Supercritical fluid chromatography	M_n, M_w, M_z	$M < 10^7$
Field-flow fractionation	M_n, M_w, M_z	$M > 10^3$

3. Colligative properties and M_n

Colligative properties of solutions are properties that depend upon the concentration of solute molecules or ions, but not upon the identity of the solute. **Colligative properties include vapor pressure lowering, boiling point elevation, freezing point depression, and osmotic pressure.**

Cryoscopy

$$\left(\frac{\Delta T_f}{C}\right)_{C=0} = \frac{RT^2}{\rho \Delta H_f \overline{M}_n} + A_2 C$$

Ebulliometry

$$\left(\frac{\Delta T_b}{C}\right)_{C=0} = \frac{RT^2}{\rho \Delta H_v \overline{M}_n} + A_2 C$$

ΔT_f : freezing-point depression,

C : the concentration in grams per cubic centimeter

R : gas constant

T : freezing point

ΔH_f : the latent heats of fusion

A_2 : second virial coefficient

ΔT_b : boiling point elevation

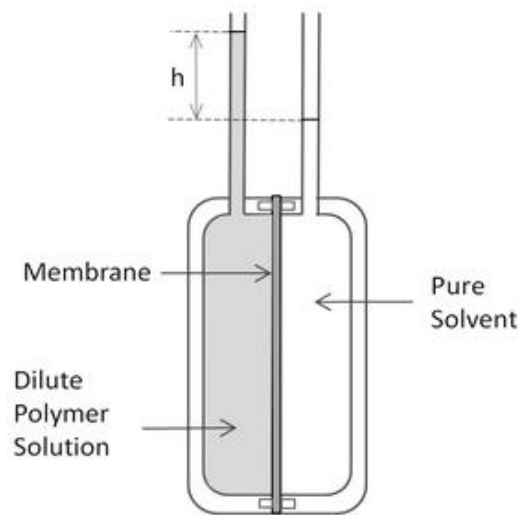
ΔH_v : the latent heats of vaporization

M_n for low molar mass (≤ 10000 g/mol)

3. Colligative properties and M_n

Membrane osmometry METHODS BASED ON COLLIGATIVE PROPERTIES

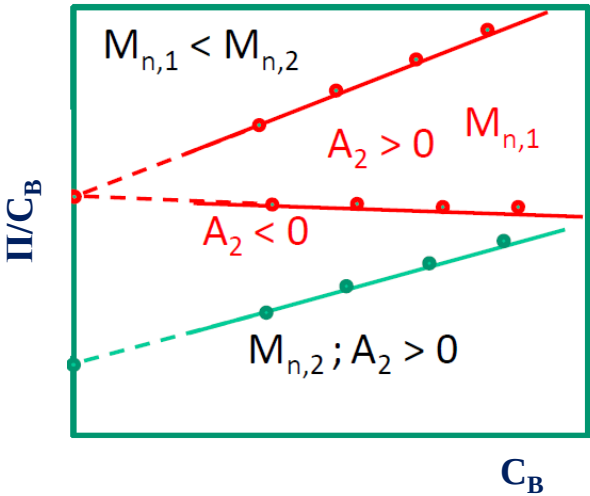
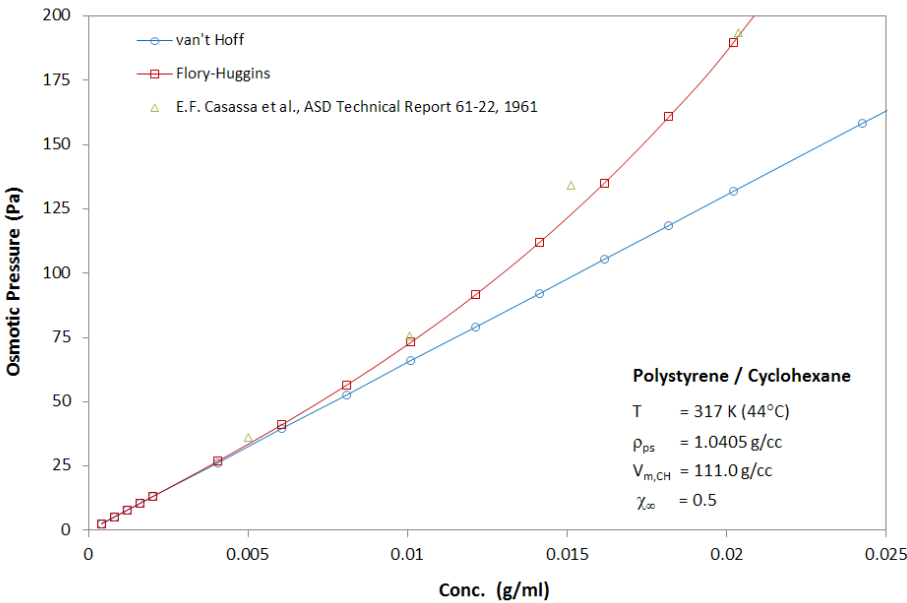
SIMPLE MEMBRANE OSMOMETER



$10^4 < M_n < 10^6$

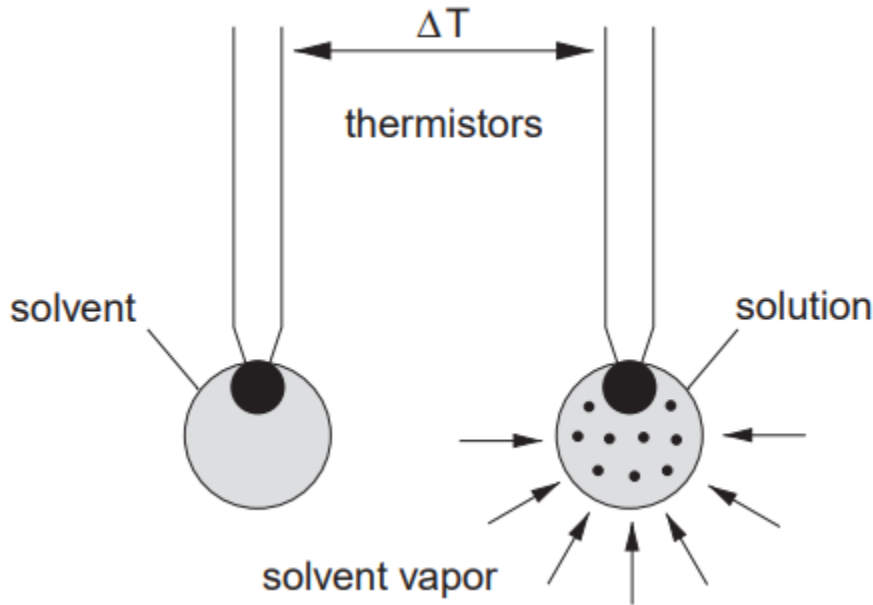
$$\frac{\pi}{c_B} = RT \left(\frac{1}{M_n} + A_2 c_B \right)$$

Determination of M_n
(interception) and A_2 (slope)



3. Colligative properties and M_n

Vapour pressure osmometry METHODS BASED ON COLLIGATIVE PROPERTIES



$$\frac{p_0 - p}{p_0} = V_s^m \frac{c}{\langle M \rangle_n}$$

$$\frac{\Delta p}{p} = \frac{\Delta T}{T} \frac{\Delta H_{\text{vap}}}{RT} = x_p = V_s^m \frac{c}{\langle M \rangle_n}$$

M_n for low molar mass ($\leq 20000 \text{ g/mol}$)

4. Equivalent method

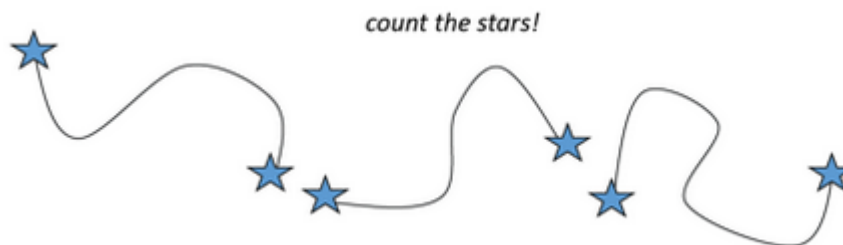
End group analysis

$$M_n \leq 50000 \text{ g/mol}$$

- Molecular weight limitation up to 50000 g/mol (the concentration of end groups has to be sufficient to get an accurate measurement)
- You have to know how many end groups there are per molecule (to find molar mass), OR you know the molar mass, and want to know number of end groups per molecule. **Limitation for branched polymers**

- End-group must have detectable species

- a. Vinyl polymer: $-\text{CH}=\text{CH}_2$
- b. Ester Polymer: $-\text{COOH}$, $-\text{OH}$
- c. Amide and urethane polymer: $-\text{NH}_2$, $-\text{NCO}$
- d. Radioactive isotopes or UV, IR, NMR detectable functional groups



4. Equivalent method

How to Determine Hyaluronic Acid Molecular Weight Using Gel Electrophoresis

Hyaluronic Acid, is a natural non-sulfated glycosaminoglycan produced in many organs and tissues. It was discovered in the 1930s and was originally thought to have no physiological function except serving as a lubrication “space filler” between joints. With additional research, hyaluronic acid is now appreciated as an important part of the extracellular matrix. Indeed, it has critical roles in many cell signaling events such as proliferation, inflammation, wound healing, and fertilization.

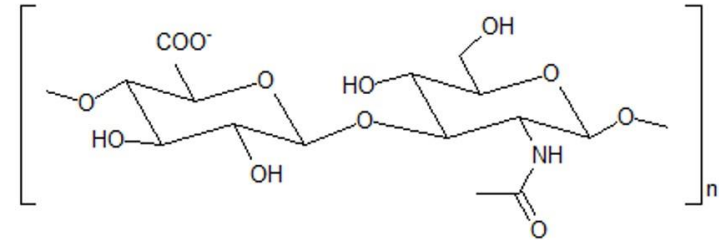


Figure 1. Repeating disaccharide units of Hyaluronic Acid

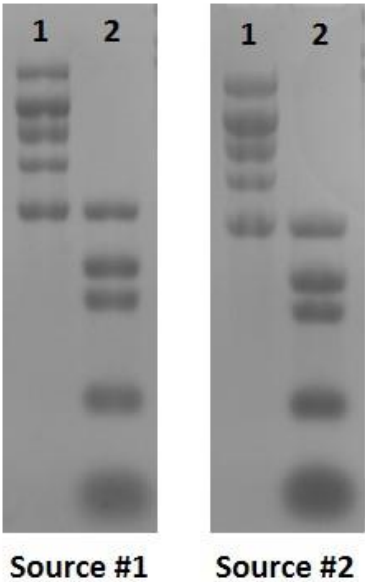


Figure 2. Source of Agarose

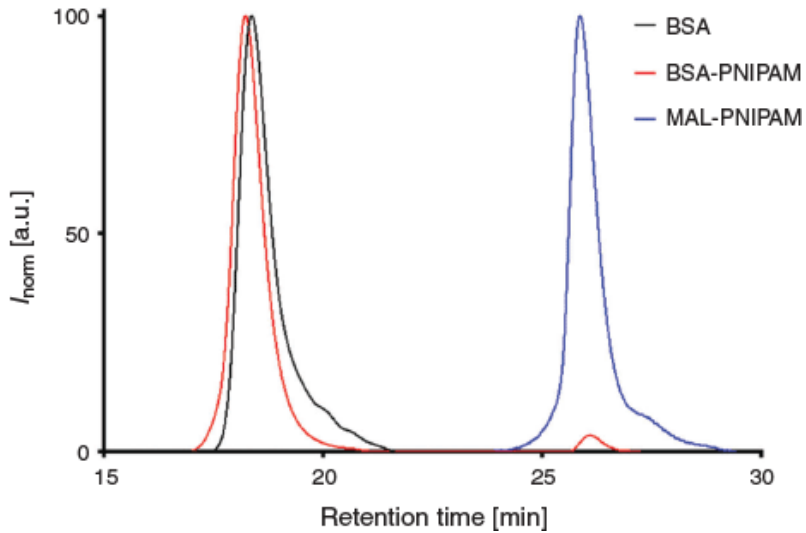
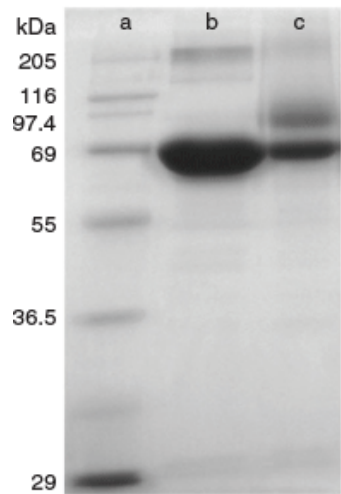
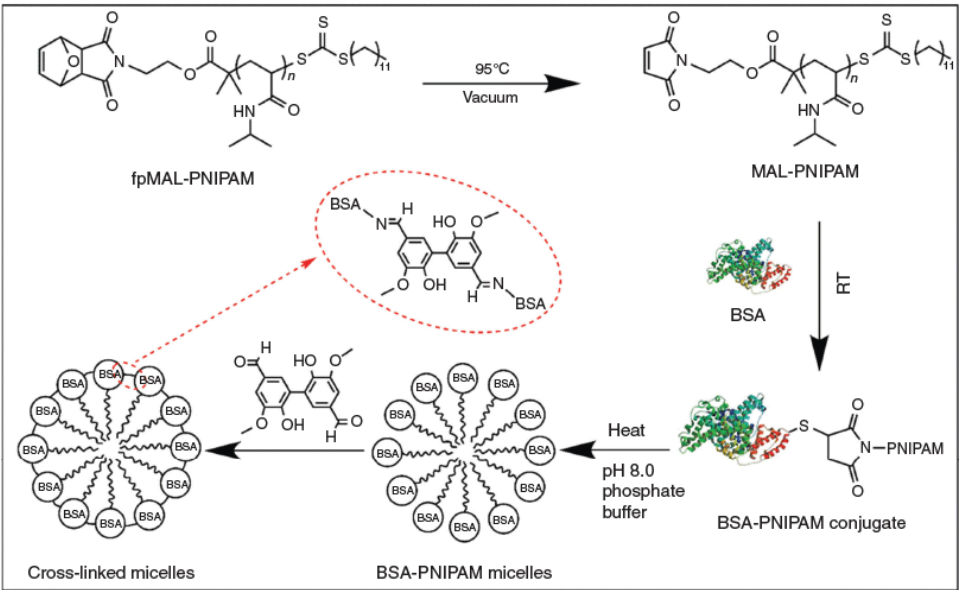
Select-HA™ Ladders were run on 1% agarose gel prepared using 2 different agarose source. Both agarose gels were run using same conditions.

Agarose gel prepared using Source #1 resulted with more defined HA bands than agarose gel prepared using Source #2.

Lane 1 – 5 μL Select-HA™ HiLadder
Lane 2 – 5 μL Select-HA™ LoLadder

4. Equivalent method

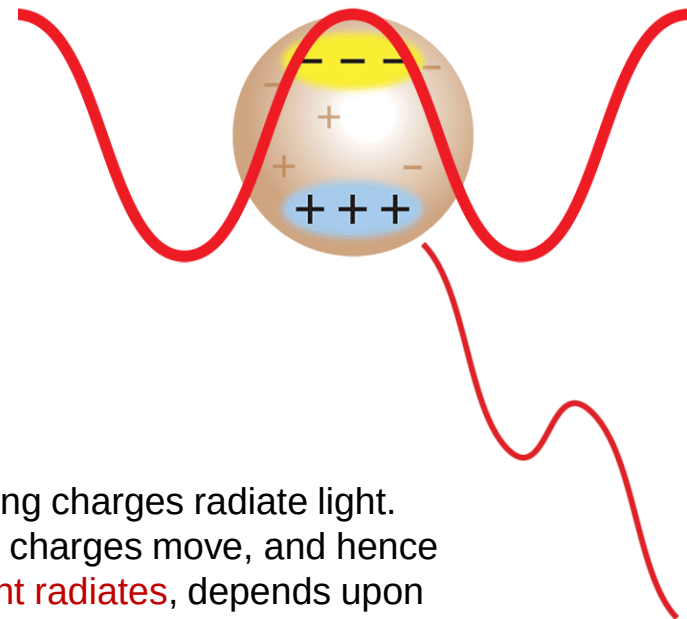
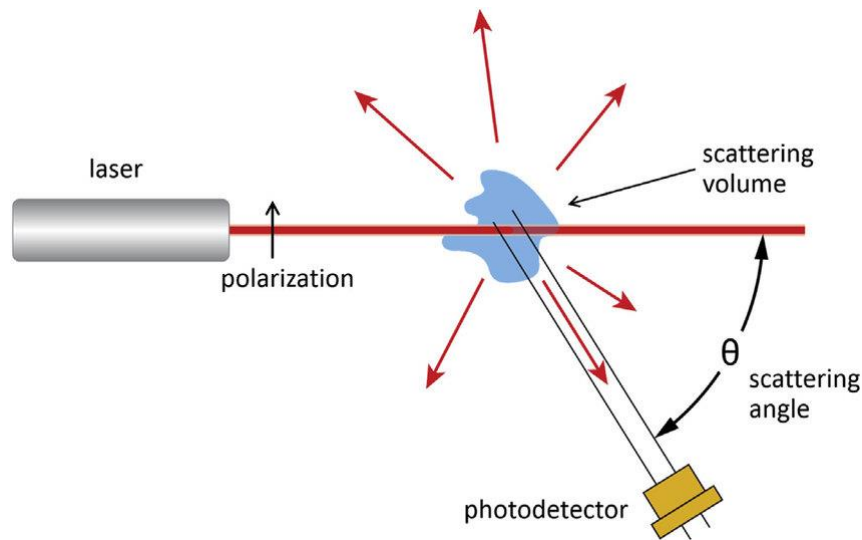
Gel Electrophoresis



5. Absolut method

Static light scattering

Why can we use it to measure molar masses?



The oscillating charges radiate light. How much the charges move, and hence how **much light radiates**, depends upon the matter's refractive index.

The intensity of scattered light is **directly proportional to the molar mass**.

The angular dependency of scattered light is proportional to the size (radius).

5. Absolut method

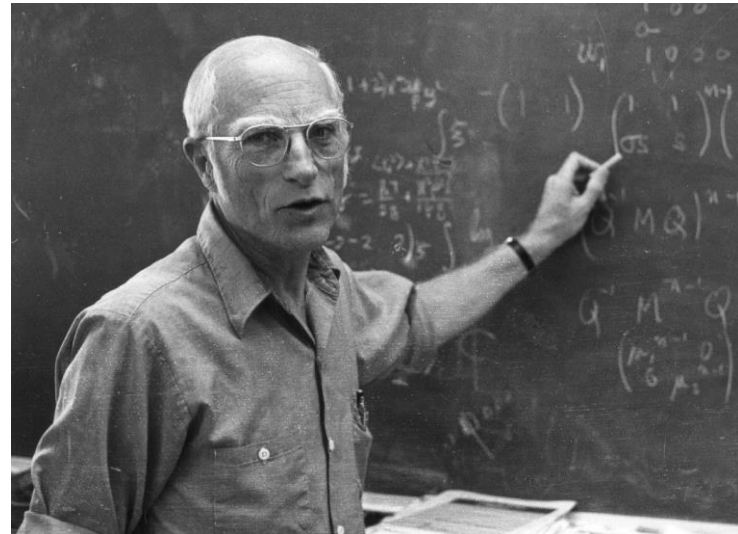
Static light scattering

From Rayleigh Ratio to molar mass

Zimm Equation, J. Chem. Phys. 16, 1093-1099 (1948).

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2c$$

$$K^* = 4\pi^2 (dn/dc)^2 n_0^2 N_A^{-1} \lambda_0^{-4}$$

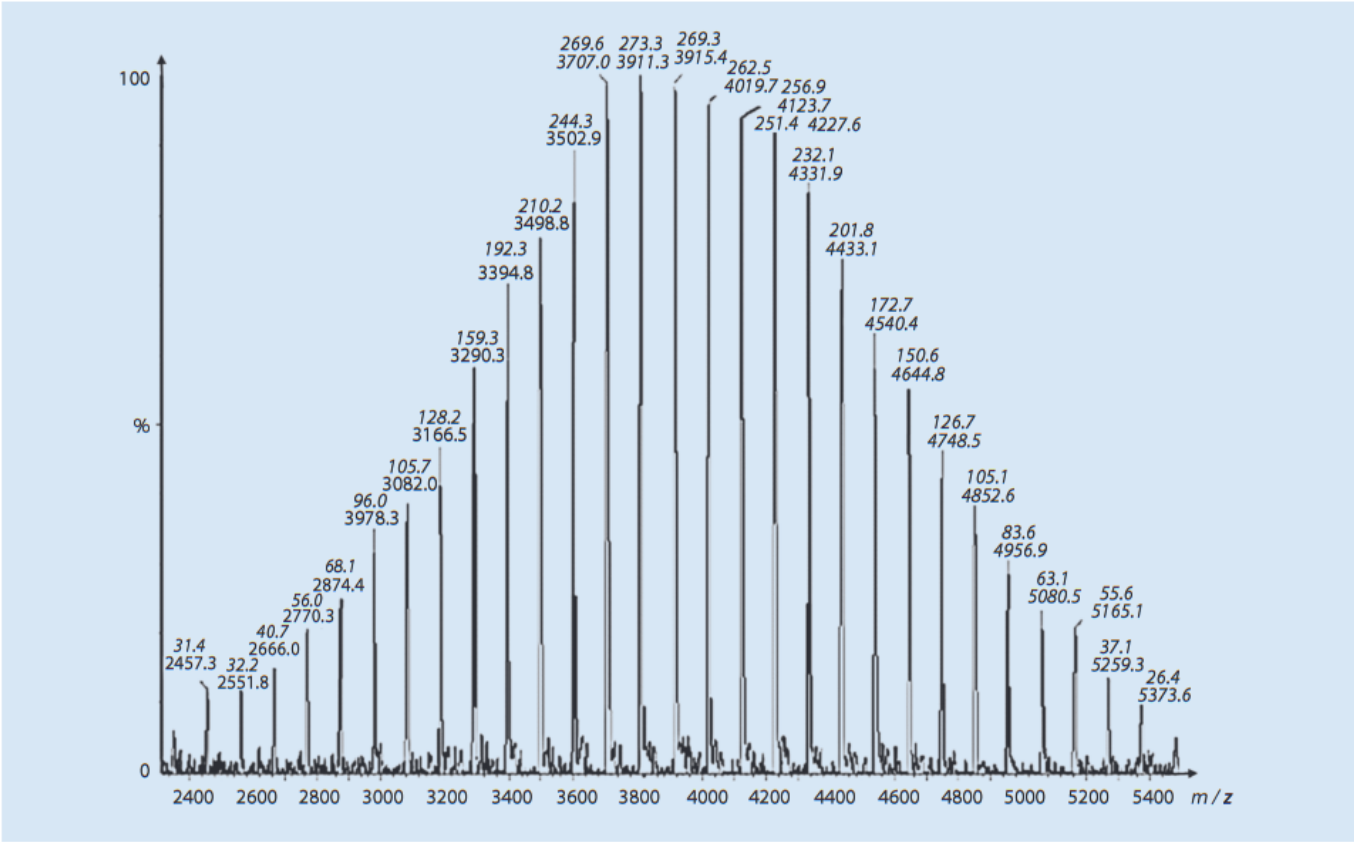


The amount of scattered light at scattering angle 0 is directly proportional to the product of **molar mass** (g/mol) and concentration (g/ml).

5. Absolut method

MALDI-TOF

The **Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectroscopy (MALDI-TOF-MS)** is a fast and sensitive absolute method for determining both the num-ber average and weight average molar masses. It has a special status in the analysis of polymers of biological origin. In an ideal case, molar masses of <300,000 g/mol can be measured with an accuracy of $\pm 0.01\%$



■ Fig. 3.16 MALDI-TOF-MS-spectrum of a polystyrene. The relative abundance (normalized to the most abundant peak) is plotted against the mass/charge ratio (m/z). The numbers above the peaks are the relative abundance and the corresponding m/z value

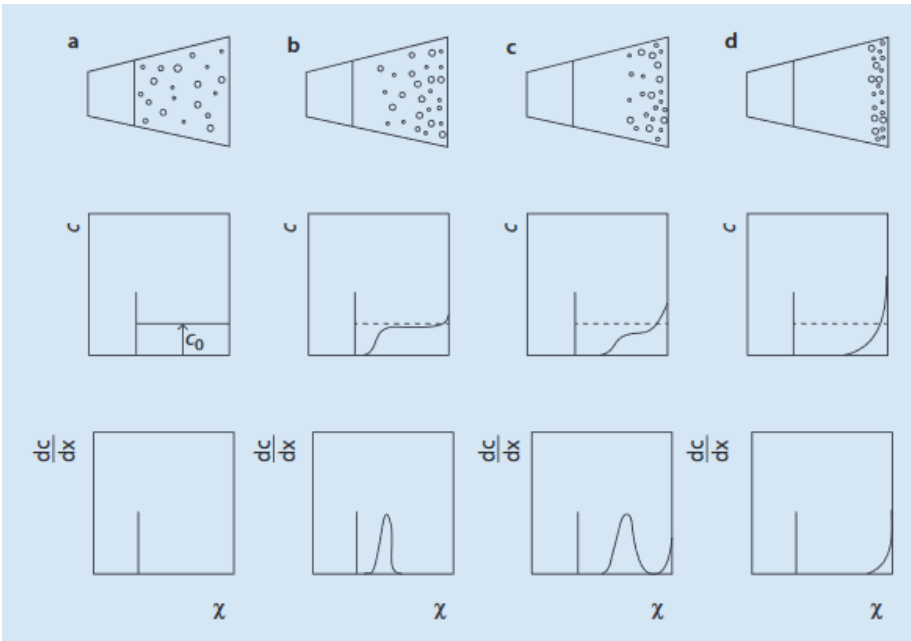
5. Absolut method

Ultracentrifuge

The ultracentrifuge (UC) is a centrifuge which rotates at very high speeds and was originally developed by Svedberg for his research on inorganic and organic colloids (Svedberg and Pedersen 1940).

Three types of experiment are possible, which allow conclusions about **the shape, conformational changes, and size distribution of dispersed particles or dissolved macromolecules.**

- (1) Analysis of Sedimentation Velocity
- (2) Measurement at Thermodynamic Equilibrium
- (3) Sedimentation Equilibrium in a Density Gradient



6. Relative method

Dilute solution viscometry

A. IUPAC suggested the terminology of solution viscosities as following.

Relative viscosity :

$$\eta_{rel} = \frac{\eta}{\eta_o} = \frac{t}{t_o}$$

$\left[\begin{array}{l} \eta : \text{solution viscosity} \\ \eta_o : \text{solvent viscosity} \\ t : \text{flow time of solution} \\ t_o : \text{flow time of solvent} \end{array} \right.$

Specific viscosity :

$$\eta_{sp} = \frac{\eta - \eta_o}{\eta_o} = \frac{t - t_o}{t_o} = \eta_{rel} - 1$$

Reduced viscosity :

$$\eta_{rel} = \frac{\eta_{sp}}{c} = \frac{\eta_{rel} - 1}{c}$$

Inherent viscosity :

$$c \quad \eta_{inh} = \frac{\ln \eta_{rel}}{c}$$

Intrinsic viscosity : $[\eta] = \left(\frac{\eta_{sp}}{c} \right)_{c \rightarrow 0} = (\eta_{inh})_{C=0}$

6. Relative method

Dilute solution viscometry

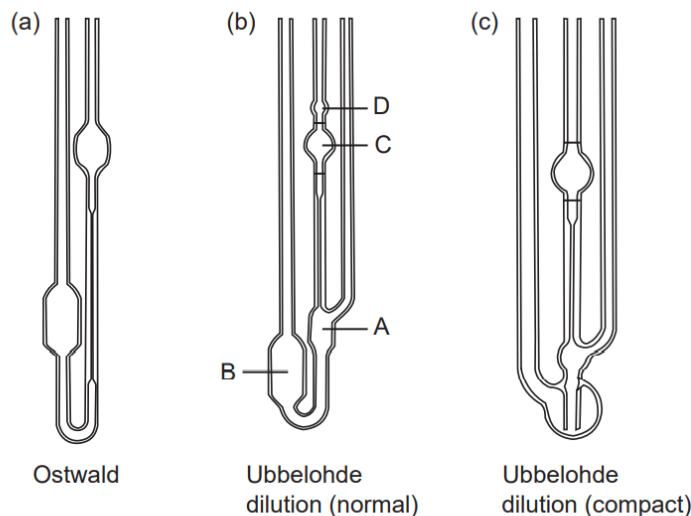


Figure 5 Sketch of the (a) Ostwald viscometer, (b) Ubbelohde dilution viscometer in the normal form, and (c) compact form.

$$\left(\frac{M^2}{[\eta]}\right)^{1/3} = A_{\eta} + B_{\eta}M^{1/2}$$

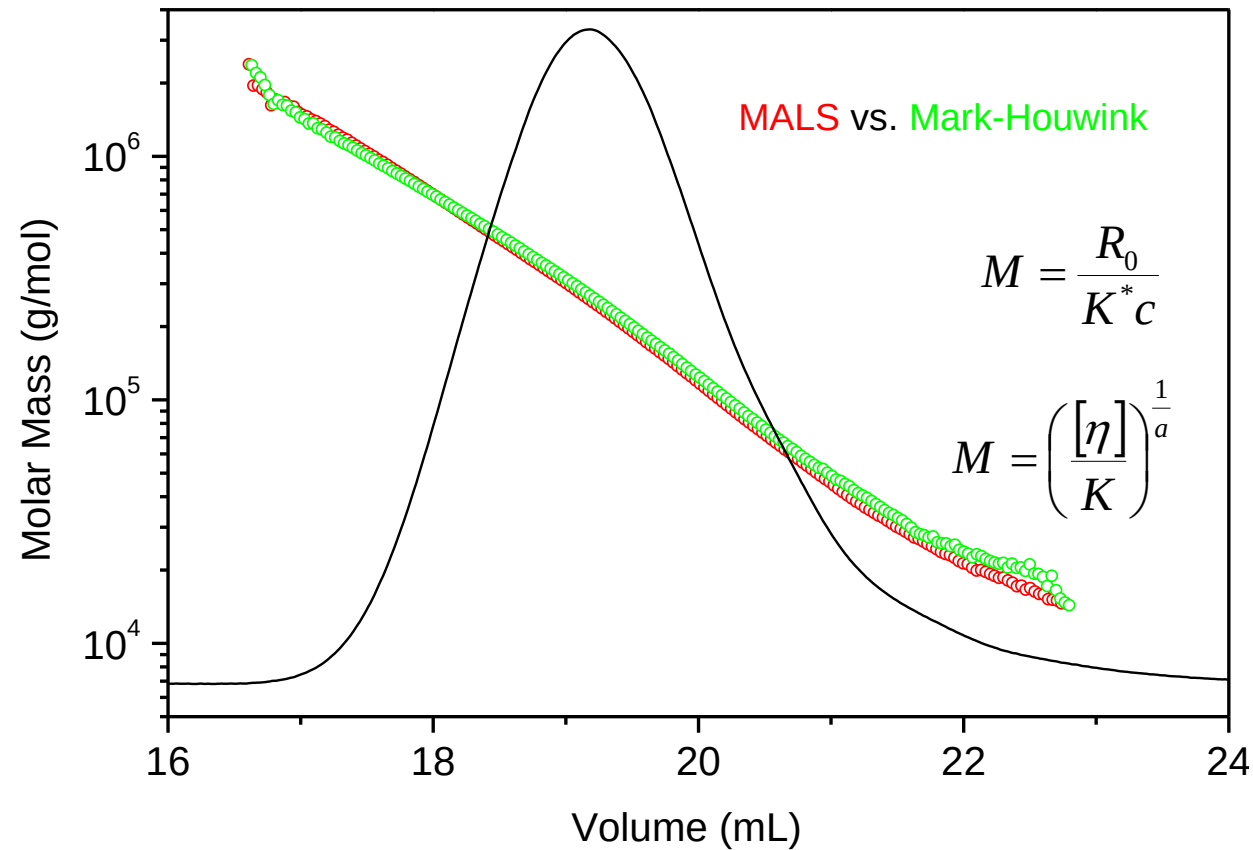
$[\eta] = KM^a$ Mark–Houwink– Sakurada equation

- $K, a =$ constants of Mark-Houwink equation for given polymer, solvent and temperature; M = molar mass
- Traditional method of the determination of molar mass
- Viscosity average (M_v) close to weight-average (M_w)

6. Relative method

Dilute solution viscometry

Molar Mass from Mark-Houwink Equation



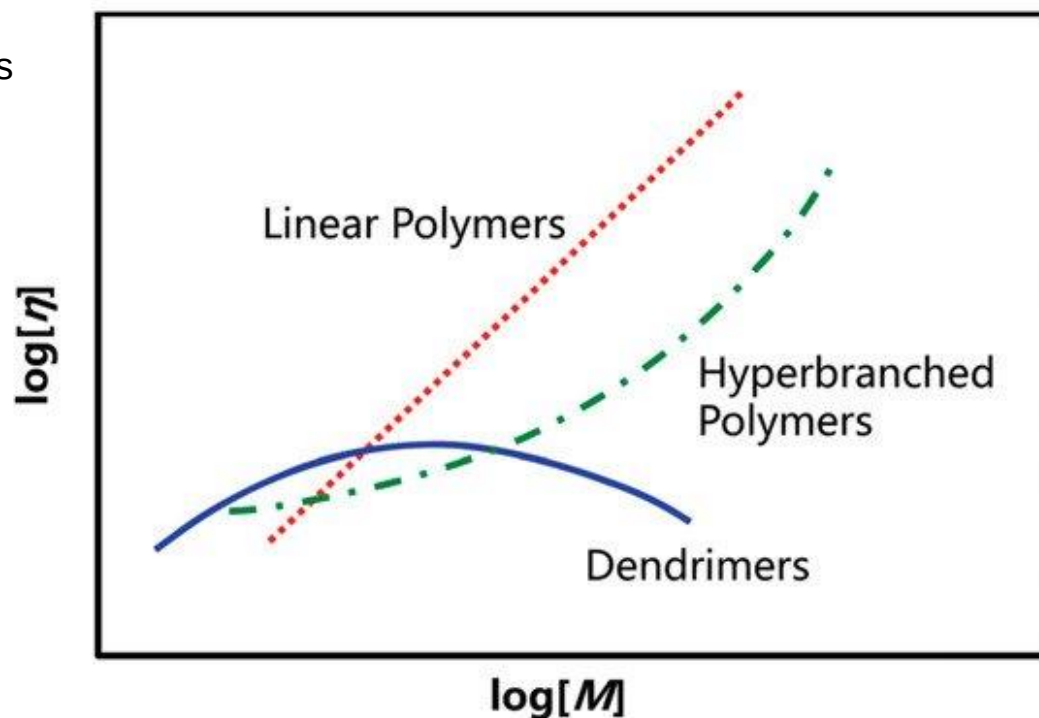
Molar mass versus elution volume plots of linear polystyrene by **MALS** and calculated from **Mark-Houwink equation**.

6. Relative method

Dilute solution viscometry

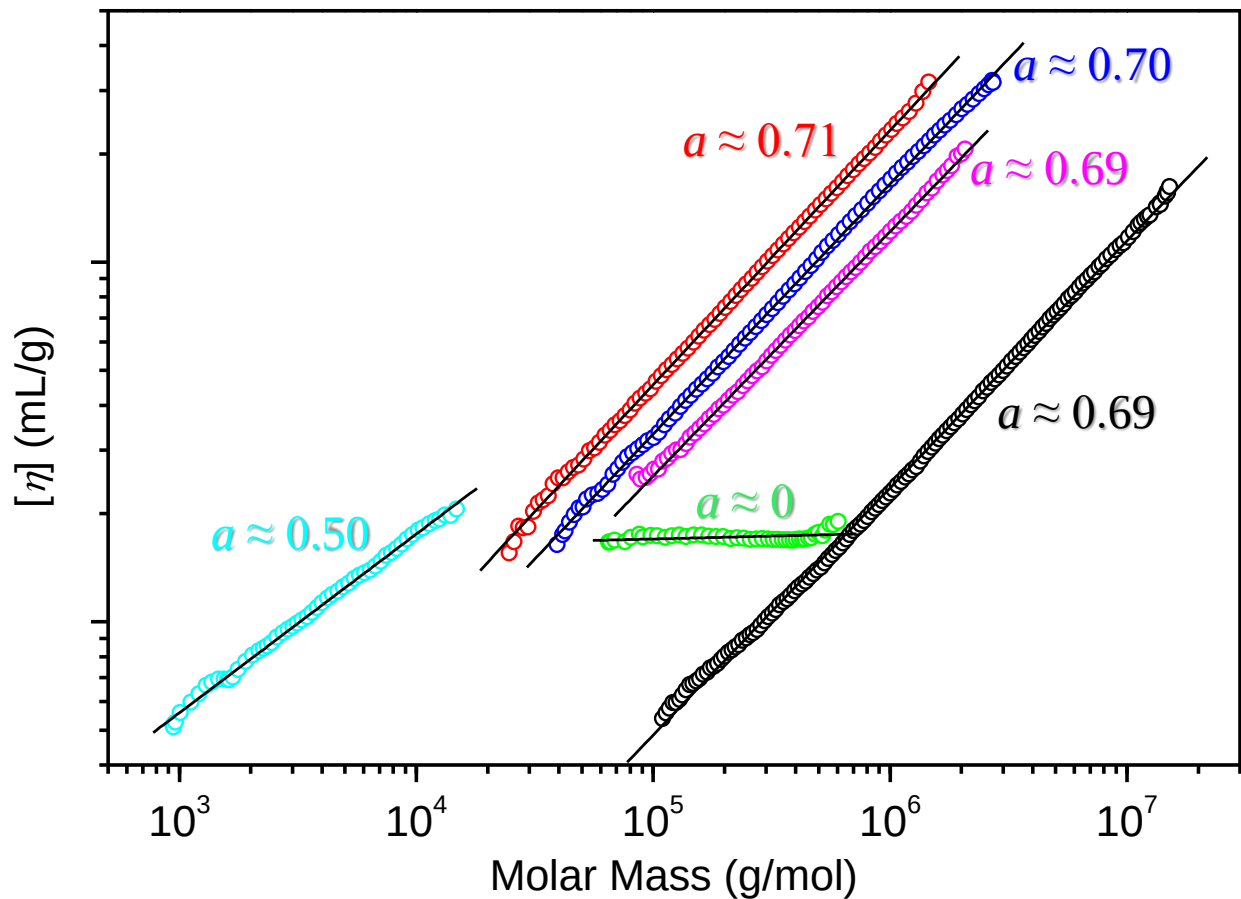
Exponent of Mark-Houwink equation

- Linear macromolecules in thermodynamically good solvents: $a \approx 0.7$
- Linear macromolecules in thermodynamically poor solvents: $a \approx 0.5$
- Oligomers: $a \approx 0.5$
- Hard spheres: $a \approx 0$
- Extended chains: $a \approx 0.8$ to ≈ 1.5
- Linear polymers have linear MH plots
- Curved plots indicates branching



6. Relative method

Dilute solution viscometry

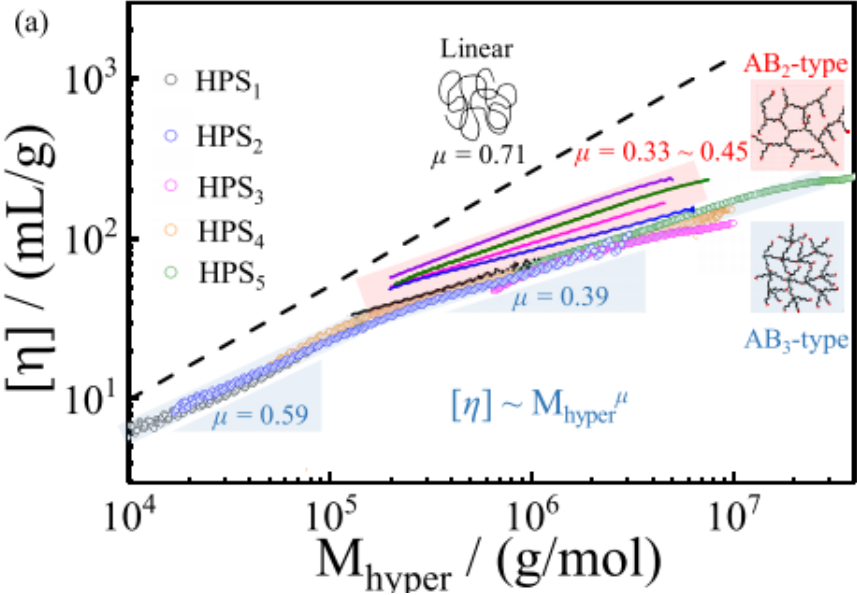


Mark-Houwink plots of epoxy resin, linear polystyrene, linear poly(methyl methacrylate, linear poly(benzyl methacrylate), linear poly(iBuPOSSMA) and star-branched poly(isobutyl methacrylate).

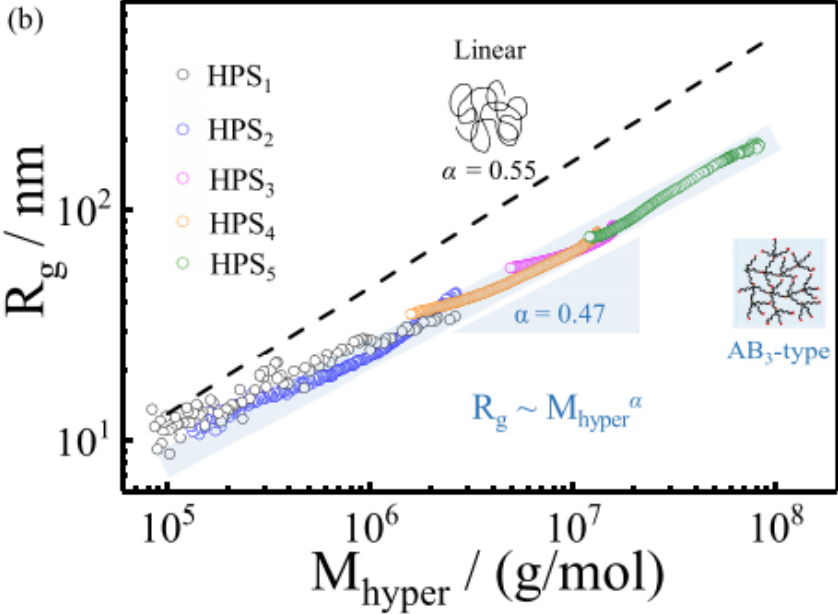
6. Relative method

Dilute solution viscometry

How Does the Branching Effect of Macromonomer Influence the Polymerization, Structural Features, and Solution Properties of Long-Subchain Hyperbranched Polymers?



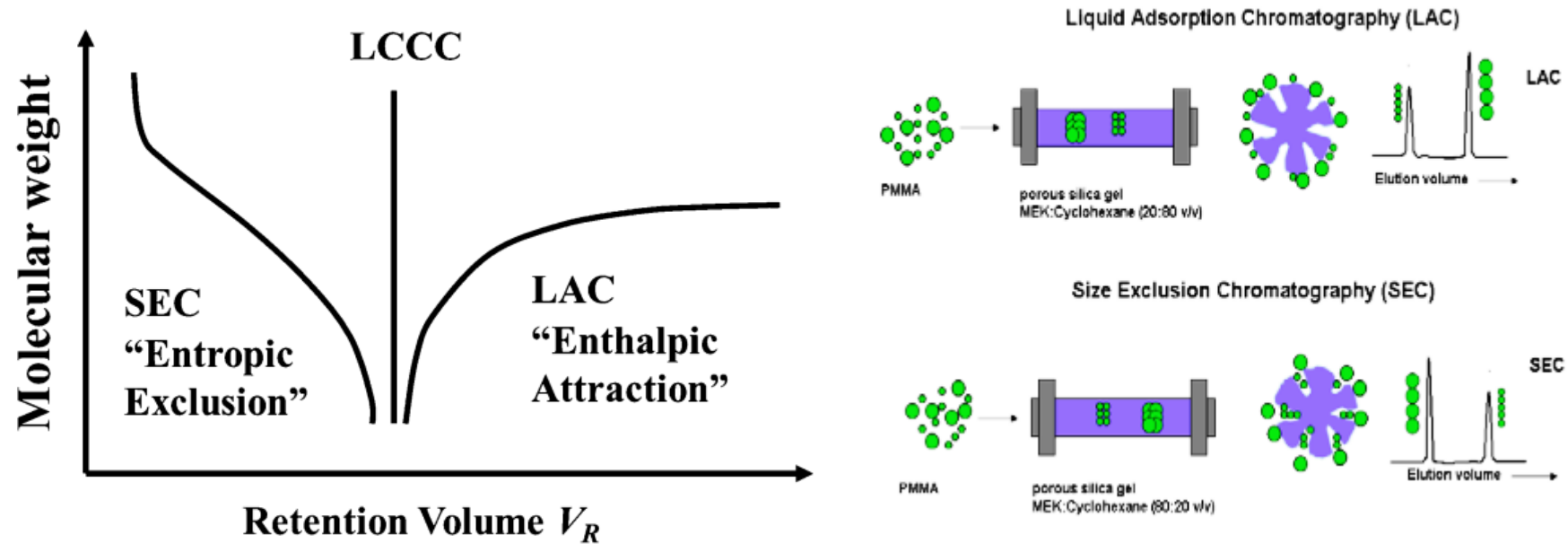
Scaling of intrinsic viscosity



Scaling of the Radius of Gyration

6. Relative method

Types of liquid chromatographic separations



Size Exclusion Chromatography (SEC)

Liquid Chromatography under Critical Conditions (LCCC)

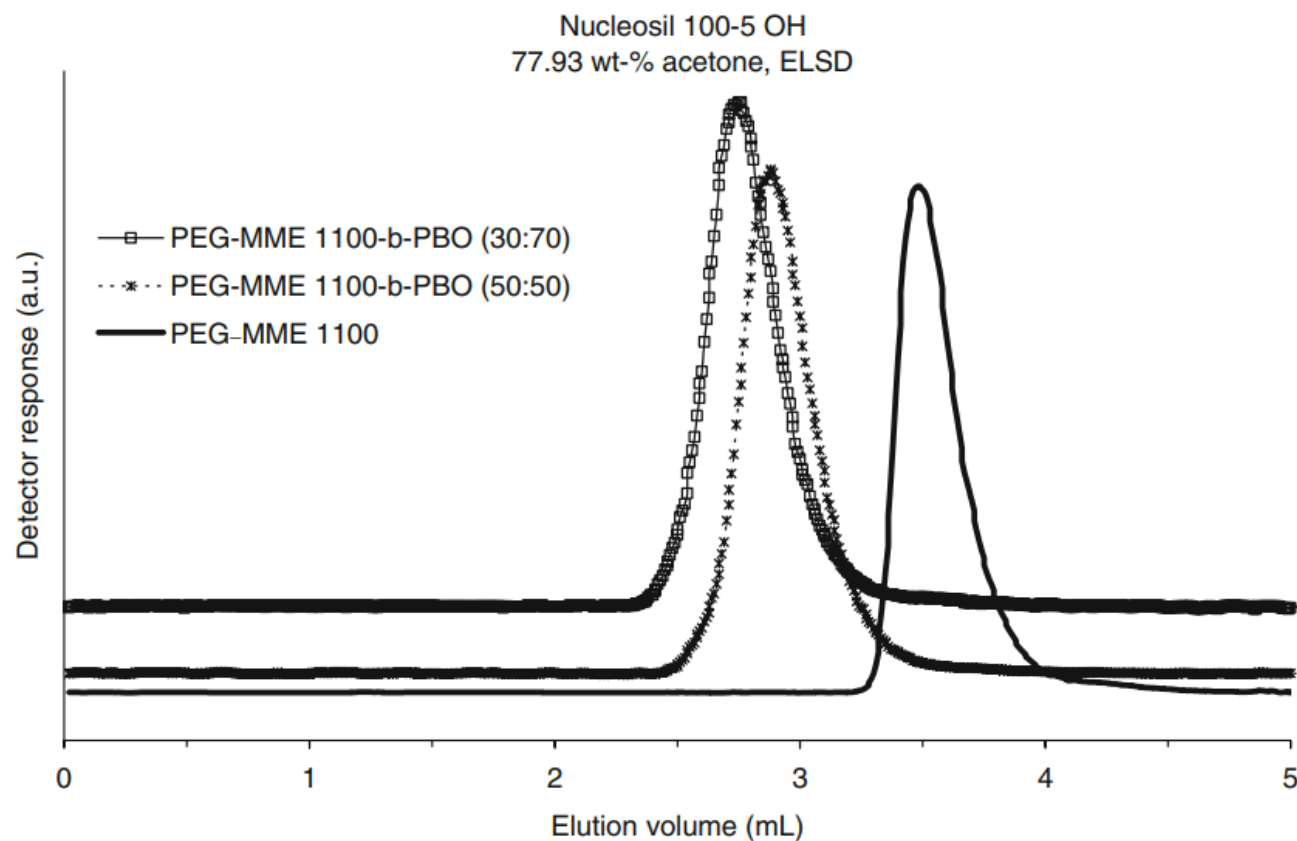
Liquid Adsorption Chromatography (LAC)

5. Relative method

Types of liquid chromatographic separations

Liquid Chromatography under Critical Conditions (LCCC)

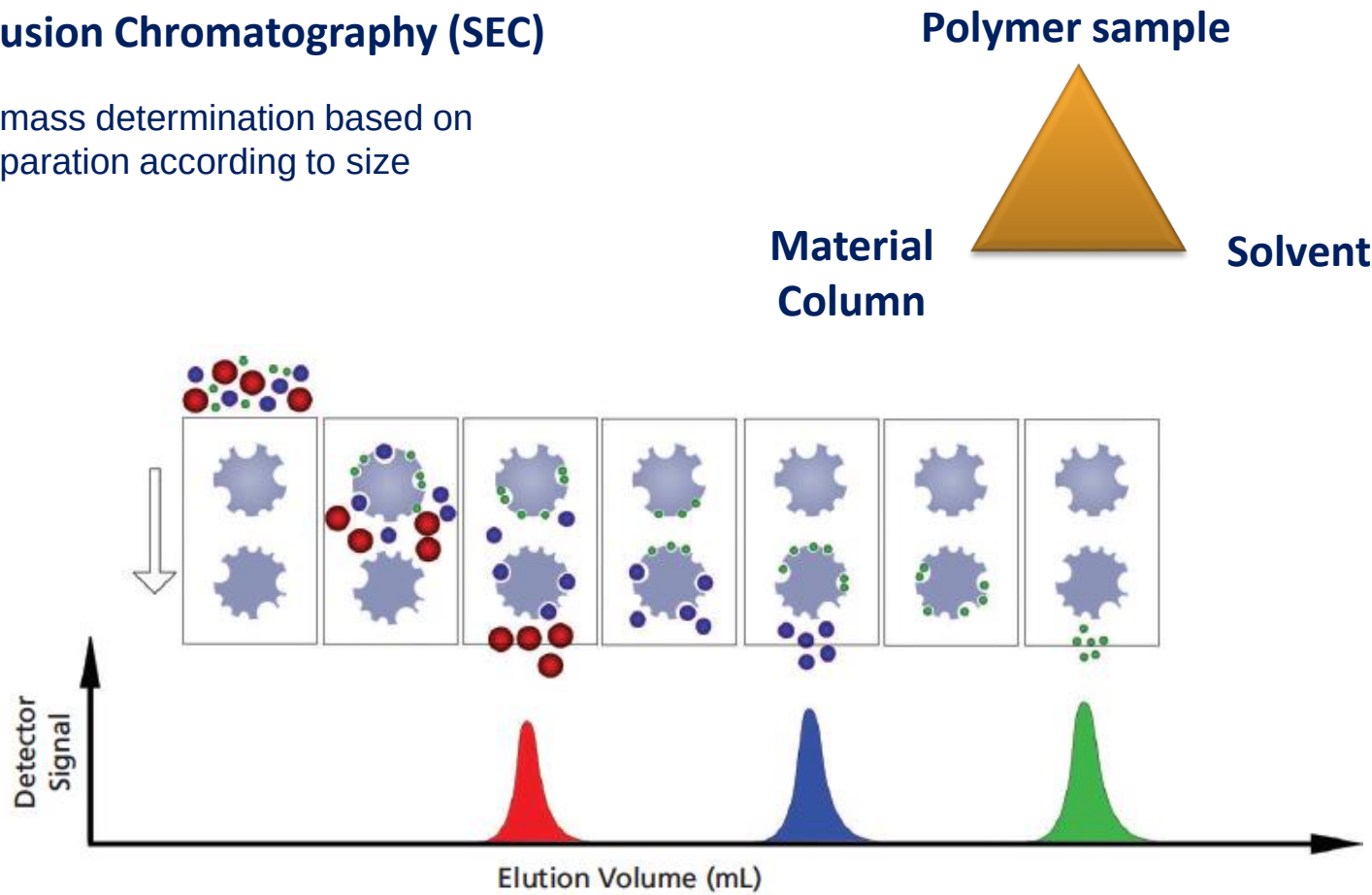
Fig. 3 LCCC (at the CAP for EO) of PEG-MME and the corresponding diblock copolymers with butene oxide (BO) (exclusion conditions for the BO block). *PBO* poly(butene oxide)



6. Relative method

Size Exclusion Chromatography (SEC)

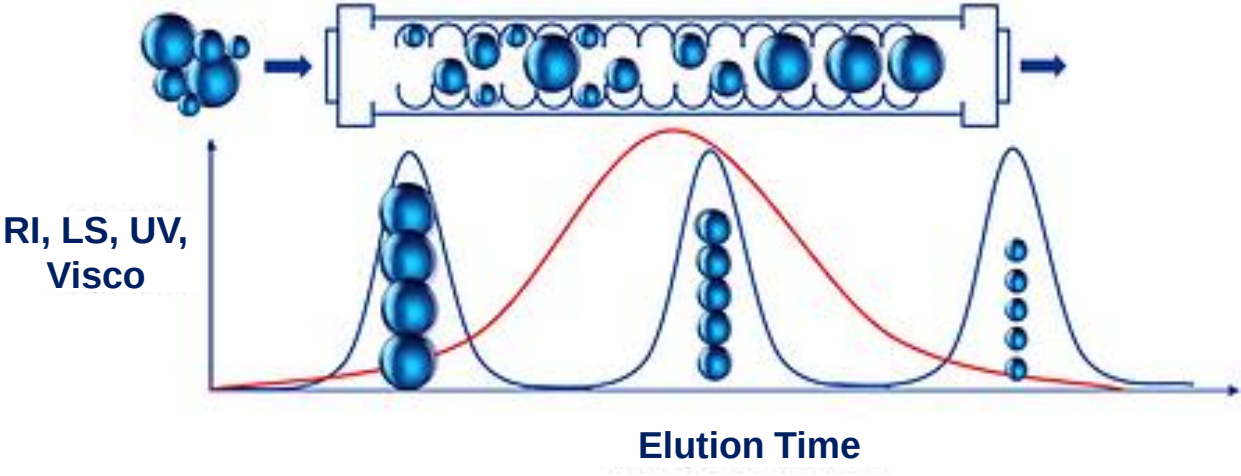
molar mass determination based on separation according to size



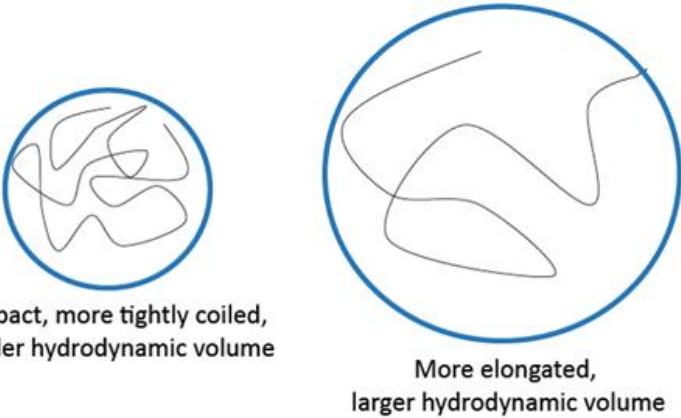
- **Large particles** cannot enter gel and **are excluded**. They have less volume to traverse and **elute sooner**
- **Small particles** can enter gel and have more volume to traverse. They **elute later**

6. Relative method

Size Exclusion Chromatography (SEC)

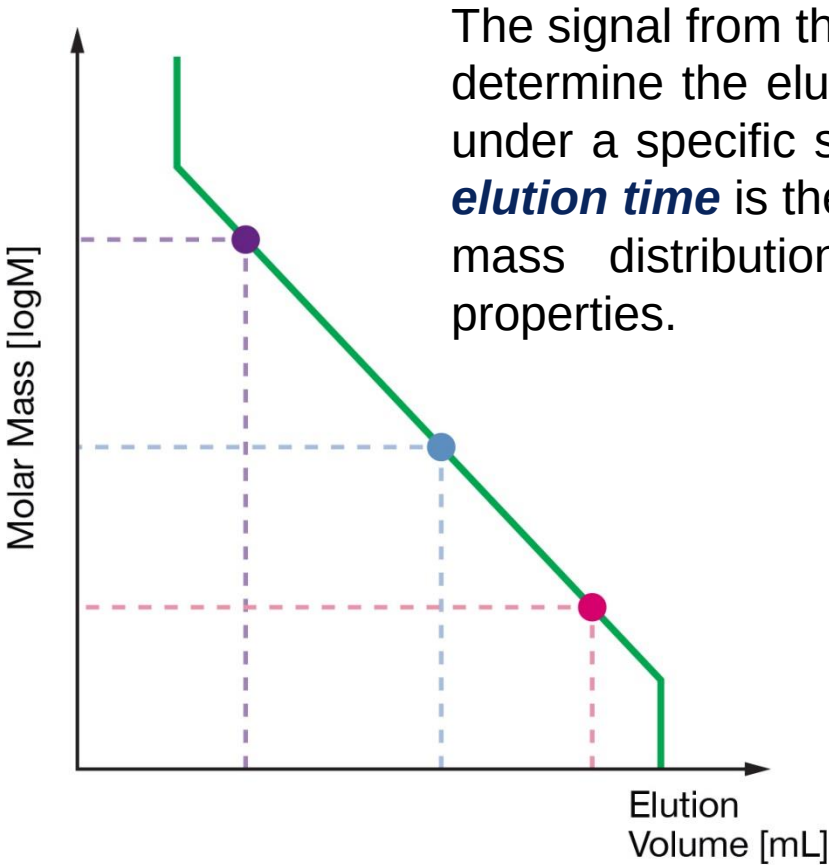


SEC is separating polymers by their **hydrodynamic volume** or **hydrodynamic radius** – which is affected by various things, in particular the polymer (chemistry and structure), solvent, solvent/polymer interactions, and temperature.



6. Relative method

Size Exclusion Chromatography (SEC)- Calibration curve



The signal from the **concentration detector** in SEC is used to determine the elution time of **known molar mass standards** under a specific set of conditions. A plot of **molar mass vs. elution time** is then generated and used to estimate the molar mass distribution of actual samples from their elution properties.

The column calibration method assumes that the sample of interest

- has the same conformation as calibration standards
- has the same density as standards
- does not interact with the column packing

6. Relative method

What is SEC-MALS?

- **SEC = Size-Exclusion Chromatography**

Size-Exclusion Chromatography (SEC) is a chromatographic method in which molecules are separated based on their size, or, in more technical terms, their hydrodynamic volume.

- **MALS = Multi-Angle Light Scattering**

Two modes of operation

$$I_s(\theta) \propto c \times M \times \left(\frac{dn}{dc} \right)^2$$

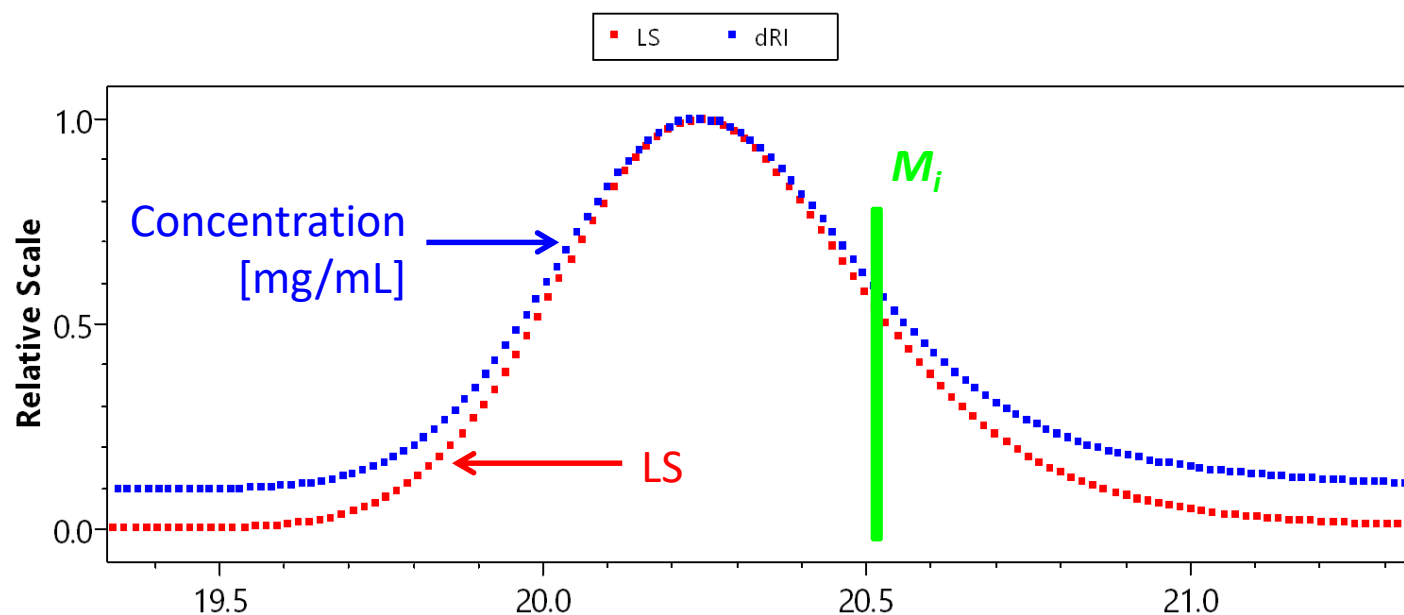
- A batch experiment measures an unfractionated sample.
 - Only the weight-averaged molar mass M_w , z-averaged RMS radius R_z , and potentially A_2 will be determined.
 - No information about the polydispersity of the sample can be obtained.
- SEC allows the sample to be chromatographically separated.
 - Molar Mass and RMS Radius moments and distributions can be assessed.

6. Relative method

SEC-MALS-UV/RI method

- SEC provides separation and the molar mass is measured by online MALS and concentration detectors. Molar mass moments (M_n , M_w , M_z), radius moments, dispersity, conformation

$$I_s(\theta) \propto c \times M \times \left(\frac{dn}{dc} \right)^2$$

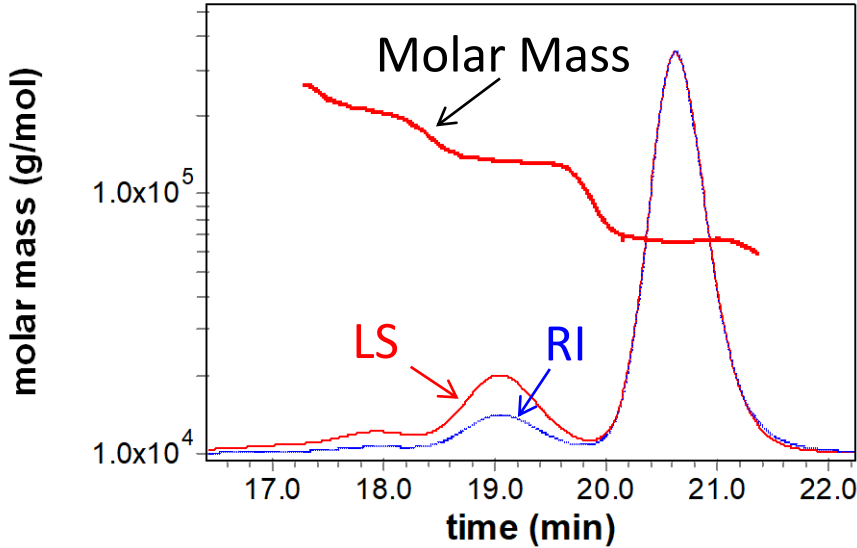


6. Relative method

SEC-MALS-UV/RI method

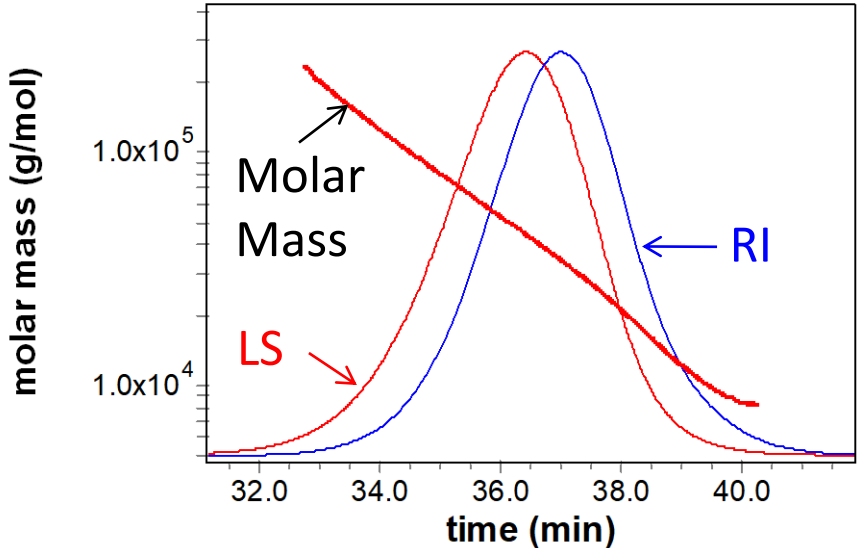
Molar Mass at each elution slice is measured

Proteins
(discrete populations)



BSA

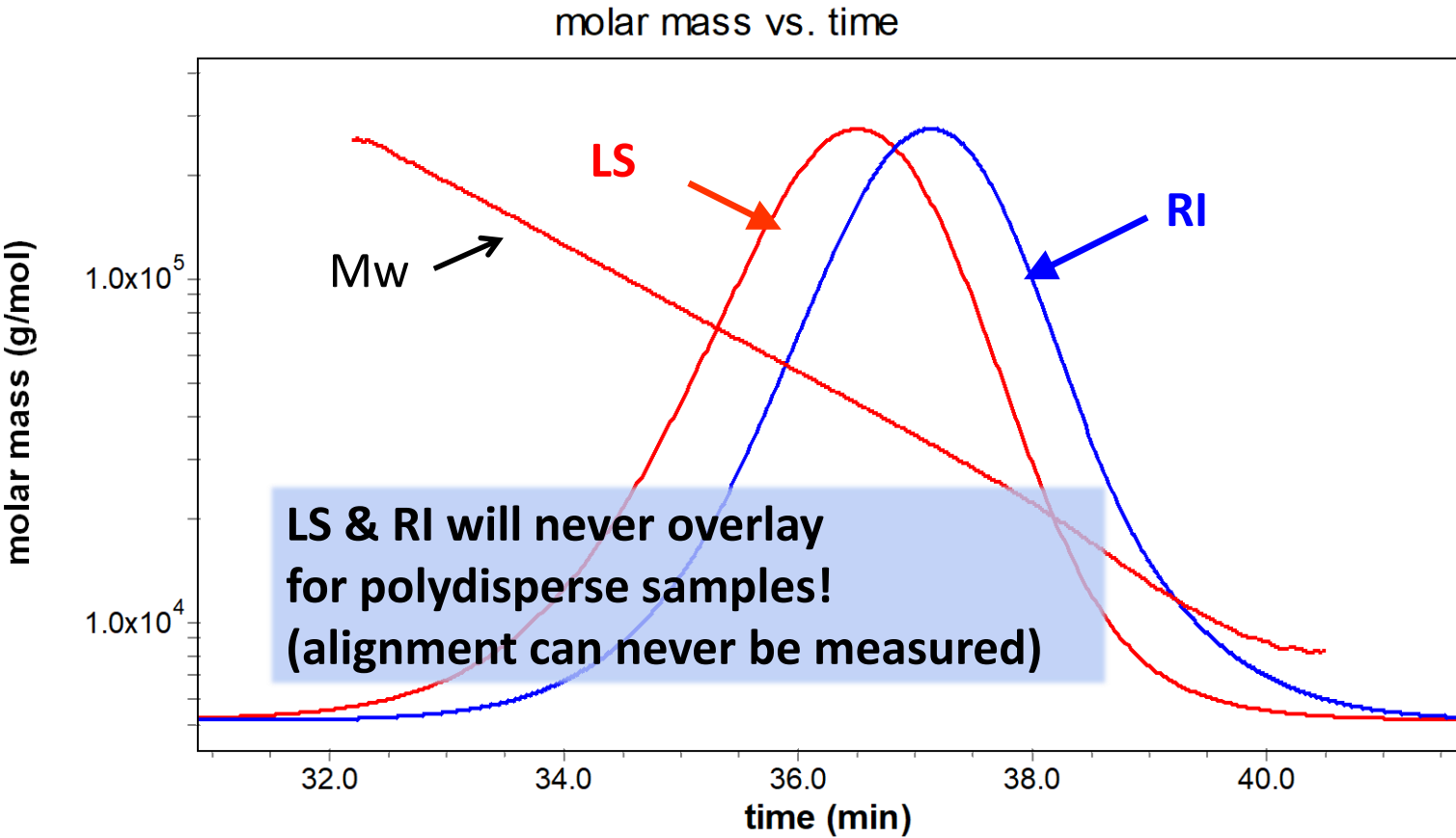
Polymers
(continuous distribution)



Dextran

5. Relative method

SEC-MALS-UV/RI method



6. Relative method

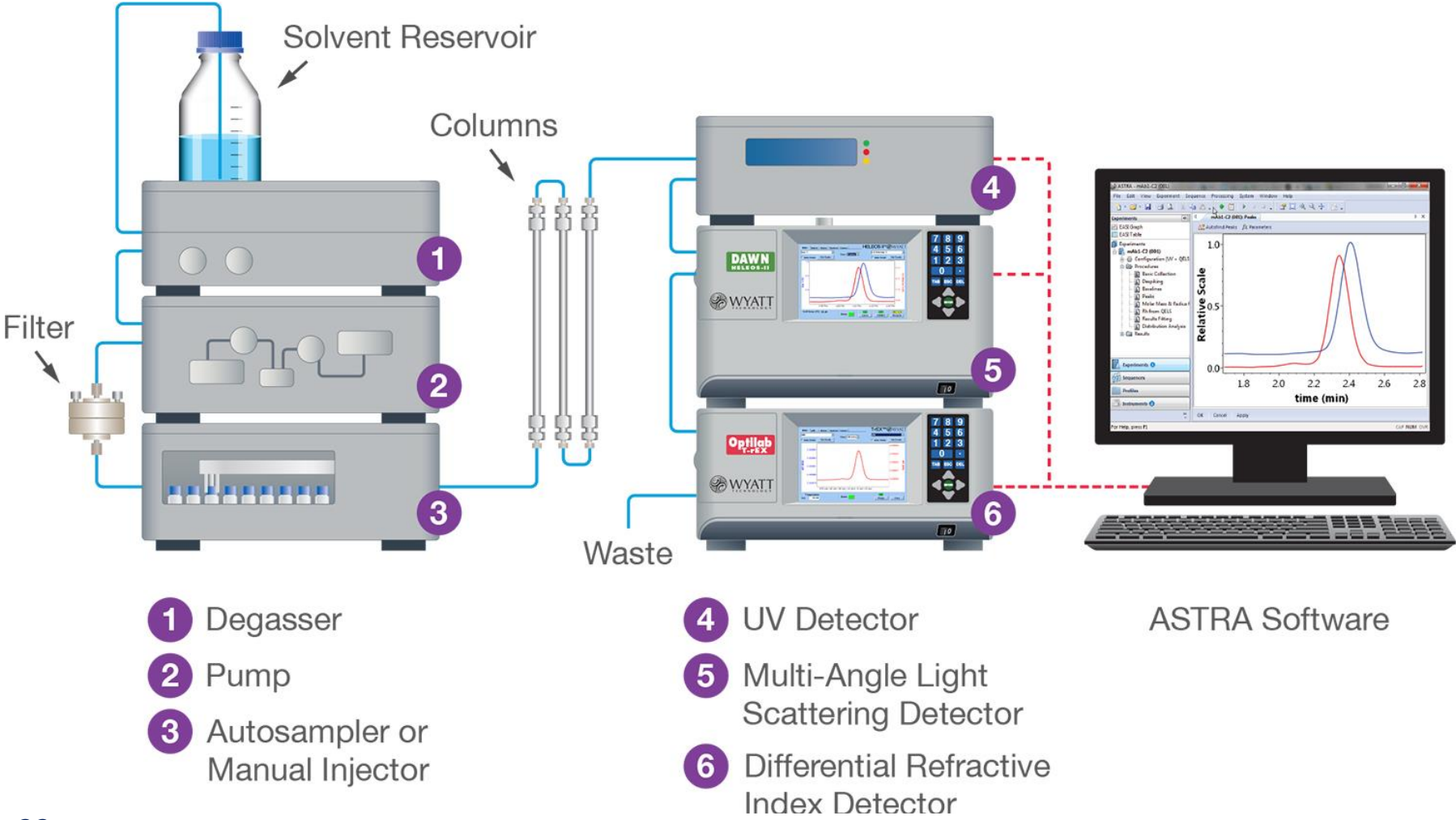
SEC-MALS-UV/RI method

- Knowledge of dn/dc is necessary for
 - Molar mass determination from light scattering measurements
 - Determining concentrations by an RI detector
 - Analog RI calibration
- The value of dn/dc depends on
 - Polymer composition
 - Solvent
 - Molar mass
 - Laser wavelength
- The dn/dc can be obtained from
 - The literature
 - Direct measurement with an RI detector (online or offline)
- For greatest accuracy, the dn/dc used in light scattering experiments should have been obtained for your polymer, in your solvent, and at the wavelength of your LS detector.
- dn/dc describes the change in the refractive index of a solution as a function of solute concentration.
- dn/dc has units of mL/g; it expresses how much the refractive index of a solution theoretically increases for every g of solute contained in a 1 ml final volume of solution.
- The dn/dc of a polymer solution should be measured at polymer concentrations appropriate for chromatography conditions; some non-linearity can occur at very high solute concentrations.
- To determine molar mass by LS, dn/dc needs to be known
 - For your solute
 - In your solvent
 - At the wavelength of your LS detector
 - Even when you are not using a concentration detector!

6. Relative method

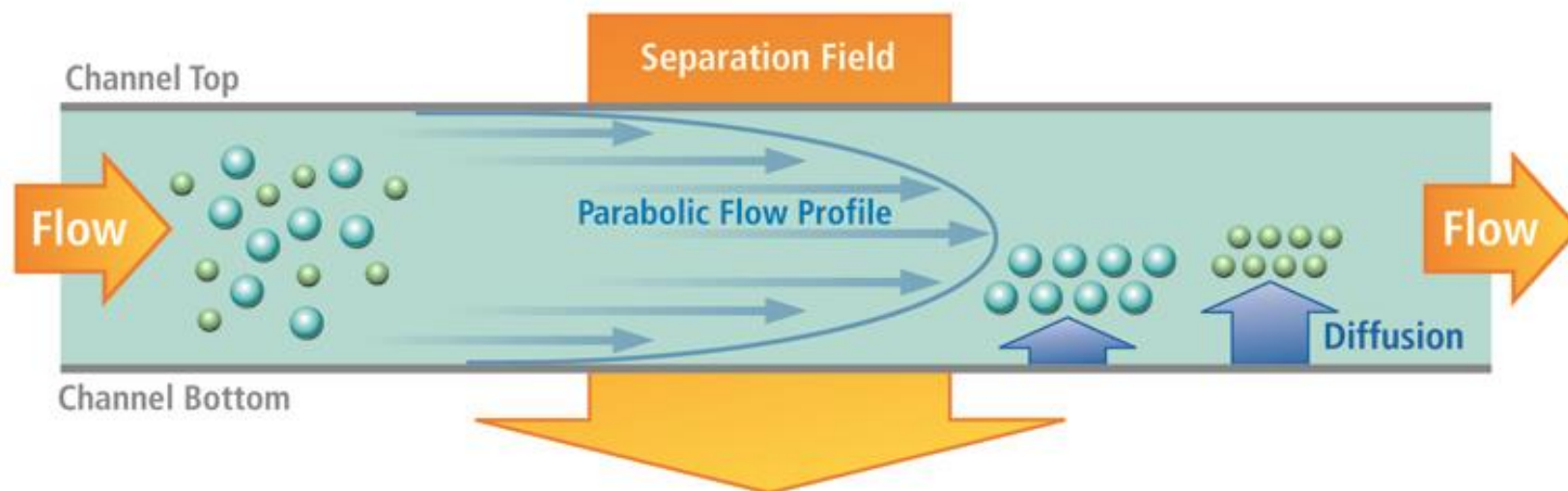


Typical SEC-MALS-hardware setup



6. Relative method

Field-flow fractionation (FFF)



It is a separation technique in which a field (thermal, electric, magnetic, hydraulic, gravitational, ...) is applied to a diluted suspension in a fluid or to a solution pumped through a long and narrow channel, perpendicular to the direction of the field, in order to cause the separation of particles present in the fluid, depending on their differing "mobilities" under the force exerted by the field. The FFF method is unique to other separation techniques because it can separate materials over a wide colloidal size range while maintaining high resolution. Although FFF is an extremely versatile technique, there is no "one size fits all" method for all applications.