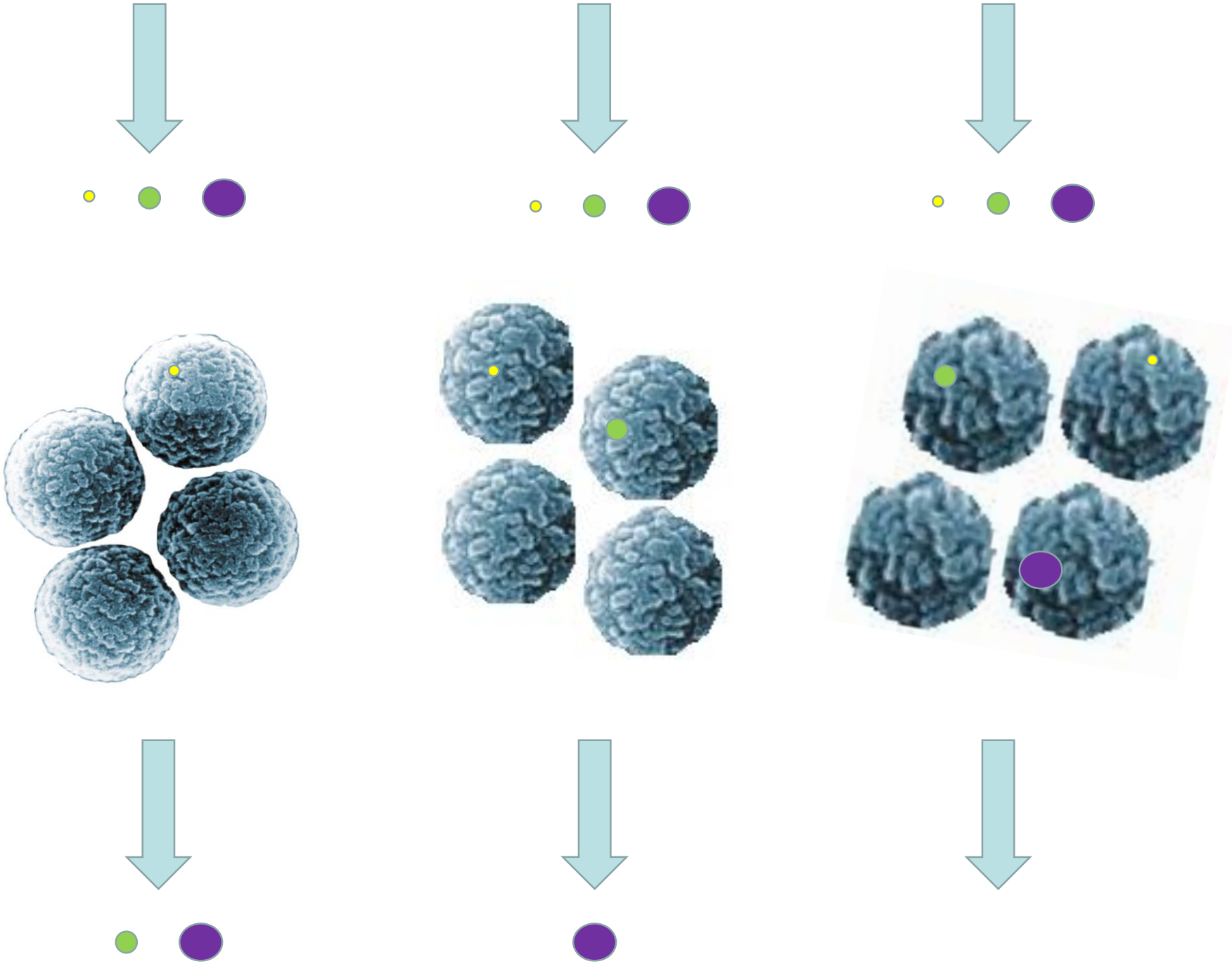
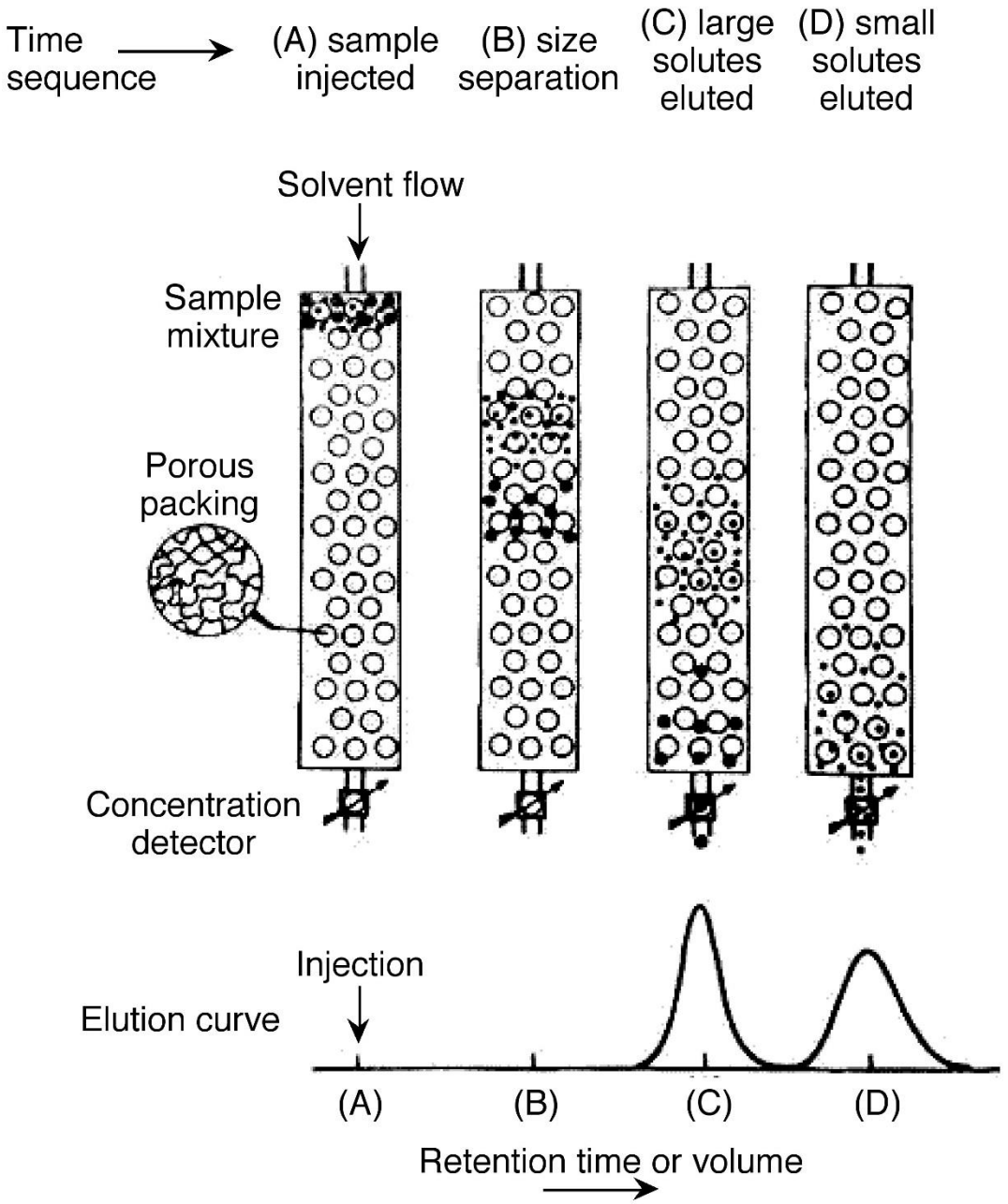
Size exclusion
or
gel permeation chromatography
(SEC or GPC)

Aim: separation of molecules based on size

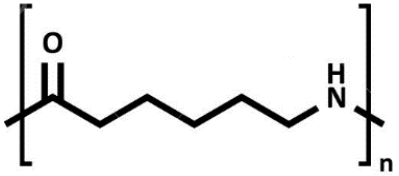
Used for:
- characterization of polymers
- protein preps
- sample desalting and cleanup



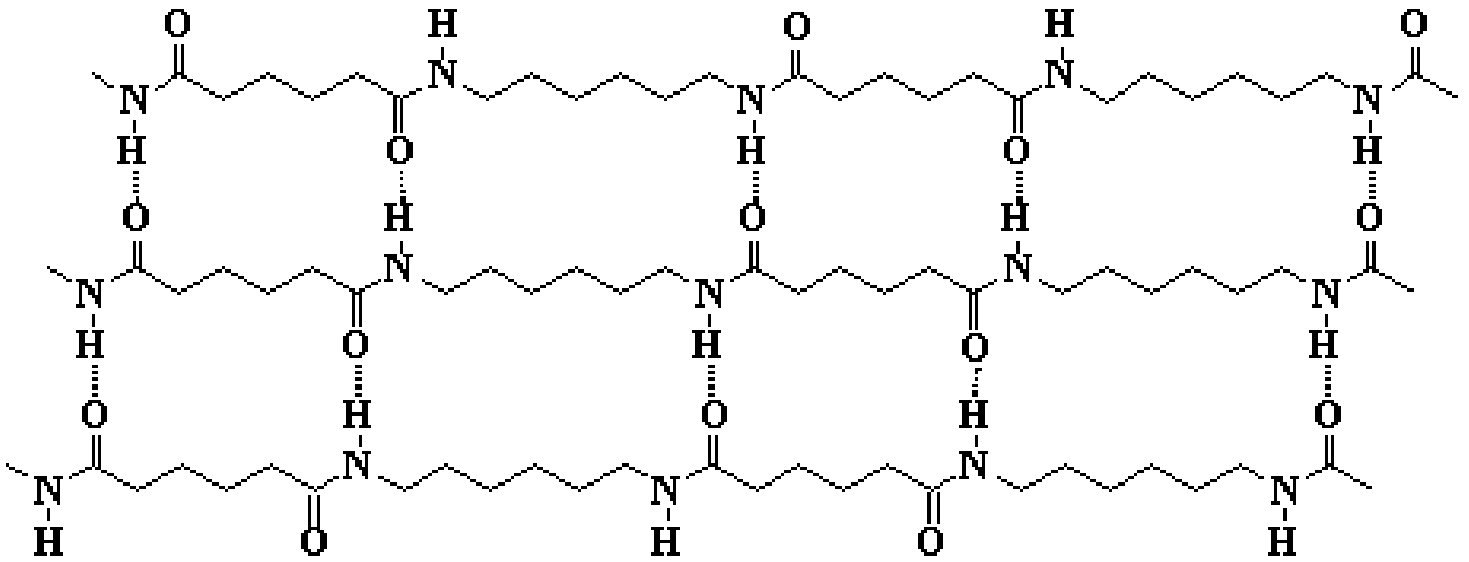


http://cnx.org/contents/ba27839d-5042-4a40-afcf-c0e6e39fb454@20.9:25/Physical_Methods_in_Chemistry_

Most common application: Determination of MW distribution of polymers (research & industry)

Example: Nylon-6,6 is made up of repeating units of 

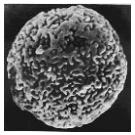
- *n* depends on the polymerization process.
- With low or large n values the polymer has different properties but is still called *nylon*.
- Synthetic polymer samples usually contain a characteristic n distribution.
- The larger n, the more robust the material.
- Nylon can be a liquid, a paste or a rigid solid.



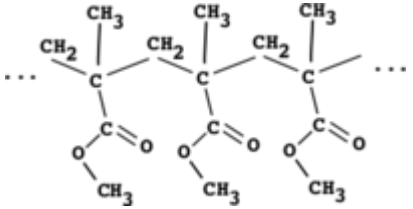
In nylon 6,6, the carbonyl oxygens and amide hydrogens can hydrogen bond with each other. This allows the chains to line up in an orderly fashion to form fibers.

Materials used for SEC beads

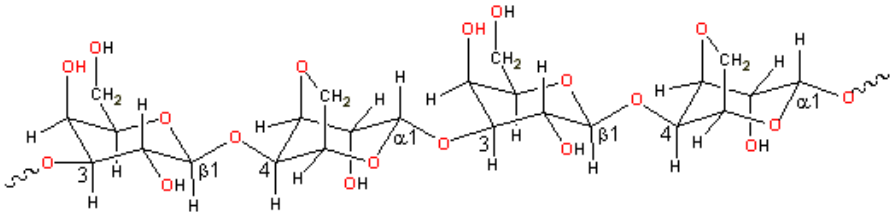
- Porous silica (SiO_2) (organic or water soluble polymers)



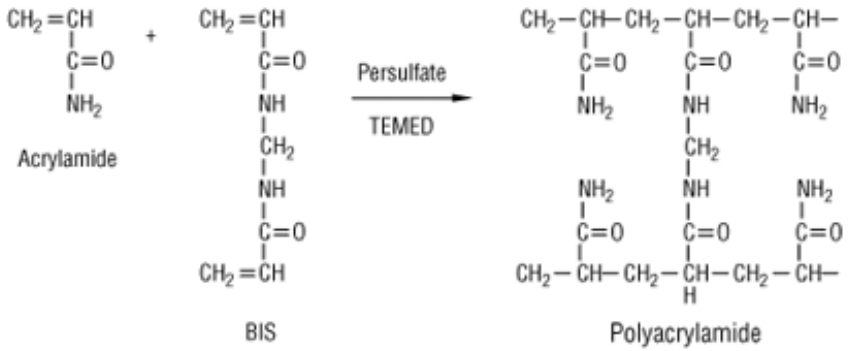
- Polymethacrylate (water soluble polymers)



- Polymethacrylate with high % of cross linking (polar organic-soluble polymers)
- Modified polystyrene (organic soluble polymers)
- Agarose gel (water soluble polymers)



- Polyacrylamide (water soluble polymers)



SEC or GPC

- “Semi-stagnant” liquid in bead pores (V_S) is part of the stationary phase, and flowing liquid is the mobile phase (V_0).
- The mobile phase flows in and out of bead pores.
- Separation is based on molecular size.

SEC parameters

V_m = total volume of mobile phase in column (outside and inside pores)

V_0 = void volume or volume of mobile phase outside particle pores

V_r = retention volume for a given analyte

V_s = volume of mobile phase inside the pores.

as $V_m = V_s + V_o$, then $V_s = V_m - V_0$

Retention of analytes is described by K_{av}

$$K_{av} = \frac{V_r - V_0}{V_m - V_0}$$

For a large molecule, $V_r = V_0$ and $K_{av} = 0$

For a small molecule freely entering the pores,
$Vr \geq Vm$ and $K_{av} \geq 1$

SEC parameters: in practice

Void volume V_0 is measured by passing a large inert molecule through the column

e.g. Blue Dextran 2000, MW = 2×10^6 Da.

V_m: calculated from the measured column bed volume of dry resin.

Example:

For a certain resin, 10 g of dry beads occupy 10 mL. Once swollen with liquid, the volume becomes 25 mL (V_t).

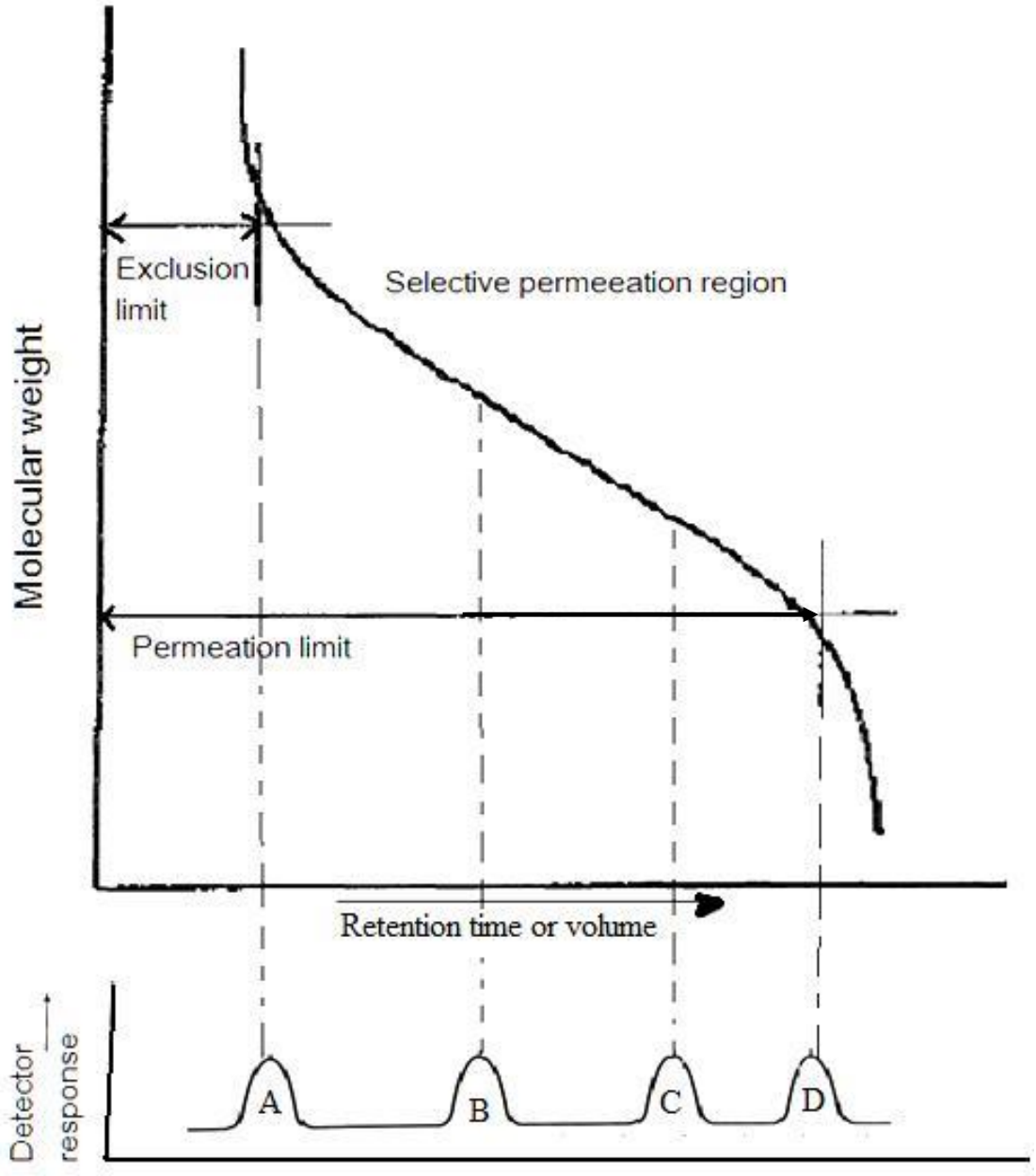
Then, V_m is 15 mL in a 25-mL column.

Equal masses of different sorbents produce widely varying volumes when swollen with solvent (specified by manufacturer).

Molecular weight determination

- SEC mainly used to separate molecules with *significant differences* in MW
- No resolution between closely related polymer species
- For each type of beads: a calibration must be performed, i.e. MW vs. retention time, elution volume, or K_{AV}
- Unknowns interpolated to find MW

MW vs retention volume



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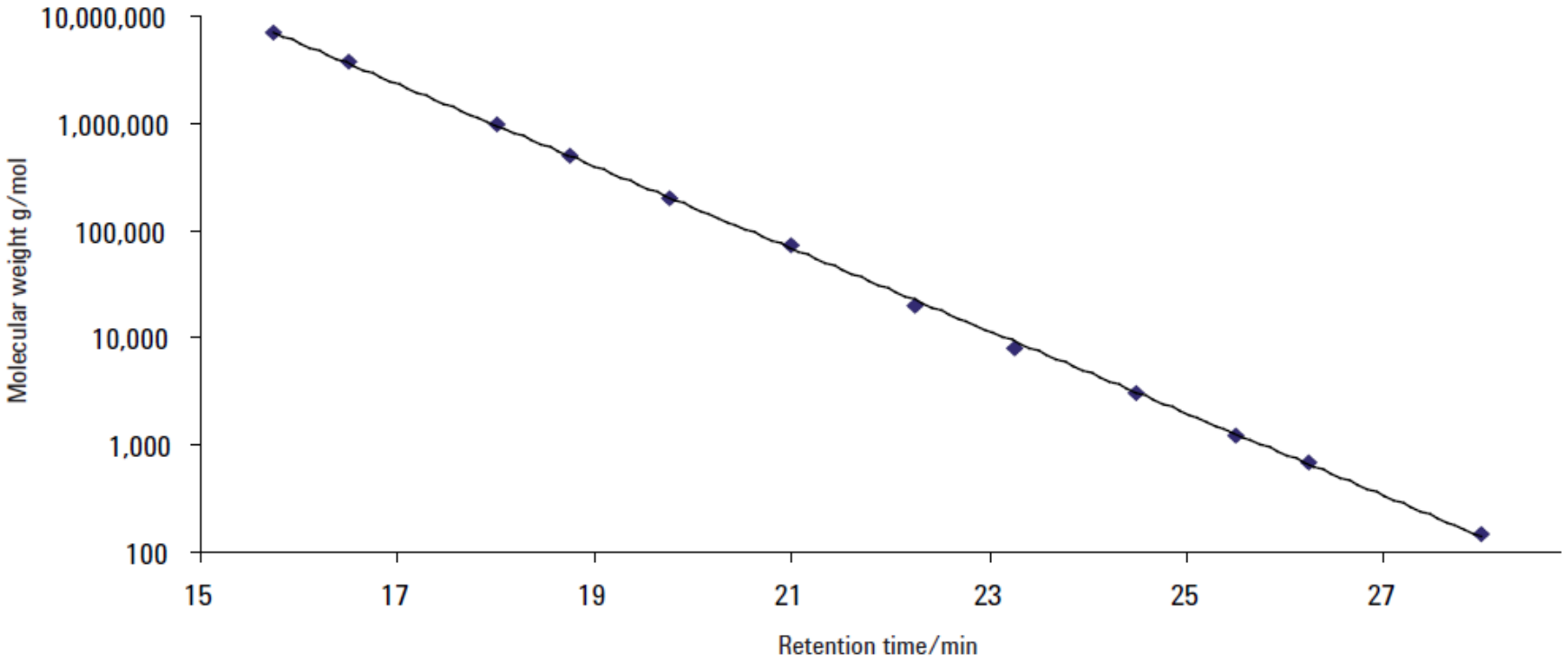
Calibration curve for polymers



https://www.agilent.com/cs/library/primers/Public/5990-6969EN%20GPC%20SEC%20Chrom%20Guide.pdf

Polymer chain mass distribution

•Once dissolved, polymer chains coil up on themselves to form sphere-like shapes.

•In SEC they behave like spheres, with size of the sphere dependent on MW.

•SEC gives information regarding the size of polymer molecules, but not about the chemistry of the sample.

Mass distribution of polymers

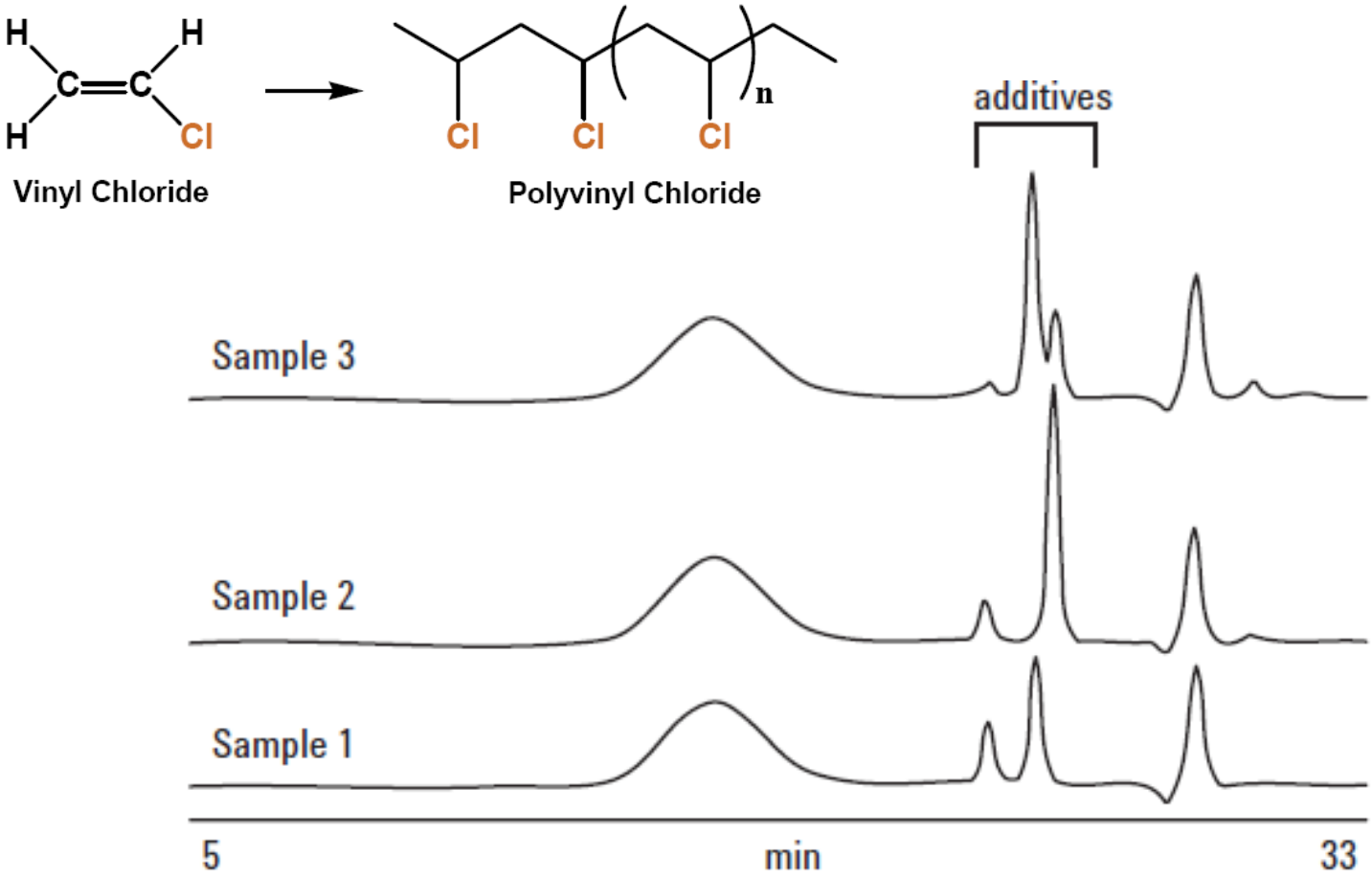


Figure 9. *Chromatograms of samples of PVC showing the presence of different additives in the low molecular weight region*

In practice

Detectors for SEC of polymers

- Refractive index
- Viscosity
- Light scattering
- UV
- IR

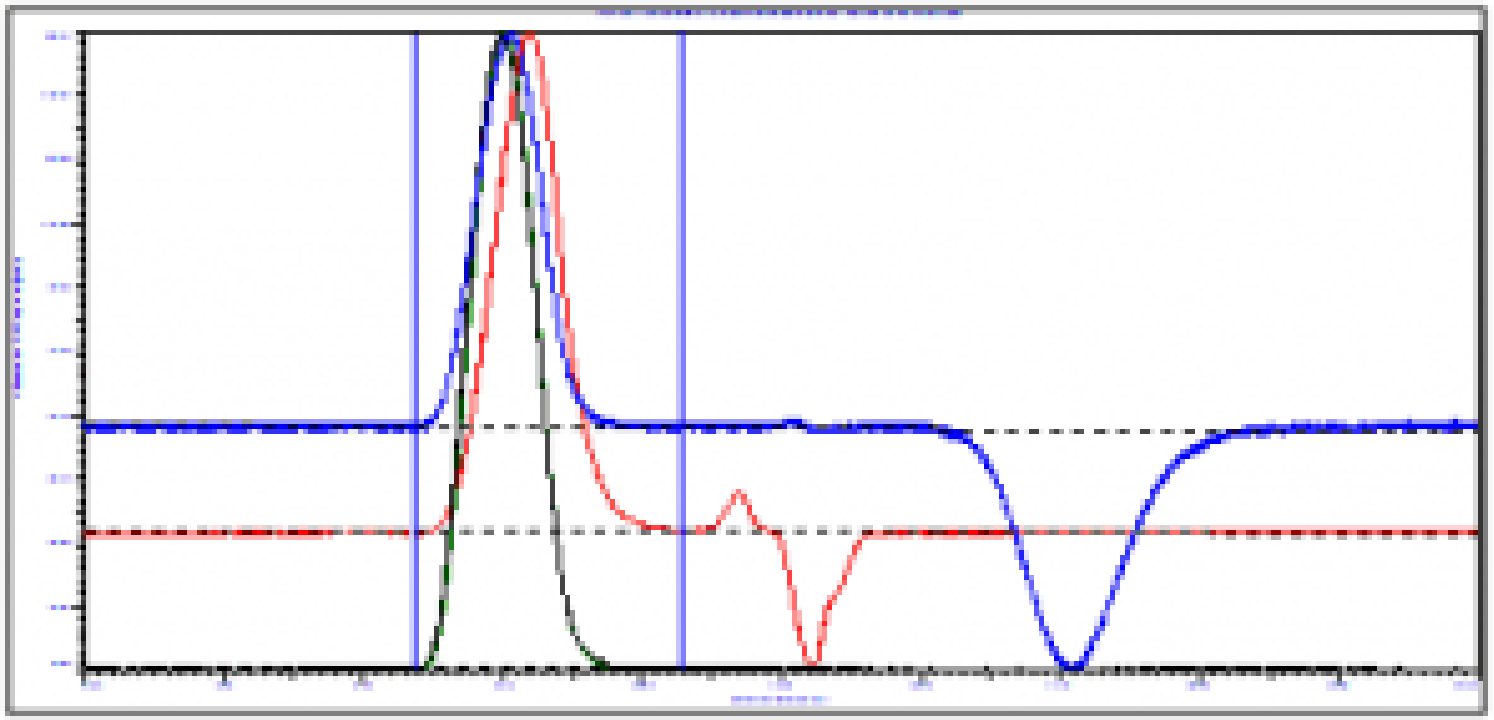


Figure 1: Refractive Index (RI) in Red, Viscometer (DP) in Blue, Right Angle Light Scattering (RALS) in Green

https://jordilabs.com/lab-testing/technique/gpc/gpc-t/

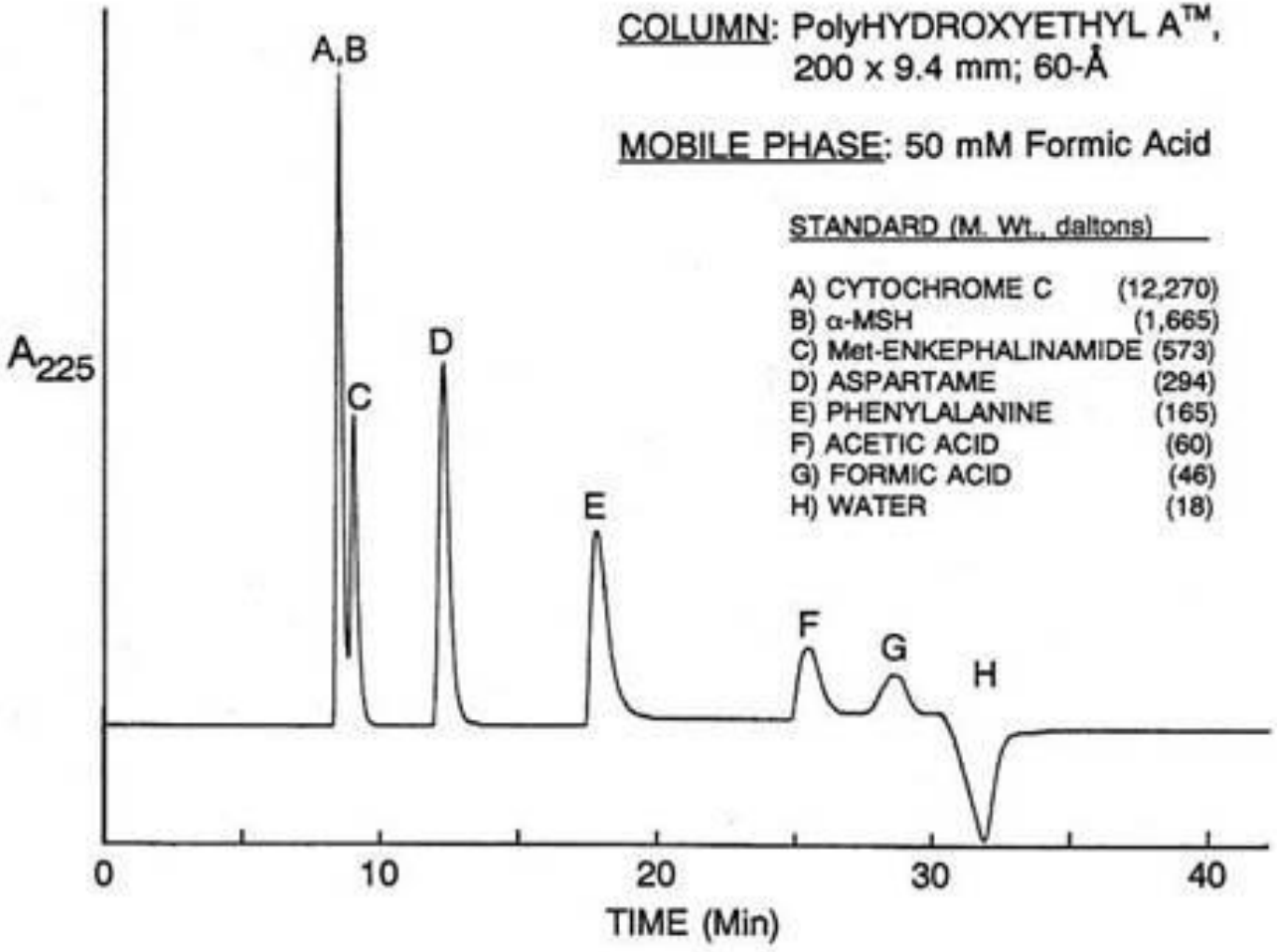
Tetra Detector Array

The Viscotek Model 302-050 Tetra Detector Array is a revolutionary, integrated multiple-detector device designed for the characterization of natural and synthetic polymers and copolymers, proteins, protein conjugates, excipients and other macromolecules. In a single GPC/SEC experiment, the Tetra Detector will provide:

- Absolute molecular weight without the assumptions, extrapolations or corrections required by alternative techniques.
- Molecular size expressed as Hydrodynamic Radius (Rh) to less than 1 nm and Radius of Gyration (Rg).
- Intrinsic viscosity or molecular density.
- Information on structure, conformation, aggregation, branching and copolymer or conjugate composition.

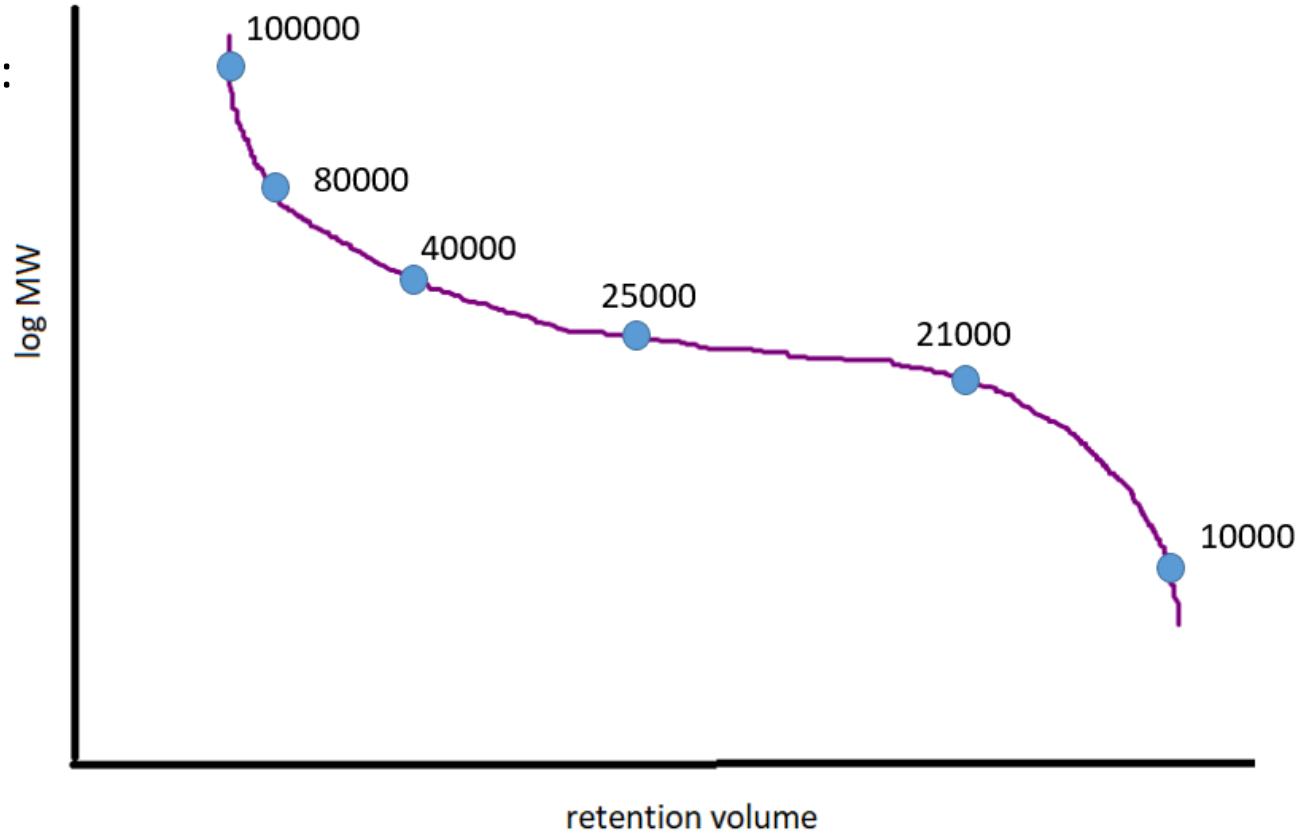
The Model 302-050 Tetra Detector Array consists of a Differential Refractive Index (RI) Detector, UV, four-capillary Differential Viscometer Detector and a Low Angle Light Scattering (LALS) Detector. All the detectors reside within a temperature-controlled compartment which also has space for 3-5 analytical GPC columns. This arrangement minimizes inter-detector volumes to reduce band-broadening effects and insures that detectors, inter-detector tubing, columns and sample reside at the same temperature throughout the course of the analysis.

Application: detection of small components in a cytochrome-c extract



Questions

1. Given this SEC curve:



- Draw a chromatogram for the following mixture of proteins:
ß-galactosidase, 120 kDa; bovine serum albumin, 67 kDa; turkey albumin, 40 kDa; phosphorylase B, 24 kDa; lysozyme, 14 kDa; insulin, 5.8 kDa, encephalin, 0.55 kDa.

- Is it possible to improve the separation and how?

2. A manufacturer indicates a 125% volume swelling when agarose gel is mixed water.

   Calculate V_m for a 1 L column.