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Liquid Chromatography of Synthetic Polymers

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LIQUID CHROMATOGRAPHY OF SYNTHETIC POLYMERS

(Short Course 2008 – ultra short version)

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Outline

- Introduction - why and how: high performance liquid chromatography of synthetic polymers (polymer HPLC)

  - scope of the short course
- Basic terms
- Retention mechanisms in polymer HPLC
- Size exclusion chromatography (entropic polymer HPLC)
- Coupled polymer HPLC (entropy-enthalpy combinations)
  - ☐ - critical conditions
  - ☐ - limiting conditions
  - ☐ - eluent gradient elution
  - ☐ - temperature gradient elution

  } "barrier" methods
- Enthalpy dominated HPLC of oligomers
- Full retention-elution procedures
- Two dimensional polymer HPLC

Synthetic polymers

End-use properties of polymeric materials depend

- on molecular characteristics of their building species
- on mutual arrangement of macromolecules in solid state (heavily affected also by the processing method)
- on nature and amount of admixtures (low molar mass substances, other macromolecules, solid particles and/or fibers ...)

⇨ Surprisingly little attention is paid in many research laboratories.

➤ - to molecular characteristics of polymers used in their studies

➤ - to exactly characterize products of their syntheses/transformations on molecular level

⇨ Usually, only size exclusion chromatography is used – and macromolecular admixtures, for example the unwanted products of polyreactions, are ignored.

Polymer solutions

- Electroneutral macromolecules vs. (potentially) charged macromolecules – polyelectrolytes; counter-ions; pseudopolyelectrolytes

⇨ Polymer solutions

- statistical coils
- worms
- rods
- globules
- .....

- Flory-Huggins interaction parameter χ

$$\chi = \frac{V_1(\delta_1 - \delta_2)^2}{RT}$$

Polymer solutions, *cont.*

- Kuhn-Mark-Houwink-Sakurada viscosity law

$$[\eta]=KM^{a}$$

where **K** and **a** are constants; exponent a describes thermodynamic quality of solvent for polymer

- $[\eta] \cdot M = V_h$ – "hydrodynamic volume" of polymer coils (Benoit) – a parameter describing hydrodynamic properties of macromolecules ("universal" SEC parameter). Chemically different **coiled** macromolecules in different solvents with equal V_h behave equally in the SEC column provided enthalpic interactions are absent

Molecular characteristics of polymers

- - molar mass [g/mol], MM (NOT molecular mass!)
- - chemical structure (composition) (CC) in terms of basic structural units (not C, H, N, O ...). Also functional groups: their nature, number per macromolecule, arrangement ...
- - physical (molecular) architecture, (MA); linear vs. branched macromolecules (vs. crosslinked systems). Short vs. long branches; stereoregularity; topology (head-to-head); length of sequences in copolymers
- - charges: nature, number per macromolecule, position and arrangement

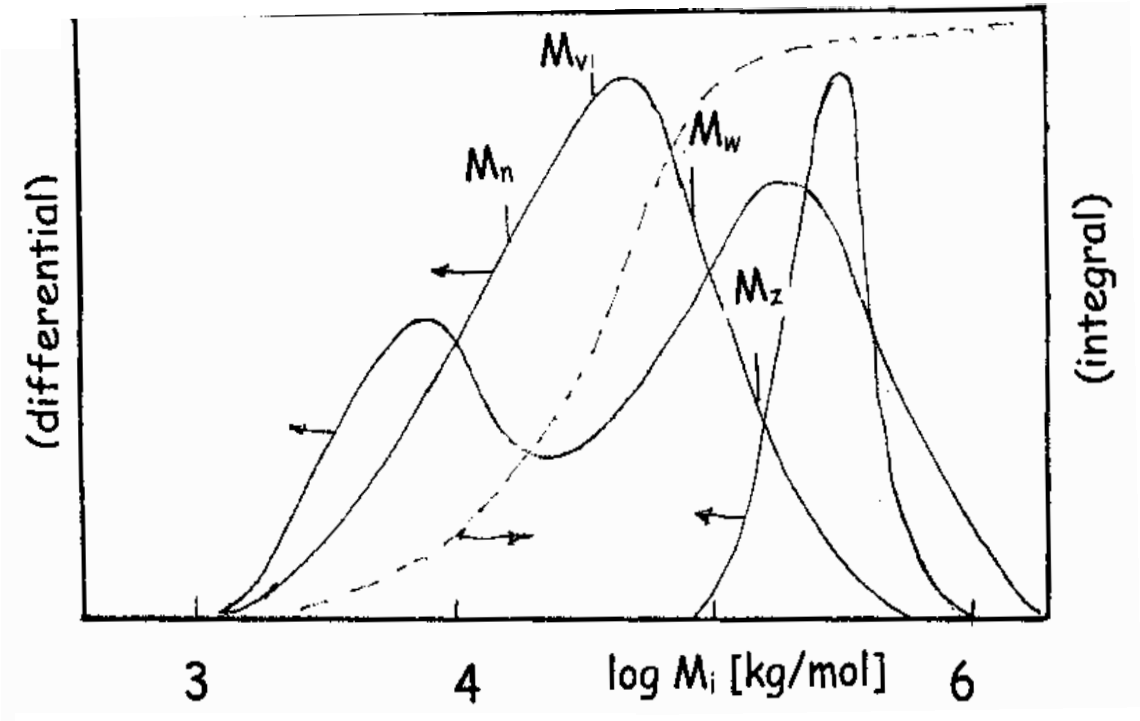
⇨ Averages and distributions:

- - mean molar mass (MMM), molar mass distribution (MMD)
- - mean chemical composition (MCC), chemical composition distribution (CCD)
- - mean molecular architecture (MMA) and its distribution (MAD), e.g. mean sequence length in copolymers and its distribution, mean tacticity and its distribution, etc.

➤ if more than one distribution is present (molar mass distribution is always present!) ➡ complex polymer systems

Molar mass distribution

- Functional dependence expressing how many (N_i = number) macromoleucles within polymeric material possess particular molar mass M_i (number fraction p_i *versus* log M_i) or what is the mass of macromolecules possessing particular molar mass M_i (mass fraction q_i *versus* log M_i).



$$p_i = \frac{N_i}{\sum N_i}$$

$$\overline{M}_n = \sum_i p_i M_i$$

$$q_i = \frac{N_i M_i}{\sum N_i M_i}$$

$$\overline{M}_w = \sum_i q_i M_i$$

Chromatography of polymers

Separation of macromolecules based on their

- hydrodynamic properties
- size
- enthalpic interactivity

} chromatography

- Gas chromatogragraphy of degradation products including products of pyrolysis or volatile low molecular admixtures
- Liquid chromatography, (high performance liquid chromatography – polymer HPLC) direct determination of mean values (averages) and distributions of molecular characteristics: parameters characterizing distributions or entire distribution functions
  - Two phases – static: layer (in TLC) or bed (in column LC)
- (Column) packing. Gel, sorbent, ion exchanger ... Carrier and (bonded) stationary phase

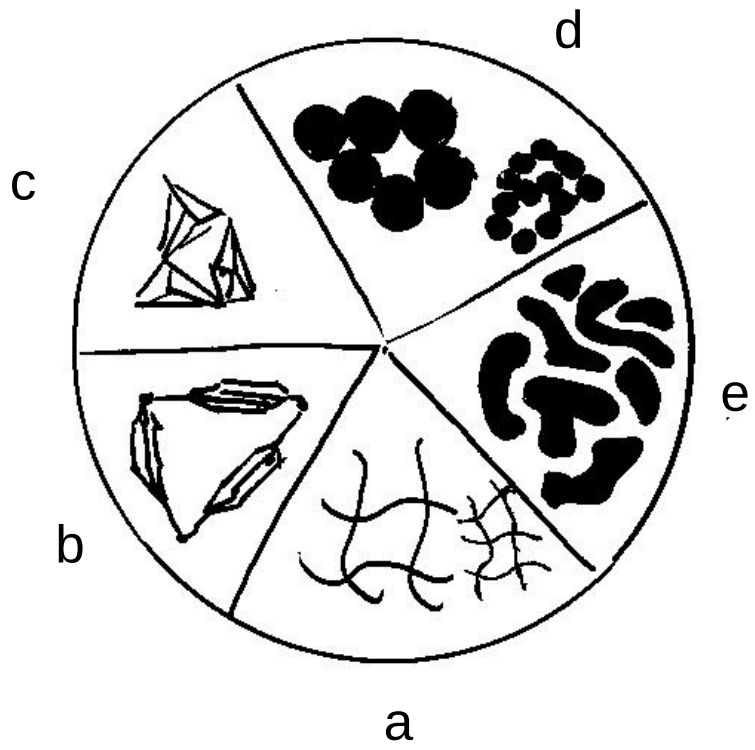
- Mobile phase (eluent) transports sample (analyte) along column

(liquid, gas, supercritical fluid, plasma)

- Selective retention of macromolecules due to differences in

  - **size** (size exclusion chromatography, gel permeation chromatography, gel filtration chromatography, ...)
  - **interactions with column packing** controlled by mobile phase and temperature (pressure): adsorption, partition, ionic effects
  - intermolecular interactions among macromolecules in samples including ionic effects: association, aggregation; coil expansion, coil collapse
  - intramolecular interactions among segments of the same macromolecule, also ionic effects
  - ionic interactions between macromolecules and packing: exchange, inclusion, exclusion
  - **interactions between macromolecules and mobile phase:** thermodynamic quality of solvent (mobile phase) toward macromolecules - shrinking or expansion of polymer coils, phase separation, enthalpic partition

Column packings for polymer HPLC
Physical structure of packing matrices



a) homogeneously crosslinked polymer
b) arrays of crystallites
c) arrays of crystalls
d) arrays of nonporous nanospheres (noduli)
e)sponge-like structure

Pore sizes above 2 nm; averages 6, 10, 30 nm (50 up to 400 nm for SEC)

Chemical structure of packing matrices

- Inorganic and organic polymers
- Inorganic: Oxides of metals – silica, zirconia, titania, alumina; alumosilicates, etc.
- Organic: Polysaccharides, poly(styrene-co-divinyl-benzene)s, poly(divinylbenzene)s, poly(acrylate)s, poly(methacrylate)s, poly(urethanes), ...)
- Carbon – (amorphous – glassy carbon), graphite
- Composites – mechanically strong (inorganic) matrices combined with organic polymers (P(S-co-DVB), poly(siloxane)s, poly(acrylate)s and poly(methacrylate)s.

- Purity of matrices – crucial for silica gels (A, B,... C ...)
- Problems with matrices suitable for SEC of cationic polymers

HPLC of synthetic polymers

- Retention mechanisms

$$V_R \sim K \frac{V_s}{V_m} = \exp\left(-\frac{\Delta G}{RT}\right)\frac{V_s}{V_m}$$

where V_R is retention volume, K is distribution coefficient defined as $K=c_s/c_m$ with c_s and c_m = concentrations of solute in stationary and mobile phase, respectively, V_s and V_m are volumes of stationary and mobile phase, respectively. ΔG is change of **Gibbs function** due to transfer of solute between mobile and stationary phases, R is gas constant and T temperature

HPLC of synthetic polymers, *cont.*

$$\ln V_R \sim \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \frac{V_s}{V_m}$$

where ΔH and ΔS are changes of enthalpy and entropy associated with solute transfer between mobile and stationary phases, respectively.

- Formally, one can speak about enthalpic and entropic contribution to retention volume.
- Division of HPLC processes into enthalpic and entropic is very useful though it alone says little about the processes taking place on molecular level within column.

Size exclusion chromatography of polymers (SEC)

The special case: $\Delta S \neq 0, \Delta H \sim 0$

Size exclusion chromatography (SEC), gel permeation chromatography, gel filtration chromatography, exclusion polymer HPLC ...

- *Vaughan* (1946), *Lathe & Ruthwen* (1951), *Porath & Flodin* (1959);
- *Moore* (1964); *Benoit* (1967); *Casassa* (1967) ...

- Column is packed with (spherical) **porous** particles. Pore sizes match sizes of macromolecules dissolved in eluent.
- Macromolecules are separated according to their **size** in solution. Size of macromolecules can be directly related to their molar mass only in few cases, e.g. for linear homopolymers.

SEC, *cont.*

- Enthalpic interactions between macromolecules and column packings are suppressed – idealized situation

- A member of HPLC family; enormous importance & popularity → dominating position in molecular characterization of synthetic polymers. Determination of molar mass averages and distributions (exact mainly for linear homopolymers); long chain branching; limiting viscosity numbers; radii of gyration; preferential solvation in mixed solvents; association and aggregation of macromolecules ...

SEC, *cont.*

- **Advantages of SEC**

  ⇨ fast, simple, relatively cheap, high intra-laboratory repeatability (precision)

- **Drawbacks of SEC**

  ⇨ low inter-laboratory reproducibility (accuracy), often caused by a "switch-on, inject, switch-out" approach and by non-qualified operators → necessity of standardization of both measurements and data processing (e.g. base line setting)!

  ⇨ low selectivity of separation,

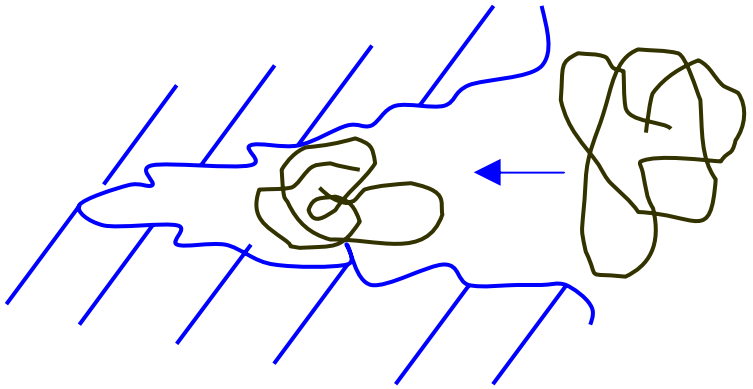
  ⇨ danger of unwanted and uncontrolled enthalpic interactions (mixed retention mechanism).

  ⇨ limited direct applicability to complex polymers exhibiting multiple distributions (molar mass, chemical structure, physical architecture)

  ⇨ low sample capacity

SEC, *cont.*

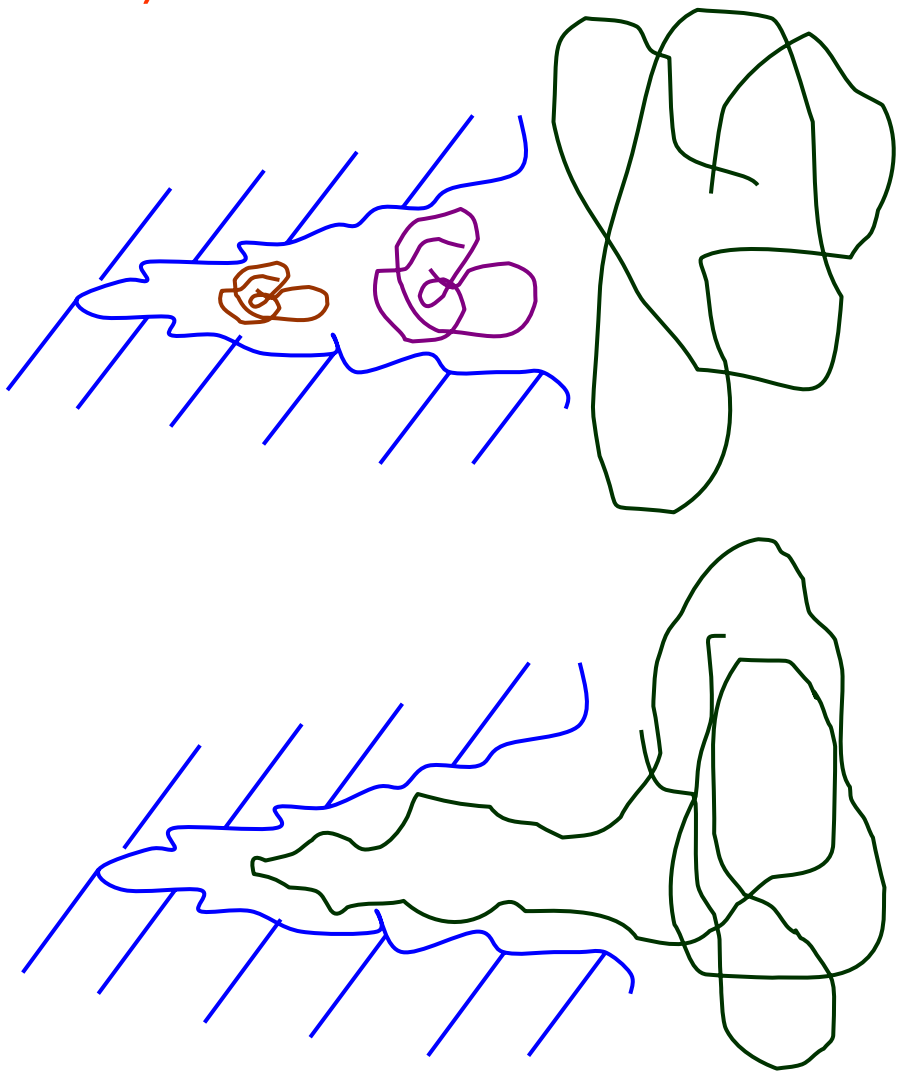
- ΔS ⇨ entropic partition of macromolecules between two chem. identical liquid phases – pore diameter vs. gyration radius of macromolecules ⇨ partial or full (?) exclusion



- In absence of attractive interactions, macromolecules are depleted from pore surface

- - mixing, as well as changes in molecular conformation (Casassa) and orientation of macromolecules
- - local concentration gradients, diffusion, flow & mixing
- - crowding of macromolecules ⇨ concentration effects

SEC, *cont.*



- *Accessible pore volume drops with increasing size of macromolecules. As result, transport of larger polymer species is faster compared to smaller one. Retention sizes decrease with size of macromolecules.*

- *Large species may partially penetrate packing pores → danger of their **degradation by shearing forces***

SEC, *cont.*
Separation mechanisms

- Basic mechanism – size exclusion
- Auxiliary mechanisms – support SEC separation
- Secondary mechanisms – enthalpic interactions - adsorption; partition; incompatibility; ion effects
- Side separation mechanisms – changes in sizes of separated macromolecules **before** or **in the course** of separation process
- Parasitic processes
- Combined effects

SEC, *cont.*

- Complicated processes in SEC columns – also partial exclusion and partial penetration

- Complicated systems ➤ dynamics of macromolecules in solution ⇨ changes of size, shape and orientation

  ➤ complicated pore sizes and shapes in real systems (fractals…)

SEC, *cont.*

Consequences

- SEC has no general and quantitative theory

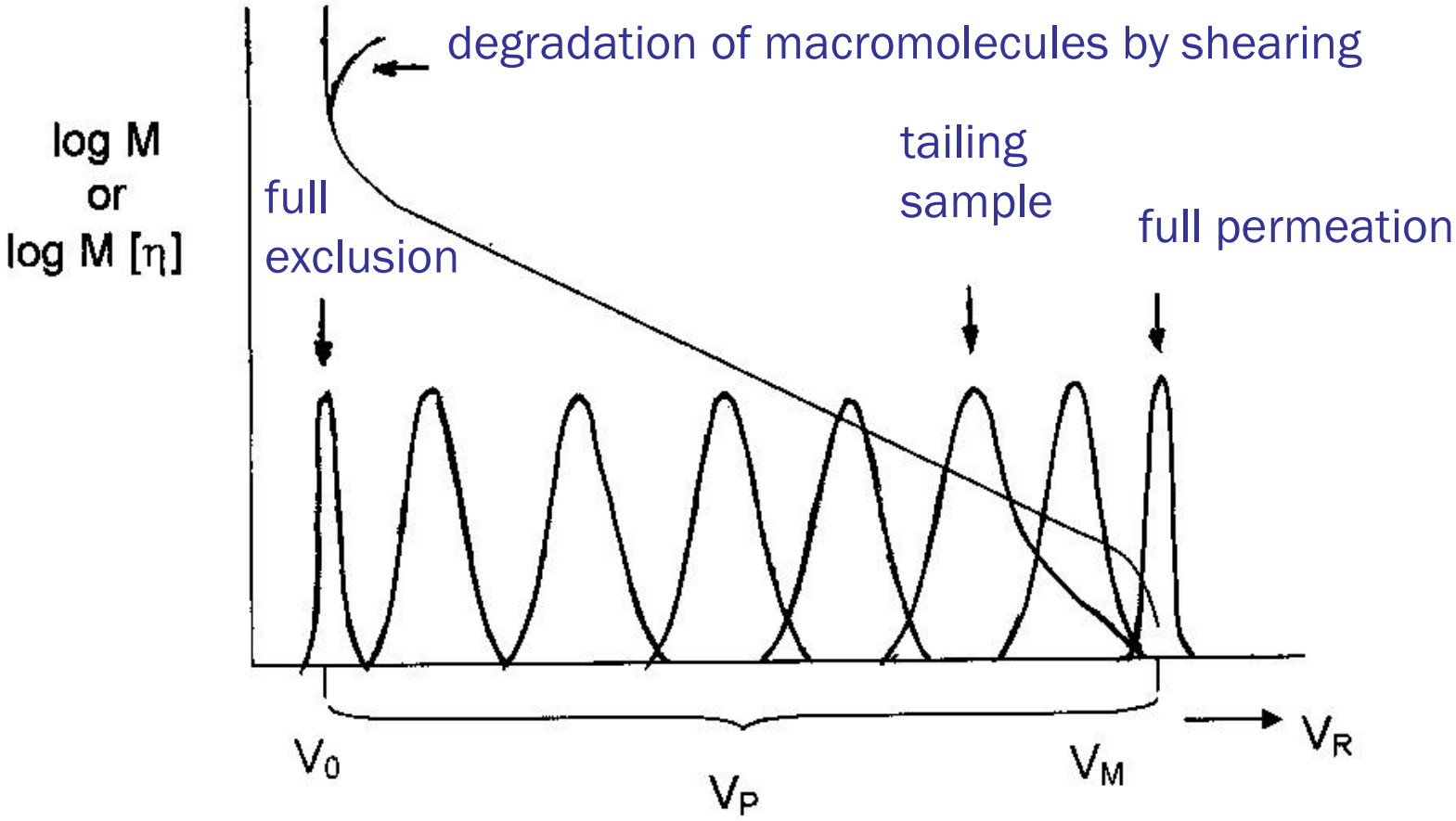
- there is no direct correlation between pore size (distribution) in column packing determined e.g. by mercury porometry and size (distribution) of macromolecules e.g. R_g determined by light scattering or viscometry (?) – [⇨ long dispute – inverse SEC]

- SEC is a relative, non-absolute method. Each column (system) must be calibrated, or molar mass of species leaving column must be monitored by special “absolute” detectors (light scattering measuring devices, viscometers, (osmometers), mass spectrometers, etc.)

SEC, *cont.*

Calibration of columns

- with a series of narrow MMD polymers with known M



SEC, *cont.* Calibration, *cont.*

In the preceding Figure:

M is the peak molar mass (most abundant in sample), $[\eta]$ is limiting viscosity number (intrinsic viscosity) of polymer and V_h is the hydrodynamic volume of given macromolecules (in given eluent, at given temperature) $M.[\eta] = V_h$, with $[\eta] = KM^a$ (for linear, coiled macromolecules) where **K** and **a** are constants for given polymer and given solvent at given temperature. They are tabelated for many systems

KMHS viscosity law is valid above M ~ 10^4 g.mol^{-1}

This is important limitation of universal calibration approach for lower molar masses

SEC, *cont.* Calibration, *cont.*

- V_h governs retention of macromolecules in SEC. It is called Benoit's universal parameter. In absence of enthalpic effects, the same dependence of log V_h vs. V_R holds for a given column for any polymer and any eluent and any temperature

- ⇨ log V_h vs. V_R is **universal calibration dependence**

- For assessment of tendencies, also plots of log M vs. V_R can be applied. Very often, however, polystyrene based log M vs. V_R calibrations are directly used for determination of molar masses and distributions of other polymers. These "polystyrene equivalent molar masses" do NOT represent absolute values!

SEC, *cont.*

Problems:

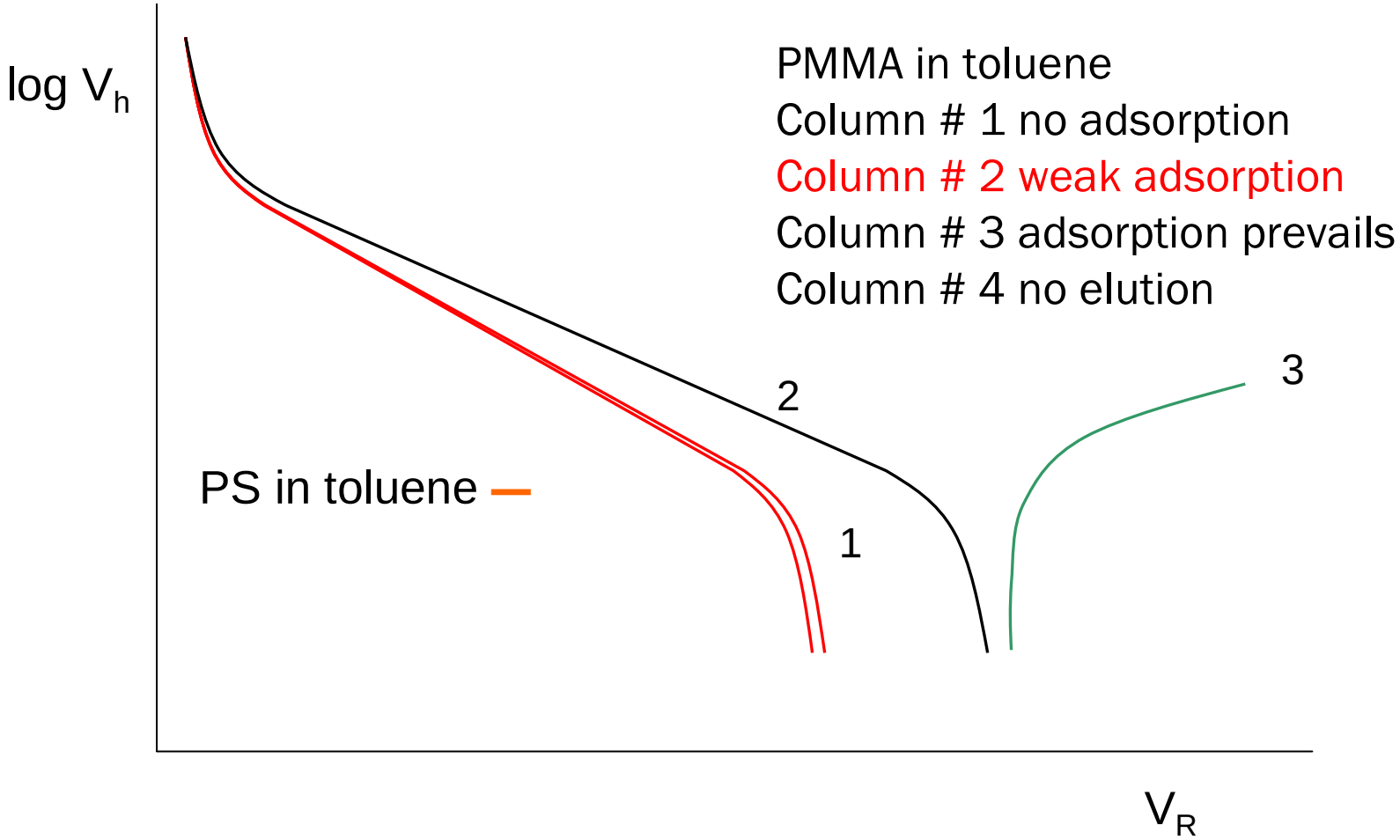
- Effects of sample volume v_i and sample concentration c_i on V_R
- Unwanted and non-controlled enthalpic interactions
- Changes in retention volumes and often also peak broadening
- ⇨ due to enthalpic effects (V_Rs usually increase)

  ⇨ due to changes in pore volume caused by macromolecules irreversibly retained within column packing (V_Rs decrease)

  Base line and peak limits setting
- Commercial columns supplied by different producers may exhibit different interactive properties even if the overall chemical compositions of their packing is “identical” – e.g. poly (styrene-co-divinylbenzene) gels.

➤ These effects are often overlooked

Calibration dependences for various polystyrene/divinylbenzene commercial SEC column packings



SEC, *cont.*

- Solutions:

⇨ Additives in eluents, which suppress enthalpic effects: a non universal remedy, appearance of system peaks, data processing problems, e.g. with MALS

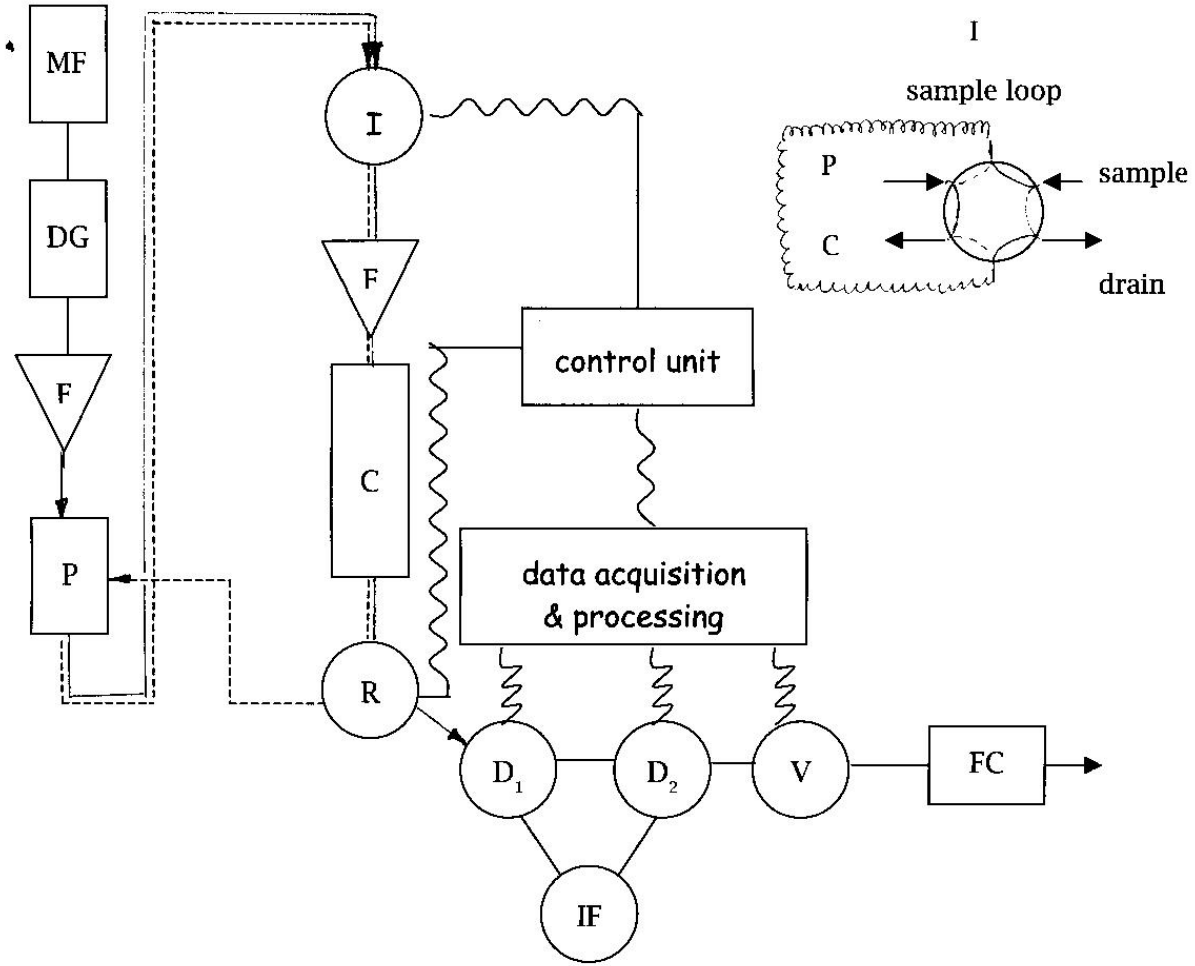
⇨ Matching column packing polarity toward both sample and eluent

⇨ Matching (single) eluent: strength and quality toward both sample and packing. Eluent must be strong and good – interacting strongly with both sample macromolecules and column packing to suppress interactions of macromolecules with column packing

⇨ Polarity of separated polymer, eluent and column packing should be similar, system should be as symmetrical as possible

- Changing column properties during their application ⇨ large errors in M values determined

A scheme of polymer HPLC (SEC) instrument



SEC, *cont.*

- Three sets of data are monitored in SEC:
  retention volume
  sample concentration } *conventional approach based on calibration*
  sample molar mass (light scattering, viscosity)

- Several other parameters are added in other methods of polymer HPLC – composition, stereoregularity, etc.

- Pumps
- Pulseless, highly precise and accurate, very constant & resettable flow rate allows working on the time scale. High pressure (30-40 MPa), corrosion resistance, low wear.

SEC, *cont.*

- Detectors
- Assessment of
  - concentration/mass of analyte in column effluent
  - specific properties of analyte
    - - composition, molar mass, stereoregularity ...

flow-through measuring cells

Differential refractometers

- Deflexion (Snellius); reflexion (Fresnel);
- Christiansen principle; interferometers

Photometers

- Ultraviolet; visible; infrared; fluorescent

NMR spectrometers

Detectors with sample transfer

- MALDI
- pyrolysis ➔ GC

Mass spectrometers - electrospray MS

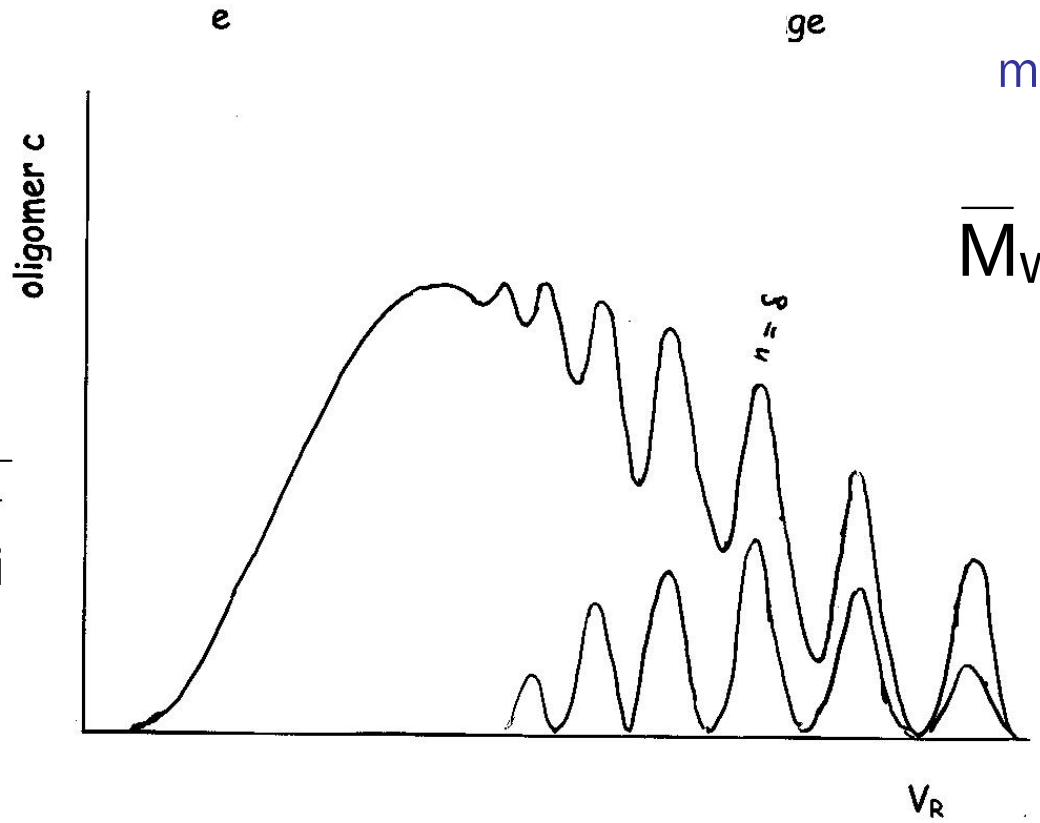
Absolute detectors - light scattering, viscometry, (osmometry)

Evaporative light scattering

SEC, *cont.* Processing of results - oligomers

number average

$$\overline{M}_n = \frac{1}{\sum_1^k c_i / M_i}$$

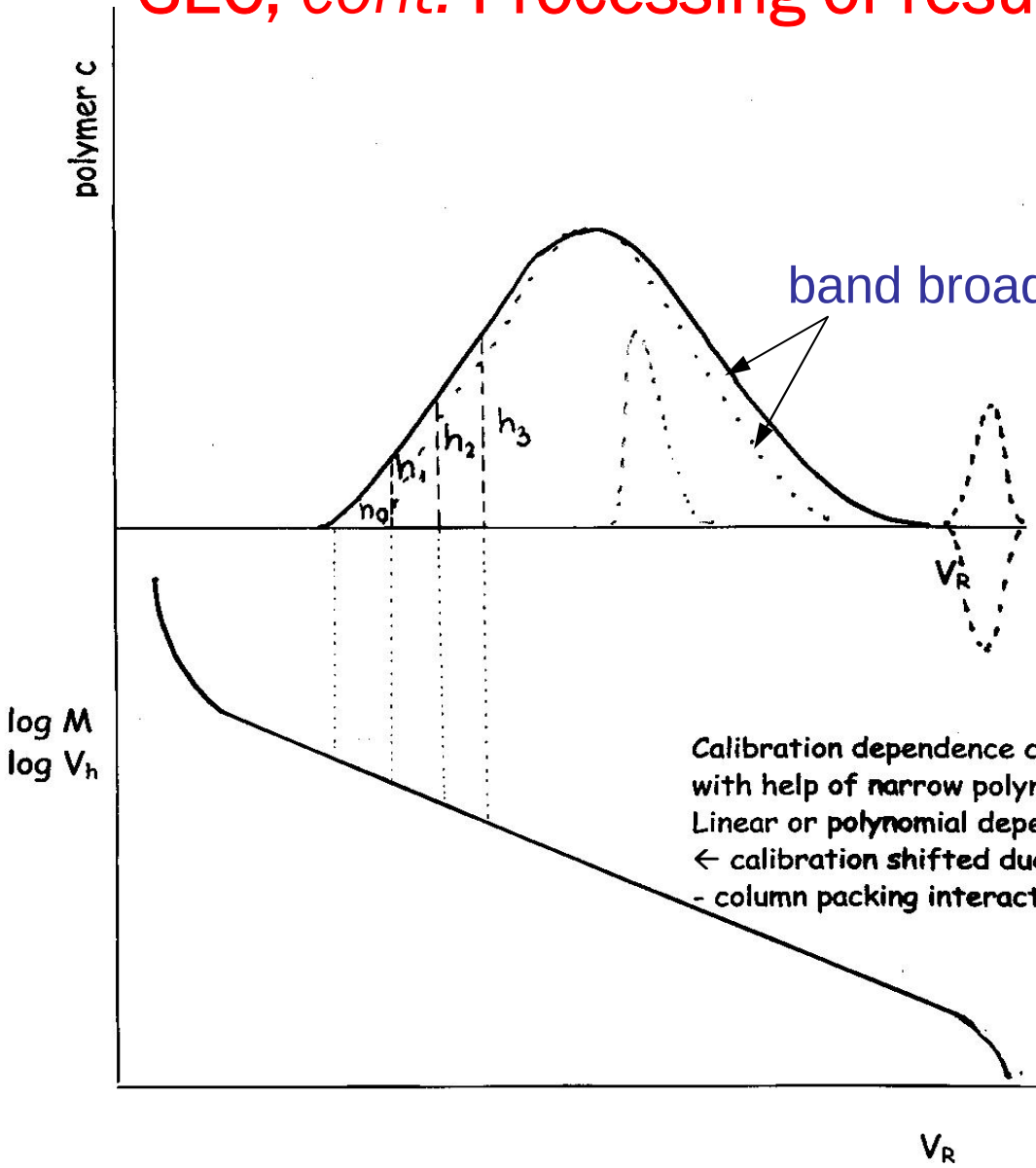


mass (weight) average

$$\overline{M}_W = \sum_1^k c_i M_i$$

Each peak belongs to a particular oligomeric species. Peak identification, deconvolution, $\overline{M}_n$, $\overline{M}_W$ calculation. End group effects: Detector response depends on M ➔ corrections needed. Extent of preferential solvation in two-component eluents changes with M ➔ corrections needed.

SEC, *cont.* Processing of results - polymers



-air,
- low molar mass impurities
- system peak

$$c_i = \frac{h_i}{\sum h_i}$$

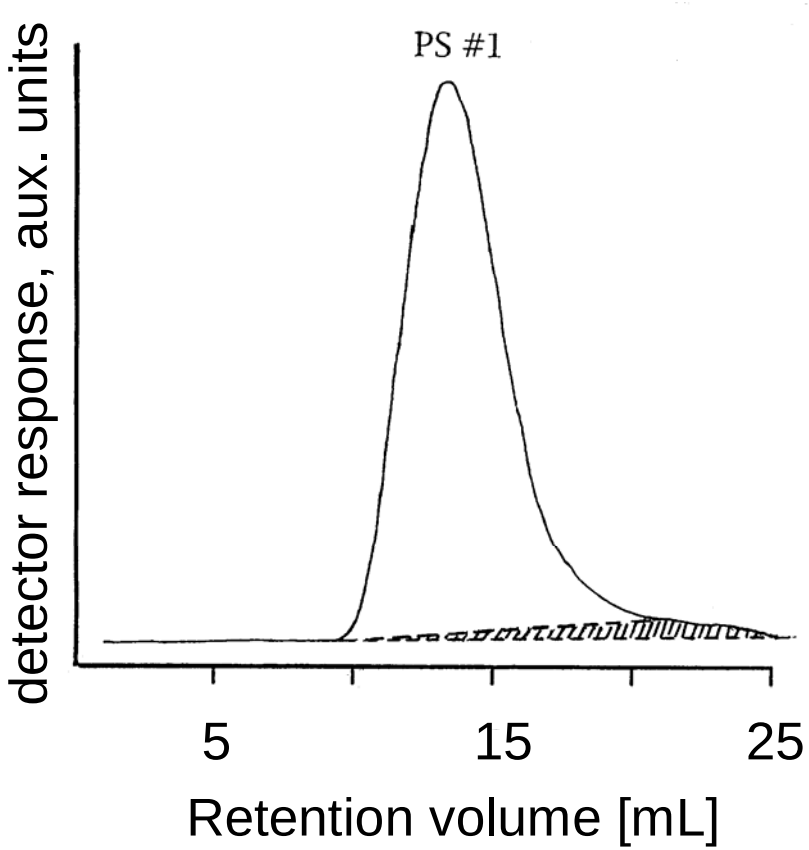
SEC, *cont.* Processing of results, *cont.*

- Calculation by computer, commercial softwares

- Correction for
  - chromatographic band (zone) broadening
  - (for branching effects)
  - for concentration effects
  - for secondary separation mechanisms

- Important problem: base line and peak limits setting
- Necessity of separation of low molar mass impurities and air (oxygen) dissolved in sample solution from macromolecules

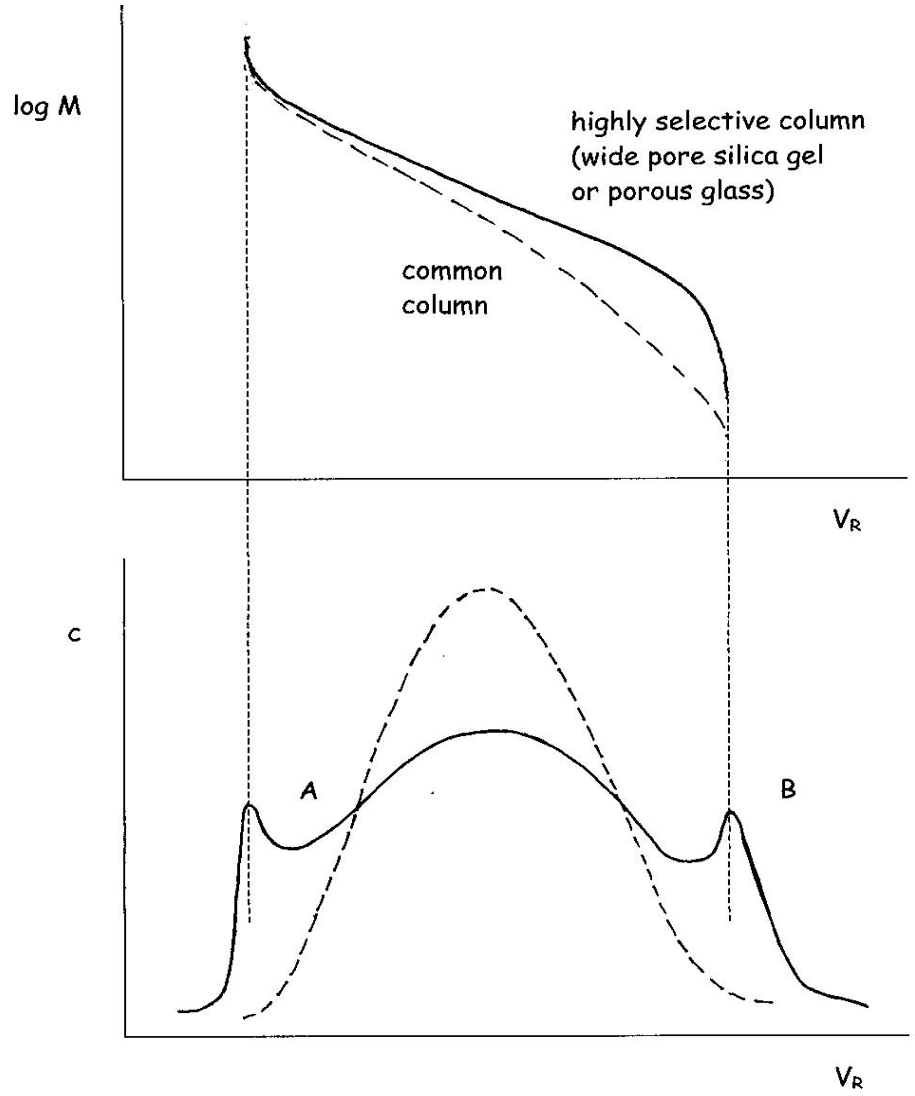
SEC, *cont.* Processing of SEC results, *cont.*



Sample contained
low molar mass
tail, down to
oligomer area. If ignored:
M_w = pract. unchanged
M_n = increased

Role of base line setting. M_n values calculated applying alternatively two base lines indicated differ 8x (800%)! Evaporative light scattering detector was used to monitor chromatogram. With a differential refractometer, peaks of low molar mass impurities and oxygen, as well as possible system peaks appeared in the V_R area of 23 mL.

SEC, *cont.* Accumulation peaks in SEC



"A" and "B" are the accumulation peaks due to inappropriate choice of the SEC column packing selectivity. "A" type of accumulation peaks is rather common. Such peaks are often (erroneously) ascribed to"sample bimodality"

SEC, *cont.* Applications of SEC

- Polymers & oligomers
- Low molar mass substances: low selectivity ⇨ only for selected applications ⇨crude oil – identification ⇨ fingerprint approach
- Preseparation – sample clean up (deleting polymers) & group separations
- Conventional applications
  - determination of $\overline{M}_w; \overline{M}_n; \overline{M}_z;$ distribution parameters, distribution functions
  - preparative fractionation
  - (production of narrow MMD polymers)

SEC, *cont.* Applications, *cont.*

- Special applications
  - determination of limiting viscosity numbers $[\eta]$ and constants K and a in the viscosity law $[\eta] = KM^a$
  - assessment of long chain branching
  - polymer mixtures, copolymers (semiquantitative data)
  - charged polymers

- Unconventional applications of SEC
- Interactions polymer – solvent: (solvation); preferential solvation in mixed solvents, second virial coefficient
- Interactions polymer – polymer: incompatibility, association, aggregation
- Interactions polymer – column packing
- Segment length in polymer crystallites
- Polymerization kinetics

SEC, *cont.* Applications, *cont.*

- *Specific conditions*
  - polymers of low solubility ➔special eluents incl. multicomponent mixtures ➔ increased temperature (e.g. polyolefins)
  - biopolymers – appropriate environment to prevent denaturation; decreased temperature
  - polymers rapidly degrading in solution (e.g. poly(hydroxy butyral) ➔ fast separation and data correction
  - ultra-high molar mass polymers degrading by flow (shear degradation, from M ~ $5x10^6$ to $1x10^7$ and up): larger particles of column packing, low flow rates
  - ultra fast separation and high sample throughput ➔ on - line process control (< 1 min analyses)
  - combinatorial material science

SEC, *cont.* Applications, *cont.*

- (Polymer structure – tacticity [indirectly])
- (Unperturbed) dimensions of macromolecules in solution
- Theta conditions
- Inverse SEC: assessment of pore size distribution in porous bodies ➔ potential and problems
- Diffusion rate of macromolecules in porous bodies
- Separation of particles <20 nm (?) (danger of full retention due to lacking Brownian motion for larger particles)

SEC, *cont.* Materials

- Mobile phases
  - sample solubility, thermodynamic quality
  - (effects on biomacromolecules)
  - suppression of sample –column packing interactions
  - detector requirements (Δn, UV or IR transparency, etc.)
  - environmental aspects, incl. toxicity
  - availability & price
- Multicomponent eluents – problems with preferential evaporation, preferential sorption on the column packing, preferential solvation of sample molecules ➔ system peaks
- Most commonly used eluents – **tetrahydrofuran (THF),** di- and trichlorobenzene, dimethylformamide, hexafluoroisopropanol, water and salt solutions incl. buffers, ...

SEC, *cont.* Materials, *cont.*

- THF, the most common SEC eluent for synthetic polymers
  - ➤ easily oxidizes ➔ explosive peroxides
  - ➤ forms charge – transfer complexes with O_2 ➔ UV absorption
  - ➤ is highly hygroscopic and absorbs water from air.

  Boiling point of THF/water azeotrope, which contains about 5 wt. % of water is only 3 ^{o}C below b.p. of pure THF !!

- Calibration standards ➤ narrow or broad molar mass distribution & known molar masses (& distribution); numerous different M; different polarities

  ➤ polystyrenes; poly(methyl methacrylate)s; poly(ethylene oxide)s; (polyolefins); dextrans; pullulans; proteins ...

SEC, *cont.*
Standardization of measurements - compromises

- Precision vs. accuracy of results
- Fast vs. conventional SEC → sacrified accuracy
- Producers of column and instruments vs. users
- Standardization: **not** in terms of instruments (mainly columns)!
- Eluent nature (mainly THF) & additives. Water content!
- Calibration standards – mainly PS → universal calibration
- Detection (RI; ELS; LS; visco; MS ...)
- Injected volume and concentration & size of column
- High concentration → concentration effects; low concentration → detection problems; large injected volume → effect and on peak width on V_R; small injected volume at low concentration → detection problems

SEC, *cont.* Increasing selectivity of separation according to molar mass

- Adding more columns – impractical (time, eluent consumption)
- Increasing pore volume – limits (mechanical stability of packings)
- Recycling – limits (first fraction catches the last one)
- Reducing column separation range

  - impractical, limits

- Ultimate limit: constituents of complex polymer system possess similar or even identical molecular size in solution. Separation according to M is often impossible Therefore:

➤ combination (coupling) of entropic (exclusion) and enthalpic retention mechanisms

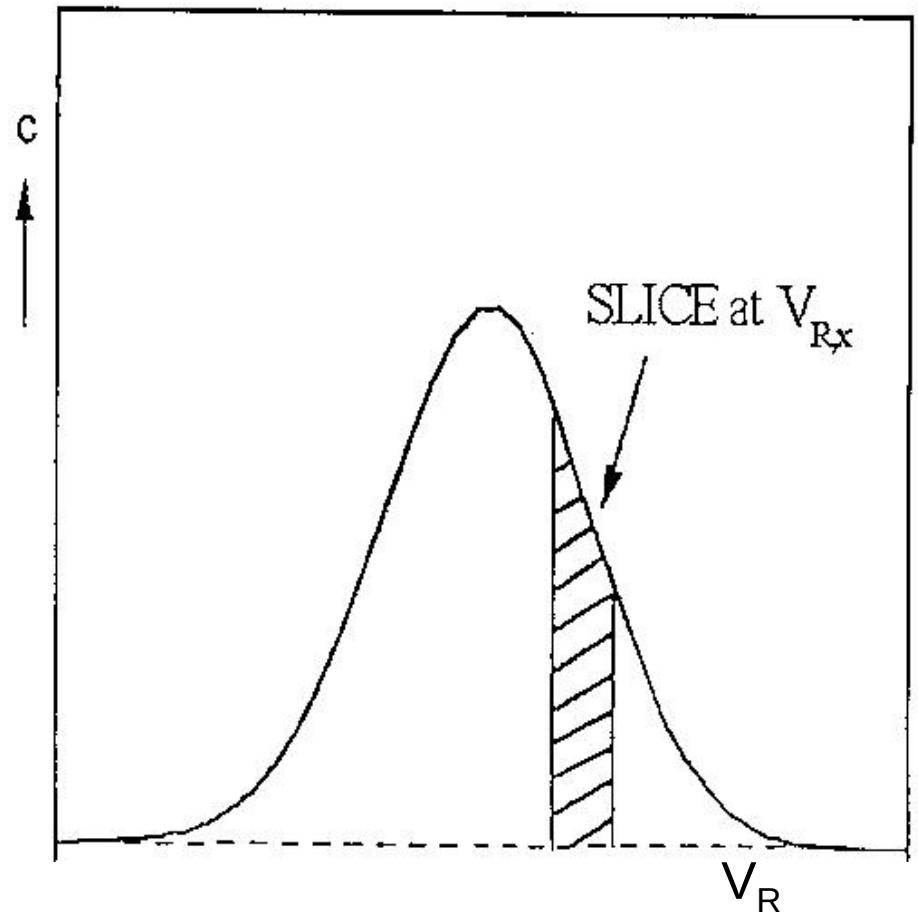
Coupled polymer HPLC methods
Combinations of entropic and enthalpic retention mechanism

Selectivity of polymer separation according molar mass, to chemical structure (copolymers, polymer blends, functional oligomers, etc.) or physical architecture (stereoregularity, etc.) can be enhanced due to controlled enthalpic interactions, or alternatively:

Effect of molar mass has to be suppressed in order to separate macromolecules mainly or exclusively according to their chemical structure or molecular architecture

Retention mechanisms based on enthalpic interactions are to be added to entropic (exclusion) retention mechanism

Limited applicability of SEC for complex polymers, *cont.*



SEC chromatogram of a copolymer. Slice contains macromolecules of the same size – but their M may differ depending on composition and on blockiness.

Coupled methods in HPLC of synthetic polymers

- The general case $\Delta S \neq 0$, $\Delta H \neq 0$

  ⇨ coupled methods of polymer HPLC (ΔS never can be zero!)

  ΔH can be either

  negative – attraction (e.g. adsorption, enthalpic partition) ⇨ increase of V_R, or

  positive – repulsion (e.g. incompatibility ion – ion repulsion) ⇨decrease of V_R

Coupled methods in polymer HPLC, *cont.*
Enthalpic interactions in coupled polymer HPLC

$\Delta H \neq 0; \Delta S \neq 0$

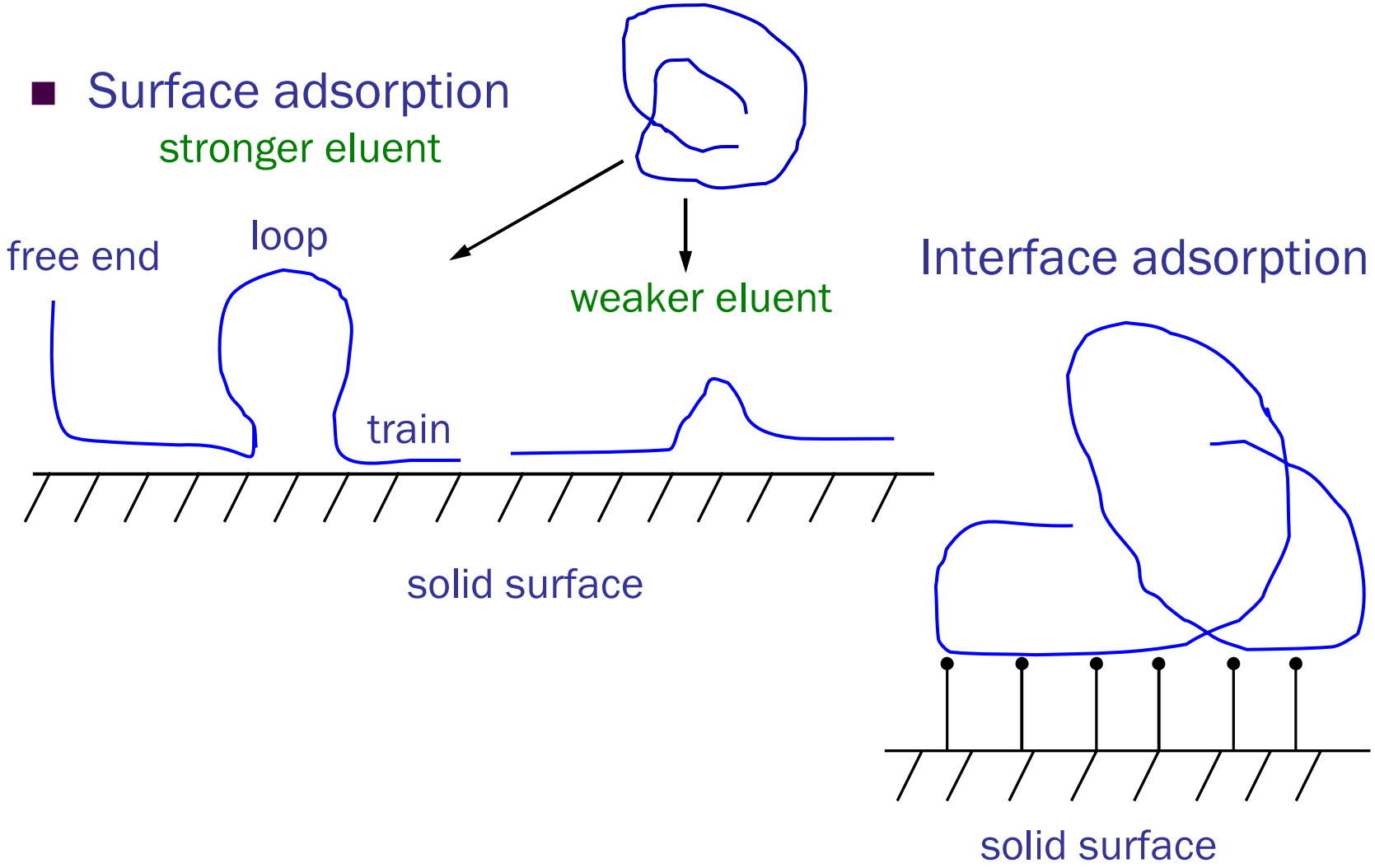
- Polymer – packing interactions ⇨ adsorption, enthalpic partition, and [ion effects] of macromolecules
- Polymer – solvent interactions ⇨ if not attractive ⇨ decreased solubility ⇨ enthalpic partition of macromolecules in favour of column packing (e.g. partition in favour of bonded phase): Macromolecules are pushed from mobile phase into the stationary (bonded) phase

➤ Insolubility of polymer ⇨ phase separation

- Packing – eluent interactions ⇨ suppress or enhance adsorption or partition

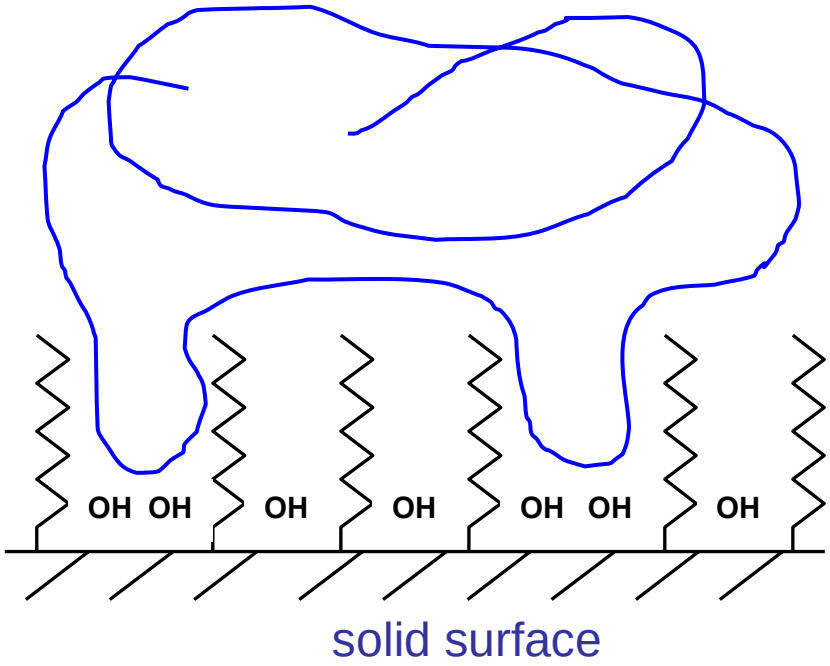
Enthalpic interactions in coupled polymer HPLC, *cont.*

- Surface adsorption

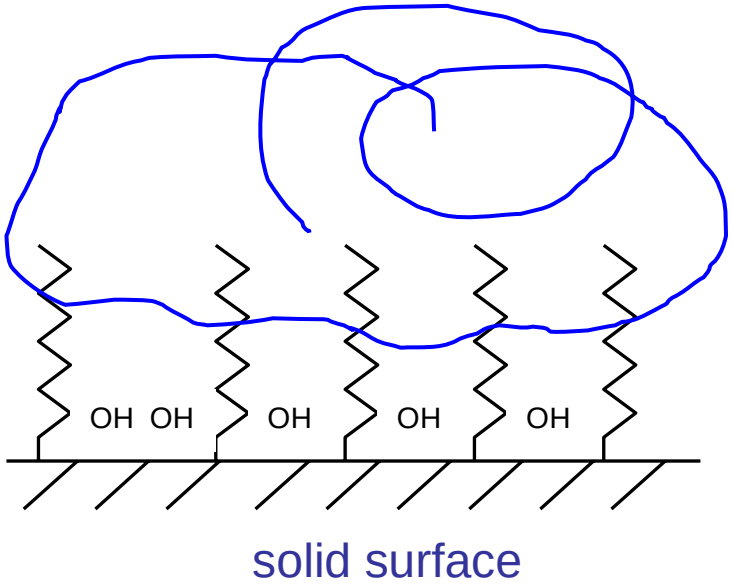


Enthalpic interactions in coupled polymer HPLC, *cont.*

U-turn adsorption



Enthalpic partition



Enthalpic interactions in coupled polymer HPLC, *cont.*

- Mobile phases

⇨ Often composed of at least two components in order to enhance sample solubility and/or stability, to improve sample detectability, to decrease price.

⇨ Sometimes unwanted admixtures present in eluent (water, impurities, products of oxidization, charge-transfer complexes with oxygen…) [salts, de-aggregating agents…]

➤ solvent strength, ε^0

➤ interactions with macromolecular samples solvent thermodynamic quality, χ, a …

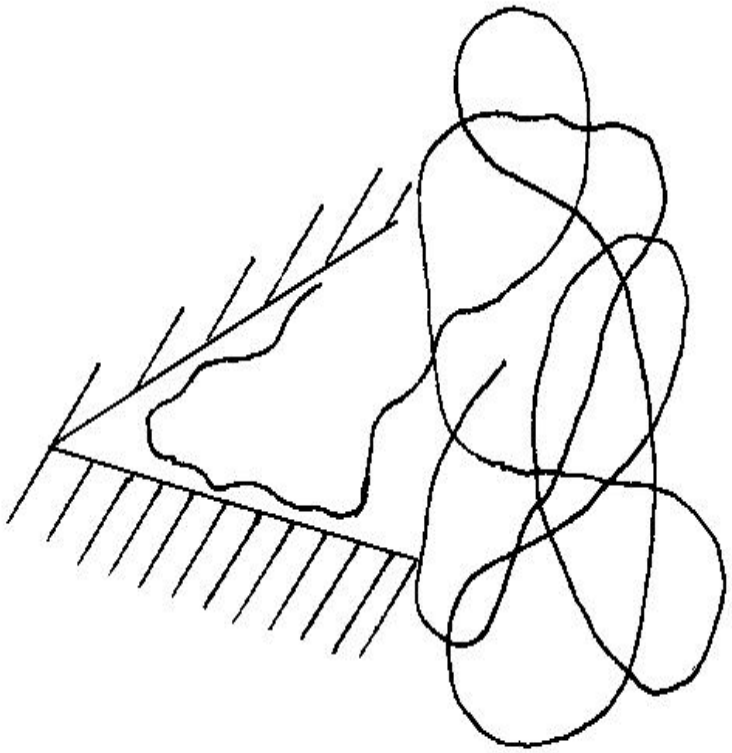
- Role of solvent – solvent interactions including temperature and pressure effects = ?

Enthalpic interactions in coupled polymer HPLC, *cont.*

- At low ε and/or high ε^0 – (ideal) SEC
- At medium ε ($\varepsilon = \varepsilon_{cr}$) entropy – enthalpy compensation: critical conditions
- At high ε ($\varepsilon > \varepsilon_{cr}$) prevailing adsorption retention mechanism – hardly applicable for isocratic elution of high polymer, however, suitable for oligomers
- The course A and B: enthalpy assisted SEC, increased selectivity of separation. Course A is applicable only for oligomers (interaction of end-groups is strong and its role diminishes with M).

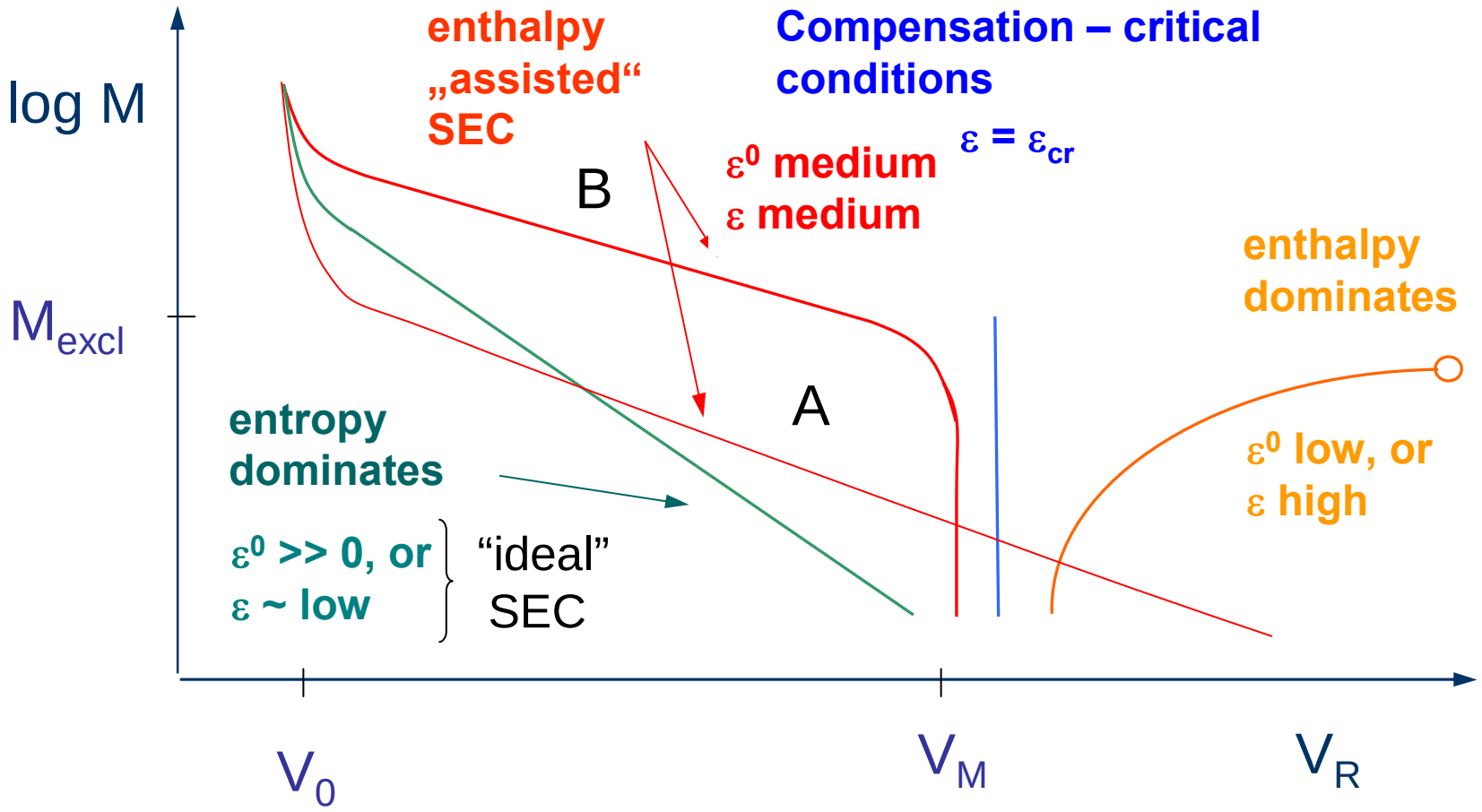
Enthalpic interactions in coupled polymer HPLC, *cont.*

If polymer-packing interactions are strong and competing interactions eluent-packing interactions are weak, macromolecules reptate even into (very) narrow packing pores



"Flower like" adsorption
(ε must be high, ε^0 rather low):
Very slow desorption of macromolecules
⇨extensive peak broadening, reduced sample recovery and changes in column retentivity ("columnhistory")

Enthalpic interactions in coupled polymer HPLC, *cont.*



Enthalpic interactions enhance or suppress size separation of macromolecules

Liquid chromatography under critical conditions *(Skt. Petersburg, 1976)*

- Critical conditions – macromolecules are chromatographically “invisible”. Their molar mass does not play any role!

- Separation of block and graft copolymers, cyclic polymers, polymer blends, oligomers according to their functionality

- Drawbacks of liquid chromatography under critical conditions: (peak broadening, sensitivity toward temperature and eluent composition variations, sample recovery = ?, ...

Barrier methods – liquid chromatography under limiting conditions, LC LC

- *Polymer Institute* SAS, Bratislava, 1995
  - Fast progression of macromolecules due to exclusion
  - Low velocity of permeating molecules of solvents

these can create slowly moving barriers „impermeable“ for macromolecules.

- Barrier can e.g. promote adsorption of polymer

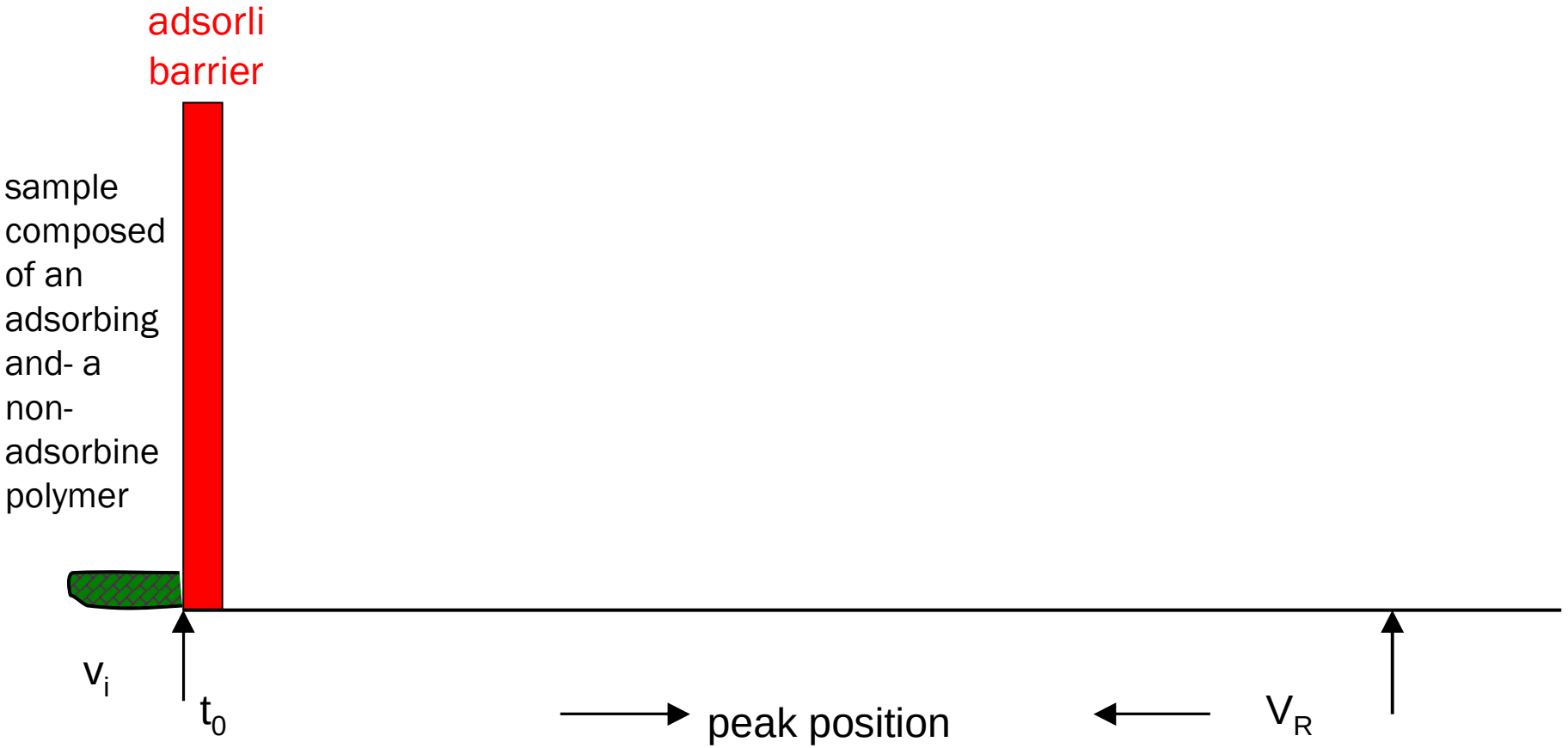
species, barrier acts as an adsorli

⇨ Eluent as a barrier, sample is injected in a desorli which prevents adsorption: LC under limiting conditions of adsorption (LC LCA).

⇨ Eluent is a desorli but sample is preceded by a zone of adsorli: LC under limiting conditions of desorption (LC LCD). Alternatively, sample is injected in an adsorli.

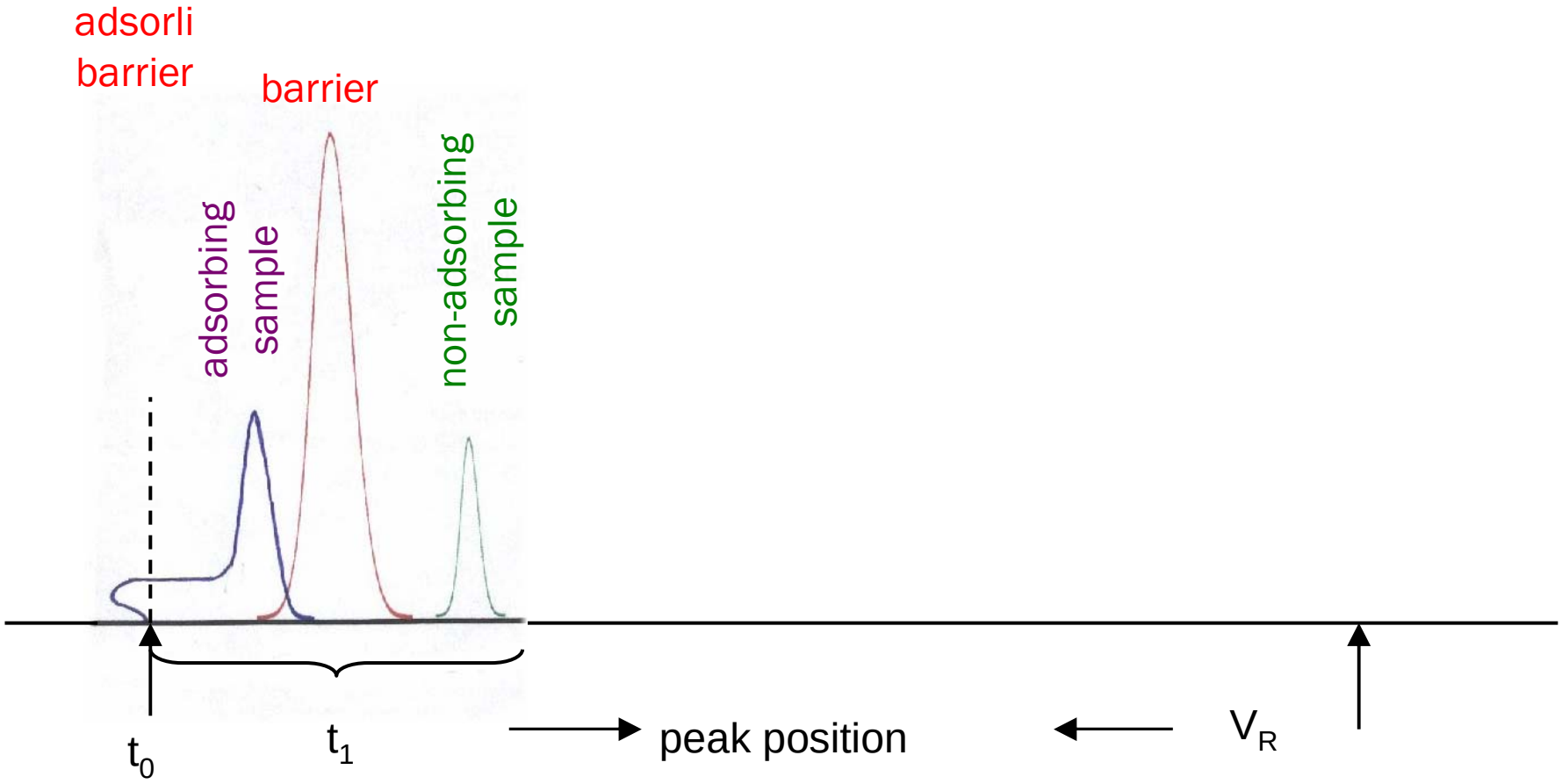
➤ Enthalpic partition and phase separation retention mechanisms can be applied, as well

Schematic representation of LC LCD separation process stages. Two component polymer blend. Column packing promotes adsorption of one polymer component.



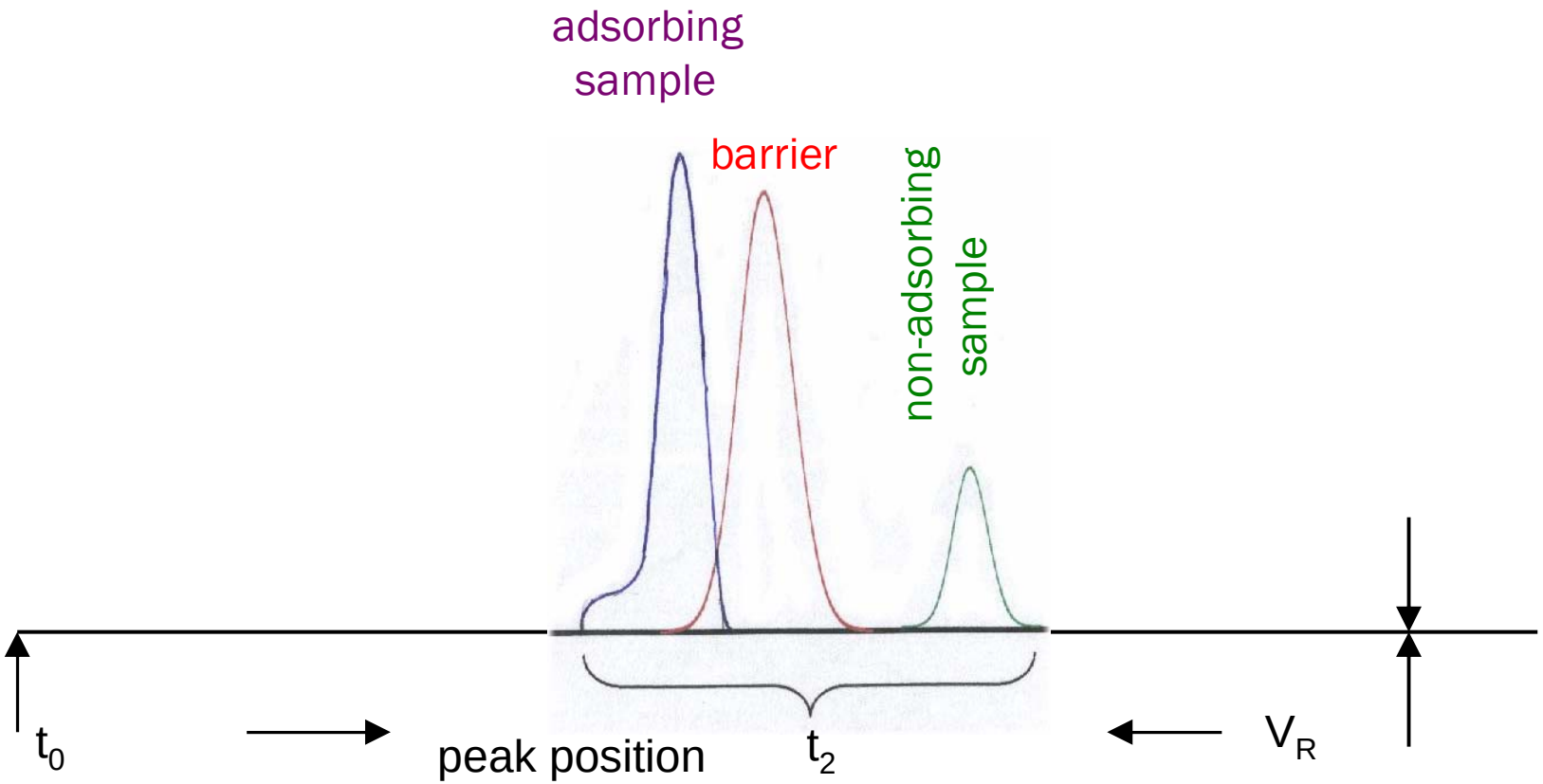
Eluent promotes sample desorption. Adsorli must be efficient enough to stop progression of one sample component due to its adsorption, while second sample component is not retained by the adsorli zone.

Schematic representation of LC LCD separation process stages. Two component polymer blend. Column packing promotes adsorption of one polymer component.



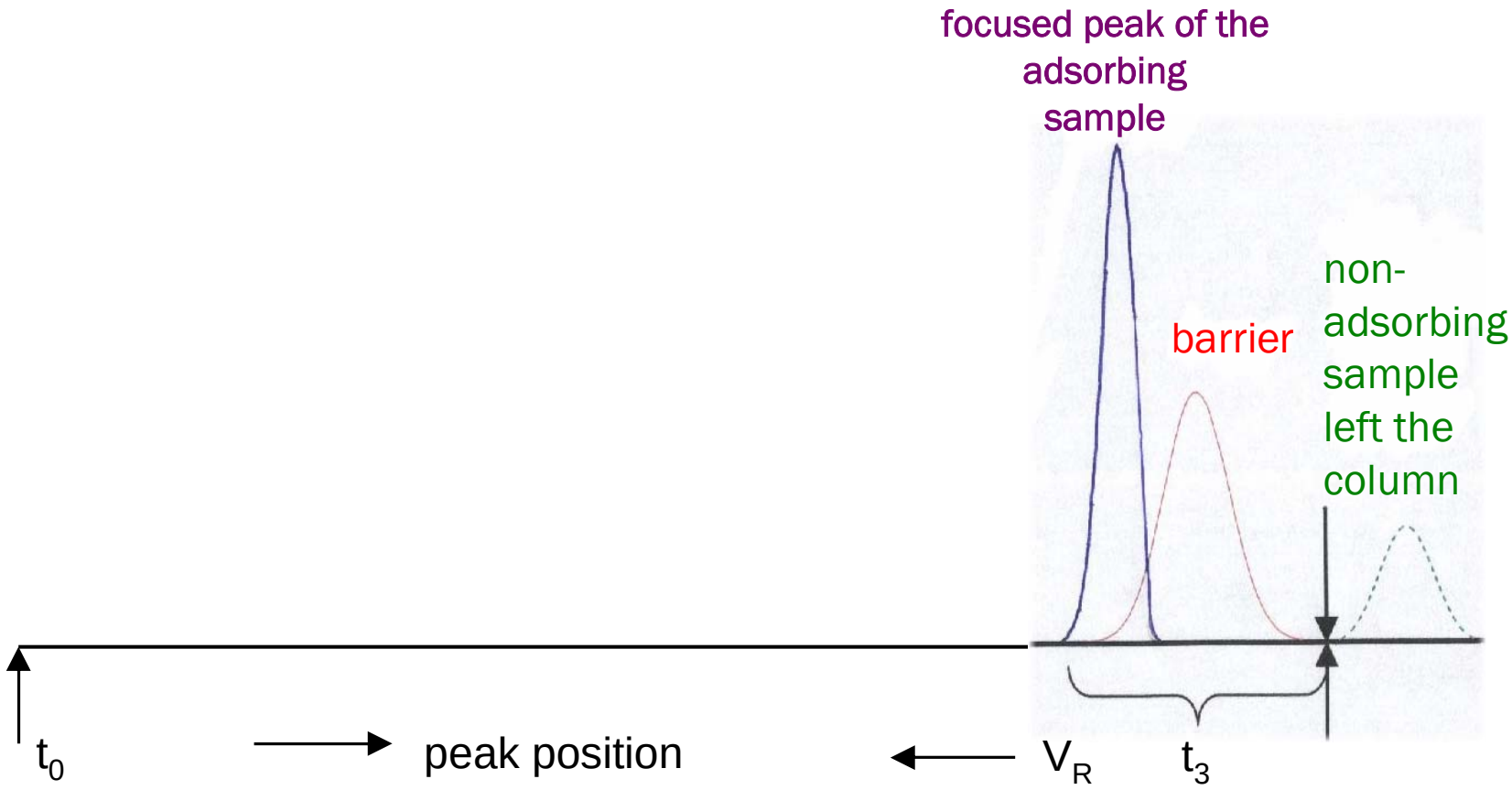
Volume of barrier is optimized by independent experiments. It should be neither too small not too large. Sample volume v_i can be extremely large, it may reach 25 % of column volume! Notice sample peak focusing and reconcentration process. Retention volume of sample does not depend on its molar mass.

Schematic representation of LC LCD separation process stages. Two component polymer blend. Column packing promotes adsorption of one polymer component.



Notice sample peak focusing and reconcentration process. Retention volume of sample does not depend on its molar mass.

Schematic representation of LC LCD separation process stages. Two component polymer blend. Column packing promotes adsorption of one polymer component.

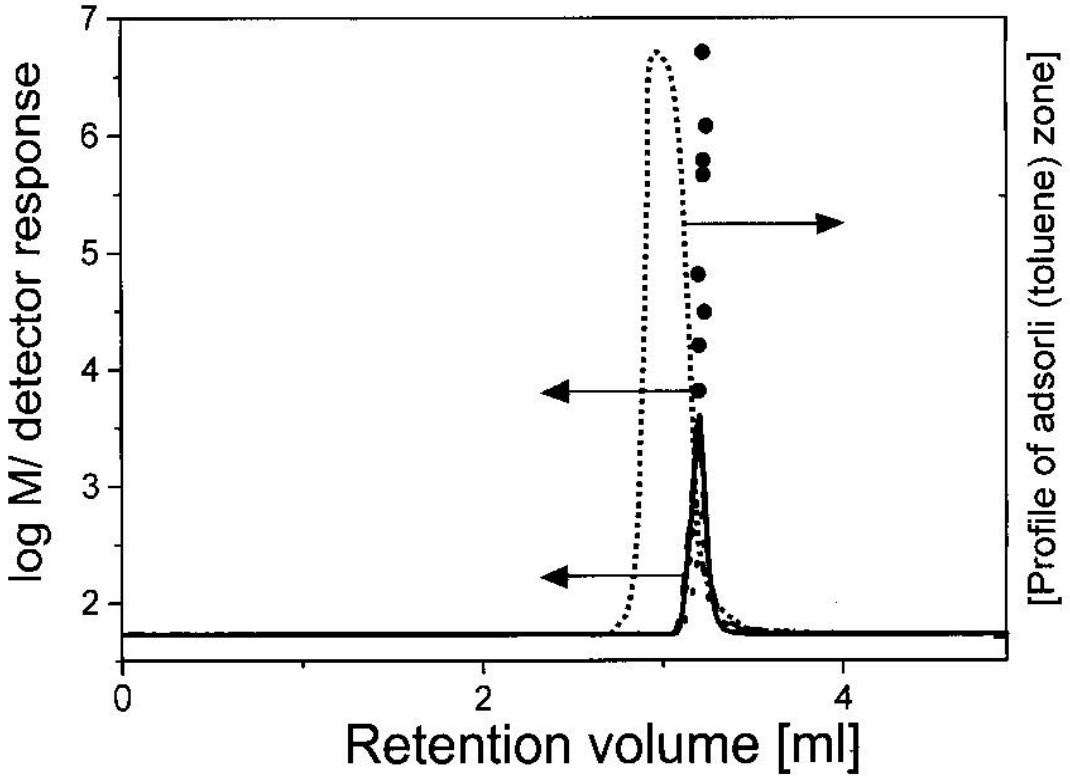


Narrow pore, large pore volume column packings are advantageous

Liquid chromatography under limiting conditions, *cont.*

Molar mass independent retention in LC LCD

Bare silica gel -
6 nm
pore diameter.
PMMA in THF/toluene
50/50 wt./wt. eluent.
Toluene sample
solvent



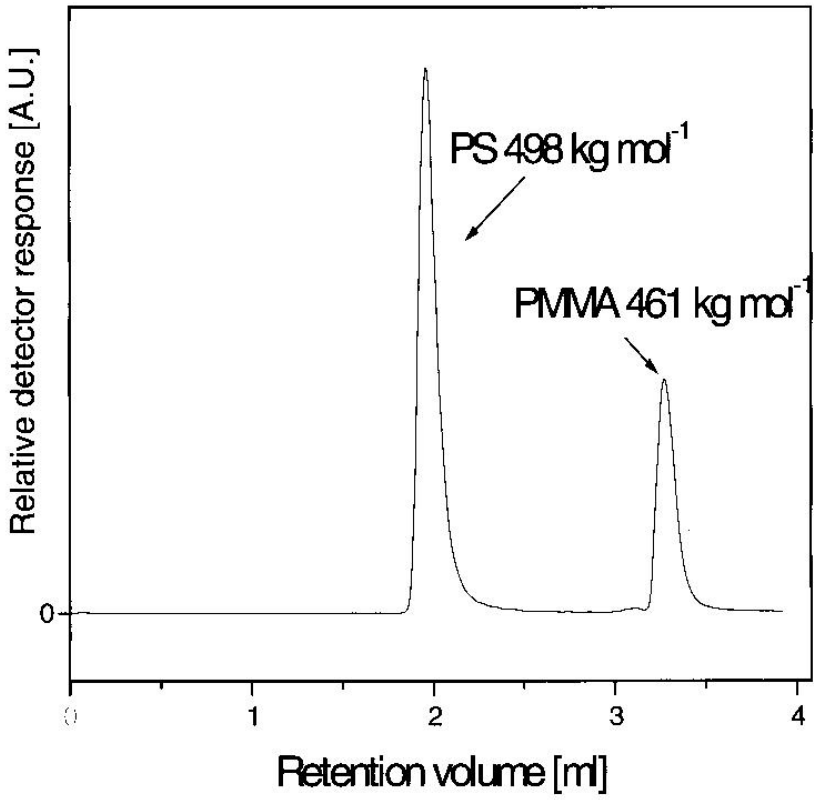
The area of molar masses which elute at the same V_R is very broad

Molar mass independent retention in LC LCD

Separation of PS and PMMA by LC LCD

Bare silica gel – 6 nm pores.

Eluent THF/toluene
50/50 wt./wt.
Toluene sample solvent.



Non-adsorbed PS and adsorbed PMMA of (almost) identical M are very well separated

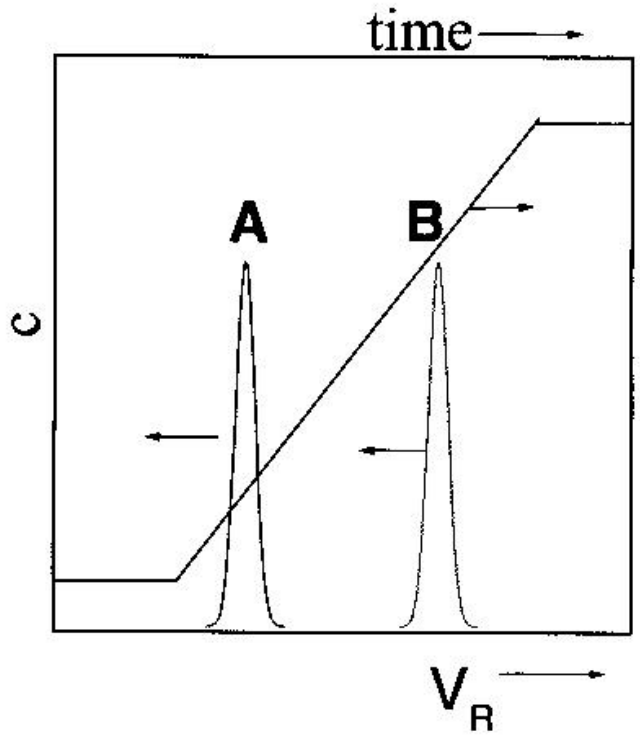
Liquid chromatography under limiting conditions (LC LC)

- Basic features – advantages over LC CC
- Broad molar mass range – no limit for very large molar masses but limits for oligomers
- Rather insensitive toward changes of eluent composition and temperature – robustness
- High sample capacity – enormously large sample volumes allowed
- Narrow, focused peaks produced – sample reconcentration possible
- LC LCD – high sample recovery
- LC LCA, LC LCI – medium recovery of interacting species, allows discrimination of minor components in polymer blends, analysis and purification of copolymers
- LC LCP, LC LCU, LC LCS limited sample recovery; broadened peaks

Coupled methods of polymer HPLC, *cont.*
Eluent gradient polymer HPLC

- (Skt. Petersburg 1968, Kyoto 1969 ➔ TLC; Tokyo 1979, Prague 1982; Tokyo 1984; Rochester 1987; ➔ column LC Tsu, Dresden
- Sample is dissolved in an appropriate liquid and injected into an eluent promoting its retention due to adsorption, enthalpic partition or phase separation. Macromolecules are retained near HPLC column inlet. Subsequently a continous or stepwise eluent gradient is applied with increasing amount of a component promoting sample elution. Macromolecules with different inherent retentivities successively start eluting. As result constituents of polymer blends-, random- and graft-copolymers with different composition, macromolecules with different architecture, etc. can be discriminated.

Eluent gradient polymer HPLC, *cont.*



elution promoting liquid

eluent composition

retention promoting liquid

A,B – polymers with different enthalpic interactivities

Each peak contains macromolecules with different molar masses

Eluent gradient polymer HPLC, *cont.*

- Important features of eluent gradient polymer HPLC: molar mass independent retention of numerous homopolymers and statistical copolymers; peak focusing; high sample capacity; high selectivity: short columns are sufficient; good repeatability provided the gradient maker applied is reliable; retention of many graft copolymers depends on the graft frequency and not on the molar mass of the backbone chain....

- Eluent gradient HPLC is an important candidate for 2D polymer LC.
- Problems: ➤ Eluent gradient repeatability and reproducibility
  ➤ Sample recovery

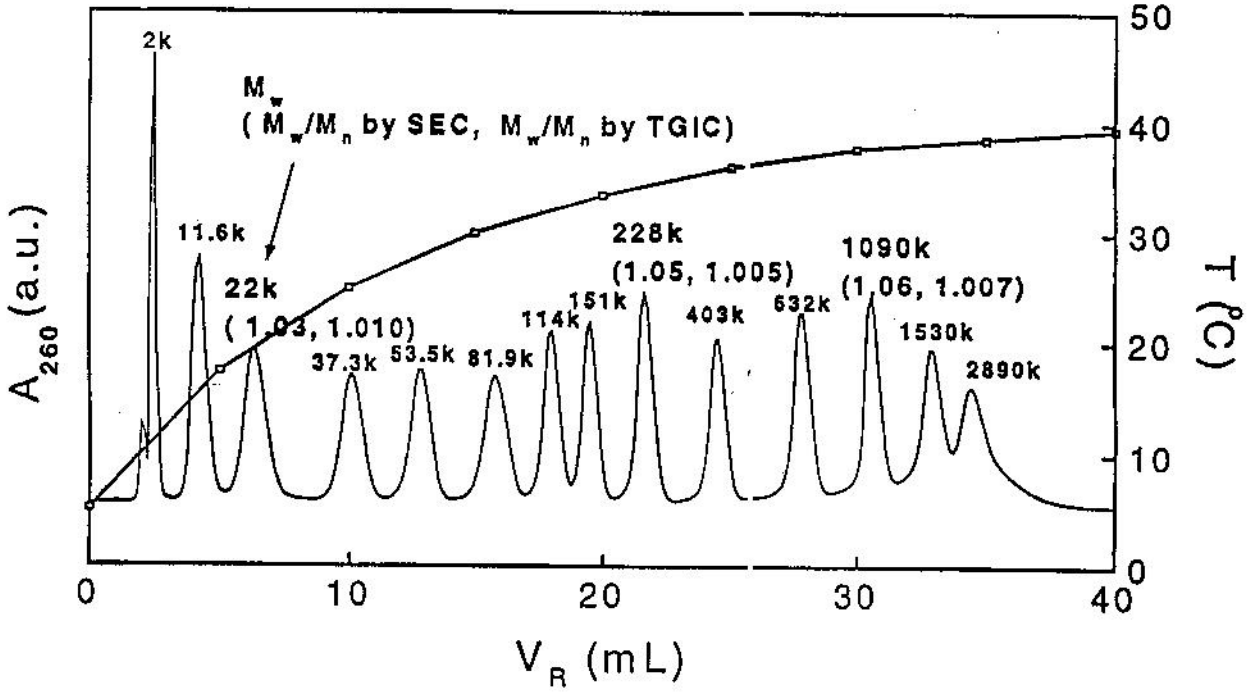
⇨ This may be a barrier method

Coupled methods in polymer HPLC, *cont.* Temperature gradient interaction liquid chromatography of macromolecules (TGIC) *(Pohang, 1996)*

- Polymer retention is controlled by temperature. System is near to its critical adsorption point where small temperature variations extensively influence retention volumes. Elution is isocratic and this allows application of non-specific (DRI) and, especially in the case of single eluents also of “absolute” (light scattering, viscosity) detectors. Selectivity of separation is very high – retention volumes strongly depend on molar mass, architecture and evidently also on slight variations in chemical composition of macromolecules. Macromolecules non-interacting with the column packing in given eluent can be easily discriminated from interacting species.

⇨ TGIC may be a barrier method

Temperature gradient interaction liquid chromatography of macromolecules (TGIC)



TGIC chromatogram of a mixture of 14 polystyrene standards. Eluent: 57/43 vol/vol CH_2Cl_2/CH_3CN. Column packings: silica gel C-18, pore sizes 10, 50 and 100 nm.

Enthalpy aided SEC

- Two different packings are applied. First one just discriminates macromolecules of different nature. Second (e.g. an SEC one) separates polymer species according to their molar mass, size in solution:

Full Retention – Elution (FRE) / SEC combination

Full Adsorption – Desorption (FAD) / SEC combination

Full retention-elution HPLC-like procedures *(Bratislava, 1995)*

(Multi-component) sample is injected into an FAD column packed by nonporous particles flushed with appropriate eluent. All (but one) components are fully retained in column

Next, retained macromolecules of one kind are quantitatively released and eluted by action of a displacer (desorli). Displacer efficiently is stepwise increased to successively release all components

Retention and release of macromolecules must by quantitative, fast, easy to control

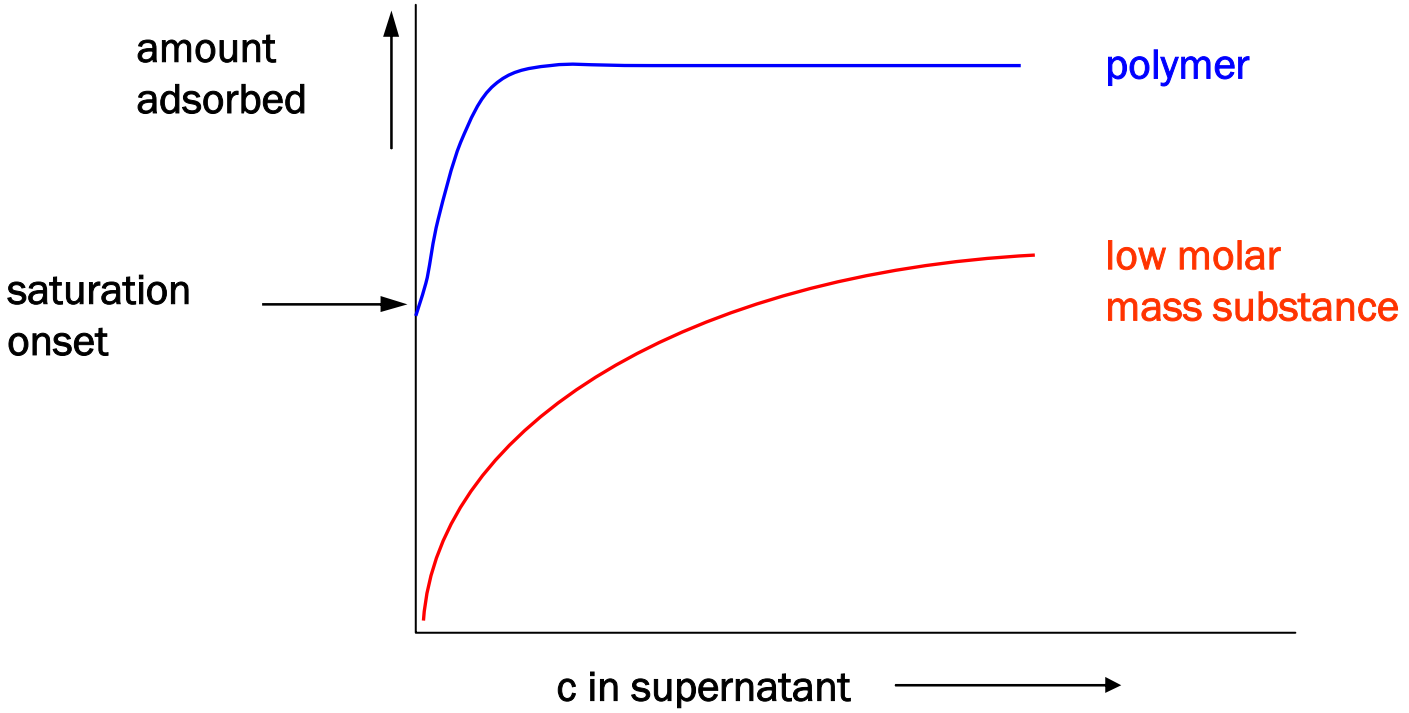
So far adsorption retention mechanism was studied in detail.

⇨ Full adsorption – desorption HPLC-like procedure – FAD – on line combination with SEC

Full Retention – Elution LC-Like Procedures *(Bratislava, 1995)*

In case of adsorption retention mechanism: full adsorption – desorption method (FAD)

High affinity adsorption isotherm for macromolecules



Full adsorption – desorption method FAD *(Bratislava, 1995)*

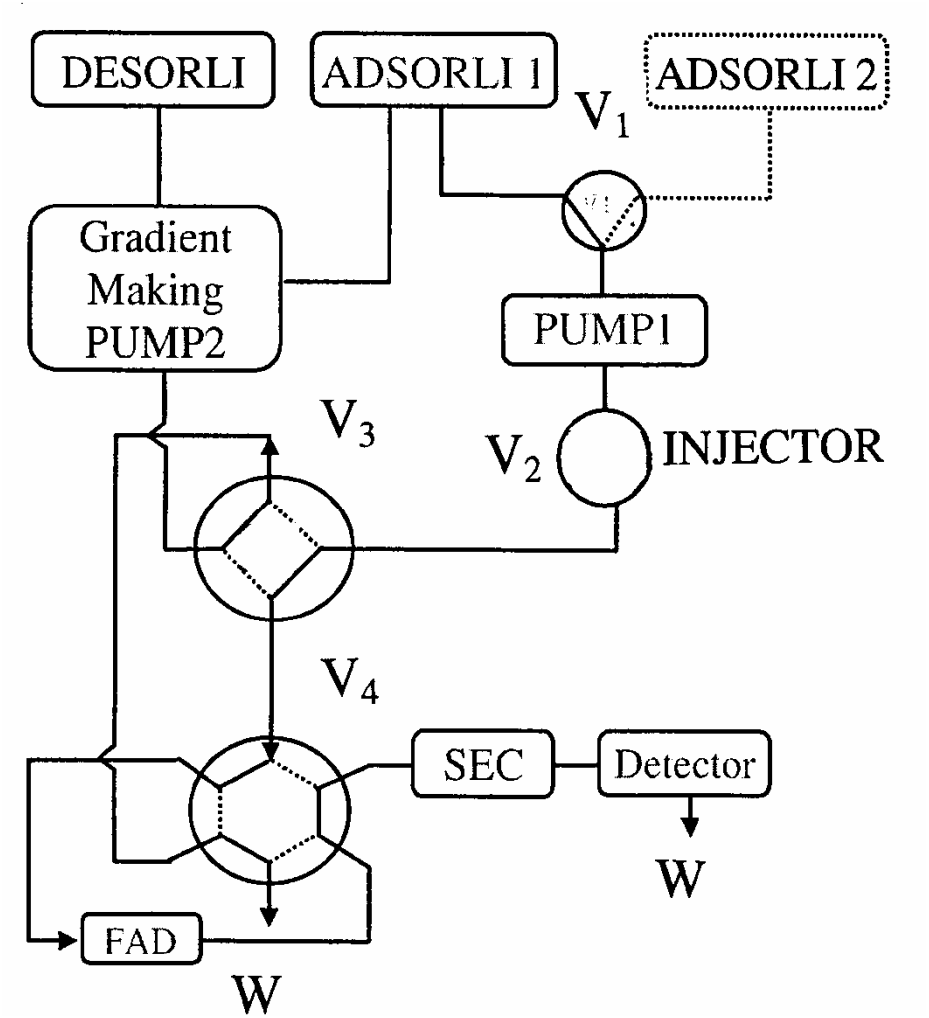
(Multi-component) sample is injected into an FAD column flushed with appropriate eluent. Macromolecules are fully retained by adsorption.

Next, adsorbed macromolecules of one kind are quantitatively desorbed by action of a displacer (desorli).

Desorli strength is stepwise increased to successively desorb all components

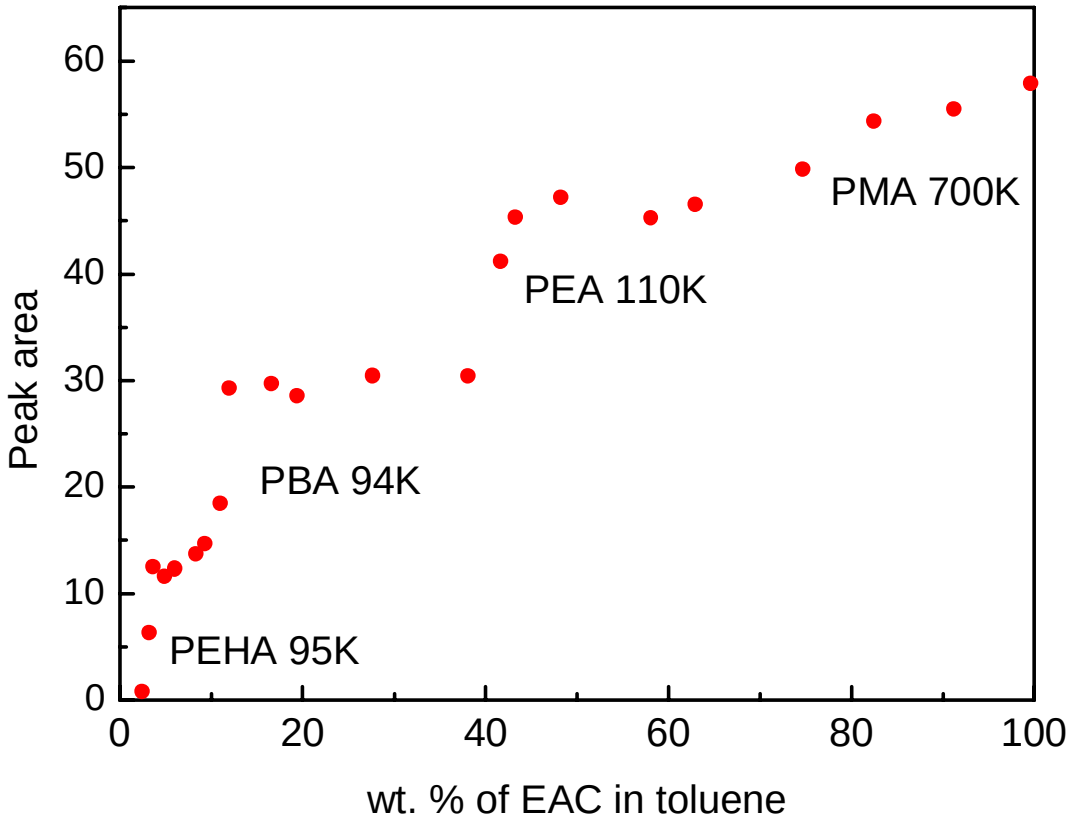
Adsorption and/or desorption must by quantitative, fast, easy to control

Scheme of an FAD instrument



or LC column

FAD separation of multi-component blend of polyacrylates



Nonporous silica
4 μm.
Adsorli: toluene
Desorli: mixtures of toluene with ethylacetate

Basic features of FAD procedures

- Very high selectivity in separation of polymers of different nature. Method is very suitable for discrimination of polymer blend constituents, including the minor ones
- Desorption step simultaneously depends on chemical composition and on molar mass of polymer species. Therefore FAD can be hardly applied for separation of a given copolymer according to its composition
- Selectivity of FAD does not allow separation of polymers according to their stereoregularity
- (Parent homopolymers in block – and graft – copolymers: ?)

Basic features of FAD procedures

- FAD allows reconcentration of (very) diluted polymer solutions; it can be used for reconcentration, storing, and re-injection of fractions leaving “first dimension” column in two- and multi-dimensional polymer HPLC

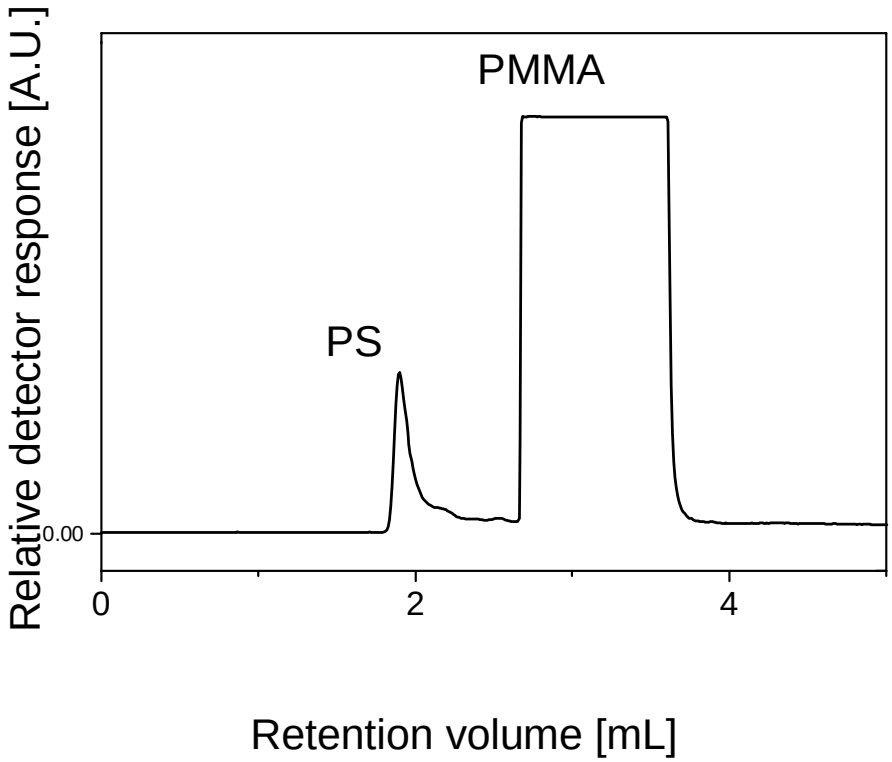
Minor components in polymer blends

- Molecular characterization of minor components (<1%) in polymer blends represents an important challenge for science and technology
  - Minor components are generated in the course of polyreactions: for example parent homopolymers in block - and graft copolymers
  - Minor components are intentionally added to major components to modify their properties. Their nature and quantity is usually not disclosed ⇨reverse engineering

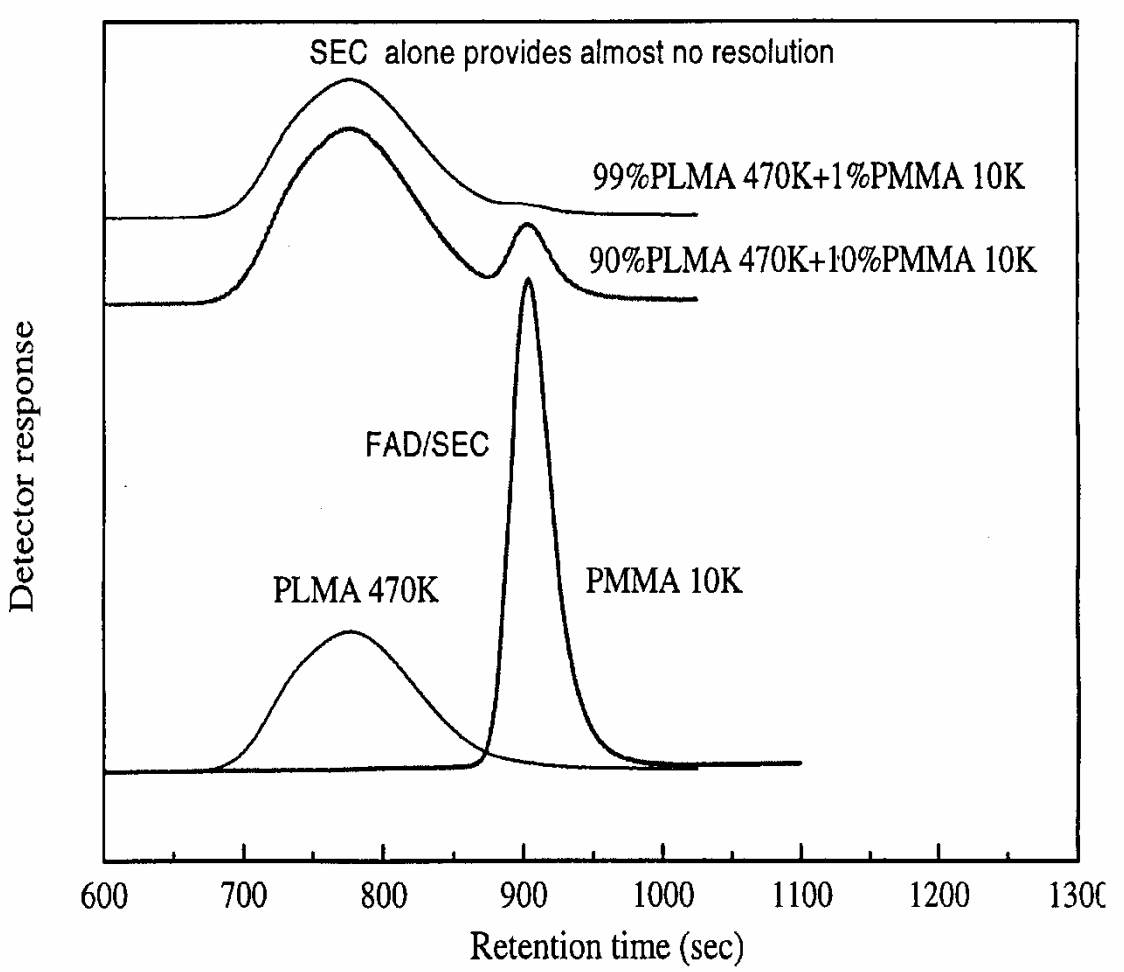
Minor components in polymer blends, *cont.*

LC LCA separation of minor component (1% of PS) from a major component (99% of PMMA)

- *Narrow pore bare silica gel*
- *column of total volume 3.14 mL*
- *PS 233, PMMA 269 kg/mol*
- *Eluent toluene adsorli plus THF desorli 65/35 wt./wt.*
- v_i = *1.0 mL*
- *ELS detector*



FAD separation and SEC characterization of minor (1%) component of polymer blend



Nonporous silica 4 μm.
Adsorli for PMMA: toluene
Desorli for PMMA: THF

General strategy for liquid chromatography (LC) separation of complex polymer systems
Two-, three-, multidimensional LC

- In the first step the effect of **ALL BUT ONE** characteristics is suppressed. Macromolecules are separated just by one characteristic. Alternatively, separation selectivity according to ONE SINGLE characteristic is strongly enhanced so that effects of other characteristics can be neglected. This is the first "dimension" of separation.

➤ How to do so? By a combination (coupling) of two or several separation mechanisms within the same single column or column system: "critical LC"; eluent gradient LC; LC LC; FAD (FRE) ...

General strategy for liquid chromatography (LC) separation of complex polymer systems. Two-, three-, multidimensional LC, *cont.*

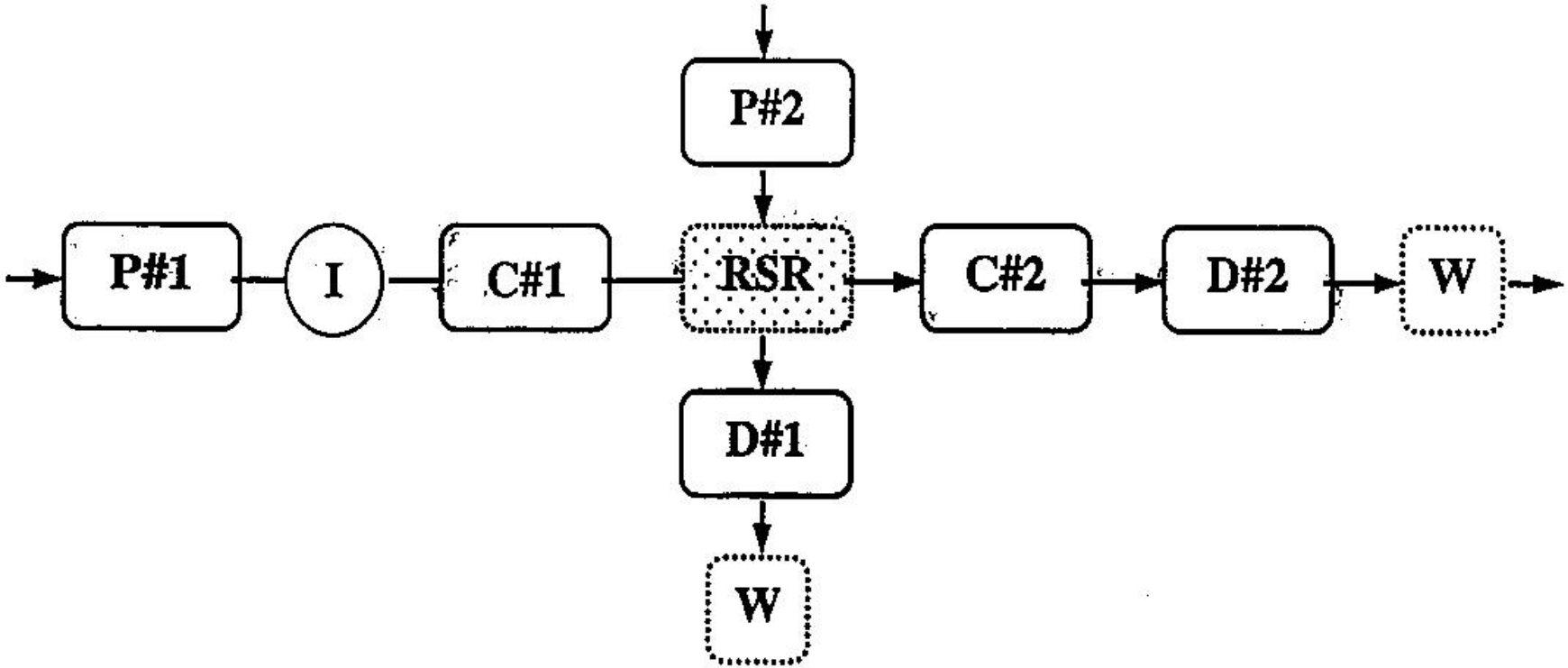
- In the second step – which may be arranged either on-line or off-line – macromolecules are separated fully or (strongly) preferentially according to the second characteristic ("second" dimension of separation) and subsequently according to the third, etc., characteristics ➔ multidimensional separation. So far, two-dimensional separations have been attempted. Problems increase exponentially with number of separation dimensions, including detection of fractions and data processing. Between particular steps, the diluted sample solutions must often be reconcentrated and sample solvents (eluents) must be exchanged.

General strategy for liquid chromatography (LC) separation of complex polymer systems. Two-, three-, multidimensional LC, *cont.*

- The last step (that is separation according to the last remaining characteristic) is done preferentially but not exclusively by SEC and if possible with a single component eluent compatible with various detectors – so that hyphenated detection can be applied.

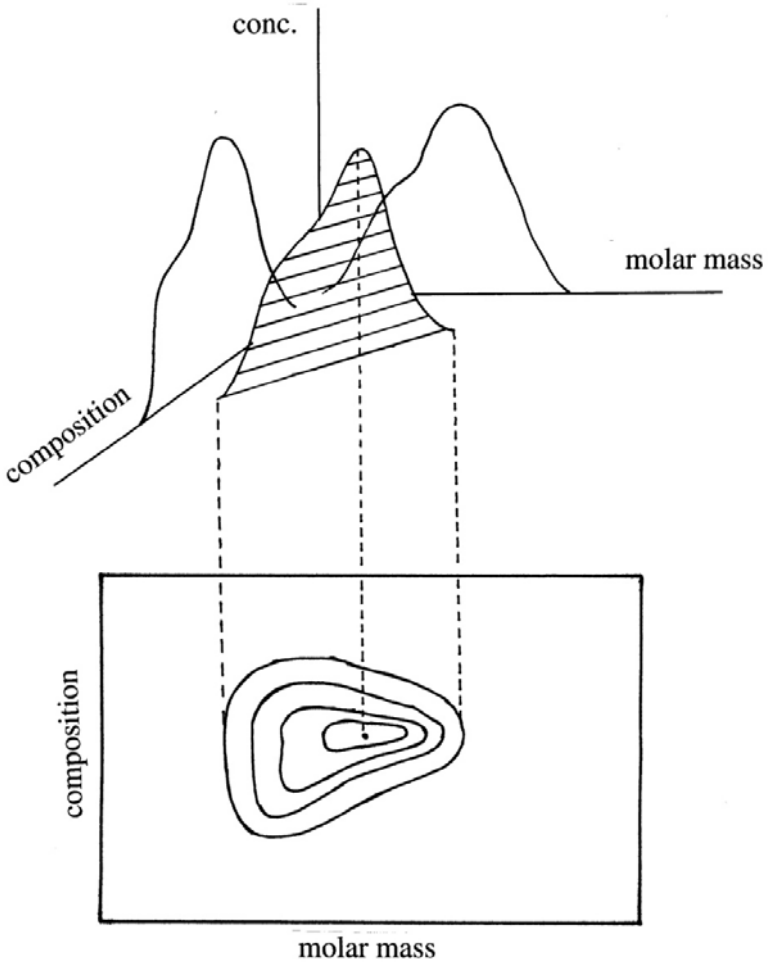
- Partners for coupling: exclusion, adsorption, partition, phase separation (solubility), ion effects ...

Two- dimensional HPLC of polymers



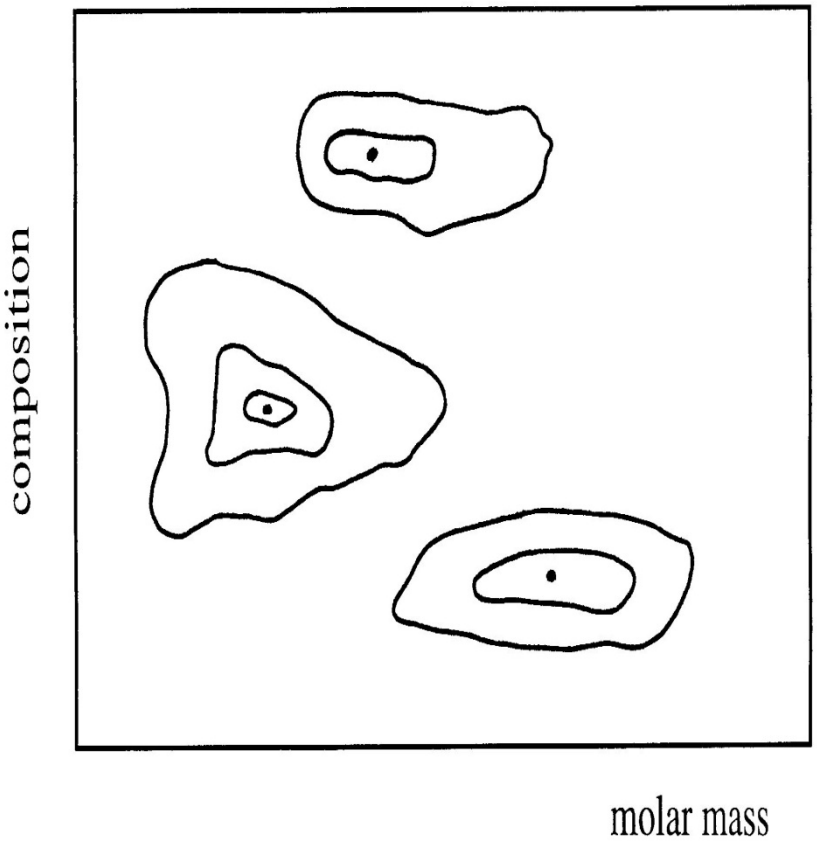
P = pump; I = injector; C = column; D = detector or system of detectors; W = waste

Two- Dimensional HPLC of polymers, *cont.*
Data representation



Three dimensional diagrams and contour plot for a copolymer with bimodal, continuous molar mass, and chemical composition distribution.
Sequence length distribution is neglected

Two- Dimensional HPLC of polymers, *cont.*
Data representation



Contour plot of a copolymer mixture exhibiting multimodal, discontinuous molar mass, and chemical composition distribution.

- Thank you for your attention –

and good luck in polymer characterization by polymer HPLC