Size-exclusion Chromatography as a Useful Tool for the Assessment of Polymer Quality and Determination of Macromolecular Properties

Peter Kilz, PSS Polymer Standards Service, 55023 Mainz, Germany

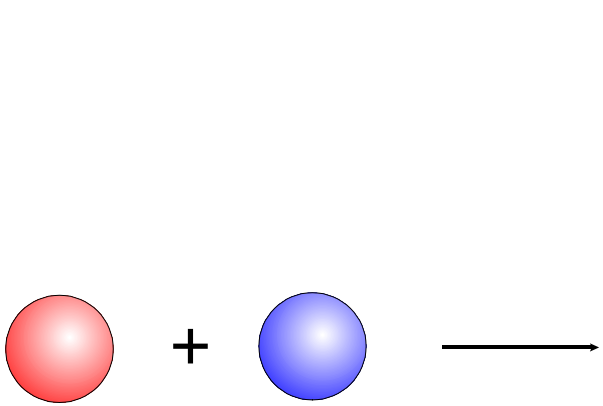
1. Introduction into Macromolecular Chromatography
2. Separation and 2-dimensional Techniques
3. Detection and Information Content
4. Summary

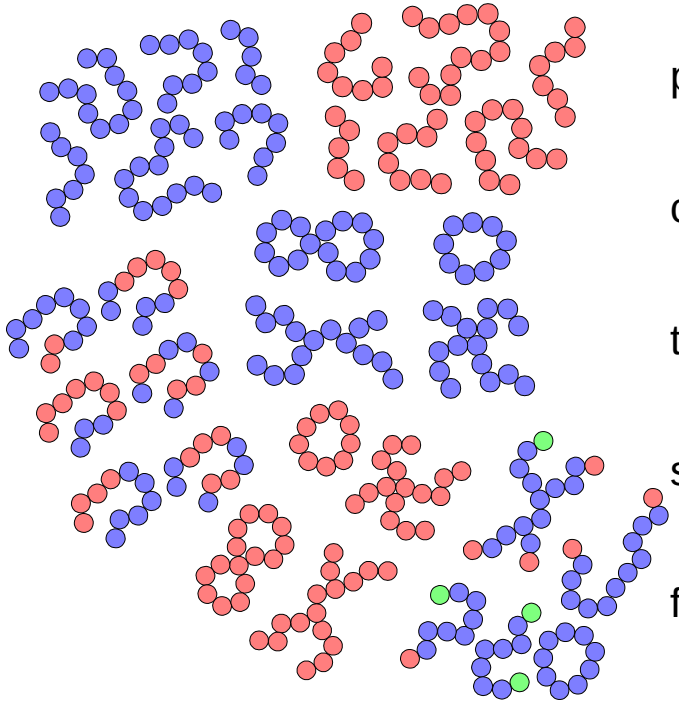
Introduction

Most polymeric materials are highly complex multi-component materials

even simple polymerization leads to products with multiple property distributions

Analytical Challenges: Determination of distributed properties





physical dispersity (MMD)

chemical dispersity (CCD)

topological dispersity (MAD)

structural dispersity

functional distribution (FTD)

Introduction

Characterization Strategy

A) Characterization of bulk materials — **access to bulk properties / property averages**

requires batch methods

e.g. Light Scattering (LS), Viscometry, Osmometry, Ultracentrifugation (AUC) NMR, IR, ...

B) Characterization of separated fractions — **access to property distributions**

requires comprehensive chromatography

1) analytical fractionation methods: E.g. Liquid chromatography (SEC, LAC, LCCC)
Ultracentrifugation (AUC)
Field flow fractionation (FFF)
(Gas chromatography: GC)
Mass sprectrometry: MALDI-ToF

2) detection techniques e.g. RI, UV, LS, Viscometry, FTIR, NMR, MS

separation - detection combinations determine which distributions can be measured

Introduction

Chromatographic Modes

a) Size exclusion mode: SEC

$K_{SEC} = \exp(\Delta S/R)$

$0 < K_{SEC} < 1$ $\Delta H = 0$

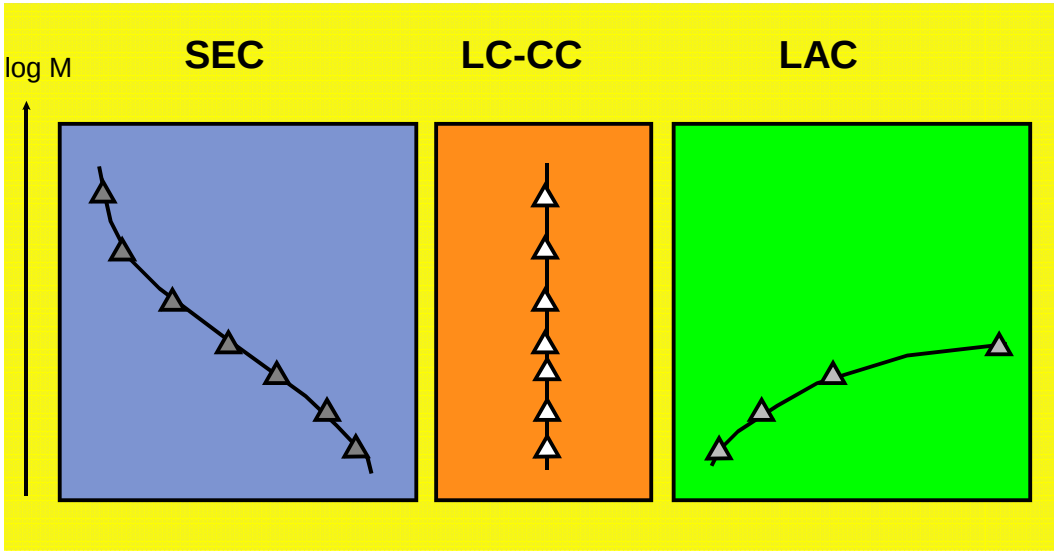
b) Adsorption mode: HPLC

$K_{HPLC} = \exp(-\Delta H/RT)$

$K_{HPLC} > 1$ $\Delta H \gg T\Delta S$

c) critical adsorption point: LC-CC

$K = 1$ $\Delta H = \Delta S$



Chromatographic Modes of Separation

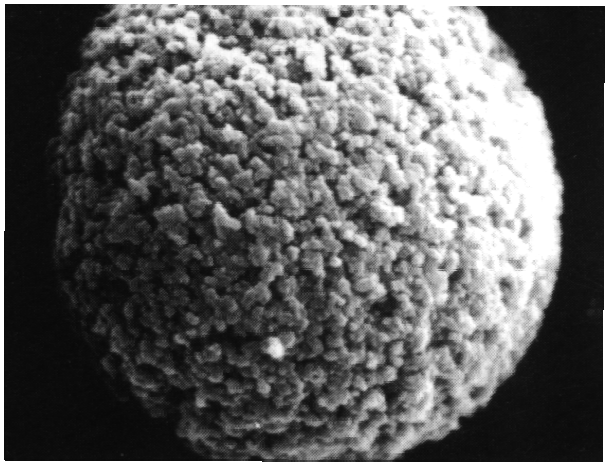
Comparison of Chromatographic Modes

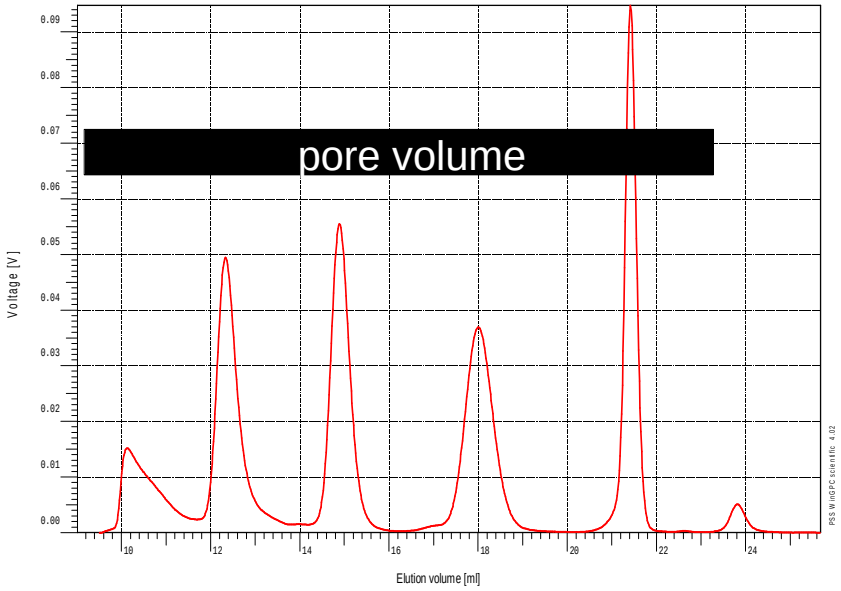
technique	separation governed by	information content	potential problems
SEC	• hydrodynamic volume • molecular size in solution *diffusion controlled process*	• molar mass (MMD) • chemical composition (CCD)	• calibration dilemma • specific interactions
LC-CC	• chain inhomogeneity • defect structures • endgroups *diffusion and adsorption controlled process*	• functionality type (FTD) • molecular architecture (MAD)	• irreversible adsorption • determination of critical adsorption point
HPLC	• chemical composition • endgroup *adsorption controlled process*	• chemical composition (CCD) • functionality type (FTD)	• molar mass influence • partial adsorption • large k'

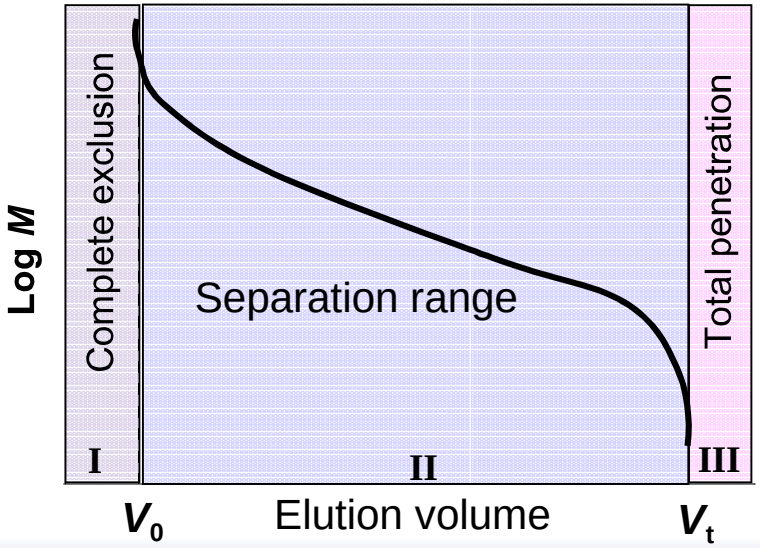
Introduction

SEC Separation Principles

- solutes diffuse between mobile phase and pores in stationary phase
- conformational entropy loss is driving force
- retention based on hydrodynamic size in solution V_h
- molar mass by rentention calibration or proper detection method







Introduction

SEC Instrumentation

Special instrumental requirements:
- solvent compatibility
- prevent clogging by solvent evaporation
- multi-detector application
- columns: mainly polymer packing
- often:
  - absolute concentrations required
  - absolute injection volume required

critical modules:
- pumps
- autosamplers
- software

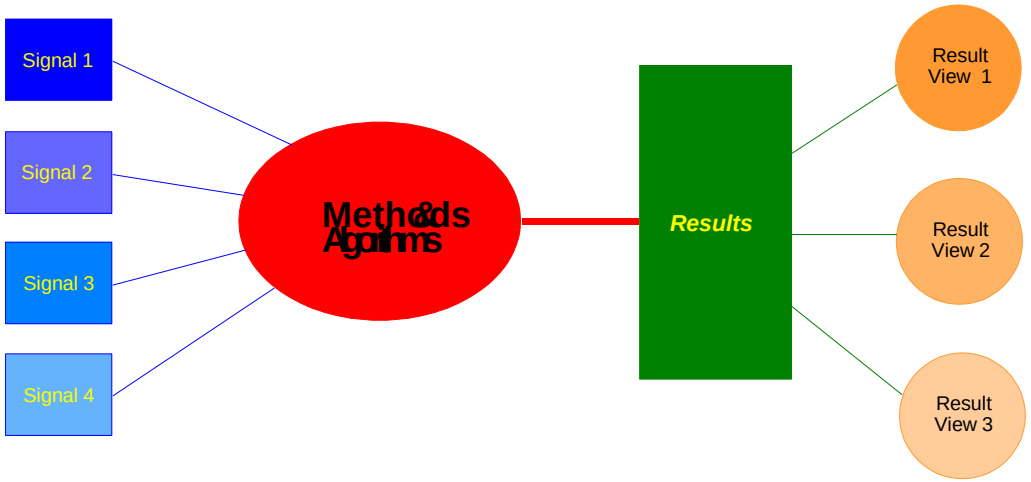
Introduction

SEC Data Systems

Special software requirements:

- long analysis times
- complex data treatment
- multi-signal processing
- determination of distributions
- combination of methods
- multiple vendor support
- integration in existing infrastructure

Plethora of Software Requirements



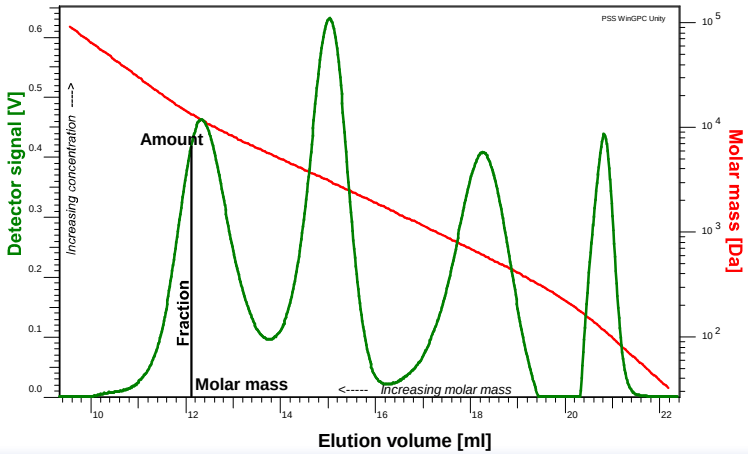
Introduction

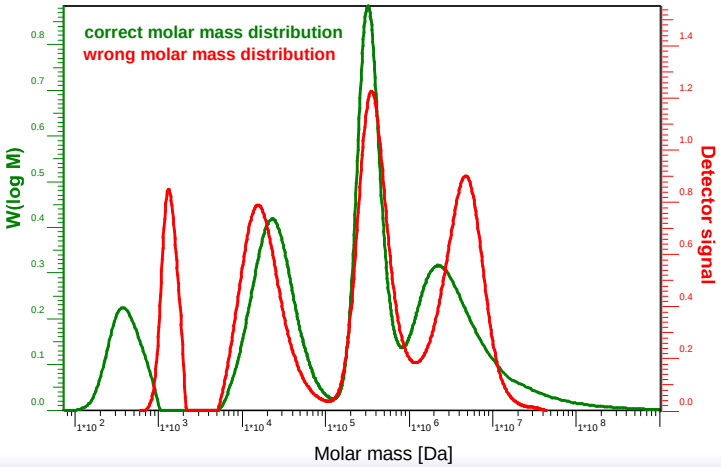
Determination of Property Distributions

complete description of properties and contributions

- accurate determination of amounts
- proper measurement/calibration of properties
- accurate results calculation and representation

Example: Conversion of raw signals to molar mass distribution





The **molecular weight averages** can be calculated from the moments, μ_i, of the molar mass distribution:

$$\mu_i = \int_0^\infty M^i \cdot w(M)\,\mathrm{d}M$$

with: μ_i the i-th moment of the molar mass distribution

The molar mass averages are defined and calculated in PSS WinGPC Unity by:

Number average molecular weight:

$$M_n = \frac{\sum h(M)\cdot M}{\sum h(M)} = \frac{\sum w(M)}{\sum w(M)/M} = \frac{\mu_0}{\mu_{-1}}$$

Weight average molecular weight:

$$M_w = \frac{\sum h(M)\cdot M^2}{\sum h(M)\cdot M} = \frac{\sum w(M)\cdot M}{\sum w(M)} = \frac{\mu_1}{\mu_0}$$

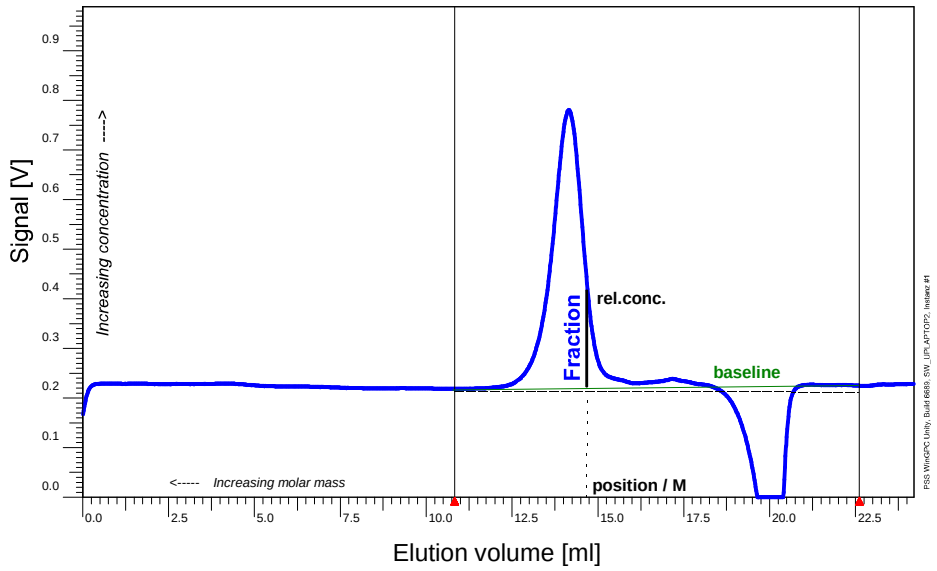
z-average molecular weight:

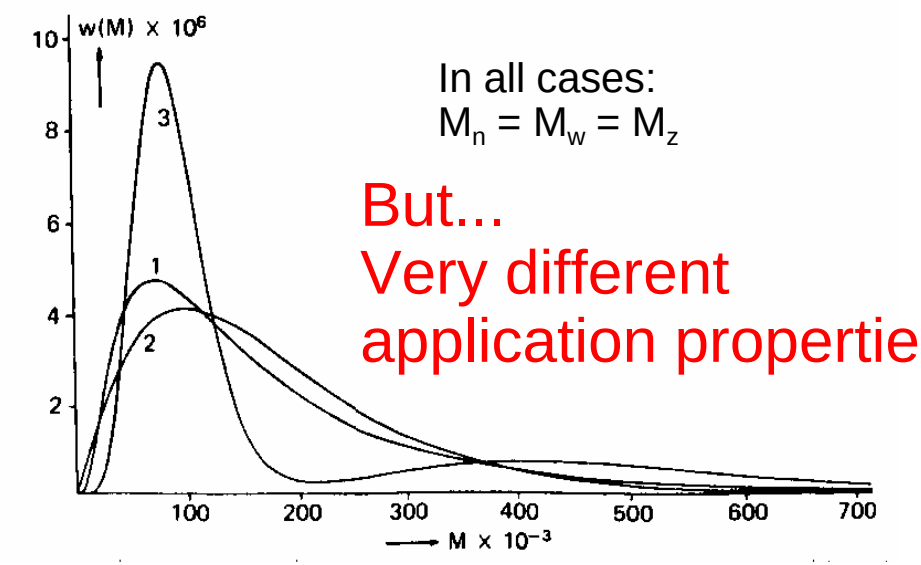
$$M_z = \frac{\sum h(M)\cdot M^3}{\sum h(M)\cdot M^2} = \frac{\sum w(M)\cdot M^2}{\sum w(M)\cdot M} = \frac{\mu_2}{\mu_1}$$

Conventional Data Analysis

Determination of fundamental parameters

Chromatogram:	relates apparant concentration to elution volume / retention time
calibration curve:	relates molar mass to chromatographic position
molar mass distribution:	shows mass fraction of molecules of given molar mass





In all cases:
$M_n = M_w = M_z$

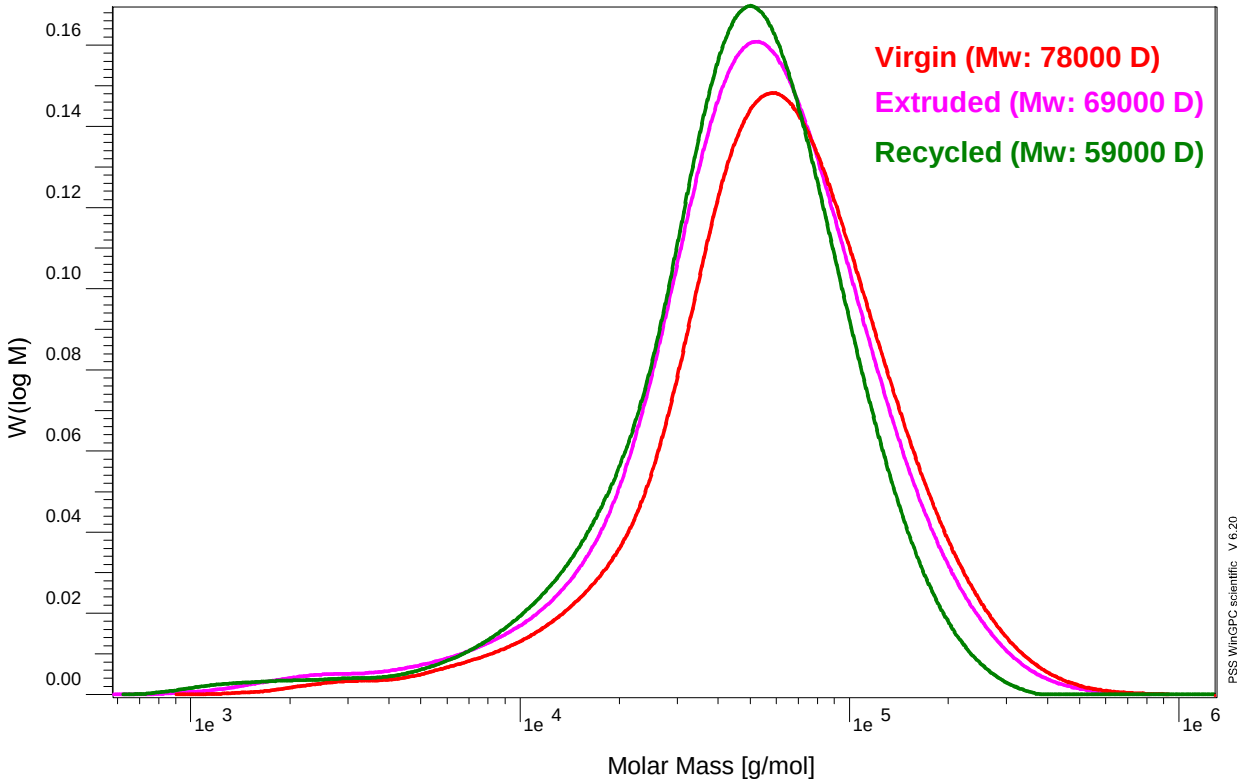
But...
Very different
application properties

Conventional Data Analysis

Polymer Degradation during Recycling Processes

conditions:

system:	PSS SECcurity GPC
eluent:	TCM/HFIP
columns:	PSS SDV 5µm
detection:	UV@260nm
software:	PSS WinGPC
analysis in:	35 min / sample



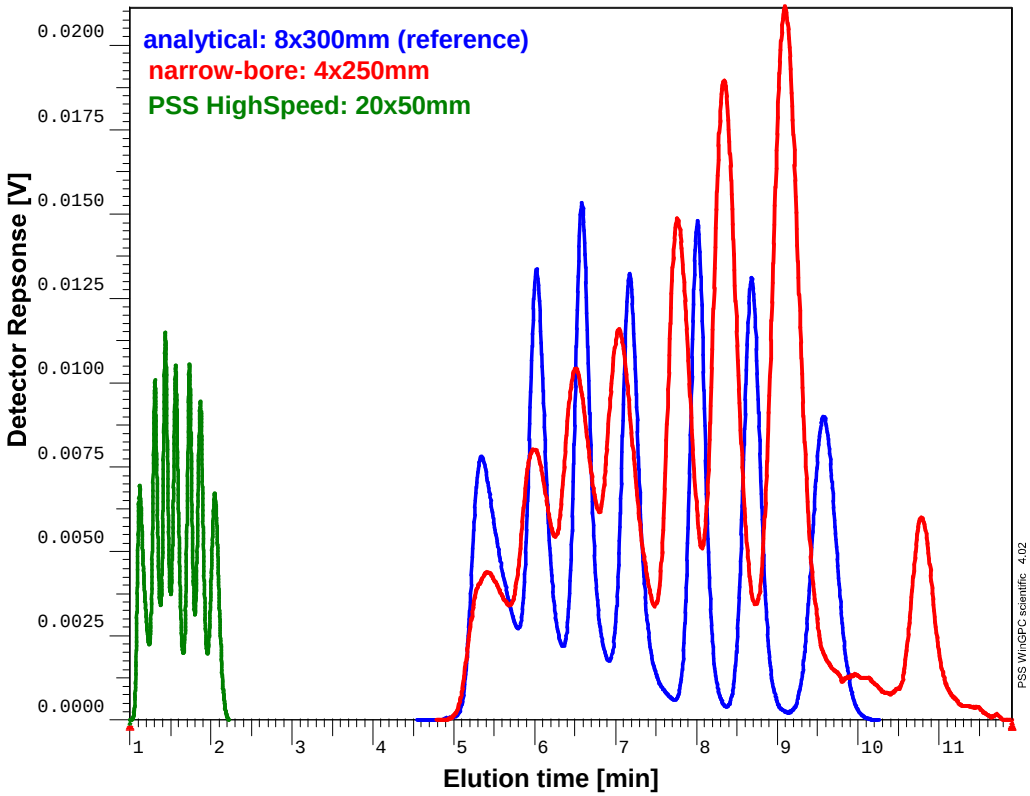
Conventional Data Analysis

Conventional and HighSpeed Analysis

Benefits of HighSpeed SEC

- short analysis time (up to 10-fold)
- no (additional) sample degradation*
- no special SEC hardware required*
- no method change*

*) only by using PSS HighSpeed column technology

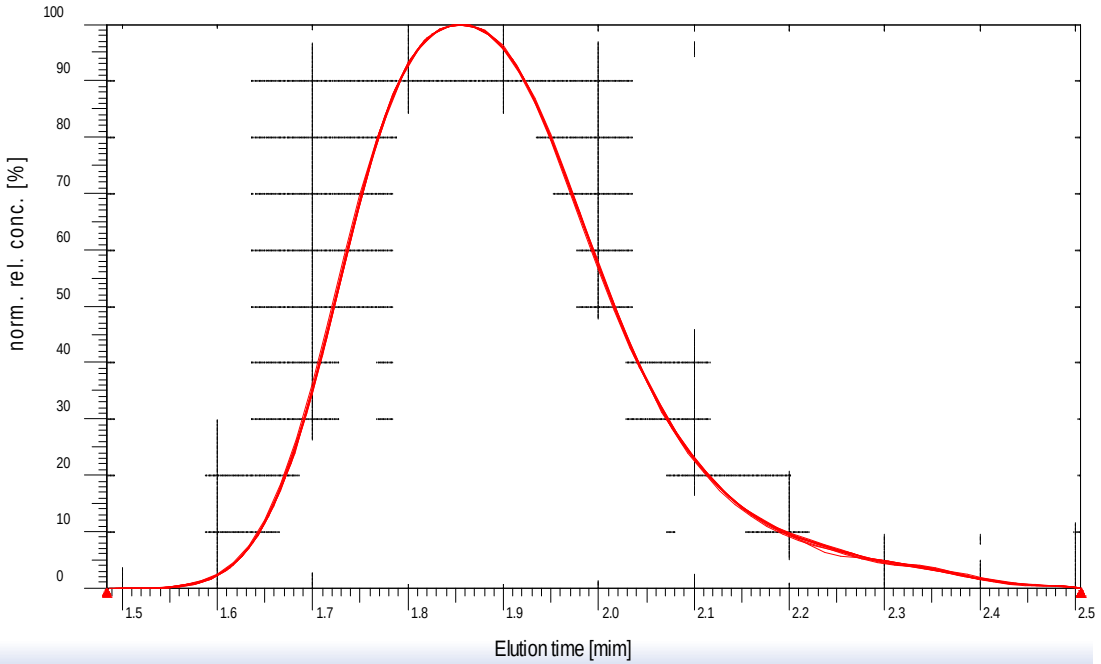


Conventional Data Analysis

Quality Assurance by HighSpeed SEC

commercial polycarbonate in THF
mw by producer: 30000 g/mol
60 repeats in 2h

column: 2x PSS SDV 5 µm HighSpeed
calibration: PSS ReadyCal PS standards
detection: UV



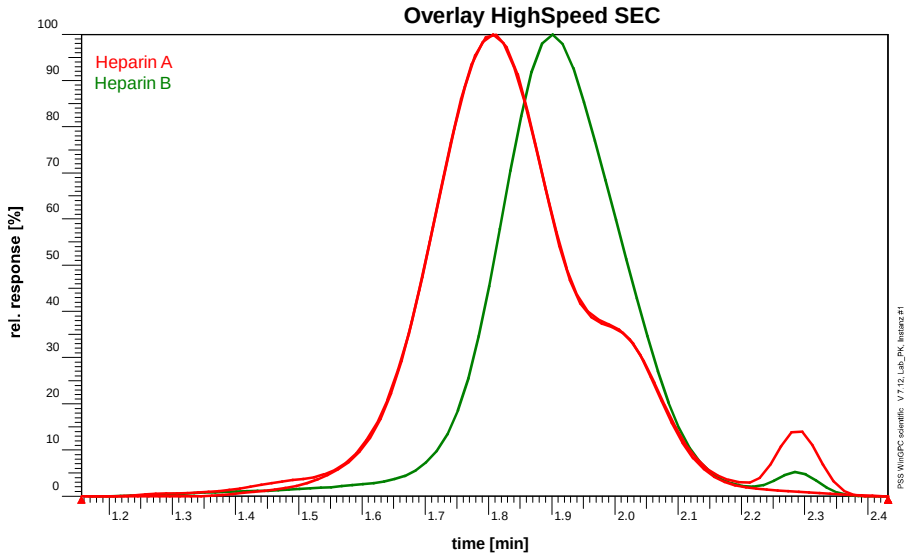
HighSpeed result:

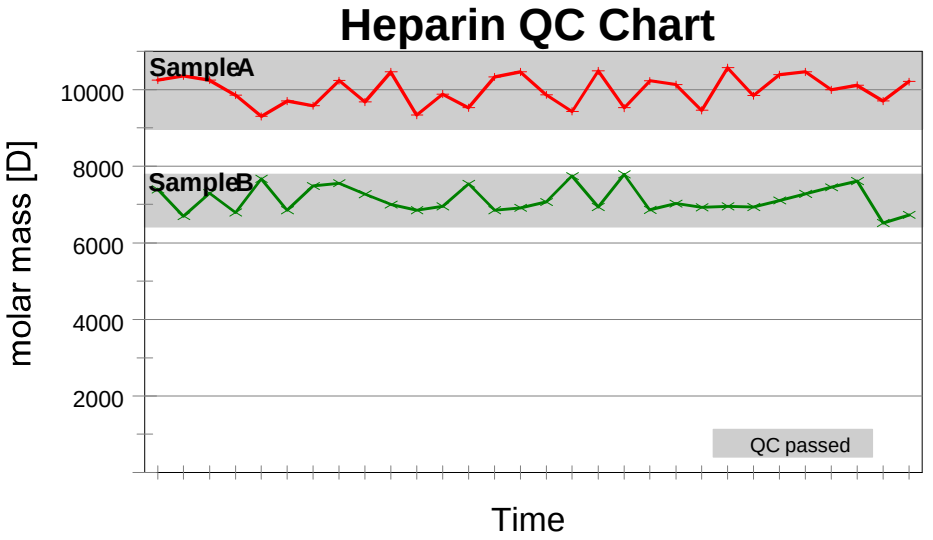
M_w: (29610±150) g/mol
RSD: 0.5%

Conventional Data Analysis

HighSpeed Heparin Quality Assurance

column: PSS HighSpeed Suprema 100, 10 µm
analysis time: 2 min
calibration: Heparin endgroup (DAB); PSS WinGPC
detection: RI





Chromatographic Modes of Separation

Potential limitations in SEC

- Molecular weight range:
  Separation range may be increased by using combination of different pore size columns

Easy to overcome

- Resolution:
  Separation efficiency may be increased by using longer or more columns of same pore size

Easy to overcome

Peak capacity**:**

$$n = 1 + \frac{\sqrt{L}}{4} \cdot \ln \frac{V_p}{V_0}$$

- Size-separation
  co-elution of different species (e.g. copolymers, branched molecules)

Can be difficult to meet
requires often different LC techniques

2-Dimensional Chromatography

n independent properties require *n*-dimensional methods for accurate (independent) characterization.

Possible multidimensional chromatography techniques:

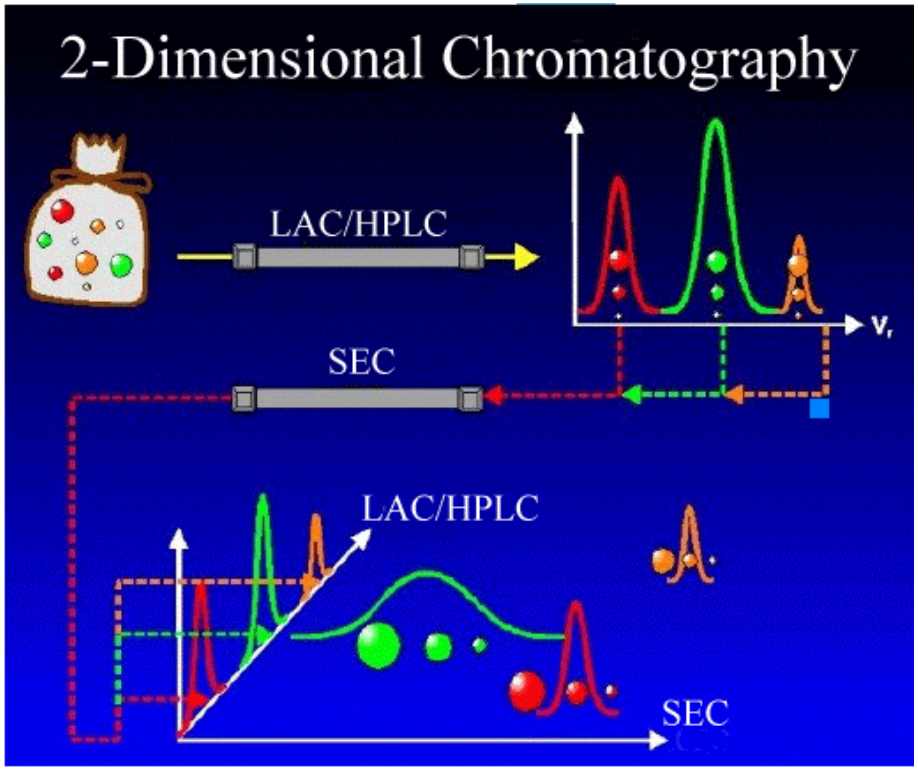
HPLC, SEC, LC-CC, GC, TREF, GPEC,.....

Example:

combination of LAC(HPLC) and SEC:

1st dimesion:
LAC/HPLC for separation according to CC

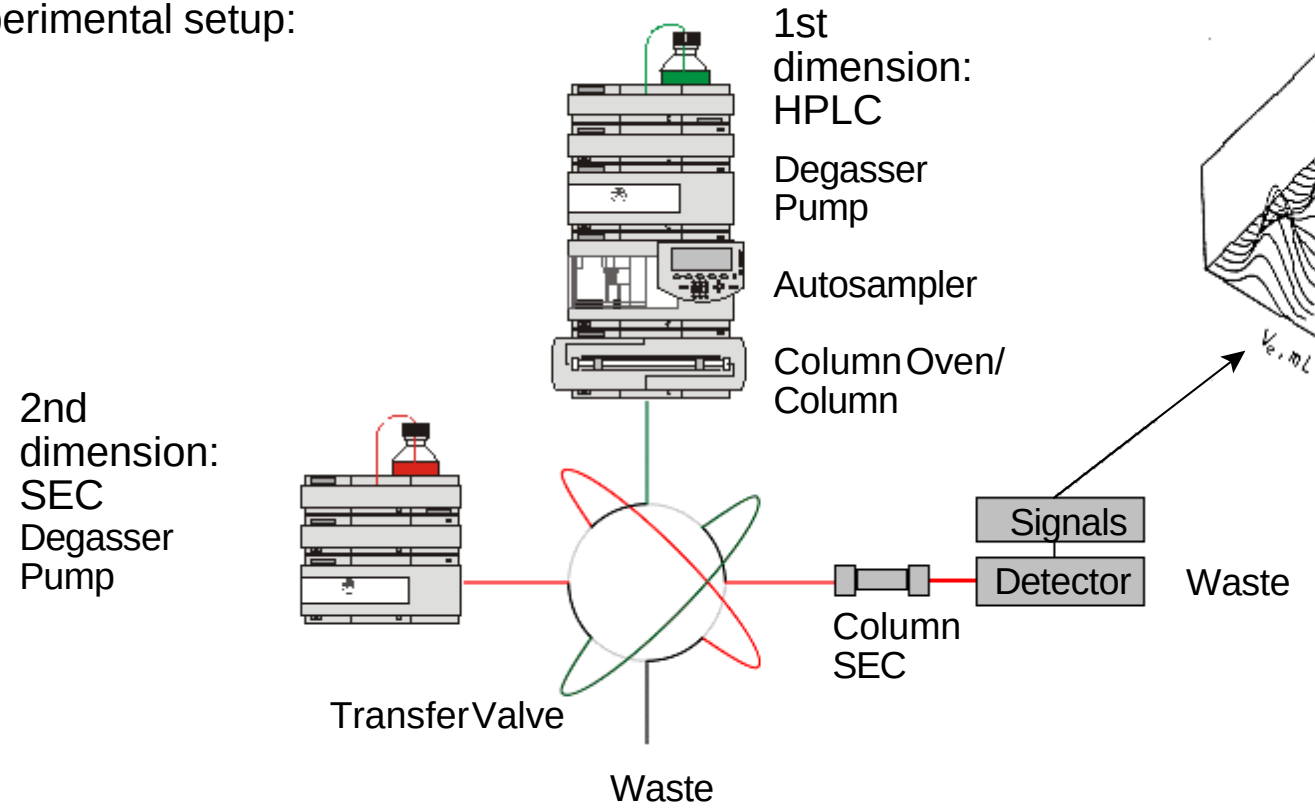
2nd dimesion:
SEC for separation according to MM



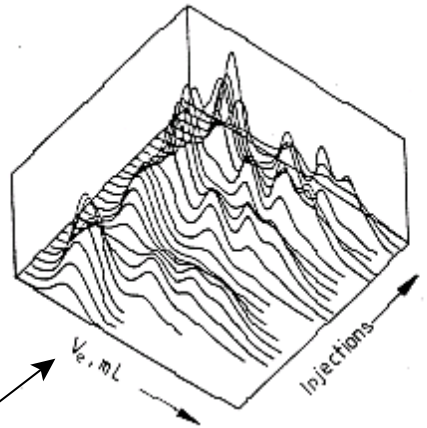
2-Dimensional Chromatography

Investigation of CCD and MMD

Experimental setup:



SEC results (chromatograms)



2D Chromatography

SEC Analysis of TPE

sampleBfailedinthefield

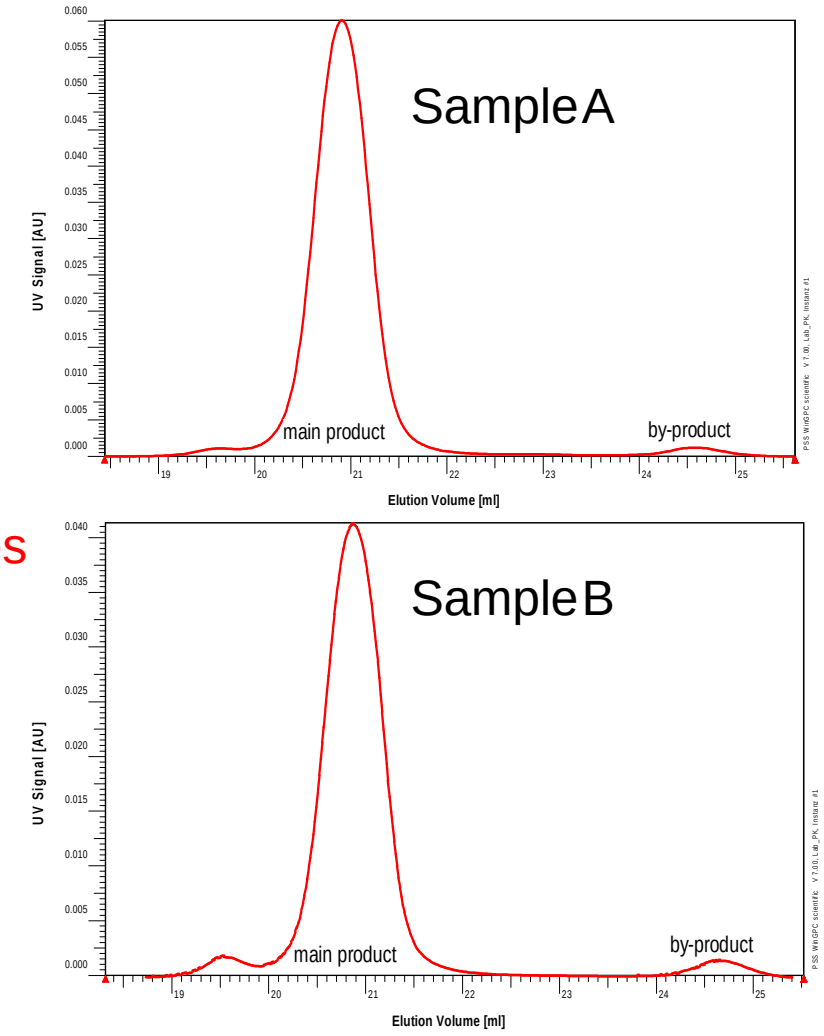
- main product looks very similar
- similar by-products present

SEC does not track performance differences

	sample A	sample B
Mn[kD]	99	90
Mw [kD]	109	103
Mw/Mn	1.08	1.14
Mp [kD]	108	104
by-product	0.8%	1.7%

molar masses by narrow PSt calibration

differences due to composition?



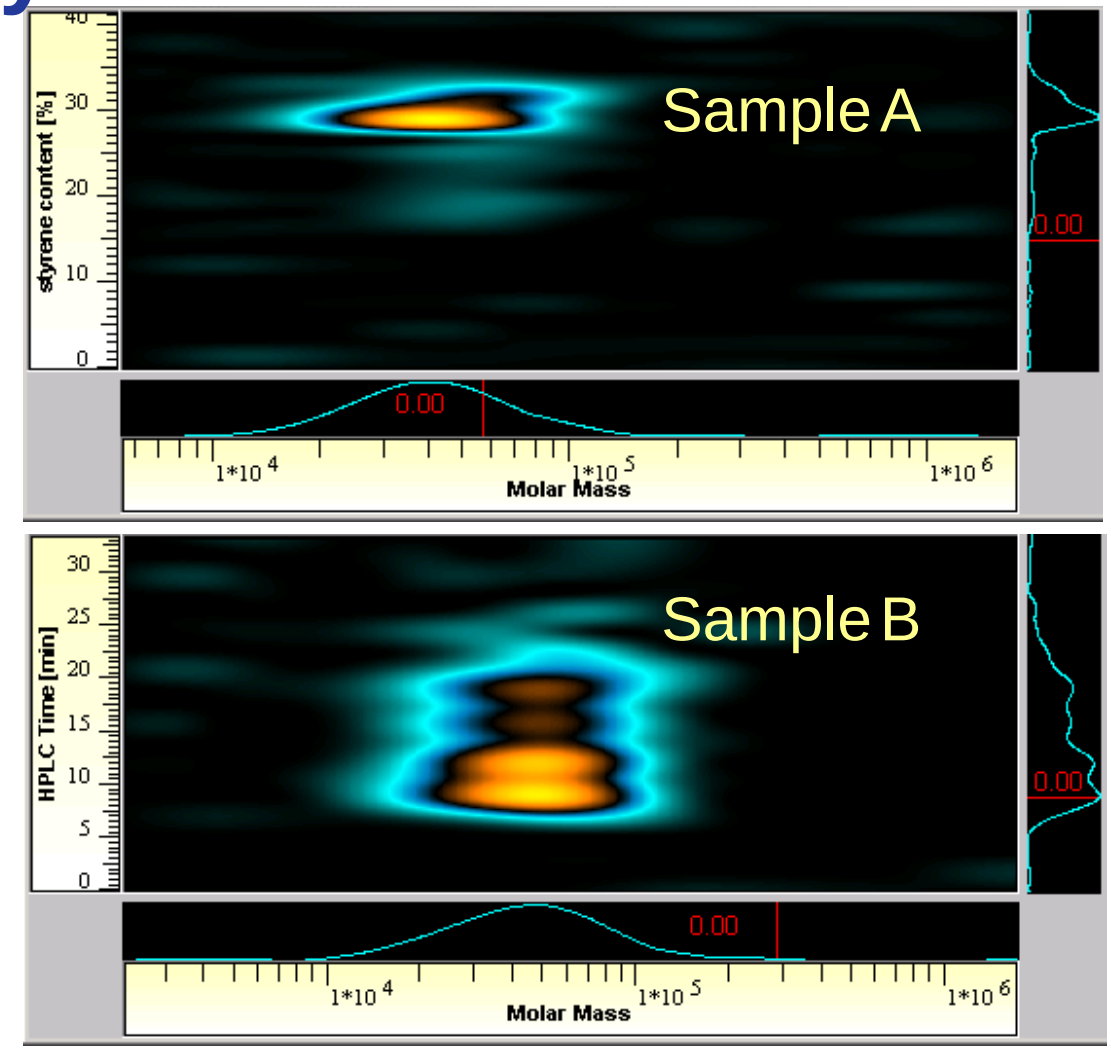
2D Chromatography

Comprehensive 2D by HPLCxSEC

HPLC tracking composition
SEC tracking molar mass

2D analysis

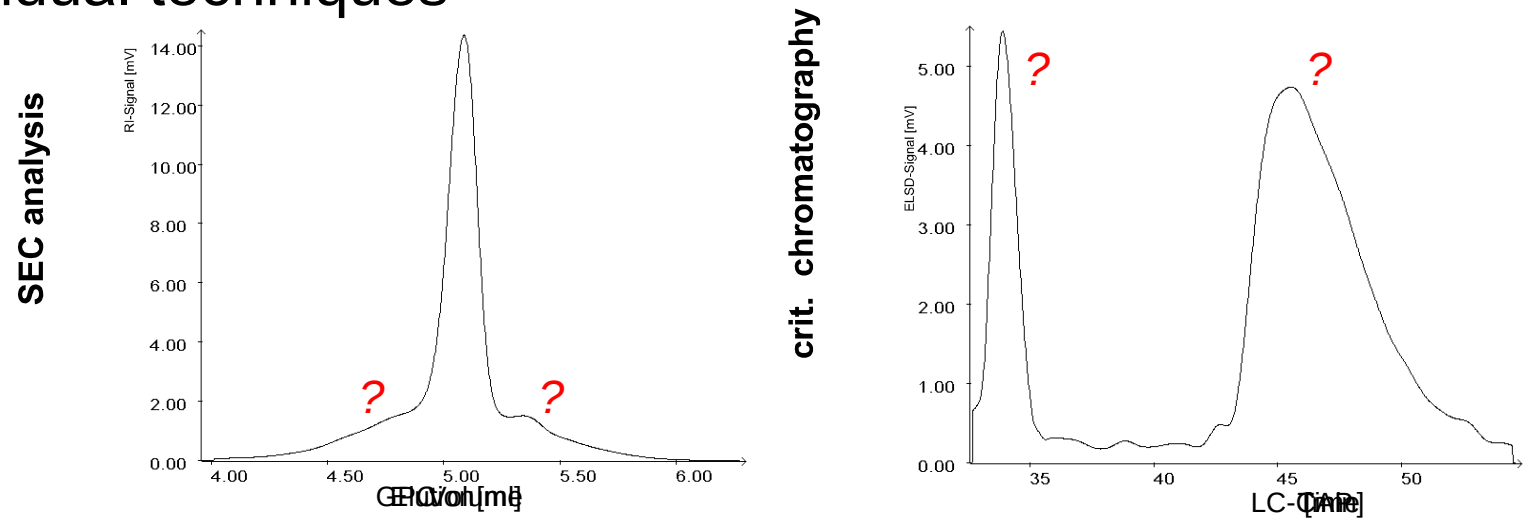
- corroborates similar MMD
- shows similar average PS content
- reveals big differences in CCD
- contour map shows
  - differences easily
  - 2D property distributions



2-Dimensional Chromatography

Investigation of by-product in motoroil additives

Individual techniques



observations difficult to explain

2-Dimensional Chromatography

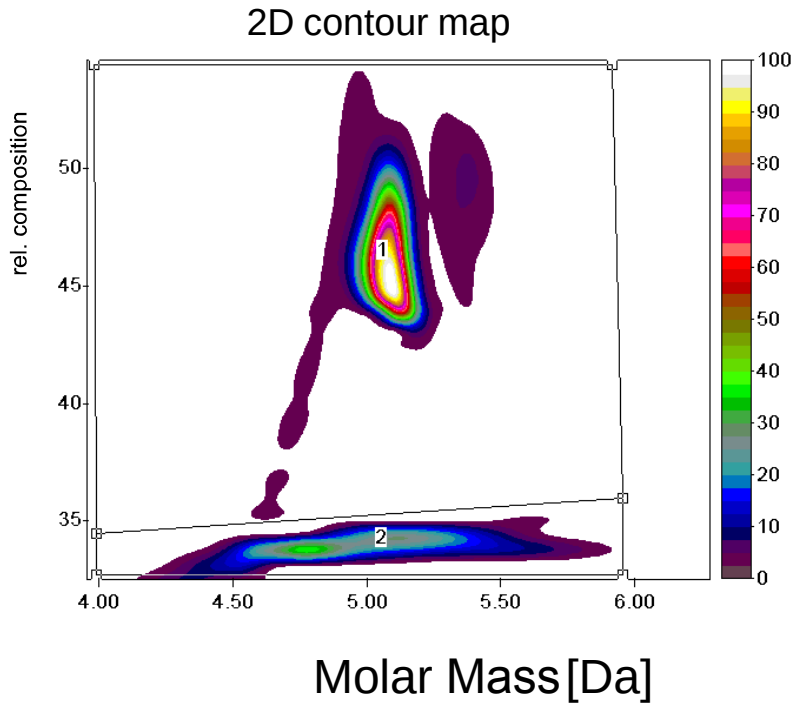
Investigation of by-product in motor oil additives

2D results

- main product (region1)
- parallel reaction forms region 2

- two different processes
- by-product is homopolymer
- by-product has broad MMD

- reaction mixture contains 60% desired product
- desired product is narrow in CCD and MMD



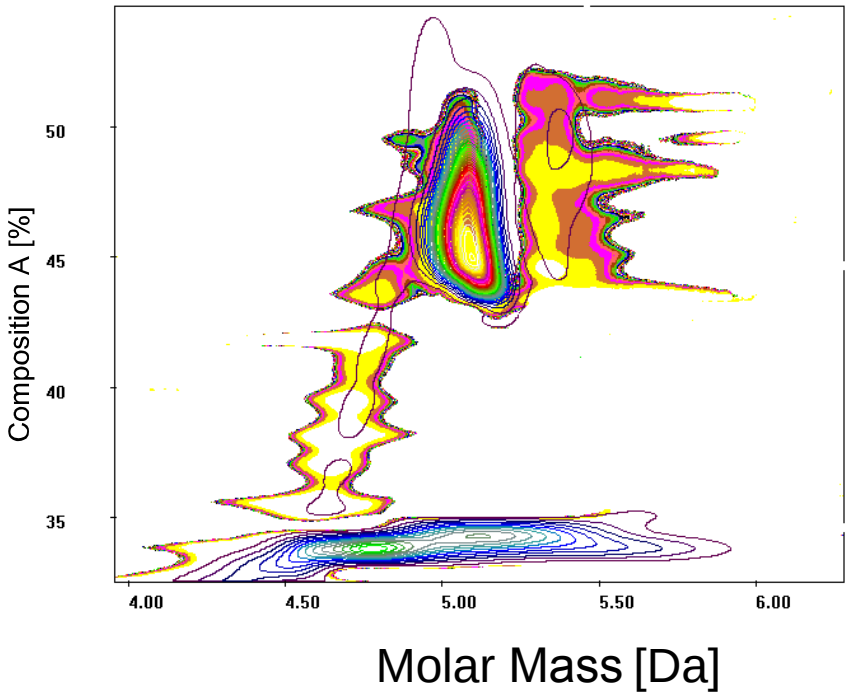
2-Dimensional Chromatography

Investigation of by-product in motor oil additives

2D compositional analysis

overlay of 2D separation
with chemical composition

supports
- two simultaneous polymerization processes
- desired product is copolymer
- by-product is homopolymer



Detection Techniques in SEC

Detector Signal Characteristics

$$U_d = K_d \times \sum_i \left(k_{Sample} \times c_{Sample} \times M^x \right)$$

U_d: Signal intensity

K_d: Instrument constant

k_{sample}: Sample dependent parameter

- for spectroscopic detectors: k_{sample} = extinction coefficient, κ
- for refractive index (RI) detectors: k_{sample} = refractive index increment, dn/dc
  - note: dependent on solvent composition, T, λ

c_{sample}: Sample concentration

M: Molar mass

x: Detector dependent

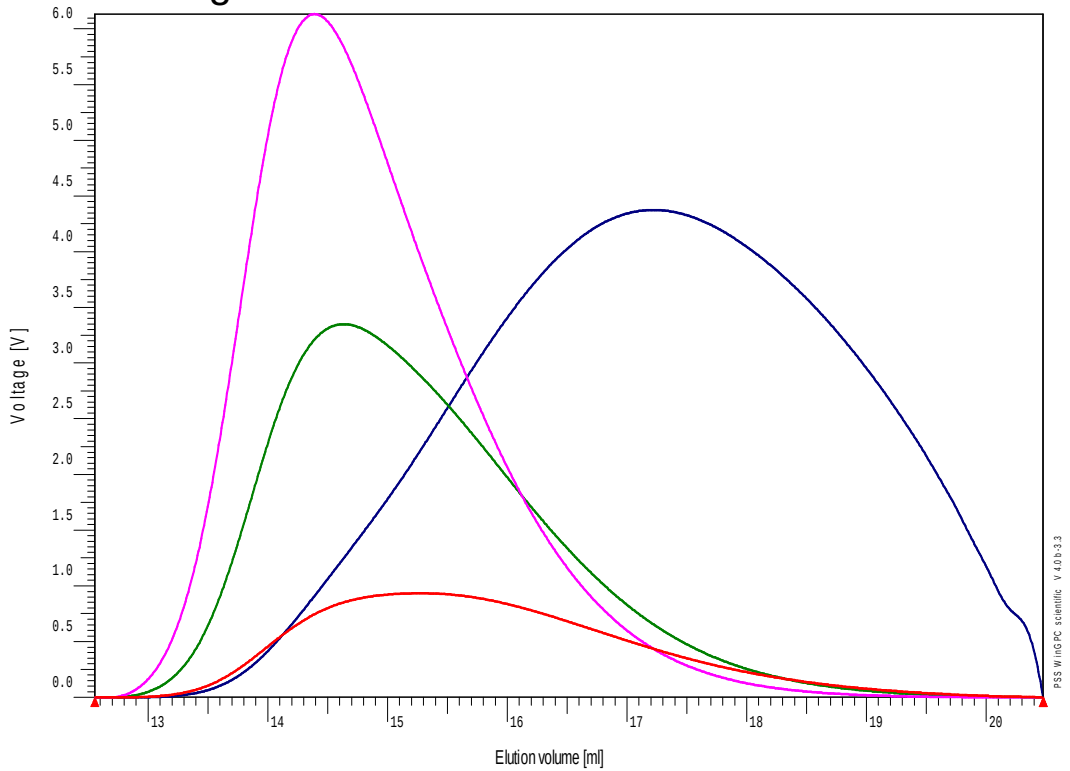
- for RI, UV, ELSD: X = 0
- for on-line LS and MS detectors: X = 1
- for on-line viscometers: X = Mark Houwink coefficient α
- for on-line NMR, osmometers* : X = -1

* not commercially available

Detection Techniques in SEC

Detector Properties

Detector Signal Characteristics



concentration detector

Refractive index detector (RI)

molar mass sensitive detectors

On-line light scattering detector

On-line viscosimeter

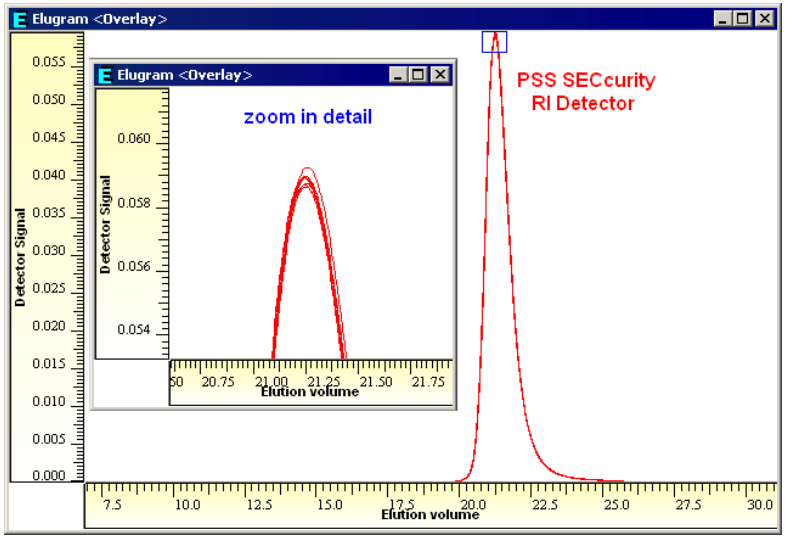
On-line mass spec (or osmometer)

advanced detector combinations provide comprehensive molecular and structural information

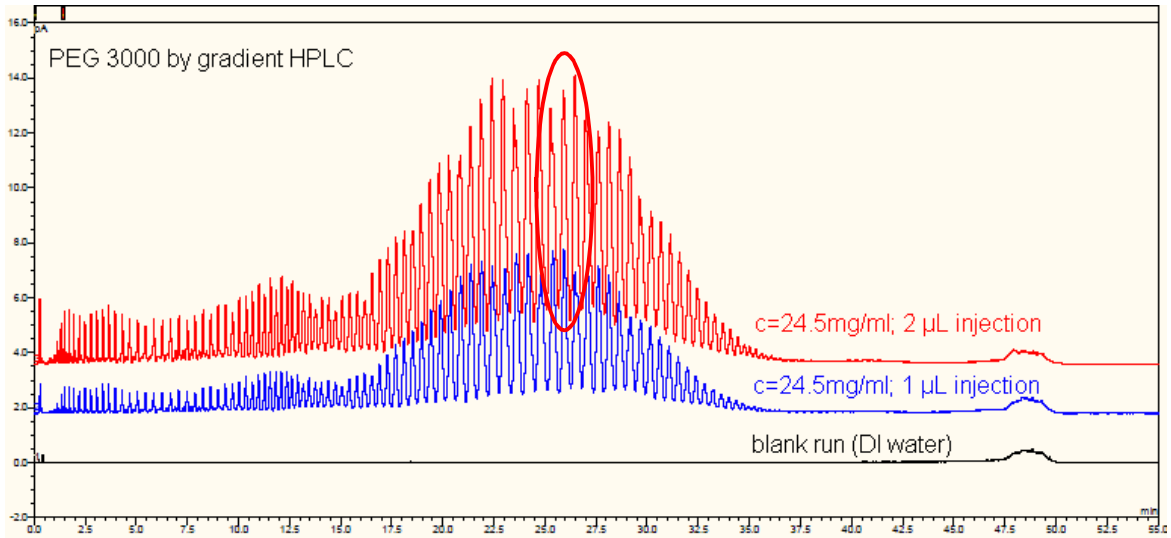
Detection Techniques in SEC

Requirements for Accurate Quantification

RI Detection



Corona (ELS) Detection



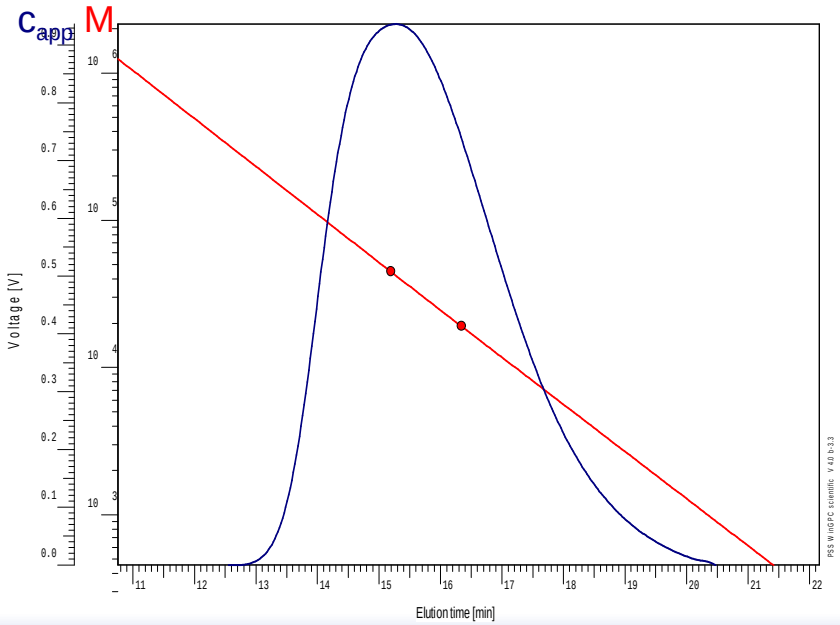
Linear response (conc-area)
Stable signal (high repeatability)
No molar mass influence
non-specific detector

Strong non-linear response (even log-log)
Poor signal stability (low repeatability)
Molar mass dependent
only non-volatiles detected

Determination of Chemical Heterogeneity

Multiple Detection in SEC Mode

What we need: $c(V), M_c(V) \rightarrow x_k(M), w(\log M_c), M_{n,c}, M_{w,c}, D_c$
What we have: $c_{app}(V), M(V)$



advantages:
- uses ordinary SEC equipment
- copolymer analysis with same injection
- no additional sample preparation

limitations:
- statistical copolymers
- graft copolymers with high graft density

Determination of Chemical Heterogeneity

Multiple Detection in SEC Mode

Approach:

~~Task1:~~ derive true c(V) from $c_{app}(V)$
needs multi-detector setup with detector calibration

$$\begin{pmatrix} U_1 \\ . \\ . \\ U_i \end{pmatrix} = \begin{pmatrix} f_{11}w_1 & .. & .. & .. \\ .. & .. & .. & .. \\ .. & .. & .. & .. \\ .. & .. & .. & f_{ik}w_k \end{pmatrix} \cdot c_{true}$$

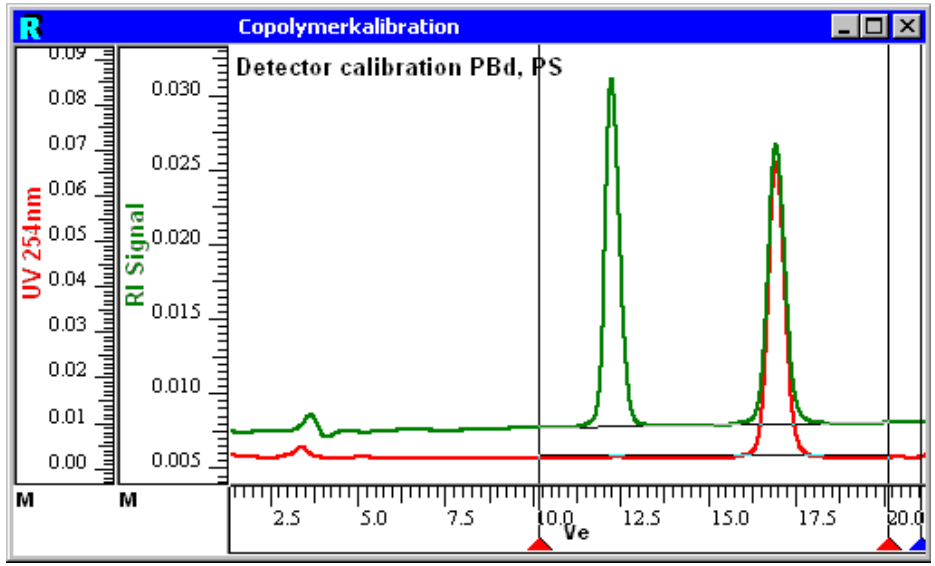
U_i response in detector *i*
f_{ik} response factor for component *k* in detector *i*
w_k weight fraction of component *k*
c_{true} concentration of sample

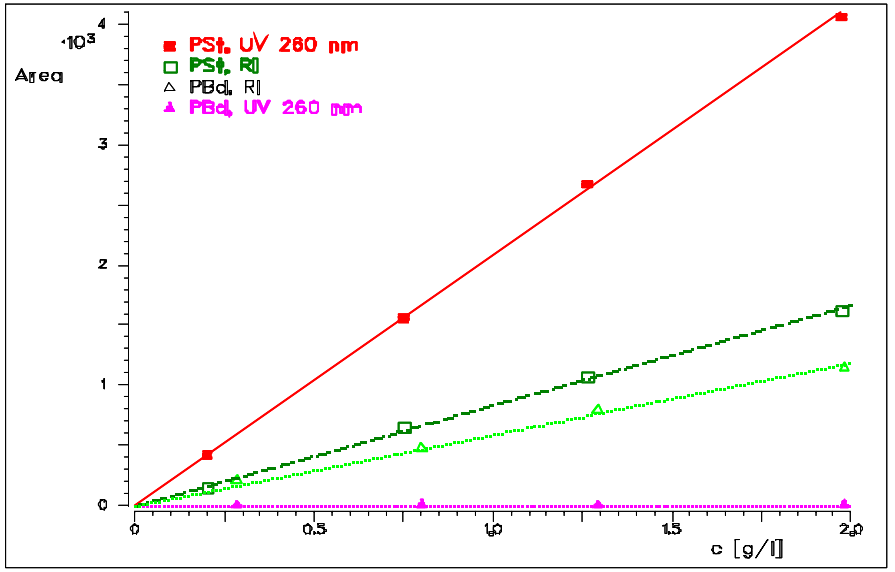
➜absolute concentration of all components *k* in sample

Determination of Chemical Heterogeneity

Multiple Detection in SEC Mode

Determination of copolymer response factors



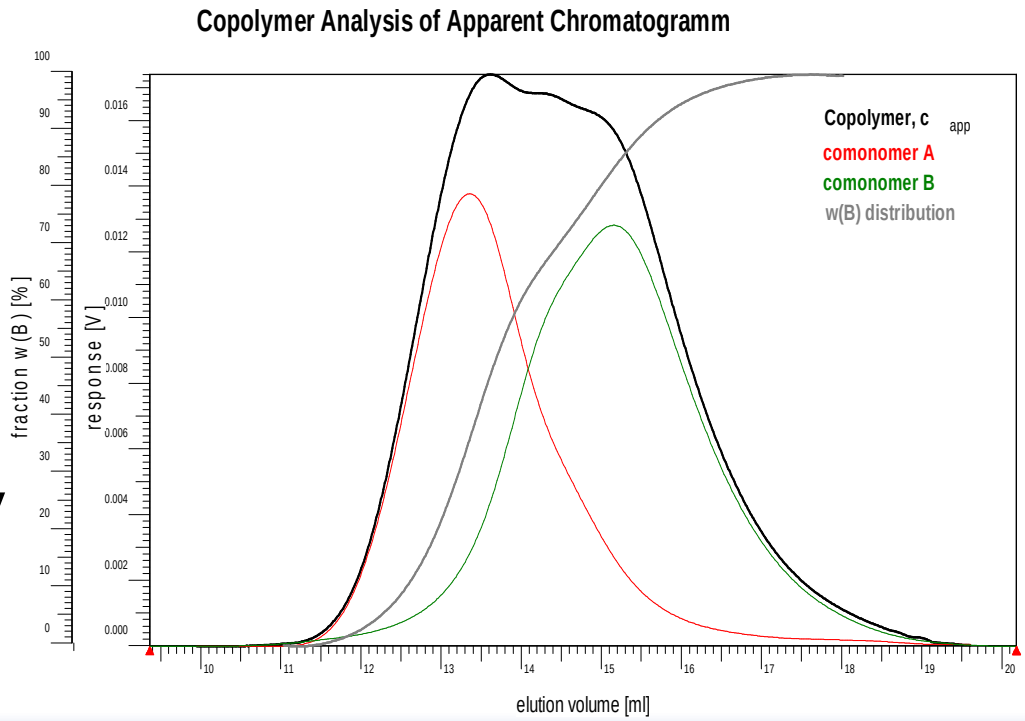


Determination of Chemical Heterogeneity

Multiple Detection in SEC Mode

Determination of comonomer concentrations

$$c_{app} = \sum_k f_{dk} \cdot c_k$$



advantages:

- universal approach
- no special equipment necessary

limitation:

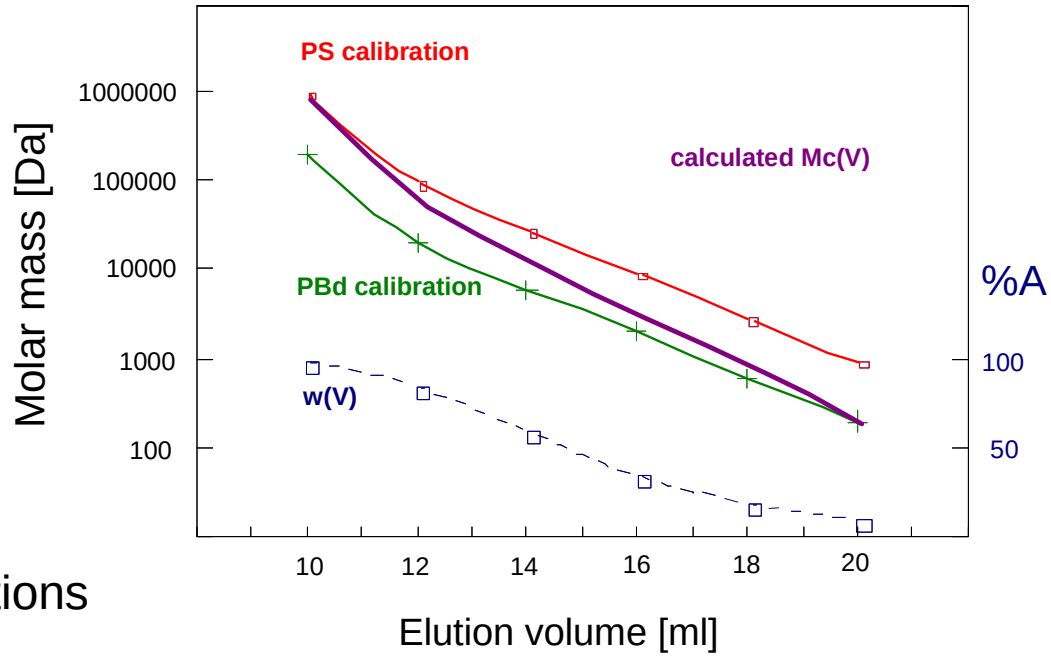
- neighbor-group effects

Determination of Chemical Heterogeneity

Multiple Detection in SEC Mode

Task 2: $M_c(V)$ from homo polymer calibration, or
$M_c(V)$ directly from molar mass sensitive detection

$$\lg M_c(V) = \Sigma\, w_k(V) \cdot M_k(V)$$



correct for negligible hetero-contact interactions

Determination of Chemical Heterogeneity

Investigation of ABA block copolymer in SEC Mode

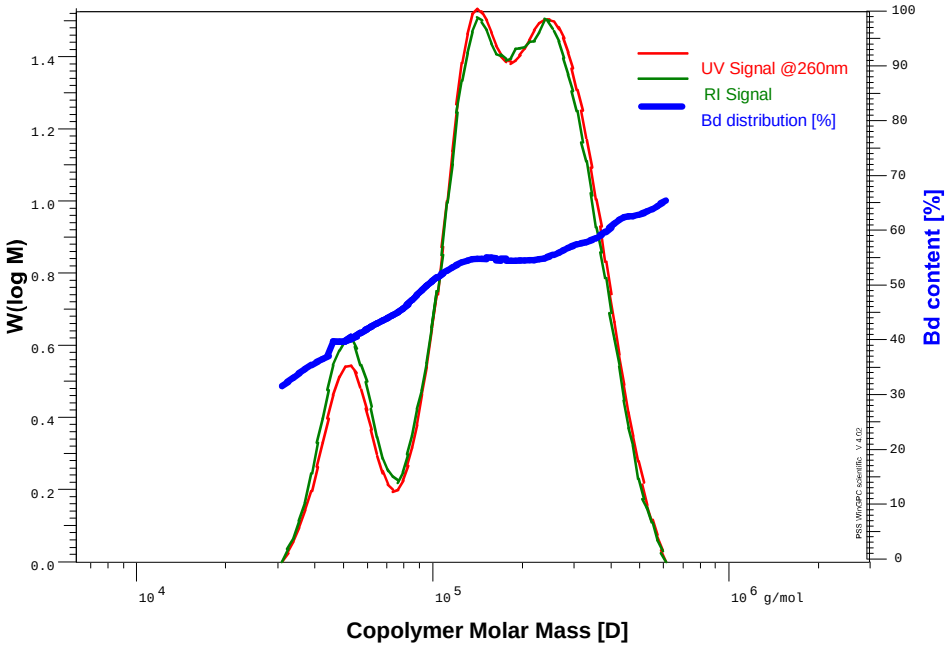
SEC results with PS standards:

Mn 127 kDa
Mw 353 kDa
PD 2.78

Copolymer results with multidetection:

Mn 76.3 kDa
Mw 222 kDa
PD 2.91

by PS and PBd calibration



Detection Techniques in SEC

SEC with a light scattering detector: MMD, MAD information

Theoretical Background Light Scattering:

for monodisperse samples, diluted solutions, particle size < $\lambda/20$

$$R(\theta) = K \cdot c \cdot M$$

K: Optical constant, includes refractive index increment $(dn/dc)^2$
M: Molar Mass
c: Concentration

for polydisperse samples with larger partical size (non-isotropic scatterer):

$$K \cdot c/R(\theta) = 1/M_w \, [1+16/3 \, \pi^2/\lambda^2 \, \langle R^2 \rangle_z \, \sin^2(\theta/2)] + 2 \, A_2 \cdot c$$

Detection Techniques in SEC

SEC with a light scattering detector: MMD, MAD information

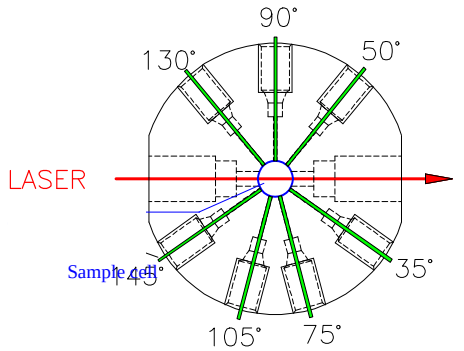
Theoretical Background Light Scattering:

$$K \cdot c / R(\theta) = 1/M_w \, [1 + 16/3 \, \pi^2/\lambda^2 \, \langle R^2 \rangle_z \sin^2(\theta/2)] + 2 \, A_2 \cdot c$$

Light scattering techniques:

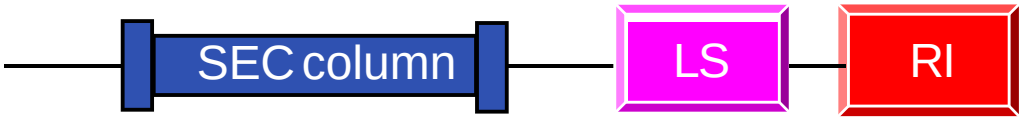
LALLS: Low angle laser light scattering
RALLS: Right angle laser light scattering
MALLS: Multi angle laser light scattering

MALLS: PSS SLD7000 detector cell



Detection Techniques in SEC

SEC with a light scattering detector: MMD, MAD information

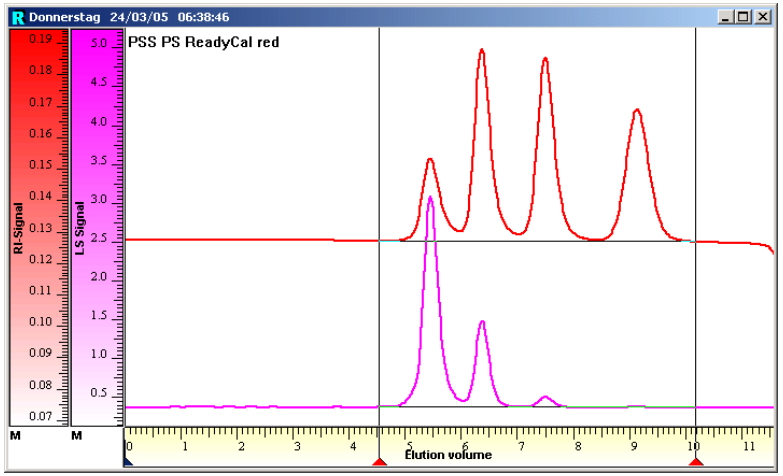


LS can be MALLS, RALLS, LALLS

LS signal: $U(LS) = K' \cdot (dn/dc)^2 \cdot c \cdot M$

RI singal: $U(RI) = K'' \cdot c$

$$\frac{LS - Signal}{RI - Signal} \rightarrow M \cdot (dn/dc)^2$$



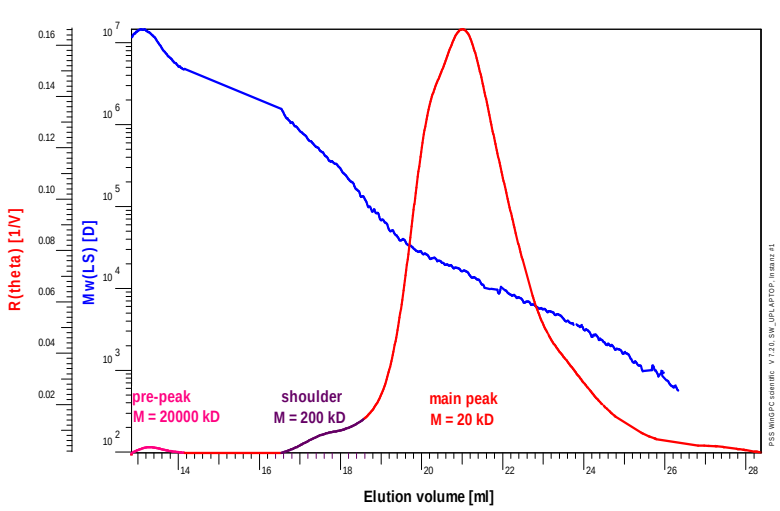
Molar Mass	1.090.000	130.000	17.800	1.620 D
Concentration	0.6334	1.2669	1.2669	1.2669 mg/ml

Detection Techniques in SEC

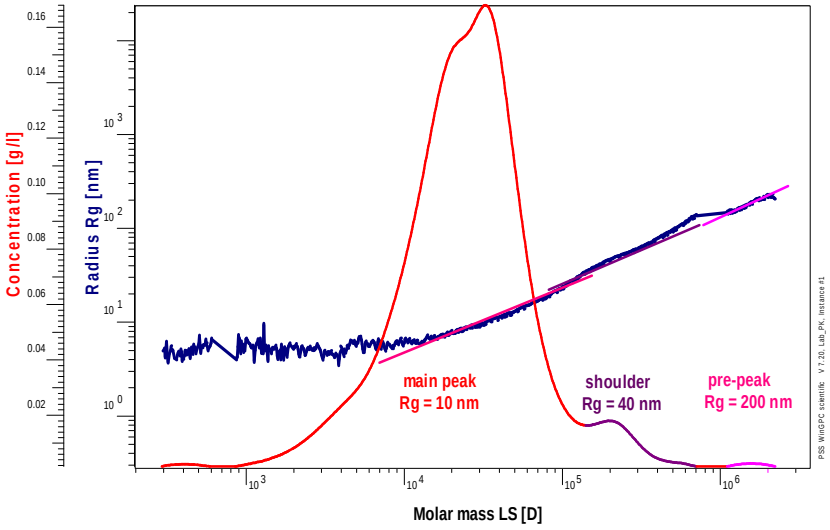
SEC with a light scattering detector: MMD, MAD information

PVB (Poly-vinyl butyral) sample: SEC-MALLS

Results on-line Zimm plot:



molar mass measured for every fraction
MMD



radius of gyration measured for every fraction
MAD

Detection Techniques in SEC

SEC with a viscometer detector: MMD, MAD information

Theoretical Background:

SEC separates according to hydrodynamic volume

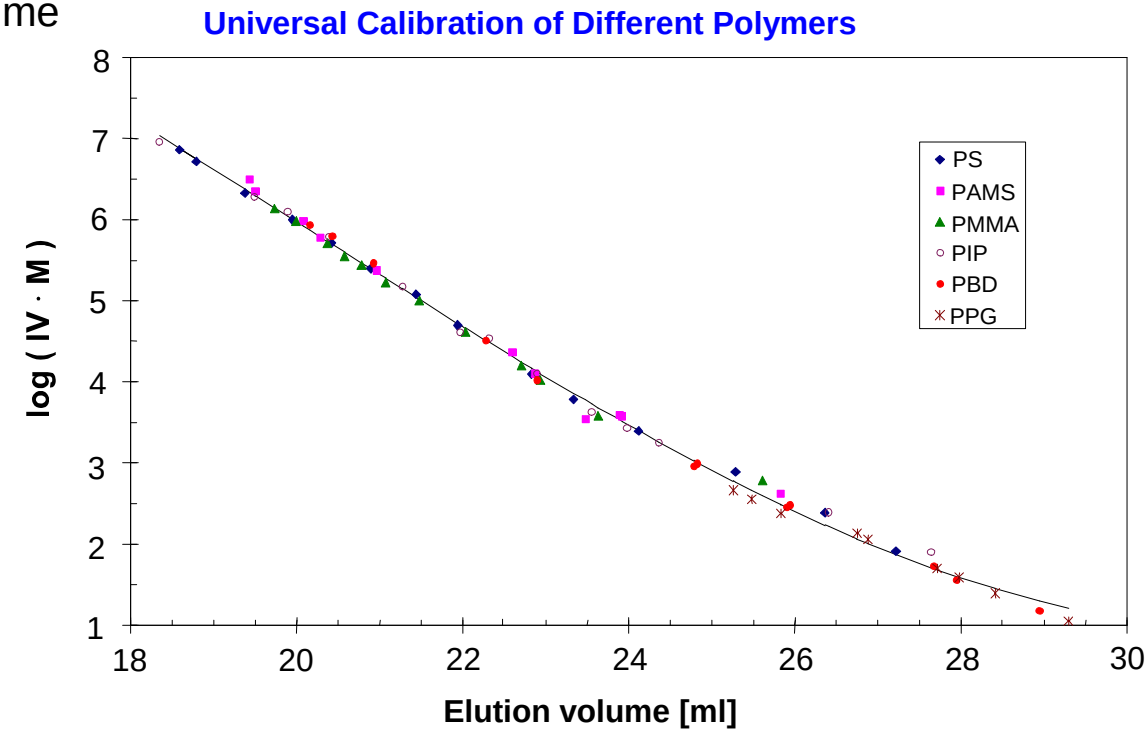
$$V_{h,1} = V_{h,2}$$

$$[\eta]_1 \cdot M_1 = [\eta]_2 \cdot M_2$$

A chance to solve the calibration dilemma:
Universal calibration curve

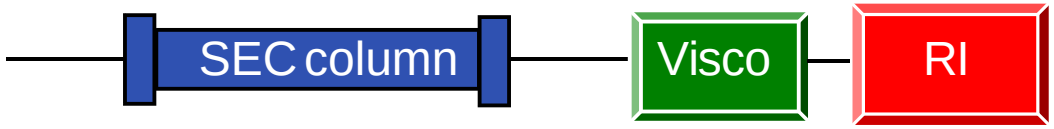
$$M_2 = [\eta]_1 \cdot M_1 / [\eta]_2$$

$$[\eta]_2 = K \cdot M_2^{\alpha}$$ Mark-Houwink equation
Structure information



Detection Techniques in SEC

SEC with a viscometer detector: MMD, MAD information

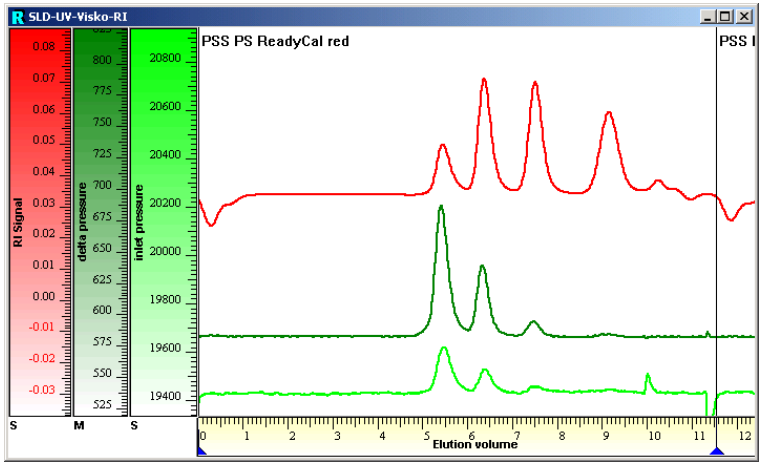


Viscometer signal: $U(V) = K' \cdot [\eta] \cdot c$

RI signal: $U(RI) = K'' \cdot c$

$$\frac{Visco - Signal}{RI - Signal} \rightarrow [\eta]_{Sample} \rightarrow$$ M from universal calibration curve

MMD



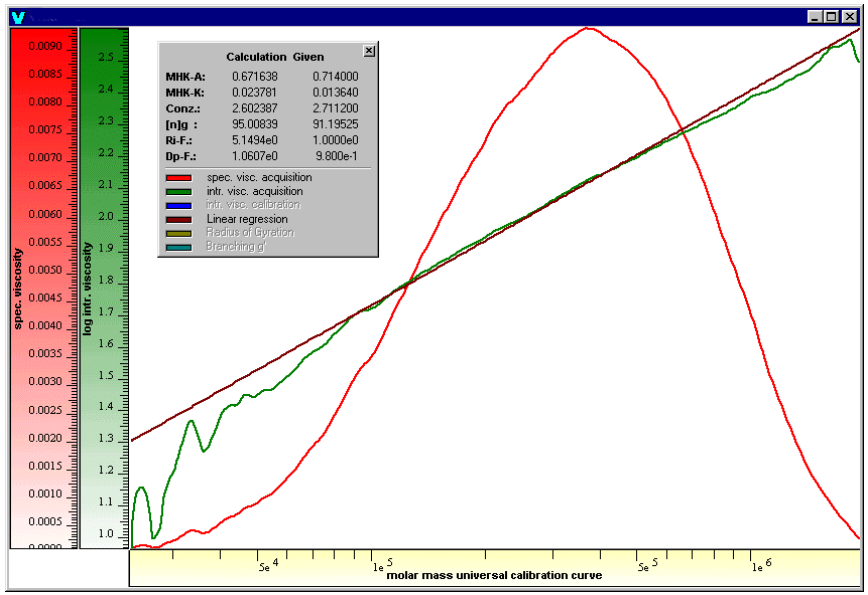
Detection Techniques in SEC

SEC with a viscometer detector: MMD, MAD information

Structure information, MAD:

$[\eta] = K \cdot M^{\alpha}$ Mark-Houwink equation

$\alpha = 2$	rigid rod
$1 > \alpha > 0{,}5$	random coil
$\alpha = 0{,}5$	random coil,Theta-conditions
$\alpha = 0$	solid sphere

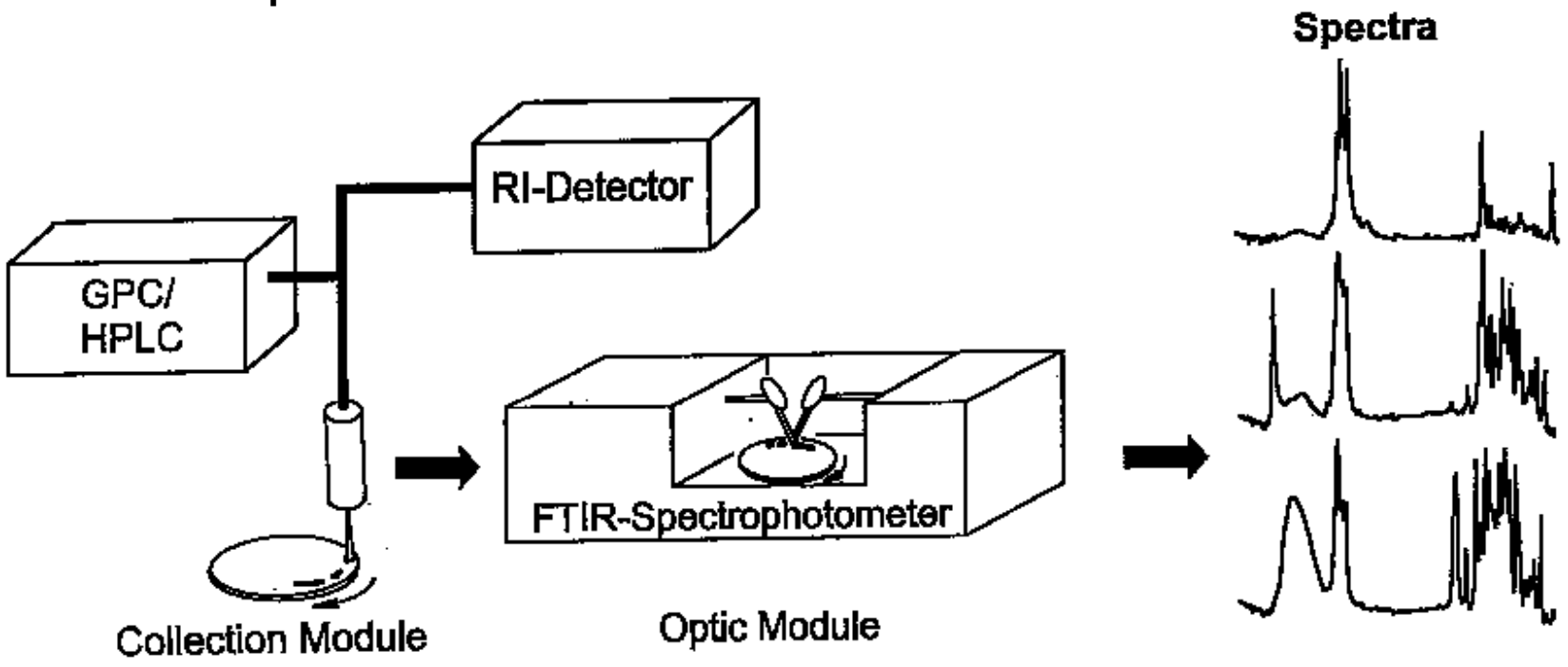


Branching coefficient g': $g' = \{[\eta]_{branched}/[\eta]_{linear}\}_M$

Detection Techniques in SEC

SEC with FTIR detection: CCD, MMD information

Simultaneous separation and identification of fractions



Detection Techniques in SEC

SEC with FTIR detection: CCD, MMD information

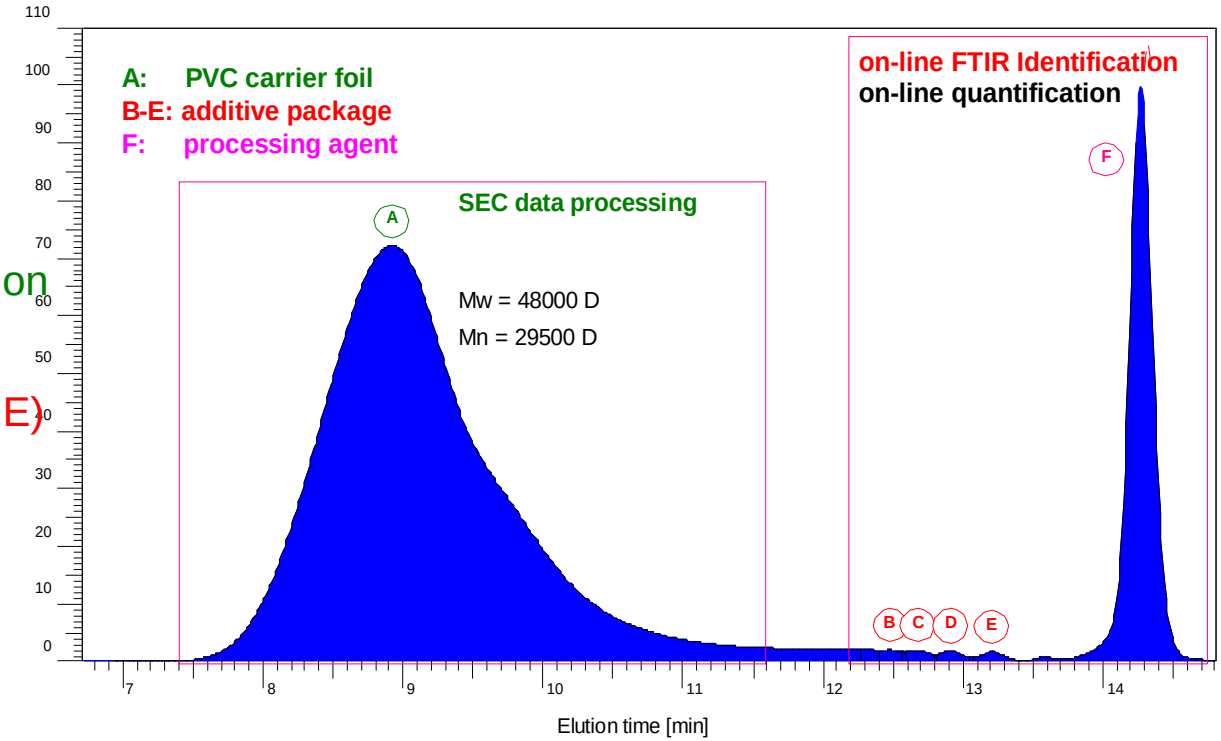
type and nature of the polymer used (peak A: PVC)

molar masses and molar mass distribution of the polymer (peak A)

identification of the additives (peaks B - E)

quantification of all additives in the packaging foil

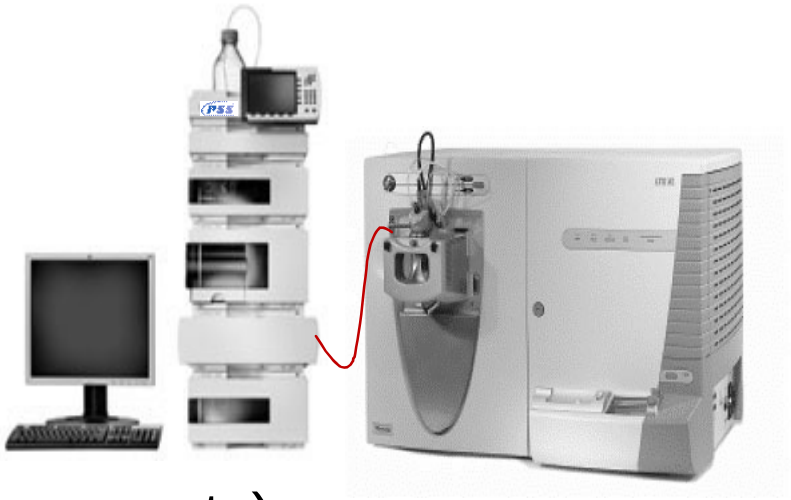
identification and quantification of the processing agent (peak F)

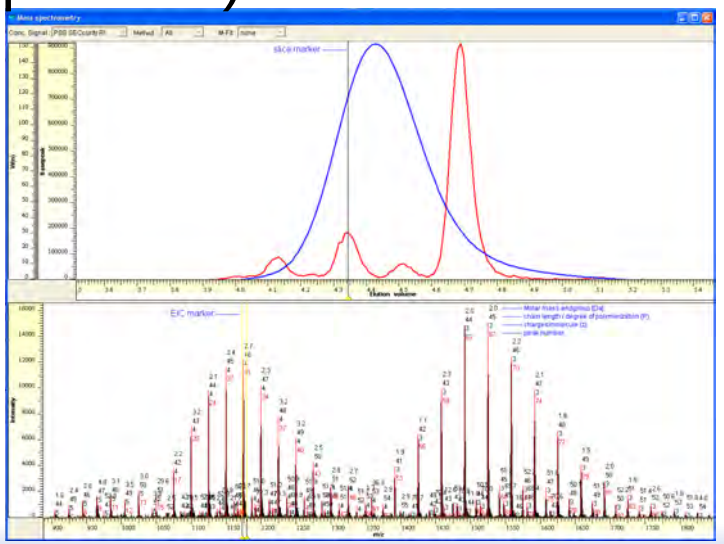


SEC with MS Detection

Potential benefits

- multiple MS techniques:
  offline: MALDI
  online: SQ, QQQ, QTOF, ion mobility
- MS measures M with highest accuracy
- MS has high resolution
- MS can resolve co-eluting species
- MS offers very high sensitivity (trace components)
- current instruments easy to use





SEC with MALDI-MS Detection

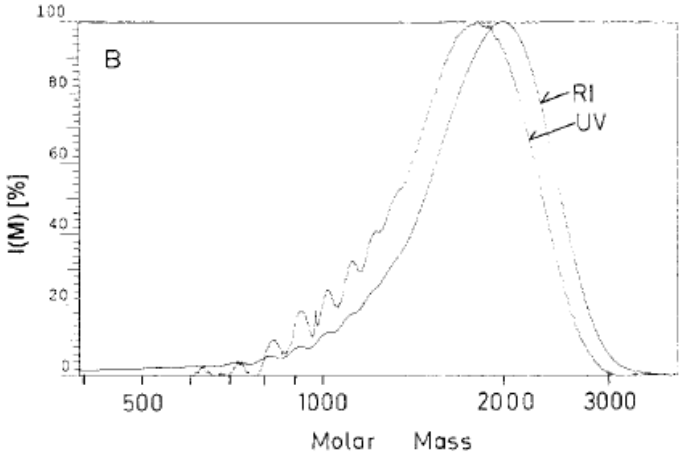
Overview

MALDI advantages:

- absolute molar mass
- repeat unit identification
- endgroup determination
- structure elucidation
- high molar mass range

disadvantages:

- matrix influnces
- discrimination in polydisperse samples
- only offline mode (spotting)
- copolymers difficult



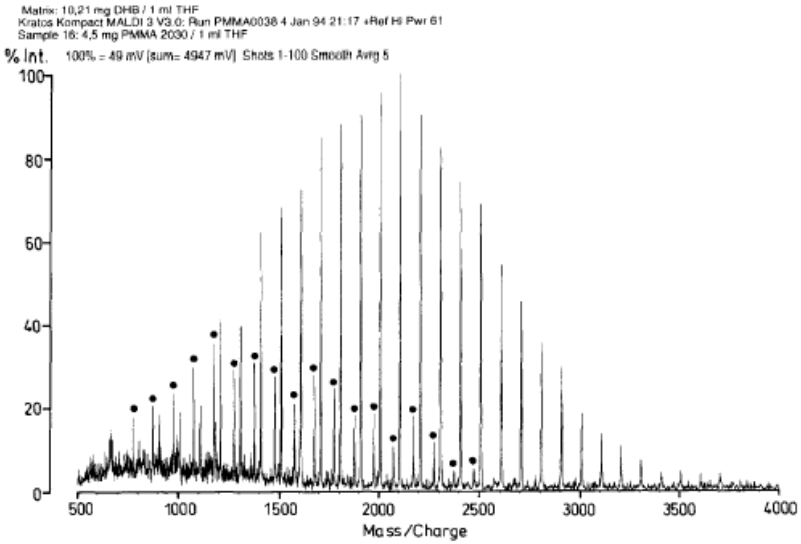


Figure 4 M.a.l.d.i.-m.s. spectrum of a PMMA calibration standard (sample 3); full circles indicate the cyclic oligomers

GPC-MALDI of PMMA
Ref.: Gores, Pasch; *Polymer* **36**, 1999

SEC with ESI-MS Detection

Overview

online MS advantages:

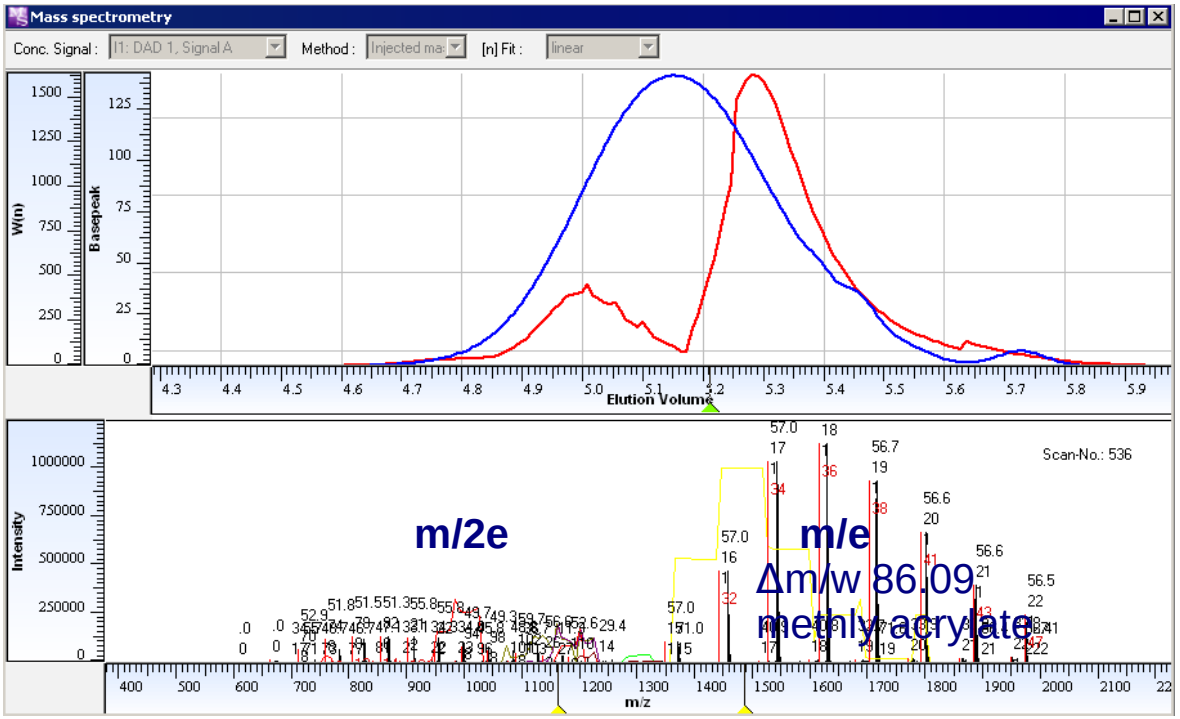
- absolute molar mass
- repeat unit identification
- endgroup determination
- structure elucidation
- identification
- high resolution

disadvantages:

- hmw limitations
- multiple charges (ESI)
- copolymers difficult

Current state of SEC-MS:

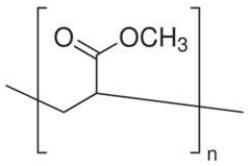
- integration in GPC/SEC software
- easy-to-use for chromatographers
- many automated workflows

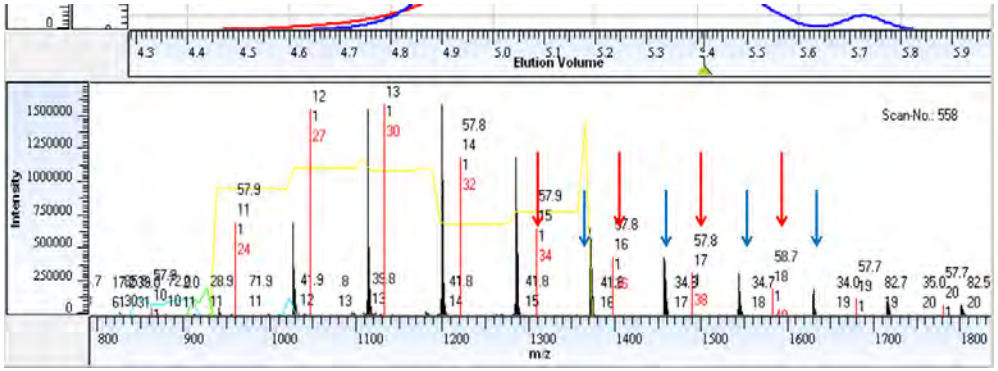


SEC with ESI-MS Detection

Analytical Results

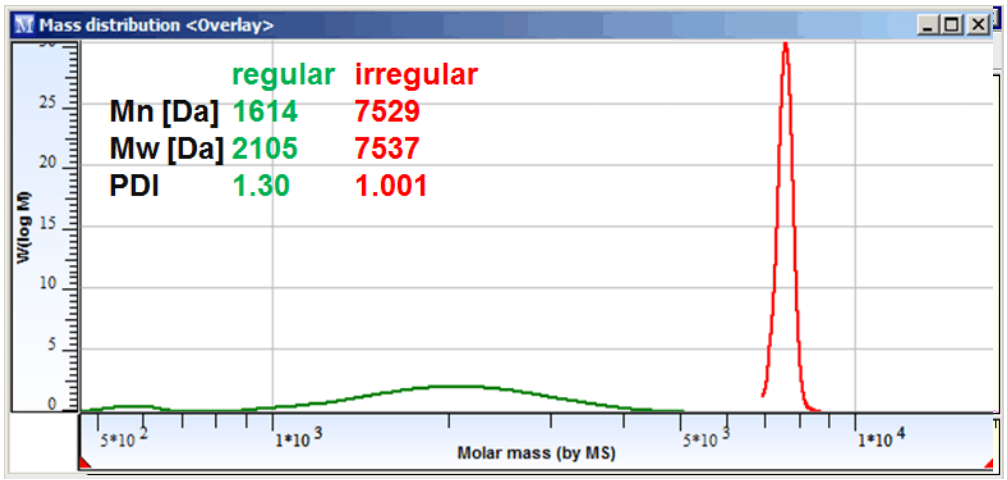
- poly(methyl acrylate)
- 2 endgroups
  propyl (43), butyl (57)
- simple charge pattern
- good mass resolution
- 2 main distributions
  same MA repeat units





SEC-MS reveals:
- mixture of species which behave differently in SEC
- GPC separation of regular chains
- no separation if irregular species

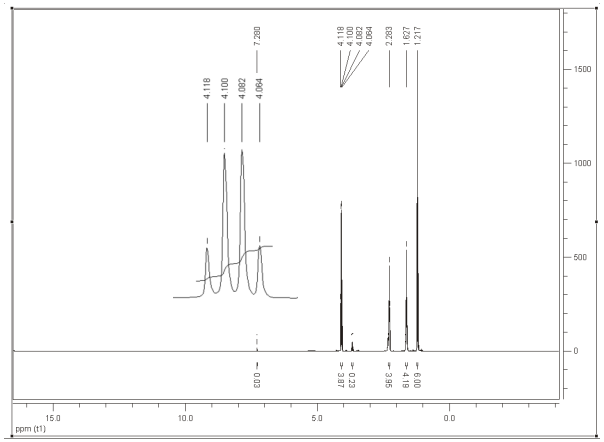
However:
still absolute M and MWD



SEC with NMR Detection

Basics

NMR can be used as a (universal) chemical detector
NMR is a chemical sensor looking at local chemical environment
ideal for structure elucidation: chemical shift, J coupling

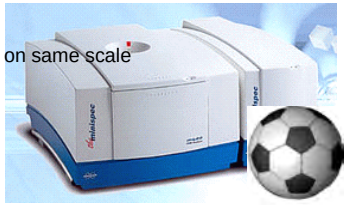


High-field NMR couling

non-destructive
super-conductivemagnet
highresolution
small differences obvious
expensive
complex
large
time-consuming
interfacing difficult
high operational cost
expert knowledge required

Low-field NMR detection

non-destructive
permanentmagnet
lowresolution
major sample characteristics
inexpensive
simple to use (detector)
smallbenchtop
low operation cost
flexible
modular setups
saves sample prep time

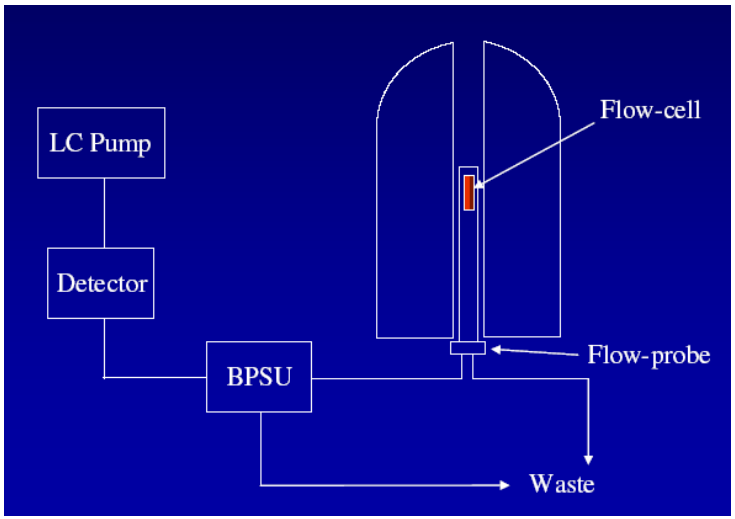


SEC with NMR Detection

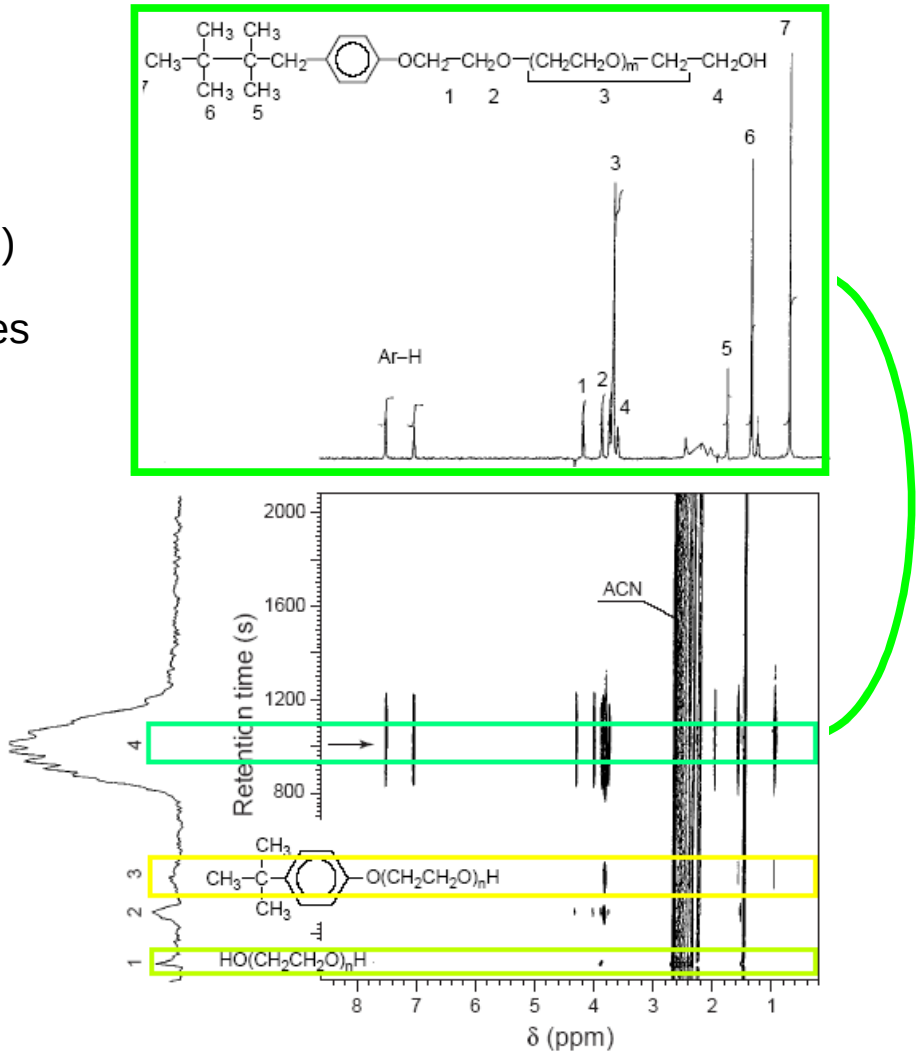
High-Field NMR Coupling to HPLC

Sample: non-ionic surfacrants

- samples have been stored in storage valve (BPSU)
  Offline NMR scans
- solvent signals eliminated by NMR pulse sequences



Ref.: Pasch/Hiller (1996), Macromolecules, 2, 6556



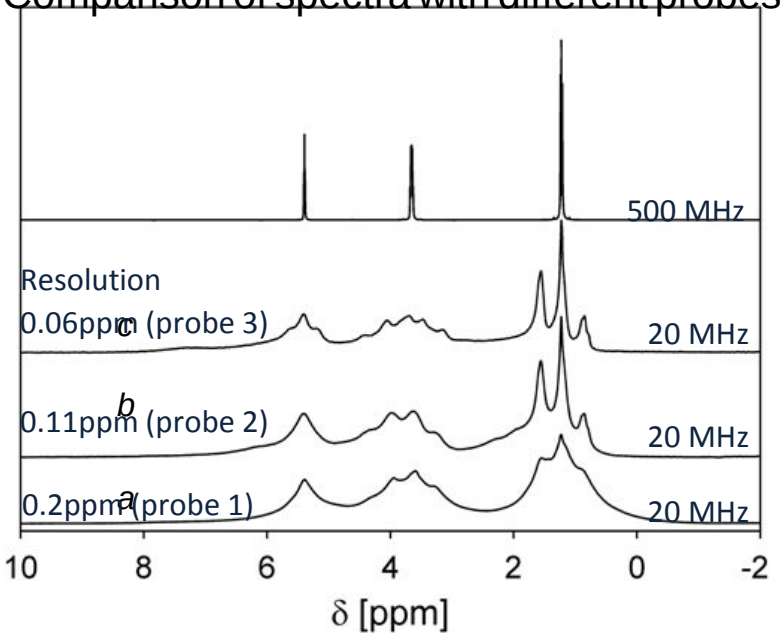
SEC with ^{1}H-NMRDetection

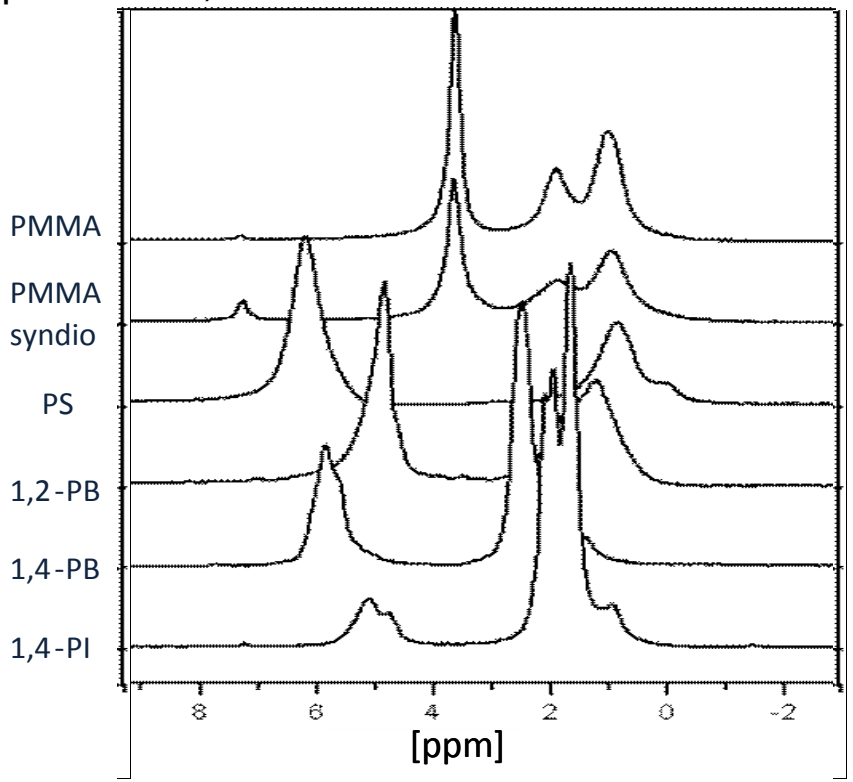
Current Status

base NMR: Bruker TopSpin, 20 MHz magnet
automatic supression of solvent peaks
0.2ml probe
scan time: 2 secs

run on: PSS SECcurity GPC system, single PSS SDV 5µm column, THF
typical SEC injection conditions

Comparison of spectra with different probes





Conclusions

- comprehensive SEC/GPC is an established and versatile method
- plethora of LC and detection methods for structure investigation
- information request determines chromatographic strategy
- in-depth characterization of MMD,CCD,FTD,MAD,etc. possible
- combination of LC modes opens new horizons
- increase of peak capacity by 2D chromatography
- unbiased investigation of property distributions
- mapping of samples or property quantification in 2D
- information-rich detectors add identification and structure elucidation to separation