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

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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
   requires batch methods
   access to bulk properties / property averages
   e.g. Light Scattering (LS), Viscometry, Osmometry, Ultracentrifugation (AUC)
   NMR, IR, ...

B) Characterization of separated fractions
   requires comprehensive chromatography
   access to property distributions
   E.g. Liquid chromatography (SEC, LAC, LCCC)
   Ultracentrifugation (AUC)
   Field flow fractionation (FFF)
   (Gas chromatography: GC)
   Mass spectrometry: MALDI-ToF

   1) analytical fractionation methods:
   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

\[ K_{\text{SEC}} = \exp (\Delta S/R) \]

\[ 0 < K_{\text{SEC}} < 1 \quad \Delta H = 0 \]

b) Adsorption mode: HPLC

\[ K_{\text{HPLC}} = \exp (-\Delta H/RT) \]

\[ K_{\text{HPLC}} > 1 \quad \Delta H \gg T\Delta S \]

c) critical adsorption point: LC-CC

\[ K = 1 \quad \Delta H = \Delta S \]
## Chromatographic Modes of Separation

### Comparison of Chromatographic Modes

<table>
<thead>
<tr>
<th>Technique</th>
<th>Separation governed by</th>
<th>Information Content</th>
<th>Potential Problems</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SEC</strong></td>
<td>• hydrodynamic volume</td>
<td>• molar mass (MMD)</td>
<td>• calibration dilemma</td>
</tr>
<tr>
<td></td>
<td>• molecular size in solution</td>
<td>• chemical composition (CCD)</td>
<td>• specific interactions</td>
</tr>
<tr>
<td></td>
<td><em>diffusion controlled process</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LC-CC</strong></td>
<td>• chain inhomogeneity</td>
<td>• functionality type (FTD)</td>
<td>• irreversible adsorption</td>
</tr>
<tr>
<td></td>
<td>• defect structures</td>
<td>• molecular architecture (MAD)</td>
<td>• determination of critical adsorption point</td>
</tr>
<tr>
<td></td>
<td>• endgroups</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>diffusion and adsorption controlled process</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HPLC</strong></td>
<td>• chemical composition</td>
<td>• chemical composition (CCD)</td>
<td>• molar mass influence</td>
</tr>
<tr>
<td></td>
<td>• endgroup</td>
<td>• functionality type (FTD)</td>
<td>• partial adsorption</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• large k’</td>
</tr>
<tr>
<td></td>
<td><em>adsorption controlled process</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 retention 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
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, \( \mu_i \), of the molar mass distribution:

\[
\mu_i = \int M^i \cdot w(M) \, dM
\]

with \( \mu_i \) 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:

\[
M_n = \frac{\sum \nu(M_i) \cdot M_i}{\sum \nu(M_i)} = \frac{\sum \nu(M_i) \cdot M_i}{\mu_1}
\]

Weight average molecular weight:

\[
M_w = \frac{\sum \nu(M_i) \cdot M_i^2}{\sum \nu(M_i) \cdot M_i} = \frac{\sum \nu(M_i) \cdot M_i^2}{\mu_1}
\]

Z-average molecular weight:

\[
M_z = \frac{\sum \nu(M_i) \cdot M_i^3}{\sum \nu(M_i) \cdot M_i^2} = \frac{\sum \nu(M_i) \cdot M_i^3}{\mu_1}
\]
Conventional Data Analysis

Determination of fundamental parameters

Chromatogram: relates apparent 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:

\[ M_w : (29610 \pm 150) \text{ g/mol} \]
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

Overlay HighSpeed SEC

Heparin QC Chart

QC passed

Sample A
Sample B

molar mass [D]

Time
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:

- **1st dimension:**
  - HPLC
  - Degasser
  - Pump
  - Autosampler
  - Column Oven/
  - Column

- **2nd dimension:**
  - SEC
  - Degasser
  - Pump

- Transfer Valve

SEC results (chromatograms)

Waste

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2D Chromatography

SEC Analysis of TPE

Sample B failed in the field
- main product looks very similar
- similar by-products present

SEC does not track performance differences

<table>
<thead>
<tr>
<th></th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn[kD]</td>
<td>99</td>
<td>90</td>
</tr>
<tr>
<td>Mw[kD]</td>
<td>109</td>
<td>103</td>
</tr>
<tr>
<td>Mw/Mn</td>
<td>1.08</td>
<td>1.14</td>
</tr>
<tr>
<td>Mp[kD]</td>
<td>108</td>
<td>104</td>
</tr>
<tr>
<td>by-product</td>
<td>0.8%</td>
<td>1.7%</td>
</tr>
</tbody>
</table>

Molar masses by narrow PSt calibration

Differences due to composition?
2D Chromatography

Comprehensive 2D by HPLC$x\times$SEC

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 motor oil additives

Individual techniques

Observations difficult to explain
2-Dimensional Chromatography

Investigation of by-product in motor oil additives

2D results

- main product (region 1)
- 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

\[ U_d = K_d \times \sum_i (k_{\text{Sample}} \times c_{\text{Sample}} \times M^x) \]

- **U_d**: Signal intensity
- **K_d**: Instrument constant
- **k_{\text{Sample}}**: Sample dependent parameter
  - for spectroscopic detectors:
    - k_{\text{sample}} = extinction coefficient, \( \kappa \)
  - for refractive index (RI) detectors:
    - k_{\text{sample}} = refractive index increment, dn/dc
    - note: dependent on solvent composition, T, \( \lambda \)
- **c_{\text{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 \( \alpha \)
  - 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**
- Linear response (conc-area)
- Stable signal (high repeatability)
- No molar mass influence
- Non-specific detector

**Corona (ELS) Detection**
- 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:

Task 1: derive true \(c(V)\) from \(c_{app}(V)\)

needs multi-detector setup with detector calibration

\[
\begin{pmatrix}
U_1 \\
\vdots \\
U_i \\
\vdots \\
U_j
\end{pmatrix} =
\begin{pmatrix}
f_{11}w_1 & \cdots & \cdots \\
\vdots & \ddots & \cdots \\
\vdots & \cdots & \ddots \\
\vdots & \cdots & f_{ik}w_k
\end{pmatrix}
\cdot c_{true}
\]

- \(U_i\) 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

\[ c_{\text{app}} = \sum_{k} f_{dk} \cdot c_{k} \]

advantages:
- universal approach
- no special equipment necessary

limitation:
- neighbor-group effects

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Determination of Chemical Heterogeneity

Multiple Detection in SEC Mode

Task 2: \( M_c(V) \) from homo polymer calibration, or \\n\( M_c(V) \) 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:

- $M_n$ 127 kDa
- $M_w$ 353 kDa
- $P_D$ 2.78

Copolymer results with multidetection:

- $M_n$ 76.3 kDa
- $M_w$ 222 kDa
- $P_D$ 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 < λ/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 particle size (non-isotropic scatterer):

\[ K \cdot c / R(\theta) = 1/M_w [1 + 16/3 \pi^2/\lambda^2 <R^2> + \sin^2(\theta/2)] + 2 A_2 \cdot c \]
Detection Techniques in SEC

SEC with a light scattering detector: MMD, MAD information

Theoretical Background Light Scattering:

\[ K \cdot c / R(\theta) = 1/M_w \left[ 1 + 16/3 \pi^2/\lambda^2 <R^2>_z \sin^2(\theta/2) \right] + 2 A_2 \cdot c \]

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

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

<table>
<thead>
<tr>
<th>Molar Mass</th>
<th>1.090.000</th>
<th>130.000</th>
<th>17.800</th>
<th>1.620 D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.6334</td>
<td>1.2669</td>
<td>1.2669</td>
<td>1.2669 mg/ml</td>
</tr>
</tbody>
</table>
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

Universal Calibration of Different Polymers

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Elution volume [ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>18</td>
</tr>
<tr>
<td>PAMS</td>
<td>20</td>
</tr>
<tr>
<td>PMMA</td>
<td>22</td>
</tr>
<tr>
<td>PIP</td>
<td>24</td>
</tr>
<tr>
<td>PBD</td>
<td>26</td>
</tr>
<tr>
<td>PPG</td>
<td>28</td>
</tr>
</tbody>
</table>

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Detection Techniques in SEC

SEC with a viscometer detector: MMD, MAD information

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

RI signal: \( U(\text{RI}) = K'' \cdot c \)

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

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 \( g' \):

\[ g' = \left( \frac{[\eta]_{\text{branched}}}{[\eta]_{\text{linear}}} \right)_M \]
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 influences
- discrimination in polydisperse samples
- only offline mode (spotting)
- copolymers difficult

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
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 coupling

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

Low-field NMR detection

- non-destructive
- permanent magnet
- low resolution
- major sample characteristics
- inexpensive
- simple to use (detector)
- small benchtop
- low operation cost
- flexible
- modular setups
- saves sample prep time
SEC with NMR Detection

High-Field NMR Coupling to HPLC

Sample: non-ionic surfactants

- 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-NMR Detection

Current Status

base NMR: Bruker TopSpin, 20 MHz magnet
automatic suppression 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

- Resolution
  - 0.06ppm (probe 3)
  - 0.11ppm (probe 2)
  - 0.2ppm (probe 1)

- Chemical shifts:
  - PMMA
  - syndio
  - PS
  - 1,2-PB
  - 1,4-PB
  - 1,4-PI

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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