INTERACTION POLYMER CHROMATOGRAPHY

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Liquid chromatography for polymer characterization

Dissolution → Separation → Detection → Data Reduction

**Separation media**
- porous particles (HPLC, UPLC)
- non-porous particles (HDC)
- monolithic columns
- capillary

**Size Exclusion Chromatography**
- SEC
  - separation by molecular size

**Interaction Polymer Chromatography**
- IPC
  - separation by **everything** but size

2D-Chromatography
- IPC/SEC, IPC/IPC, SEC/IPC
Molecular properties of polymers & other nanostructures

Molar mass, size and shape
- End-groups
  - Linear
  - Telechelic
- Topology
  - Star
  - Comb

Chemical Composition and Microstructure
- Homopolymers
  - Linear
  - Telechelic
- Copolymers
  - Statistical
  - Alternating
  - Block
  - Gradient

Biomolecules, nanoparticles, micelles, vesicles

Molecular heterogeneities (distributions): strong effect on end-use properties

Synthesis - Molecular Structure - Property
Open literature

1. SEC is by far the **most popular** LC technique for polymer characterization

2. IPC: mostly **isocratic separations** (LCCC+ barrier techniques)

3. HPLC practitioner: **do not use** isocratic mode in separation of big molecules!

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1. IPC/SEC: **50/50(%)**

2. IPC: **gradient** approaches (including TGIC) **>90%**

3. IPC: practically all separations problems are more effectively solved in gradient mode (vs. isocratic)

Perception: more difficult to control the mechanism of separation in gradient mode
How to control separation

- Order of elution (selectivity of separation) is determined by **mechanism of retention**
- Retention is result of **interaction** between molecules and solid surface of porous particles
- **Right balance** between different types of interaction is the key to **separation mechanism**

Steric Interaction (SEC)

Critical point of adsorption (LCCC)

IPC

Adsorption (HPLC)  Phase Transition / Partition (Precipitation LC)

**LCCC**: LC at critical conditions, or LC at **critical point of adsorption** (CPA)

Enthalpy gain  Entropy loss

**Mutual compensation effect**: MW-independent elution
Elution of Narrow Polystyrenes on Symmetry® C18

THF suppresses non-polar interaction

SEC mode at $\Phi = 100\%$THF : big elutes first

Non-polar interaction prevails

Adsorption mode at $\Phi = 45\%$THF : big elutes last (or does not elute at all)
Elution of Narrow Polystyrenes at Symmetry® C\textsubscript{18}

**Critical Point of Adsorption (CPA)**

<table>
<thead>
<tr>
<th>THF-ACN (48/52)</th>
<th>474</th>
<th>37,900</th>
<th>186,000</th>
</tr>
</thead>
</table>

Transition mode: MW-independent elution at $\Phi = \Phi_{cr}$ (48\% THF)

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**Elution at different compos. $\Phi$ (THF,%)**

- **SEC**
- **LCCC**
- **LAC**

- $\Phi_{CR} = 48\%$
- $45$
- $40$
- $80$
- $100$

Elution volume $V_R$ (mL)

- $V_0$
- $V_{liq}$

- $t_R$, min (flow rate 0.25 ml/min)
Applications

• Separation by functional groups
• Separation of block-copolymers by length of individual blocks
• Separation of homopolymer blends
• Not applicable to statistical copolymers

Limitations

• Often limited to oligomers/low-M polymer separations in wide-pore columns
• Very sensitive to experimental conditions
• Temperature/solvent composition fluctuations resulting in peak splitting, limited mass recovery

LCCC of 186K at different flow rates

Flow rate (ml/min):

Nova-Pak C\textsubscript{18} Column
(60A, 4 \(\mu\)m, 3.9 x 300 mm)
THF-ACN (49/51)

Let’s discuss after Falkenhagen’s and Hiller’s talks
Elution of linear PEG (MW=40K) with different end groups at CPA on Symmetry® C₄

Critical point of adsorption (CPA): 50% ACN in H₂O on Symmetry® C₄

$$K_{LCCC} = 1 + q$$

$$\Delta t = 0.13$$

- mono-brominated PEG
- PEG standard with 2 OH ends

$$\Phi = \Phi_{cr} = 50\%$$ ACN
Barrier Techniques

- **Liquid Chromatography under Limiting Conditions (D. Berek, et al)**
  - of adsorption (LGA): \( \Phi_{\text{sample}} > \Phi_{\text{CR}}, \Phi_{\text{eluent}} < \Phi_{\text{CR}} \)
  - of solubility (LCS): \( \Phi_{\text{sample}} > \Phi_{\text{SOL}}, \Phi_{\text{eluent}} < \Phi_{\text{SOL}} \)
  - of desorption (LCD): \( \Phi_{\text{sample}} < \Phi_{\text{CR}}, \Phi_{\text{eluent}} > \Phi_{\text{CR}} \)

- **Limitations**: local solvent gradient depends on viscosity, column hydrodynamics, chromatographic conditions (flow rate, injection volume, concentration), separation is limited to column volume

- **SEC-Gradients method (W. Radke)**

**Let’s discuss after Radke’s talk**
Chromatographic characterizations of polymer blend:
polystyrene / styrene-acrylonitrile copolymer / styrene-butadiene copolymer

**SEC: separation by size**

Isocratic separation by size in THF at SEC column set

**Gradient IPC: MW-independent elution**

Gradient separation by composition in ACN-THF gradient at C_{18} RP column
Elution of narrow polystyrenes at Symmetry® C18

ACN – THF gradient (0%-100%) over 10 min

Elution time, min (flow rate 1 ml/min)

Eluent composition \( \Phi \) (THF, %)

Oligomers

\( \Phi_{CR} \)

Eluent gradient

9,100

43,900

190,000

355,000

710,000

1,260,000

Gradient Elution (GE) at CPA

LAC

\( \Phi_{CR} = 48\% \) THF

Log \( M \)

0 10 20 30 40

0 2 4 6

Practical advice: even if you want to use LCCC, run gradient first: gradient elution is the best way to find critical point of adsorption

Brun, Alden, J. Liq. Chrom 2002
Solvent gradient methods

**LAC**
separation by MW, chemistry, etc.

**GE at critical point of adsorption (CPA)**
separation by chemistry, topology

**Precipitation-redissolution chromatography (elution at dissolution point) (GPEC)**
separation by MW and chemistry

**CPA does not exist**
Elution of narrow polystyrenes at Nova-Pak® C₈

Different modes of gradient separation

Gradient elution at CPA

ACN – THF (0 -100%) over 10 min

\[ \Phi^*_{sol} < \Phi_{CR} \]: elution at CPA

Gradient elution at redissolution point (GPEC)

MeOH – THF (0 -100%) over 10 min

\( \Phi_{CR} \) does not exist: elution at redissolution

Brun, Alden, J. Liq. Chrom 2002
Elution of polystyrenes at Nova-Pak® C₈

X: MeOH (■) - elution at redissolution

ACN (◇) – elution at CPA

Only elution at CPA is MW-independent!
Gradient elution at redissolution point (GPEC)

Liquid-liquid phase *transition in the presence of solid surface*: $\Delta F \sim M (\Phi - \Phi^*_{\text{sol}})$

Usually, $\Phi^*_{\text{sol}} > \Phi_{\text{sol}}$

Threshold depends on polymer molecular weight and concentration

$\Phi^*_{\text{sol}}$ vs. $\Phi_{\text{inj}} < \Phi^*_{\text{sol}}$

Gradient elution at CPA can occur only if $\Phi^*_{\text{sol}} < \Phi_{\text{CR}}$

Otherwise, $\Phi_{\text{CR}}$ does not exist and elution is MW-dependent

(Armstrong et.al., 1984)
How to control the transition from LAC to GE at CPA

Transition point: \( Q \sim 1 \)

\( Q < 1 \) - retention depends on molar mass \( M \) (LAC)

\( Q > 1 \) (CPA)

\[
Q = \frac{2R_g^2}{\alpha D} \times \frac{d\varepsilon}{d\Phi} \times \Phi'
\]

Transition to CPA occurs faster (at lower \( M \)) for:

- narrow pores (low \( D \))
- steep gradient (high \( \Phi' \))
- high selectivity (high \( \frac{d\varepsilon}{d\Phi} \))

\( R_g \) - radius of gyration

\( D \) - pore diameter

\( \varepsilon \) - segment interaction energy

\( \Phi' \) - gradient rate
Effect of operating conditions

Elution of narrow polystyrenes at NovaPak® Silica

Hexane – THF gradient (0 -100%) over 10 min

Theory vs. Experiment

Effect of gradient rate: shallower gradient – later transition to CPA
- IPC at adsorption conditions (LAC): separation by M, not size, but for oligomers only

- $\Phi_{CR}$ depends on $T$, but $d\varepsilon/dT << d\varepsilon/d\Phi$

- Temperature gradient should be much more effective in separation by MW
Temperature vs. solvent gradients

TGIC of narrow polystyrenes at Symmetry® C18

T' = 2°C/min, flow rate 1 ml/min, Φ_{isocr} = 44% THF, T_{CR} = 60°C

- Able to resolve high M species
- Solvent gradient is still useful to identify critical conditions
- Intrinsic relation between solvent & temperature gradient
- TGIC could be coupled to SEC in 2D setting: SEC-IPC for branching analyses
Gradient elution at CPA

Applications

• Separation of polymer blends
• Separation of telechelic polymers by functional groups
• Separation of copolymers by chemical composition and microstructure, e.g. Blockiness
• Copolymer purification by flesh chromatography based on IPC
• Separation of nanoparticles by surface chemistry

Benefits

• Easiest way to find CPA
• Broad range of eluents (including non-solvents)
• Practically no limitation on MW
• Superb resolution, especially at narrow pore columns
• No peak splitting or some other chromatographic artefacts

Potential problems

• Break-through effect
• Multiple interaction mechanisms for complex polymers
• Competition with dissolution mechanism of retention for non-solvents
Separation of poly(alkyl methacrylate) blend (MW ~ 300K) at Symmetry® C_{18}

ACN – THF gradient (0 – 100%) over 30 minutes

Brun, Alden, J. Liq. Chrom 2002
Efficiency and resolution on narrow pores ($D \sim 6 \text{ nm}$)

Separation of ultra-high $M (> 10^6)$ polyacrylates at Nova-Pak® $C_{18}$

ACN – THF (0 -100%) over 10 min

Elution time, min

Eluent composition (THF, %)

Acrylate copolymers (core-shell) blend

Only “flower” conformations contributes to retention

Brun, Alden, J. Liq. Chrom 2002
Separations by end-groups: isocratic vs. gradient

4-arm PEGs with OH- and Cl-ends

**Water-ACN at XTerra® C₁₈**

**Isocratic elution (LCCC)**

- **water/ACN (60.1/39.9, v/v)**
- **number of chlorine ends**

**Gradient elution**

- **water – ACN gradient: 20%ACN -100%ACN**
- **number of chlorine ends**
Theoretical prediction of gradient elution of PEGs with various end-groups.
• Critical point of adsorption for statistical copolymers

• Separation by chemical composition (chemical composition distribution)

• Separation by microstructure (blockiness)
• **Statistical copolymer**
  - Contains a single CPA (like in homopolymer)
  - CPA of statistical copolymers depends on chemical composition and microstructure (blockiness) of polymer chains

• **Block-copolymer**
  - Does not contain a single CPA, but each individual block has its own CPA
  - Retention in gradient elution depends on MW and composition
  - Could be separated by block-length in gradient mode

• **Effect of blockiness on retention in gradient elution**
  - Always increases retention
  - Elution time at same chemical composition: \textit{alternating < statistical < block-copolymer}

• **Practical implication**
  - Gradient elution allows for separation by chemical composition and microstructure

Y. Brun, J. Liq. Chrom., 1999
Y. Brun, P. Foster, JSS, 2010
Chromatographically, statistical copolymers behave similar to homopolymers: they contain CPA.

Silasorb 600 Silica, different hexane-CHCl₃ compositions

Y. Brun, J. Liq. Chrom., 1999
RAFT polymerization of 2-EHA/n-BA (50/50) statistical copolymers

\[
\text{dwt/d(logM)}
\]

\[
\begin{align*}
\log M & : 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6 \\
\end{align*}
\]

\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{Sample} & \text{Mn} & \text{Mw} & \text{PD} & \text{Conv (\textsuperscript{1}H-NMR)} \\
\hline
\text{Stat-1} & 6450 & 7278 & 1.13 & <1 \\
\text{Stat-2} & 8694 & 9617 & 1.11 & <1 \\
\text{Stat-3} & 11585 & 12638 & 1.09 & 3 \\
\text{Stat-4} & 13240 & 14657 & 1.11 & 5 \\
\text{Stat-5} & 14540 & 15856 & 1.09 & 12 \\
\text{Stat-6} & 15289 & 16734 & 1.09 & 73 \\
\text{Stat-7} & 16065 & 17704 & 1.10 & 89 \\
\text{Stat-8} & 17107 & 18918 & 1.11 & 95 \\
\hline
\end{array}
\]

\[
\begin{array}{c}
\text{CH}_3 \text{COOCH}_{2} \text{CH}_{2} \text{CH}_{2} \text{CH}_{2} \text{CH}_{3} \\
\text{CH}_{2} \text{CH} \quad \text{H}_{3} \text{CCH}_{2} \quad \text{CH} \text{CH}_{2} \text{CH}_{2} \text{CH}_{2} \text{CH}_{2} \text{CH}_{3} \\
\end{array}
\]
Critical Point of Adsorption (CPA)

THF-ACN (60/40)

$\Phi_{CR, \text{stat}} = 60\% \text{THF}$

Elution time, min (flow rate 0.25ml/min)

Elution at different compos. $\Phi$ (THF,\%):

- THF-ACN (60/40)
- $\Phi_{CR, \text{stat}} = 60\% \text{THF}$
- Critical Point of Adsorption (CPA)
- Elution at different compositions $\Phi$ (THF,\%):
XTerra® C8, ACN – THF (0 -100%) over 10 min

Elution time, min (flow rate 1 ml/min)

\[ \Phi_{CR, PBA} = 42\% \]
\[ \Phi_{CR, stat} = 60\% \]
\[ \Phi_{CR, PEHA} = 68\% \]

Effect of MW on retention
Stat. 2-EHA/n-BA copolymers at different C₁₈ columns

ACN – THF (0 -100%) at different gradients

**Effect of Gradient Rate**

Column: XTerra ® 127A
Gradient time: 5 min, 10 min, 15 min, 20 min

**Effect of Pore Size**

Columns: Symmetry ® 300A, XTerra ® 127A, Nova-Pak ® 60A
Gradient time: 10 min
Ethylene-Methyl Acrylate Copolymers at Nova-Pak® C₁₈
mobile phase gradient: EtAc/MeOH (50/50)-toluene (50 - 100%) over 10 min

Y. Brun, M. Pottiger, Macrom. Symp., v. 282, 2009
RAFT polymerization of A/B block-copolymers

A (2-EHA)

B (n-BA)

Sample Mn Mw PD % 2-EHA

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mn</th>
<th>Mw</th>
<th>PD</th>
<th>% 2-EHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 1 (Homopol A)</td>
<td>7117</td>
<td>7786</td>
<td>1.094</td>
<td>100.00</td>
</tr>
<tr>
<td>Block 2</td>
<td>7548</td>
<td>8569</td>
<td>1.135</td>
<td>91</td>
</tr>
<tr>
<td>Block 3</td>
<td>8482</td>
<td>9660</td>
<td>1.139</td>
<td>81</td>
</tr>
<tr>
<td>Block 4</td>
<td>9256</td>
<td>10629</td>
<td>1.148</td>
<td>73</td>
</tr>
<tr>
<td>Block 5</td>
<td>10169</td>
<td>11811</td>
<td>1.161</td>
<td>66</td>
</tr>
<tr>
<td>Block 6</td>
<td>11293</td>
<td>13666</td>
<td>1.210</td>
<td>57</td>
</tr>
<tr>
<td>Block 7</td>
<td>11801</td>
<td>14585</td>
<td>1.236</td>
<td>53</td>
</tr>
<tr>
<td>Block 8</td>
<td>12127</td>
<td>15473</td>
<td>1.276</td>
<td>50</td>
</tr>
</tbody>
</table>
Composition affects retention stronger than MW
Effect of copolymer microstructure on gradient elution

Statistical and block- 2-EHA/n-BA (50:50) copolymers

**Novo-Pak\textsuperscript{\textregistered} Silica**, Hexane – THF (0 -100\%) over 10 min

**XTerra\textsuperscript{\textregistered} C\textsubscript{18}**, ACN-THF (0 -100\%) over 10 min

CPA depends on microstructure: separation by CPA = separation by blockiness

Blockiness always increases retention
Effect of copolymer microstructure on gradient elution

Statistical and block styrene/MMA (50/50) copolymers

**Nova-Pak® Silica**, Hexane – THF (0-100%) over 10 min

- PS (107K)
- Stat (91K)
- PMMA (103K)

**Nova-Pak® C_{18}**, ACN-THF (0-100%) over 10 min

- Block (93K)
- Block (407K)
- PMMA
- Stat (91K)
- PS

Retention of block-copolymers increases with MW

Brun, Foster, JSS, 2010
How to control microstructure of condensation copolymers?

"Monomer first": traditional copolycondensation should produce statistical copolymer

"Oligomer first": chain extension in melt may produce blocky copolymers if transesterification is suppressed by phase separation

Gradient elution of “monomer first” copolymers

FluoroFlash F₈, water-HFIP

UV chromatograms

- mol %F₁₆–iso:
  - 10
  - 25
  - 50
  - 90
  - 100

- Minutes

Chemical composition calibration curve (MW independent)

- F₁₆–iso in copolymer (mol%)
- MP composition, HFIP%
- Polynomial fit

Separation of statistical copolymers by chemical composition (elution at CPA)
Chromatographic identification of copolymer microstructure

PTT

Statistical “monomer first”
50 mol%

Blocky “oligomer first”
50 mol%
Purification based on step-wise flash IPC

Purification of St/MMA copolymer from residual homopolymers

ELSD Chromatograms in Hexane-THF Gradient on Silica column
Free polymer chains grafted within the monolith pores provide higher degree of interaction between nanoparticle and column.

- Monolith column produce better recovery for nanoparticles
- Rigid surface provides minimal tangential nanoparticle/column interaction
- Currently commercially available monoliths from styrene-divinyl crosslinked gel contain relatively short chains. Monoliths with grafted flexible chains are needed
- Recently done at the Molecular Foundry at Berkley National lab (Dr. Frantisek Svec)
Separation of colloidal silica coated with mix of phenyl- and aminophenylsilane

**PhTMS / APhTMS ratio:**

- **Mobile phase:** toluene-DMAc gradient
- **Column:** BMA/EDMA monolith grafted with PMMA chains (F. Svec, Berkley)

- Long and flexible polymer chain functional groups (bonded phase) produce “reversed” critical point of adsorption.
- Nanoparticle is “swallowed” by these chains through multiple attachment mechanism

*First reported at HPLC 2012*