Polymer Characterization by Temperature Gradient Interaction Chromatography: SEC vs. TGIC

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How well do we know the polymers we are working with?

Various molecular heterogeneities in synthetic polymers

- Molar mass
- Chain Architecture
- Functionality
- Chemical Composition
- Stereoregularity, Monomer sequence, etc.
Content

• Introduction
  Size Exclusion Chromatography vs. Interaction Chromatography

• Temperature Gradient Interaction Chromatography

• Precision Analysis of Molecular Weight Distribution

• Nonlinear Polymers
  Branched polymer, Ring polymer

• Chemical Composition
  Mixture, Block Copolymer, Functionality

• Stereoregularity, Isotope

Chromatography separation

In a chromatographic separation, analyte molecules are subjected to consecutive equilibrium in the distribution between the mobile phase and stationary phase while they pass through the column.

\[
K = \frac{c_s}{c_m}
\]

\[
V_R = V_m + K V_s
\]
**Porous packing materials for HPLC columns**

- SEM images of PS/DVB gel (top: 10^3, bottom: 10^2)
- Cross-sectional TEM images of a silica particle for IC (pore diameter = 300 Å)

**Size Exclusion Chromatography (SEC)**

- Flow

\[
K_{SEC} = \exp\left(\frac{\Delta G_{SEC}^o}{RT}\right) \equiv \exp\left(\frac{\Delta S_{SEC}^o}{R}\right)
\]

\[
\alpha \leq K_{SEC} = \frac{c_x}{c_i} \leq 1
\]

- Development of the technique: J. Moore, Dow Chemical Company (1962)
- First commercial instrument: Waters Associate (1963)
Limitations in SEC analysis

1. SEC separates polymers by hydrodynamic size only
2. Hydrodynamic size decreases with chain branching

Polymer Mixture  Copolymer  Non-linear Polymer

Intrinsic Band broadening – particularly serious in SEC

SEC vs. IC

Size Exclusion Chromatography

Flow

Interstitial space

Exclusion $\Delta S$

Solid phase

Pore

$K_{SEC} = \exp \left( \frac{\Delta G_{SEC}^o}{RT} \right) = \exp \left( \frac{\Delta S_{SEC}^o}{R} \right)$

$\alpha \leq K_{SEC} = \frac{c_p}{c_i} \leq 1$

Interaction Chromatography

Flow

Interstitial space

Exclusion $\Delta S$

Solid phase

Pore

Interaction $\Delta H$

Stationary phase

$K_{IC} = \exp \left( -\frac{\Delta G_{IC}^o}{RT} \right) = \exp \left( \frac{\Delta S_{IC}^o}{R} - \frac{\Delta H_{IC}^o}{RT} \right)$

$\alpha \leq K_{IC} = \frac{c_i}{c_m} \leq \infty$
IC Analysis of Polymers - History

- Separation of Copolymers in terms of Chemical Composition
  

- Separation of Homopolymers in terms of Molecular Weight
  
  TGIC: Chang & coworkers *Polymer* 37 5747 (1996)

- Separation at Chromatographic Critical Condition (LCCC)
  
  *Vysokomol. Soedin.* A19 657 (1977)

Temperature & Solvent effect on HPLC retention

Column: Nucleosil C18, 5 μm, 100 Å, 250 x 4.5 mm

Eluent: CH₂Cl₂/CH₃CN mixture at 0.5 mL/min

<table>
<thead>
<tr>
<th>Temperature Effect (57) vs (43)</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C</td>
<td>502,000</td>
</tr>
<tr>
<td>45°C</td>
<td>165,000</td>
</tr>
<tr>
<td>40°C</td>
<td>502,000</td>
</tr>
<tr>
<td>35°C</td>
<td>29,000</td>
</tr>
<tr>
<td>30.5°C</td>
<td>12,000</td>
</tr>
<tr>
<td>28°C</td>
<td>2,500</td>
</tr>
<tr>
<td>25°C</td>
<td>2,500</td>
</tr>
</tbody>
</table>

Three HPLC separation modes for polymers

SEC: Exclusion >> Interaction ~ 0

IC: Exclusion < Interaction

LCCC: Interaction ~ Exclusion

Two Dimensional Liquid Chromatography (2D-LC)
**Martin’s Rule**

**Martin’s rule:** \( \Delta G^\circ_{\text{polym}} = n \cdot \Delta G^\circ_{\text{unit}} \),  
\( n \): degree of polymerization

Then \( K_{\text{polym}} = K_{\text{unit}}^n \) or \( V_{\text{fi}} = V_{\text{m}} + K_{\text{unit}}^n \cdot V_{\text{s}} \)

Retention of polymer molecules changes exponentially with the degree of polymerization.

In order for a polymer sample with a wide molecular weight distribution to be eluted in a reasonable experimental period, it is necessary to change \( K \) during the elution.

One way to control the retention is to change \( \Delta G^\circ \) itself: **Solvent gradient** interaction chromatography.

Alternative way to control the retention is to change \( T \): **Temperature gradient** interaction chromatography (TGIC).

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  - Stereoregularity, Isotope

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**Column Temperature Control**

- Column: Nucleosil C18, 3 μm, 100 Å, 50 x 4.6 mm
- Eluent: CH₂Cl₂/CH₃CN = 57/43 (v/v)
- 10 mL/min
- \( \frac{dT}{dt} = 8^\circ C/min \)

**Temperature effect in IC Separation**

- PS Samples: 5.1, 15.3, 30.9, 55.2, 98.9, 200, 648 (kg/mol)

- In TGIC, retention is controlled by changing the column temperature.
- How does the temperature program affect the retention?
- Can we predict the polymer retention with given T-program?

**Thermodynamics in IC separation**

\[ V_R = V_m + K V_s, \quad K = c_s / c_m \]

\[ K = \exp\left(-\frac{\Delta G^o}{RT}\right) = \exp\left(-\frac{-\Delta H^o}{RT} + \frac{\Delta S^o}{R}\right) \]

\[ k = \frac{(t_R - t_o)}{t_o} = K \left(V_s / V_m\right) \]

\[ \ln k = \ln\left[\frac{(t_R - t_o)}{t_o}\right] = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} + \ln \phi, \quad \phi = V_s / V_m \]

\[ t_R = t_o \left(\exp\left(\frac{a}{T} + b\right) + 1\right) \]

**Assuming that** \( V_s \) **and** \( V_m \) **are invariant, the solute retention is a function of the thermodynamic parameters,** \( \Delta H^o \) **and** \( \Delta S^o \), **and temperature,** \( T \).

**In solvent gradient elution, retention is controlled by changing the mobile phase composition (thus changing** \( \Delta H^o \) **and** \( \Delta S^o \) **) while in TGIC, retention is controlled by changing the column temperature.**

At a fixed temperature, \( T \)

\[ \ln k = \ln\left[\frac{(t_R - t_o)}{t_o}\right] = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} + \ln \phi = \frac{a}{T} + b \]

**Definition**

\( v(T) \): migration rate of polymer (T dependent)

\( t_R \): retention time of polymer

\( t_o \): solvent elution time (T independent)

\( T(t) \): column temperature at time \( t \)

\( L \): column length

\( \gamma \): column heating rate (K/min)

\[ v(T) = \frac{L}{t_R} = \frac{\frac{L}{t_o \left(\exp\left(\frac{a}{T} + b\right) + 1\right)}}{t_R} \quad \text{and} \quad \int_0^{t_R} v(T)dt = L \]
For a linear $T$ ramp with a gradient $\gamma$, $T = T(0) + \gamma t$.

\[ dT = \gamma dt \quad \text{or} \quad dt = dT/\gamma, \quad \text{and} \]

At $t = 0$, $T = T(0)$ and at $t = t_R$, $T = T(0) + \gamma t_R$.

\[ \int_0^t v(T)dt = \int_{T(0)}^{T(0)+\gamma t_R} v(T) \times \frac{1}{\gamma} dT = L \]

Since $v(T) = \frac{L}{t_o \left( \exp(a/T + b) + 1 \right)}$

\[ \int_{T(0)}^{T(0)+\gamma t_R} \frac{L}{\gamma t_o \left( \exp(a/T + b) + 1 \right)} dT = L \]

\[ \int_{T(0)}^{T(0)+\gamma t_R} \frac{1}{\gamma \left( \exp(a/T + b) + 1 \right)} dT = t_o \]

• Using the integral equation above, we can calculate $t_R$ numerically if we know $a$ and $b$, which depend on $M$.

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**For a multistep linear $T$-ramp**

\[ T(t_3) = T(0) + \gamma_1(t_3-t_1) + \gamma_2(t_3-t_2) \]

\[ T(t_2) = T(0) + \gamma_1(t_2-t_1) \]

\[ T(t_1) = T(0) \]

\[ t = \frac{t_1}{\exp(-\Delta H/RT(0) + \Delta S/R) + 1} + \frac{t_2}{\gamma_1 \exp(-\Delta H/RT + \Delta S/R) + 1} + \frac{t_3}{\gamma_2 \exp(-\Delta H/RT + \Delta S/R) + 1} \]

• We can solve this integral equation numerically to obtain $t_R$. 
**Experimental verification**

Column: Nucleosil C18, 3 μm, 100 Å, 50 x 4.6 mm
Eluent: CH₂Cl₂/CH₃CN = 57/43 (v/v), 0.7 mL/min

van’t Hoff plot of polystyrene

First, the thermodynamic parameters are obtained by van’t Hoff plot.

**Temperature Gradient Interaction Chromatography (TGIC)**

Column: Nucleosil C18, 3 μm, 100 Å, 50 x 4.6 mm
Eluent: CH₂Cl₂/CH₃CN = 57/43 (v/v) at 0.7 mL/min

**How polymers migrate inside the column?**

- **Column**: Nucleosil C18, 3 μm, 100 Å
  - 50 x 4.6 mm
- **Eluent**: CH₂Cl₂/CH₃CN = 57/43 (v/v)
- **Flow rate**: 0.7 mL/min
- **T-program**: linear log MW vs. tᵣ

- High MW polymers essentially do not move at all until it reaches a certain temperature.
- Above the temperature, they start to move rapidly.
- It tells us that most of the separation of high MW polymers occurs in a short distance from the column inlet.

**Advantage of TGIC over solvent gradient elution**

Solvent gradient elution causes significant background signal drift that makes it difficult to use many useful detection methods for polymer analysis such as differential refractometer, light scattering, and viscometry. Temperature change also causes background signal drift, but it is smaller than solvent gradient and, in principle, temperature can be readjusted at the detector.

**TGIC of PS with RI & LS detection**

- PS: 32.7k, 74.4k, 127k, 213k, 394k, 683k
**Advantage of TGIC over SEC**

Much lower band broadening and MW sensitive separation!

<table>
<thead>
<tr>
<th>Column</th>
<th>Eluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 C18 silica column, 100 Å, 250 x 4.5 mm</td>
<td>THF</td>
</tr>
<tr>
<td>4 DVB columns, 10^5, 10^4, 10^3 Å, mixed, 250 x 10 mm</td>
<td>CH₂Cl₂/CH₃CN (57/43)</td>
</tr>
</tbody>
</table>

9 PS standards: 2.0 k, 11.6 k, 30.7 k, 53.5 k, 114 k, 208 k, 502 k, 1090 k, 2890 k


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  - Chemical Composition
    - Mixture, Block Copolymer, Functionality
  - Stereoregularity, Isotope
TGIC separation of 14 Polystyrene Standards

Sample: 14 PS standards
Column: C18 100 Å (Nucleosil, 250 x 2.1 mm)
Eluent: CH₂Cl₂/CH₃CN = 57/43 (v/v)

Mw (Mw/Mn by SEC, Mw/Mn by TGIC)

<table>
<thead>
<tr>
<th>Mw (k)</th>
<th>Mw/Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>2890</td>
<td>1.06</td>
</tr>
<tr>
<td>1530</td>
<td>1.007</td>
</tr>
<tr>
<td>1090</td>
<td>1.06</td>
</tr>
<tr>
<td>632</td>
<td>1.005</td>
</tr>
<tr>
<td>403</td>
<td>1.05</td>
</tr>
<tr>
<td>228</td>
<td>1.03</td>
</tr>
<tr>
<td>151</td>
<td>1.010</td>
</tr>
<tr>
<td>114</td>
<td>1.010</td>
</tr>
<tr>
<td>81.9</td>
<td>1.005</td>
</tr>
<tr>
<td>63.5</td>
<td>1.010</td>
</tr>
<tr>
<td>42.2</td>
<td>1.010</td>
</tr>
<tr>
<td>22</td>
<td>1.010</td>
</tr>
</tbody>
</table>

How Narrow is really Narrow?

MWD of polymer of <DP>ₙ = 1,000

degree of polymerization
**SEC and TGIC Characterization of Polystyrenes**

Styrene

- sec-BuLi
- Cyclohexane, 45 °C

Take out aliquots and terminate with i-propanol at different polymerization times.

**SEC**

- $A_{260} (\text{a.u.})$
- $M_w/M_n$ (Mw)

**TGIC**

- $A_{260} (\text{a.u.})$
- $W_i$ (a.u.)

**MWD of Polystyrenes by SEC and TGIC**

**SEC**: 2 x PL mixed C, THF

**TGIC**: 1 x Nucleosil C18 (100Å), CH$_2$Cl$_2$/CH$_3$CN 57/43 (v/v)

Poisson distribution: $W_j = \frac{i^j e^{-\nu}}{(\nu + 1)! (i - 1)!}$

<table>
<thead>
<tr>
<th>Poly'n time</th>
<th>$M_w/M_n$ (Mw) SEC</th>
<th>$M_w/M_n$ (Mw) TGIC</th>
<th>Poisson</th>
</tr>
</thead>
<tbody>
<tr>
<td>238s</td>
<td>1.08 (4.3k)</td>
<td>1.06 (4.0k)</td>
<td>1.02</td>
</tr>
<tr>
<td>888s</td>
<td>1.04 (21.3k)</td>
<td>1.02 (21.0k)</td>
<td>1.003</td>
</tr>
<tr>
<td>1626s</td>
<td>1.05 (35.0k)</td>
<td>1.02 (34.4k)</td>
<td>1.002</td>
</tr>
<tr>
<td>2296s</td>
<td>1.05 (42.9k)</td>
<td>1.01 (43.4k)</td>
<td>1.002</td>
</tr>
<tr>
<td>3098s</td>
<td>1.05 (50.3k)</td>
<td>1.008 (50.2k)</td>
<td>1.002</td>
</tr>
<tr>
<td>4220s</td>
<td>1.05 (56.0k)</td>
<td>1.006 (55.2k)</td>
<td>1.002</td>
</tr>
<tr>
<td>14345s</td>
<td>1.05 (62.0k)</td>
<td>1.005 (62.0k)</td>
<td>1.002</td>
</tr>
</tbody>
</table>

**PS standards (RP-TGIC x SEC)**

**Calibration curve**

PS standards: 6 k, 15.3 k, 30.9 k, 54 k, 93 k, 170 k, 384 k, 1160 k

1D RP-TGIC

Nucleosil C18 150 x 4.6 mm (100 Å)

C<sub>H</sub>2Cl<sub>2</sub>/CH<sub>3</sub>CN = 57/43 (v/v)

0.05 mL/min

2D SEC

PolyPore (250 x 4.6 mm)

THF: 1.65 mL/min, T = 110 °C

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Star-shaped PS by linking with divinyl benzene

Styrene + sec-BuLi → PS\(^{\oplus}\)\(^{\ominus}\) DVB

Highly Branched Species

2D-LC of star-shaped PS (RP-TGIC x SEC)

SEC t\(_R\) (min)

2D SEC
Bare Silica 150 × 4.6 mm (100 Å) + 50 × 4.6 mm (300 Å) +150 × 4.6 mm (1000 Å)
Eluent: THF
Flow rate: 1.8 mL/min at 130 °C

1D RP - TGIC
Nucleosil C18 250 × 4.6 mm (500 Å)
Eluent: CH\(_2\)Cl\(_2\)/CH\(_3\)CN = 57/43 (v/v)
Flow rate: 0.05 mL/min

Im et al. J. Chromatogr. A 1216 4606 (2009)
**Synthesis of Exact PB combs**

Nikos Hadjichristidis, Univ. of Athens

**SEC & TGIC analysis of comb-shaped Polybutadiene**

Column: Polypore(300 x 7.5mm, 5μm) 2EA
Eluent: THF, 0.8mL/min

Column: Lunasil C18 150 x 4.6 (i.d.) mm, 500Å, 7μm
Eluent: 1,4-dioxane, 0.5mL/min

---

Synthesis of H-shaped Polybutadiene

\[
\begin{align*}
\text{s-BuLi} & + \text{Butadiene} \rightarrow 20k \text{ (arm)} \\
\text{Butadiene} & \xrightarrow{\text{Tritration}} \text{H-PBd} \xrightarrow{\text{s-BuLi}} 120k \text{ (H)} \\
\end{align*}
\]

SEC & TGIC characterization of H-shaped PBd

(a) linear PBd arm  
(b) star PBd (½ H)  
(c) H-PBd (HA20B40).

Chen et al. Macromolecules, 44, 7799 (2011)
**PSPI₃ Miktoarm Star Copolymer**

Styrene + sec-BuLi → Li

Isoprene + sec-BuLi → Li

Li + SiCl₄ (excess) → SiCl₃ + excess SiCl₄

SiCl₃ + Li (excess) → + side products (fractionation)

Possible side products: PSPI₂SiOR, PSPISi(OR)₂, HomoPI, etc.

**SEC and TGIC Analysis of PSPI₃**

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**Synthetic scheme of Ring PS**

(1) (CH$_3$)$_2$SiCl$_2$

(2) CH$_3$OH

**Possible byproducts**

---

**LCCC separation of Rings from Linear Precursors**

**SEC**
- Column: 2 x PL mixed-C
- Eluent: THF
- Column temperature: 40 °C

**LCCC for linear PS**
- Column: Nucleosil C18AB
- Eluent: CH$_2$Cl$_2$/CH$_3$CN=57/43
- Column temperature: 42 °C

**Fractionation results by SEC & MALDI-TOF MS**

**SEC**
- As-received Ring PS
- Fractionated Ring PS

**MALDI-TOF MS**
- Linear precursor
- Ring PS As-received
- Ring PS Fractionated by LCCC

**Before and After LCCC Fractionation**
- DP = 38

Property of Purified Ring PS – 4.6 kg/mol

![Graph showing Tg vs Mn (kg/mol) with data points for PS ring, PS ring (theory), and linear PS.](image)

Segmental Relaxation Time

![Graph showing segmental relaxation time for PS ring and linear PS.](image)


Stress Relaxation of Entangled Polymers

![Graph showing stress relaxation for R198, R161, and linear polymers.](image)

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### TGIC Separation of Various Polymers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Column: C18 silica, 5 μm, 100 Å pore</th>
<th>Eluent: CH₂Cl₂/CH₃CN = 80/20(v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyisoprene</td>
<td><a href="polyisoprene.png">Graph</a></td>
<td></td>
</tr>
<tr>
<td>Polystyrene</td>
<td><a href="polystyrene.png">Graph</a></td>
<td></td>
</tr>
<tr>
<td>Poly(methyl methacrylate)</td>
<td><a href="pmma.png">Graph</a></td>
<td></td>
</tr>
</tbody>
</table>

---

- Column: C18 silica, 5 μm, 100 Å pore
- Eluent: CH₂Cl₂/CH₃CN = 57/43(v/v)

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2015-10-26

25
**Fractionation of Mixture by Simultaneous SEC/IC**

**Size Exclusion Chromatography**

FLOW
SEC condition

**SOLVENT**

**Interaction Chromatography**

FLOW
IC condition

**Temperature Dependence of PS/PMMA Retention**

Column: 3 Nucleosil C18, 5 μm
100Å, 500Å, 1000Å pore
Eluent: CH₂Cl₂/CH₃CN = 57/43 (v/v)
SEC condition for PMMA
IC condition for PS

\[
K_{\text{SEC}} = \exp \left( \frac{\Delta G_{\text{SEC}}}{RT} \right) = \exp \left( \frac{\Delta S_{\text{SEC}}}{R} \right)
\]

T- insensitive

\[
K_{\text{IC}} = \exp \left( \frac{\Delta G_{\text{IC}}}{RT} \right) = \exp \left( \frac{\Delta S_{\text{IC}}}{R} - \frac{\Delta H_{\text{IC}}}{RT} \right)
\]

T- sensitive

5 PMMA standards: 2k ~ 1,500k
11 PS standards: 1.7k ~ 2,890k
Simultaneous SEC/TGIC fractionation of PS/PMMA mixture

Column: 3 Nucleosil C18, 5 μm
100Å, 500Å, 1000Å pore
Eluent: CH₂Cl₂/CH₃CN = 57/43 (v/v)

<table>
<thead>
<tr>
<th>Code</th>
<th>Polymer</th>
<th>Mₚ (kg/mol)</th>
<th>Mₚ/Mₚ (SEC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PMMA</td>
<td>1500</td>
<td>1.11</td>
</tr>
<tr>
<td>2</td>
<td>PMMA</td>
<td>501</td>
<td>1.09</td>
</tr>
<tr>
<td>3</td>
<td>PMMA</td>
<td>77.5</td>
<td>1.06</td>
</tr>
<tr>
<td>4</td>
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<td>a</td>
<td>PS</td>
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<td>1.08</td>
</tr>
<tr>
<td>k</td>
<td>PS</td>
<td>2890</td>
<td>1.09</td>
</tr>
</tbody>
</table>


Simultaneous SEC/TGIC Fractionation of Polymer Mixtures

Column: Nucleosil C18, 250 x 4.6 mm
1000, 500, 100 Å pore
Eluent: CH₂Cl₂/CH₃CN = 80/20(v/v)

SEC condition for PS and PMMA
TGIC condition for PI

<table>
<thead>
<tr>
<th>Code</th>
<th>PI</th>
<th>PS</th>
<th>PMMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,700</td>
<td>2,890,000</td>
<td>2,150,000</td>
</tr>
<tr>
<td>2</td>
<td>10,400</td>
<td>501,500</td>
<td>365,000</td>
</tr>
<tr>
<td>3</td>
<td>28,100</td>
<td>140,000</td>
<td>78,700</td>
</tr>
<tr>
<td>4</td>
<td>56,800</td>
<td>15,300</td>
<td>17,900</td>
</tr>
<tr>
<td>5</td>
<td>199,000</td>
<td>3,600</td>
<td></td>
</tr>
</tbody>
</table>

**NP- and RP-TGIC of PS**

NP-TGIC
Column: Nucleosil bare silica
100 Å pore, 250 x 2.1 mm
Eluent: i-octane/THF = 55/45 (v/v)

RP-TGIC
Column: Nucleosil C18 bonded silica
100 Å pore, 250 x 2.1 mm
Eluent: CH₂Cl₂/CH₃CN = 57/43 (v/v)

<table>
<thead>
<tr>
<th>PS (kg/mol)</th>
<th>Mw (kg/mol)</th>
<th>Mw/Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>RP</td>
<td>NP</td>
</tr>
<tr>
<td>32.7</td>
<td>68.1</td>
<td>68.5</td>
</tr>
<tr>
<td>112</td>
<td>112.3</td>
<td>113.0</td>
</tr>
<tr>
<td>213</td>
<td>205.7</td>
<td>200.3</td>
</tr>
<tr>
<td>394</td>
<td>395.5</td>
<td>385.4</td>
</tr>
<tr>
<td>683</td>
<td>653.4</td>
<td>648.3</td>
</tr>
<tr>
<td>1530</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NP- vs. RP-TGIC: Simultaneous SEC and TGIC of PS and PI**

NP-TGIC
Column: 3 Nucleosil bare silica
100, 500, 1000 Å pore, 250 x 4.6 mm
Eluent: i-octane/THF = 55/45 (v/v)

RP-TGIC
Column: 3 Nucleosil C18 bonded silica
100, 500, 1000 Å pore, 250 x 4.6 mm
Eluent: CH₂Cl₂/CH₃CN = 80/20 (v/v)

Samples (kg/mol)
PS: 10.3, 32.7, 68.1, 112, 213, 394, 683, 1530
PI: 2.7, 11.9, 53.0, 199

Lee et al. J. Chromatogr. A. 910 51 (2001)
Content

• Introduction
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  Mixture, Block Copolymer, Functionality
• Stereoregularity, Isotope

MW & Composition Distribution in Block Copolymers

Effects of MW and composition distribution on the BCP phase behavior.

Various structures of diblock copolymer

- Body Centered Cubic (BCC)
- Hexagonally Packed Cylinder (HEX)
- Lamella (LAM)
- Double Gyroid (DG)
- Modulated Lamella (MLAM)
- Hexagonally Perforated Lamella (HPL)
**Fractionation of Homopolymer from BCP**

**Size Exclusion Chromatography**

**Interaction Chromatography**

**PS-b-PMMA/PS Mixtures**

**Fractionation of homo-PS (Multiple injection)**

---

**NPLC separation of PS-b-PMMA**

- **Column**: Bare Silica (100 x 22 mm)
- **Eluent**: THF/isooctane = 65/35 (v/v)
- **Flow rate**: 1.5 mL/min

**Multiple Injection**

- **Column**: Bare Silica (100 x 22 mm)
- **Eluent**: THF/isooctane = 60/40 (v/v)
- **Flow rate**: 1.5 mL/min

---

**Fractionation of individual block of BCP**

**NP & RPLC Separation of Low MW PS-b-PI**

**NP & RPLC Separation of Low MW PS-b-PI**

Matrix, dithranol
Salt: AgTFA
Solvent: CH2Cl2/CH3CN = 53/47 (v/v)
Column: LUNA C18 4.6 mm ID, 100 Å
Detection: Reflection mode

**NP & RPLC Separation of Low MW PS-b-PI**

Matrix, dithranol
Salt: AgTFA
Solvent: CH2Cl2/CH3CN = 53/47 (v/v)
Column: Diol bonded silica 7.8 mm ID, 100 Å
Eluent: i-Octane/THF = 97/3 (v/v)
0.7 mL/min

**NP & RPLC Separation of Low MW PS-b-PI**

Matrix, dithranol
Salt: AgTFA
Solvent: CH2Cl2/CH3CN = 53/47 (v/v)
Column: Diol bonded silica 7.8 mm ID, 100 Å
Eluent: i-Octane/THF = 97/3 (v/v)
0.7 mL/min
2D NP-TGIC & RPLC Separation set-up


**PS-b-PI (NP-TGIC x RPLC)**

C18 silica, 150 x 4.6 mm
Eluent: CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>CN=53/47 (v/v)
Flow rate: 1.5 mL/min

Dioctyl-bonded silica
250 x 7.8 mm
Eluent: isoctane/THF = 97/3 (v/v)
Flow rate: 0.05 mL/min

Fractionation of High MW PS-b-PI

RPLC fractionation
PS-b-PI 12k/24k (21k/12k)
Column : C18, 100Å (4.6mm ID)
Eluent: CH₂Cl₂/ACN (80/20, 74/26)
T = 2°C (3°C)

NPLC fractionation
PS-b-PI 12k/24k (21k/12k)
Column : Diol, 100Å (4.6mm ID)
Eluent: isooctane/THF (75/25, 70/30)
T = 5°C (18°C)

Fractionation according to PI block
Fractionation according to PS block

**PS-b-PMMA at Air/Water Interface - surface micelles**

Structure of surface micelles of amphiphilic block copolymers at air/water interface

Homo-polymer free PS-b-PMMA mother:
- Weight of PS = 0.73
- $M_w/M_n = 1.05$

Chung et al. Macromolecules 38 6122 (2005)

**Morphology of PS-b-PMMA Surface Micelles**

PS-b-PMMA mother: 72.8 kg/mol
- wtPS = 0.73
- $M_w/M_n = 1.05$ (SEC)

Chung et al. Macromolecules 38 6122 (2005)
Note: The image contains a diagram and a textual content. The diagram includes a chemical reaction and a graph showing molecular weight distribution. The textual content outlines the content of the presentation, including sections on introduction, temperature gradient interaction chromatography, precision analysis of molecular weight distribution, nonlinear polymers, chemical composition, and stereoregularity.

**Content**

- Introduction
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- Stereoregularity, Isotope

**OH end-functional PS**

Styrene + sec-BuLi → PS\(_{Li}\)\(\oplus\)

(1) Ethylene oxide
(2) i-propanol

i-propanol

PS\(_{Li}\)\(\oplus\)

H

CH\(_2\)CH\(_2\)OH

<table>
<thead>
<tr>
<th>SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x PL-mixed B</td>
</tr>
<tr>
<td>Eluent: THF</td>
</tr>
<tr>
<td>Flow rate: 1.5 mL/min</td>
</tr>
</tbody>
</table>

Graph showing molecular weight distribution with peaks at different molecular weights.
**NP- vs. RP-TGIC: Separation by a single -OH end group**

**NP-TGIC**
- Column: Nucleosil bare silica 100 Å pore, 250 x 2.1 mm
- Eluent: i-octane/THF = 55/45 (v/v)

**RP-TGIC**
- Column: Nucleosil C18 bonded silica 100 Å pore, 250 x 2.1 mm
- Eluent: CH$_2$Cl$_2$/CH$_3$CN = 57/43 (v/v)

---

**End group analysis by 2D-LC**

**1D NPLC**
- Bare silica 50 x 2.1 mm (100 Å)
- Eluent: i-octane/THF = 55/45 (v/v)
- Temperature: 34.2 °C
- Flow rate: 0.015 mL/min

**2D SEC**
- PolyPore 250 x 4.6 mm
- Eluent: THF
- Temperature: 110 °C
- Flow rate: 0.7 mL/min

Switching valve loop: 30 μL


Styrene + sec-BuLi → $\text{PS}^{\ominus}\text{Li}^{\ominus}$

CDMSS (less than 1 eq.)

**Synthetic Scheme of Branched PS with CDMSS**

- **Exp 1**: CDMSS/sec-BuLi = 0.87 (BS 87)
- **Exp 2**: CDMSS/sec-BuLi = 0.65 (BS 65)
- **Exp 3**: CDMSS/sec-BuLi = 0.45 (BS 45)

**SEC and TGIC separation of branched PS**

- **SEC**: PL mixed C x 2
  - Eluent: THF
  - Flow rate: 0.8 mL/min

- **TGIC**: Luna C18, 100 Å, 5 mm, 250 x 4.6 mm
  - Eluent: CH$_2$Cl$_2$/CH$_3$CN = 55/45 (v/v)
  - Flow rate: 0.6 mL/min

Star-shaped PS with arms of broad MWD

BS 65: CDMSS/n-BuLi = 0.65
BS 49: CDMSS/n-BuLi = 0.45

RP-TGIC
Kromasil C18, 150 × 4.6 mm (100 Å)
Eluent: CH₂Cl₂/CH₃CN = 53/47 (v/v)
Flow rate: 0.5 mL/min

LCCC (T = 53.3°C)
Kromasil C18, 150 × 4.6 mm, 5 μm, 100 Å
Eluent: CH₂Cl₂/CH₃CN = 53/47 (v/v)
Flow rate: 0.5 mL/min

2-D LC (TGIC x LCCC) analysis of MW & branching

Kromasil C18, 150 × 4.6 mm, 100 Å
Eluent: CH₂Cl₂/CH₃CN = 53/47 (v/v)

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SEC vs. TGIC of the three different tactic PEMAs

SEC
Column: PL-mixed C × 2
Eluent: THF
Flow rate: 0.8 mL/min

RPLC
Column: LUNA C18 4.6 mm ID, 100Å
Solvent: CH₃CN/CH₂Cl₂ = 70/30 (v/v)
Flow rate: 0.45 mL/min

T. Kitayama, Osaka Univ.
**MALDI-TOF MS Spectra of mother PEMAs & Fractions**

MALDI-TOF (Reflection mode)
- Matrix: IAA (indoleacrylic acid)
- Solvent: THF
- Salt: NaI

**SEC vs. TGIC of the three different tactic PEMAs**

**SEC**
- Column: PL-mixed C × 2
- Eluent: THF
- Flow rate: 0.8 mL/min

**RPLC**
- Column: LUNA C18 4.6 mm ID, 100Å
- Solvent: CH₃CN/CH₂Cl₂ = 70/30 (v/v)
- Flow rate: 0.45 mL/min

---

SEC calibration curve of stereoregular PEMA

Column: PL mixed C × 2
Eluent: THF
Flow rate: 0.8 mL/min


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PS Comb - Synthetic Scheme

h-styrene + sec-BuLi $\rightarrow$ h-PS bar (200k)

chloromethylation

d-styrene + sec-BuLi $\rightarrow$ d-PS arm (85k)

SEC & Light scattering detection

PL mixed C×2, THF
Light scattering detection (Viscotek TDA 300)

Average branch number

$$\langle N_b \rangle_n = \frac{M_{n,comb} - M_{n,backbone}}{M_{n,branch}} = 4.2$$

C.M. Fernyhough, Univ. of Sheffield
NP-TGIC Chromatogram of PS Comb

Column: Bare, 7 μm, 500 Å, 250×2.1 mm
Eluent: Hexane/THF = 57/43 (v/v)
Flow rate: 0.3 mL/min


NP-TGIC Analysis of PS comb

Assumption - Backbone: 200 k
Branch: 85 k

<table>
<thead>
<tr>
<th>PS Comb</th>
<th>( \langle N_\text{br} \rangle \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>From average MW</td>
<td>4.1</td>
</tr>
<tr>
<td>From distribution</td>
<td>4.0</td>
</tr>
</tbody>
</table>
**RP-TGIC Analysis of PS comb**

Column: C18, 7 μm, 500 Å, 150 × 4.6 mm  
Eluent: CH₂Cl₂/CH₃CN=57/43 (v/v)  
Flow rate: 0.5 mL/min

**Isotope effect in IC separation**

<table>
<thead>
<tr>
<th>SEC</th>
<th>NP-TGIC</th>
<th>RP-TGIC</th>
</tr>
</thead>
</table>
| Column: PL mixed C×2  
Eluent: THF  
Flow rate: 0.8 mL/min | Bare silica 250×2.1 mm, 5 μm, 500 Å  
Eluent: i-octane/THF=57/43 (v/v)  
Flow rate: 0.3 mL/min | Kromasil C18 150×4.6 mm, 5 μm, 100 Å  
CH₂Cl₂/CH₃CN=57/43 (v/v)  
Flow rate: 0.5 mL/min |

**Graphs and Data**

1. **RP-TGIC Analysis of PS comb**
   - Temperature (T) vs. Retention time (tᵣ)
   - Log M vs. A₂₆₀ (a.u.)

2. **Isotope effect in IC separation**
   - Molar mass (M) vs. retention time (tᵣ)
   - A₂₆₀ vs. retention time (tᵣ)
**RP-TGIC × SEC of comb PS**

Nucleosil C18 150 × 4.6 mm (500 Å)  
Eluent: CH₂Cl₂/CH₃CN = 57/43 (v/v)  
Flow rate: 0.05 mL/min

**RP-TGIC & NP-TGIC analysis of PS comb**

RP-TGIC

NP-TGIC
Things need to be considered:

1. Eluent compatibility: NPLC first, trapping and eluent exchange

   ![Diagram showing switching valve condition]

   - Pre-column: Diol, 50 × 4.6 mm, 100 Å, 7 μm
   - Loop: 100 μL

2. Speed: Solvent gradient elution of 2nd - RPLC

   ![Solvent gradient profile]

   - Initial state
   - Re-stabilization time
   - Separation time

NP-TGIC x RPLC of comb PS

- NP-TGIC
  - Nucleosil bare silica, 50 × 4.6 mm, 500 Å
  - Eluent: THF/iso-octane = 48/52 (v/v)
  - Flow rate: 0.05 mL/min

- RPLC
  - Nucleosil C18 150 × 4.6 mm (500 Å)
  - Eluent: CH2Cl2/CH3CN = 57/43 (v/v) → 59/41 (v/v)
  - Flow rate: 1.5 mL/min

SEC analysis of fractionated hPS-b-dPS

Column: Polypore (300 x 7.5mm, 5μm) 2EA
Eluent: THF at 0.8mL/min
Detector: Viscotek (Triple detector)

Elution of hPS-b-dPS at CC of hPS

Lunasil C18, 150 x 4.6 mm, 100Å, 5μm; CH$_2$Cl$_2$/CH$_3$CN = 57/43 (v/v), 0.5 mL/min

Expected $M_p$ | Measured $M_p$ (error)
--- | ---
Precursor | - | -
hPS-b-dPS 18k-17k | 16.9 k | 10.1 k (40.2%)
hPS-b-dPS 18k-38k | 37.7 k | 33.5 k (11.4%)
hPS-b-dPS 18k-80k | 80.2 k | 74.8 k (6.7%)

Calibration with homo-dPS

- At CC of hPS, hPS-b-dPS elutes in the decreasing order of MW while they elute before the solvent peak (SEC region).
- MW of dPS block (homo-dPS calibration) deviates significantly from the true value, particularly for low MW dPS block.
Elution of hPS-b-dPS at CC of dPS

Lunasil C18, 150×4.6 mm, 100Å, 5µm; CH₂Cl₂/CH₃CN = 57/43 (v/v), 0.5 mL/min

At CC of dPS, hPS-b-dPS elutes in the decreasing order of MW while they elute after the solvent peak (IC region).

Again, the MW of hPS block (homo hPS calibration) deviates significantly from the true value, particularly at high MW dPS block.

2D-LC (RP-TGIC x RP-LCCC) of hPS-b-dPS

As expected, hPS is retained more strongly than dPS in the RPLC separation.

At CC of dPS, hPS-b-dPS elutes in the order of decreasing MW while they elute after the solvent peak (IC region).

Full resolution of 3 hPS-b-dPS and a number of byproducts is realized by 2D-LC.

Lee et al. Macromolecules. 46 9114 (2013)
Summary

Size Exclusion Chromatography

Interstital space → Solid phase
Exclusion ∆S

Interaction Chromatography

Interstital space → Solid phase
Exclusion ∆S
vs.

Flow

SEC
Exclusion >> Interaction ~ 0
IC
Exclusion < Interaction
LCCC
Interaction ~ Exclusion


High Resolution Branched Polymer

Cyclic Polymer

Mixture

Block copolymer
Summary

- Multivariate distribution in synthetic polymers
  Mol. Wt., Composition, Branching, Functionality, etc.

- 2D-LC helps! Even isotope effect can help!
POSTECH was ranked 1st in Times Higher Education 100 under 50 Rankings for last 3 consecutive years, 2012–2014.

Pohang University of Science and Technology (POSTECH)

4th generation light source (x-ray free electron laser) is under construction to be operational in 2016.