Polymer Separations by Chemical Composition Using SEC-Gradients

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Agenda

- Polymer Chromatography: Who is my neighbour?
- Gradient Chromatography/ Breakthrough Peaks/ Barrier Methods
- Concept of SEC-Gradients
- Proof of Concept (PMMA/PS)
- Separation of Polyamides
- Application of SEC-Gradients to Copolymer Separations
  - Poly(methyl methacrylate – stat – methacrylic acid)
  - Poly(n-butyl acrylate – stat – acrylic acid)
- Conclusions
Polymer Chromatography
Who is my neighbor?
Ideal Gradient Chromatography

Polymer dissolved in initial eluent

Flow direction

Injected solvent

Sample

www.pss-polymer.com
Polymers are soluble in only some solvents
Solvent differs from initial eluent

Flow direction

Breakthrough Peaks
Barrier Methods
LC LCA

Solvent differs from initial eluent
– n.b. injection at strong eluent (SEC) conditions

Sample component 1
Sample component 2
SEC Gradients
The concept

- $\vec{v}_P > \vec{v}_E$
- $\vec{v}_P = \vec{v}_E$
- $\vec{v}_P < \vec{v}_E$

AdSORPTION THRESHOLD

Flow direction →
SEC Gradients
The concept

\[ \vec{v}_P > \vec{v}_E \]
\[ \vec{v}_P = \vec{v}_E \]
\[ \vec{v}_P < \vec{v}_E \]

Flow direction →

Adsorption threshold
### Comparing conventional vs. SEC gradients

<table>
<thead>
<tr>
<th>Conventional gradient</th>
<th>SEC gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample dissolved in weak eluent</td>
<td>Sample dissolved in strong eluent</td>
</tr>
<tr>
<td>Sample injected at start of gradient</td>
<td>Sample injected at end of gradient</td>
</tr>
<tr>
<td>Sample experiences LAC conditions when injected</td>
<td>Sample experiences SEC conditions when injected</td>
</tr>
<tr>
<td>Desorption threshold approaches sample</td>
<td>Sample approaches adsorption threshold</td>
</tr>
<tr>
<td>Sample fully adsorbed upon injection</td>
<td>Sample only at verge of adsorption</td>
</tr>
</tbody>
</table>
ZONE PRECIPITATION

By Dr. JERKER PORATH
Institute of Biochemistry, University of Uppsala

IN preparative and analytical biochemistry pre-
cipitation and crystallization have been largely
superseded by more sophisticated procedures such as
counter-current extraction, zone electrophoresis and
various kinds of chromatography. Solubility is a
powerful ‘separation parameter’ that deserves to be
utilized also when efficient fractionation is attempted.
It is the purpose of this article to describe a column
procedure based on precipitation-dissolution pro-
cesses.

Suppose a precipitating agent moves in a sorbent
bed with a slower speed than the substance to be
precipitated. If adsorption or molecular sieving
do not affect the latter, solubility would be the only
factor to cause their differential migration. Such
conditions prevail, for example, when proteins move
in beds of particulate, highly cross-linked detergents
in which the concentration of ammonium sulphate
gradually increases from the top downwards.

If a protein is filtered through such a column it
moves faster than the ammonium sulphate, and a
region is reached where precipitation occurs. Clearly
the critical concentration of ammonium sulphate for
formation of a precipitate depends on the nature and
concentration of the protein. When the particles have
grown to a certain size their migration ceases.
As the solvent passes the precipitate will soon be
back in an unsaturated environment. Dissolution
starts and the substance moves again. These events
are presumably repeated to yield a continuous migra-
tion of a solute-precipitate containing zone. When a
protein mixture is passed through a bed the com-
ponents will precipitate in different parts of the
column according to their relative solubilities. The
zones thus formed move through the column and the
substances appear separated in the eluate. The
principle is schematically illustrated in Fig. 1.

The separation efficiency may be reduced by the
fact that the crystals or particles have grown large
until they are entrapped in the bed. The choice of
the gel in the present investigation has been made
on the assumption that a limited penetrability of the
proteins may be advantageous, since nuclei will then
be formed in the gel phase where extensive growth is
impossible.

The term ‘zone precipitation’ is tentatively sug-
gested for the method, which is not limited to gel
filtration only.

Some exploratory experiments have been made,
two of which are described here. They were performed
under primitive conditions since adequate instrumen-
tation were not available either for the production of a
continuous linear gradient or for convenient recording
of the ammonium sulphate concentration in the
effluent. Gradients covering intervals of 20-35
per cent saturated ammonium sulphate solution
seem to give the best results. Extraction has been
made with decreasing ammonium sulphate, but this
fact does not appear to have affected the fractiona-
tion to any extent.

(1) Human serum was fractionated on a ‘Sephadex
G-200’ column as described by Frömter and Illantad
A fraction corresponding to the substances present in
peak 3 and the rear part of peak 2 was concentrated
to one-fourth of the original volume of the serum.

A chromatographic column tube (3-5 x 40 cm)
was closed at the bottom with a porous plate and
filled with a suspension of the dextran gel ‘Sephadex
G-100’ (140-200 mesh, obtained from Pharmacia
Uppsala, Sweden) in a solution of 85 per cent satu-
rated ammonium sulphate containing 0-5
hydroxy-anisylamine-sulphonic and adjusted to
pH 8-8. The ‘Sephadex’ particles were
allowed to settle. A bed 35 cm high was obtained.
A solution above the sediment was removed
millilitres portions of ammonium sulphate
solution were washed into the bed in a similar
manner to produce a stepwise gradient from 35
per cent saturated ammonium sulphate above
These millilitres of the serum fraction were washed
into the column followed by ammonium sulphate
buffer solution. The ammonium sulphate concen-
tration was continuously lowered from 55 to 20
per cent saturated ammonium sulphate.

Fig. 1. The principle of zone precipitation exemplified for three
zones A, B and C of different solubilities. A, starting pre-
cipitation; B, the first phase of washing the substances, B, C
appears later in the gradient elution. After reaching the
gradient they display different behaviour: A moves independent
of the gradient, A and B are separated to different extents.
Proof of Concept
PMMA standards

THF (SEC)

ELSD-signal/V

Note inverse volume axis!

PSS Proteema column, SEC gradient THF → CHCl₃ Polymer view!!
Proof of Concept
PMMA standards

PSS Proteema column, SEC gradient THF $\rightarrow$ CHCl$_3$
Proof of Concept
Separation of PS/PMMA blend

PSS Proteema column, SEC gradient 100 THF → CHCl₃
Separation of Polyamides

PSS Proteema column, SEC gradient 75/25 → 55/45 Toluene/TFE Precipitation based system!

![Graph showing separation of polyamides](image-url)
Separation of Polyamides
Number of Amide Groups / Carbon

PSS Proteema column, SEC gradient 75/25 → 55/45 Toluene/TFE
Poly(methyl methacrylate-stat-methyacrylic acid)

PMMA
\[
\begin{array}{c}
C - C \\
\bigg( \begin{array}{c}
CH_2 \\
C - C \\
O - CH_3
\end{array} \bigg) \quad n
\end{array}
\]

PMMA
\[
\begin{array}{c}
C - C \\
\bigg( \begin{array}{c}
CH_2 \\
C - C \\
O - O - H
\end{array} \bigg) \quad n
\end{array}
\]

Tablet coating

Dissolution speed depends on AA content

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mw / cmol(^{-1})</th>
<th>D</th>
<th>MAA content / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>161000</td>
<td>2,4</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>176000</td>
<td>2,8</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>164000</td>
<td>3,3</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>172000</td>
<td>2,3</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>97000</td>
<td>4,4</td>
<td>48</td>
</tr>
</tbody>
</table>
SEC

PSS Proteema column
SEC Gradient
100%DMAc $\rightarrow$ 100%Chloroform

Samples dissolved in DMAc
Optimization
Changing gradient slope 1

Adsorption thresholds

Flow direction
SEC Gradient

Samples dissolved in DMAc
Optimization

Changing gradient slope 2

Adsorption thresholds

Flow direction
Samples dissolved in DMAc
Chemical Composition Distribution

content methacrylic acid/ wt% vs. w(%)
Samples dissolved in DMAc / CHCl₃ with 4-10% DMAc

SEC Gradient vs. Conventional Gradient

6 min. SEC-Gradient 5% - 50% DMAc

6 min. conventional-Gradient 5% - 50% DMAc
Poly(butyl acrylate-stat-acrylic acid)

Acrylic acid content 0-100%
6 min. SEC gradient 100% to 4% DMAc
Samples dissolved in DMAc
Elution Volume vs. Composition

The graph shows the elution volume in mL plotted against the acrylic acid content in % for two solvents: Solvent 1 (black squares) and Solvent 2 (red circles). The elution volume increases with the acrylic acid content for both solvents, indicating a positive correlation.
Sample Differences

Similar composition

Incorrect composition

ELSD Signal [V]

Elutionsvolumen [mL]
Conclusions

- Proof of concept
  - Polymer can be retarded by gradient introduced bevor the sample injection
  - If retardation by adsorption threshold, molar mass independent elution (above critical molar mass)
- Separation of polymer blends (PS/PMMA) possible
- Separation of Polyamides according to Amide content
- New separation method for poly(methyl methacrylate-\textit{stat}-methyacrylic acid)
  - Similar selectivity as conventional gradient, but no breakthrough peaks
- New separation method for poly(butyl acrylate-\textit{stat}-acrylic acid)
  - Differences in CCD or composition can be easily detected
- Easier adjustment to 2D-chromatography (same column materials @ larger pore size)
Acknowledgements

- Martin Schollenberger
  - Proof of concept
  - Separation of PS/PMMA, PMMA/PtBA, PMMA/PnBA blends
- Helena Maier
  - Separation of poly(methyl methacrylate-stat-methyacrylic acid)
  - Separation of poly(butyl acrylate-stat-acrylic acid)
- Nico Apel
  - Separation of Polyamides
- TESA SE
  - Samples poly(butyl acrylate-stat-acrylic acid)