Introduction

The investigation of many different mobile phase components is of critical importance in optimization of preparative chromatography due to their strong influence on the separation and on the production rate of the process.

In enantioselective chromatography, certain solvents such as ethyl acetate and THF have not been available for use with conventionally coated polysaccharide CSPs. Although they are good solvents for the samples, they are also excellent solvents for the chiral polymers. The development of immobilized polysaccharide-based chiral stationary phases (CSPs) such as CHIRALPAK® IA™ has allowed the study of these previously “forbidden” mobile phases.

Use of such solvents can influence profoundly the adsorption isotherms for the separations, leading to major improvements in production rate. This poster concludes an ongoing study of solvent effects on SMB separations by presenting the SMB separation of the enantiomers of 1-methyl-1-phenylsuccinimide (MPS) using THF- based mobile phase.
Solvent selection in Preparative LC

- High selectivity
- Short retention times
- High sample solubility*
- Easy to evaporate
- Promote high loading*
  - Need to avoid bi-Langmuir isotherms
  - Need high saturation capacity

*Not always easy for chiral separations!
Case Study –
α-Methyl-α-Phenylsuccinimide

Multiple separation opportunities
Also separates with conventional solvents on CHIRALPAK IA

CHIRALPAK IA, 250 x 4.6 mm
Flow rate 1 ml/min
UV detection 254 nm
Previously Reported Results
Loading Studies – ACN/IPA; MTBE

CHIRALPAK IA, 250 x 4.6 mm
15% IPA in Acetonitrile, 1ml/min
(Solubility 70 g/l)

- Bi-Langmuir isotherm
- Overloads rapidly

CHIRALPAK IA, 250 x 4.6 mm
MTBE, 1ml/min

(Solubility 35 g/l)

- Bi-Langmuir/Quadratic Isotherm
- Difficult to model
Loading Studies – EtOAc; CHCl$_3$

- Short retention times
- Good loading

- Long retention times
- Overloads rapidly
Current Study
Analytical Injection – 1:1 THF-Hexane

CHIRALPAK IA (20 µm) 250 x 4.6mm;
Mobile phase: 50% THF in hexane
1 ml/min, 25°C

Selectivity: 3.35
Loading Studies – 1:1 THF / Hexane

Overlaid chromatograms from the loading study.

Vinj = 5 – 150 µl

CHIRALPAK IA 250 x 4.6mm;
Mobile phase: 1:1 THF-Hexane, 1 ml/min
The isotherm parameters were estimated by adjustment of the saturation capacities to fit simulated retention times to the experimental data.
SMB Parameters: Initial Conditions for a 5 cm System

The software calculates the Morbidelli triangle assuming Langmuir isotherm behavior from input data and uses this to generate SMB conditions for a user-selected point in the operating space. These conditions are used for the computer simulation of the process...
SMB Parameters:
Optimized Conditions for a 5 cm System

Note: Green trace is predicted polarimeter output
Simulation Results:
Final Prediction for a mini-SMB System

8 columns (4.6 x 100 mm)  7.32 g CSP
Feed Flow  0.11 ml/min
Feed Concentration  94 g/l
Recycle Flow  3.91 ml/min
Extract Flow  2.06 ml/min
Raffinate Flow  0.22 ml/min
Eluent Flow  2.17 ml/min
Switch Time  0.82 min
Zone 1 Flow  3.91 ml/min
Zone 2 Flow  1.85 ml/min
Zone 3 Flow  1.95 ml/min
Zone 4 Flow  1.74 ml/min
Average Flow Rate  2.36 ml/min
Extract Purity  99.03 %e.e.
Extract Concentration  2.47 g/l
Raffinate Purity  99.50 %e.e.
Raffinate Concentration  23.48 g/l
Production Rate  7.35 g/day enantiomer
Productivity  1.00 kg/kg/day enantiomer
Experimental Set-Up

- **Columns:** Eight 100 x 4.6 mm (each containing 0.915g)

- **Initial SMB Run** (optimized for an operating pressure of 35-40 bar)
  - Feed Flow Rate = 0.15 ml/min (Feed at 94 g/l)
  - Eluent Flow Rate = 5.50 ml/min (↔ to Section I Flow)
  - Extract Flow Rate = 2.90 ml/min
  - Raffinate Flow Rate = 0.31 ml/min
  - Switch Time = 35 Seconds
  - Recycle stream initially discarded.

- **Sample Collection after Reaching Steady State**
  - At outlet ports (Raffinate, Extract and Recycle) during a full cycle.
  - At online sampling loop (twice every switch) for a full cycle.

- **Fraction Analysis:** Agilent 1100 System
  - Column: CHIRALPAK®IA®, 20 μ, 250 x 4.6 mm
  - Mobile phase: 50% THF in Hexane, Flow = 1 mL/min, P = 4 bar, T = 25°C
  - Injection Volume = 10 μl, Detection: UV at 270 nm
Experimental Internal Profile for MPS/IA System

CHIRALPAK IA / 1:1 Hexane : THF
# Experimental Optimal SMB Parameters (THF/Hexane)

## SMB Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental</th>
<th>From Simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone1 (Eluent)</td>
<td>9.20 ml/min</td>
<td>8.87 ml/min</td>
</tr>
<tr>
<td>Feed (@ 94 g/l)</td>
<td>0.24 ml/min</td>
<td>0.25 ml/min</td>
</tr>
<tr>
<td>Extract</td>
<td>5.03 ml/min</td>
<td>4.68 ml/min</td>
</tr>
<tr>
<td>Raffinate</td>
<td>0.75 ml/min</td>
<td>0.50 ml/min</td>
</tr>
<tr>
<td>Switch Time</td>
<td>0.58 min</td>
<td>0.36 min*</td>
</tr>
</tbody>
</table>

*Note: Poor correlation of simulation with experiment*

## Other Parameters

- Amount of CSP in 6 columns: 5.49 g
- Column Dimension: 100 x 4.6 mm
- Number of Columns: 6
- Operating Pressure: 32-41 bar

**Productivity (kg\text{enantiomer} / kg\text{CSP} / Day) = 2.96**
## Comparison of Separations - SMB

<table>
<thead>
<tr>
<th>Mobile Phase:</th>
<th>MTBE</th>
<th>ACN/IPA</th>
<th>EtOAc</th>
<th>CHCl₃</th>
<th>THF/Hex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (mPa.s)</td>
<td>0.27</td>
<td>0.63</td>
<td>0.45</td>
<td>0.57</td>
<td>0.38</td>
</tr>
<tr>
<td>Solubility (g/l)</td>
<td>35</td>
<td>70</td>
<td>200</td>
<td>200</td>
<td>102</td>
</tr>
<tr>
<td>Selectivity</td>
<td>3.62</td>
<td>6.83</td>
<td>5.17</td>
<td>3.17</td>
<td>3.35</td>
</tr>
<tr>
<td>Sat. Capacity (g/l)</td>
<td>***</td>
<td>54/22</td>
<td>250/59</td>
<td>55/45</td>
<td>120/40</td>
</tr>
<tr>
<td>Pressure (bar)</td>
<td>34*</td>
<td>30-37**</td>
<td>35-41**</td>
<td>35*</td>
<td>32-41**</td>
</tr>
<tr>
<td>SMB Productivity (kg/kg/day)</td>
<td>2.16*</td>
<td>0.98**</td>
<td>4.97**</td>
<td>0.69*</td>
<td>2.96**</td>
</tr>
</tbody>
</table>

* Values from experiment normalized for pressure

** Values from experiment

*** Saturation capacity is a meaningless parameter for the isotherm observed

+ Values from simulations only
Conclusions

- Use of immobilized CSPs in Prep chromatography allows:
  - Choice of solvent to give high solubility
  - Choice of solvent to give best selectivity
  - Choice of solvent/CSP combination with best isotherm properties to maximize production

- The best choice of solvent is not always obvious!