Chiral Selector Screening and Regeneration of Novel Brush and Polysaccharide-Type Zirconia Chiral Stationary Phases

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Specialists in High Efficiency, \textcolor{red}{Ultra-Stable} Phases for HPLC
• A New Approach to Chiral HPLC Columns
  – Surface Chemistry
  – Building a zirconia-based CSP
• Brush-Type Chiral Stationary Phases (CSPs) on Zirconia
• Stability Study of Brush-Type CSPs
• New Cellulosic CSPs on Zirconia
• Column Regeneration Study
• **Key Conclusion** – A carefully selected anchor group allows for a stable CSP under routine conditions that can be stripped off under high pH condition and regenerated. This general approach allows for a variety of different regenerable CSPs based on a zirconia particle platform.
A Novel Approach to Attaching Chiral Selectors\textsuperscript{1} to Zirconia\textsuperscript{2}

2. Phase II SBIR Grant (NIH).
A Modified Approach to Attaching Chiral Selectors\textsuperscript{1} to Zirconia\textsuperscript{2}

2. Phase II SBIR Grant (NIH).
Zirconia chemistry is dominated by Lewis acid-base reactions

Lewis Acid: $\text{Zr(IV)}: \text{H}_2\text{O} + \text{RPO}_3^{2-} \rightleftharpoons \text{Zr(IV)}: \text{RPO}_3^{2-} + \text{H}_2\text{O}$

Other Lewis base examples: $\text{PO}_4^{3-}$, $\text{RCO}_2^-$, Catechol
### Interaction Strength of Lewis Bases with Zirconia\(^1\)

<table>
<thead>
<tr>
<th>Interaction Strength</th>
<th>Lewis Base (L)</th>
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<tbody>
<tr>
<td>Strongest</td>
<td>Hydroxide, Phosphate, Fluoride, Citrate, Sulfate, Acetate, Formate, Nitrate, Chloride, Water</td>
</tr>
<tr>
<td>Weakest</td>
<td>Small Lewis bases with high electron density and low polarizability interact more strongly with Zr atoms.</td>
</tr>
</tbody>
</table>

A Bidentate Phosphonate Anchor— the Key to Improved Stability

1. Phase II SBIR (NIH).

Pamidronic acid (PDA)

Aminopropylphosphonic acid (APPA)

Bidentate anchor
Zirconia CSP 2-Step Synthesis with Bidentate Anchor (PDA)

Lewis acid-base reaction

CS-COOH

EEDQ coupling reaction

CS = Chiral Selector
Chiral Selectors Evaluated

1. Phase II SBIR (NIH)
Changing (S) to (R)-Phenylglycine CSP on Same Zr Column

Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, F=1 ml/min, rm °C, 254 nm, Column: ZirChrom PDA-(S)-PG, S/N SPG122005D and ZirChrom PDA-(R)-PG, S/N RPG020806A (100 × 4.6 mm, 3 µm, Running HPLC coated on PHASE110805A, batch#: 52-132). Solute: 1,3,5-Tri-t-butyl-benzene, (R orS)-2,2,2-Trifluoro-1-(9-anthryl) EtOH. 5 µl injection.

2-Step Load (S)-PG CS
k’(less) = 2.84
k’(more) = 3.81
α = 1.34

Strip (S)-PG CS
No separation.

2-Step Load (R)-PG CS
k’(less) = 2.92
k’(more) = 3.83
α = 1.34
Carboxylate Modified Cellulose Based CSP on Zirconia
Phosphonate Modified Cellulose Based CSP on Zirconia

1) NaH, DMF
2) Br

Dimedone
Pd(PPh₃)₄
THF

ZrO₂
Cellulose Phase Regeneration

1) 50:50 1M NaOH:THF, 1h 60 °C
2) H₂O, 1h
3) 1M HNO₃, 1h
4) H₂O, 1h

1) Cellulose-PO₃H₂, THF, 16h
2) THF, 1h
Cellulose Phase Regeneration

Pre-mixed 90/10 Hexane/IPA, F=1 ml/min, rm °C, 254 nm, Column: ZirChrom-CelluloZe, S/N R020907W (100 × 4.6 mm, 5 µm, batch 67-C46). Solute: α-Burke, 10 µl injection.

Initial run

After CSP stripping

After regeneration

~24 hours total!

α=1.55

α=1.54

mAU

0 25 50 75 100 125

1 2 3 4 5 6 min
Comparison of Zirconia and Silica Cellulosic Phases

Columns, (A) CelluloZe™ (Celul022006A), 100 × 4.6 mm, 3 µm Zirconia, (B) Silica-based column, 150 × 4.6 mm, 5 µm Silica, Solute (RS)-(±)-2,2,2-Trifluoro-1-(9-anthryl) EtOH, Mobile phase 90 / 10 Hexane / IPA, Flow Rate, 1 mL/min, Column temperature, ambient.
Separation of Basic Drugs on Zirconia Phosphonated Cellulose CSP

Column, CelluloZe™ (Celu022006A), 100 × 4.6 mm, 3 µm Zirconia,
Mobile phase, = 50/50 Heptane/IPA (100 mM NH₄OAc in IPA),
Flow Rate, 1 mL/min, Column temperature, ambient.
Effect of Ionic Strength on Zirconia Phosphonated Cellulose CSPs

Increasing ammonium acetate increases the selectivity and decreases retention and improves peak shape for Pindolol. This is likely due to suppression of cation-exchange retention mechanism that occurs for basic molecules.
Effect of Ionic Strength on Zirconia Cellulosic CSPs

41-C54, J04-175, 3,5-dimethylphenyl, -C_{11}H_{22}PO_{3}H

<table>
<thead>
<tr>
<th>Ion Strength/Selectivity</th>
<th>Ammonium Acetate in IPA (mM)</th>
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<tbody>
<tr>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Pindolol</td>
<td>2.87</td>
</tr>
<tr>
<td>Propranolol</td>
<td>1.55</td>
</tr>
<tr>
<td>Atenolol</td>
<td>1.26</td>
</tr>
<tr>
<td>Nadolol</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Increasing ammonium acetate increases enantio-selectivity.

LC Conditions: Agilent 1100 with Chemstation, flow rate 0.5 mL/min., UV 254, mobile phase = 100% IPA with specified concentration of ammonium acetate, temperature = ambient, column dimension 10 cm x 4.6 mm id, 3 micron particles.
Conclusions

- Brush-type CSPs were attached to zirconia using a multi-dentate chelate, pamidronic acid (PDA).
- Zirconia-based CSPs were shown to be reproducible, stable and have comparable chromatographic performance to commercial silica-based Brush-type CSPs for a range of chiral compounds.
- The new zirconia-based cellulosic CSPs showed similar resolving power to commercial silica-based cellulosic CSPs for selected chiral compounds; increased ionic strength improved resolution of basic chiral compounds by suppressing cation exchange.
- Zirconia-based CSPs can offer users the ability to replace or regenerate the chiral stationary phase.
References


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Thanks very much for listening!