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

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Specialists in High Efficiency, Ultra-Stable Phases for HPLC
Outline

• 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 I 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:** \( \text{PO}_4^{3-}, \text{RCO}_2^{-}, \text{Catechol} \)
Interaction Strength of Lewis Bases with Zirconia

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

A Bidentate Phosphonate Anchor– the Key to Improved Stability\textsuperscript{1}

Aminopropylphosphonic acid (APPA)

Bidentate anchor

Pamidronic acid (PDA)\textsuperscript{1}
(Phase II Anchor)

1. Phase II SBIR (NIH).

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

Lewis acid-base reaction

CS-COOH
EEDQ coupling reaction

CS = Chiral Selector

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Chiral Selectors Evaluated

1. Phase II SBIR (NIH)

(S)-DNB-L-Leucine  
[(S)-Leu]

(S)-DNB-L-Phenylglycine  
[(S)-PG]

(S)-N-[1-(1-naphthyl)ethyl]succinamic acid  
[(S)-NESA]
Stability of Zr-(S)-NESA at pH 2

Zirconia (PDA) CSPs are compatible with reversed phase conditions


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Stability of Zr-(S)-NESA at pH 2


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Stability of Zr-(S)-DNB-Leu at pH 8

Changing (S) to (R)-Phenylglycine CSP on Same Zr Column

2-Step Load (S)-PG CS
\[ k'(less) = 2.84 \]
\[ k'(more) = 3.81 \]
\[ \alpha = 1.34 \]

Strip (S)-PG CS
No separation.

2-Step Load (R)-PG CS
\[ k'(less) = 2.92 \]
\[ k'(more) = 3.83 \]
\[ \alpha = 1.34 \]

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.

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Carboxylate Modified Cellulose Based CSP on Zirconia

1) NaH/DMF

2) Br\(\cdot\)COONa

Carbamate

Anchor
Phosphonate Modified Cellulose Based CSP on Zirconia

1) NaH / DMF
2) \( \text{HO-} \underset{\text{Br}}{\text{P}} \text{O}_{11} \text{HO} \)

Carbamate

Anchor

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Selectivity Comparison of Previous and New Zirconia Based Cellulosic CSPs


- **Carr Ref.** 1.36% C
- 41-C47, 1.44% C - (C5-COOH)
- 41-C54, 1.13% C – (C11-PO$_3$H$_2$)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Condition</th>
<th>Chromatogram Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-stilbene Oxide</td>
<td>a</td>
<td>α=1</td>
</tr>
<tr>
<td>Trifluoracryl Ethanol</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>1-phenyl-1-propanol</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>1-phenyl-2-propanol</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>r-(2-naphthyl)-r-butyrolactone</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>3-phenyl-1-butanol</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>trans-stilbene Oxide</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>a-(trifluoromethyl)benzyl alcohol</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>3,5-dinitro-N-(1-phenylethyl)benzamide</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Burke O2</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>a-methylbenzyl cyanide</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>a-Methyl-1-napthalenone methanol</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Napropamide</td>
<td>a</td>
<td></td>
</tr>
</tbody>
</table>

* α = 90/10 hexane/IPA
  β = 98/2 hexane/IPA

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Selectivity Comparison Between Zirconia and Silica Cellulosic CSPs

41-C54, J04-175, 3,5-dimethylphenyl, -C_{11}H_{22}PO_{3}H_{2}
Commercial Silica CSP column

Undecylphenyl carbamate modified cellulosic CSP has good selectivity compared to a commercial silica column.

a: 90/10 hexane/IPA
b: 98/2 hexane/IPA

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Comparison of Zirconia and Silica Cellulosic Phases

Columns, (A) CelluloZe™ (Celu022006A), 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.
Comparison of Zirconia and Silica Cellulosic Phases

Columns, (A) CelluloZe™ (Celu022006A), 100 × 4.6 mm, 3 µm Zirconia, (B) Silica-based column, 150 × 4.6 mm, 5 µm Silica, Solute, trans stilbene oxide, Mobile phase 90 / 10 Hexane / IPA, Flow Rate, 1 mL/min, Column temperature, ambient.
Comparison of Zirconia and Silica Cellulosic Phases

Columns, (A) CelluloZe™ (Celuo22006A), 100 × 4.6 mm, 3 µm Zirconia, (B) Silica-based column, 150 × 4.6 mm, 5 µm Silica, Solute Napropamide, Mobile phase 90 / 10 Hexane / IPA, Flow Rate, 1 mL/min, Column temperature, ambient.

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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.

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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\textsubscript{11}H\textsubscript{22}PO\textsubscript{3}H

<table>
<thead>
<tr>
<th>Ion Strength/Selectivity</th>
<th>Ammonium Acetate in IPA (mM)</th>
</tr>
</thead>
<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!