Chiral Separations on Zirconia-Based Chiral Stationary Phases

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Specialists in High Efficiency, Ultra-Stable Phases for HPLC
Surface Chemistry of Zirconia

Zirconia chemistry is dominated by Lewis acid-base reactions

Lewis Acid: \( \text{Zr}^{4+}: \text{H}_2\text{O} + \text{RPO}_3^{2-} \rightleftharpoons \text{Zr}^{4+}: \text{RPO}_3^{2-} + \text{H}_2\text{O} \)

Other Lewis base examples: \( \text{PO}_4^{3-}, \text{RCO}_2^{-}, \text{Catechol} \)
A Novel Approach to Attaching Chiral Selectors\textsuperscript{1} to Zirconia\textsuperscript{2}

\begin{itemize}
\item[2.] 2. Phase I SBIR Grant (NIH).
\end{itemize}
Interaction Strength of Lewis Bases with Zirconia\(^1\)

<table>
<thead>
<tr>
<th>Interaction Strength</th>
<th>Lewis Base (L)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strongest</strong></td>
<td>Hydroxide</td>
<td></td>
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<tr>
<td></td>
<td>Phosphate</td>
<td></td>
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<tr>
<td></td>
<td>Fluoride</td>
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<tr>
<td></td>
<td>Citrate</td>
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<td></td>
<td>Sulfate</td>
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<td></td>
<td>Acetate</td>
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<tr>
<td></td>
<td>Formate</td>
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<tr>
<td></td>
<td>Nitrate</td>
<td></td>
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<tr>
<td></td>
<td>Chloride</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td></td>
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<tr>
<td><strong>Weakest</strong></td>
<td></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\(^1\)

Aminopropylphosphonic acid (APPA)

Pamidronic acid (PDA)\(^1\) (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

CS
Chiral Selectors Evaluated\(^1\)

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]
Chiral Separation on Zr (S)-Leu
(\(\pi\)-acceptor phase)

Trifluoranthryl Ethanol
\(\alpha = 1.15\)
Conditions: 99/1 Hexane/IPA,
Flow=1

1-Naphthyl-Leucine Ester
\(\alpha = 16.8\)
Conditions: 99/1 Hexane/IPA,
Flow=1

Napropamide
\(\alpha = 1.47\)
Conditions: 99/1 Hexane/IPA,
Flow=1
Chiral Separation on Zr (R)-PG
(Another $\pi$-acceptor phase)

**Trifluoranthryl Ethanol**

$\alpha = 1.18$

Conditions: 99/1 Hexane/IPA, Flow=1, 30 °C

**1-Naphthyl-Leucine Ester**

$\alpha = 2.98$

Conditions: 99/1 Hexane/IPA, Flow=1, 30 °C

**Napropamide**

$\alpha = 1.14$

Conditions: 99/1 Hexane/IPA, Flow=1, 30 °C
Chiral Separations on Zr (S)-NESA
\((\pi\text{- donor phase})\)

(R/S)-3,5-Dinitro-N-(1-phenylethyl)benzamide.
Conditions: Pre-mixed 88.9/11/0.1
Hexane/IPA/TFA, F=1 ml/min, 30 °C

\(\alpha = 2.18\)

(R/S)-N-3,5-dintrobenzoyl-a-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate.
Conditions: Pre-mixed 88.9/11/0.1
Hexane/IPA/TFA, F=1 ml/min, 30 °C

\(\alpha = 1.28\)

(R/S)-(3,5-dinitrobenzoyl)-phenylglycine
Conditions: Machine mixed 15/85 (99.9/0.1
MeOH/TFA) / (89/11 Hexane/IPA), F=1 ml/min, 30 °C

\(\alpha = 1.65\)
Methanol Effect on Zr (S)-NESA

Sample: (R/S)-N-3,5-dintrobenzoyl-a-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate

Conditions: 89/11 Hexane/IPA, F=1 ml/min, 30 °C.

\[ \alpha = 1.59 \]
\[ N_2 = 971 \]

Conditions: 90 / 2 / 8 (99/1 Hexane/IPA) / MeOH / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

\[ \alpha = 1.42 \]
\[ N_2 = 6,425 \]

Conditions: 80 / 10 / 10 (99/1 Hexane/IPA) / MeOH / (70/30 Hexane/IPA), F=1 ml/min, 30 °C

\[ \alpha = 1.25 \]
\[ N_2 = 13,315 \]
Stability of Zr-(S)-NESA at pH 2

Zirconia CSPs are compatible with reversed phase conditions

Initial

After 7000 column volume flush


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

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

Production Scale-up of Zr (S)-NESA

- 7.5 g zirconia per batch used for coating
- 4X scale-up 30 g zirconia per batch used for coating

**Napropamide**
Conditions: 90/10 Hexane/IPA, Flow=1
2-Step Synthesis of Zirconia CSPs for Chiral Selector Screening

- ZrO$_2$
- Lewis Base (Anchor)
- Chiral Selector

Zirconia

Anchor adsorption

CSP Stripping

Chromatographic Test

CS bonding
2-Step Online Zirconia CSP Synthesis for Chiral Screening

1) Lewis Acid-base Anchor Attachment (10 mg/mL aq. PDA, recycle for 16 hrs at 1 mL/min and 60°C)

2) EEDQ Coupling Reaction (CS reagent in THF overnight (16 hrs) at 30°C)

3) Chromatographic Test

4) CSP Column Stripping (1M NaOH for 2 hrs at 60°C, water and 1M HNO₃ finish)

Process not optimized for time

ZrO₂-PDA-CS

(Pamidronic acid)

ZrO₂-PDA-CS

CS (Chiral Selector)
Changing Chiral Selectors

(S)-DNB-L-Phenylglycine (S-PG)    (R)-DNB-L-Phenylglycine (R-PG)

Pamidronic acid derivatives
Stripping Experiment: (S)-PG CSP

Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, flow rate=1 ml/min, ambient temperature, 254 nm, Column: ZirChrom PDA-(S)-PG, S/N SPG122005D (100 × 4.6 mm, 3 µm, Running HPLC coated on PHASE110805A, batch#: 52-132).

Solute: (1) 1,3,5-Tri-t-butyl-benzene, (2) (S)-2,2,2-Trifluoro-1-(9-anthryl) ethanol , (3) (R)-2,2,2-Trifluoro-1-(9-anthryl) ethanol 5 µl injection.

After coating

After stripping

(1) (2+3)

(2) (3)
1- Original column.
2- Column flushed with 15/85 ACN/pH 12 NH₄OH for 10 column volumes, then 10 column volumes of water, 10 column volumes of 1.0 M nitric acid, and 10 column volumes of water.
3- Column then flushed with 50 column volumes of 20/80 ACN/1 M NaOH, then 10 column volumes of water, 10 column volumes of 1 M nitric acid and 10 column volumes of water.
4- Column then flushed with 20/80 ACN/1 M NaOH for 50 column volumes at 60 °C, then flushed with 10 column volumes of water, 10 column volumes of 1 M nitric acid, and 10 column volumes of water.
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
1-Step Synthesis of Zirconia CSPs for Fast Chiral Screening

Zirconia

Stripping-off

CS with Anchor Attached

Chromatographic Test

= ZrO₂

= Chiral Selector with Anchor Group
Example 1-Step Attachment and Detachment Cycle

- Pass a solution of 20 mM N-(4-nitrobenzoyl)-L-glutamic acid (CSP) in tetrahydrofuran for 10 minutes at a column temperature of 60°C and a flow rate of 1 mL/min.
- Flush column with 100% THF for 10 minutes at 2 mL/min at ambient temperature.
- Separate a racemic solution of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol.
- Strip the CSP by flushing the column with a 50 mM solution of tetramethylammonium hydroxide solution (pH 12) for 20 minutes at 60°C using a flow rate of 1 mL/min.
- Repeat procedure using the same CSP.
Comparison between the initial and final separation of (±)-2,2,2-trifluoro-1-(9-anthyl)ethanol leucine ester during a single CSP screening cycle.

Chromatographic conditions: mobile phase: 99/1 hexane/IPA; flow rate: 1 ml/min; temperature: 30 °C, solute concentration = 1mg/mL, 5 μL injection.
Development of a New Class of Regenerable Cellulosic Coated Zirconia Stationary Phases
Carboxylate Modified Cellulose Based CSP on Zirconia

1) NaH/DMF

2) Br\(_5\)COONa

[Chemical Structures]

Carbamate

Anchor
Phosphonate Modified Cellulose Based CSP on Zirconia

1) NaH / DMF
2) HO-P\textsubscript{Br}\textsubscript{11}

Carbamate

Anchor

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Retention Comparison of Hexanoic Acid Modified and Undecylphosphonic Acid Zirconia Based Cellulosic CSPs


(2) Batch C54 is slightly less retentive than C47.
Selectivity Comparison of Previous and New Zirconia Based Cellulosic CSPs

Retention Comparison Between Alkylphenyl Modified Cellulosic CSPs and Commercial Silica CSPs

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

Commercial Silica CSP column

New phase has less retention than commercial Silica-based column likely due to lower loading of CSP and anchor group.

a: 90/10 hexane/IPA
b: 98/2 hexane/IPA
Selectivity Comparison Between Undecylphenyl Carbamate Modified Cellulosic CSPs and Commercial Silica CSPs

41-C54, J04-175, 3,5-dimethylphenyl, -C₁₁H₂₂PO₃H₂
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
Effect of Ionic Strength on the Separation of Basic Chiral Pharmaceuticals on Undecylphosphonic Acid Modified 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 Actate 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.
Effect of Ionic Strength on Undecylphosphonic Acid Modified Cellulosic CSPs

Increasing ammonium acetate increases the selectivity and decreases retention and improves peak shape for Pindolol. This is likely due to supression of cation-exchange retention mechanism that occurs for basic molecules.
Comparison of Silica and Zirconia 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.

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Columns, (A) CelluloZe™ (Celu022006A), 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.
Comparison of Silica and Zirconia 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, trans stilbene oxide, Mobile phase 90 / 10 Hexane / IPA, Flow Rate, 1 mL/min, Column temperature, ambient.
Separation of Basic Drugs on Phosphonated Cellulose Zirconia

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.
Conclusions

• Brush-type CSPs were attached to zirconia using multi-dentate 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.
• Zirconia-based CSPs offer the user the potential to regenerate the chiral stationary phase online.
• 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.
References


Acknowledgement: National Institutes of Health Grant (Phase II SBIR) 2R44HL070334-02A2.
Thanks very much for listening!

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