Synthesis of Stable Zirconia Based Chiral Stationary Phases for Enantiomer Separations and Fast Chiral Selector Screening

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Specialists in High Efficiency, Ultra-Stable Phases for HPLC.
Goal: To Make Zirconia Based Chiral Stationary Phases for Fast Chiral Selector Screening

- Why Zirconia?
- Synthetic Approach
  - Surface Chemistry
  - Building a zirconia-based CSP
  - Proof of concept
- Chiral Separations on Zirconia Based CSPs
- Effect of Mobile Phase Additives on $\alpha$, $k'$ and $N$
- Stability Study
- Conclusion – Careful selection of an anchor group results in a stable CSP that can be stripped off and reattached under high pH condition. This offers the possibility of regeneration or use for Chiral Selector Screening.
Surface Chemistry of Zirconia

**Brönsted Acid:**  \[ \text{ZrOH} + \cdot\text{OH} \rightleftharpoons \text{ZrO}^- + \text{H}_2\text{O} \]

**Brönsted Base:**  \[ \text{Zr} \rightleftharpoons \text{Zr} \quad + \quad \text{H}^+ \rightleftharpoons \text{Zr} \rightleftharpoons \text{Zr} \]

**RPO}_3^{2-} \text{ or Catechol**

**Lewis Acid:**  \[ \text{Zr}^{4+}: \text{H}_2\text{O} + \text{R-COO}^- \rightleftharpoons \text{Zr}^{4+}:\cdot\text{OOC}-\text{R} + \text{H}_2\text{O} \]
New Way to Attach Chiral Selectors to Zirconia Surface

\[ \text{ZrO}_2 \] = Lewis Base (Anchor) \[ \triangle \] = Chiral Selector
Example Attachment and De-attachment 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.
• Flushed column with 100% THF for 10 minutes at 2 mL/min at ambient temperature.
• Separate a racemate 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 microliter injection.
Anchors

1) APPA (Aminopropylphosphonic acid)
2) DHNP (3,4-Dihydroxynorephedrine)
3) ASPA (Aspartic acid)

PDA (Pamidronic acid)

Phase I Anchors

Phase II Anchor
Three-Point Interactions

Example of Lewis Acid-Base Modified Zirconia CSPs

Lewis acid-base reaction

EEDQ coupling reaction

CS = Chiral Selector
Chiral Selectors

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

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

(S)-N-[1-(1-naphthyl)ethyl]succinamic acid ((S)-NESA)
Example of Lewis Acid-Base Modified Zirconia CSPs

Lewis acid-base reaction

(Pamidronic acid)

Steric Site

EEDQ coupling reaction

H-donor

π-acceptor
Selectivity Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu

Selectivity for both anchors is very similar.
Retention Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu

Retention for both anchors is different.
Efficiency Comparison Between PDA Anchored Zr (S)-Leu and APPA Anchored (S)-Leu

Efficiency on PDA anchored Zr (S)-Leu is much better than on APPA anchored Zr (S)-Leu.
Chiral Separation on Zr (S)-Leu (pi-acceptor phase)

Trifluoranthryl Ethanol
Conditions: 99/1 Hexane/IPA, Flow=1
\[ \alpha = 1.15 \]

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

Napropamide
Conditions: 99/1 Hexane/IPA, Flow=1
\[ \alpha = 1.47 \]
Chiral Separations on Zr (S)-NESA (pi-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)- (R/S)-N-3,5-dintrobenzoyl-\(\alpha\)-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
  - $\alpha = 1.65$
Mobile Phase Effect of adding MeOH on Separation of (R/S)-N-3,5-dintrobenzoyl-\(\alpha\)-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate on Zr (S)-NESA

1. Conditions: 89/11 Hexane/IPA, F=1 ml/min, 30 °C.
   - \(\alpha=1.59\)
   - N2=971

2. Conditions: 90 / 2 / 8 (99/1 Hexane/IPA) / MeOH / (70/30 Hexane/IPA), F=1 ml/min, 30 °C
   - \(\alpha=1.42\)
   - N2=6,425

3. Conditions: 80 / 10 / 10 (99/1 Hexane/IPA) / MeOH / (70/30 Hexane/IPA), F=1 ml/min, 30 °C
   - \(\alpha=1.25\)
   - N2=13,315
Stability of Zr-(S)-NESA at pH 2

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

Scale-up the Production 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
Conclusions

• Five new CSPs were attached to zirconia using the PDA anchor, including:
  \[\pi\text–acceptors}: \text{Zr (S)}\text{-Leu, Zr (R)}\text{-PG, and Zr (S)}\text{-PG}\]
  \[\pi\text–donors}: \text{Zr (R)}\text{-NESA, Zr (S)}\text{-NESA}\]
• Small amounts of methanol in the mobile phase had a large effect on efficiency, retention, and selectivity.
• The new Zirconia-based CSPs were found to be fairly stable in reversed-phase mobile phase from pH 2 to pH 8.
• The CSP synthesis is reproducible.
• Chiral selector screening is possible on the new zirconia-based CSPs.
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Thanks very much for listening!

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