Chiral Separations on Novel Brush and Polysaccharide-Type Zirconia Chiral Stationary Phases

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Daniel Nowlan¹, Clayton V. McNeff¹, Bingwen Yan¹, Richard A. Henry¹
Yini Wang², Shengxiang Ji², Thomas R. Hoye²

¹ ZirChrom Separations, Inc. 617 Pierce St., Anoka, MN 55303
² University of Minnesota, 207 Pleasant Street SE, Minneapolis, MN 55455

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
  - Building a zirconia-based CSP
  - Proof of concept
- Chiral Separations on Zirconia Based CSPs
- Stability Study
- Column Regeneration
- 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.
Example of Lewis Acid-Base Modified Zirconia CSPs

Lewis acid-base reaction

(Pamidronic acid)

Steric Site

H-donor

π-acceptor

EEDQ coupling reaction
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.
Chiral Separation on Zr (S)-Leu (pi-acceptor phase)

Trifluoranthryl Ethanol
Conditions: 99/1 Hexane/IPA, Flow=1 ml/min
$\alpha=1.15$

1-Naphthyl-Leucine Ester
Conditions: 99/1 Hexane/IPA, Flow=1 ml/min
$\alpha=16.8$

Napropamide
Conditions: 99/1 Hexane/IPA, Flow=1 ml/min
$\alpha=1.47$
Chiral Separations on Zr (S)-NESA (π-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.

α=2.18

(R/S)- (R/S)-N-3,5-dinitrobenzoyl-α-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.

α=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.

α=1.65
Mobile Phase Effect of adding MeOH on Separation of (R/S)-N-3,5-dintrobenzoyl-α-amino-2,2-dimethyl-4-pentenyl dimethyl phosphonate on Zr (S)-NESA

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

\[ \alpha = 1.25 \]
\[ N_2 = 13,315 \]
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)

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

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

Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, F=1 ml/min, 30 °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)
Phosphonate Modified Cellulose Based CSP on Zirconia
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.

- 41-C54
- Commercial silica-based column

k'

trans-stilbene Oxide 1-phenyl-1-propanol 3-phenyl-1-butanol 3,5-dinitro-N-(1-phenylethyl)benzamide 1-phenyl-2-propanol 1-phenyl-2-propanol trans-stilbene Oxide trans-stilbene Oxide

a: 90/10 hexane/IPA
b: 98/2 hexane/IPA
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
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: a-Burke, 10 μl injection.

**Cellulose Phase Regeneration**

**Original Cellulose**
- k'(less) = 1.73
- k'(more) = 2.67
- α = 1.54

**Remove Cellulose**
- No separation.

**Reload Cellulose**
- k'(less) = 1.59
- k'(more) = 2.47
- α = 1.55
Conclusions

- Five new CSPs were attached to zirconia using the PDA anchor, including:
  - \(\pi\)-acceptors: Zr (S)-Leu, Zr (R)-PG, and Zr (S)-PG
  - \(\pi\)-donors: Zr (R)-NESA, Zr (S)-NESA
- Polysaccharide based CSPs were attached to zirconia using a phosphonate anchor.
- Zirconia based CSPs can be regenerated online allowing for fast screening of chiral phases with only one column.
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Thanks *very much* for listening!

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