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

Richard A. Henry[1], Clayton V. McNeff[1], Bingwen Yan[1], Daniel Nowlan[1], Shengxiang Ji[2], Thomas R. Hoye[2]

1. ZirChrom Separations, Inc. 617 Pierce St., Anoka, MN 55303,

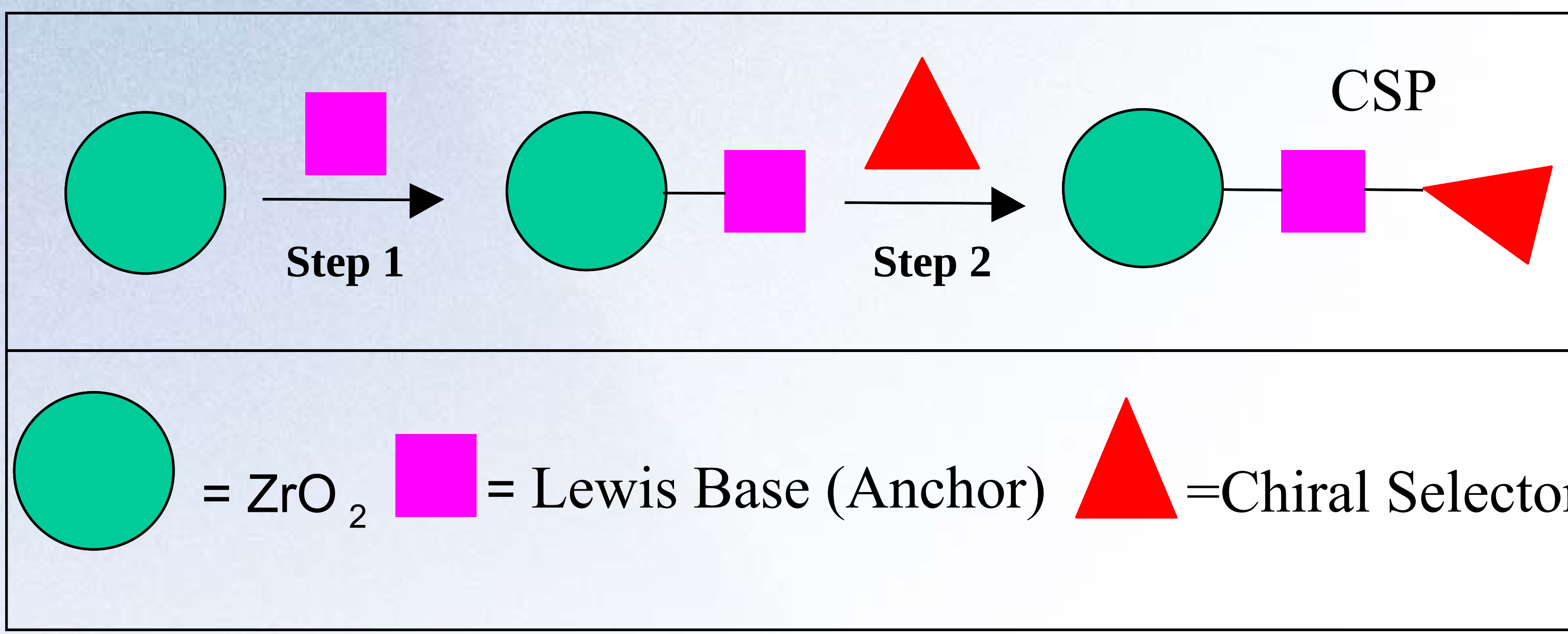
2. University of Minnesota, 207 Pleasant Street SE, Minneapolis, MN 55455.

Specialists in High Efficiency, Ultra-Stable Phases for HPLC

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.

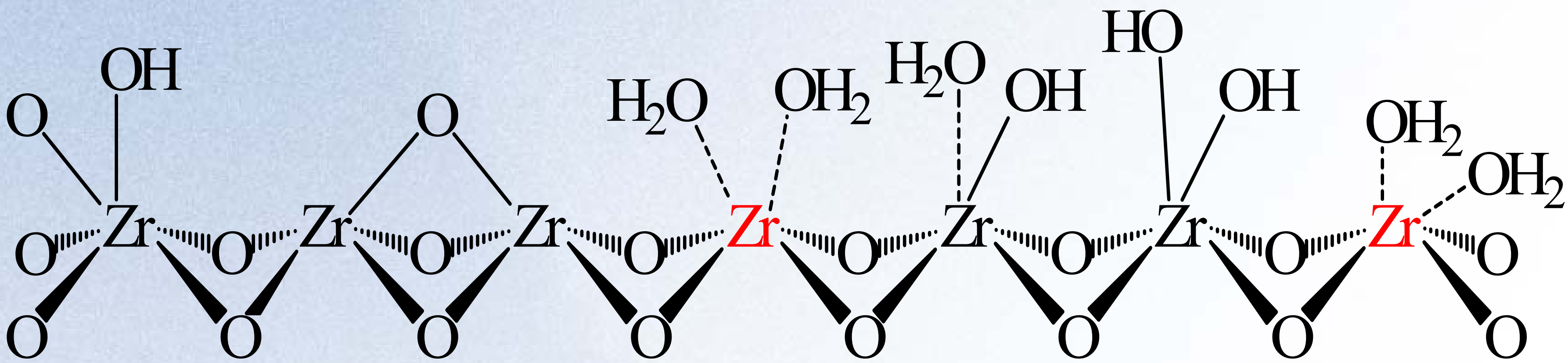
A Novel Approach to Attaching Chiral Selectors[1] to Zirconia[2]



1. William H. Pirkle, et. al., J. Chromatogr., 316 (1984) 585.

2. Phase I SBIR Grant (NIH).

Surface Chemistry of Zirconia



Zirconia chemistry is dominated by Lewis acid-base reactions

Lewis Acid: Zr(IV): H_2O + RPO_3^{2-} $\rightleftharpoons$ Zr(IV): RPO_3^{2-} + H_2O

Other Lewis base examples: PO_4^{3-}, RCO_2^- , Catechol

ZirChrom®

Interaction Strength of Lewis Bases with Zirconia[1]

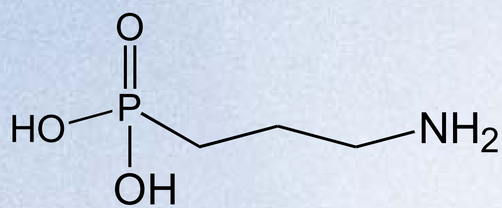
Interaction Strength	Lewis Base (L)
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
	Nitrate
	Chloride
Weakest	Water

Small Lewis bases with high electron density and low polarizability interact more strongly with Zr atoms.

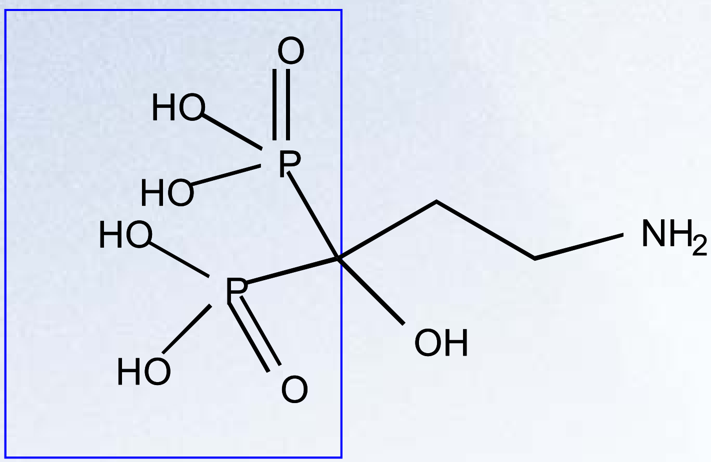
1. J.A. Blackwell and P.W. Carr, "Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide," Anal. Chem. 64, 863-73 (1992).

ZirChrom

A Bidentate Phosphonate Anchor– the Key to Improved Stability[1]



Aminopropylphosphonic acid (APPA)



Bidentate anchor

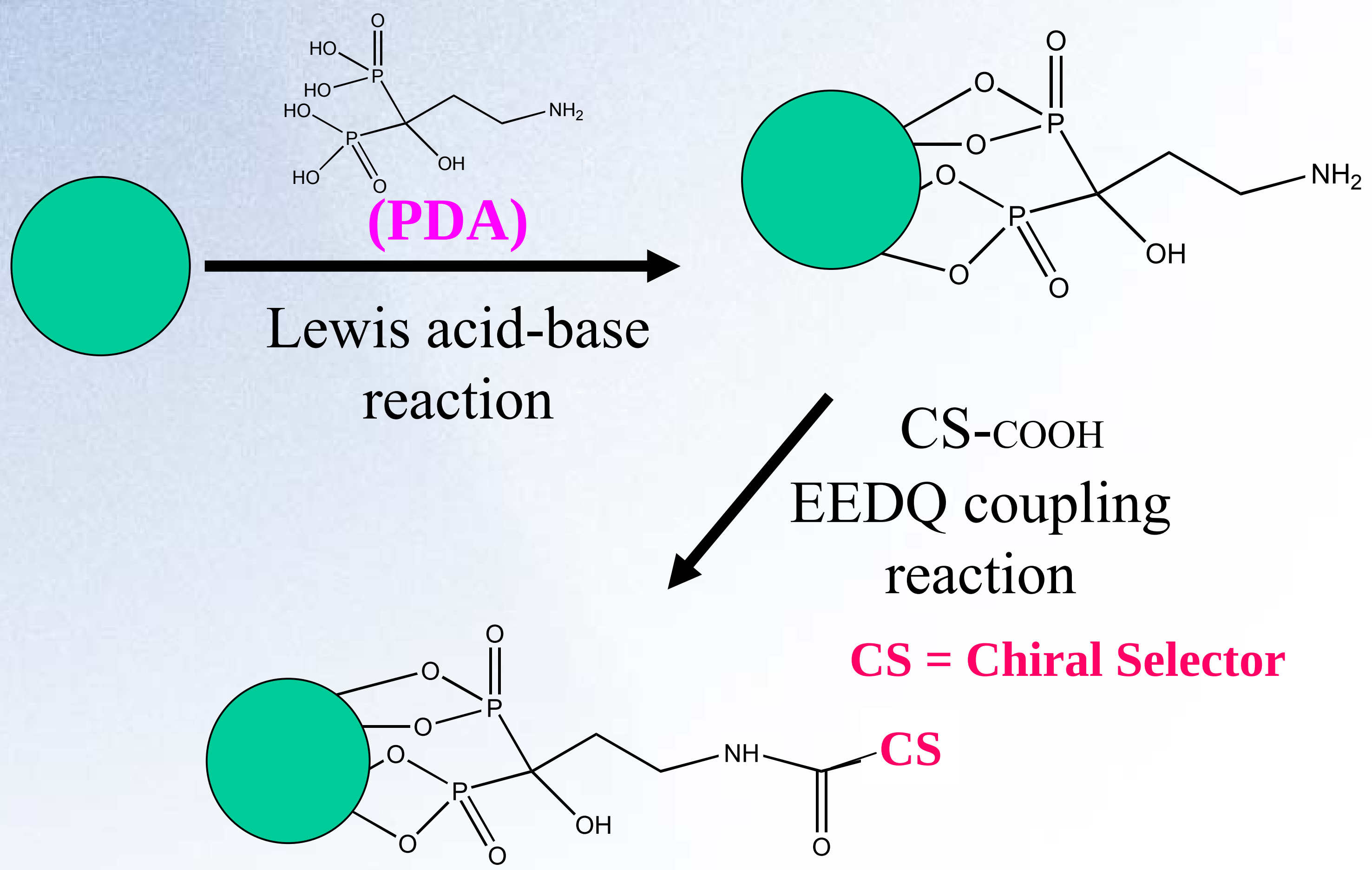
Pamidronic acid (PDA)[1]

(Phase II Anchor)

1. Phase II SBIR (NIH).

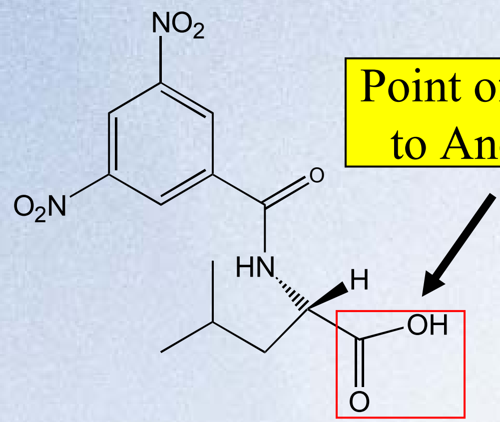
ZirChrom®

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



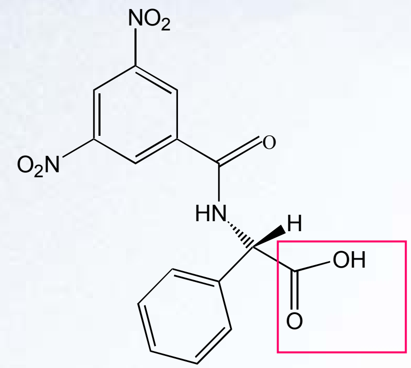
Chiral Selectors Evaluated[1]

Point of Attachment to Anchor Group

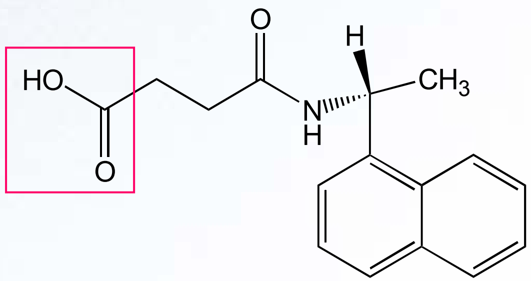


(S)-DNB-L-Leucine

[(S)-Leu]



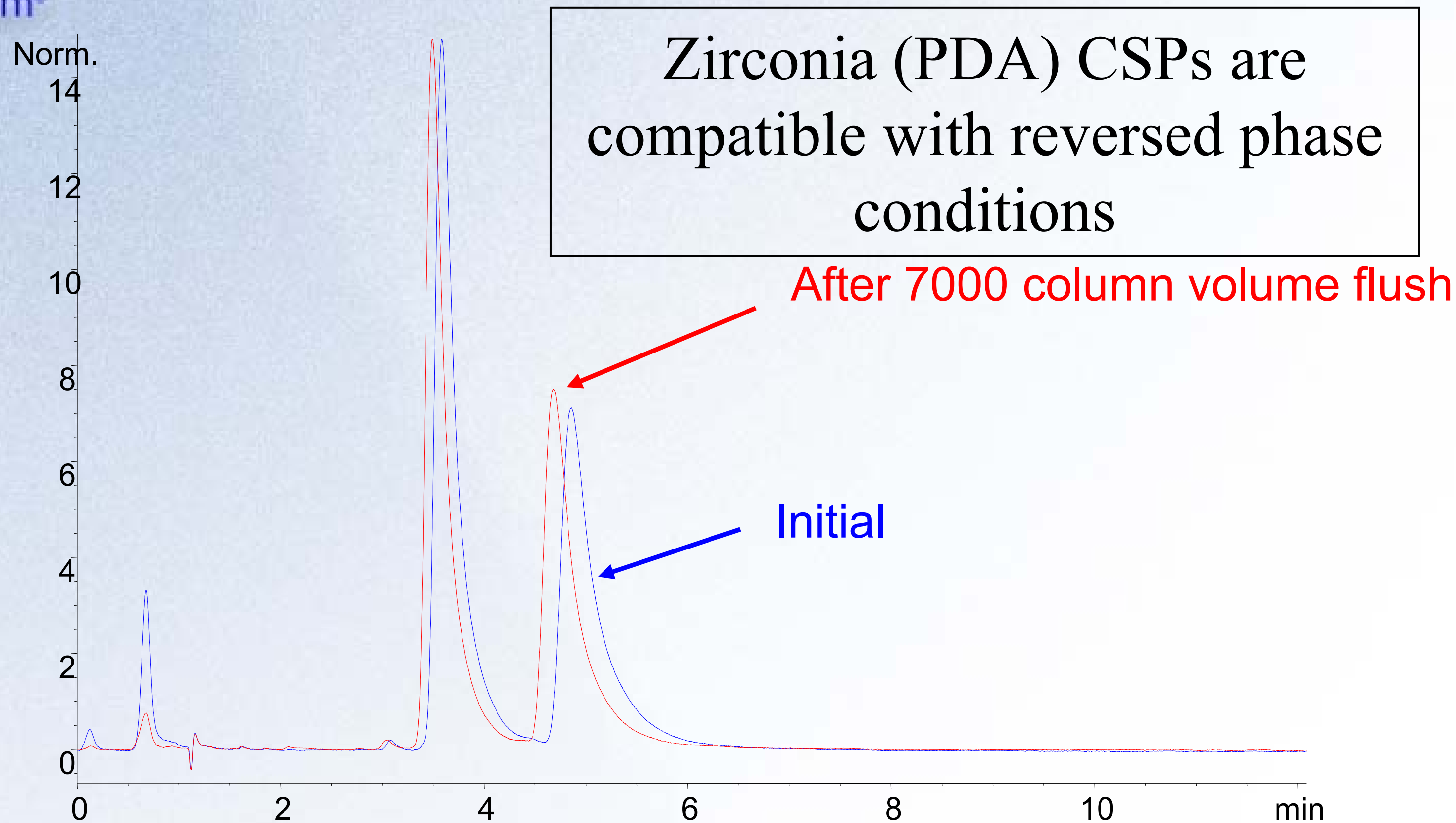
(S)-DNB-L-Phenylglycine
[(S)-PG]



(S)-N-[1-(1-naphthyl)ethyl]succinamic acid
[(S)-NESA]

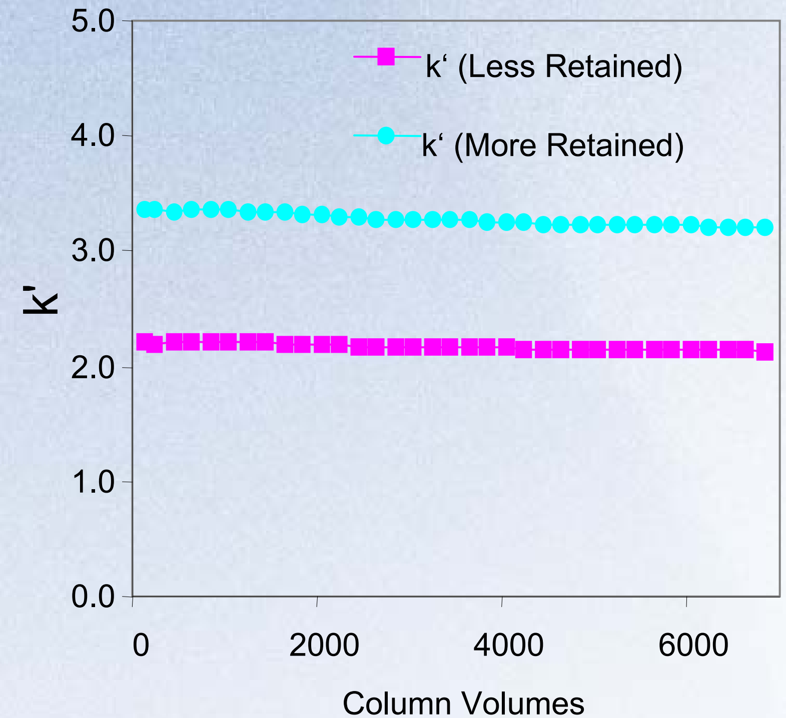
1. Phase II SBIR (NIH)

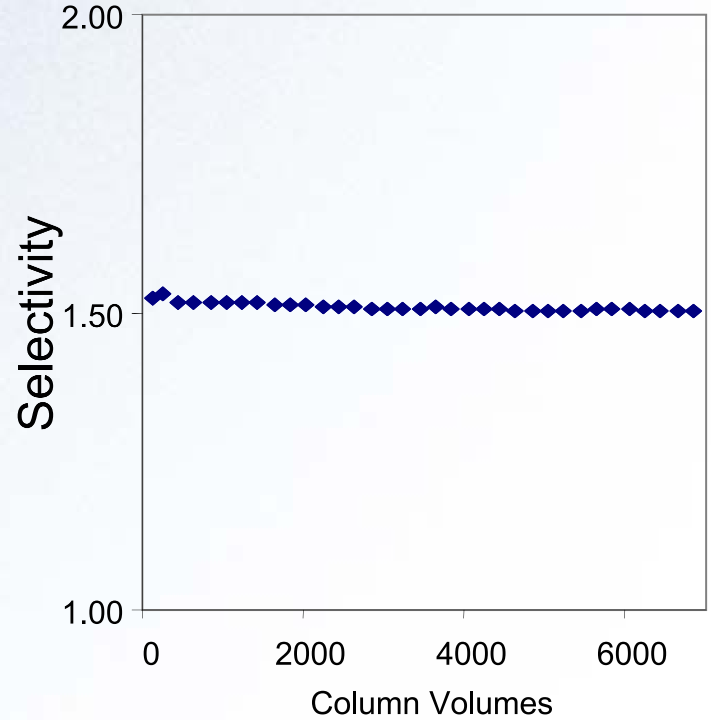
Stability of Zr-(S)-NESA at pH 2



Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

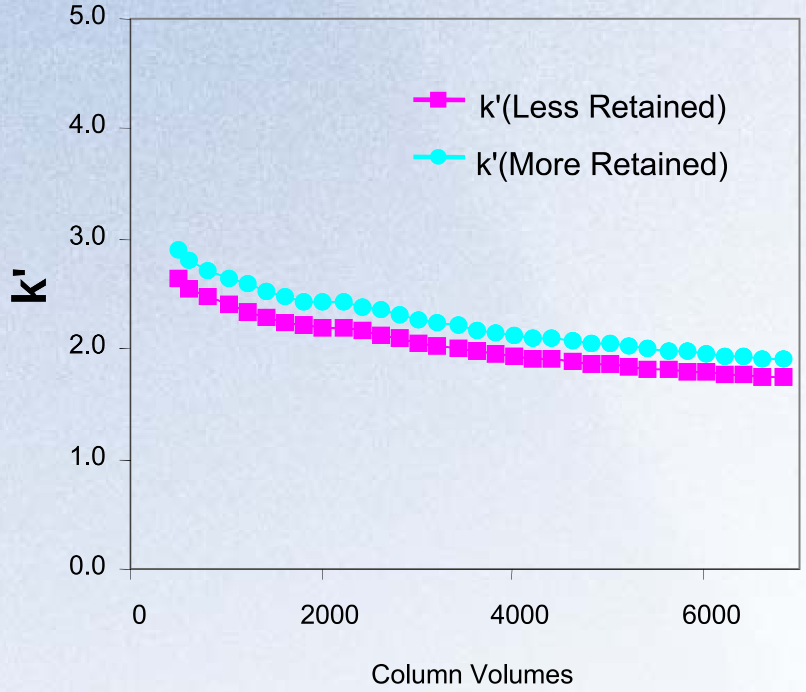
Stability of Zr-(S)-NESA at pH 2

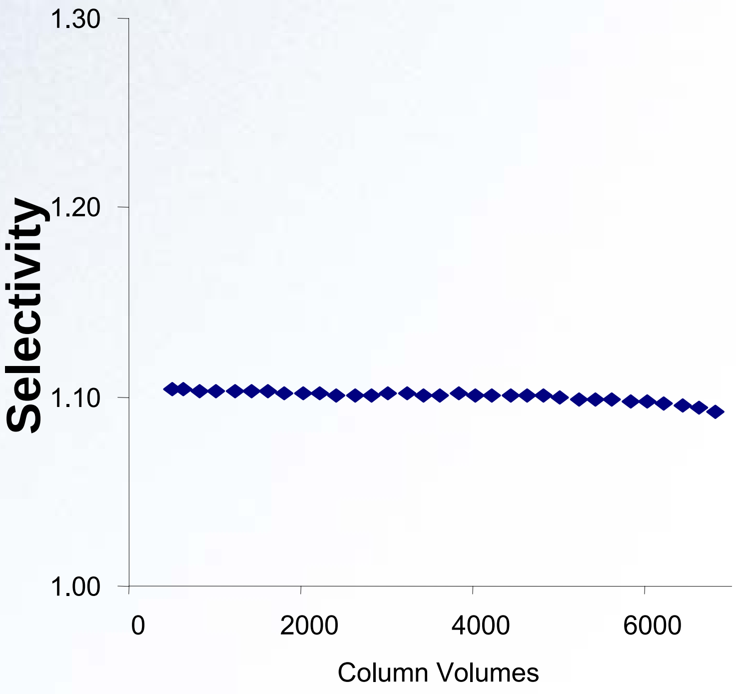




Column ID: ZrCSP051605C, Mobile phase: 15/85 ACN/0.01 mM TFA pH 2, Temperature: 30 ^{o}C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-3,5-dinitro-N-(1-phenylethyl)benzamide.

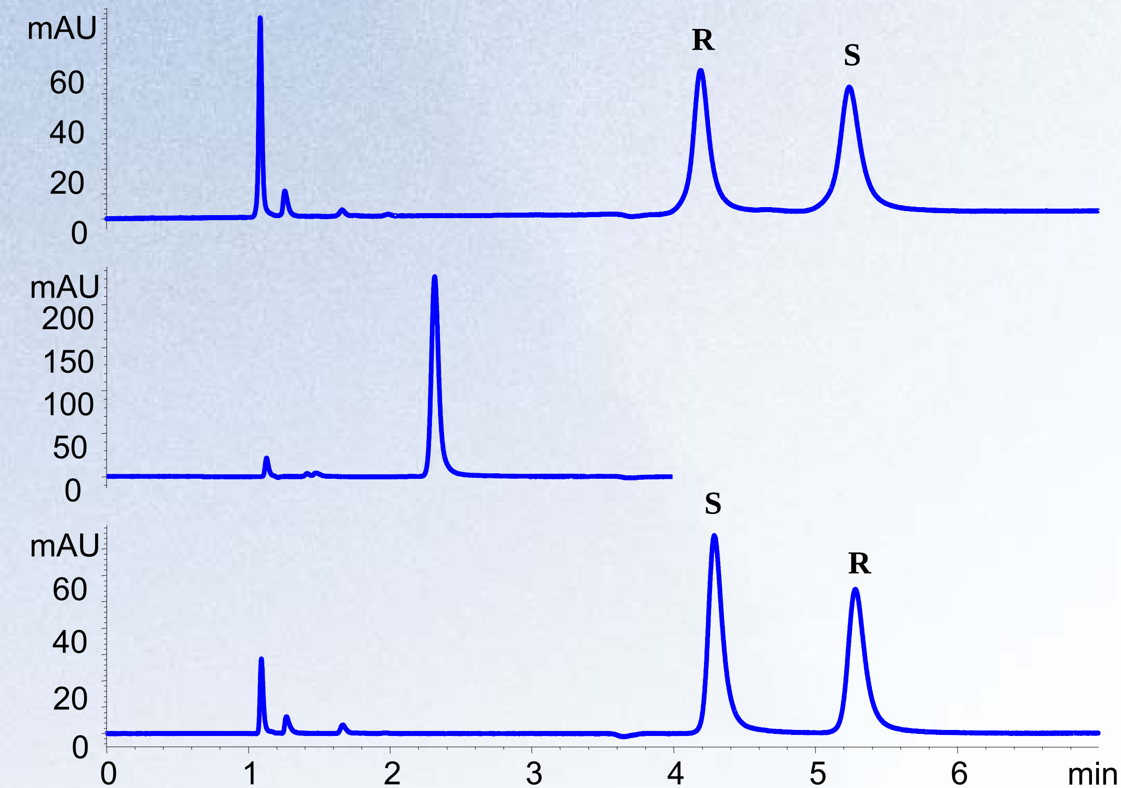
Stability of Zr-(S)-DNB-Leu at pH 8





Column ID: ZrCSP032805A, Mobile phase: 15/85 ACN/5 mM ammonium hydrogencarbonate pH 8.0, Temperature: 30 °C. Injection volume: 5 ul, Wavelength: 254 nm. Probe solutes:(R/S)-2, 2, 2-trifluoro-1-(9-anthryl)ethanol

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

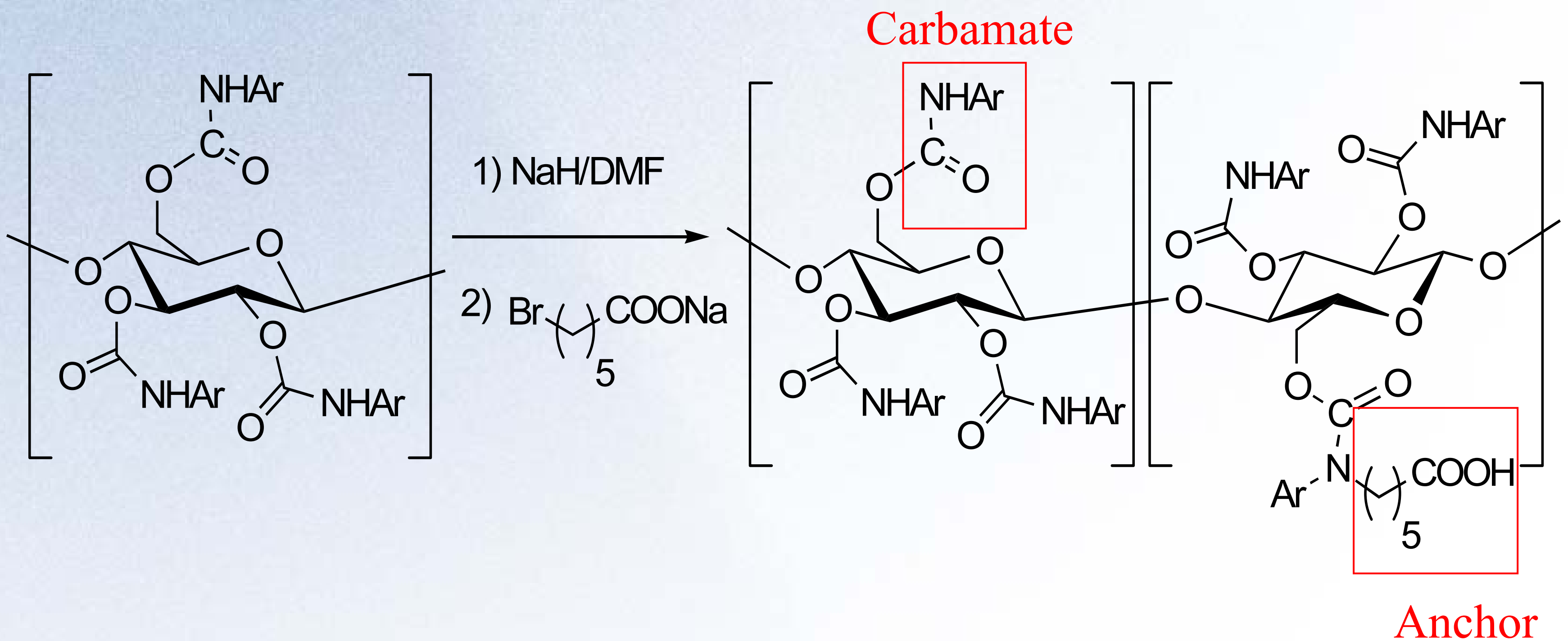
k'(less) = 2.92

k'(more) = 3.83

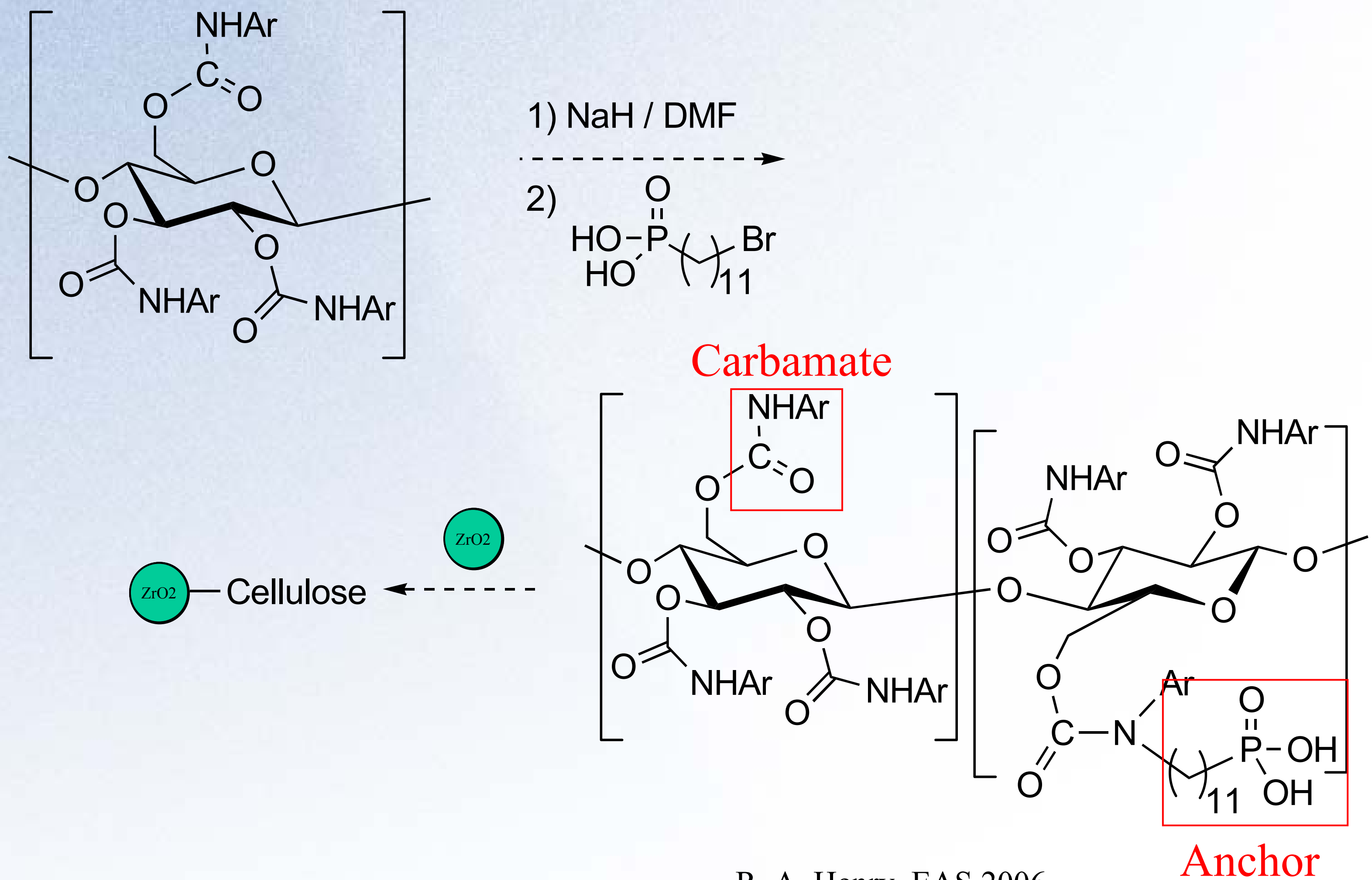
α = 1.34

Pre-mixed 98/0.5/1.5 Hexane/TFA/IPA, F=1 ml/min, 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.

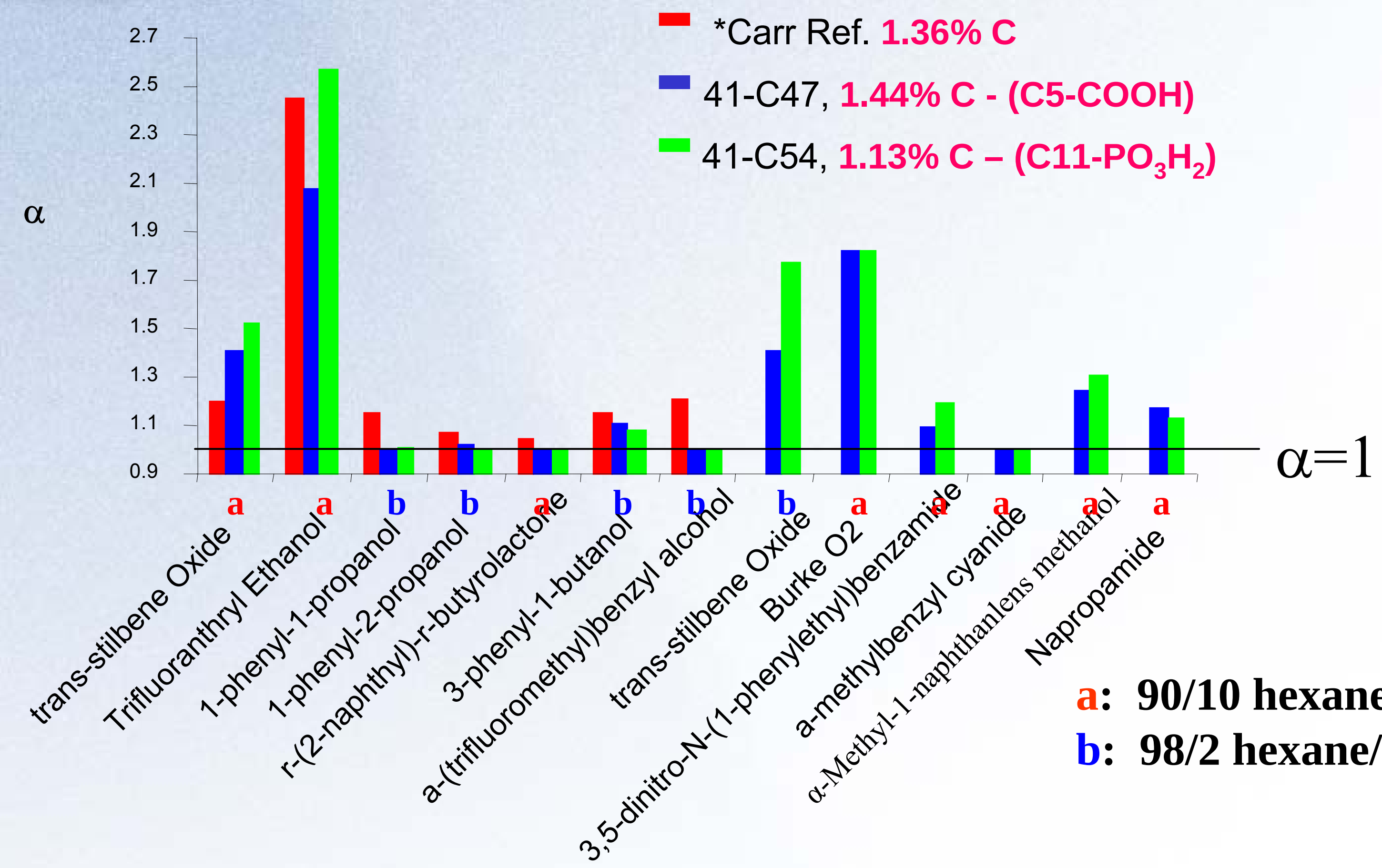
Carboxylate Modified Cellulose Based CSP on Zirconia



Phosphonate Modified Cellulose Based CSP on Zirconia



Selectivity Comparison of Previous and New Zirconia Based Cellulosic CSPs

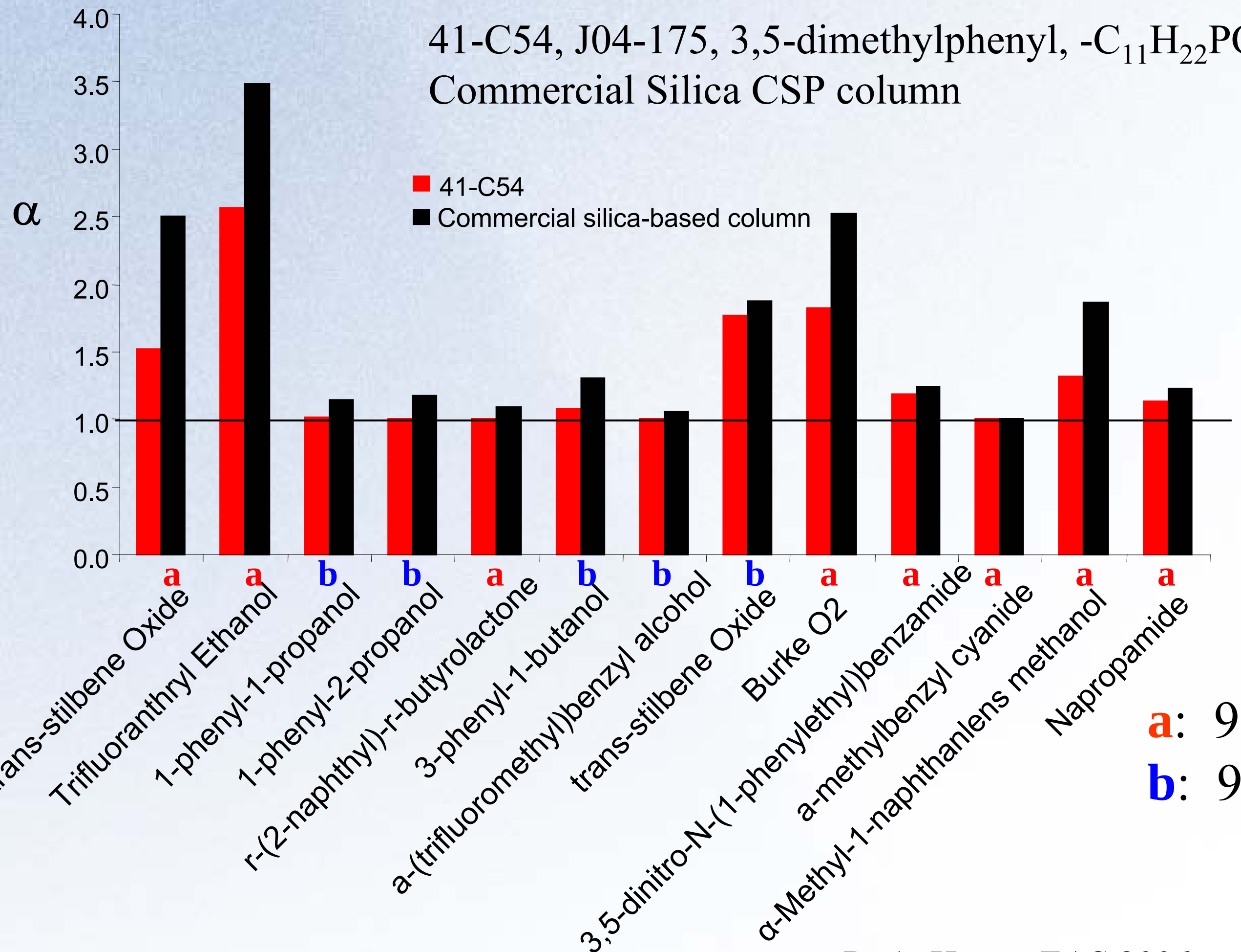


a: 90/10 hexane/IPA
b: 98/2 hexane/IPA

***Data of α from Carr, et al., Anal. Chem., 71 (1999) 3013-3021**

Selectivity Comparison Between Zirconia and Silica Cellulosic CSPs

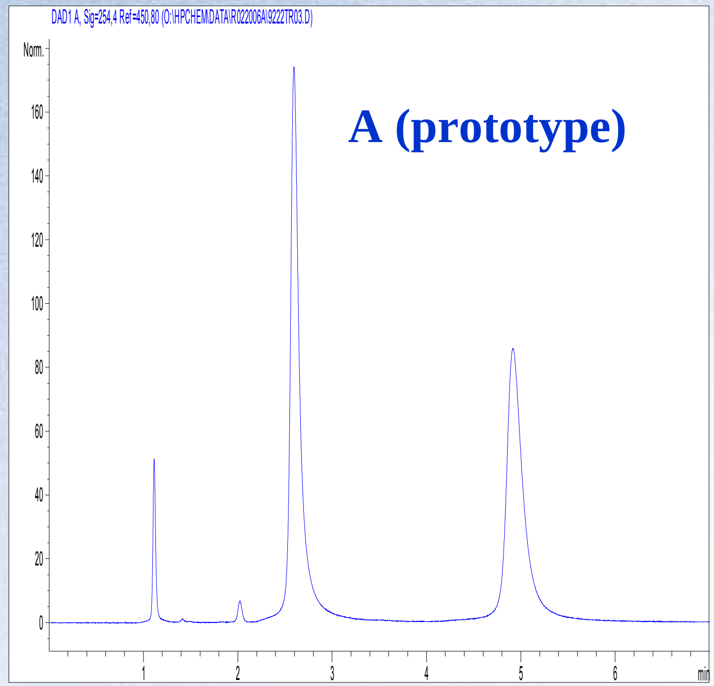
ZirChrom®

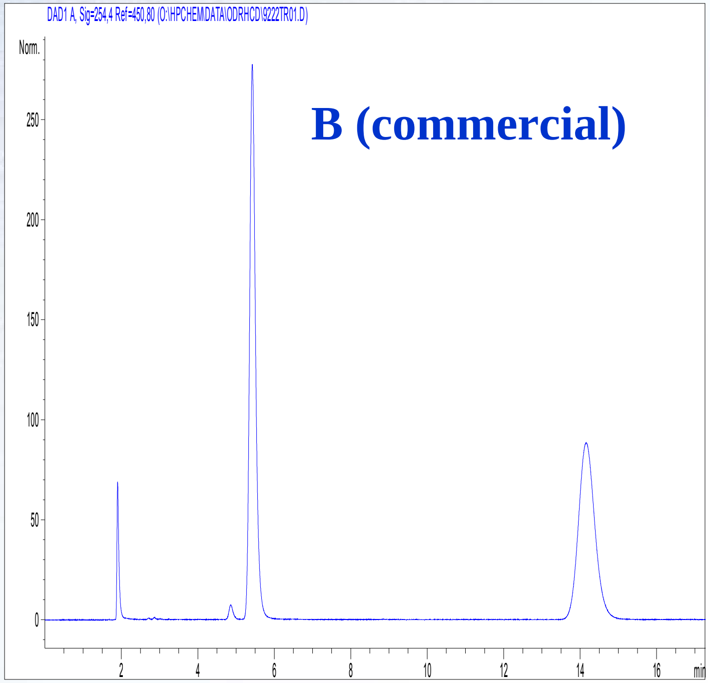


Undecylphenyl carbamate modified cellulosic CSP has good selectivity compared to a commercial silica column.

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

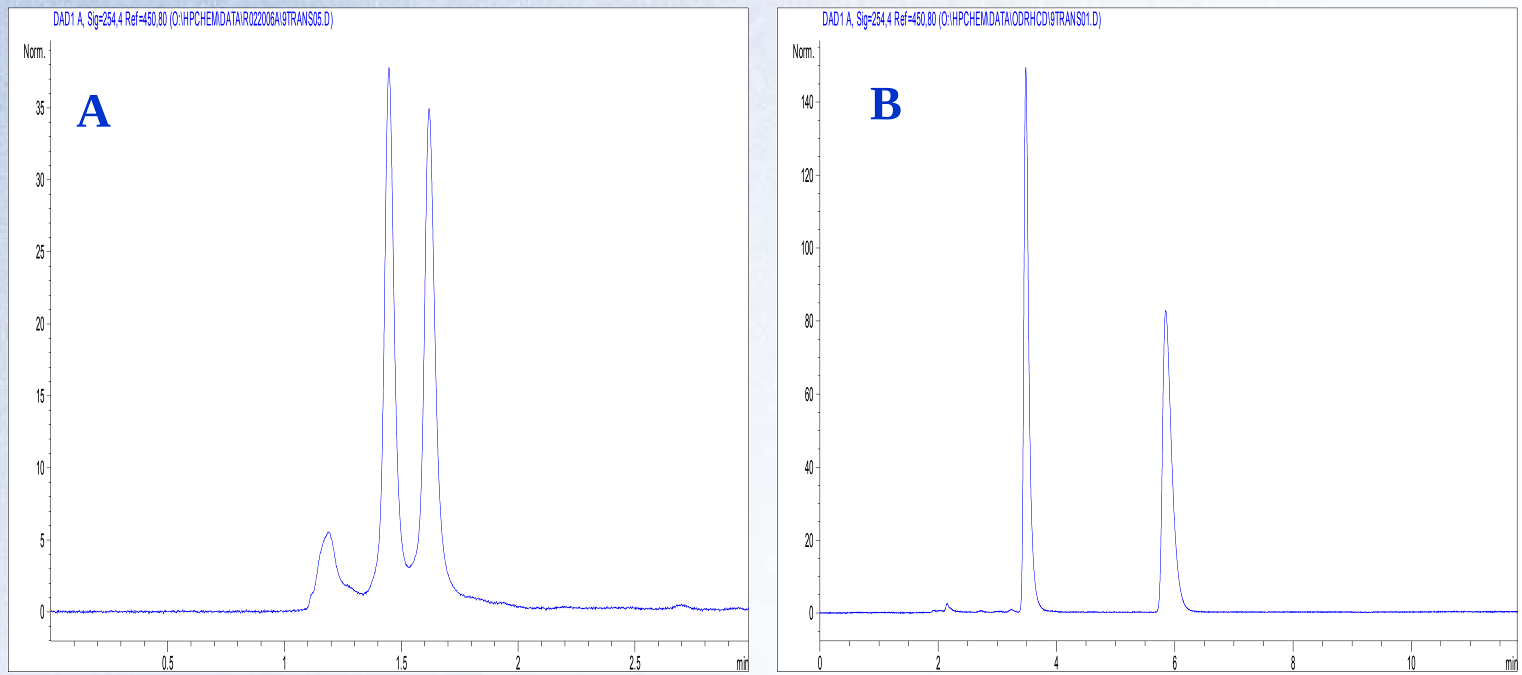
Comparison of Zirconia and Silica Cellulosic Phases





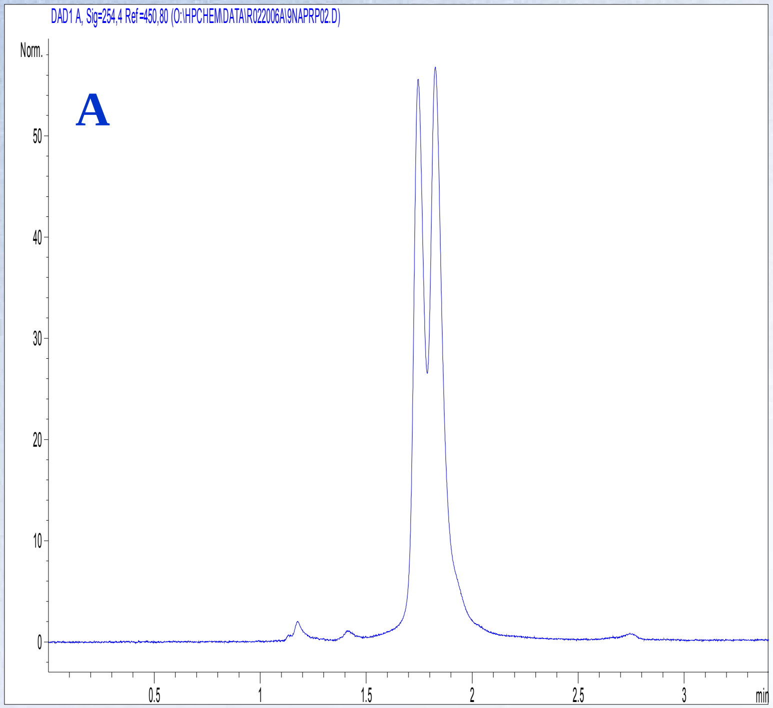
Columns, (A) **CelluloZe™** (Celu022006A), 100 × 4.6 mm, 3 µm Zirconia, (B) Silica-based column, 150 × 4.6 mm, 5 µm Silica, **Solute (RS)-(±)-2,2,2-Trifluoro-1-(9-anthryl) EtOH**, **Mobile phase** 90 / 10 Hexane / IPA, **Flow Rate**, 1 mL/min, **Column temperature**, ambient.

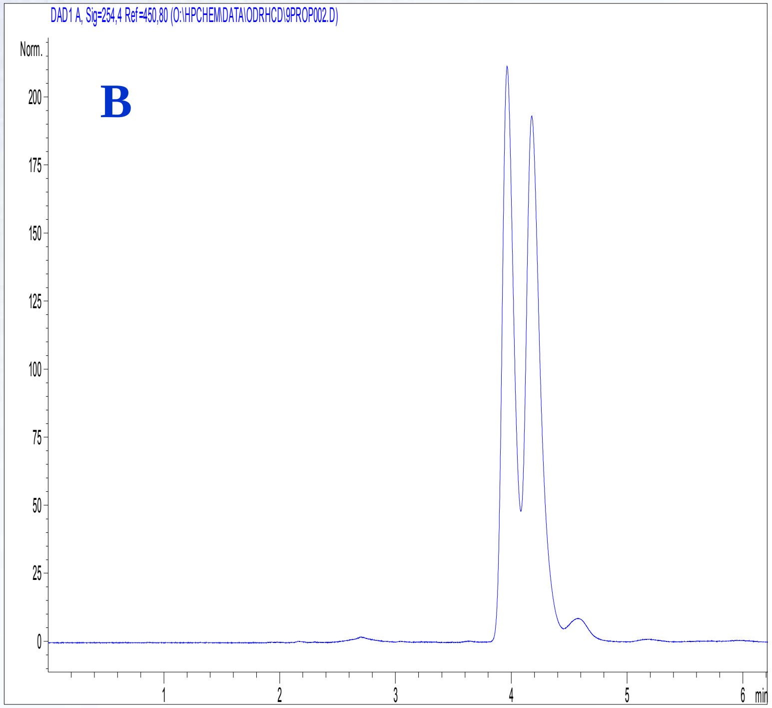
Comparison of Zirconia and Silica Cellulosic Phases



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

Comparison of Zirconia and Silica Cellulosic Phases

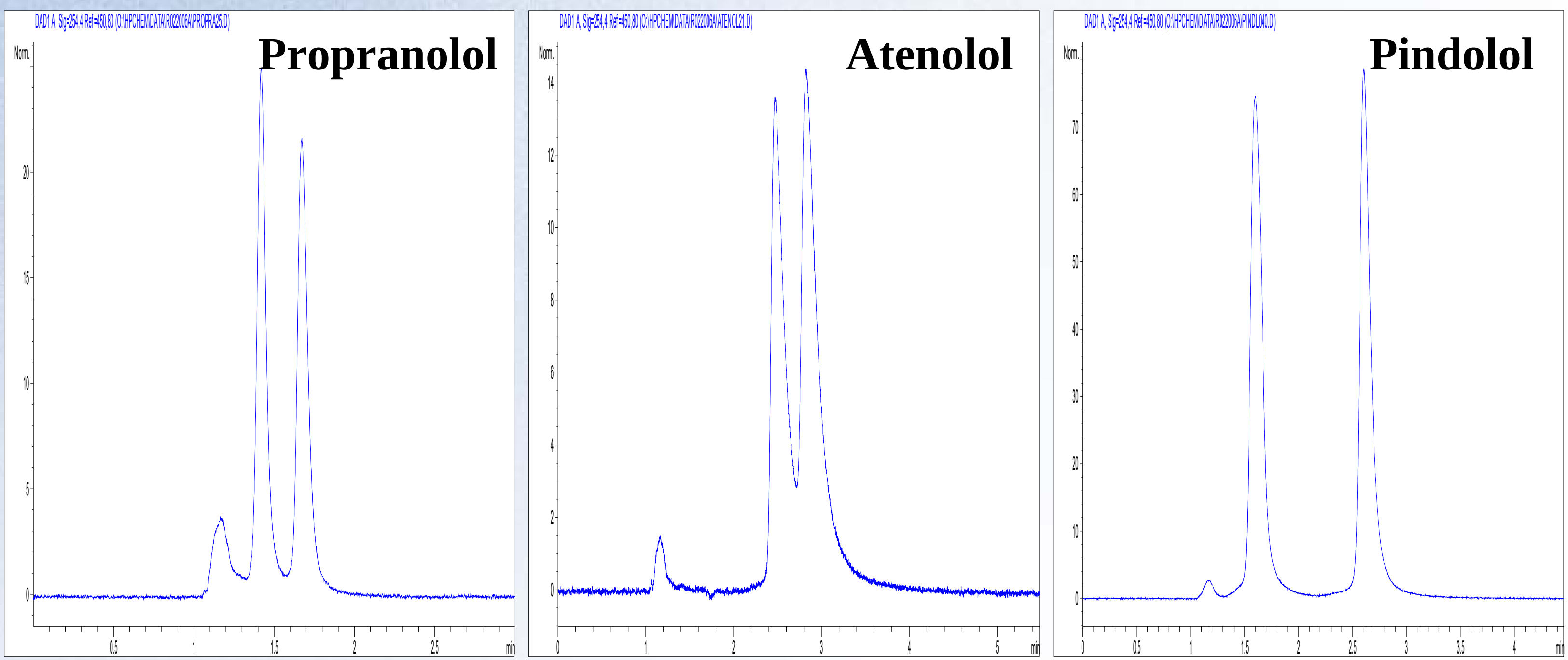




Columns, (A) **CelluloZe™** (Celu022006A), 100 × 4.6 mm, 3 µm Zirconia, (B) Silica-based column, 150 × 4.6 mm, 5 µm Silica, **Solute Napropamide**, **Mobile phase** 90 / 10 Hexane / IPA, **Flow Rate**, 1 mL/min, **Column temperature**, ambient.

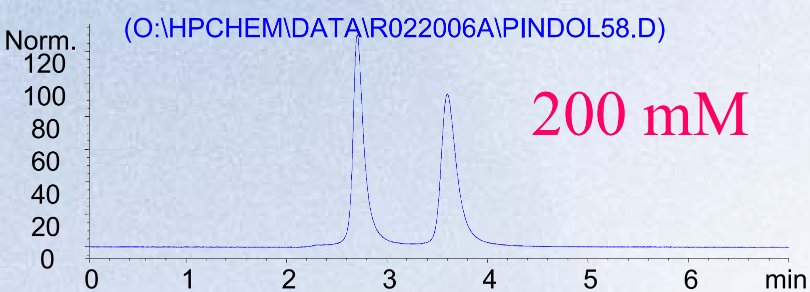
ZirChrom®

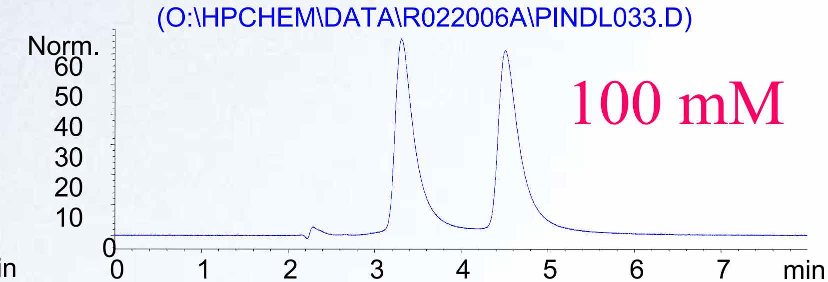
Separation of Basic Drugs on Zirconia Phosphonated Cellulose CSP

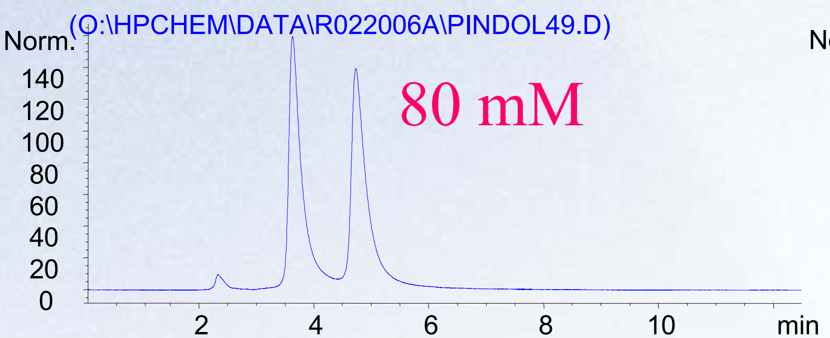


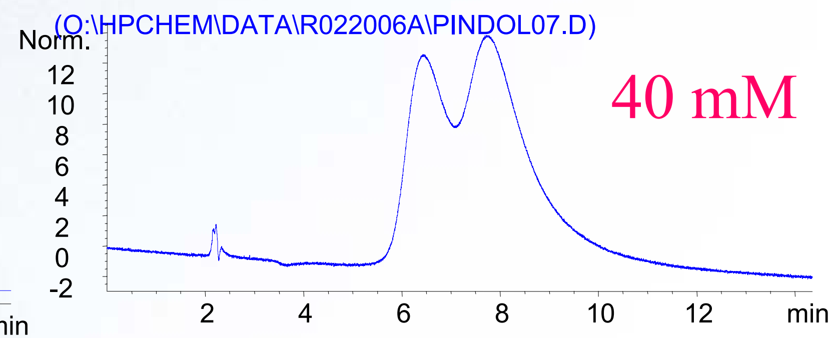
Column, **CelluloZe™** (Celu022006A), 100 × 4.6 mm, 3 µm Zirconia,
Mobile phase, = 50/50 Heptane/IPA (100 mM NH_4OAc in IPA),
Flow Rate, 1 mL/min, **Column temperature**, ambient.

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, $-C_{11}H_{22}PO_3H$

Ion Strength/ Selectivity	Ammonium Actate in IPA (mM)			
	200	100	80	40
Pindolol	2.87	2.10	1.79	1.30
Propranolol	1.55	1.53	1.35	1.10
Atenolol	1.26	1.12	1.09	1.00
Nadolol	1.00	1.00	1.00	1.00

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

1. C. B. Castells and P. W. Carr, Anal. Chem., 1999, *71*, 3013-3021.
2. C. B. Castells and P. W. Carr, Chromatographia, Vol. 52, No. 9/10, November 2000, 535-542.
3. C. B. Castells and P. W. Carr, J. of Chromatogr. A (2000) 904, 17-33.

Acknowledgement: ***National Institutes of Health Grant***
(Phase II SBIR) 2R44HL070334-02A2.

Thanks *very much* for listening!