Development of Novel Bonded Carbon-Based Stationary Phases for Chiral HPLC

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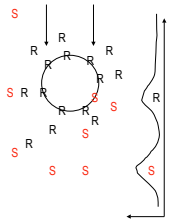
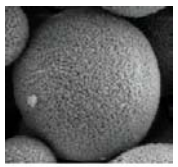
Drivers for the Chromatographic Separation of Enantiomers



- Retention is strongly dependent on thermodynamics of partition of analytes between mobile and stationary phase
- Separation is driven by the specific interactions of analytes with the chiral selector
- To enhance the separation most non-specific interactions with the support need to be minimized
- In reality, the type of support dictates:
  - residual interactions between solutes and stationary phase (e.g. silanol interactions)
  - the chemistry that can be used to bond the selectors
  - the operating range of the CSP
- Carbon has the broadest operating range of existing supports (in reversed phase: pH 1-14, T up to 200 ºC)
- **Objective: Develop CSPs based on Carbon**

The Base ZirChrom-Carb Particle

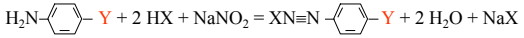


Particles are coated with a thin layer of carbon using a chemical vapor deposition process

Characteristic	Property
Surface Area (m^2/g)	22
Pore Volume (cc/g)	0.13
Pore Diameter (Å)	250-300
Porosity	0.45
Density (g/cc)	5.8 (2.5x silica)
Particle Diameter (µ)	5.0

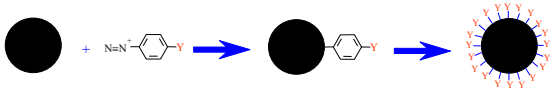
Surface Modification of Carbonaceous Particles

Step 1



$$H_2N\text{–}\bigcirc\text{–}Y + 2\,HX + NaNO_2 = XN{\equiv}N\text{–}\bigcirc\text{–}Y + 2\,H_2O + NaX$$

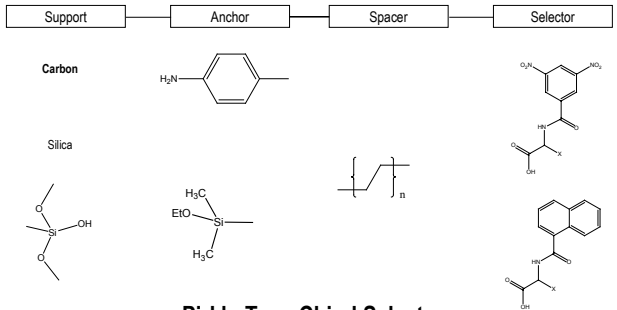
Step 2



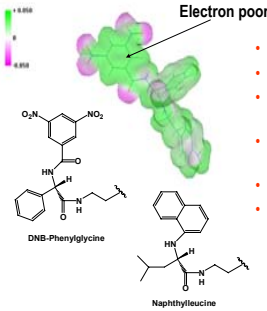
Current Applications:

- DIAMOND BOND-C18 HPLC Columns (ZirChrom Separations Inc)
- Cabot Inkjet Colorants

Building a Chiral Stationary Phase

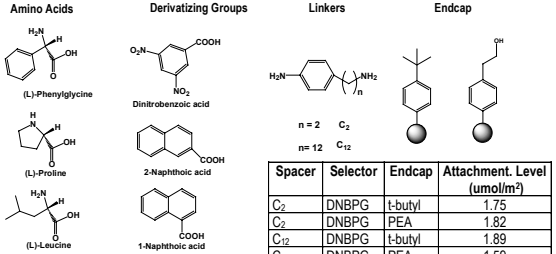


Pirkle-Type Chiral Selectors



- Concepts invented by Prof. William Pirkle in the early 80's
- Products mainly offered by Regis Technologies
- Small organic molecules such as derivatized amino acids are used as chiral selectors
- Separation mechanism is based on p-p interactions and hydrogen-bonding between target molecules and CSPs
- p-electron acceptor: 3,5-Dinitrobenzoyl (DNB)
- p-electron donor: Naphthyl

Chemistries That Are Being Explored



- A range of derivatized aminoacids were successfully attached covalently to ZirChrom-Carb particles
- The surface density of attached chiral selectors compares well with reported values for silica-based CSPs with similar selectors

Spacer	Selector	Endcap	Attachment. Level (umol/m^2)
C_2	DNBPG	t-butyl	1.75
C_2	DNBPG	PEA	1.82
C_{12}	DNBPG	t-butyl	1.89
C_{12}	DNBPG	PEA	1.59
C_2	DNBPro	t-butyl	N/A
C_2	DNBPro	PEA	N/A
C_{12}	DNBPro	t-butyl	1.15
C_{12}	DNBPro	PEA	1.85
C_2	***DNBPG***	***None***	*2.29*
C_2	***DNBPro***	***None***	*2.82*

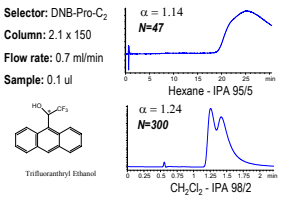
Chiral Chromatography Results

1. Separation Factors (a)

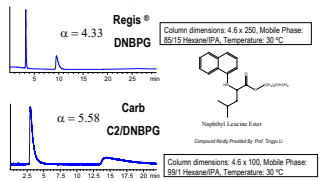
Analyte	C_2-DNBPG	C_2-DNBPro	Regis® DNBPG
Trifluoroanthryl ethanol	1.18	1.30	1.51
1,1'- bis-2-naphthol	1.20	1.23	1.82
1-naphthyl-leucine ester	5.58	1.08	4.33

2. Chromatograms

1,2,3-trifluoroanthryl ethanol



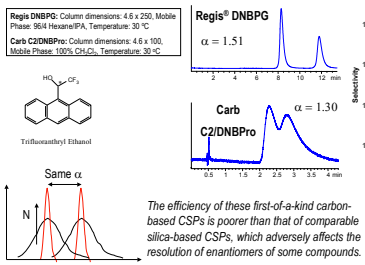
Naphthyl-leucine ester



Mobile phase selection is very important for achieving the best separation. Methylene chloride gave better results than hexane on the carbon CSPs for some analytes.

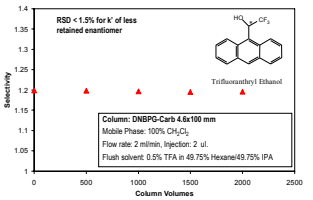
For some analytes the carbon based CSPs gave better separations than the silica-based columns with the same functionality, indicating that the choice of support can affect the separation.

3. Efficiency of the Carbon Chiral Columns



The efficiency of these first-of-a-kind carbon-based CSPs is poorer than that of comparable silica-based CSPs, which adversely affects the resolution of enantiomers of some compounds.

4. Stability of the Carbon Based Columns



Carbon-based columns are ultra-stable, even when exposed to 0.5% TFA, because of the ultra-stable covalent bonding to the surface.

Conclusions

- Novel chiral stationary phases based on bonded carbon clad zirconia chromatographic particles have been developed
- The effect of spacer length and precap type is not as important as the surface density and type of the chiral selector
- For some molecules the carbon based columns have higher selectivity than similar silica-based commercial columns
- The carbon based columns are stable under more aggressive HPLC conditions, promising more flexibility in chiral method development
- **FUTURE WORK:**
  - The eluent composition that works best with carbon based supports needs to be optimized
  - The efficiency of the carbon-based chiral columns must be improved

Acknowledgement: NIH SBIR Grant 1 R43 HL70334-01