The Development and Applications of Polymer and Carbon Coated Zirconia-based Supports for Reversed Phase LC

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Outline

• Advantages of Stable Phases
• Selectivity Comparison of Zirconia based phases to ODS Silica
• Development of a New Type of Reversed Phase Column: DiamondBond®-C18
• Applications
• Summary
Why Stable Phases?

Advantages of Extraordinary Chemical Stability

pH Stability
- pH < 1
  - Clean with conc. Acids
  - Suppress Ions for Acids
  - Suppress Ions for Amines
  - Sanitation Depyrogenation
- pH > 13
  - Sanitation

Thermal Stability
- Lower Pressure Drop
- Less Organic Solvent
- Thermally Optimize Selectivity
- Less Wear and Tear
- Higher Flow Fast Analysis
- More Robust Analysis
- Easier Method Development

Stable Stationary Phases have advantages in terms of Selectivity, Column Lifetime, and Analysis Time
Improving the Stability of HPLC Phases: History

- Pure organic polymers
- Silica “hardened” by coating with alumina or zirconia
- Pure carbon
- Silica improvements
  - Sterically bulk & bidentate ligand
  - Polymer coated silica
  - Hybrid organic-inorganic silicaceous composite phase
- Polymer coated porous alumina and zirconia
- Carbon coated zirconia
- Chemically bonded carbon-coated zirconia
# ZirChrom® Particle Properties

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface Area (m²/g)</td>
<td>22</td>
</tr>
<tr>
<td>Pore Volume (cc/g)</td>
<td>0.13</td>
</tr>
<tr>
<td>Pore Diameter (Å)</td>
<td>250-300</td>
</tr>
<tr>
<td>Porosity</td>
<td>0.45</td>
</tr>
<tr>
<td>Density (g/cc)</td>
<td>5.8 (2.5x silica)</td>
</tr>
<tr>
<td>Particle Diameters (µ)</td>
<td>3.0, 5.0, 10.0</td>
</tr>
</tbody>
</table>

**ZirChrom®-Carb** particles are prepared by coating base particles with a thin layer of carbon using a chemical vapor deposition process.

**ZirChrom®-PBD** particles are prepared by coating base with a layer of highly crosslinked polymer.

**DIAMOND BOND® - C18**

Bonding Reaction on Carbon Clad Zirconia

\[
\text{NH}_2\text{-Y} + 2\text{HA} + \text{NaNO}_2 = \text{AN}_2\text{N}^+\text{-Y} + 2\text{H}_2\text{O} + \text{NaA}
\]

Carbon Clad Zirconia  Diazenium Salt  Modified Carbon Clad Zirconia
Adjusting Selectivity in HPLC

- Mobile Phase Composition (B%)
- Mobile Phase Type (ACN, MeOH, THF)
- Stationary Phase Type (C18-SiO2, C-ZrO2, PBD-ZrO2)
- Temperature

➢ Poor correlations in the \(\kappa-\kappa\) plot indicate changes in selectivity.

\[
\begin{align*}
\alpha_1 &= 1.03 \\
\alpha_2 &= 2.00
\end{align*}
\]

\(\kappa-\kappa\) plot

\[
\begin{align*}
r^2 &= 1.00, \text{s.d.} = 0.00 \\
r^2 &= 0.66, \text{s.d.} = 0.70
\end{align*}
\]
22 Non-Ionizable Solutes

<table>
<thead>
<tr>
<th>Nonpolar</th>
<th>Polar</th>
<th>HB Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>Bromobenzene</td>
<td>Benzyl alcohol</td>
</tr>
<tr>
<td>Toluene</td>
<td>p-Dichlorobenzene</td>
<td>3-Phenyl Propanol</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>Anisole</td>
<td>N-Benzyl Formamide</td>
</tr>
<tr>
<td>p-xylene</td>
<td>Methylbenzoate</td>
<td>Phenol</td>
</tr>
<tr>
<td>Propylbenzene</td>
<td>Napthalene</td>
<td>p-Chlorophenol</td>
</tr>
<tr>
<td>Butylbenzene</td>
<td>Acetophenone</td>
<td></td>
</tr>
</tbody>
</table>

Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Detection at 254nm; 5 ul Injection volume.
Selectivity Comparison

log (k/k' benzene)

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0

Benzene **
Toluene
Ethylbenzene
p-xylene
Propylbenzene
n-Butylbenzene
Naphthalene **
Bromobenzene **
p-Dichlorobenzene
p-Chlorotoluene
Anisole **
Benzonitrile
Nitrobenzene
Methylbenzoate **
Acetophenone
p-Nitrobenzyl chloride
N-benzylformamide
Benzyl alcohol
Phenylpropanol
Phenol
p-Chlorophenol

Diamondbond-C18
ODS-1
Unmodified Carbon coated zirconia
Comparison of Variables Affecting Selectivity

Stationary Phase Type
Carbon-ZrO₂ vs. PBD-ZrO₂

R²=0.986
SD=0.17

MeOH vs. THF

R²=0.896
SD=0.05

Stationary phase type has a large effect on selectivity.
Regression Data from log $k'$ vs. log $k'$ Plots vs ODS

<table>
<thead>
<tr>
<th>Column</th>
<th>$R^2$</th>
<th>Selectivity Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZirChrom®-PBD</td>
<td>0.985</td>
<td>12</td>
</tr>
<tr>
<td>DiamondBond®-C18</td>
<td>0.889</td>
<td>33</td>
</tr>
<tr>
<td>ZirChrom®-Carb</td>
<td>0.549</td>
<td>67</td>
</tr>
</tbody>
</table>

- For **non-ionizable** solutes:
  - ZirChrom-Carb and Diamondbond-C18 columns have very different selectivities from traditional C18-Silica HPLC columns
  - ZirChrom-PBD has selectivity similar to C18-Silica

- For **ionizable** solutes the picture is very different

*S=100(1-R²)⁰.⁵, as described by U. Neue at FACSS meeting
Zirconia Has Unique Surface Chemistry

- Zirconia by itself has very rich surface chemistry
- Coated zirconia phases (Carbon and PBD) have mixed surface properties
- The retention of various basic and acidic analytes can be fine tuned by changing pH, buffer, salt concentration, in addition to mobile phase modifier concentration and type
Zirconia Features Tunable Surface Properties

<table>
<thead>
<tr>
<th>Interaction Strength</th>
<th>Lewis Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongest</td>
<td>Hydroxide, Phosphate, Fluoride, Citrate, Sulfate, Acetate, Formate, Nitrate, Chloride</td>
</tr>
<tr>
<td>Weakest</td>
<td></td>
</tr>
</tbody>
</table>

- The choice of buffer and pH on zirconia columns affects the surface charge and the elution properties of ionizable analytes.
The selectivity of zirconia based columns towards ionizable compounds becomes very different from that of traditional silica columns when Lewis base buffers are used.

The content includes a mobile phase composition and a comparison of absorbance data for C18-SiO2 and ZirChrom-PBD columns. The selectivity is quantified with a correlation coefficient R²=0.147 and a selectivity index S=92.
DiamondBond®: A New Family of Stable Phases

- ZrO$_2$ DB-C18
- ZrO$_2$ DB-C8
- ZrO$_2$ DB-Amide18

HPLC Columns
Why are Bonded Carbons Unique?

- Carbon surfaces have π-electrons which increase retention of certain types of analytes:
  - fused polyaromatics (e.g. naphthalene, etc)
  - polar molecules (e.g. amides, ketones, alcohols, etc)
- Surface modified carbon surfaces combine some of these interactions with interactions specific to the bonded surface groups.
- Bonded Carbons maintain the high pH and thermal stability that is inherent in the C-C bond.
Shape Makes a Difference

\[ \alpha_{\text{ODS}} = 1.03 \]
\[ \alpha_{\text{CARB}} = 1.58 \]
\[ \alpha_{\text{DB-C18}} = 1.22 \]
Shape Selectivity: Anabolic Steroids

**LC Conditions:** Column, 150 x 4.6 DiamondBond-C18; Mobile phase, 60/40 ACN/Water; Flow rate, 2.0 ml/min.; Temperature, 100 °C; Injection volume, 10ul; Detection at 215nm; Solutes: 1=Epietiocholanolone, 2=Etiocholanolone, 3=Androsterone, 4=Epiandrosterone

Anabolic Steroids Separation in less than 3 minutes!
Test Chromatogram on DB-C18

Analytes
1: Acetone
2: Benzonitrile
3: Ethylbenzene
4: Methylbenzoate

DiamondBond-C18

ZirChrom-Carb

>100000 plates/m

LC Conditions: Column dimension, 50 x 4.6 mm id.; Mobile phase, 37.5/5/57.5 ACN/THF/Water; Temperature, 60 °C; Flow rate, 1.0 ml/min.; Injection volume, 5 µl; Detection at 254 nm.
Effect of Polar Embedded Amide

Silica Amide vs. C18

Carbon Amide vs. C18

RPAmide shows increased retention of HB Donors on silica and carbon-based phases.
DiamondBond-C18 Stability

LC Conditions:
**Base Stability**—DiamondBond™ Phase A, 30 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm.

**Acid Stability**—DiamondBond™ Phase A, 50 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm.

**Temperature Stability**—DiamondBond™ Phase B, 50 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm.
High pH Stability - Beta Blockers

Analytes
1 - Atenolol
2 - Metoprolol
3 - Oxprenolol
4 - Alprenolol

LC Conditions:
20/20/60 ACN/THF/200 mM TMAH and 200 mM NaCl, pH 13.3
Flow Rate: 1 ml/min. Temperature: 75 °C. Injection Volume: 5 ul
Detection: 254 nm.
High Temperature Stability - Speed

Resolution (min; 3,4) = 2.2
Temperature = 21 °C
Flow Rate = 1.4 ml/min.
ACN/Buffer = 18.5/81.5
Analysis Time = 43 min.

Resolution (min; 3,4) = 2.2
Temperature = 80 °C
Flow Rate = 3.2 ml/min.
ACN/Buffer = 8.8/91.2
Analysis Time = 21 min.

Analytes:
1 = Barbital
2 = Butabarbital
3 = Pentobarbital
4 = Carbromal
5 = Secobarbital
6 = Methohexital

Mobile Phase: ACN/5mM Ammonium phosphate, pH 7.0
Pressure drop = 195 bar
Application in LC/MS/MS

THC in Saliva by LC/MS/MS†

- Blue – THC (tetrahydrocannabinol parent drug)
- Red – D3 THC (Internal Standard)
- Column – 50mm X 4.6mm DBC18
  - 80°C @ 1.5 mL/min
  - Solvent A – 20mM NH₄CH₃CO₂ in 70% Acetonitrile, 30% aqueous (0.1% acetic acid, pH 4.5)
  - Solvent B – Acetonitrile
- Isocratic 35% A, 65%B – 25 ul injection

† Data Courtesy R. Clouette - Clinical Reference Laboratories
Summary

- All zirconia-based supports have surface chemistry that enables unique separations

- Even bonded zirconia-based phases are extremely stable:
  - Low pH and High pH applications
  - High Temperature / Fast HPLC

- Both “normal” and high pH, high temperature applications are possible on these new materials
  - LC/MS pharmaceutical applications enabled by this technology