Efficient Separation of Important Quaternary Drugs
On Zirconia HPLC columns

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Review of Zirconiia Particles

- Rigid and very stable over wide range of conditions
- Physical properties of analytical particles:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore Diameter (Å)</td>
<td>250-300</td>
</tr>
<tr>
<td>Porosity</td>
<td>0.45</td>
</tr>
<tr>
<td>Particle Diameter (µm)</td>
<td>3, 5, 10</td>
</tr>
<tr>
<td>Density (gm/cc)</td>
<td>5.8 (2.5 x silica)</td>
</tr>
<tr>
<td>Pore Volume (cc/g)</td>
<td>0.13</td>
</tr>
<tr>
<td>Surface Area (m²/g)</td>
<td>22</td>
</tr>
<tr>
<td>Lewis Acid Site (µmol/m²)</td>
<td>4-5</td>
</tr>
</tbody>
</table>

- Various surface modifications available: Discovery® Zr-PS, Discovery® Zr-PBD, Discovery® Zr-Carbon, Discovery® Zr-CarbonC18
Surface properties of zirconia oxide of chromatographic relevance:

- **Charge:**
  \[
  \text{Zr-OH}_2^+ \quad \overset{pK_a = 4.0}{\leftrightarrow} \quad \text{Zr-OH} + \text{H}^+ \quad \overset{pK_a = 8.1}{\leftrightarrow} \quad \text{Zr-O}^- + \text{H}^+
  \]

- **Lewis acid* sites:**
  - Coordinately unsaturated zirconium (IV) sites
  - Very reactive toward Lewis bases

*Base is electron donor; acid is electron acceptor; (more general definition than Brønsted)
Zirconia Lewis Acid-Base Chemistry

<table>
<thead>
<tr>
<th>Interaction Strength</th>
<th>Lewis Base Anion (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongest</td>
<td>Hydroxide (cleaning method)</td>
</tr>
<tr>
<td></td>
<td>Phosphate</td>
</tr>
<tr>
<td></td>
<td>Fluoride</td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
</tr>
<tr>
<td></td>
<td>Sulfate</td>
</tr>
<tr>
<td></td>
<td>Acetic acid</td>
</tr>
<tr>
<td></td>
<td>Formic acid</td>
</tr>
<tr>
<td></td>
<td>Nitrate</td>
</tr>
<tr>
<td></td>
<td>Chloride</td>
</tr>
</tbody>
</table>

Ligand Exchange: \( \text{Zr-L} + A^- \leftrightarrow \text{Zr-A}^- + L \)

Strongest Lewis Acid Site Dominates Surface Chemistry

- When ligand is charged, surface becomes charged!
Cation-Exchange Behavior of Zirconia

• Coordinated Lewis base can make surface an effective cation-exchanger. This is particularly the case with phosphate at near neutral pH; only hydroxide is stronger Lewis base.

  - If surface is to be regenerated, must strip off phosphate with hydroxide solution (high pH) devoid of phosphate.
Mixed-Mode Behavior of Coated Zirconia Phases

- Polymer coating: **hydrophobic retention**
- Coordinated Lewis Base ions: **ion-exchange retention**
  - $\text{Zr-L}^-:X^+ + A^+ = \text{Zr-L}^-:A^+ + X^+$ (very important mechanism)
- Zirconols:
  - $\text{Zr-O}^-:X^+ + A^+ = \text{Zr-O}^-:A^+ + X^+$ (unimportant mechanism)

$A^+$ = analyte cation, $X^+$ = counterion, $L^-$ = adsorbed Lewis base anion
Mixed-Mode Behavior of Coated Zirconia Phases

• Retention of ionic analytes modulated by pH, choice of buffer/salt, buffer/salt concentrations, and temperature

• Selectivity affected by these mobile phase parameters as well
Zirconia Displays Unique Ion Exchange (IEX) Selectivity

Mixed-mode zirconia can provide separation that is difficult to accomplish on high-purity C18-silica²

- Discovery C18 is >30x more hydrophobic than Discovery Zr-PS as measured by retention of butylbenzene
- To what extent, is IEX responsible for retention of the herbicides on the coated zirconia phase?

Separation of paraquat and diquat on Discovery® C18 vs. Discovery® Zr-PS
C18-silica conditions: Discovery C18, 15cm x 4.6mm, 3µm particles; 5% CH₃CN in 25mM H₃PO₄ (to pH 7 with NH₄OH); 35°C, 1mL/min, UV 290nm
Zr-PS conditions: Discovery Zr-PS, 7.5cm x 4.6mm, 3µm particles; 50% CH₃CN in 25mM H₃PO₄, 25mM NH₄F, (to pH 8 with NH₄OH); 65°C, 3mL/min.
Quantitative Retention Parameters in IEX LC³

- Thermodynamic retention model* where $k$ is HPLC retention factor, Const related to packing capacity and ion-exchange equilibrium constant, and $y/x$ is ratio of net charges on analyte and displacement ions.

$$\log k_A = \text{Const} - \frac{y}{x} \log \left[ C_m \right]$$

- This relationship predicts a linear relationship between $\log k$ and $\log$ displacement ion concentration with a slope of -1 when analyte and displacement ion have the same net charge, and analyte behaves as point charge.

* model assumes thermodynamic equilibrium, unit activity coefficients and absence of analyte interaction by forces other than coulombic attraction.
Experimental Approach

- The objective is to quantitate the potential contribution of IEX to the retention mechanism of the mixed-mode phase Discovery Zr-PBD.
- The analytes will be chromatographed at a constant buffer concentration (and constant pH) while varying the concentration of counterion (sodium, in this case); this will be repeated at three different concentrations of acetonitrile.

Conditions:

- Column: Discovery Zr-PBD; 3µ, 2.1 x 100 mm
- Mobile Phase A: 50:x:y, (10 mM sodium phosphate, pH 7) : x : y
- Mobile Phase B: 50:x:y, (10 mM sodium phosphate, pH 7, 42 mM NaCl) : x : y
  - notes: pH-7 buffer prepared from calculated amounts of mono- and dibasic sodium phosphate
  - x + y = 50 (i.e. 50% of final volume)
  - x = water; y = acetonitrile, either 10, 20, or 30 total conc of sodium in mobile phase B, is 50 mM.
- Flow: 0.3 mL/min
- Temperature: 80° C
- Inj: 1 µL
- Detection: 225 nm
Example of Data Analysis

Retention as function of Na\(^+\) conc at 10% acetonitrile

Data Summary

<table>
<thead>
<tr>
<th>Analyte</th>
<th>10% MeCN</th>
<th>20% MeCN</th>
<th>30% MeCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>diquat</td>
<td>-2.114</td>
<td>-2.166</td>
<td>-2.180</td>
</tr>
<tr>
<td>paraquat</td>
<td>-2.064</td>
<td>-2.096</td>
<td>-2.110</td>
</tr>
</tbody>
</table>

• Recall, slope is parameter that indicates degree of IEX as retention mechanism.

• With analyte charge of 2 and counterion charge of 1, slope of -2 indicates that the charges on analyte are fully accessible to the IEX sites on the chromatographic surface.

• IEX is very effective as mechanism of retention on this phase (polymer-coated zirconia).
Hydrophobic interaction on the polymer-coated zirconia is a component of retention for diquat and paraquat, but not as dramatic as that of IEX.

Neither level of organic nor concentration of mobile phase counterion impacts selectivity to a significant degree.

Level of organic and concentration of mobile phase counterion control retention, therefore either type of gradient can be effective.
Anticholinergics as Probes for IEX Retention

- With the exception of scopolamine, these are all quaternary ammonium compounds.
- The quats have a charge of +1
- Scopolamine $pK_a = 7.75$
- pH of mobile phase = 7
Data Analysis: log plots

Example of Data Analysis

Retention as function of Na⁺ conc at 10% acetonitrile

Data Summary

- With counterion and analyte of equivalent valence, a slope of -1 indicates charges on analyte are fully accessible to the charges on the chromatographic surface, and retention mode is IEX.

- Results with the quaternary analytes indicate that IEX can be largely responsible for retention of cationic analytes on the zirconia phase, esp at high organic conc.

- Scopolamine, with smaller formal charge, is less retained by IEX, and less retained overall.

- At low levels of organic, less of retention is accounted for by IEX, but IEX is still most significant.

<table>
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<tr>
<th>Analyte</th>
<th>10% MeCN</th>
<th>20% MeCN</th>
<th>30% MeCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>propantheline</td>
<td>-0.781</td>
<td>-0.950</td>
<td>-0.962</td>
</tr>
<tr>
<td>pipenzolate_3</td>
<td>-0.871</td>
<td>-0.968</td>
<td>-0.991</td>
</tr>
<tr>
<td>pipenzolate_2</td>
<td>-0.865</td>
<td>-0.961</td>
<td>-0.991</td>
</tr>
<tr>
<td>mepenzolate</td>
<td>-0.871</td>
<td>-0.974</td>
<td>-0.991</td>
</tr>
<tr>
<td>ipratropium</td>
<td>-0.845</td>
<td>-0.981</td>
<td>-1.030</td>
</tr>
<tr>
<td>methscopolamine</td>
<td>-0.884</td>
<td>-1.010</td>
<td>-1.060</td>
</tr>
<tr>
<td>scopolamine</td>
<td>-0.500</td>
<td>-0.548</td>
<td>-0.512</td>
</tr>
</tbody>
</table>
IEX Retention Models\textsuperscript{4}

• Pure IEX

\[
k_{IEX} = \frac{\beta_{IEX}}{[C^+]}
\]

\(\beta_{IEX}\) is a constant that accounts for the phase ratio and distribution ratio of the IEX process as well as the total number of available ion-exchange sites per unit area.

Plot of \(k\) vs \(1/[C^+]\), will be linear with an extrapolated intercept of 0 (i.e. at infinitely high \([C^+]\), retention will be zero).

• Mixed RP and IEX

- One-site  (RP & IEX take place simultaneously at single site)

\[
k = k_{RP} \cdot \frac{K}{[C^+]} \quad K \text{ is a constant that accounts for the IEX equilibrium and the total number of available ion-exchange sites per unit area}
\]

Plot of \(k\) vs \(1/[C^+]\), will be linear with an extrapolated intercept of 0 (i.e. at infinitely high \([C^+]\), retention will be zero).

- Two-site  (RP & IEX take place at spatially distinct sites)

\[
k = k_{RP} + \frac{\beta_{IEX}}{[C^+]} \]

Plot of \(k\) vs \(1/[C^+]\), will be linear with an extrapolated finite intercept.
Data Analysis: reciprocal plots

- At low organic, Finite (> 0) intercepts on k axis, indicate two-site retention mechanism.
- At low organic, propantheline exhibits significant retention by reversed-phase; much less so for other compounds.
- At higher organic, virtually all reversed-phase retention is suppressed.
Conclusions

• Zirconia is an attractive LC matrix because of it’s physical and chemical stability; furthermore, it can be readily modified with hydrophobic coatings, and derivatized by virtue of it’s reactive Lewis acid sites.

• Phosphate is an effective Lewis base in conferring cation-exchange properties to the zirconia surface; ion-exchange can be a very significant component of retention on phosphate-derivatized zirconia phases.

• Mixed-mode cation-exchange zirconia phases (polymer-coated, phosphate-derivatized) provide an attractive option for resolution of cationic analytes.

• Ion-exchange retention is typically modulated by level of counterion and choice of counterion; be sure to consider solubility of counterion/salt in mobile phase. Temperature can be significant effector of retention.

• Mixed-mode ion-exchange zirconia phases are amenable to elution by ionic gradients, or organic gradients, or combinations thereof.
References