Zirconia: the Ideal Substrate for Ion-Exchange LC and LC-MS

Richard A. Henry* and David S. Bell

SUPELCO, 595 North Harrison Road, Bellefonte, PA 16823

* Consultant, 983 Greenbriar Drive, State College, PA 16801
Areas Where Ion Separations are Important

<table>
<thead>
<tr>
<th>Biochemicals</th>
<th>Pharmaceuticals</th>
<th>Chemicals*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>Amines</td>
<td>Inorganic ions</td>
</tr>
<tr>
<td>Peptides</td>
<td>Quaternary amines</td>
<td>Quaternary amines</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Carboxylic acids</td>
<td>Sulfates</td>
</tr>
<tr>
<td>Nucleotides</td>
<td>Etc.</td>
<td>Phosphates</td>
</tr>
<tr>
<td>Nucleosides</td>
<td></td>
<td>Surfactants</td>
</tr>
<tr>
<td>Phospholipids</td>
<td></td>
<td>Etc.</td>
</tr>
<tr>
<td>Etc.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Ion-exchange chromatography (IEC) has been shortened to ion chromatography (IC) in the chemical industry.
Ion-Exchange LC (HPLC)

- Most ion-exchange LC has been done with polymer-based support particles.
  - Polymers often exhibit poor efficiency and are subject to dimensional change when exposed to high temperature, high pressure, high flow rate and organic solvents.

- Silica has not been highly successful as a base for ion-exchange.
  - Substrate has weak cation exchange properties which are not easy to reproduce and control; silanols overload easily and reduce column performance.
  - Coated and bonded phases can exhibit limited stability in aqueous solution over range of pH and temperature conditions needed for ion-exchange.

- Zirconia may be an ideal particle for ion-exchange.
  - Zirconia is rigid and very stable over a wide range of operating conditions.
  - Both cation and anion exchange versions are readily prepared.
  - Rugged industrial and biochemical ion separations may be developed or transferred by following standard rules for IEC or IC method development.

- Challenge- can ion-exchange LC-MS become a routine tool?
Review: Ion Exchange Principles
Cation Exchange

- Cation exchange (proteins, basic drugs, quaternary amines, inorganic metal ions, etc.)
  - Weak (WCX) can be any (covalently) attached or adsorbed carboxylate.
  - Strong (SCX) can be any attached or adsorbed sulfonate, phosphonate, phosphate, etc.
Anion Exchange

- Anion exchange (carboxylates, sulfates, phosphates, inorganic ions, etc.)
  - Weak (WAX) can be any attached or adsorbed free amine (DEAE, polymer coating such as polyethyleneimine, etc.).
  - Strong (SAX) can be quaternary amine made from any WAX chemistry.
Weak vs Strong Ion-Exchangers

- Weak ion-exchangers can be “switched off” by changing pH; this is determined by the titration curve (pK value) for the weak functional group.
- Strong ion-exchangers have a much broader, useful range of ionic behavior.
Retention Relationships in IE-HPLC²

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

$$\log k_A' = C - \frac{y}{x} \log [E_m]$$

- 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.

* model assumes thermodynamic equilibrium, unit activity coefficients and absence of analyte interaction by forces other than coulombic attraction.
Review: Zirconia Chemistry¹

- While zirconia use for RPC may be a niche application, its use for IEC could become a primary application.
Evolution of Zirconia HPLC Packings

- Realization that polymer and silica packings have significant limitations for certain applications in HPLC.
- Research into use of physical and chemical stability advantages of zirconia for reversed phase HPLC.
- Extensive comparison of polymer-coated zirconia to silica RP phases show where selectivity is similar and different.
- Observation that a very reproducible, controllable cation exchange component exists for amine drugs and other bases on zirconia RP phases such as PBD zirconia.
- Use that knowledge about how zirconia works to create stable ion-exchangers that are potential improvements over what now exists.
- While zirconia use for RPC may be a niche application, its use for IEC could become a primary application.
Popular HPLC Substrates

Inorganic Oxides
- Silica (silicon oxide)
- Zirconia (zirconium oxide)
- Titania (titanium oxide)
- Alumina (aluminum oxide)

Polymers
- PS/DVB
- PVA
- Polymethacrylates

Carbon
# Properties of Analytical Zirconia

<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>
Zirconia Lewis* Acid-Base Chemistry

- Ligand exchange interaction: \( Zr-L + A^- \leftrightarrow Zr-A^- + L \)
- When ligand is charged, surface becomes charged!

* Base is electron donor; acid is electron acceptor; more general than Bronsted.
### Interaction Strength of Lewis Base Anions with Lewis Acid Sites on Zirconia

<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>Weakest</td>
<td>Chloride</td>
</tr>
</tbody>
</table>
## Eluotropic Strength for Lewis Bases

<table>
<thead>
<tr>
<th>Lewis Base Eluent (effect of competing bases on elution of substituted benzoic acids)*</th>
<th>Ave k (Probes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydroxide</td>
<td>0.0</td>
</tr>
<tr>
<td>phosphate</td>
<td>0.0</td>
</tr>
<tr>
<td>fluoride</td>
<td>0.02</td>
</tr>
<tr>
<td>ethylphosphate, malate, citrate, EDTA, oxalate</td>
<td>0.15-0.28</td>
</tr>
<tr>
<td>aspartate, succinate, maleate, glutarate, adipate, malonate, pimelate</td>
<td>0.42-0.69</td>
</tr>
<tr>
<td>sulfate, glycolate, borate, NTA, tartrate, subacate, thiosulfate</td>
<td>1.0-1.9</td>
</tr>
<tr>
<td>iminodiacetate, sebacate, acetate, TRIS, formate, sulfamate, butyrate</td>
<td>2.1-10.2</td>
</tr>
<tr>
<td>urea, bromide, butanesulfonate, guanidine, nitrate, chloride</td>
<td>12.1-18.2</td>
</tr>
<tr>
<td>thiocyanate, ethylene glycol, thiourea</td>
<td>22.6-27.8</td>
</tr>
</tbody>
</table>

* organic amines are very weak Lewis bases
Review: Dual Mode Behavior of Zr-RP

- Early efforts to harness the advantages of zirconia for HPLC focused on polymer coatings to impart RP behavior; a strong ion-exchange component was noted for charged solutes, especially cations.
- Cation retention can be attributed to adsorbed anions from the buffer.
- Dual-mode properties of polymer-coated zirconia gives it very unique selectivity for cations compared to silica-based RP packings.
Dual-Mode Mechanism on Silica-C18

- Bonded C\textsubscript{18} Chains—Reversed-Phase (RP) Moieties
- Ionized Silanol Groups — Ion-Exchange (IEX) Sites

\[ \text{SiO}^{-}:X^{+} + A^{+} = \text{SiO}^{-}:A^{+} + X^{+} \quad (\text{only ionic mechanism}) \]

A\textsuperscript{+}: analyte cation, X\textsuperscript{+}: counterion (displacement ion)

Ion-exchange is a nuisance rather than a tool with silica phases.
**Dual-Mode Mechanism on Zr-PBD**

- **PBD Coating — Reversed-Phase (RP)**
- **Lewis Base Anions — Ion-Exchange (IEX)**
  - \( \text{Zr-L}^{-}:X^{+} + A^{+} = \text{Zr-L}^{-}:A^{+} + X^{+} \) (very important mechanism)
- **Zirconols**
  - \( \text{Zr-O}^{-}:X^{+} + A^{+} = \text{Zr-O}^{-}:A^{+} + X^{+} \) (unimportant mechanism)

\( A^{+} = \text{analyte cation}, \ X^{+} = \text{counterion}, \ L^{-} = \text{adsorbed Lewis base anion.} \)
Cation-Exchange Character of Zr-PBD

Retention of p-propylbenzylamine demonstrates the presence of more ion-exchange sites on Discovery® Zr-PBD compared to C18-silicas.

Conditions: 55% CH₃OH in ammonium phosphate (pH, 6.0); 35°C; 1.0 mL/min.; UV 254 nm
Buffer Concentration Effect on IEC

![Graph showing the effect of phosphate concentration on retention factor for PBD-ZrO₂ and C18-Silica.](graph.png)
Drylab® Window Diagram for Antihistamines

**ODS**

**ZirChrom®-PBD**

<table>
<thead>
<tr>
<th>Phosphate Concentration (mM)</th>
<th>Minimum Resolution, Rs</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.6</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
</tr>
<tr>
<td>30</td>
<td>0.4</td>
</tr>
<tr>
<td>40</td>
<td>0.3</td>
</tr>
<tr>
<td>50</td>
<td>0.2</td>
</tr>
<tr>
<td>60</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Best Rs on ODS-Silica is < 0.5**

<table>
<thead>
<tr>
<th>Phosphate Concentration (mM)</th>
<th>Best Rs on PBD-ZrO₂ is &gt; 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>4.0</td>
</tr>
<tr>
<td>20</td>
<td>3.0</td>
</tr>
<tr>
<td>30</td>
<td>2.0</td>
</tr>
<tr>
<td>40</td>
<td>1.0</td>
</tr>
<tr>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>60</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Best Rs on PBD-ZrO₂ is > 3.0**
Selectivity Difference\(^3\) Caused by IEC Mode

**LC Conditions:** Mobile phase, 72/28 MeOH/25 mM ammonium phosphate, pH 6; Temperature, 35 °C; Flow rate, 1.0 ml/min; Detection at 254 nm; Solutes: 1=chlordiazepoxide, 2=desipramine, 3=nortriptyline, 4=doxepin, 5=imipramine, 6=thiothixene, 7=amitriptyline, 8=hydroxyzine, 9=buclizine, 10=thioridazine, 11=perphenazine.
Zirconia Shows Unique IEC Selectivity

**Dual-mode can provide separation that single-mode C18-silica can only achieve with ion-pair additives.**

Silica-C18:
- mainly reversed-phase

Zirconia-PS:
- mainly ion-exchange

Separation of paraquat and diquat on C18-silica 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.

- no retention of polar amines
- retention of polar amines due to ion-exchange
Zirconia for Ion-Exchange LC
Zirconia: Surface Properties

- Retention dominated by Lewis acid sites when no polymer coating is present.
  - Attracts strong electrophiles
  - 4-5 \( \mu \text{mol/m}^2 \) active sites
Possible Approaches to Zirconia-IEC

- **No modification or mobile phase modification (in-situ)**
  - Unmodified zirconia is a strong Lewis acid and has “irreversible” adsorptive behavior toward Lewis bases; when Lewis base anions (such as phosphate, fluoride, etc.) are present in the mobile phase, the packing takes on negative charge and becomes a very useful cation exchanger.
  - Organic acid anions (acetate, formate, etc.) are weak Lewis bases that can easily be displaced by stronger anions such as fluoride, phosphate or hydroxide.

- **Chelates (organic)**
  - Strongly bound to electropositive Zirconium; can only be removed by strong base.
  - Effectively deactivate adsorptive Lewis acid sites on zirconia.
  - Impart IEC properties depending on chemical structure of chelate.
  - Hydrophobic chelates (and phosphate surfactants) impart dual-mode behavior.

- **Polymer coatings (organic)**
  - Well-researched area allows positive and negative charges to be incorporated as a stable, cross-linked polymer coating (PEI, etc.).
Four Zirconia-based Options for IEC

- **Bare zirconia**
  - Phosphate, fluoride and other anionic additives that are replaceable.
  - Primarily a strong cation exchanger (SCX)

- **Zirconia with EDTPA chelator modification**
  - Multidentate attachment
  - Very stable, but can be replaced or restored
  - Strong cation exchanger (SCX)

+ **Zirconia with PEI coating**
  - Cross-linked to resist removal even under extreme conditions
  - Weak anion exchanger (WAX)

- **Zirconia with quaternized PEI coating**
  - Cross-linked to resist removal even under extreme conditions
  - Strong anion exchanger (SAX)
Development of Surface Modified Zirconia for Cation Exchange Chromatography
Chelating Ligand Modification

- EDTPA treatment (reflux particles in EDTPA solution)
  - Strong Lewis base chelate attaches to the surface
  - Probably multidentate attachment - very strongly held
  - Blocks undesirable Lewis acid/base interactions
  - Imparts cation exchange (SCX) properties to zirconia
  - Minimal RP behavior

Ethylenediamine-N,N’-tetra(methyleneephosphonic) acid = EDTPA
Nitrogen Porosimetry of Zr-EDTPA

- No pore blockage
- No loss of surface area
Comparison of EDTPA and Phosphate

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Zr-PO₄ µmol P/m²</th>
<th>Zr-EDTPA µmol P/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.57</td>
<td>2.18</td>
</tr>
<tr>
<td>4</td>
<td>5.82</td>
<td>2.24</td>
</tr>
<tr>
<td>8</td>
<td>9.41</td>
<td>2.01</td>
</tr>
<tr>
<td>16</td>
<td>----</td>
<td>2.10</td>
</tr>
</tbody>
</table>
Proteins on Zr-PO₄ and Zr-EDTPA

Mobile phase: 50 to 500 mM K₂HPO₄ in 20 min., pH 7.0. Flow Rate 1 mL/min. UV detection at 280 nm.
Proteins on Zr-EDTPA

Gradient elution of cytochrome c from horse heart. Mobile phase: 4 mM EDTPA, 20 mM MES [2-(N-morpholino)ethanesulfonic acid], pH 5.5 with a linear gradient of 0 to 1 M NaCl in 30 min. UV detection at 410 nm.
Pyridines on Zr-EDTPA

LC Conditions: Column: ZirChrom®-PEZ, 150 x 4.6 mm, Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 254 nm, Injection volume: 5 ul, Mobile Phase: 20 mM MES [2-(N-morpholino)ethanesulfonic acid] buffer, 5 mM NaCl, 2 mM EDTPA, pH = 5.5 (volatile buffers such as ammonium acetate should work also).

Analytes:
1. Hydroxypyridine
2. Ethylpyridine
3. Dimethylaminopyridine
4. Diaminopyridine
Small-Scale Purification of MAB IgG$_{2a}$ Contaminated with BSA

**LC Conditions:** ZirChrom PEZ, 5 x 4.6mm, 100 $\mu$l injection of BSA (6.0 mg/ml) contaminated MAB (1.0 mg/ml) eluted by salt step gradient. Mobile phase: 20 mM MES, 4 mM EDTPA, 0.05 M-to-1.0 M NaCl pH=5.5. Flow rate: 2.0 ml/min. Temperature: 30°C. Detection: 280 nm.

**Note:** BSA is not retained under sample loading conditions.
Development of Surface Modified Zirconia for Anion Exchange Chromatography¹
Zirconia-PEI (Zr-PEI) for Anion Exchange

- Coat and crosslink polyethyleneimine (PEI) for weak anion exchange (WAX).

- Quaternize with methyl iodide for strong anion exchange (SAX).

Lewis acid sites can compete for anions unless blocked by a stronger Lewis base.
SEC Study of PEI-Coated Zirconia

No change in pore volume for protein standards after PEI coating.
Small Anions on Zr-PEI

Addition of organic solvent may improve peak shape of organic anions by eliminating any RP effect.

LC Conditions:
Column, ZirChrom®-WAX, 150 x 4.6 mm i.d.; Mobile Phase, 45mM ammonium phosphate dibasic at pH 8.2; Flow Rate, 1.0 ml/min; 240 nm Detection, Column Temperature = 40°C. Solutes: 1 = bromate, 2 = nitrite, 3 = benzoic acid, 4 = nitrate, 5 = p-chlorobenzoic acid, 6 = p-bromobenzoic acid, 7 = iodide, 8 = p-fluorobenzoic acid, 9 = p-iodobenzoic acid.
Inorganic Anions on Zr-PEI

Must have stronger Lewis base in the mobile phase or anion targets may adsorb.

Analytes
1 - Bromate
2 - Nitrite
3 - Nitrate
4 - Iodide

LC Conditions: Column: ZirChrom®-WAX, 150 x 4.6 mm, Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 240 nm, Injection volume: 5 ul, Mobile Phase: 100% 50mM sodium phosphate, 75mM NaCl at pH 7.0
Water-Soluble Vitamins on Zr-PEI-Q

1 - Thiamine (Vit. B₁)
2 - Pyridoxine (Vit. B₆)
3 - Nicotinamide (form of Vit. B₃)
4 - Riboflavin (Vit. B₂)
5 - Nicotinic acid (form of Vit. B₃),
6 - Ascorbic acid (Vit. C)

LC Conditions: Column: ZirChrom®-SAX, 150 x 4.6 mm i.d., Mobile Phase: 50 mM Ammonium dihydrogenphosphate, pH 4.5, Flow rate: 1.0 ml/min. Temperature: 30 °C, Injection Vol.: 5.0 ml, Detection: UV at 254 nm
Trace Iodide on Zr-PEI-Q

Analytes
2M nitrate sample matrix plus iodide

LC Conditions:
Column: ZirChrom®-SAX, 50 x 4.6 mm,
Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 226 nm, Injection volume: 5 ul, Mobile Phase: 25mM ammonium phosphate, 275mM NaCl at pH 8.0
Fertilizer Plant Effluent on Zr-PEI-Q

Analytes
1 - Cyanate (1.0 mg/ml)
2 - Nitrate (.02 mg/ml)

LC Conditions: Column: ZirChrom®-SAX, 150 x 4.6 mm,
Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 205 nm, Injection volume: 5 ul, Mobile Phase: 25mM sodium fluoride, 175mM sodium chloride at pH 10.0
Inorganic Sulfate on Zr-PEI-Q

Column: 4.6 mm x 150 mm ZirChrom-SAX, Mobile Phase: 2mM EDTPA, 20mM 2-(N-morpholino)ethane sulfonic acid (MES), 5mM Sodium Chloride, Injection Vol.: 10 ul, Detection: UV at 220 nm, Flow Rate: 2.5 ml/min, Temperature: 50 ºC.

Drinking Water

Indirect UV detection

200 ppm Na₂SO₄
Oligonucleotides on Zr-PEI-Q

Poly (G) hydrolysate

LC Conditions:
Column, ZirChrom™-SAX, 50 x 4.6 mm i.d.; Mobile Phase, A = 0.02 M potassium phosphate dibasic and 0.04 M NaCl @ pH 8.5, B = 0.20 M potassium phosphate dibasic and 1.0 M NaCl @ pH 8.5; Gradient, 5 to 95 % B over 90 minutes; Flow Rate, 1.0 ml/min; 254 nm Detection, Temperature = 100°C; Solute, Poly (G) hydrolysate; Injection size, 25 microliters.
Can Ion-Exchange LC-MS (IE-MS) Become Routine?

- IE-MS can be a powerful tool that complements other modes of LC-MS.
- IEC selectivity should be orthogonal to RPC and therefore very valuable for multidimensional separations of complex mixtures.
Interfacing Ion-Exchange to LC-MS

• **Special challenges: nonvolatile mobile phase additives**
  - Traditional ion-exchange mobile phases employ nonvolatile, inorganic acids, bases and salts to control pH and adjust ionic strength.
  - High buffer and salt concentrations can suppress MS response to analytes (ionic strength gradients are often required for elution).

• **Two general solutions to the LC-MS interface problem exist**
  - Replace the typical nonvolatile inorganic mobile phase additives with volatile ones (ammonium acetate, ammonium formate, ammonium carbonate, ammonium hydroxide, etc.).
  - Remove or replace nonvolatiles post-column before MS detection.
    • this has been done for organic ions by employing an RP column in a stream—switching multidimensional scheme.
  - Both approaches can be successful.
NSAIDS on Zr-PBD-EDTPA

- RP retention and good peak shape without phosphate.
- EDTPA blocks Lewis acid sites that might coordinate with carboxylate.

**LC Conditions:**
- Column, 150 mm x 4.6 mm i.d. ZirChrom®-EZ
- Mobile phase, A = 20mM ammonium acetate, pH 5.0, B = ACN
- Flow rate, 1.0 ml/min.
- Temperature, 35 °C
- Injection volume, 10 ml
- Detection at 254 nm
- Solutes:
  - 1=Acetaminophen
  - 2=Naproxen
  - 3=Ketoprofen
  - 4=Fenoprofen
  - 5=Indomethacin

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>
NSAIDS on Zr-PEI-Q

Nonsteroidal Anti-inflammatory drugs:
(1) Acetaminophen, (2) Ibuprofen, (3) Naproxen, (4) Impurity, (5) Ketoprofen

Primarily ion-exchange in 80% acetonitrile and ammonium formate. Suppress the RPC and use the IEC. Increase IEC by raising pH.

Column: ZirChrom®-SAX (PEI-based), 50 x 4.6 mm i.d., Mobile Phase: 80/20 ACN/15 mM ammonium formate, pH = 4.0 (adjusted with formic acid), Flow rate: 1.0 ml/min., Temperature: 35°C, Injection Vol.: 1.0 ml, Detection: UV at 254 nm
LC-MS of Bases on Zr-EDTPA-PBD

**LC Conditions:** Column, ZirChrom®-MS, 5 x 2.1 mm i.d. (3 micron particles). Waters Alliance 2795 LC, Flow rate, 0.2mL/min, Mobile phases channel C=10mM ammonium acetate at pH 5, channel D=10mM ammonium acetate at pH 5:acetonitrile (10:90, v/v), Linear gradient 5% D to 100% D in 6 minutes, hold 100% 6-7.4 min, 100 to 5% D 7.4-8.1min, hold 5% D 8.1-13.0 min. Temperature, 35°C. Waters/Micromass ZQ single quadrupole interfaced with the LC using an electrospray ionization (ESI) interface. Positive ion mode (XIC) from full scan acquisitions from m/z 120-700. Solute concentrations = 10mg/mL, 2mL injections.

- Dual RP/SCX mechanism
- Fast gradients to 90% acetonitrile
- Ionic strength gradients may also be useful
- PEZ could work well in this volatile mobile phase
Beta-Blockers on Zr-EDTPA-PBD\(^4\)

- Fast gradients to 90% acetonitrile
- Same conditions as previous slide

**Chemical Structures:**
- Pindolol
- Atenolol
- Acebutolol
- Alprenolol
Zirconia IEC Conclusions

• Zirconia is a very attractive LC substrate because of its unparalleled mechanical and chemical stability (especially in aqueous solution).

• It has great potential to become a primary substrate for both preparative and analytical ion-exchange LC with UV, conductivity, electrochemical and other common detectors that can tolerate phosphate, fluoride, chloride, sulfate and other nonvolatile additives.
  - Retention rules for ions are easily understood.
  - Ionic strength gradients are tolerated by the packing (chlorides, sulfates, nitrates, etc.).
  - Zirconia and the IEC mechanism are both tolerant of organic solvent to counter excessive hydrophobic solute retention or to elute hydrophobic interferences at the solvent front.

• Zirconia is potentially very useful for ion-exchange LC-MS of both positive ions (SCX mode) and negative ions (WAX and SAX modes) using volatile mobile phases or online clean-up.
  - Ammonium acetate, ammonium formate, ammonium carbonate and ammonium hydroxide additives should be useful; effect of additives and ionic strength gradients on MS signal requires further investigation.
  - IEC mode tolerates high organic to reduce RP effects and maximize MS-ESI signal.
References

4. Data supplied by Supelco, Division of Sigma-Aldrich, Supelco Park, Bellefonte, PA (www.sial.com).
Acknowledgements

• The assistance of Clayton McNeff, Steven Rupp and Bingwen Yan of ZirChrom Separations, Inc. is greatly appreciated.
• Data presented is part of an ongoing collaboration between Supelco and ZirChrom to fully develop the potential of zirconia-based packings for analytical and preparative HPLC.
• Copies of the presentation may be requested at the Supelco Booth or from rhenry@psualum.com.