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

EAS 2005

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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: Zirconia Chemistry

- Ligand exchange interaction: $\text{Zr-L} + \text{A}^- \ll \text{Zr-A}^- + \text{L}$
- When ligand is charged, surface becomes charged!

* Base is electron donor; acid is electron acceptor; more general than Brönsted.
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)
Chelating Ligand Modification

Ethylenediamine-N,N’-tetra(methyleneephosphonic) acid = EDTPA

- 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
Proteins on Zr-PO₄ and ZirChrom-PEZ (EDTPA)

1. Myoglobin
2. Ribonuclease
3. Chymotrypsin
4. Lysozyme
5. Cytochrome c

Mobile phase, 50 to 500 mM K₂HPO₄ in 20 min., pH 7.0; Flow Rate, 1 mL/min; Detection, 280 nm.
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; Detection, 410 nm.
Small-Scale Purification of MAB IgG$_{2a}$ Contaminated with BSA

**LC Conditions:** ZirChrom-PEZ, 5 x 4.6mm (part#ZR08-0546); 100 μl 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.
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.
Small Anions on ZirChrom-WAX

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. (part# ZR05-1546); Mobile Phase, 45mM ammonium phosphate dibasic at pH 8.2; Flow Rate, 1.0 ml/min; Detection, 240 nm; 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.
Trace Iodide Separation on ZirChrom®-SAX

Analytes:
2M nitrate sample matrix and Iodide

LC Conditions:
Column, ZirChrom®-SAX, 50 x 4.6 mm, (part# ZR06-0546); Mobile Phase: 25mM ammonium phosphate, 275mM NaCl at pH 8.0; Flow Rate, 1.0 mL/min; Temperature, 30°C; Detection, 226 nm; Injection volume: 5 ul.
“Green” Analysis of Diet Soft Drinks Containing Caffeine and Aspartame

LC Conditions:
Column, ZirChrom®-SAX, 100 x 3.0 mm (part# ZR06-1030); Mobile Phase, 10mM Ammonium phosphate, 5mM Ammonium carbonate, pH 6.6; Flow rate, 1.0 ml/min; Temperature, 50 °C; Injection Vol., 5.0 ml; Pressure Drop, 205 bar; Detection, 210 nm.
LC/MS Compatible Separation of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) on ZirChrom®-SAX

LC Conditions:
Column, ZirChrom®-SAX, 50 x 4.6 mm i.d. (part number: ZR06-0546); 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, 254 nm.

1-Acetaminophen
2-Ibuprofen
3-Naproxen
4-Impurity
5-Ketoprofen
Water-Soluble Vitamin Analysis on ZirChrom®-SAX

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)

Vitamin C is largely retained on ZirChrom-SAX

LC Conditions:
Column: ZirChrom®-SAX, 150 x 4.6 mm i.d. (part number: ZR06-1546); Mobile Phase, 50 mM Ammonium dihydrogenphosphate, pH 4.5; Flow rate, 1.0 ml/min; Temperature, 30 °C; Injection Vol., 5.0 ml; Detection, 254 nm.
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.
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For more information and web access to the free Buffer Wizard: www.zirchrom.com