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

EAS 2005

Bingwen Yan[1], Clayton V. McNeff[1], Richard A. Henry[2], and David S. Bell[2]

[1]ZirChrom Separations, Inc., 617 Pierce Street, Anoka, MN 55303

[2]Supelco, 595 North Harrison Road, Bellefonte, PA 16823

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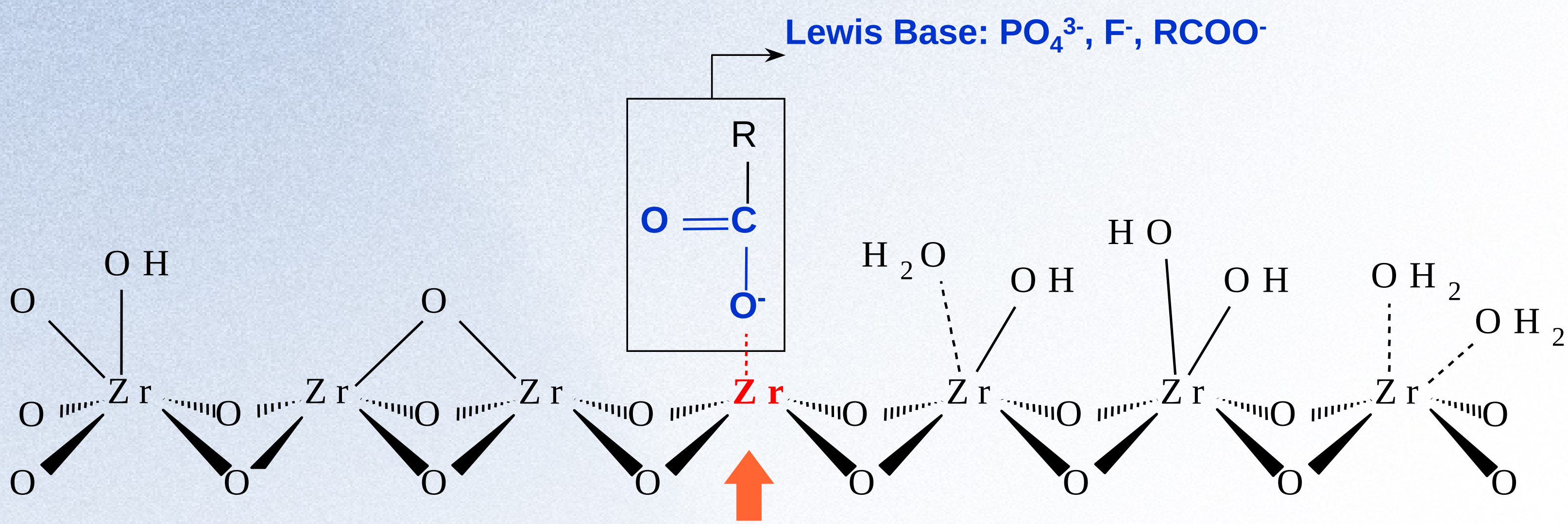
Ion-Exchange LC (HPLC)

- **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: Zirconia Chemistry

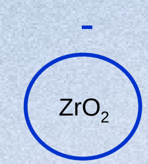


Strong Lewis Acid Site Dominates Chemistry

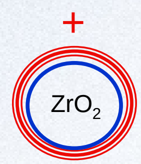
- **Ligand exchange interaction : Zr-L + A$^-$ « Zr-A$^-$ + L**
- **When ligand is charged, surface becomes charged!**

* Base is electron donor; acid is electron acceptor; more general than Brönsted.

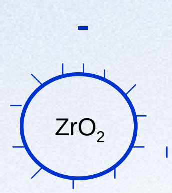
Four Zirconia-based Options for IEC



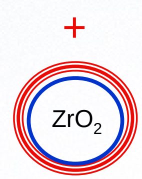
- **Bare zirconia**
- Phosphate, fluoride and other anionic additives that are replaceable.
- primarily a strong cation exchanger (SCX)



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

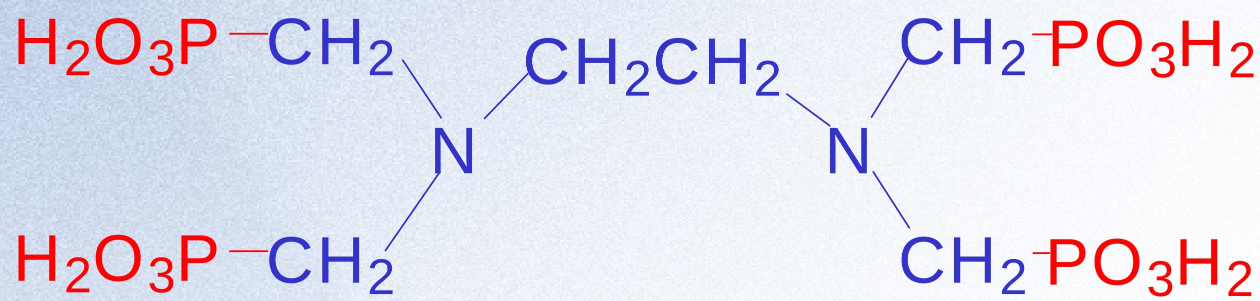


- **Zirconia with EDTPA chelator modification**
- Multidentate attachment
- Very stable, but can be replaced or restored
- Strong cation exchanger (SCX)



- **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(methylenephosphonic) 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

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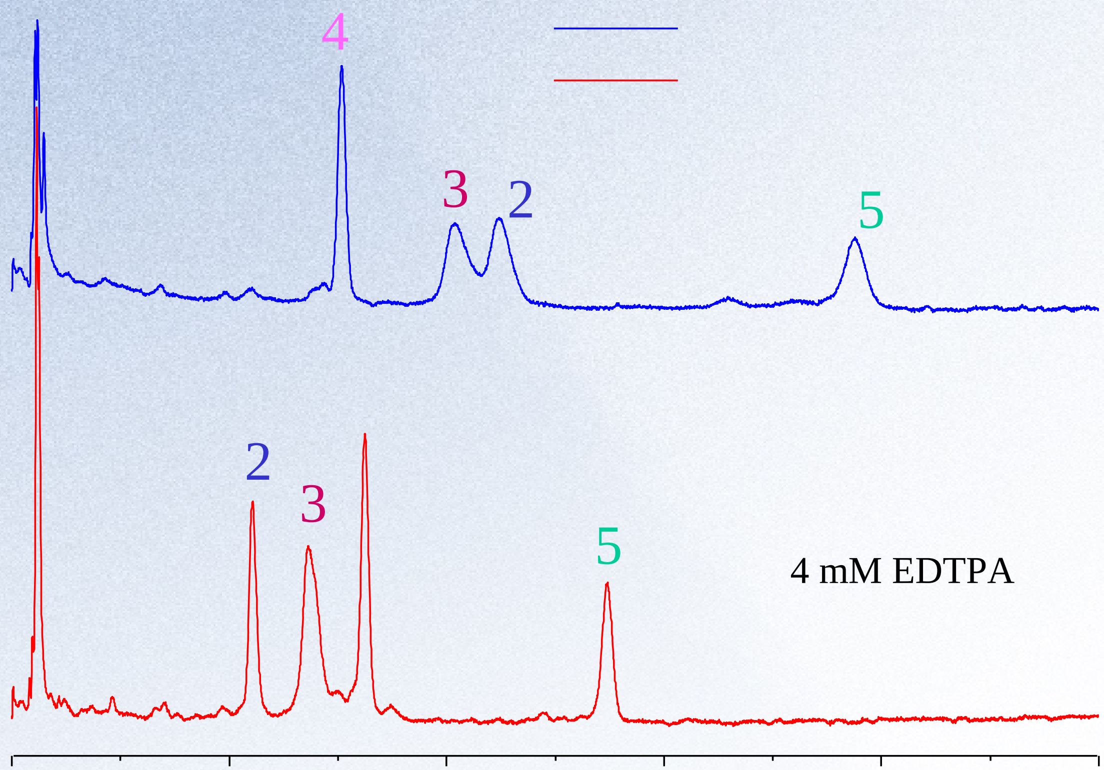
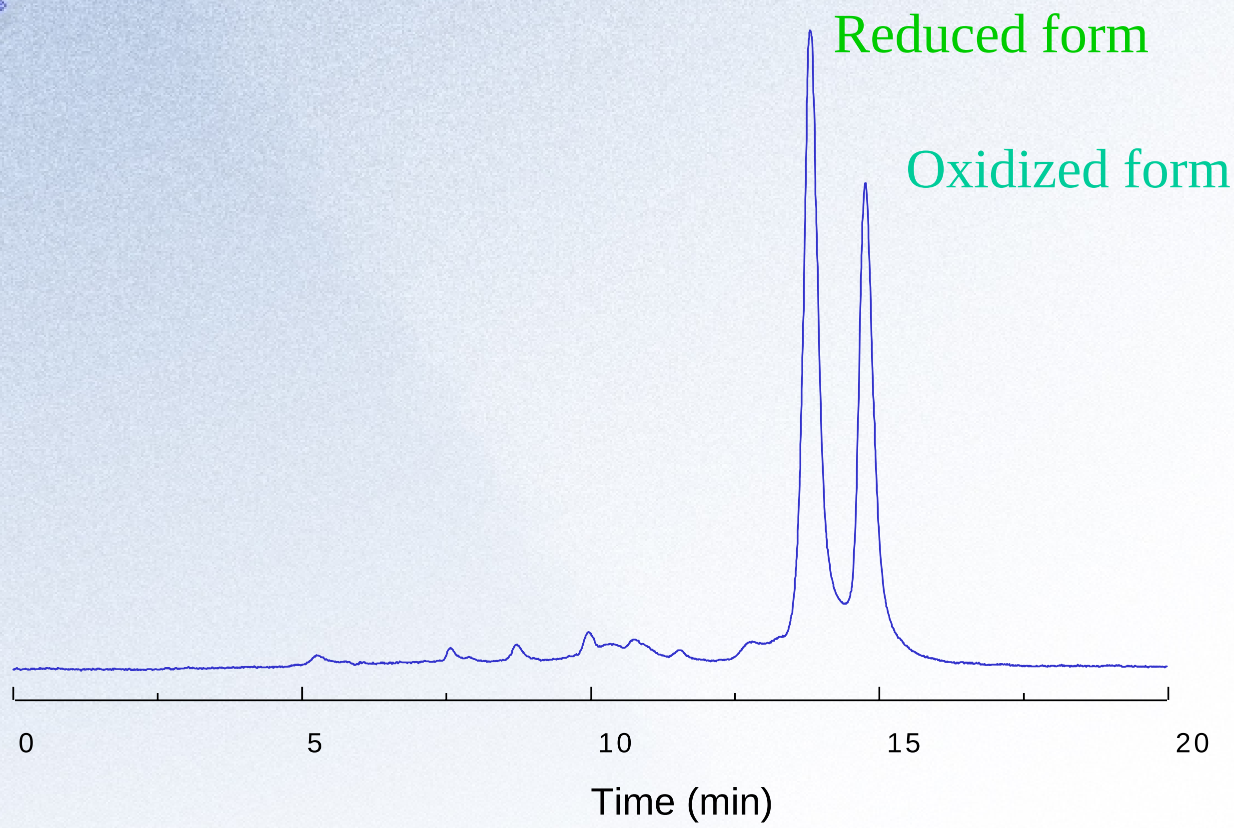
Proteins on Zr-PO_4 and ZirChrom-PEZ (EDTPA)



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

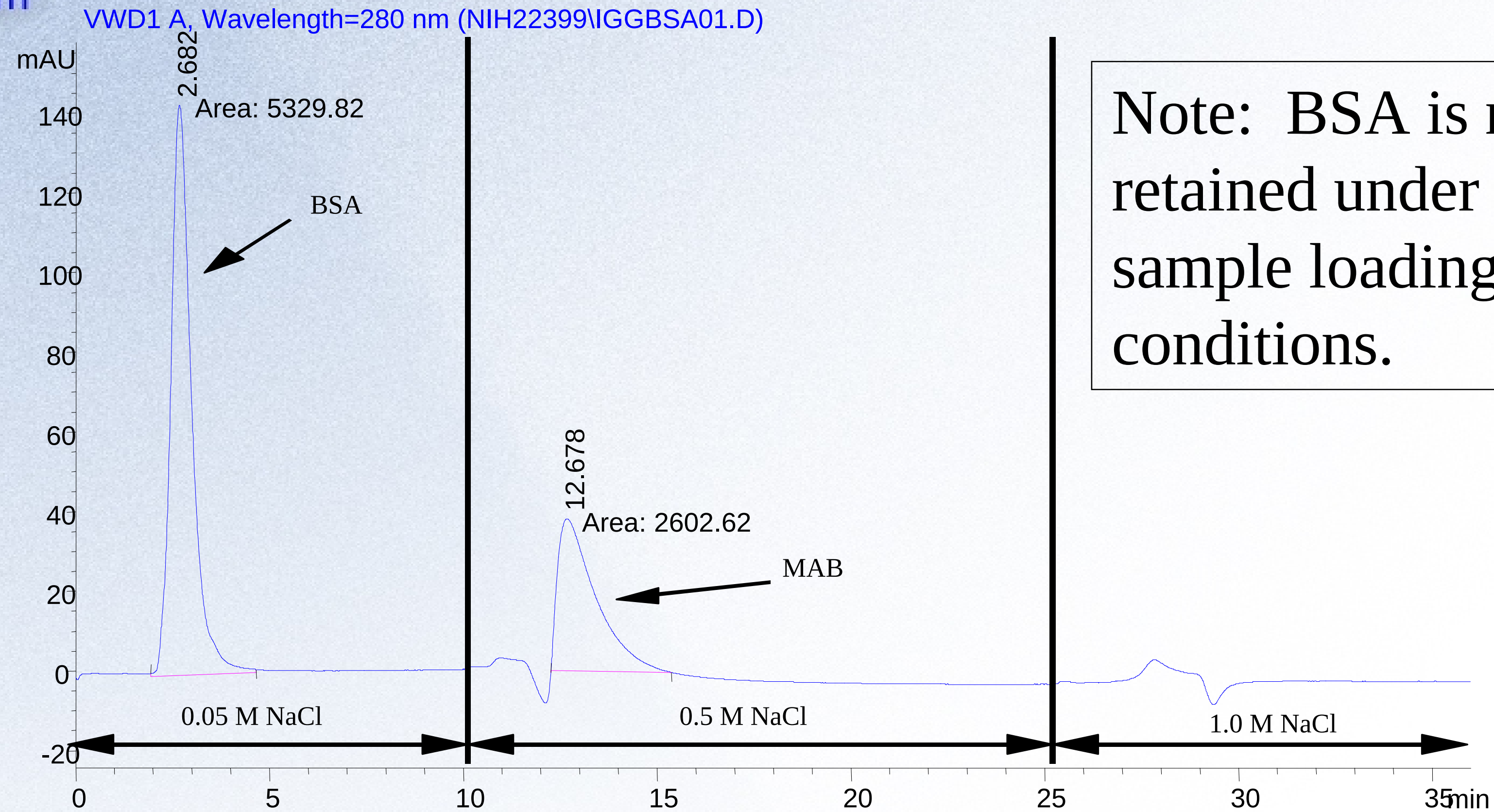
Mobile phase, 50 to 500 mM K_2HPO_4 in 20 min., pH 7.0; Flow Rate, 1 mL/min; Detection, 280 nm.

Proteins on ZirChrom-PEZ



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



Note: BSA is not retained under sample loading conditions.

<u>LC Conditions</u>: ZirChrom-PEZ, 5 x 4.6mm (part#ZR08-0546); 100 *u*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.

Zirconia-PEI (Zr-PEI) for Anion Exchange

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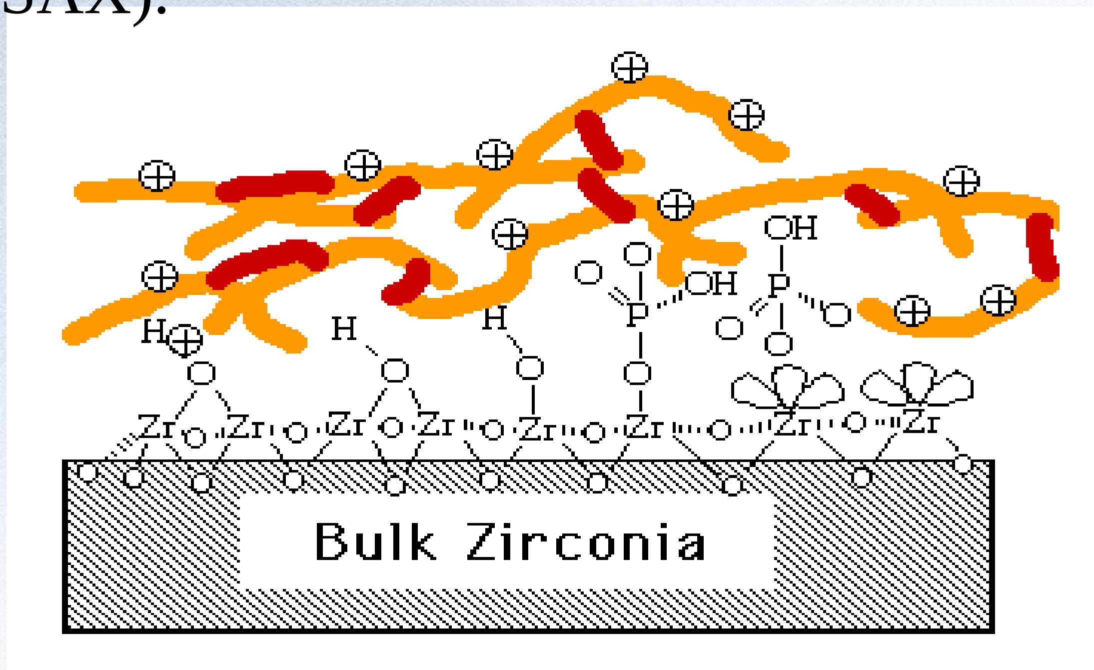
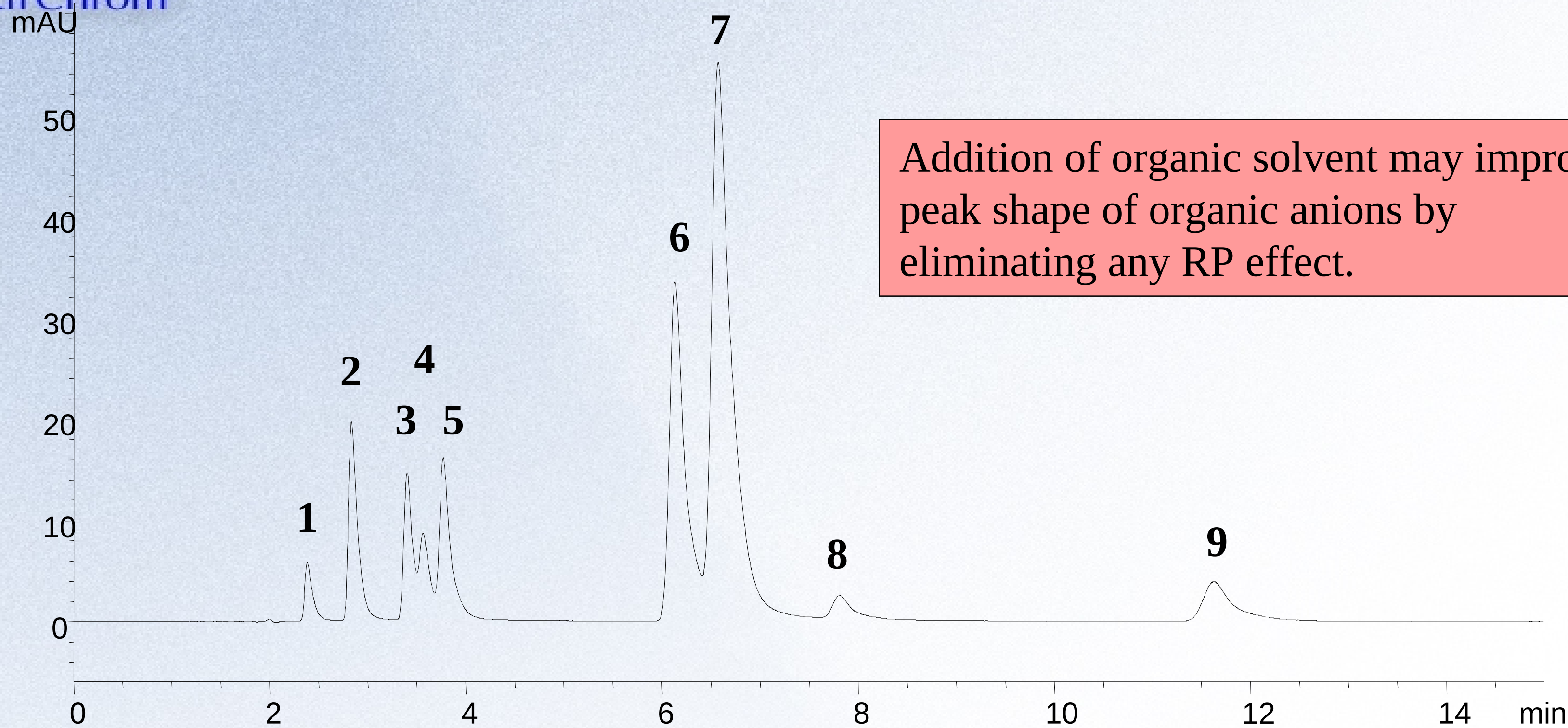
- Coat and crosslink polyethyleneimine (PEI) for weak anion exchange (WAX).
- Quaternize with methyl iodide for strong anion exchange (SAX).



Lewis acid sites can compete for anions unless blocked by a stronger Lewis base.

Small Anions on ZirChrom -WAX

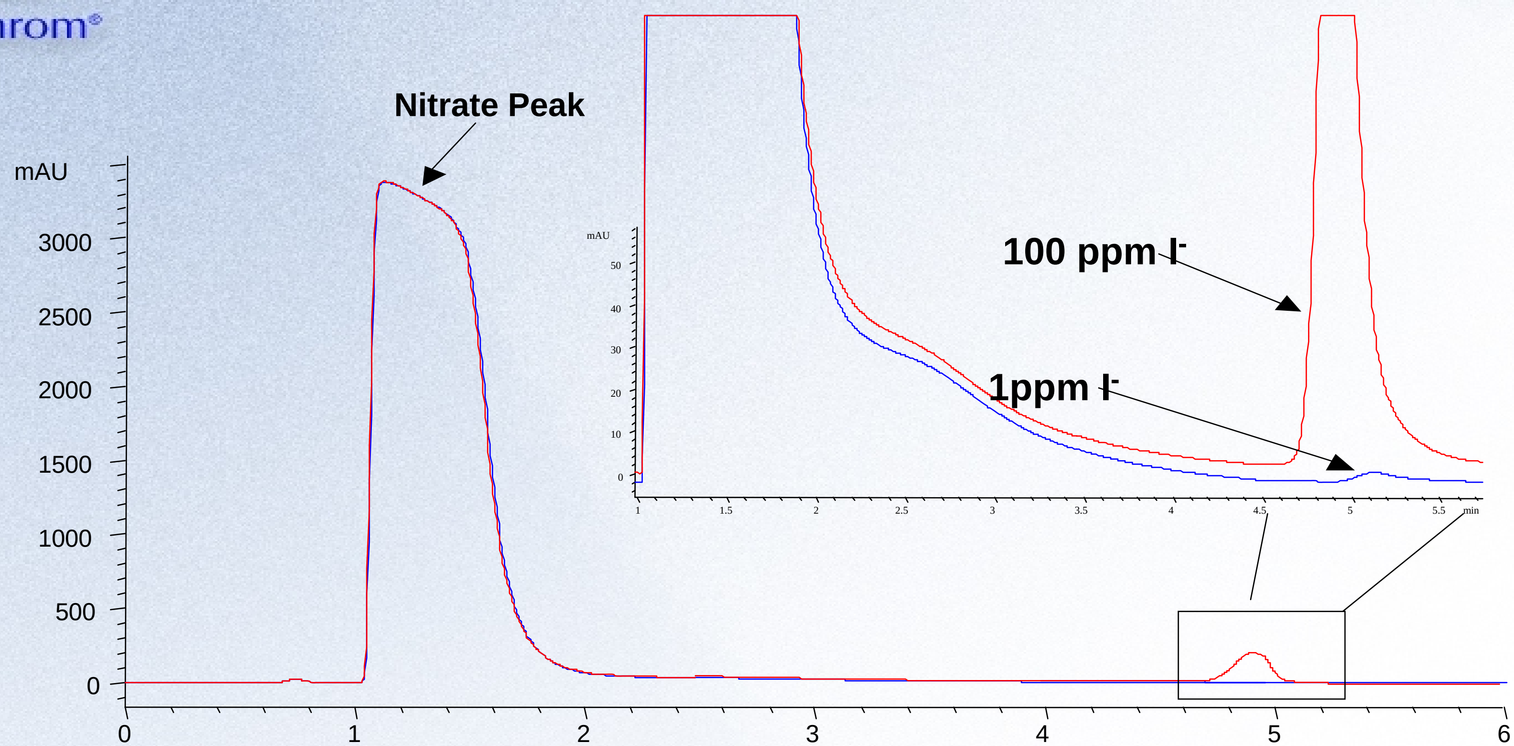


Addition of organic solvent may improve peak shape of organic anions by eliminating any RP effect.

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



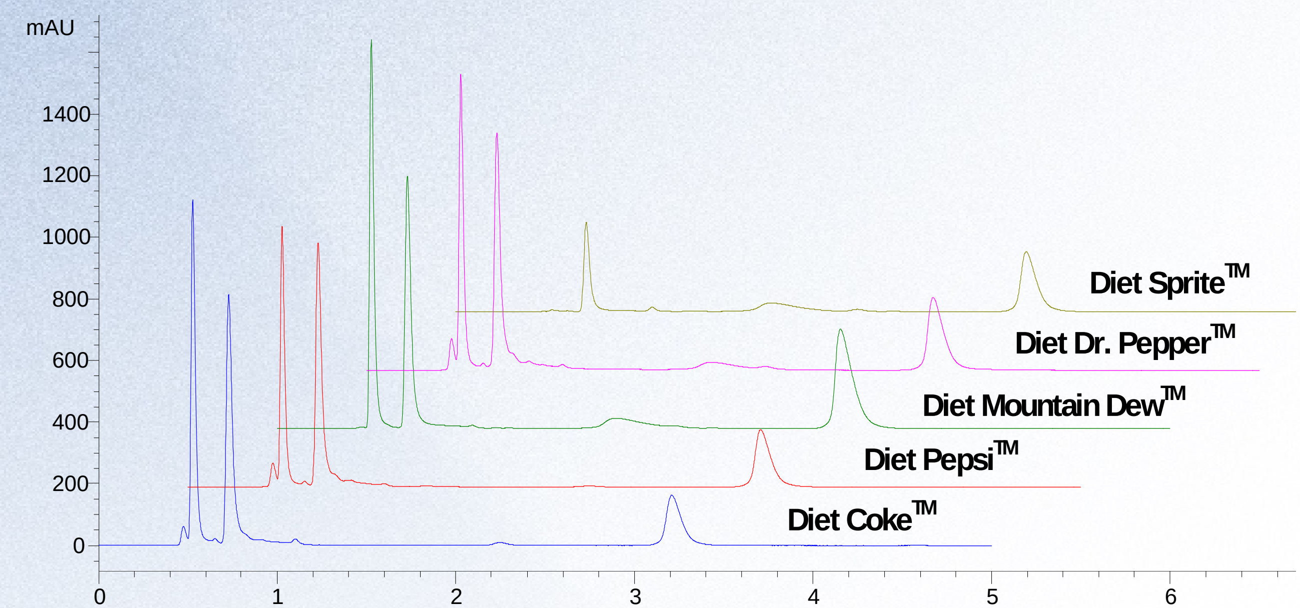
Analytes:

2M nitrate sample matrix and Iodide

LC Conditions:

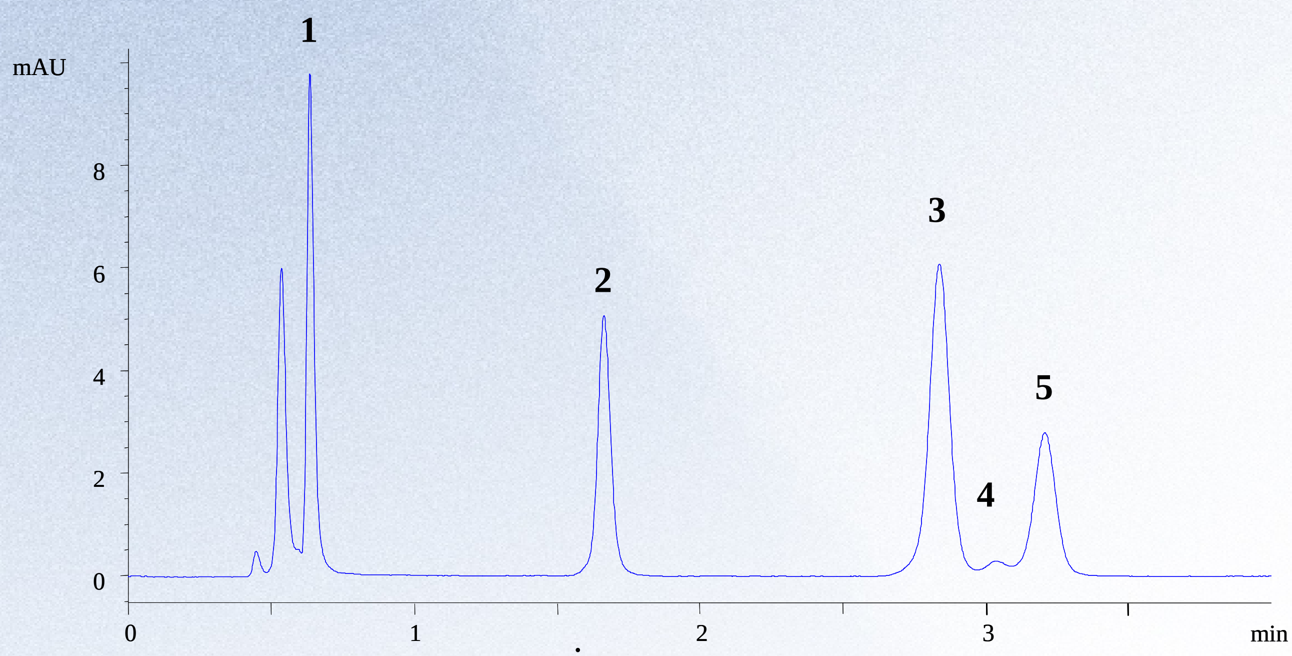
Column, ZirChrom®-SAX, 50 x 4.6 mm, (part# ZR06-0546); Mobile Phase: 25mM ammonium phosphate, 275mM NaCl at pH 8.0; Flow Rate, 1.0 mL/min; Temperature, 30°C; Detection, 226 nm; Injection volume: 5 ul.

“Green” Analysis of Diet Soft Drinks Containing Caffeine and Aspartame



<u>LC Conditions:</u>
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 ºC; 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



1-Acetaminophen

2-Ibuprofen

3-Naproxen

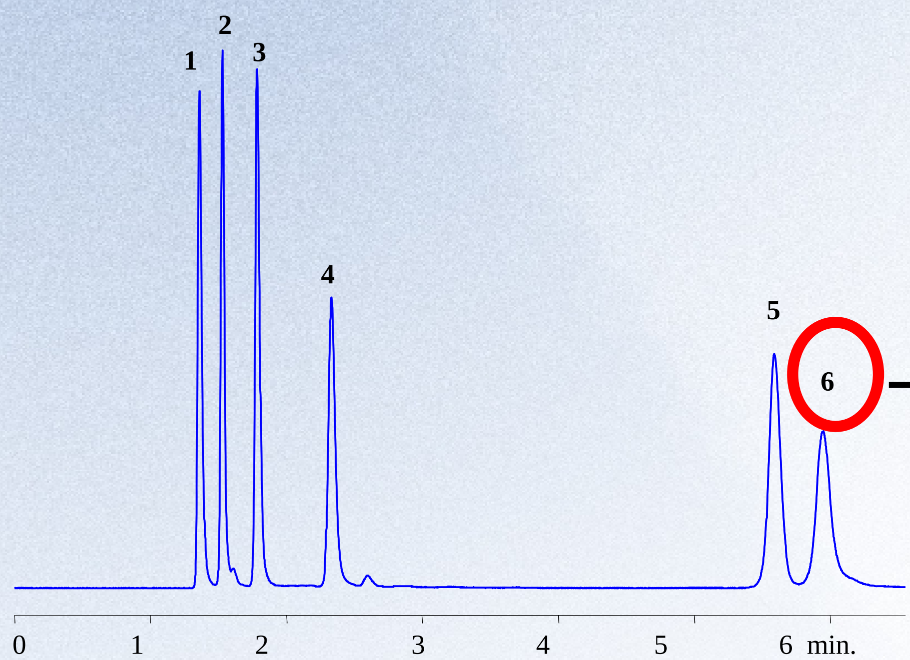
4-Impurity

5-Ketoprofen

<u>LC Conditions:</u>

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 ^{o}C; Injection Vol., 1.0 ml; Detection, 254 nm.

Water-Soluble Vitamin Analysis on ZirChrom®-SAX



1 - Thiamine (Vit. B_1)
2 - Pyridoxine (Vit. B_6)
3 - Nicotinamide (form of Vit. B_3)
4 - Riboflavin (Vit. B_2)
5 - Nicotinic acid (form of Vit. B_3),
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 ºC; 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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