



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

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Areas Where Ion Separations are Important

Biochemicals	Pharmaceuticals	Chemicals*
Proteins Peptides Amino acids Nucleotides Nucleosides Phospholipids Etc.	Amines Quaternary amines Carboxylic acids Etc.	Inorganic ions Quaternary amines Sulfates Phosphates Surfactants Etc.

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



Ion-Exchange LC (HPLC)

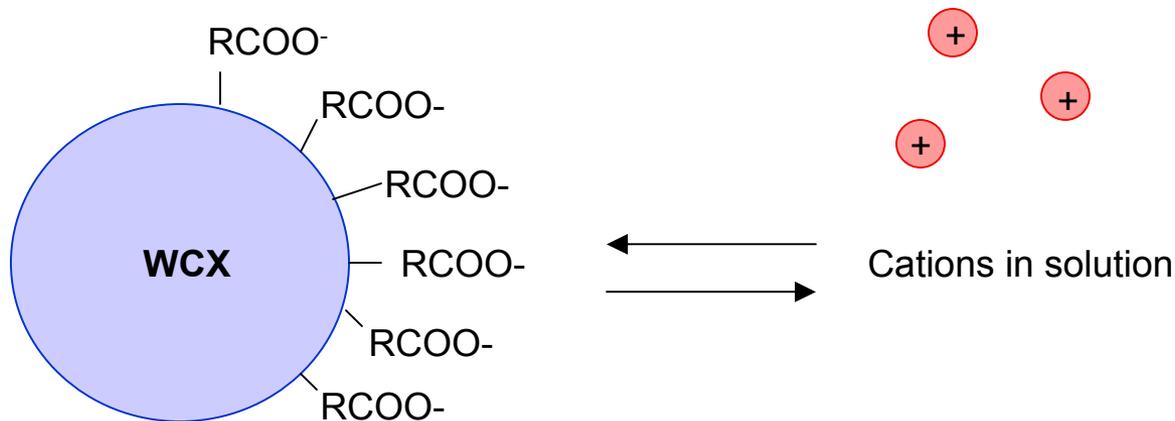
- **Most ion-exchange LC has been done with polymer-based support particles.**
 - Polymers often exhibit poor efficiency and are subject to dimensional change when exposed to high temperature, high pressure, high flow rate and organic solvents.
- **Silica has not been highly successful as a base for 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

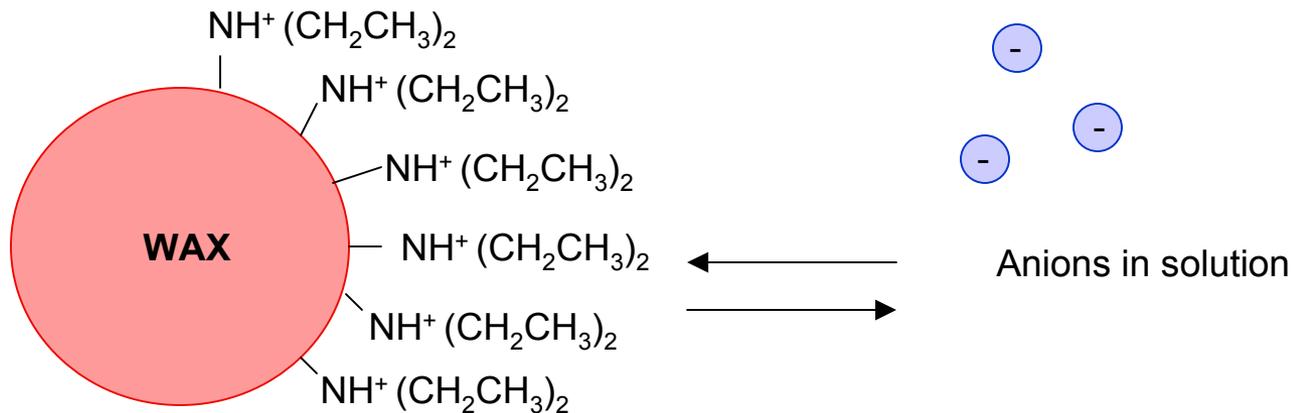
Cation Exchange

- **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.



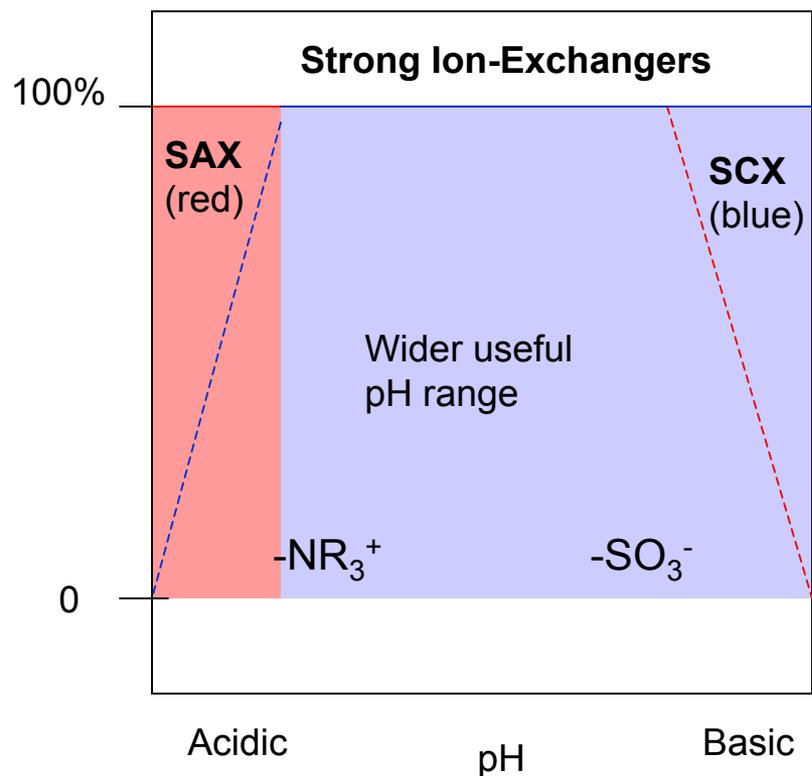
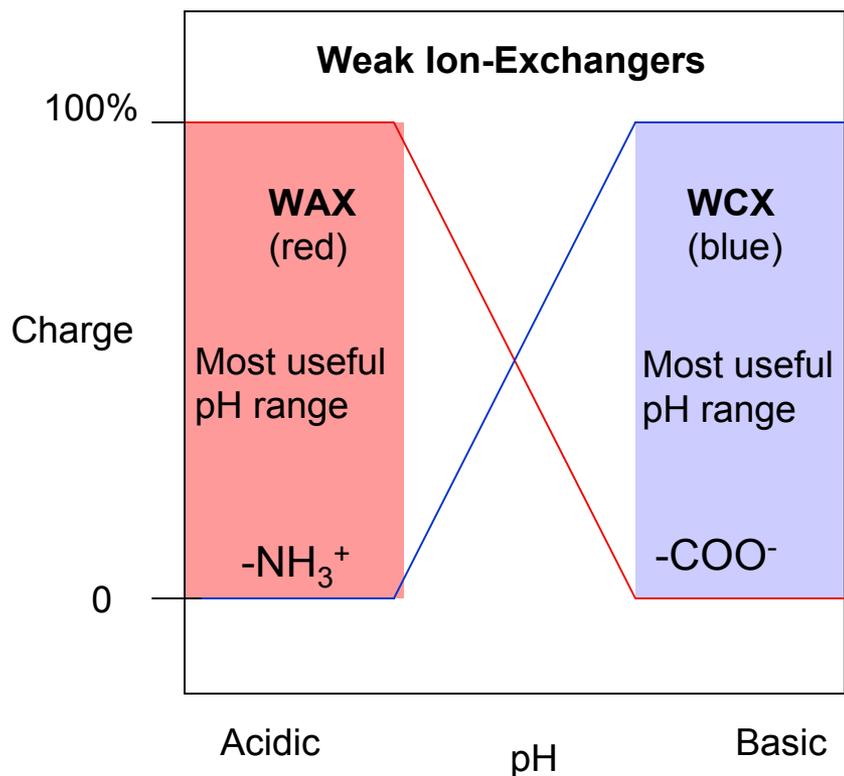
Anion Exchange

- **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.



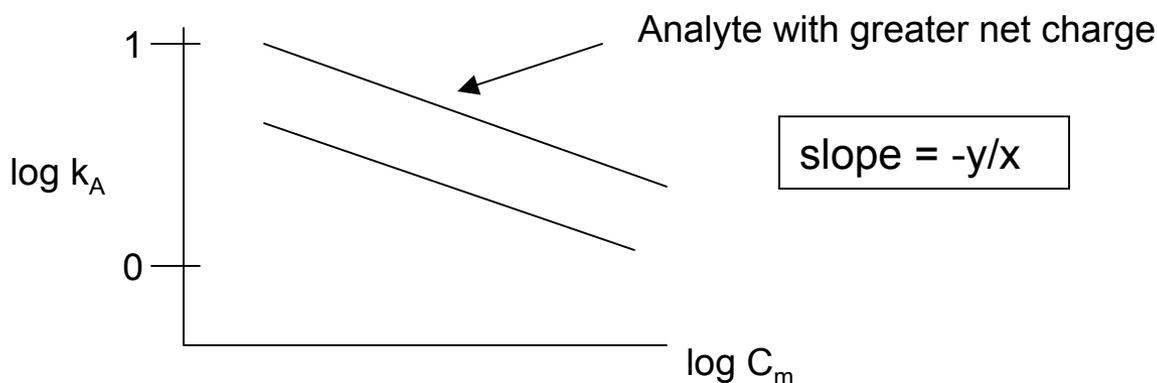
$$\log k_A' = C - \frac{y}{x} \log [E_m]$$

Retention Relationships in IE-HPLC²

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

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

- 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)

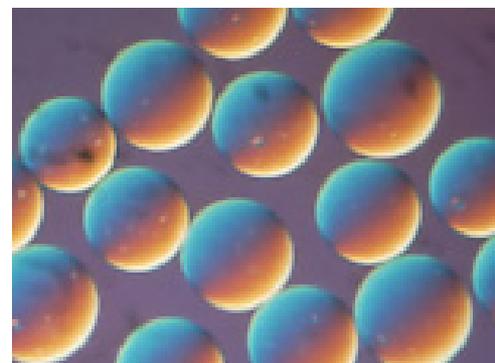
Polymers

- PS/DVB
- PVA
- Polymethacrylates

Carbon



zirconia

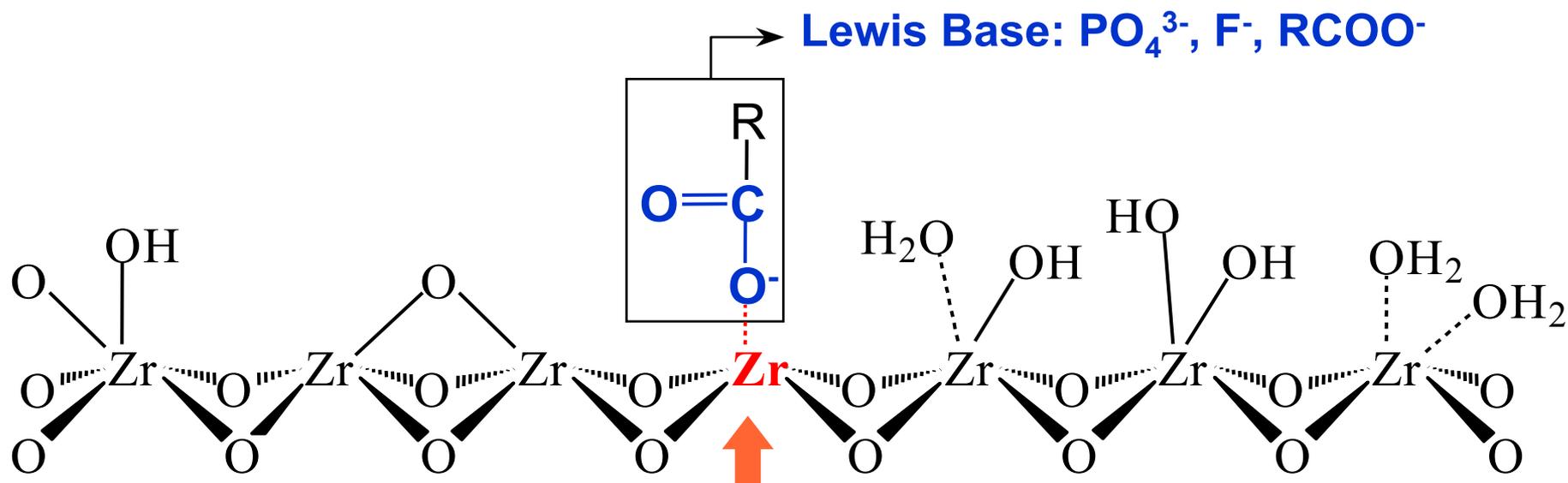


polymer

Properties of Analytical Zirconia

Parameter	Property
Pore Diameter (Å)	250- 300
Porosity	0.45
Particle Diameter (µm)	3, 5, 10
Density (gm/cc)	5.8 (2.5 x silica)
Pore Volume (cc/g)	0.13
Surface Area (m ² /g)	22
Lewis Acid Site (µmol/m ²)	4-5

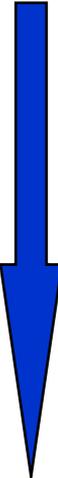
Zirconia Lewis* Acid-Base Chemistry



- Ligand exchange interaction : $\text{Zr-L} + \text{A}^- \leftrightarrow \text{Zr-A}^- + \text{L}$
- 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

Interaction Strength	Lewis Base Anion (A ⁻)
Strongest  Weakest	Hydroxide (cleaning method) Phosphate Fluoride Citric acid Sulfate Acetic acid Formic acid Nitrate Chloride

Eluotropic Strength for Lewis Bases

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

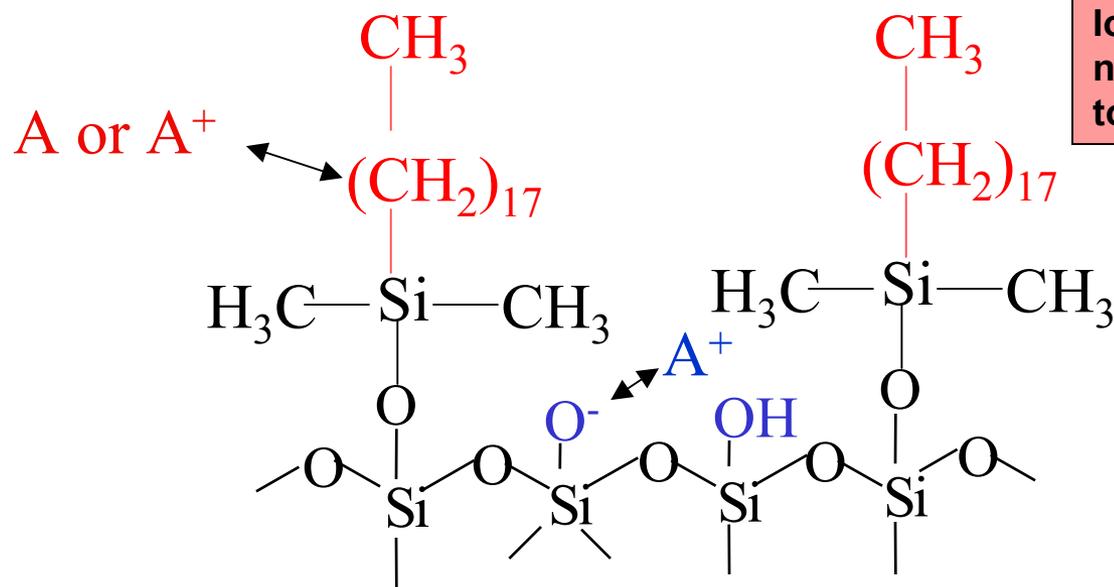
* organic amines are very weak Lewis bases



Review: Dual Mode Behavior of Zr-RP

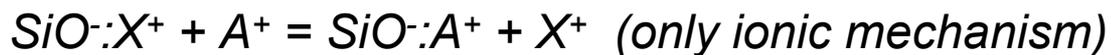
- **Early efforts to harness the advantages of zirconia for HPLC focused on polymer coatings to impart RP behavior; a strong ion-exchange component was noted for charged solutes, especially cations.**
- **Cation retention can be attributed to adsorbed anions from the buffer.**
- **Dual-mode properties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

Dual-Mode Mechanism on Silica-C18



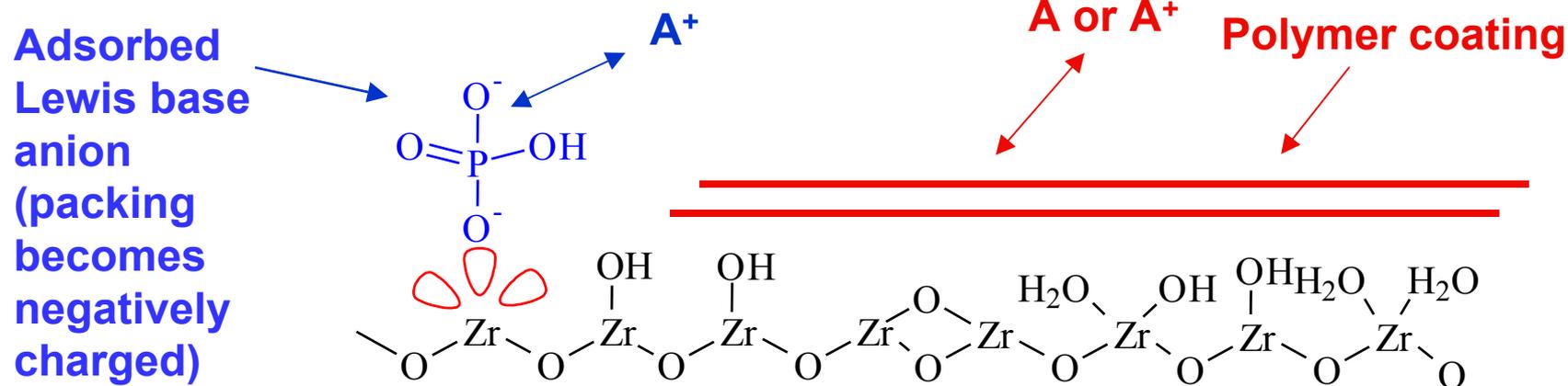
Ion-exchange is a nuisance rather than a tool with silica phases.

- Bonded C₁₈ Chains—**Reversed-Phase (RP)** Moieties
- Ionized Silanol Groups — **Ion-Exchange (IEX)** Sites



A⁺: analyte cation, X⁺: counterion (displacement ion)

Dual-Mode Mechanism on Zr-PBD

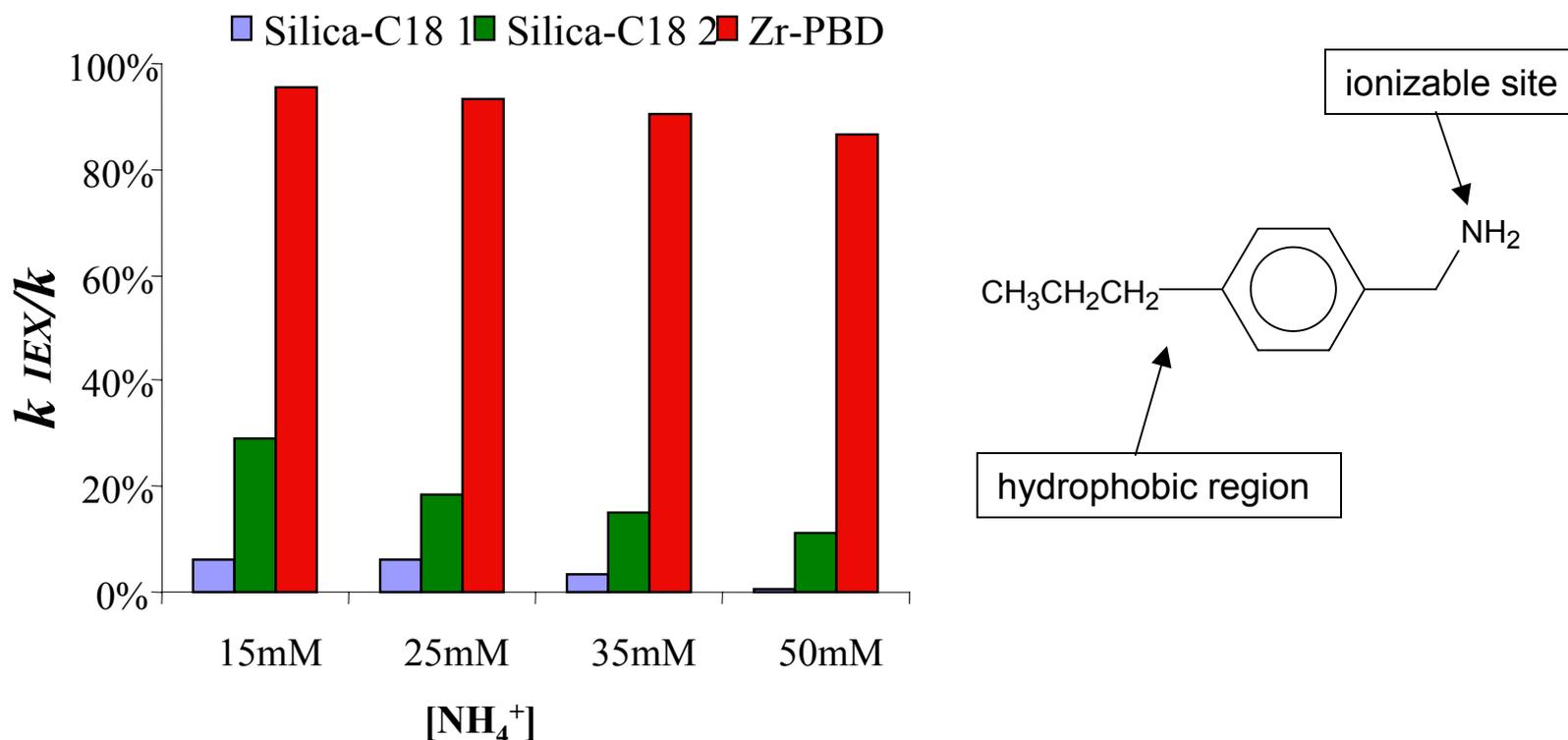


- PBD Coating — **Reversed-Phase (RP)**
- Lewis Base Anions — **Ion-Exchange (IEX)**
 - $Zr-L^-:X^+ + A^+ = Zr-L^-:A^+ + X^+$ (*very important mechanism*)
- Zirconols
 - $Zr-O^-:X^+ + A^+ = Zr-O^-:A^+ + X^+$ (*unimportant mechanism*)

A^+ = analyte cation, X^+ = counterion, L^- = adsorbed Lewis base anion.

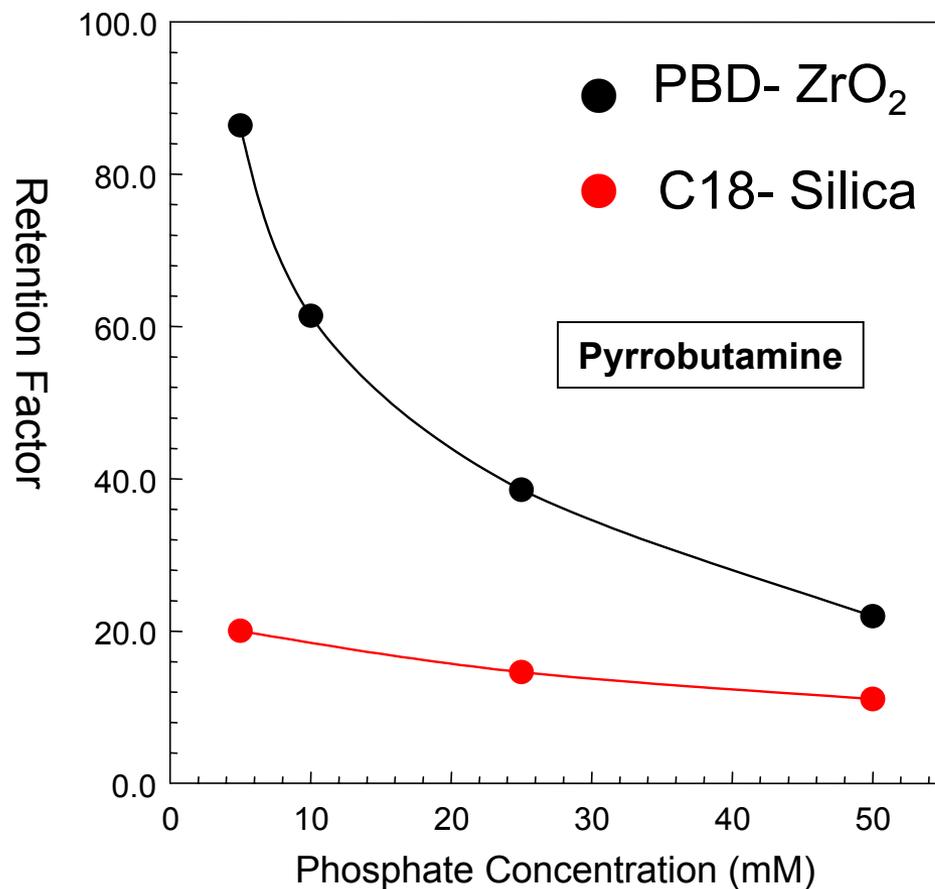
Cation-Exchange Character of Zr-PBD

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



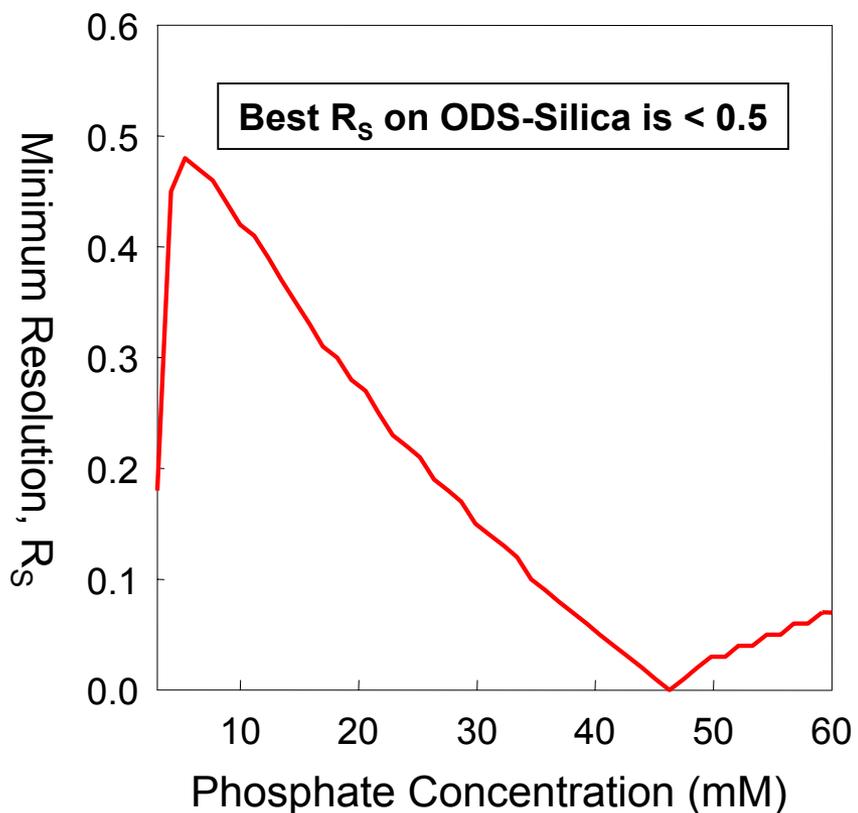
Conditions: 55% CH₃OH in ammonium phosphate (pH, 6.0); 35°C; 1.0 mL/min.; UV 254 nm

Buffer Concentration Effect on IEC

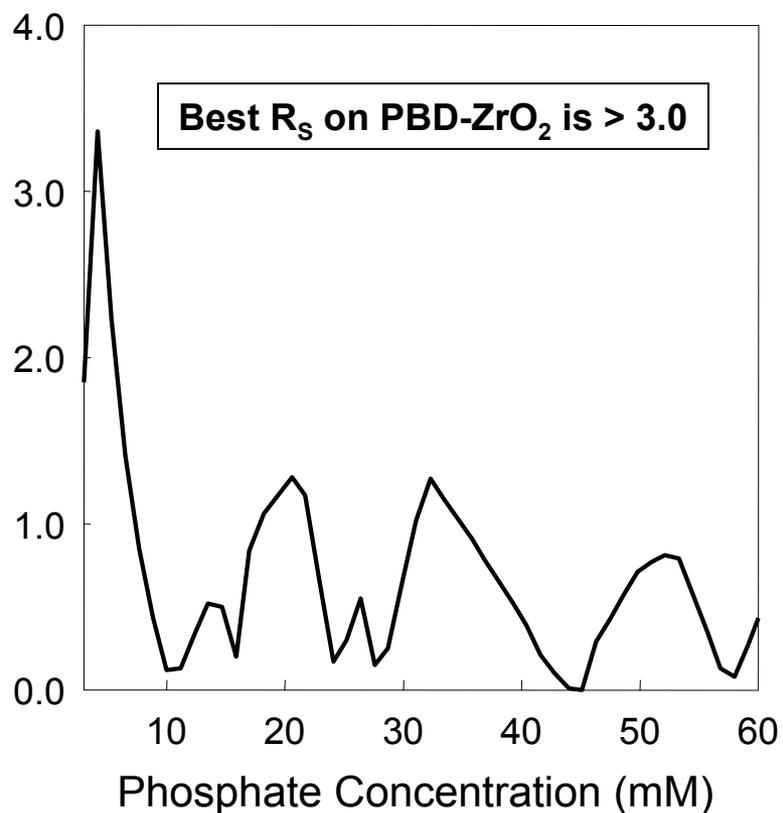


Drylab[®] Window Diagram for Antihistamines

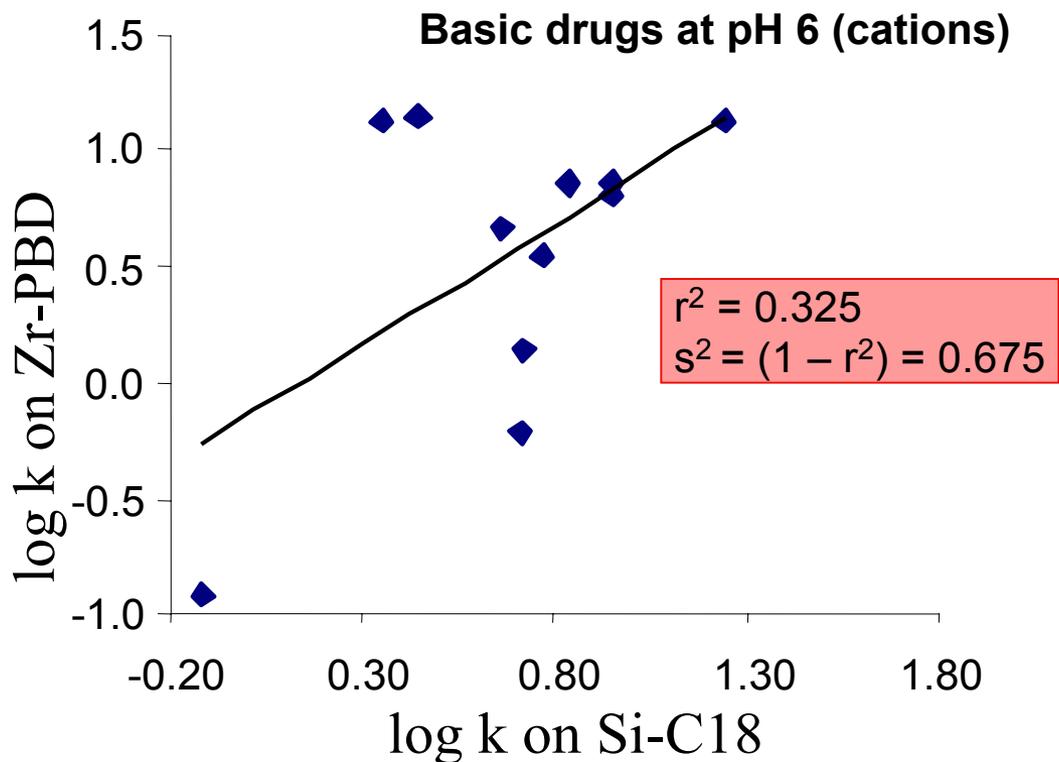
ODS



ZirChrom[®]-PBD



Selectivity Difference³ Caused by IEC Mode

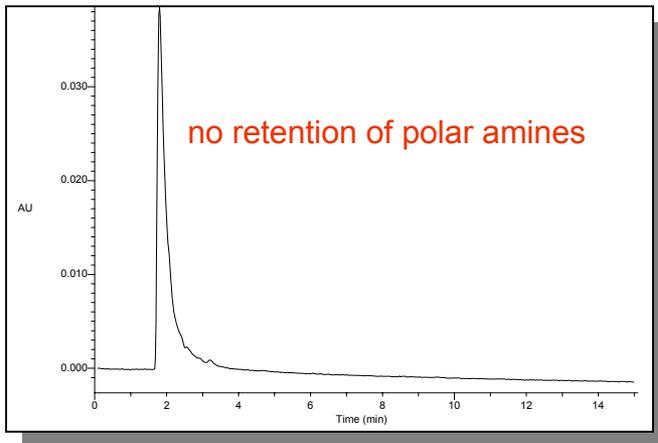


LC Conditions: Mobile phase, 72/28 MeOH/25 mM ammonium phosphate, pH 6; Temperature, 35 °C; Flow rate, 1.0 ml/min; Detection at 254 nm; Solutes: 1=chlordiazepoxide, 2=desipramine, 3=nortriptyline, 4=doxepin, 5=imipramine, 6=thiothixene, 7=amitriptyline, 8=hydroxyzine, 9=buclizine, 10=thioridazine, 11=perphenazine.

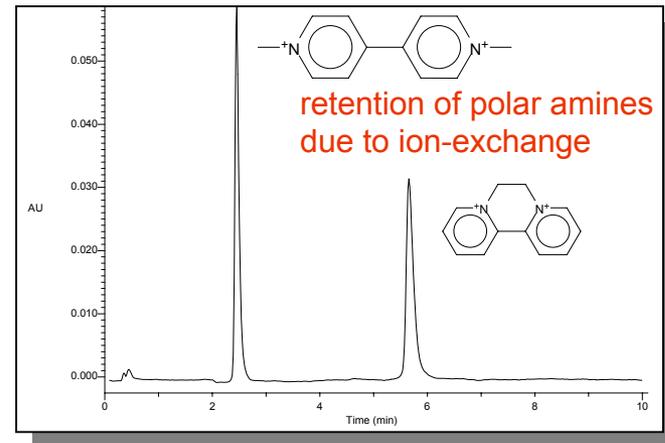
Zirconia Shows Unique IEC Selectivity⁴

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



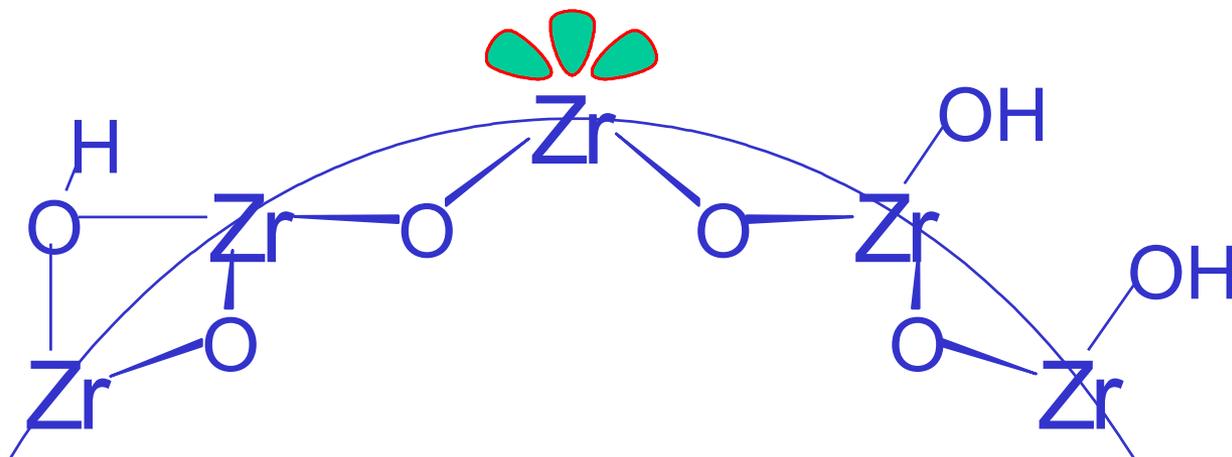
Separation of paraquat and diquat on C18-silica vs. Discovery[®] Zr-PS

C18-silica conditions: Discovery C18, 15cm x 4.6mm, 3 μ m 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, 3 μ m particles; 50% CH₃CN in 25mM H₃PO₄, 25mM NH₄F, (to pH 8 with NH₄OH); 65°C, 3mL/min.



Zirconia for Ion-Exchange LC

Zirconia: Surface Properties



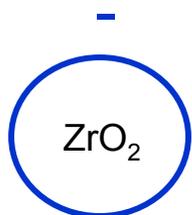
- **Retention dominated by Lewis acid sites when no polymer coating is present.**
 - Attracts strong electrophiles
 - 4-5 $\mu\text{mol}/\text{m}^2$ active sites



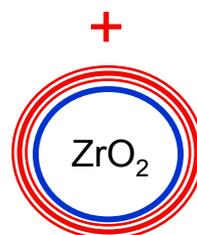
Possible Approaches to Zirconia-IEC

- **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)¹**
 - Well-researched area allows positive and negative charges to be incorporated as a stable, cross-linked polymer coating (PEI, etc.).

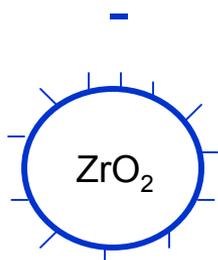
Four Zirconia-based Options for IEC



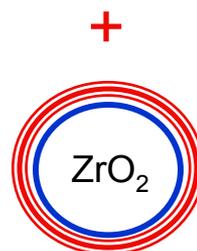
- **Bare zirconia**
- Phosphate, fluoride and other anionic additives that are replaceable.
- primarily a strong cation exchanger (SCX)



- **Zirconia with 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)

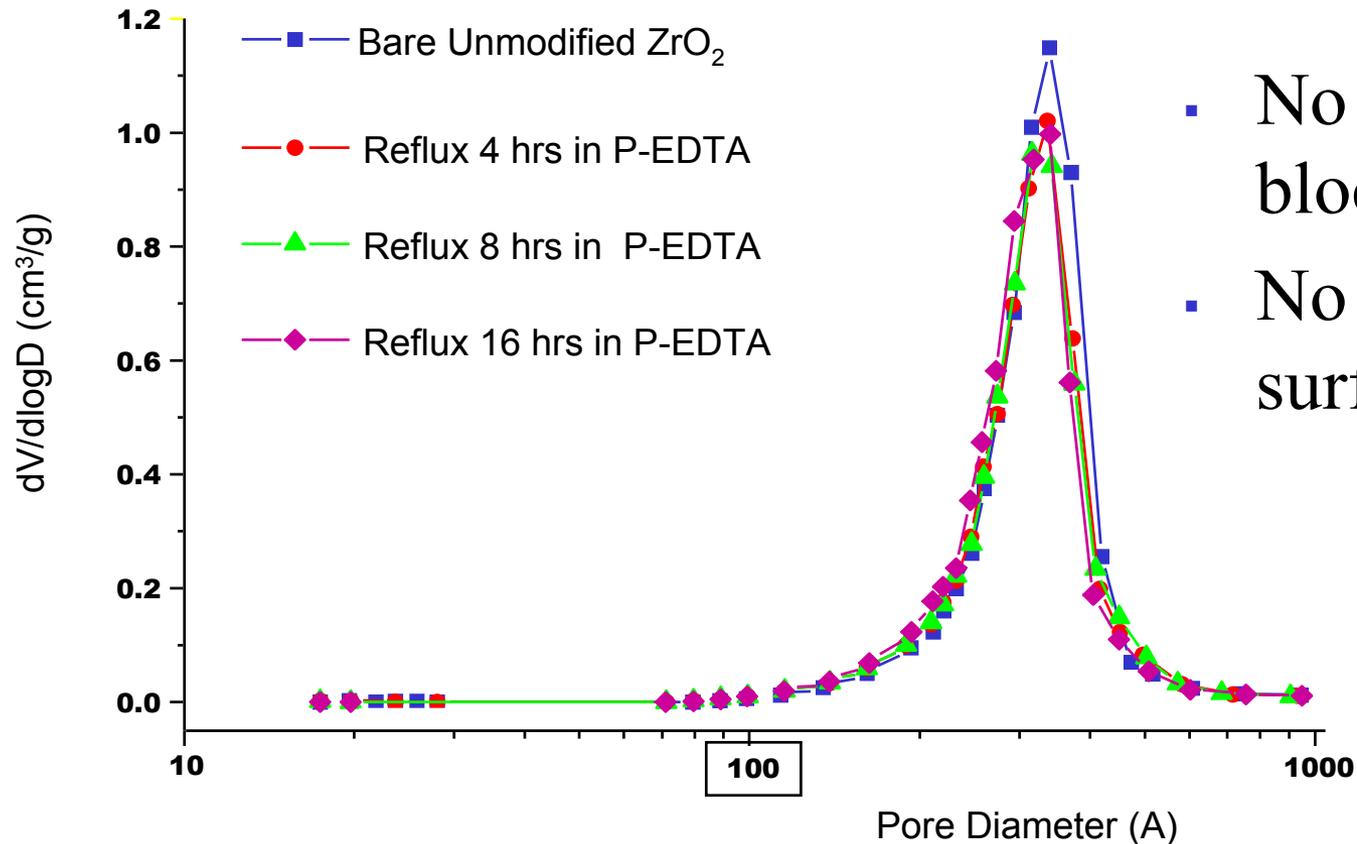


- **Zirconia with quaternized PEI coating**
- Cross-linked to resist removal even under extreme conditions
- Strong anion exchanger (SAX)



Development of Surface Modified Zirconia for Cation Exchange Chromatography

Nitrogen Porosimetry of Zr-EDTPA

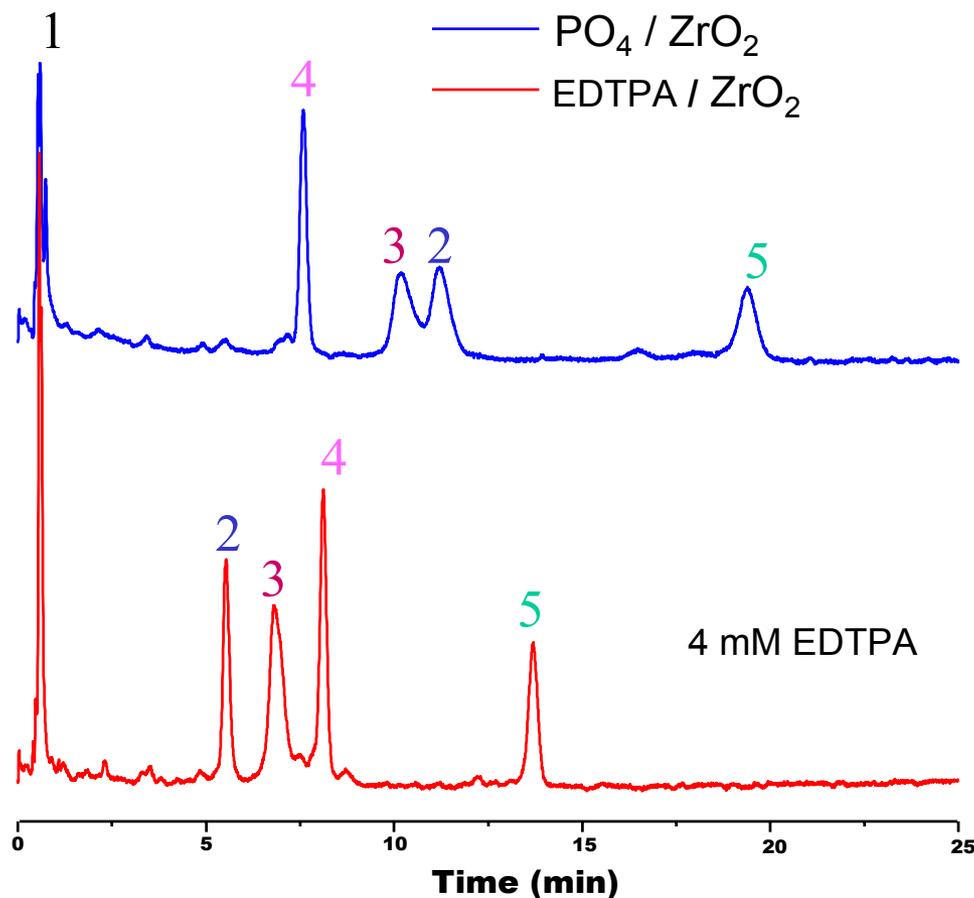


- No pore blockage
- No loss of surface area

Comparison of EDTPA and Phosphate

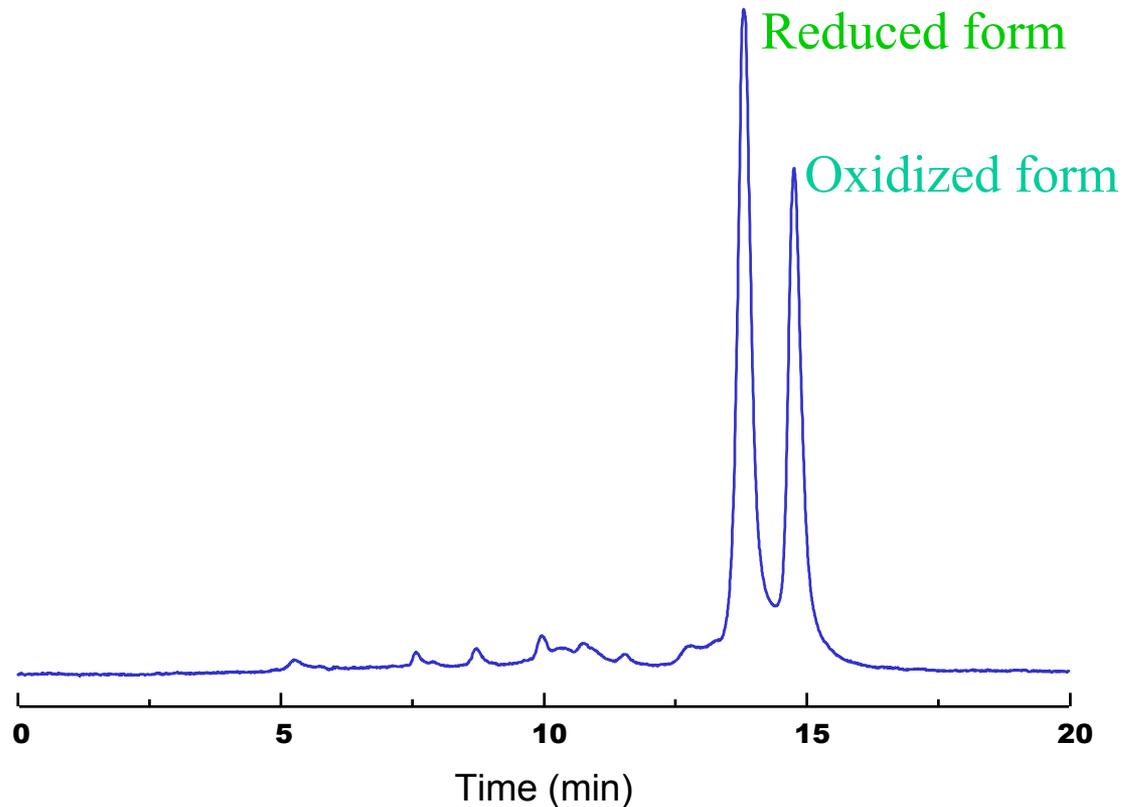
Time (hrs)	Zr-PO4 $\mu\text{mol P/m}^2$	Zr-EDTPA $\mu\text{mol P/m}^2$
2	4.57	2.18
4	5.82	2.24
8	9.41	2.01
16	-----	2.10

Proteins on Zr-PO₄ and Zr-EDTPA



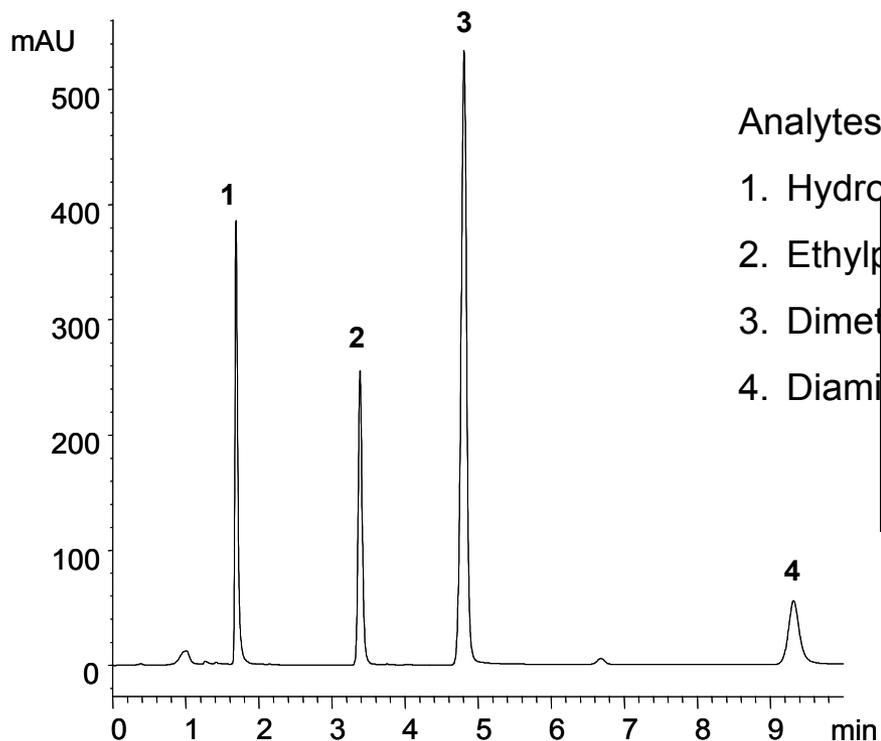
Mobile phase: 50 to 500 mM K₂HPO₄ in 20 min., pH 7.0. Flow Rate 1 mL/min. UV detection at 280 nm.

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

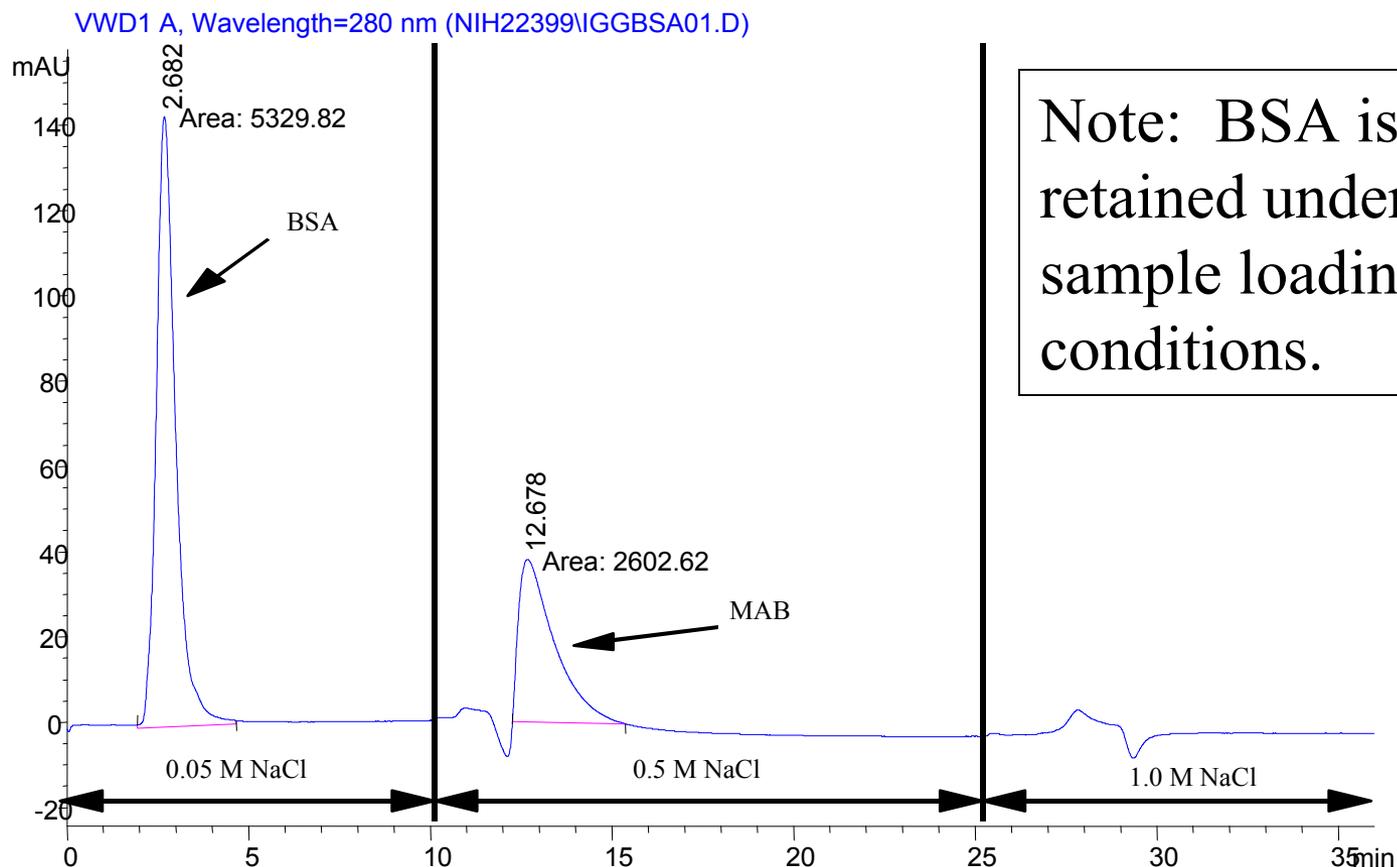


Analytes:

1. Hydroxypyridine
2. Ethylpyridine
3. Dimethylaminopyridine
4. Diaminopyridine

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).

Small-Scale Purification of MAB IgG_{2a} Contaminated with BSA



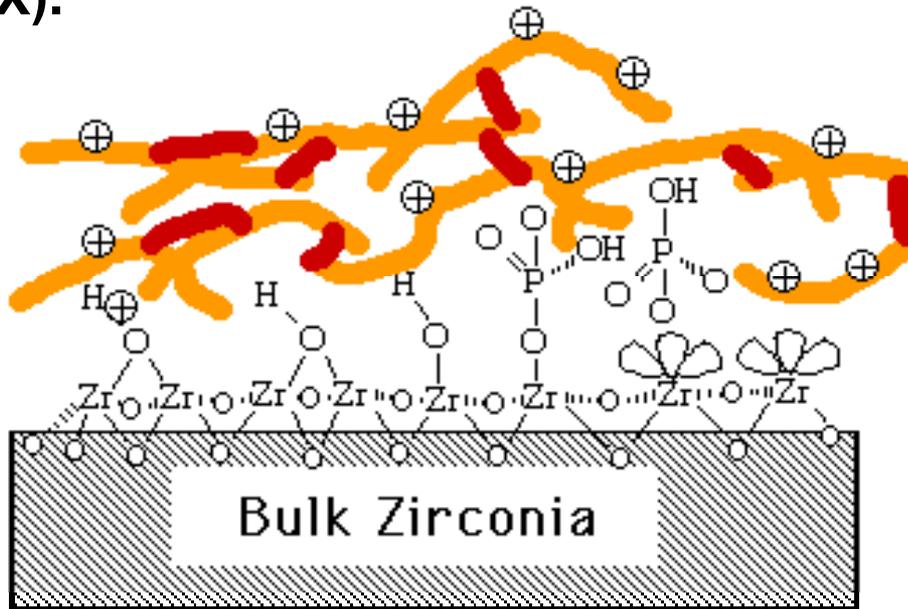
LC Conditions: ZirChrom PEZ, 5 x 4.6mm, 100 μ l injection of BSA (6.0 mg/ml) contaminated MAB (1.0 mg/ml) eluted by salt step gradient. Mobile phase: 20 mM MES, 4 mM EDTPA, 0.05 M-to-1.0 M NaCl pH=5.5. Flow rate: 2.0 ml/min. Temperature: 30°C. Detection: 280 nm.



Development of Surface Modified Zirconia for Anion Exchange Chromatography¹

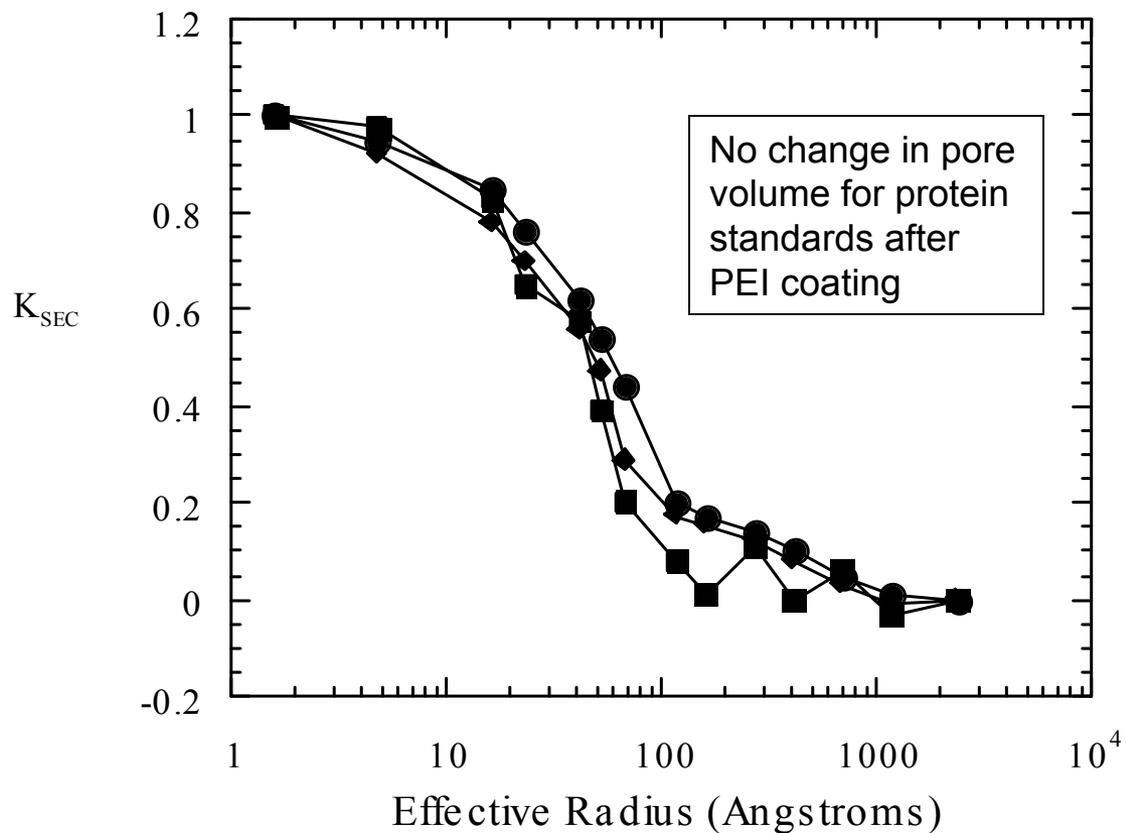
Zirconia-PEI (Zr-PEI) for Anion Exchange

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

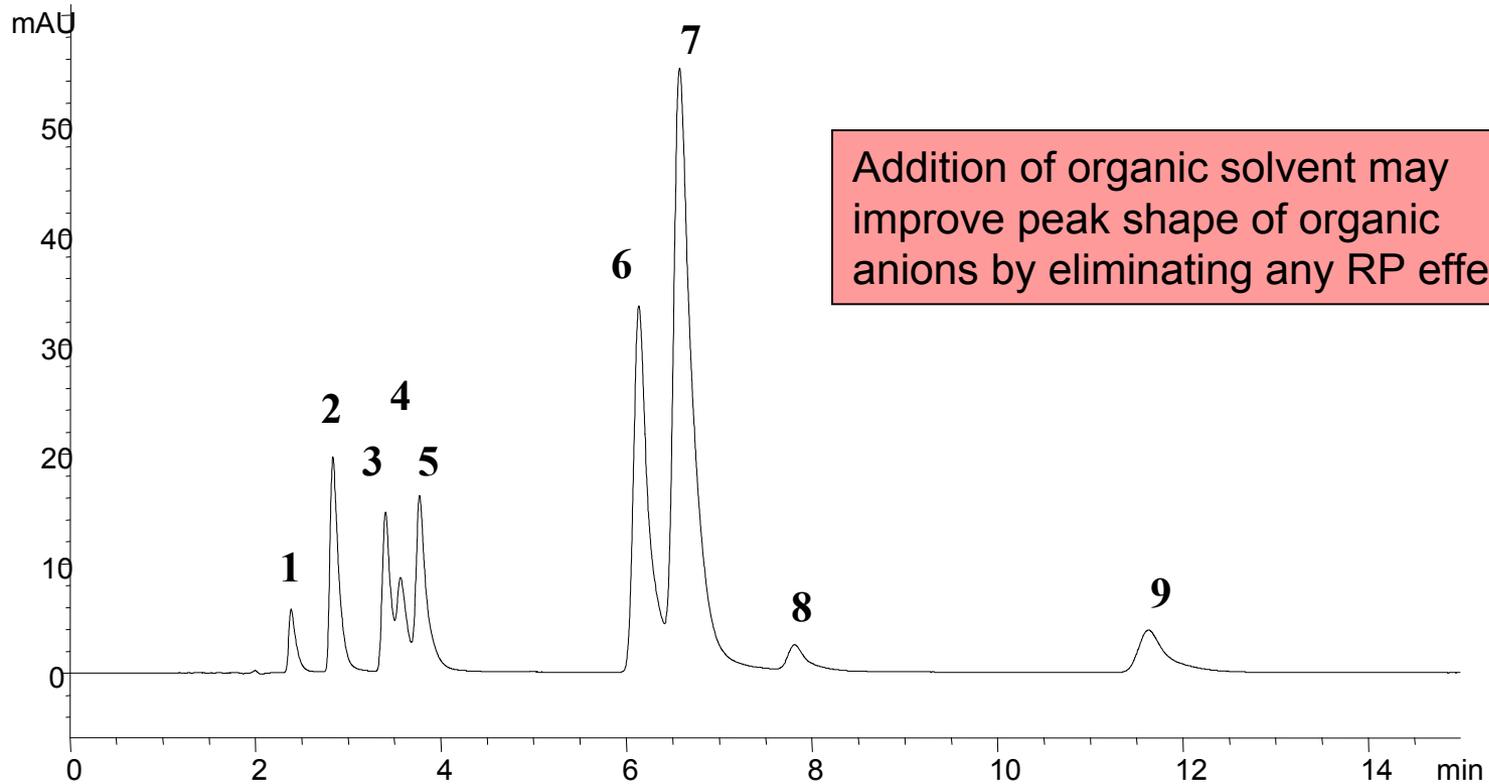


Lewis acid sites can compete for anions unless blocked by a stronger Lewis base.

SEC Study of PEI-Coated Zirconia



Small Anions on Zr-PEI

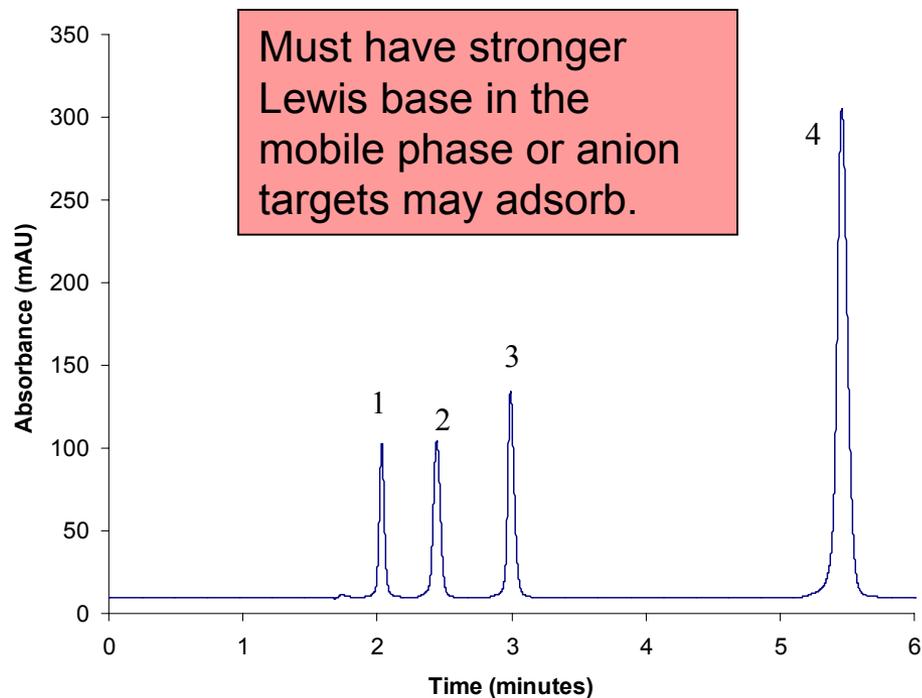


Addition of organic solvent may improve peak shape of organic anions by eliminating any RP effect.

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.

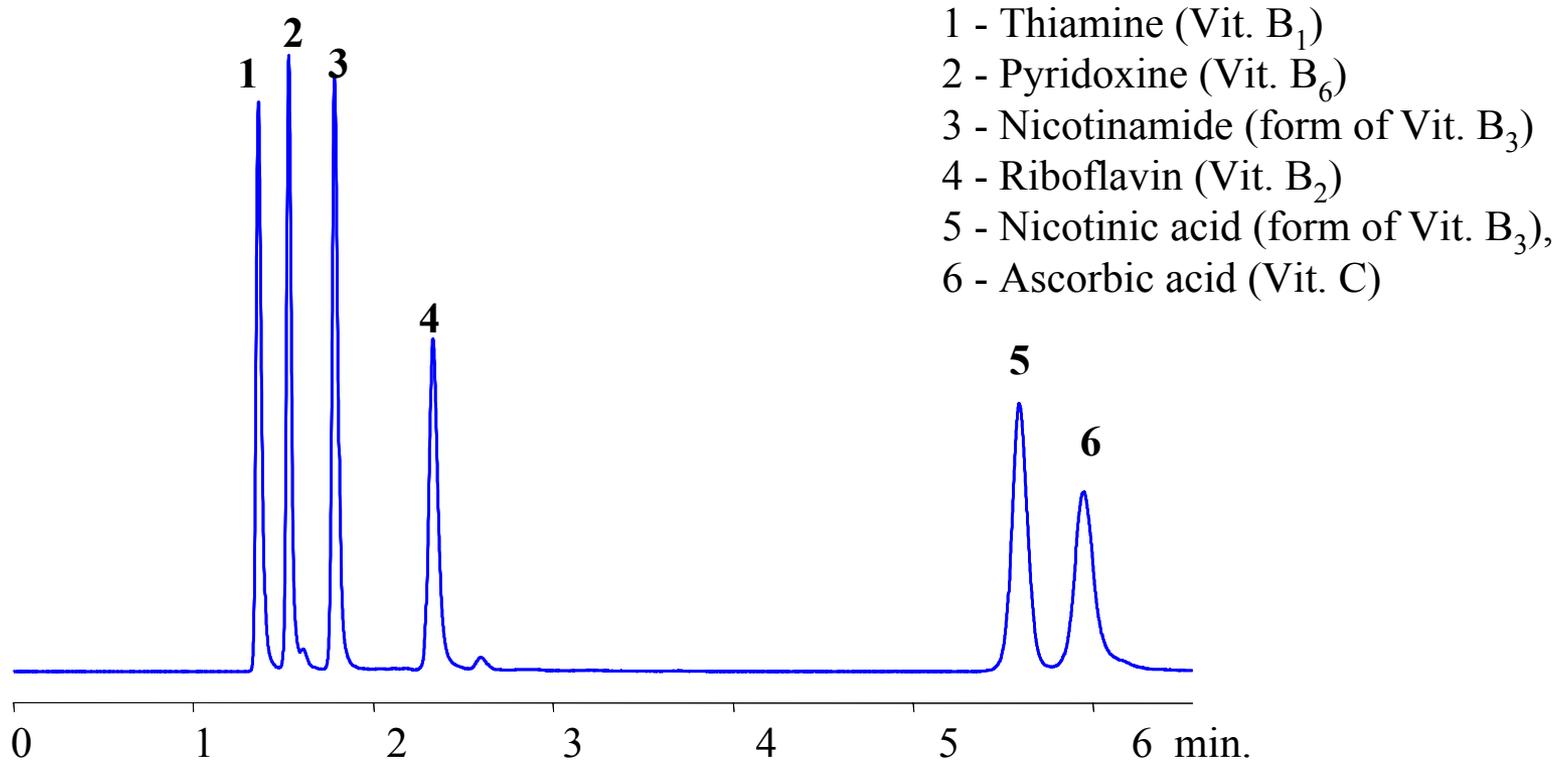
Inorganic Anions on Zr-PEI



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

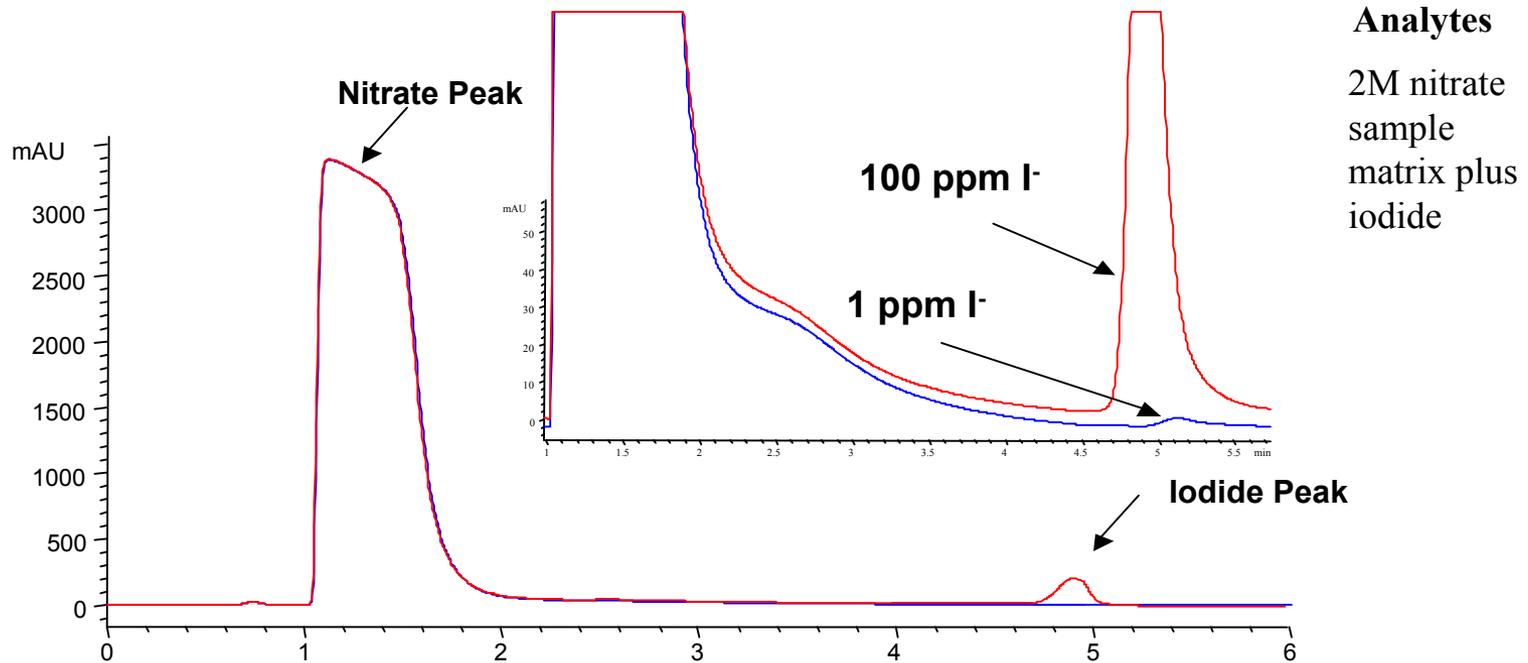
LC Conditions: Column: ZirChrom®-WAX, 150 x 4.6 mm,
Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 240 nm, Injection volume: 5 ul, Mobile
Phase: 100% 50mM sodium phosphate, 75mM NaCl at pH 7.0

Water-Soluble Vitamins on Zr-PEI-Q



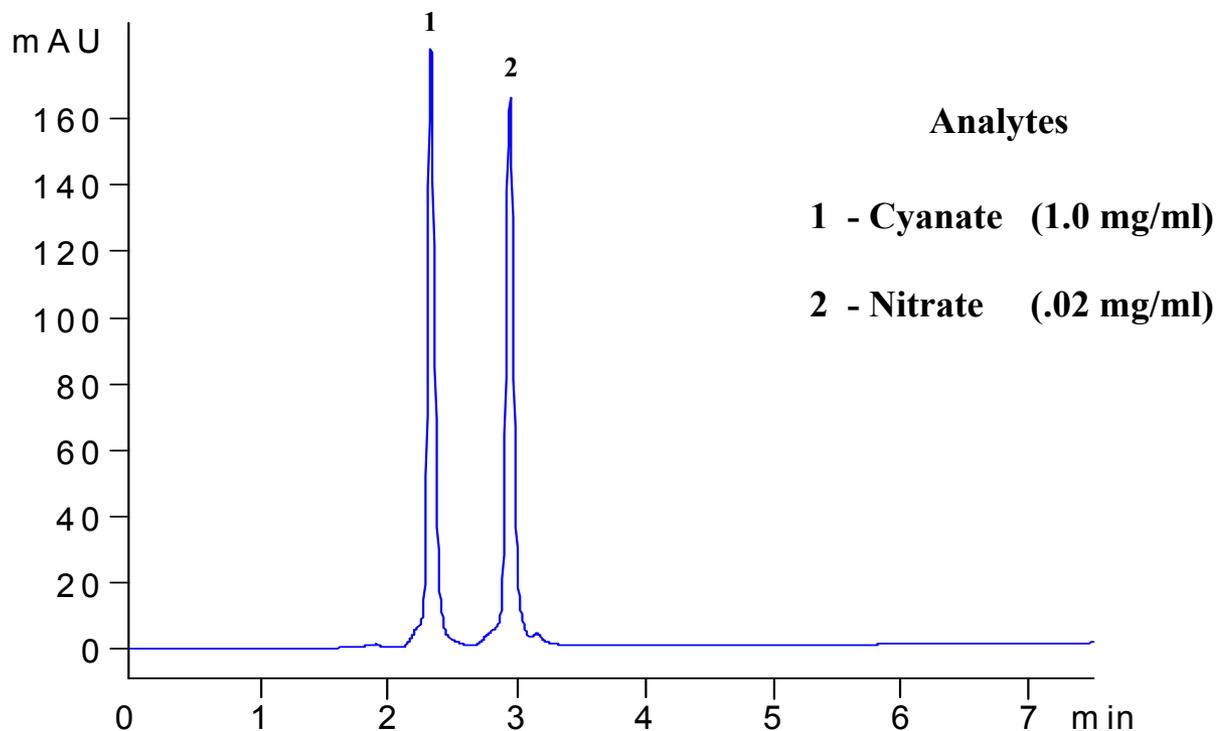
LC Conditions: Column: ZirChrom®-SAX, 150 x 4.6 mm i.d., Mobile Phase: 50 mM Ammonium dihydrogenphosphate, pH 4.5, Flow rate: 1.0 ml/min. Temperature: 30 °C, Injection Vol.: 5.0 ml, Detection: UV at 254 nm

Trace Iodide on Zr-PEI-Q



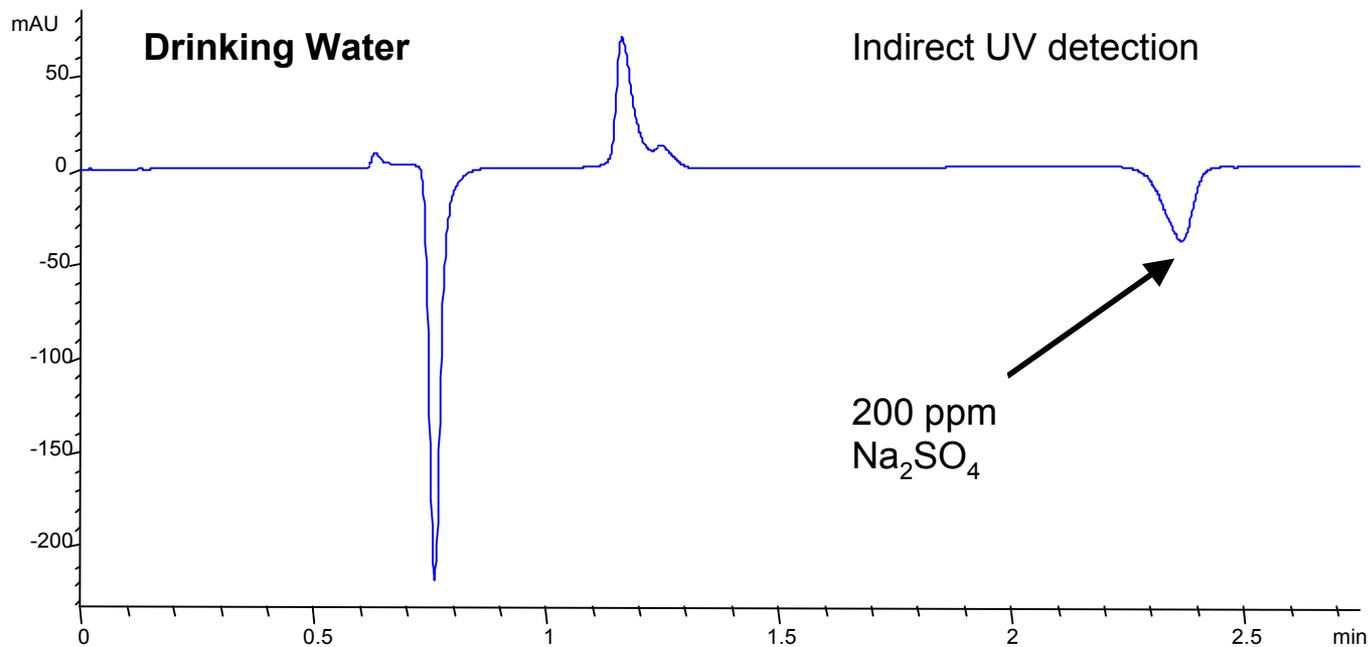
LC Conditions: Column: ZirChrom®-SAX, 50 x 4.6 mm,
Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 226 nm, Injection volume: 5 ul, Mobile
Phase: 25mM ammonium phosphate, 275mM NaCl at pH 8.0

Fertilizer Plant Effluent on Zr-PEI-Q



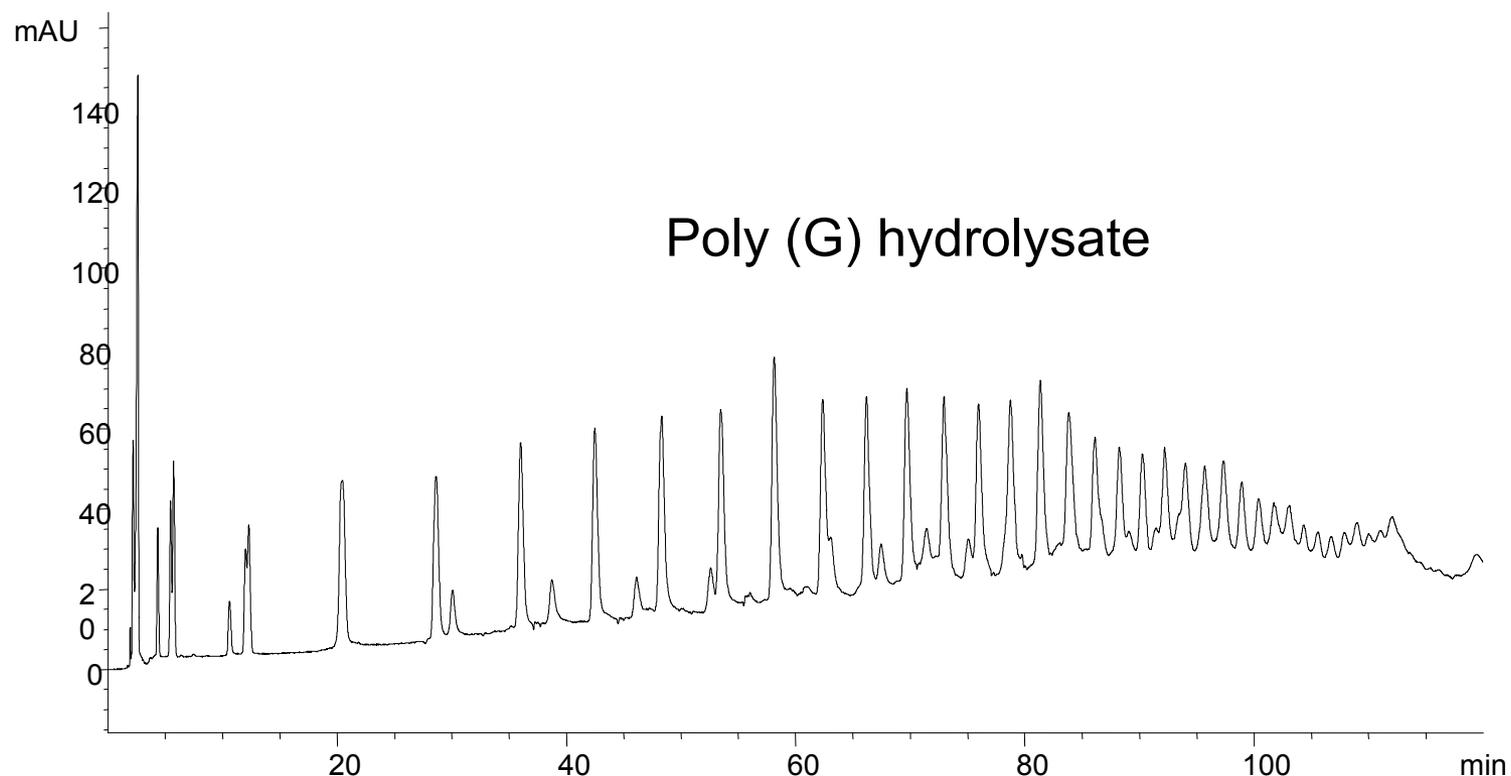
LC Conditions: Column: ZirChrom®-SAX, 150 x 4.6 mm,
Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 205 nm, Injection volume: 5 ul, Mobile
Phase: 25mM sodium fluoride, 175mM sodium chloride at pH 10.0

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 °C.**

Oligonucleotides on Zr-PEI-Q



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?

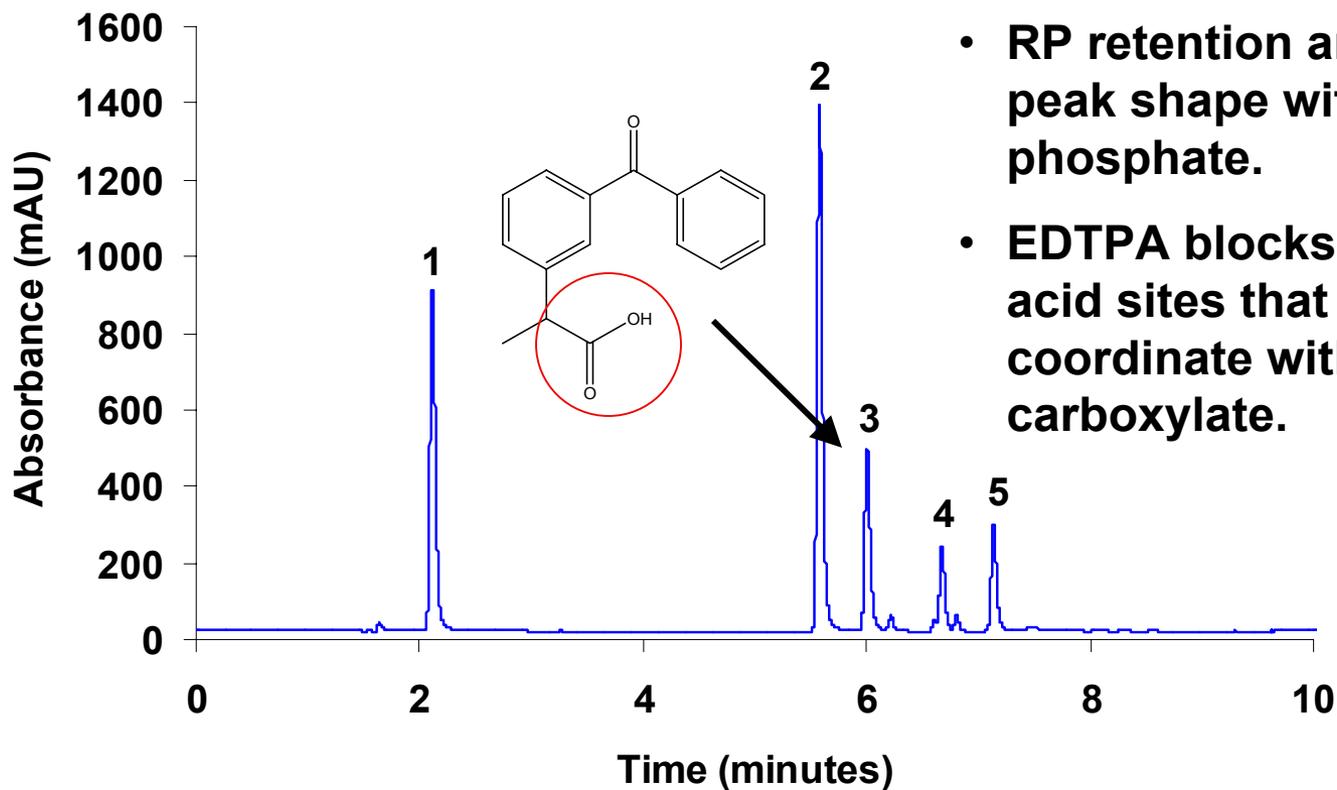
- IE-MS can be a powerful tool that complements other modes of LC-MS.
- IEC selectivity should be orthogonal to RPC and therefore very valuable for multidimensional separations of complex mixtures.



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

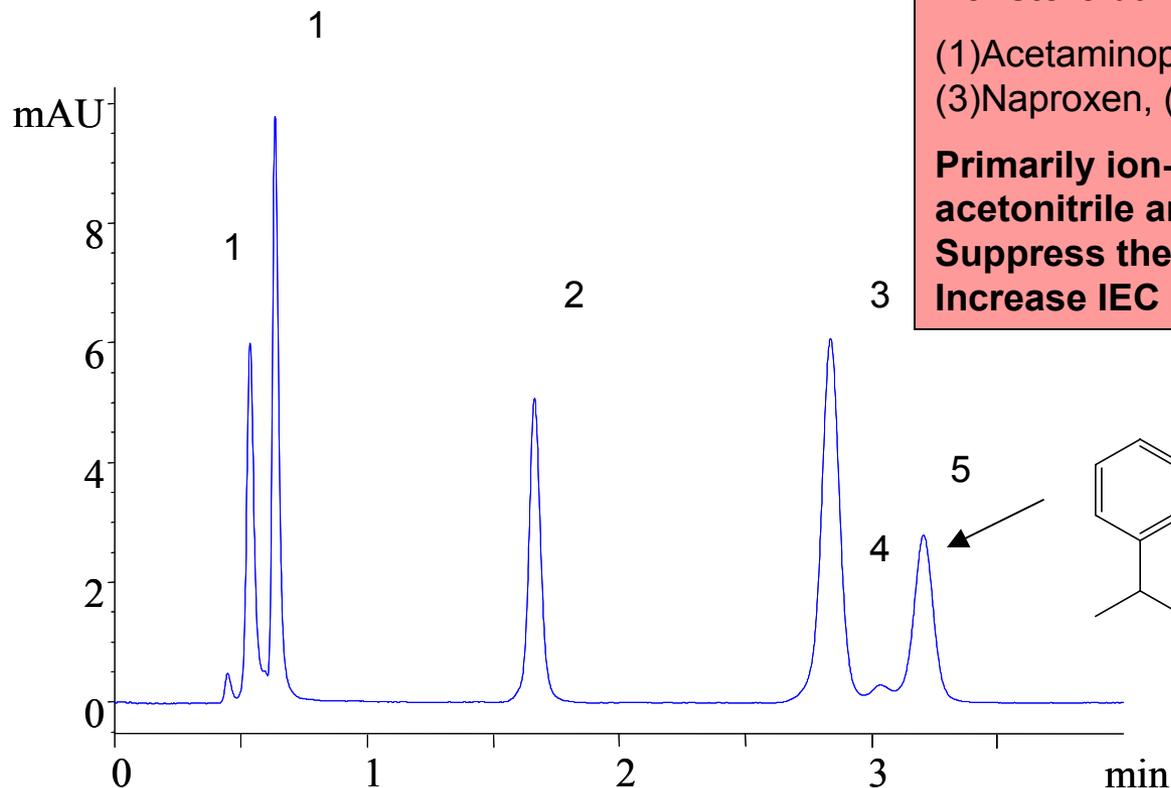


- RP retention and good peak shape without phosphate.
- EDTPA blocks Lewis acid sites that might coordinate with carboxylate.

Time (min.)	%A	%B
0	90	10
10	10	90

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

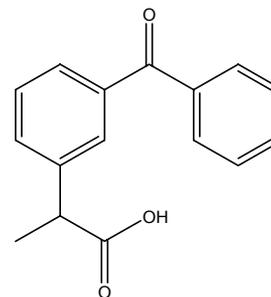
NSAIDs on Zr-PEI-Q



Nonsteroidal Anti-inflammatories:

(1)Acetaminophen, (2)Ibuprofen, (3)Naproxen, (4)Impurity, (5)Ketoprofen

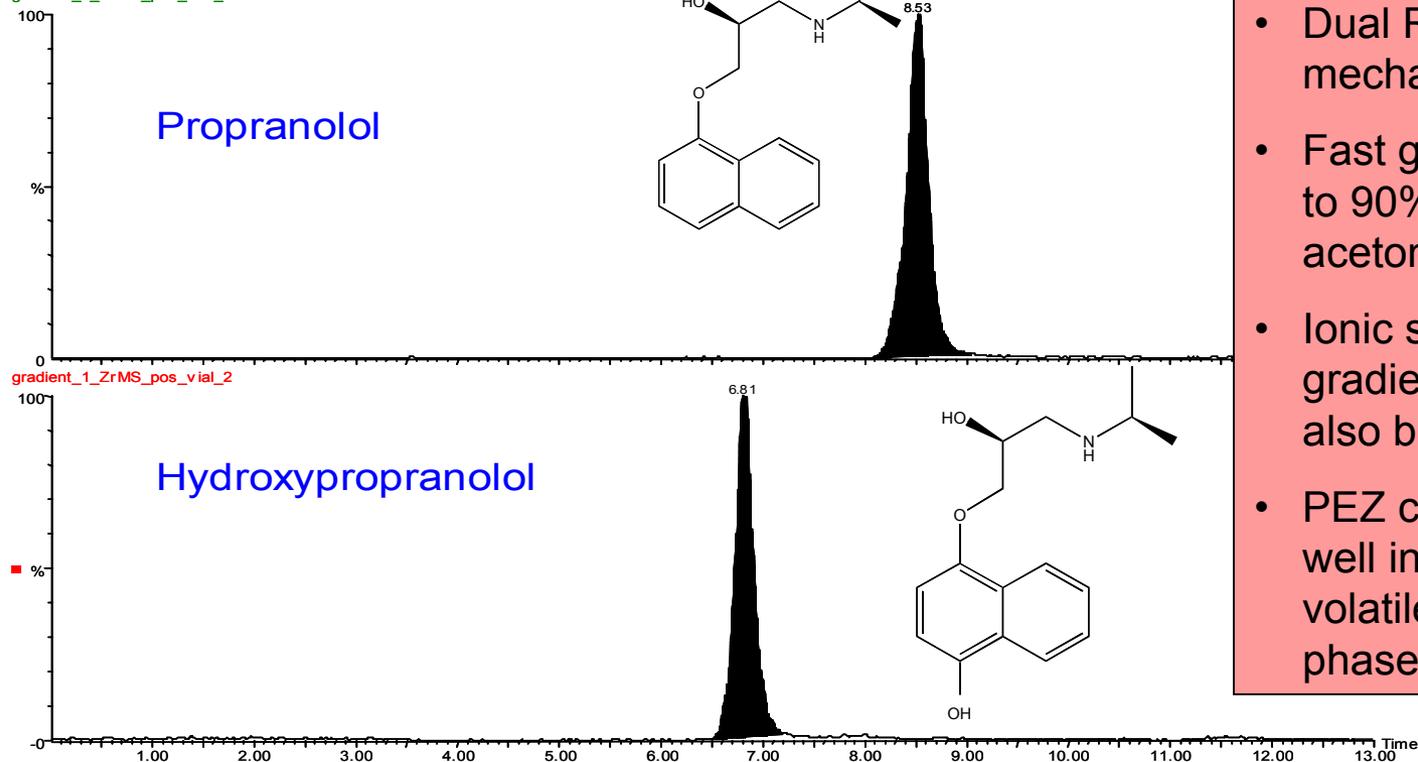
Primarily ion-exchange in 80% acetonitrile and ammonium formate. Suppress the RPC and use the IEC. Increase IEC by raising pH.



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-PBD⁴

10mM AmAc_pH5
gradient_1_ZrMS_pos_vial_2

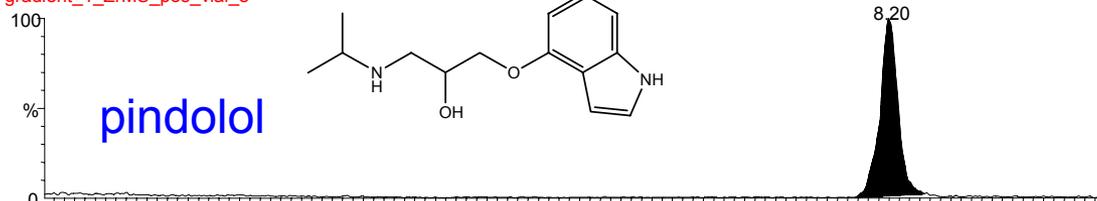


- Dual RP/SCX mechanism
- Fast gradients to 90% acetonitrile
- Ionic strength gradients may also be useful
- PEZ could work well in this volatile mobile phase

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.

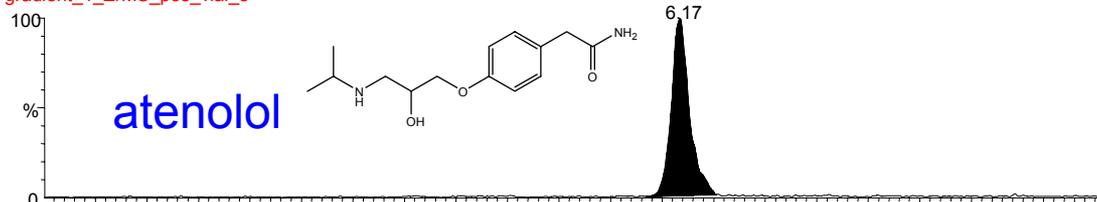
Beta-Blockers on Zr-EDTPA-PBD⁴

10MMAmAc_pH5
gradient_1_ZrMS_pos_vial_8

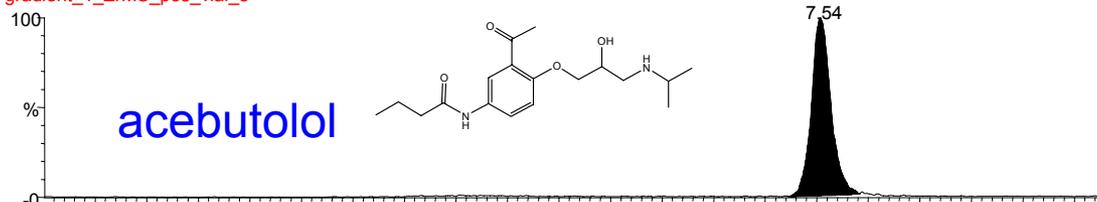


1: Scan ES+
249
1.28e8

gradient_1_ZrMS_pos_vial_8

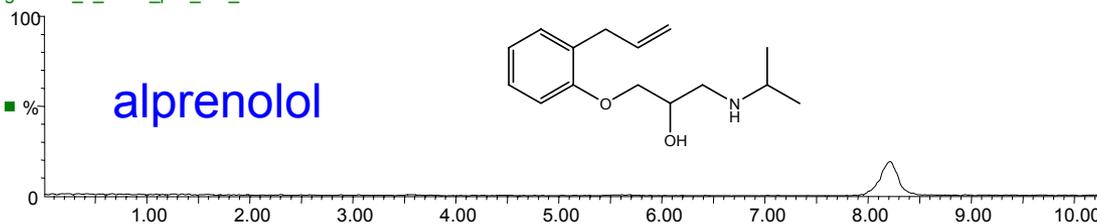


gradient_1_ZrMS_pos_vial_8



337
1.65e8

gradient_1_ZrMS_pos_vial_8



1: Scan ES+
250
2.02e8

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



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

- 1.** Data supplied by ZirChrom Separations Inc., Anoka, MN (www.zirchrom.com).
- 2.** H. F. Walton and R. D. Rocklin, *Ion Exchange in Analytical Chemistry*, CRC Press, Boca Raton, FL (1990).
- 3.** U. D. Neue, E. S. Grumbach, J. R. Mazzeo, K. V. Tran and D. M. Wagrowski-Diehl, Chapter 6 in *Bioanalytical Separations* (I. D. Wilson, Ed.), *Handbook of Analytical Separations, Vol 4*, 2003, Elsevier Science B. V. (presented by Uwe Neue at FACSS 2002).
- 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.**