

Efficient Separation of Important Quaternary Drugs On Zirconia HPLC columns

Richard A Henry* and Hillel K. Brandes

Supelco/Sigma Aldrich, 595 N Harrison Rd, Bellefonte, PA



1

* Consultant, 983 Greenbriar Dr, State College, PA



SIGMA-ALDRICH

Review of Zirconia Particles

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

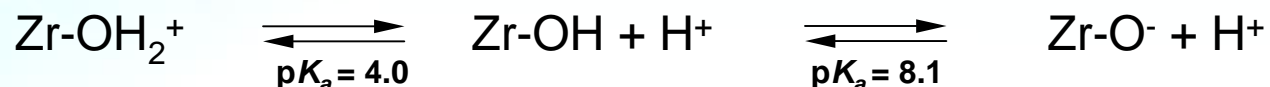
| Parameter | Property |
|--|--------------------|
| Pore Diameter (Å) | 250- 300 |
| Porosity | 0.45 |
| Particle Diameter (µm) | 3, 5, 10 |
| Density (gm/cc) | 5.8 (2.5 x silica) |
| Pore Volume (cc/g) | 0.13 |
| Surface Area (m ² /g) | 22 |
| Lewis Acid Site (µmol/m ²) | 4-5 |

- Various surface modifications available: Discovery[®] Zr-PS, Discovery[®] Zr-PBD, Discovery[®] Zr-Carbon, Discovery[®] Zr-CarbonC18

Zirconia Surface¹

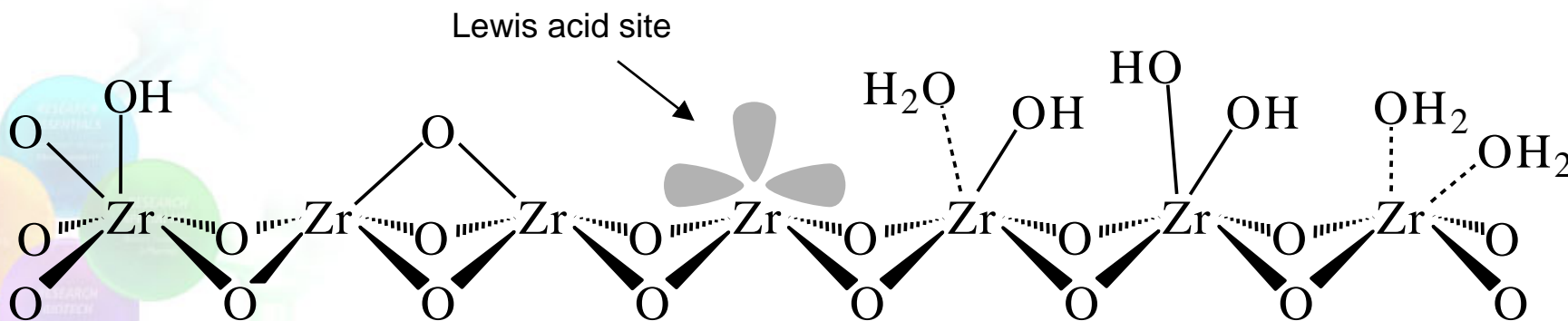
Surface properties of zirconia oxide of chromatographic relevance:

- Charge:



- 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)

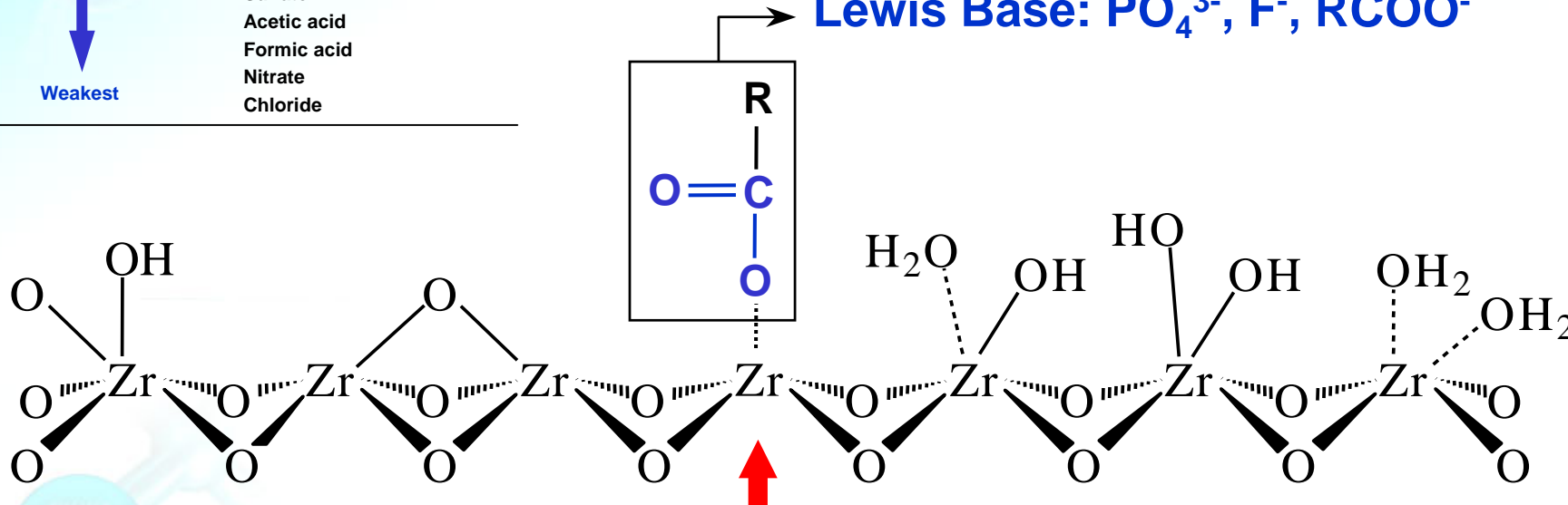


SIGMA-ALDRICH

Zirconia Lewis Acid-Base Chemistry

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

Lewis Base: PO_4^{3-} , F^- , RCOO^-

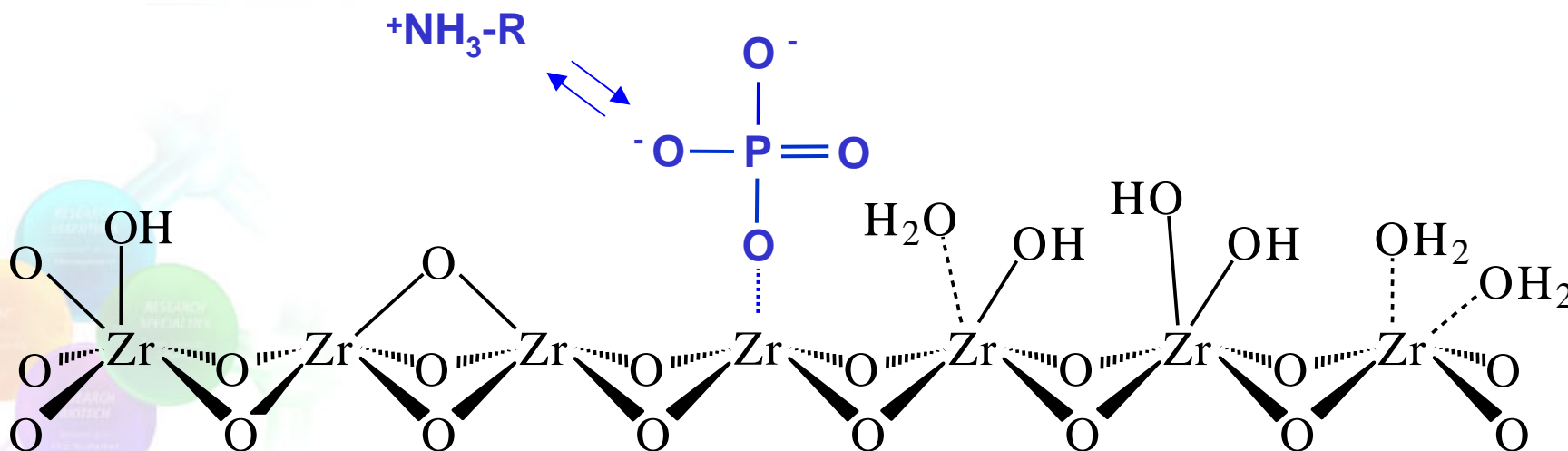


Strong Lewis Acid Site Dominates Surface Chemistry

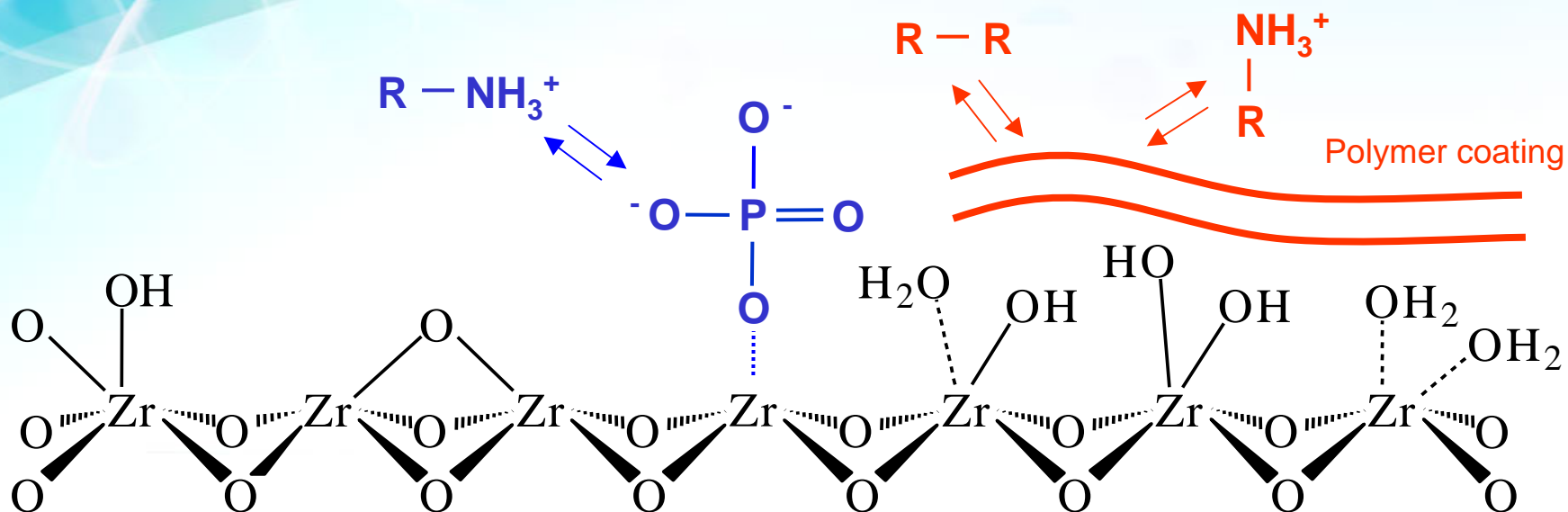
- Ligand Exchange: $\text{Zr-L} + \text{A}^- \rightleftharpoons \text{Zr-A}^- + \text{L}$
- When ligand is charged, surface becomes charged!

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



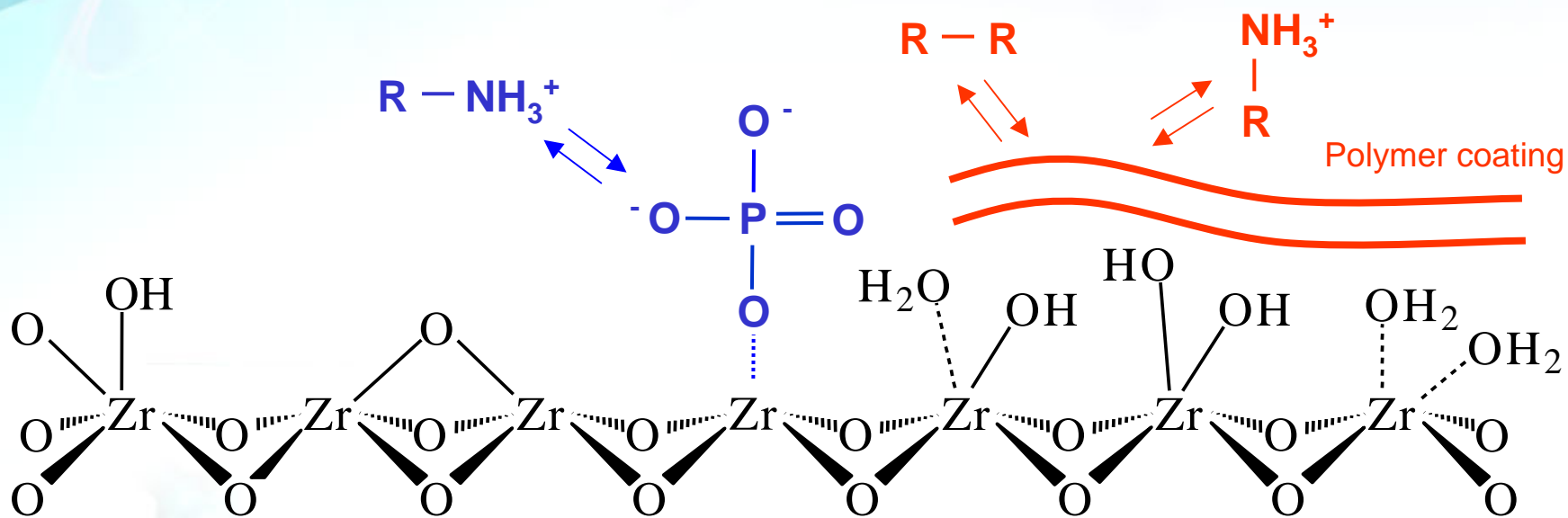
- Polymer coating: **hydrophobic retention**
- Coordinated Lewis Base ions: **ion-exchange retention**
 - $Zr-L^-:X^+ + A^+ = Zr-L^-:A^+ + X^+$ (*very important mechanism*)
- Zirconols:
 - $Zr-O^-:X^+ + A^+ = Zr-O^-:A^+ + X^+$ (*unimportant mechanism*)

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



SIGMA-ALDRICH

Mixed-Mode Behavior of Coated Zirconia Phases

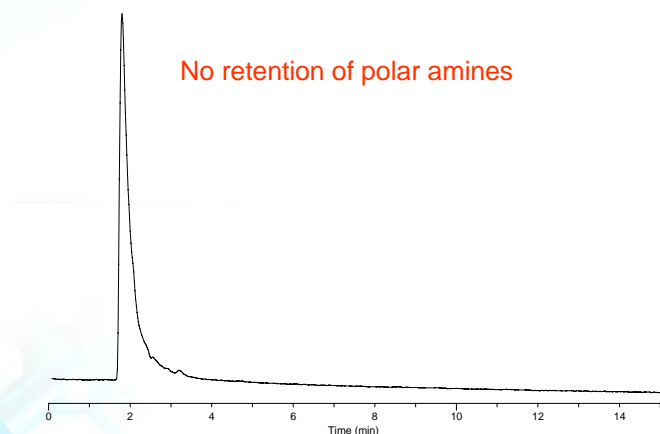


- Retention of ionic analytes modulated by pH, choice of buffer/salt, buffer/salt concentrations, and temperature
- Selectivity affected by these mobile phase parameters as well

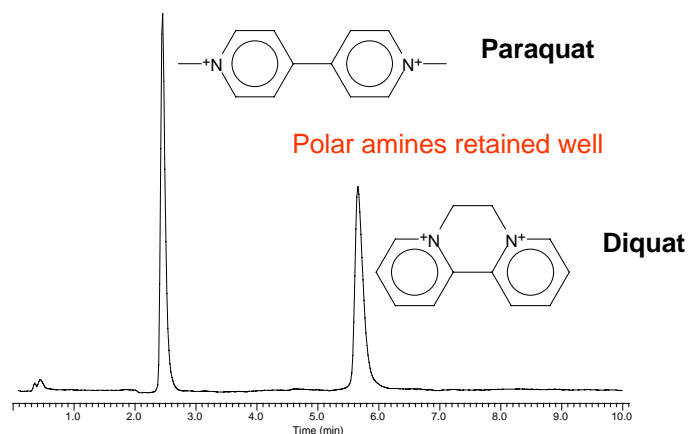
Zirconia Displays Unique Ion Exchange (IEX) Selectivity

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

Discovery[®] C18



Discovery[®] Zr-PS or Discovery[®] Zr-PBD



- 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, 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.

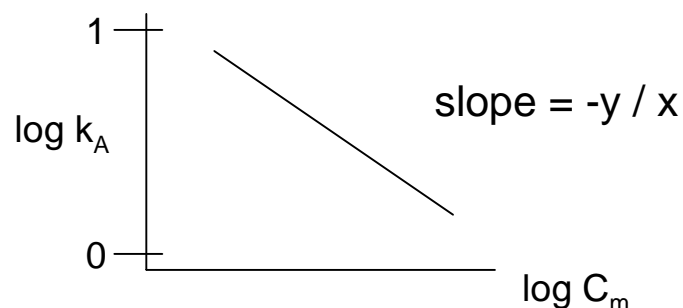


Quantitative Retention Parameters in IEX LC³

- 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.

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

- 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, 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; 3 μ , 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)

$x = \text{water}$; $y = \text{acetonitrile}$, either 10, 20, or 30

total conc of sodium in mobile phase B, is 50 mM.

Flow: 0.3 mL/min

Temperature: 80° C

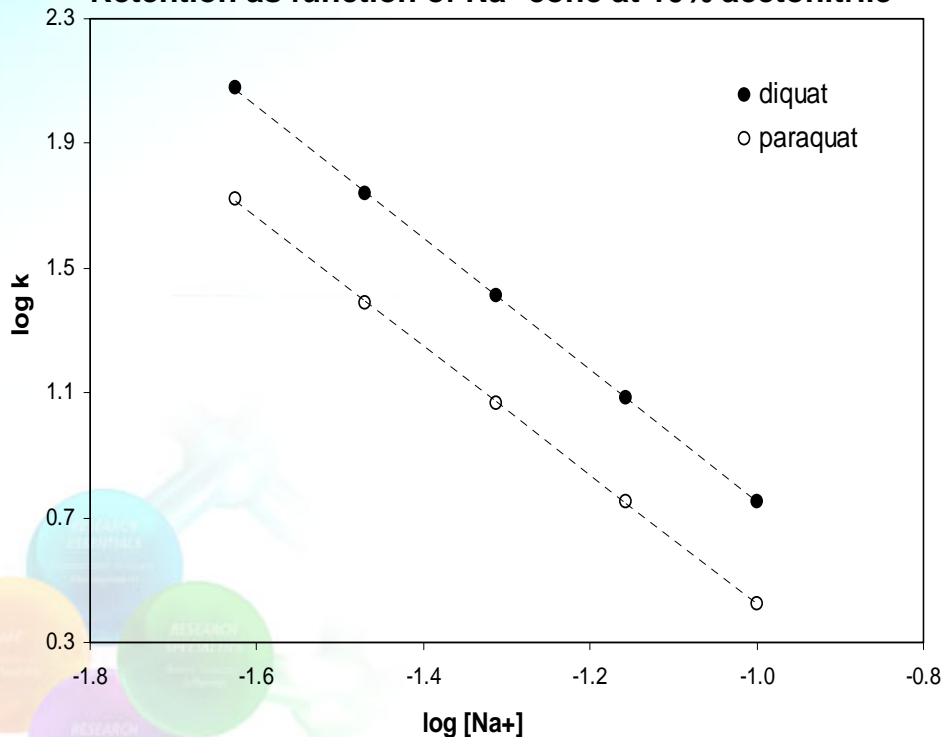
Inj: 1 μ L

Detection: 225 nm

Data Analysis of Quaternary Herbicides: log plots

Example of Data Analysis

Retention as function of Na⁺ conc at 10% acetonitrile

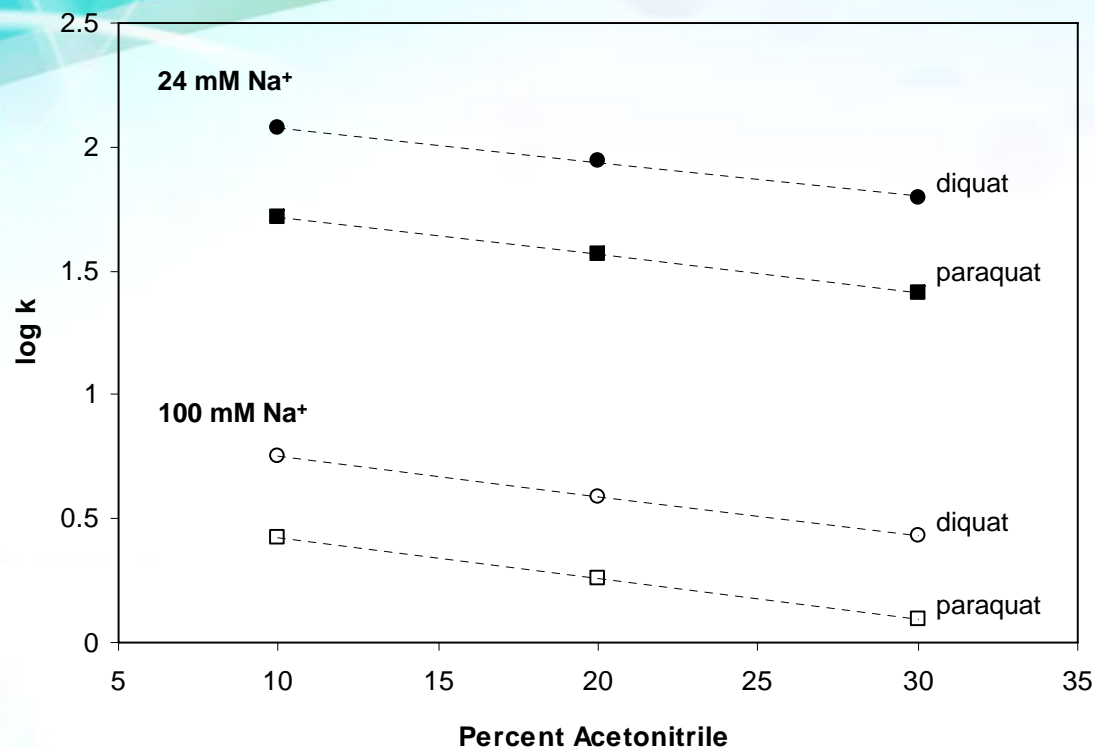


Data Summary

| Analyte | Slope | | |
|----------|----------|----------|----------|
| | 10% MeCN | 20% MeCN | 30% MeCN |
| diquat | -2.114 | -2.166 | -2.180 |
| paraquat | -2.064 | -2.096 | -2.110 |

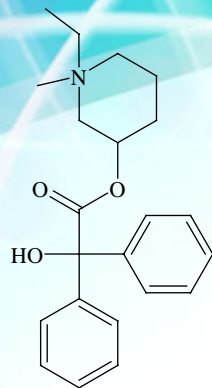
- Recall, slope is parameter that indicates degree of IEX as retention mechanism.
- With analyte charge of 2 and counterion charge of 1, slope of -2 indicates that the charges on analyte are fully accessible to the IEX sites on the chromatographic surface.
- IEX is very effective as mechanism of retention on this phase (polymer-coated zirconia).

Retention of Herbicides vs Percent Organic

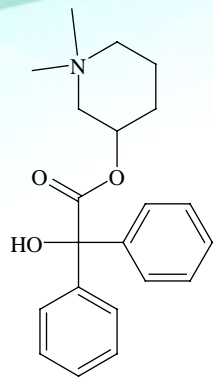


- Hydrophobic interaction on the polymer-coated zirconia is a component of retention for diquat and paraquat, but not as dramatic as that of IEX.
- Neither level of organic nor concentration of mobile phase counterion impacts selectivity to a significant degree.
- Level of organic and concentration of mobile phase counterion control retention, therefore either type of gradient can be effective.

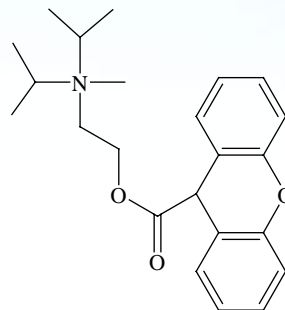
Anticholinergics as Probes for IEX Retention



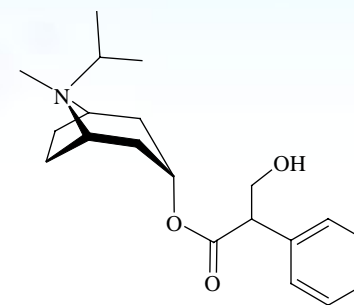
piperizolate



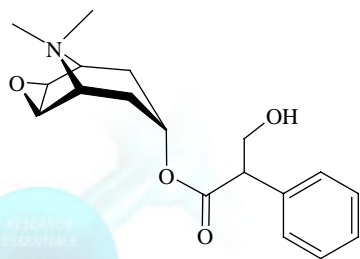
mepenzolate



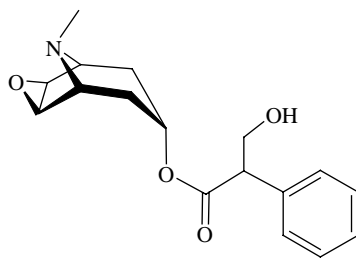
propantheline



ipratropium



methscopolamine



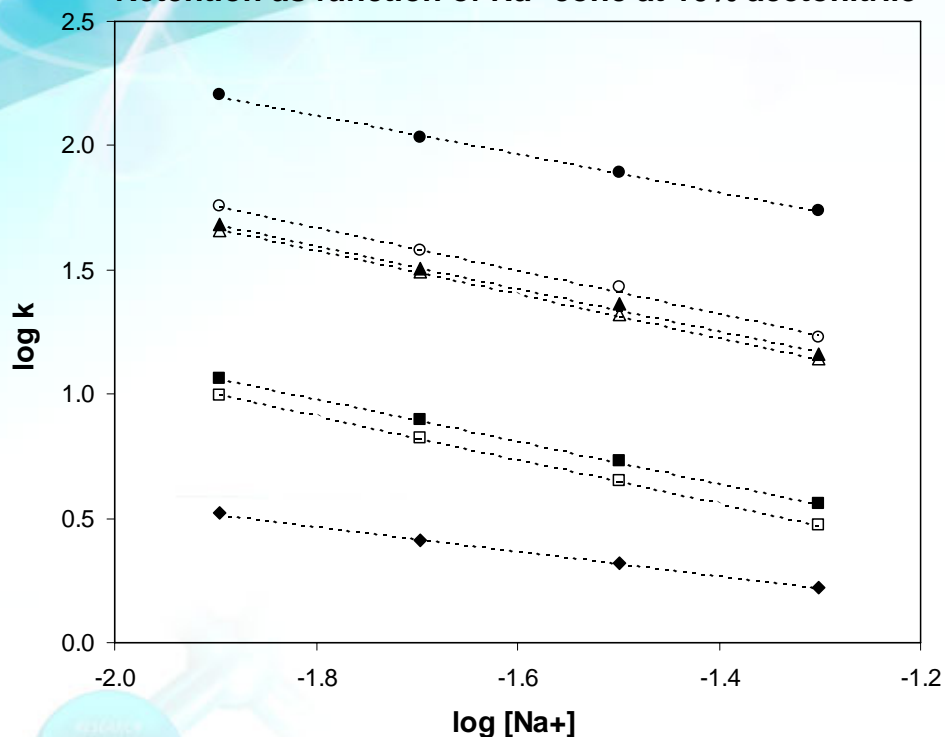
scopolamine

- 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

| Analyte | Slope | | |
|-----------------|----------|----------|----------|
| | 10% MeCN | 20% MeCN | 30% MeCN |
| proprantheline | -0.781 | -0.950 | -0.962 |
| pipenzolate_3 | -0.871 | -0.968 | -0.991 |
| pipenzolate_2 | -0.865 | -0.961 | -0.991 |
| mepenzolate | -0.871 | -0.974 | -0.991 |
| ipratropium | -0.845 | -0.981 | -1.030 |
| methscopolamine | -0.884 | -1.010 | -1.060 |
| scopolamine | -0.500 | -0.548 | -0.512 |

- 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.

IEX Retention Models⁴

- Pure IEX

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

β_{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)

$$k = k_{RP} \cdot \frac{K}{[C^+]}$$

K is a constant that accounts for the IEX equilibrium and the total number of available ion-exchange sites per unit area

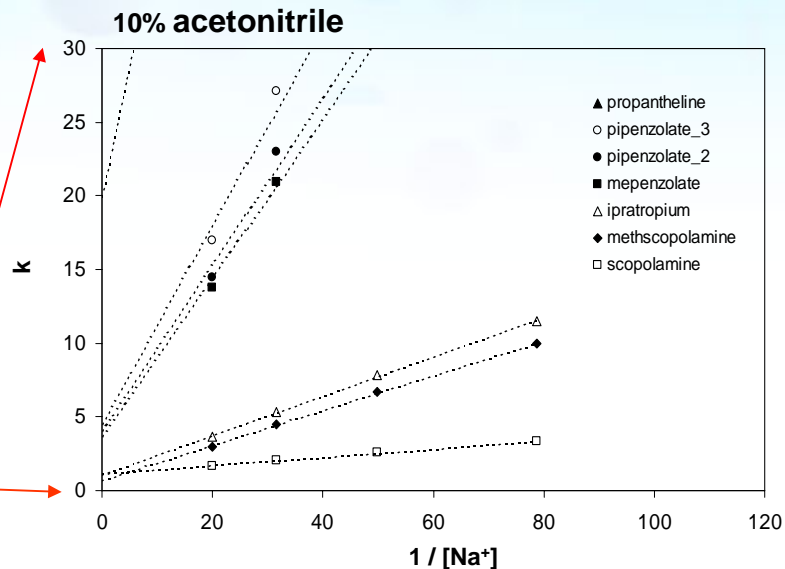
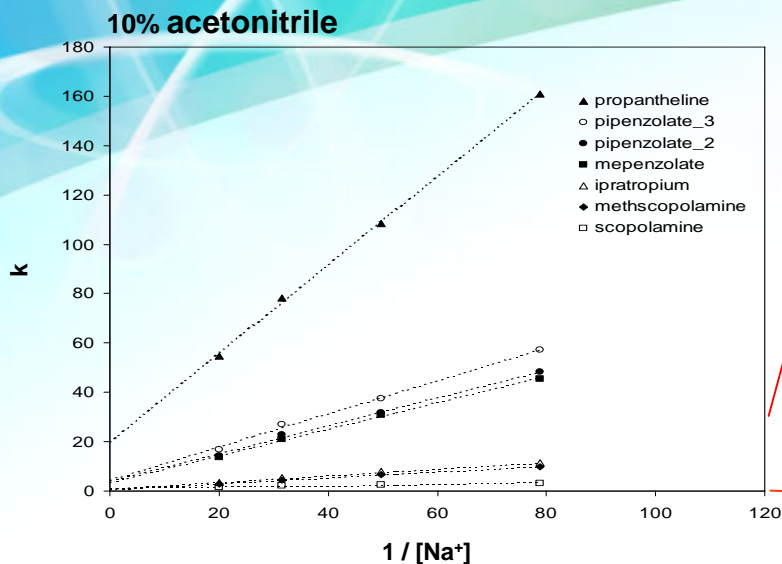
Plot of k vs $1/[C^+]$, will be linear with an extrapolated intercept of 0 (i.e. at infinitely high $[C^+]$, retention will be zero).

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

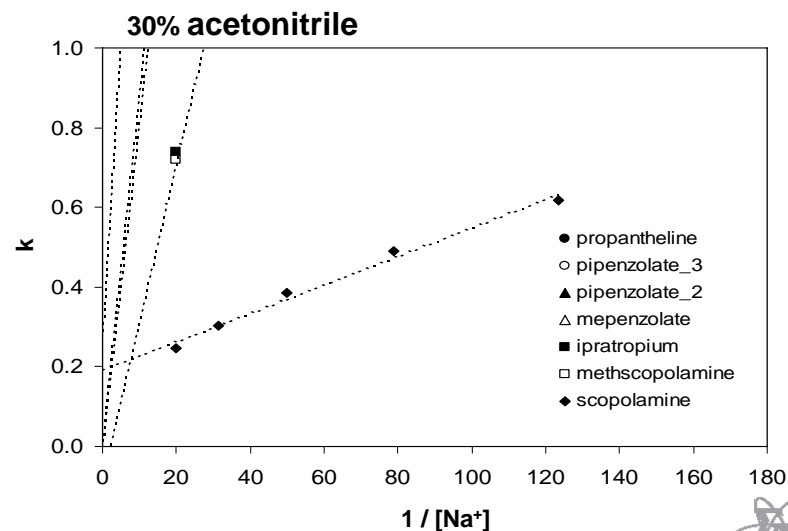
$$k = k_{RP} + \frac{\beta_{IEX}}{[C^+]}$$

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 its physical and chemical stability; furthermore, it can be readily modified with hydrophobic coatings, and derivatized by virtue of its 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

1. Blackwell, J.A. & Carr, P.W. 1991. *J Liq Chrom* 14: 2875
2. Henry, R.A. et. al. 2005. EAS presentation.
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. Yang, X. et. al. 2003. *J Chrom A* 996: 13.
5. Hu, Y, et.al. 2002. *J Chrom A* 968: 17.
6. Yang, X., et. al. 2003. *Anal Chem* 75: 3153.

