Efficient Separation of Important Quaternary Drugs On Zirconia HPLC columns

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Review of Zirconia Particles

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

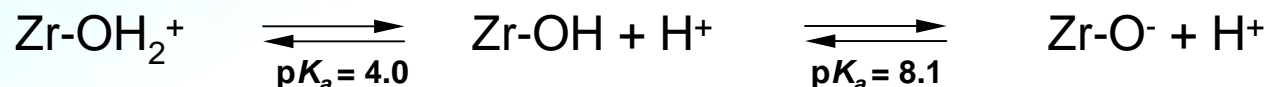| Parameter	Property
Pore Diameter (Å)	250- 300
Porosity	0.45
Particle Diameter (μm)	3, 5, 10
Density (gm/cc)	5.8 (2.5 x silica)
Pore Volume (cc/g)	0.13
Surface Area (m^2/g)	22
Lewis Acid Site (μmol/m^2)	4-5

- Various surface modifications available: Discovery® Zr-PS, Discovery® Zr-PBD, Discovery® Zr-Carbon, Discovery® Zr-CarbonC18
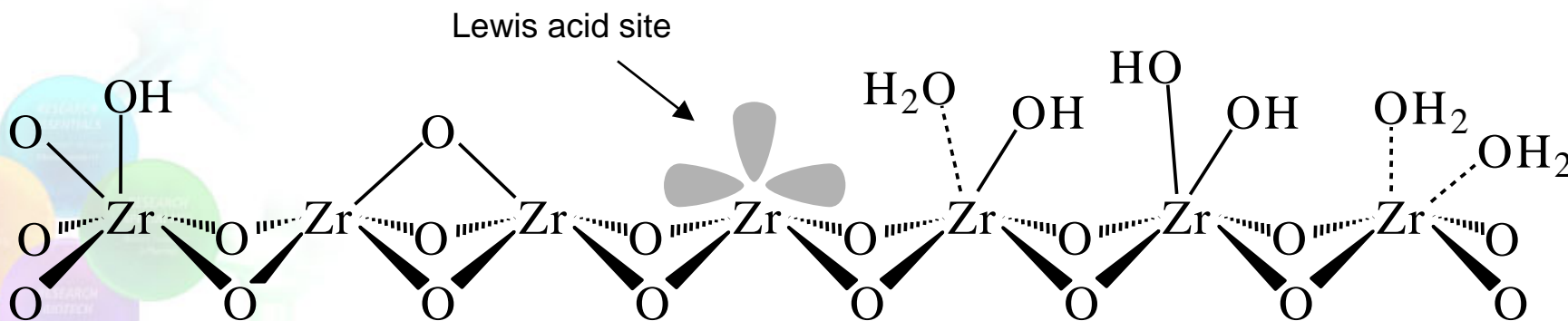
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Zirconia Surface[1]

Surface properties of zirconia oxide of chromatographic relevance:

- Charge:

$$Zr\text{-}OH_2^+ \quad \xrightleftharpoons{\quad pK_a = 4.0 \quad} \quad Zr\text{-}OH + H^+ \quad \xrightleftharpoons{\quad pK_a = 8.1 \quad} \quad Zr\text{-}O^- + H^+$$

- Lewis acid* sites:

  • Coordinately unsaturated zirconium (IV) sites
  • very reactive toward Lewis bases

Lewis acid site

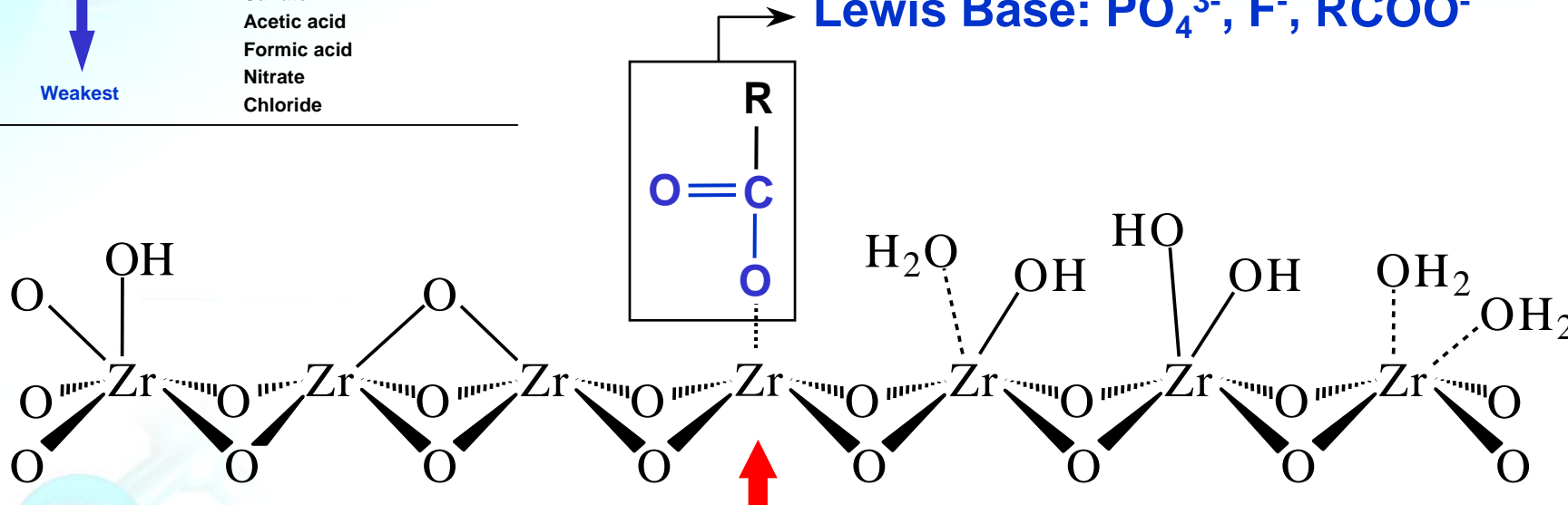*Base is electron donor; acid is electron acceptor; (more general definition than Brønsted)

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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: Zr-L + A$^-$ $\longleftrightarrow$ Zr-A$^-$ + L
- When ligand is charged, surface becomes charged!

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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.

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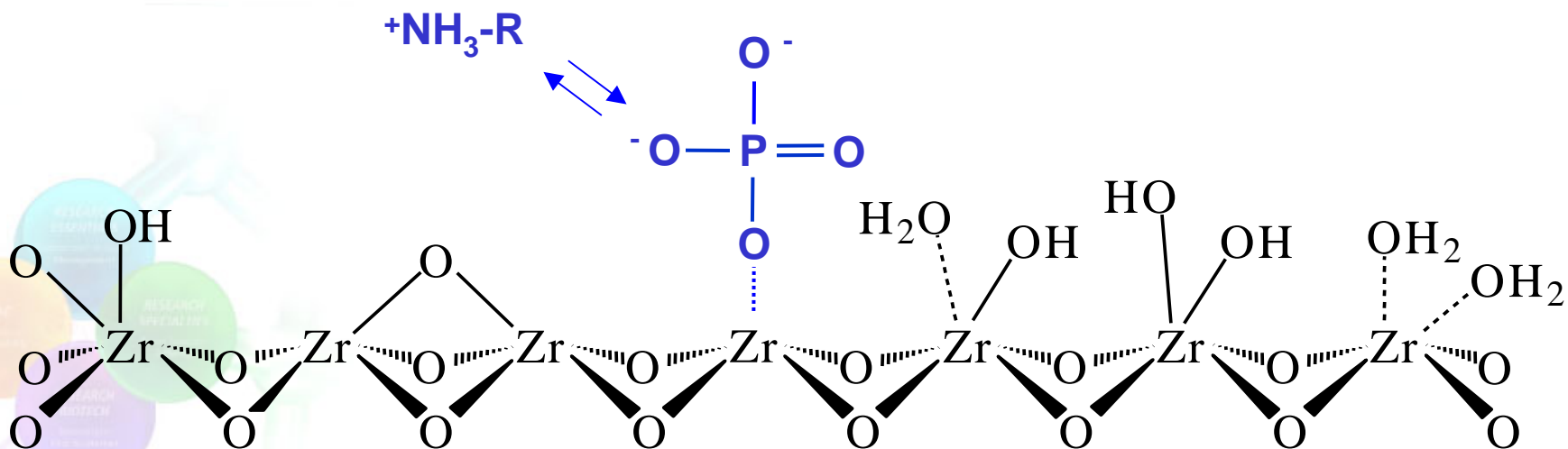
Mixed-Mode Behavior of Coated Zirconia Phases
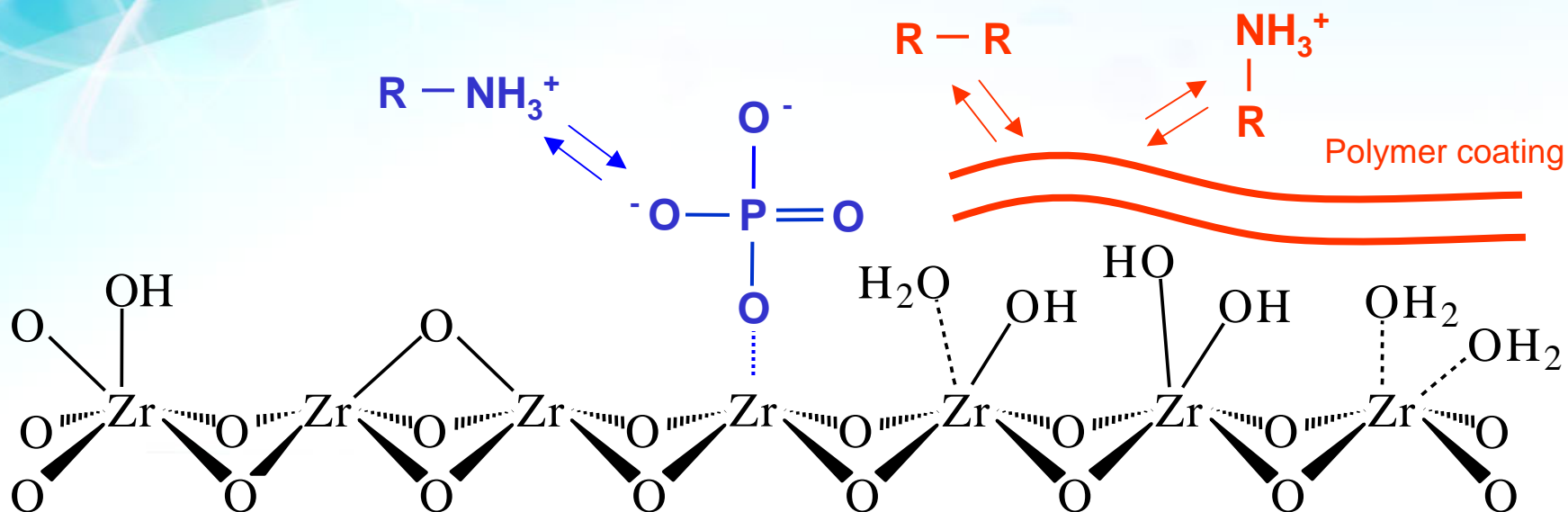
- Polymer coating: hydrophobic retention
- Coordinated Lewis Base ions: ion-exchange retention
  - $Zr-L^-:X^+ + A^+ = Zr-L^-:A^+ + X^+$ (very important mechanism)
- Zirconols:
  - $Zr-O^-:X^+ + A^+ = Zr-O^-:A^+ + X^+$ (unimportant mechanism)

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

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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
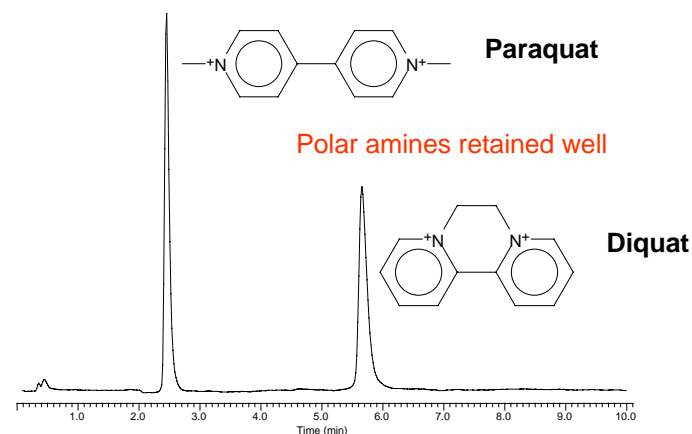
Zirconia Displays Unique Ion Exchange (IEX) Selectivity

Mixed-mode zirconia can provide separation that is
difficult to accomplish on high-purity C18-silica[2]

Discovery® C18

No retention of polar amines
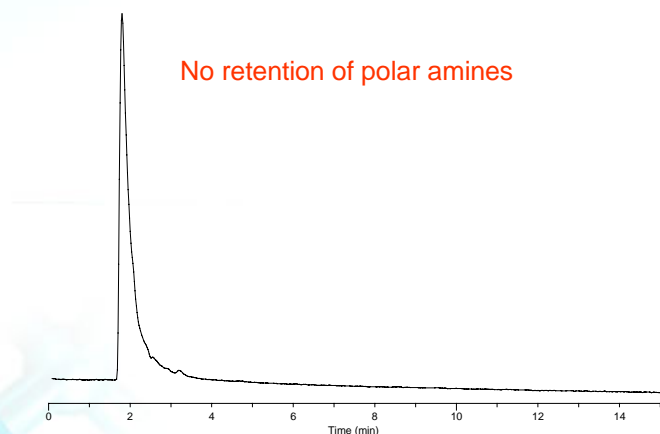
Discovery® Zr-PS or Discovery ® Zr-PBD

Paraquat

Polar amines retained well

Diquat

- •**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, 3µm particles; 5% CH_3CN in 25mM H_3PO_4 (to pH 7 with NH_4OH);
35°C, 1mL/min, UV 290nm
Zr-PS conditions: Discovery Zr-PS, 7.5cm x 4.6mm, 3µm particles; 50% CH_3CN in 25mM H_3PO_4, 25mM NH_4F, (to pH 8 with
NH_4OH); 65°C, 3mL/min.

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Quantitative Retention Parameters in IEX LC[3]

- 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.

slope = -y / x

log k_A

log C_m

1

0
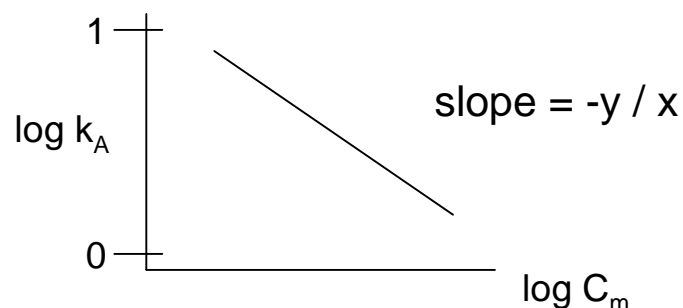
* model assumes thermodynamic equilibrium, unit activity coefficients and absence of analyte interaction by forces other than coulombic attraction.

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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; 3µ, 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 = water; y = 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 µL
Detection:	225 nm

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Data Analysis of Quaternary Herbicides: log plots

Example of Data Analysis

Retention as function of Na$^+$ conc at 10% acetonitrile
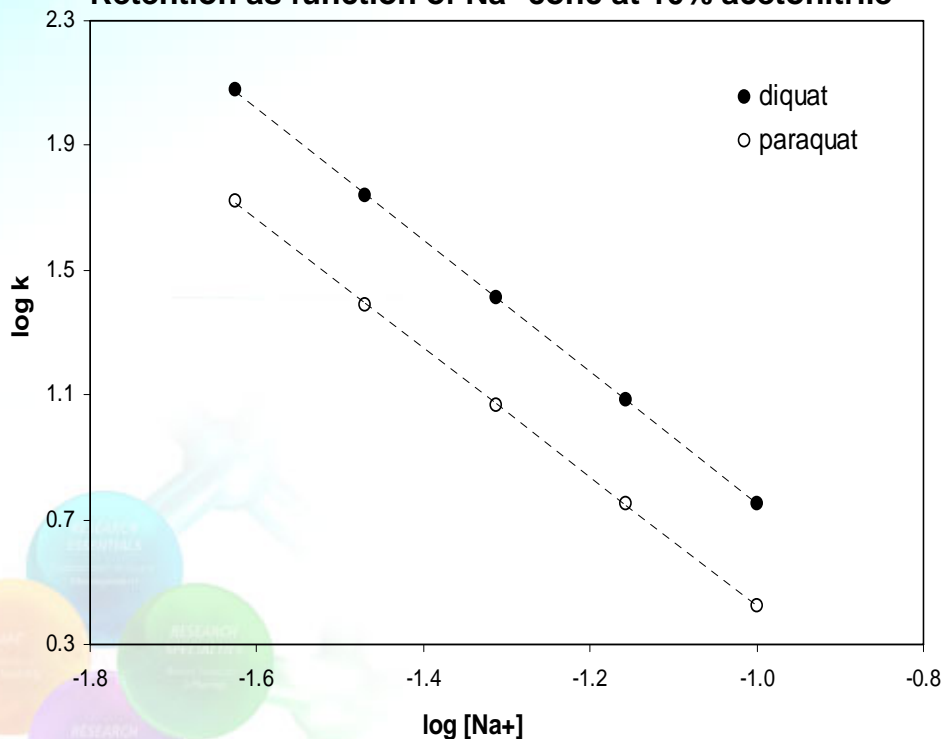

Data Summary

| 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).

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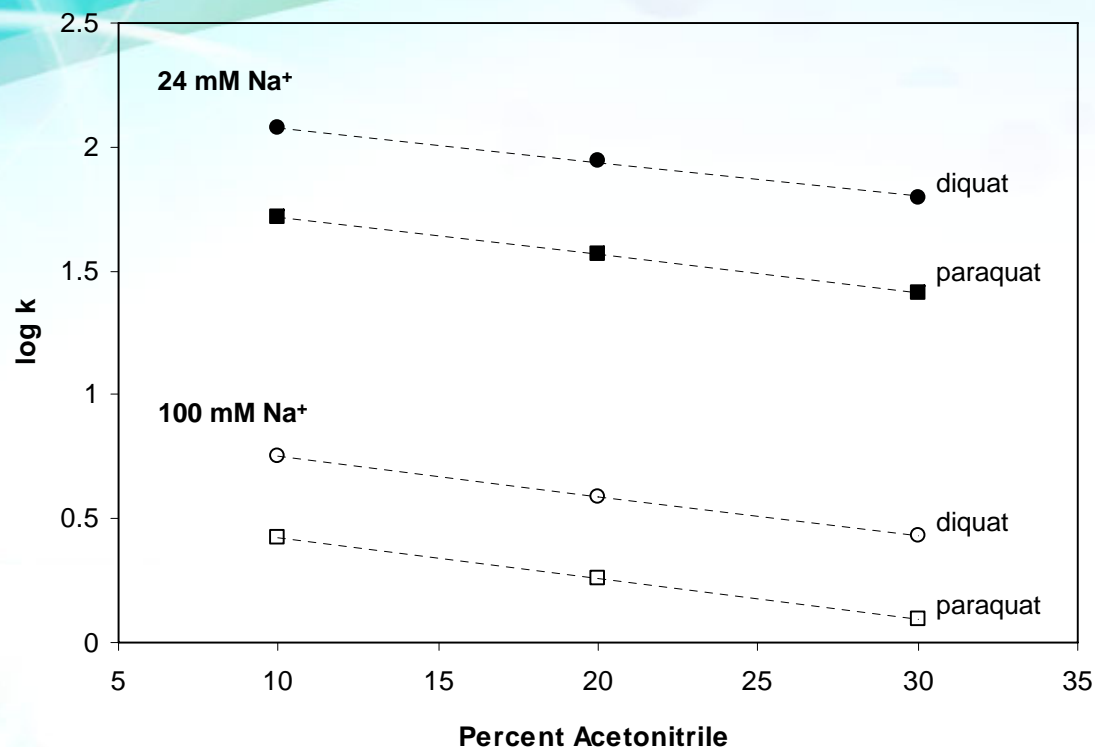
- Hydrophobic interaction on the polymer-coated zirconia is a component of retention for diquat and paraquat, but not as dramatic as that of IEX.
- Neither level of organic nor concentration of mobile phase counterion impacts selectivity to a significant degree.
- Level of organic and concentration of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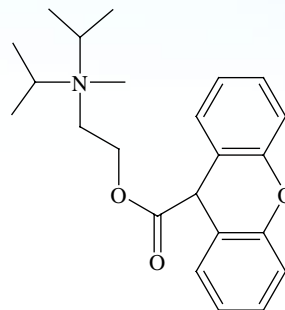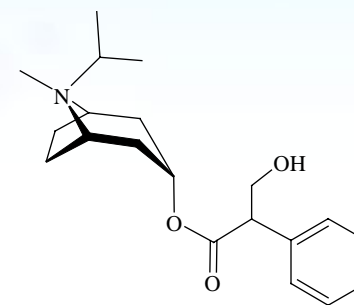
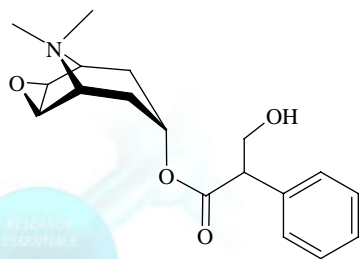# Anticholinergics as Probes for IEX Retention
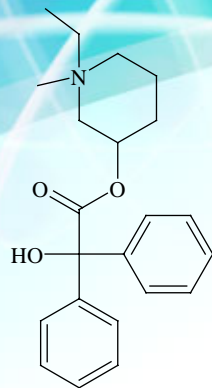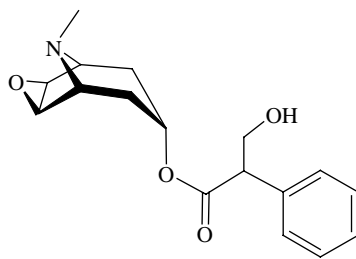
pipenzolate
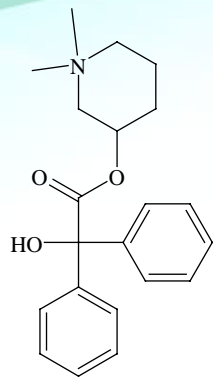
mepenzolate

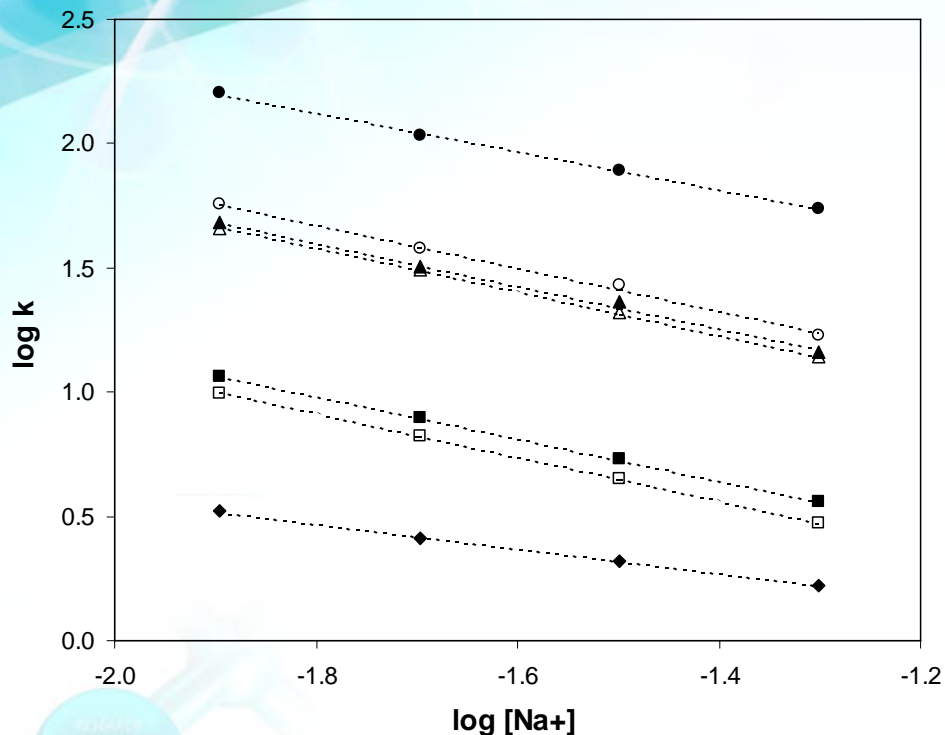**propantheline**

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

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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
propantheline	-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.

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IEX Retention Models[4]

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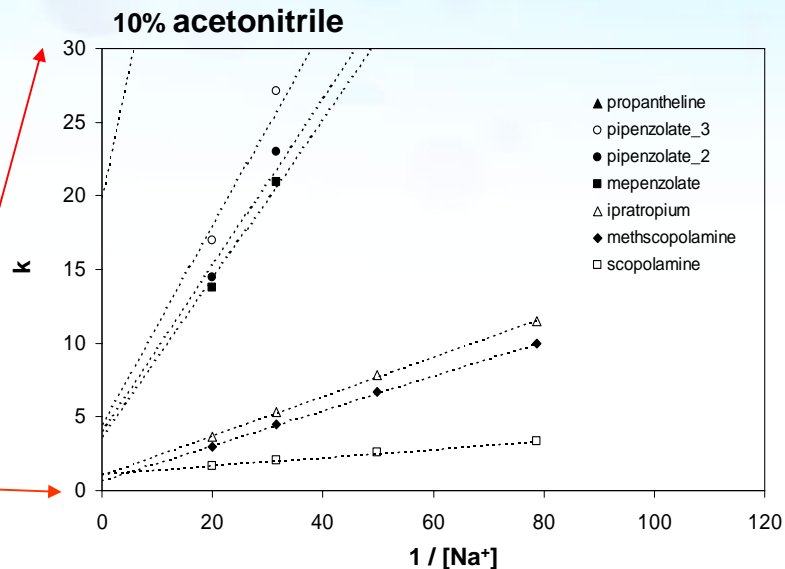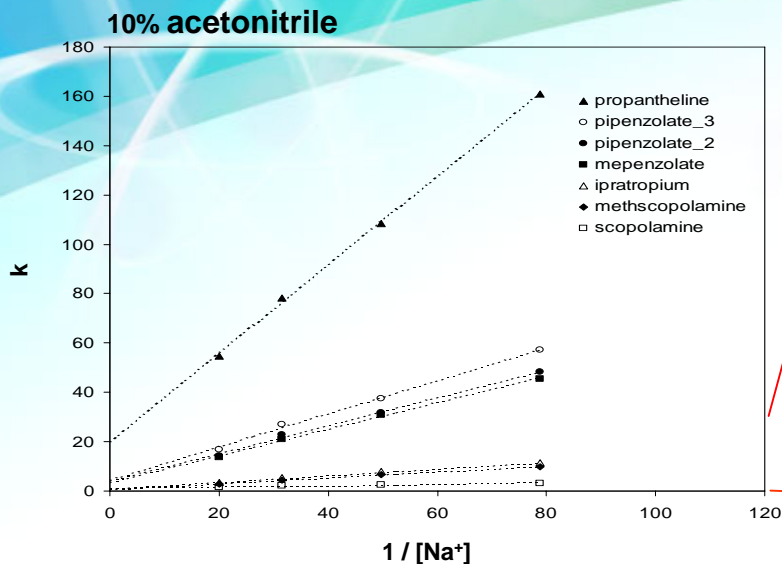
  Plot of k vs $1/[C^+]$, will be linear with an extrapolated intercept of 0 (i.e. at infinitely high $[C^+]$, retention will be zero).

 - 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.

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Data Analysis: reciprocal plots

10% acetonitrile

10% acetonitrile

- 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 supressed.

30% acetonitrile

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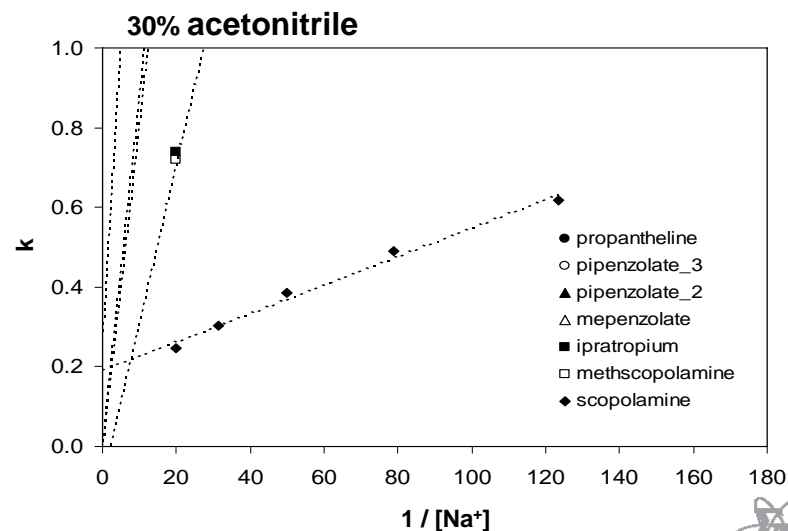
Conclusions

- Zirconia is an attractive LC matrix because of it's physical and chemical stability; furthermore, it can be readily modified with hydrophobic coatings, and derivatized by virtue of it's 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-dervivatized) 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.

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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.

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