Evaluation of 1.7µm Porous Zirconia Particles for UHPLC

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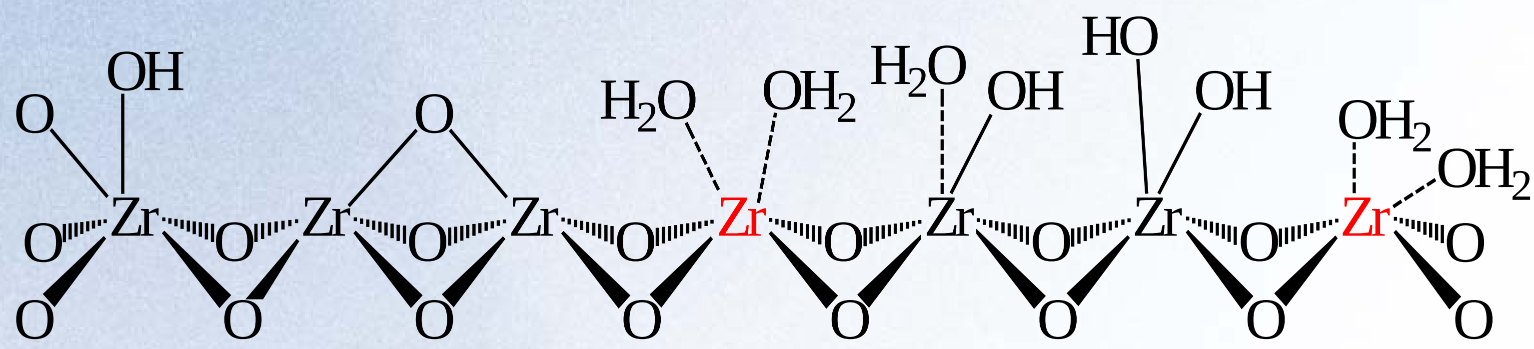
Outline

- **Chemistry of zirconia-based HPLC columns**
  - **Surface chemistry**
  - **Eluotropic series**
  - **Polymer coated zirconia**
- **Zirconia phases for Multi-Mode applications and unique selectivity**
  - **Zr-PBD and Zr-PS [RP and SCX in phosphate mobile phases]**
  - **Zr-MS [RP and SCX in any mobile phase (MS friendly)]**
  - **Zr SAX [RP and SAX in any mobile phase]**
- **Evaluation of sub-2µm Zr-PBD for UHPLC**
  - **Specifications**
  - **Performance (van Deemter Plots)**
  - **Applications**

Surface Chemistry of Zirconia



Zirconia chemistry is dominated by Lewis acid-base reactions (electron donor-acceptor) involving empty d-orbitals.

Lewis Acid: Zr(IV): H_2O + RPO_3^{2-} $\rightleftharpoons$ Zr(IV): RPO_3^{2-} + H_2O

Other Lewis base examples: PO_4^{3-}, RCO_2^- , F^-, Catechol, etc.

Interaction Strength of Lewis Bases with Zirconia[1]

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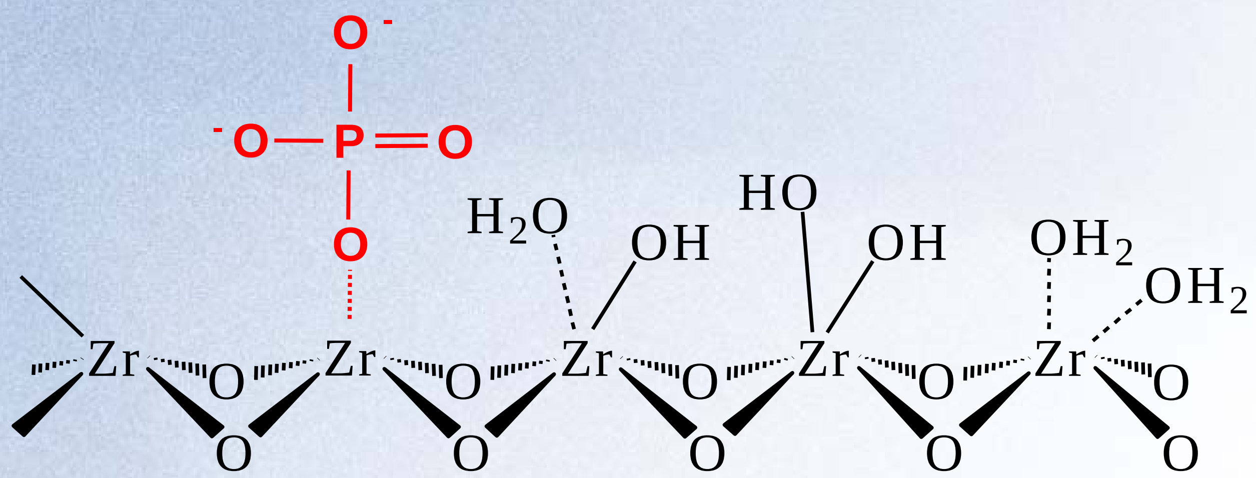
Interaction Strength	Lewis Base (L)
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
	Nitrate
	Chloride
Weakest	Water

Small Lewis base (anions) with high electron density and low polarizability interact more strongly with Zr atoms.

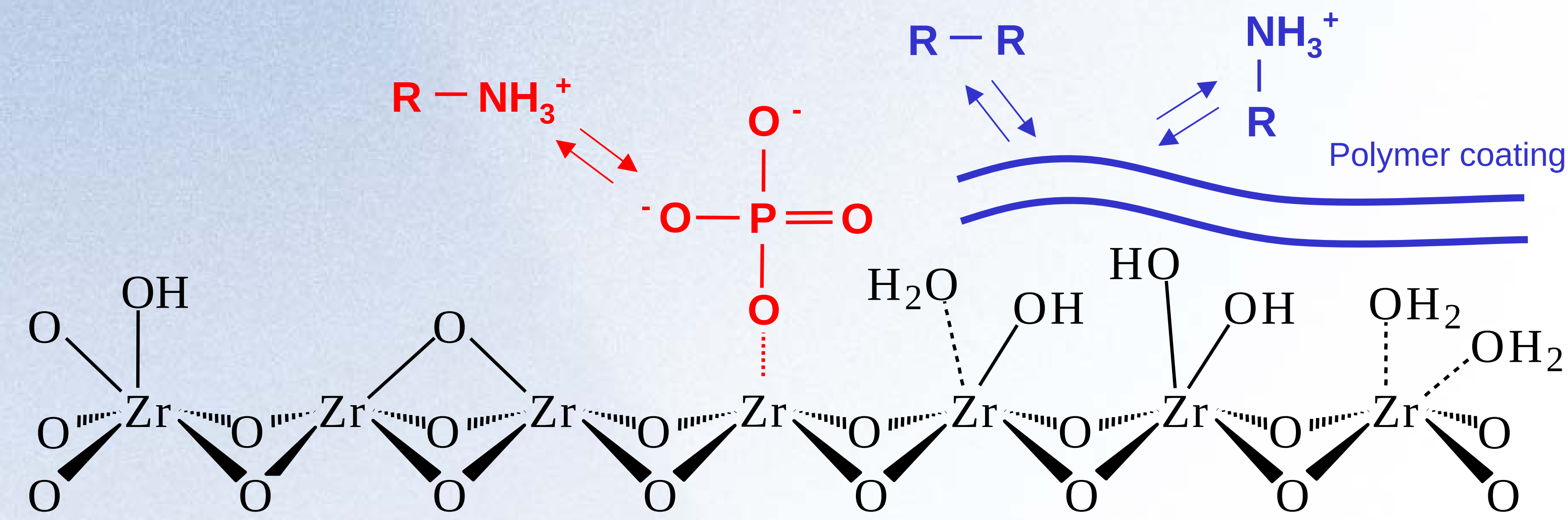
Strong Lewis base solutes adsorb to Zr atoms unless displaced by stronger bases in mobile phase.



Multi-Mode Behavior of Zirconia

- Zirconia substrate exhibits reproducible polar and ionic solute interaction.
- With stable organic coatings, reproducible reversed-phase behavior can be added.
- Extreme resistance to temperature, pH and mechanical stress are unique advantages.

Addition of RP Behavior in Coated Zirconia Phases[2]



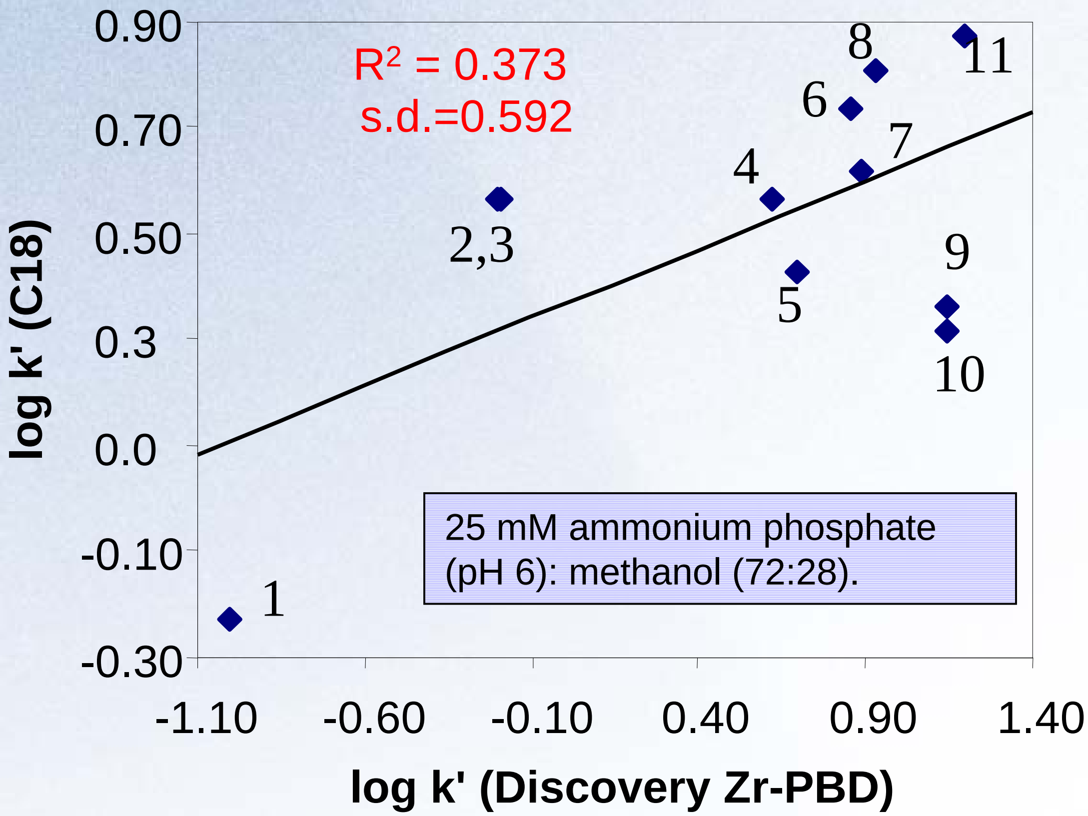
- Retention (and selectivity) of ionic analytes modulated by pH, buffer/salt type and concentrations, and temperature.
- Retention of neutral solutes modulated by organic solvent.

Definition of Multi-Mode HPLC

- Certain columns have two or more HPLC retention modes working simultaneously.
- Can be called Multi-Mode, Dual-Mode or Mixed-Mode.
- Typically, modes include Reversed-Phase, Ion-Exchange (anion or cation) and Normal-Phase (or HILIC in aqueous mobile phases).
- These modes are orthogonal to one another (analytes usually elute in different order).

C18 and Zr-PBD are Orthogonal for Basic Drugs[2]

C18 (RP) columns separate mainly by hydrophobic forces and Zr-PBD columns separate by a combination of ionic and hydrophobic forces



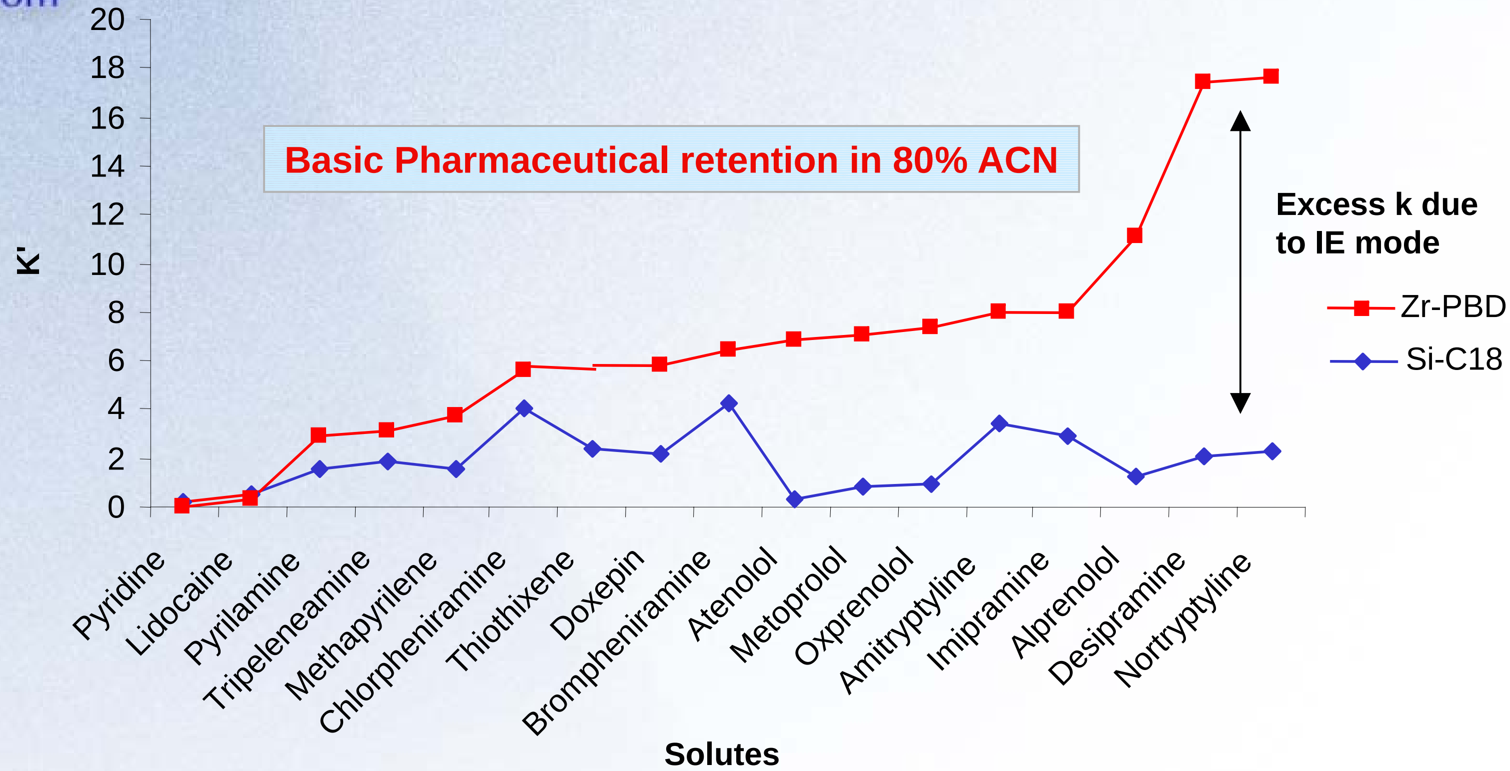
Zr-PBD and Si-C18 have very different selectivity for ionic drugs under phosphate conditions due to the SCX component of ZrO_2.

Solutes

1. **Chlordiazepoxide**
2. **Hydroxyzine**
3. **Buclizine**
4. **Thiothixene**
5. **Doxepin**
6. **Amitriptyline**
7. **Imipramine**
8. **Perphenazine**
9. **Nortriptyline**
10. **Desipramine**
11. **Thioridazine**

Data provided by Sigma-Supelco

IEX Mode Increases Retention for Zr-PBD vs Si-C18[2]



LC Conditions: Machine-mixed 80/20 ACN/10 mM ammonium acetate pH=6.7 without pH adjustment; Flow rate, 1.0 mL/min.; Injection volume 0.1 µL; Temperature, 35 ^{o}C; Detection at 254 nm; Columns, Zr-PBD, 50 x 4.6 mm i.d. (3 µm particles); Silica-C18 150 x 4.6 mm i.d., (3.5 µm particles).

Ion-Exchange Mode on Zirconia[3]

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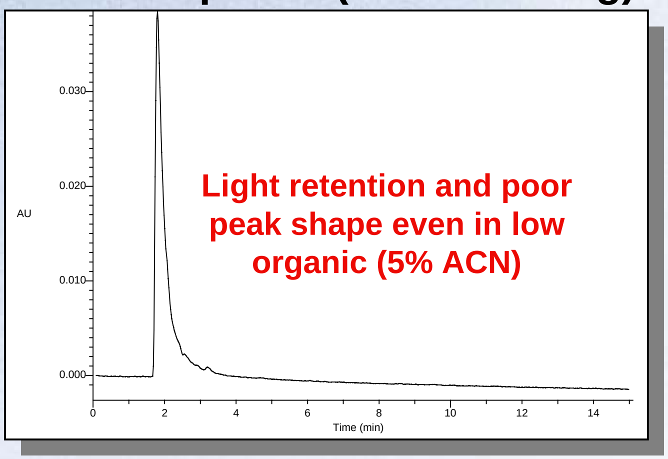
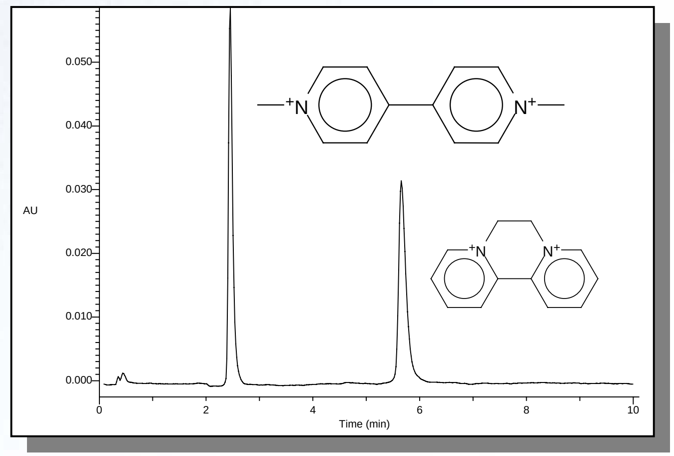
Quaternary amines paraquat and diquat are retained and resolved on Zr-PS (also Zr-PBD or bare ZrO_2) due to a cation exchange mechanism; 50% ACN is useful to suppress phase retention by RP mode.

<u>Silica-C18:</u>
reversed-phase (silanol tailing)



column: Discovery C18, 15 cm x 4.6 mm I.D., 3µm
mobile phase: 5% acetonitrile in 25 mM phosphate (pH 7)
flow rate: 1 mL/min.
temp.: 35 °C
det.: UV 290 nm

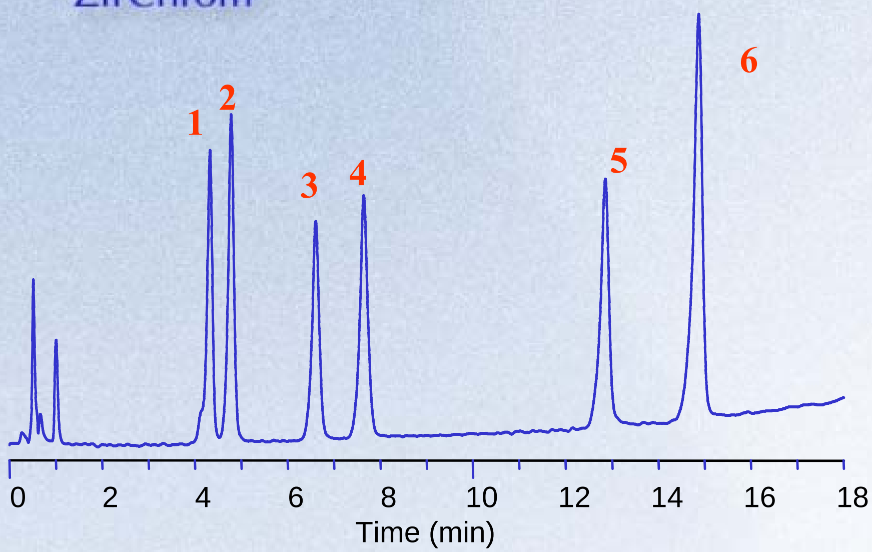
<u>Zirconia-PS:</u>
primarily ion-exchange



column: Discovery Zr-PS, 7.5 cm x 4.6 mm, 3µm
mobile phase: 50% acetonitrile in 25 mM phosphate (pH 7)
flow rate: 3 mL/min.
temp.: 65 °C
det.: UV 290 nm

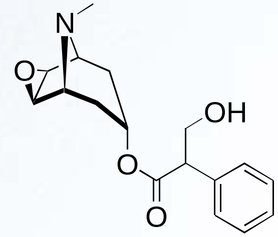
Data provided by Sigma-Supelco

Anticholinergics on Zr-PBD[3]

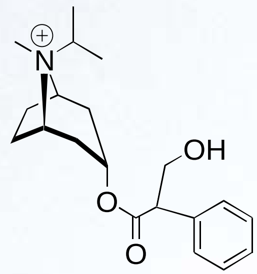


Quaternary amines (except 2)

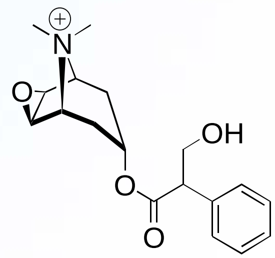
1, Pipenzolate (20 mg/L)



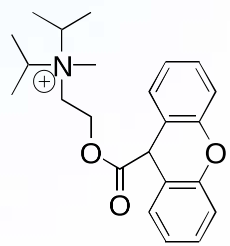
2, Scopolamine (100 mg/L)



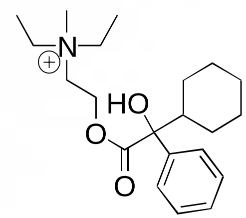
3, Ipratropium (100 mg/L)



4, Methscopolamine (100 mg/L)



5, Propantheline (20 mg/L)



6, Oxyphenonium (100 mg/L)

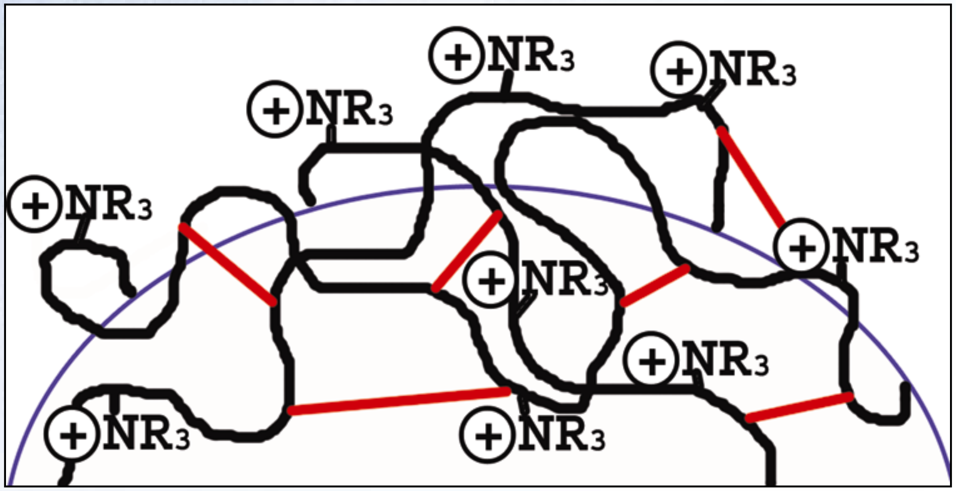
LC Conditions

Discovery Zr-PBD, 100mm x 2.1mm i.d., 3 µm
Mobile Phase A: 50:50 [20 mM H_3PO_4, pH 7.0 w/ NH_4OH]:water
Mobile Phase B: 50:30:20 [20 mM H_3PO_4, pH 7.0 w/ NH_4OH]:water:ACN
Gradient: 10-100% B over 18 minutes
Temp: 80 ^oC, Flow: 0.3 mL/min, Inj vol: 2 µL in 60% MeOH, UV@225 nm

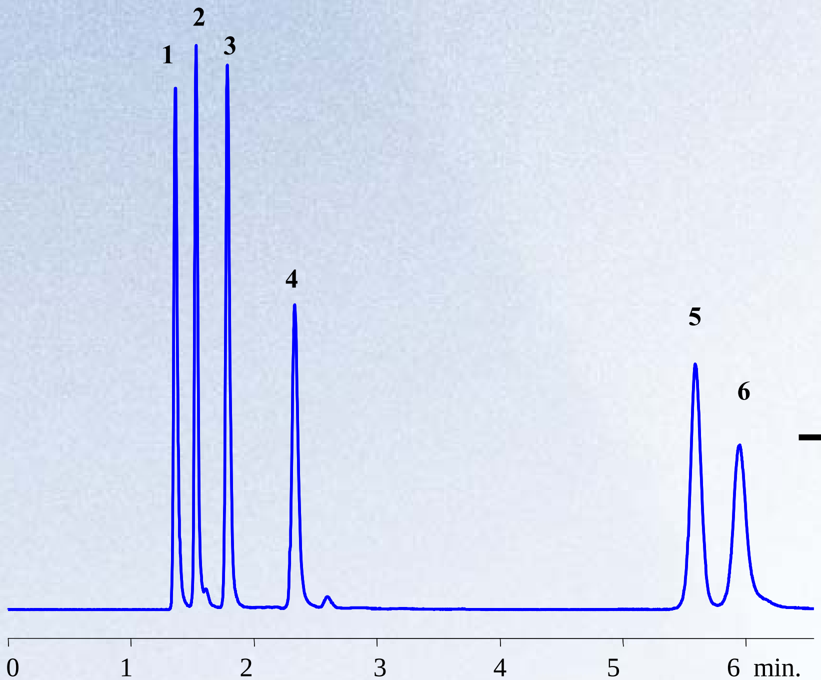
Data provided by Sigma-Supelco

Surface Chemistry and Retention Mechanisms of QPEI-Zirconia[4]

- **Anion-exchange**
- **Hydrophobic interactions**
- **Lewis acid-base interactions**



Water-Soluble Vitamin Analysis on ZirChrom®-SAX[4]



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)

→ Acid anions well retained on 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 ^{o}C, Injection Vol.: 5.0 µL, Detection: UV at 254 nm

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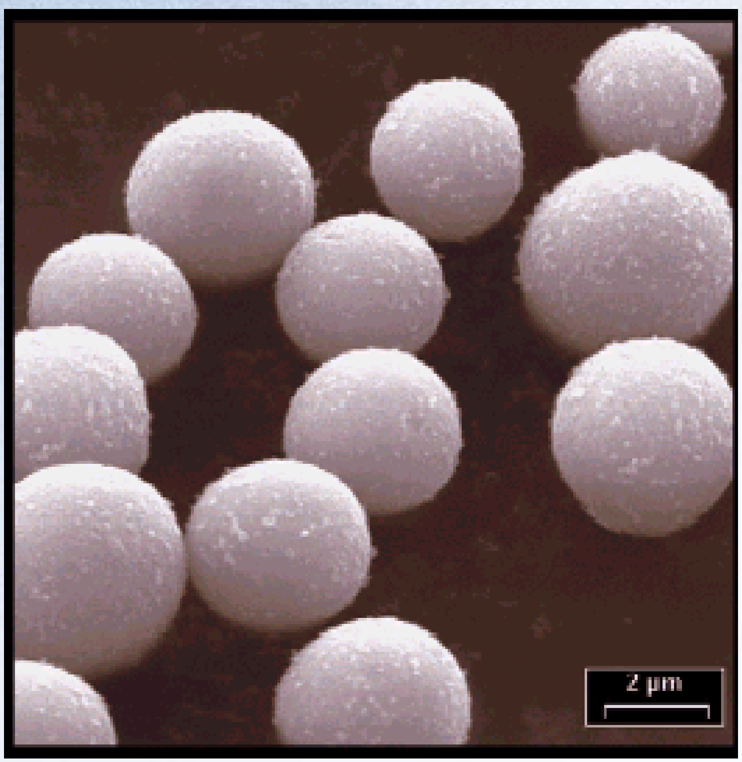
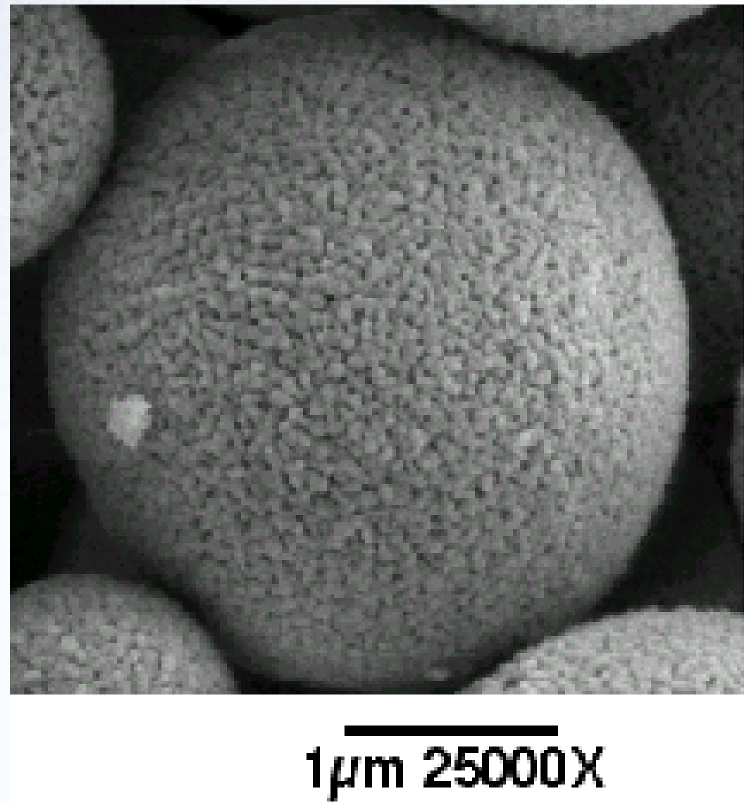
Evaluation of sub-2μm Porous Zirconia Particles for UHPLC

- Since about 1970, there has been a steady effort to develop smaller particle particles that improve HPLC column efficiency. Most recently, columns with sub-2μm particles have been given the name Ultra-HPLC (UHPLC).
- The performance of a sub-2μm zirconia particle coated with PBD has been investigated.

Analytical Diameter Porous Zirconia Particles

Particles are produced by a sol-gel process using ca. 1000Å sol.

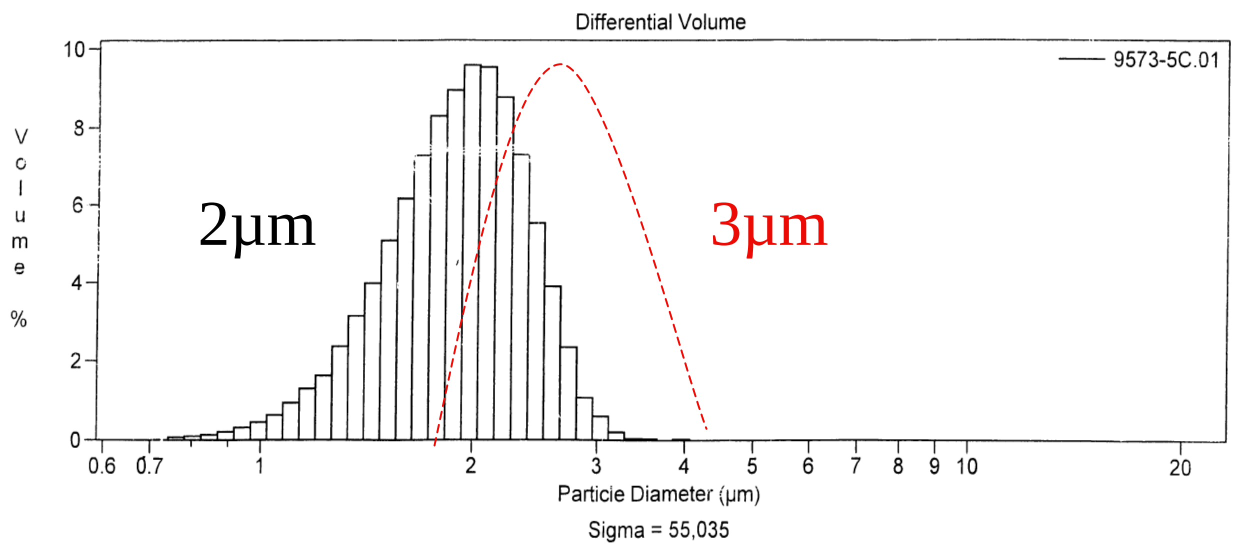




Properties of Porous Analytical Zirconia

Characteristic	Property
Surface area (m^2/g)	22
Pore volume (cc/g)	0.13
Pore diameter (Å)	250-300
Porosity	0.45
Density (gm/cc)	2.6 (2.5x silica)
Particle diameter (μm)	3.0 (and sub-2)

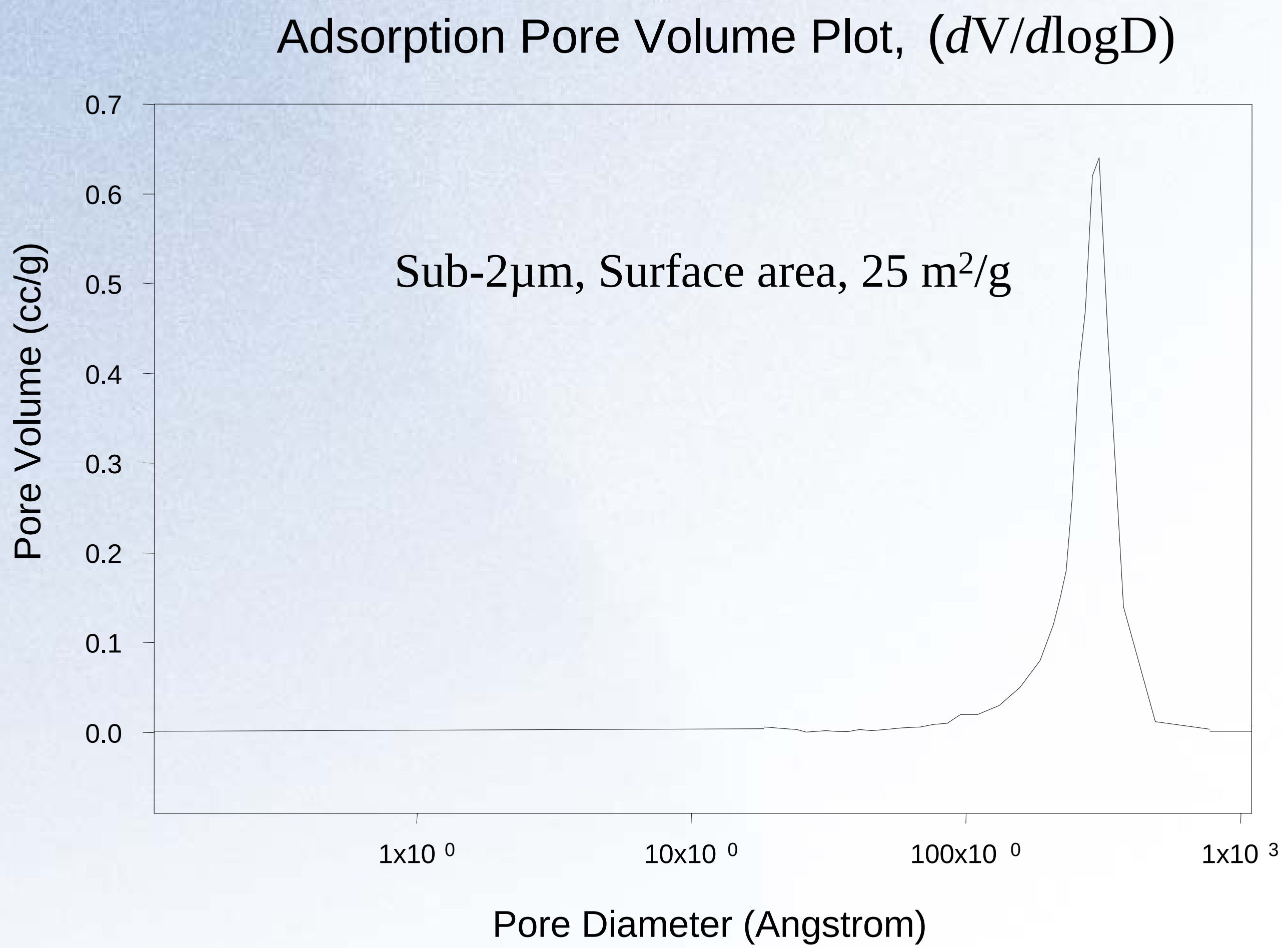
Sub-2µm Particle Size Distribution Compared to 3µm



Volume Statistics (Arithmetic) 9573-5C.01

Volume:	100%		
Mean:	1.923 µm	95% Conf. Limits:	1.919-1.927 µm
Median:	1.919 µm	S.D.:	0.426 µm
Mean/Median ratio:	1.002	Variance:	0.182 $µm^2$
Mode:	1.984 µm	C.V.:	22.2%

Sub-2μm BET Data*

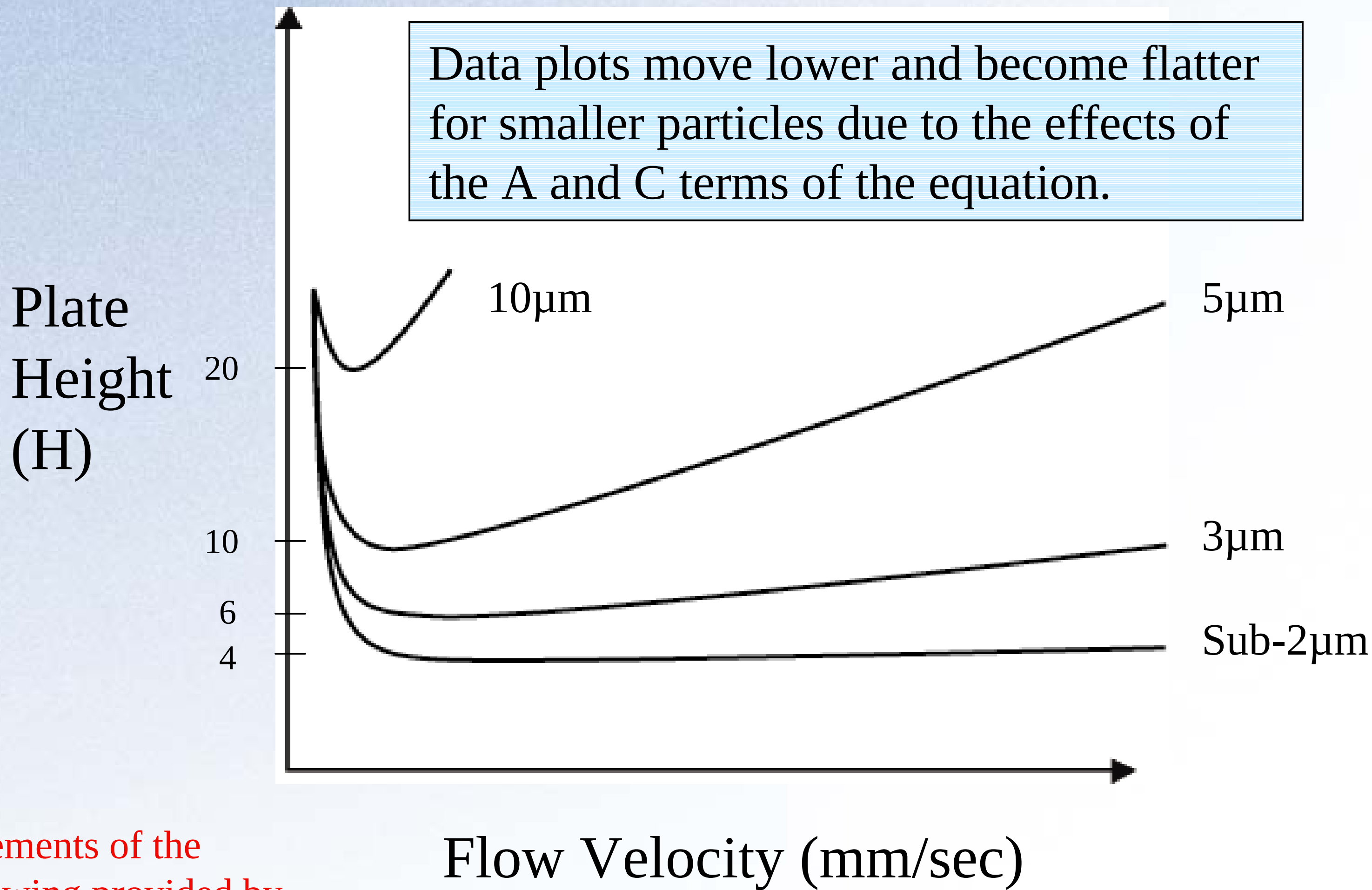


*Data identical to 3μm zirconia

Idealized van Deemter Plots

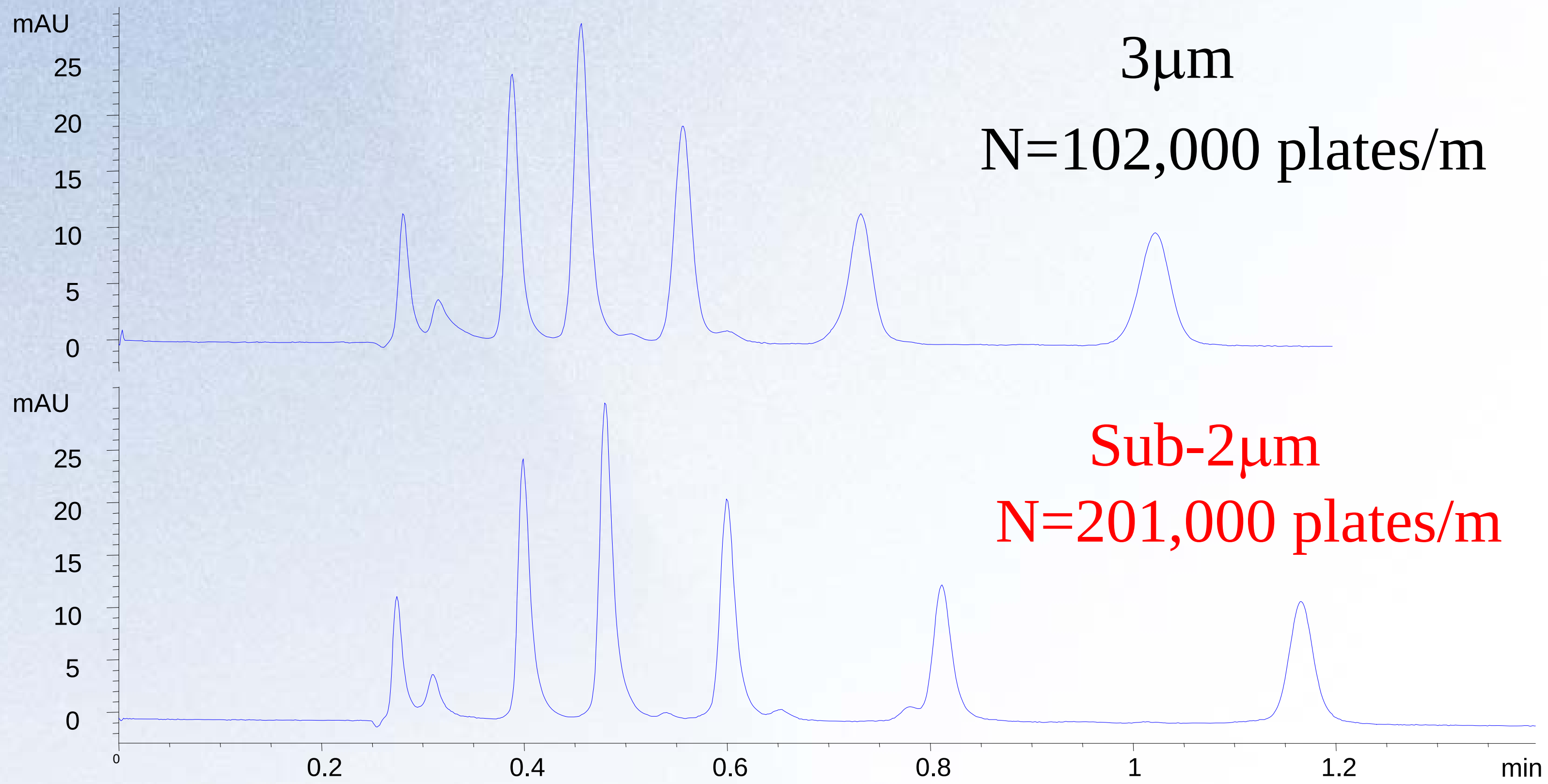
$$H = A + B/v + Cv$$

Data plots move lower and become flatter for smaller particles due to the effects of the A and C terms of the equation.



Elements of the drawing provided by Sigma-Supelco

Comparison Between Sub-2μm and 3μm Particles: Alkylbenzenes



Columns: ZirChrom PBD, 50 x 4.6mm; Mobile phase: 50/50 ACN/water; Flow 2.0 mL/min (0.53 cm/sec); Temperature: 30 ºC; UV@254nm; Agilent 1100 with micro flow cell.

Flow Studies on 3μm Zr-PBD: Alkylbenzenes

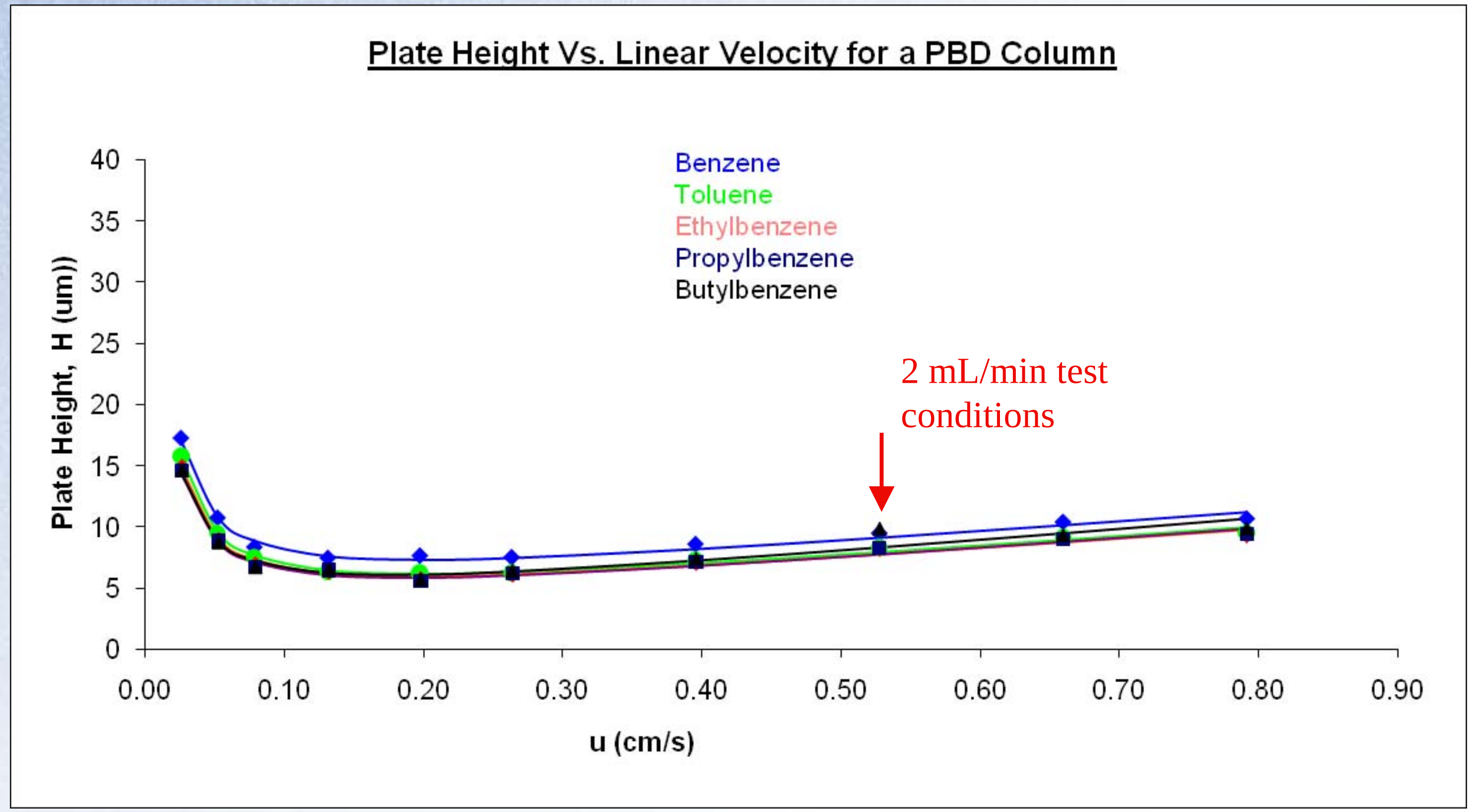


Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 ºC, Mobile phase: 55/45 ACN/water, Column: ZirChrom PBD, 50 x 4.6mm, Agilent 1100/UV with Micro Cell (0.007''i.d. tubing).

Flow Studies on Sub-2μm Zr-PBD: Alkylbenzenes

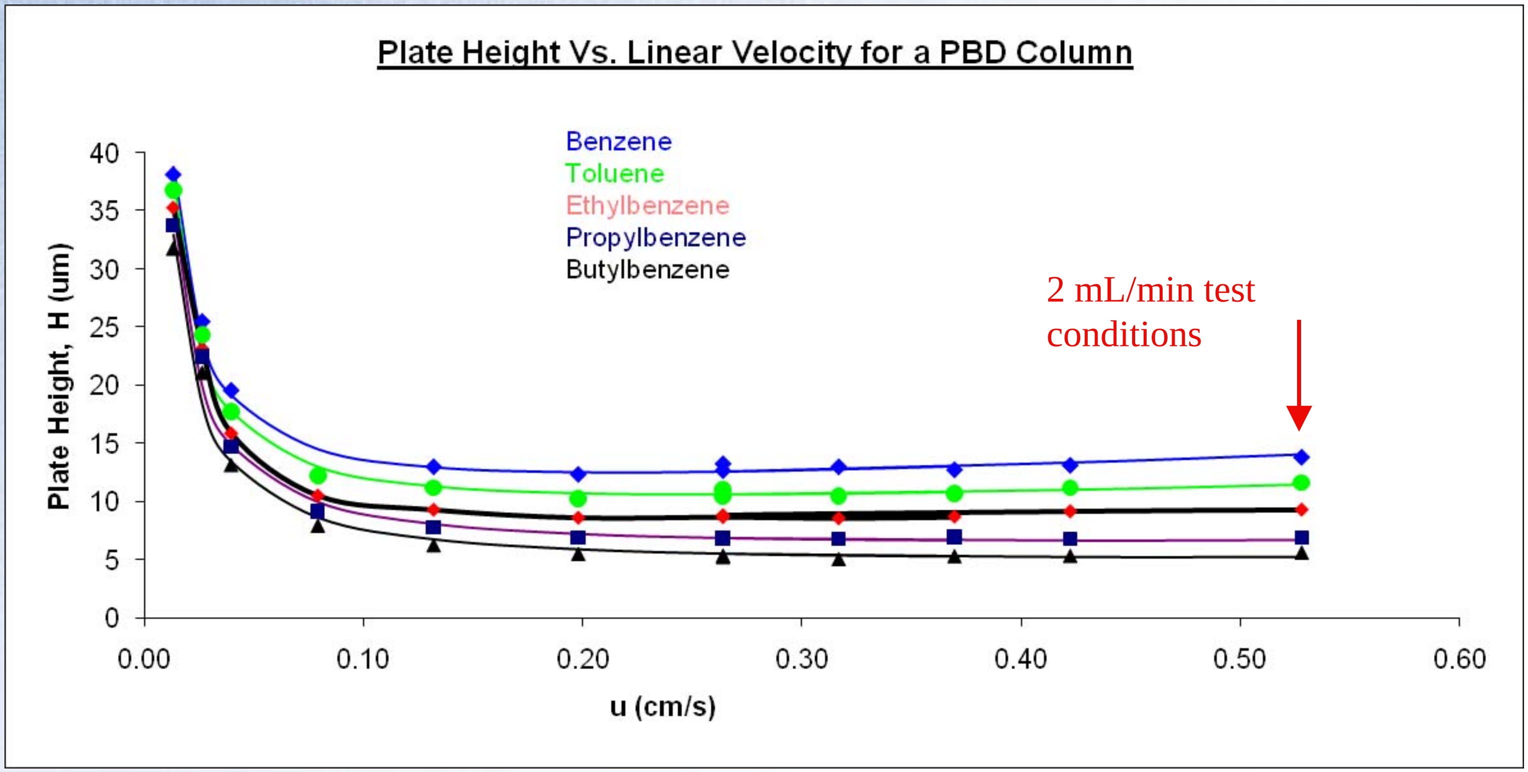
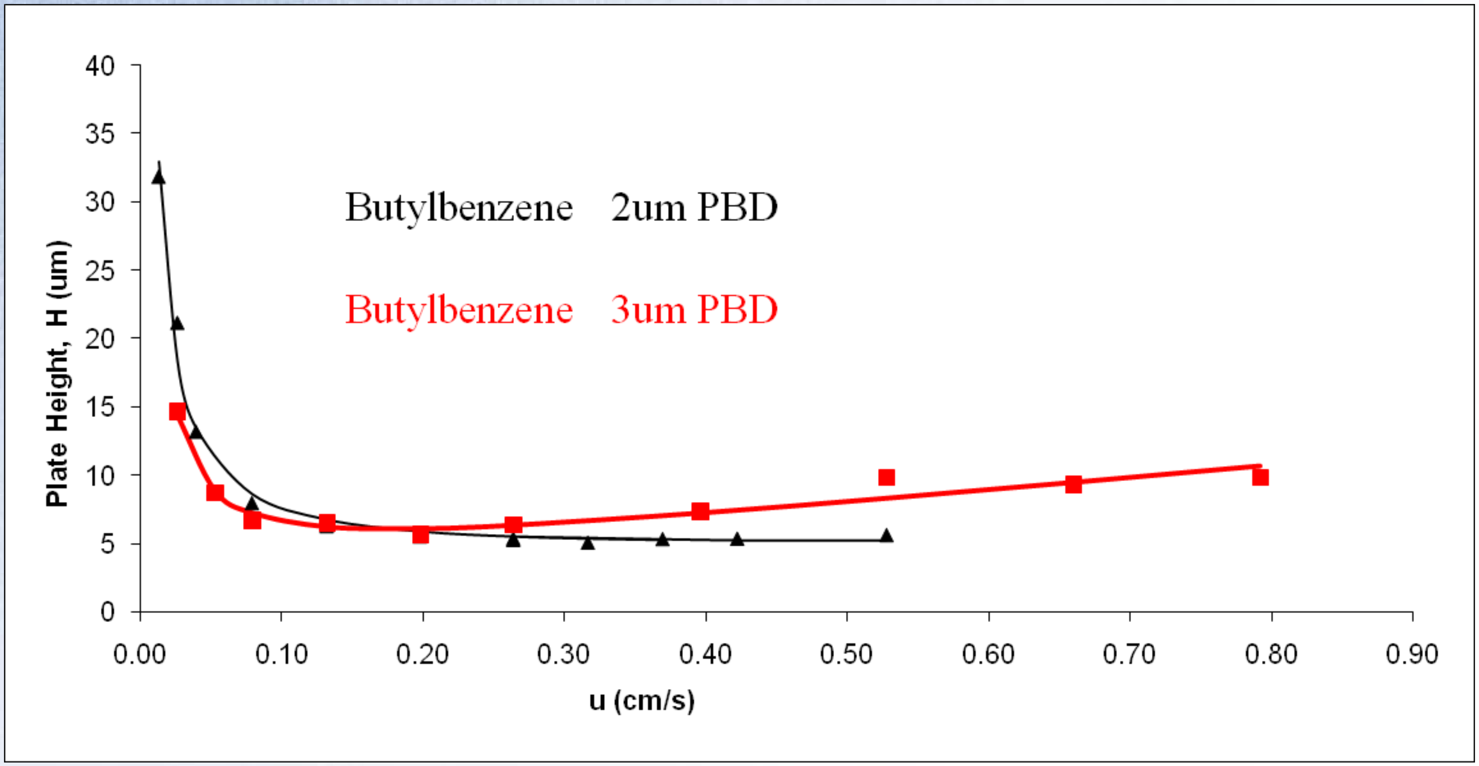


Plate height based on van Deemter Equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 ºC, Mobile phase: 50/50 ACN/water (keep k' in the same range as 3μm particles), Column: 50 x 4.6mm, Agilent 1100/UV with Micro Cell (0.007'' i.d. tubing).

Comparison Between Sub-2μm and 3μm Zr-PBD



Resistance to mass transfer is reduced as the particle size is decreased. Fast analysis can be achieved at higher flow rate with minimal efficiency loss.

Zr-PBD Separation of Catecholamines: Sub-2µm vs 3µm

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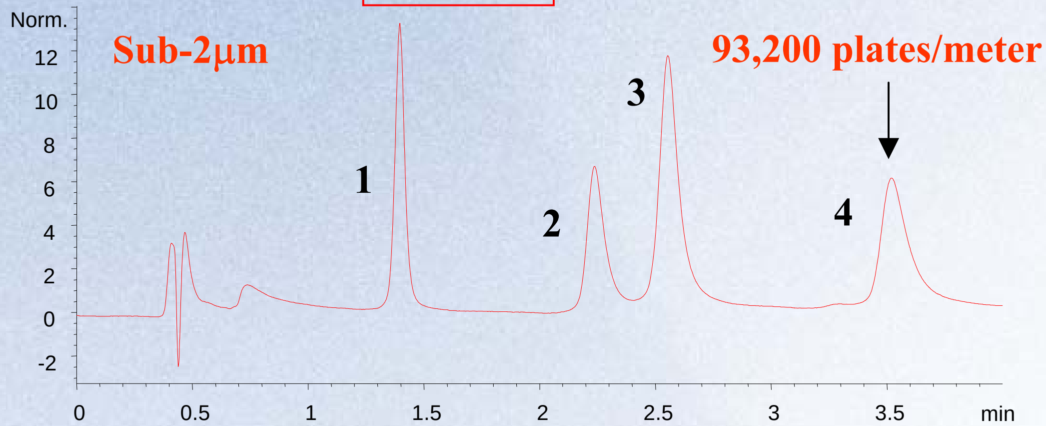
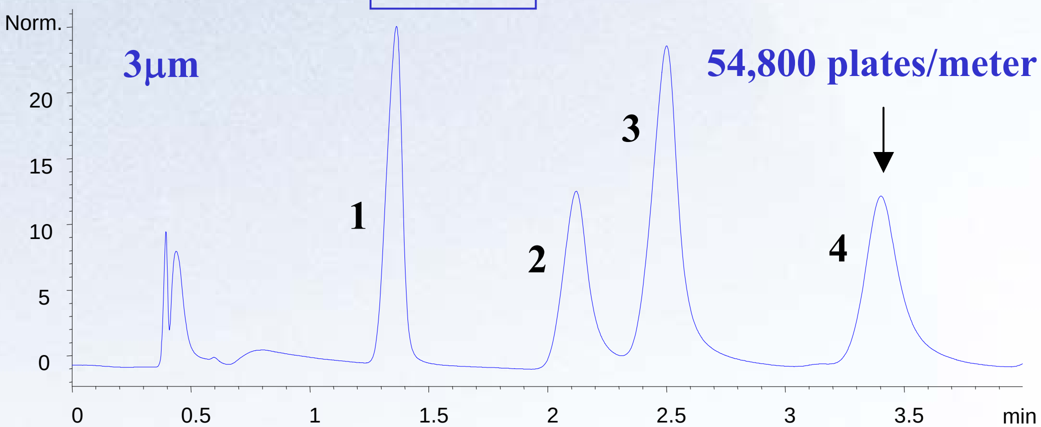
130 bar



50 bar



Conditions

Column: ZirChrom-PBD 50 x 4.6mm

Mobile Phase: 85/15 ACN/30mM NH_4OAc, 10mM $NH_4H_2PO_4$ adjusted to pH=3.4 w/ HCl

Flow rate = *1.5 mL/min*

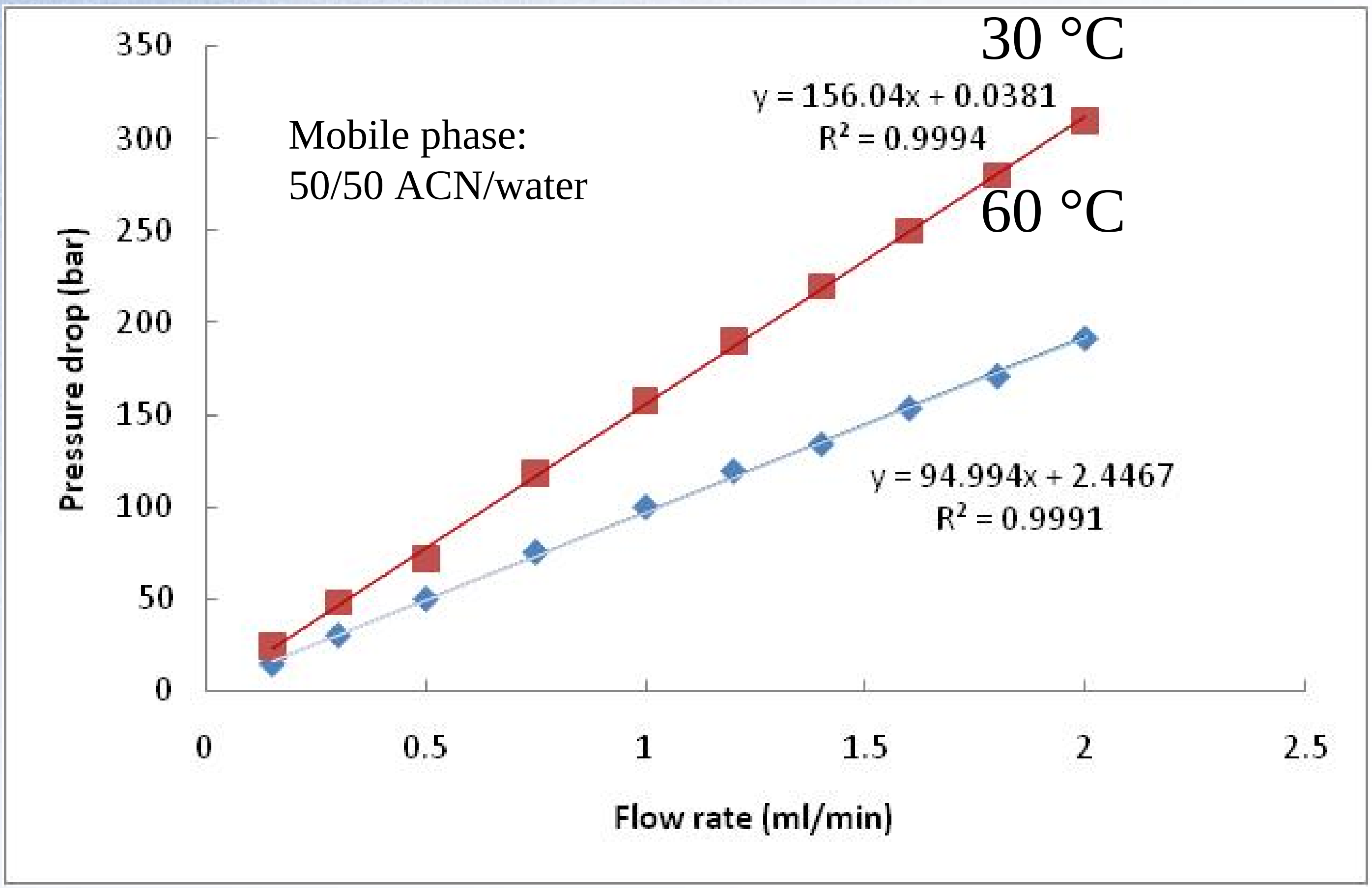
Temperature = 30°C

Inj Vol = 5 µL

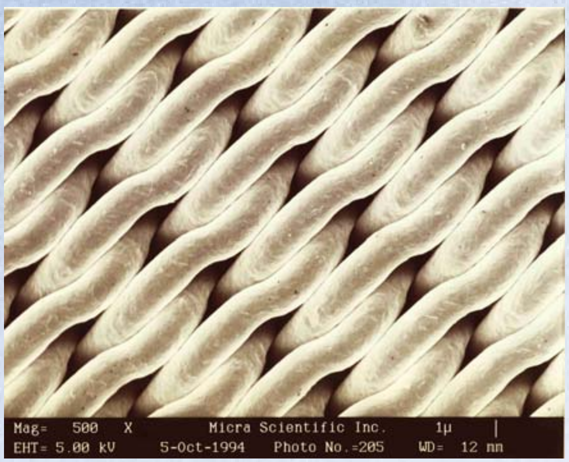
Elution order: **1**=Tyramine, **2**=Epinephrine, **3**=Dopamine, **4**=3,4-dihydroxynorephedrine

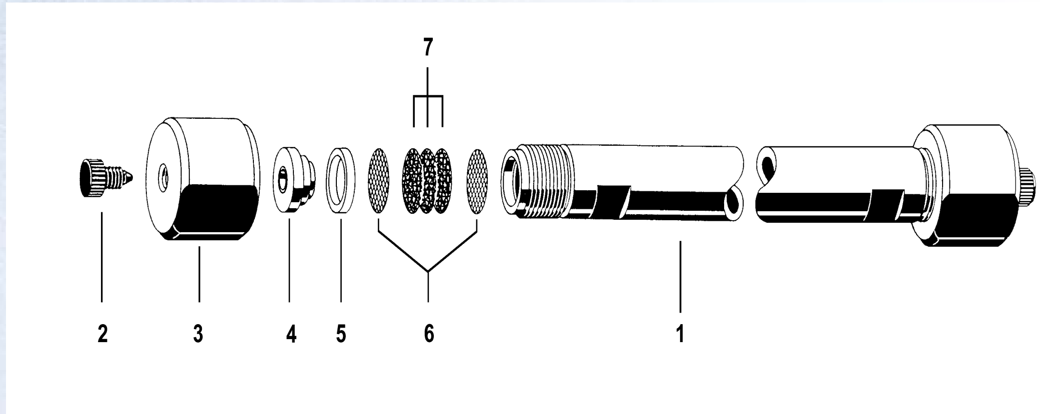
- **Smaller particle shows increased efficiency**
- **Identical selectivity to 3µm**
- **Separation time can readily be decreased to <1min for sub-2µm columns.**

Sub-2µm Pressure Drop at Different Temperatures



UHPLC Column Hardware





- Column hardware must withstand very high packing and operating pressures.
- Frits must contain particles in the 0.5µm range or smaller.
- Composite frits are composed of 0.5µm glass fiber mats sandwiched between 2-3µm stainless wire meshes.

Summary and Conclusions

- Multi-mode HPLC columns have become popular for difficult applications where compounds have ionic character and vary widely in chemical nature.
- Several ZirChrom phases are ideal and popular for multi-mode applications, including Zr-PBD, Zr-PS, Zr-MS and Zr-SAX, and are stable over a much wider range of pH and temperature than any silica-based phase.
- ZirChrom phases are currently available in high efficiency 3µm analytical particles.
- A new sub-2µm particle has been developed for preparing UHPLC columns that produce very high efficiency in excess of 200,000 N/M with PBD polymer-coated phase. Additional phase coatings are being investigated.

References and Acknowledgements

1. J. A. Blackwell and P. W. Carr, "Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide," Anal. Chem. 64, 863-73 (1992).
2. R. A. Henry, H. K. Brandes, D. S. Bell and C. T. Santasania, 30th Annual HPLC Meeting, Oral Presentation, 2006, San Francisco, CA.
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4. B. Yan. C. V. McNeff. R. A. Henry and D. Nowlan, Eastern Analytical Symposium, Poster, 2008, Somerset, NJ.

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