Evaluation of Sub-2µm Zirconia-PBD Particles for Multi-Modal UHPLC

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Introduction

- Lately, efficiency has received most of the attention in HPLC. As we study and debate optimum particle geometry and instrument design, higher efficiency columns are being adopted by analysts to improve *resolution, peak capacity, speed, sensitivity and solvent economy*.

- Most of the progress with small particles has been made with silica RP columns so it is important to investigate whether the high efficiency observed with ultra-small silica RP particles can be translated to other substrates and phases, which may retain and separate by other selective modes.

- Zirconia phases often separate by a multi-modal mechanism so they are good candidates to see if the performance advantages of sub-2µm particles can be observed (at ambient or elevated temperature) for other packings.
Brief Review of Multi-Modal Zirconia Behavior

Due to strong electropositive nature, Zr surface readily picks up negative charge

- Zirconia substrate exhibits polar and ionic solute interaction: mainly cation-exchange.
- With stable organic coatings, reversed-phase interaction creates Multi-Modal behavior.
- Extreme resistance to temperature, pH and mechanical stress are potential advantages.
Addition of RP Behavior with Coated Zirconia Phases

- Ionic solute retention (and selectivity) is modulated by pH, buffer/salt type and concentrations, and temperature.
- RP solute retention is modulated by organic solvent.
- *Five* important mobile phase variables must be controlled.
Zr-PBD and Si-C18 are Orthogonal for Basic Drugs\textsuperscript{2}

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

Data provided by Sigma-Supelco
Plot technique by Horvath, Carr, others

**Graph:**
- **Log $k'$ (C18)** vs. **Log $k'$ (Discovery Zr-PBD)**
- **R\textsuperscript{2} = 0.373**
- **s.d. = 0.592**

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

**Conditions:**
- 25 mM ammonium phosphate (pH 6): methanol (72:28)
SCX Mode Can Create Zr-PBD Retention Even in High Organic

Basic Pharmaceutical retention in 80% ACN

Excess k related to SCX mode

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 °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).
Difficult Compounds for Silica Often Separate on Zirconia

Quaternary amines paraquat and diquat are retained and resolved on Zr-PS (also Zr-PBD or bare ZrO₂) due to the cation exchange mechanism; 50% ACN is useful to suppress or regulate retention by RP mode.

Silica-C18: reversed-phase

- 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

Zirconia-PS: 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

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: 10 mM NH₄PO₄, pH 7.0
Mobile Phase B: 80/20 20 mM NH₄PO₄, pH 7.0/ACN
Gradient: 10-100% B over 18 minutes
Temp: 80 °C,
Flow: 0.3 mL/min
Inj vol: 2 μL in 60% MeOH
Detector: UV@225 nm

Data provided by Sigma-Supelco
Evaluation of Smaller Diameter Porous Zirconia Particles

Specifications:
- Particles produced by a sol-gel process with 1000Å sol
- Pore diameter 250-300Å
- Density: 2.6 g/cc (2.5X silica)
- Surface area: 25 m²/g
- Particle diameters: 3µm and sub-2µm
- Totally porous (porosity: 0.45)
van Deemter Plots Reveal Column Performance

\[ H = A + \frac{B}{\nu} + Cv \] (shown below for a single solute)

Data plots move lower and become flatter for small particles due to combined effects of the equation terms.

Flow velocity, \( \nu \) (mm/sec)

Plate Height, \( H \) (\( \mu m \))

10\( \mu m \)

5\( \mu m \)

3\( \mu m \)

Sub-2\( \mu m \)

Idealized plot provided by Sigma-Supelco

goal: ca. 250,000 N/m
Comparison Between Sub-2μm and 3μm Zr-PBD

Efficiency for the larger particle is about right, but the smaller particle should be better (lower H). Could be the phase coating or the column, but instrument dispersion cannot be ruled out with this data.

Severity of an instrument dispersion problem can be missed if one looks only at single solutes.

Pressure limit of inlet connection:
- Butylbenzene, 2μm PBD: 193 bar
- Butylbenzene, 3μm PBD: 327 bar
Sub-2µm Pressure Drop at Different Temperatures*

Mobile phase:
50/50 ACN/water

30 °C
\[ y = 156.04x + 0.0381 \]
\[ R^2 = 0.9994 \]

60 °C
\[ y = 94.994x + 2.4467 \]
\[ R^2 = 0.9991 \]

* 3µm particles (not shown) have about half the pressure drop
Initial Comparison Between Sub-2μm and 3μm Zr-PBD Particles

Alkylbenzenes

3μm
N=102,000 plates/m

Sub-2μm
N=201,000 plates/m

Columns: ZirChrom PBD, 50 x 4.6mm; Mobile phase: 50/50 ACN/water; Flow 2.0 mL/min (0.53 cm/sec); Temp.: 30 °C; UV@254nm; Agilent 1100.
Plate height based on van Deemter Equation vs linear velocity at various temperatures for retained solutes: Alkylbenzenes, Temperature: 30 °C, Mobile phase: 50/50 ACN/water, Column: ZirChrom PBD, 50 x 4.6mm, Agilent 1100/UV micro cell (0.007” i.d. tubing).
Flow Studies on Sub-2μm Zr-PBD: Alkylbenzenes

“..but we used a micro flow cell!”

Plate height based on van Deemter equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: 50/50 ACN/water (keep k in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV micro cell (0.007” i.d. tubing).

Plate height based on van Deemter equation vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, Mobile phase: 50/50 ACN/water (keep k in the same range as 3µm particles), Column: 50 x 4.6mm, Agilent 1100/UV micro cell (0.007” i.d. tubing).

Conclusion: systematic investigation of instrument dispersion needed.
Flow Studies on Sub-2µm Zr-PBD: Factory Instrument at Ambient

Plate height 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 Standard Cell and 0.007” i.d. tubing.
Flow Studies on Sub-2μm Zr-PBD: Add Micro Cell

Plate Height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, 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 and 0.007” i.d. tubing.
Plate height vs linear velocity for retained solutes: Alkylbenzenes, Temperature 30 °C, 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 and optimized 0.005” i.d. tubing.
Flow Studies on Sub-2µm Zr-PBD: Add Heat Exchanger and Fitting

Plate Height vs. Linear Velocity for a PBD Column

- Benzene
- Toluene
- Ethylbenzene
- Propylbenzene
- Butylbenzene

H = 4-8 µm (range)

1.8 mL/min test conditions

Plate height 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, high pressure fitting and heat exchanger.
Optimization and Configuration for Elevated Temperature Operation with High-Pressure Column Fitting
ZirChrom-PBD sub-2μm; Reduced Instrument Volume

Alkylbenzenes

N=244,000 plates/m

ZirChrom-PBD
50mm x 4.6mm, sub-2μm
50/50 ACN/H₂O
F=1 mL/min
UV=254nm
T=30 ºC
Drug Mix* Separation on PBD sub-2μm vs 3μm – Ambient

Non-optimized Agilent 1100 with micro cell and heat exchanger

Analytes
1=Labetalol
2=Atenolol
3=Acebutolol
4=Metoprolol
5=Oxprenolol
6=Lidocaine
7=Quinidine
8=Alprenolol
9=Propranolol

Column: ZirChrom®-PBD, 50 x 4.6 mm i.d., sub-2μm;
Mobile phase: 24/76 ACN/20 mM K₃PO₄ at pH=12; Flow rate: 1.0 mL/min; Temp.: 30 °C; Injection vol.: 2.0 μL;
Detection: UV at 254 nm

* Mainly beta-blockers
Drug Mix on ZirChrom-PBD
sub-2μm, High Temperature

246 bar (3660 psi)
Background pressure: ca. 25 bar

Agilent 1100
ZirChrom-PBD
50mm x 4.6mm, sub-2μm
22/78 ACN/20mM K₃PO₄ at pH=12
F=2.5 mL/min
UV=254nm (0.5 sec response)
T=75 ºC

ca. 163,000 plates/m
Drug Mix on ZirChrom-PBD sub-2μm, High Temp, Faster Detector Response

246 bar (3660 psi)
Background pressure: ca. 25 bar

Significant increase in sensitivity
140 to 200 mAu

Agilent 1100
ZirChrom-PBD
50mm x 4.6mm, sub-2μm
21/79 ACN/20mM K₃PO₄ at pH=12
F=2.5 mL/min
UV=254nm (0.12 sec response)
T=75 °C

c.a. 200,000 plates/m
Calibrating Background Pressure Drop for Optimized Agilent 1100

<table>
<thead>
<tr>
<th>100% H₂O at 30 ºC</th>
<th>100% H₂O at 75 ºC</th>
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<tr>
<td>Flow (mL/min)</td>
<td>BP (bar)</td>
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<tr>
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<td>2</td>
<td>50</td>
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* Reference point: Waters Acquity (0.005” ID inlet / 0.0025” ID outlet), 60/40 ACN/water, 0.5 mL/min, background pressure = 1700 psi (113 bar).
Conclusions and Plans for Further HPLC Development with Zirconia

- Performance results with a sub-2µm Zr-PBD column in an Agilent 1100 are encouraging.
- The study of ultra-high speed applications using sub-2µm Zr-PBD, especially at higher pH and temperature ("extreme conditions for silica") will be continued; generic conditions for LC-MS will be investigated.
- Additional advantages of optimizing the IBW of an Agilent Model 1100 HPLC instrument using a high performance (Model 1200) heat exchanger will be studied.
- Other sub-2µm Zr phases (such as CARB) will be prepared and compared to Zr-PBD under ambient and extreme conditions.
References and Acknowledgements


The assistance of Supelco Division of Sigma-Aldrich is gratefully appreciated, including the use of a high-pressure column fitting.