Speed and Resolution with 5-Micron Superficially Porous Particles

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Abstract

Columns packed with modern superficially porous (core-shell) particles (SPP) exhibit extraordinarily high performance compared to columns packed with totally porous particles of the same size. Many published reports by independent researchers verify that 2.5 – 2.7 micron SPP particles can produce columns with the efficiency of sub-2micron particle columns with nearly half the back pressure of the smaller particles. The success of the SPP particles has generated interest in the development of SPP particles of other sizes. Sub-2-micron SPP for separating small molecules have recently been introduced. However, columns packed with these particles exhibit the very high back pressures consistent with their very small particle sizes. This pressure requirement limits column lengths and operating flow rates for users with HPLC equipment that cannot operate above 400 - 600 bar. All sub-2-micron particle columns, whether SPP or totally porous, provide high separation efficiencies in short column lengths for high-speed, highthroughput applications, but require ultra high pressure instrumentation for maximum utility with longer columns. Recent introductions of nominally 5-micron SPP particles take the superficially porous particle technology in a different direction. Columns of these larger-particle SPP show significant performance advantages over totally porous particles of similar size. These 5-micron SPP deliver the separation efficiency of 3micron totally porous particle columns at nearly half the back pressure. This report examines the technology of 5-micron SPP columns with a variety of bonded phases to demonstrate the performance and advantages for larger particle SPP.

SEM Photo of 5-micron HALO-5 Particles



SEM Photo of FIB Sliced HALO-5 Particle

(FIB = Focused Ion Beam)



EHT = 3.00 kV WD = 4.9 mm Signal A = SESI System Vacuum = 1.11e-004 Pa FIB Probe = 30KV:120pA FIB Imaging = SEM ESB Grid = 809 V Scan Speed = 4 Mag = 29.62 K X ²⁰⁰ nm FIB Lock Mags = Yes

Improved Size Distributions for 5-Micron Particles



Measured on a Multisizer 3 Coulter Counter

Effect of Particle Size on Plate Height

Columns: 4.6 x 150 mm; Temperature: 30 ^oC Mobile phase: 50% acetonitrile/50% water Solute: 1-Cl-4-nitrobenzene; Injection: 1 mL Instruments: <400 bar, Agilent 1100; >400 bar, Agilent 1200



Plate height decreases with decreased particle size, as expected. 2.2, 2.7 μm column limit – 600 bar; 2.2 μm does not reach plate height minimum

Effect of Particle Size on Reduced Plate Height

Columns: 4.6 x 150 mm; Temperature: 30 ⁰C Mobile phase: 50% acetonitrile/50% water Solute: 1-Cl-4-nitrobenzene: Injection: 1 mL Instruments: <400 bar, Agilent 1100; >600 bar, Agilent 1200



h values lower for 5 μ m HALO (h = 1.2) - more homogeneously packed bed structure

3 µm Performance with 5 µm Pressure



Use 5 µm SPP Columns to Achieve High Resolution at Low Pressure



PEAK IDENTITIES (in order): Formaldehyde-2,4-DNPH, Acetaldehyde-2,4-DNPH, Acetone-2,4-DNPH, Acrolein-2,4-DNPH, Propionaldehyde-2,4-DNPH, Crotonaldehyde-2,4-DNPH, 2-Butanone-2,4-DNPH, Methacrolein-2,4-DNPH, Butyraldehyde-2,4-DNPH, Benzaldehyde-2,4-DNPH, Valeraldehyde-2,4-DNPH, m-Tolualdehyde-2,4-DNPH, and Hexaldehyde-2,4-DNPH Note: Small peaks preceding labeled aliphatic aldehyde peaks are minor geometric isomers (syn/anti).

Different Stationary Phases for Modifying Selectivity



C18 (octadecyl)



C8 (octyl)





Easy Method Transfer from UHPLC to HPLC



- 9. Tolbutamide
- 10. 4-Chloro-3-nitroanisole

<u>Comparable Selectivity between HALO-5 and</u> <u>HALO 2.7 µm Penta-HILIC Phases</u>



TEST CONDITIONS:

Column 1: 4.6 x 100 mm, HALO 5 µm Penta-HILIC Part Number: 95814-605 Column 2: 4.6 x 100 mm, HALO 2.7 µm Penta-HILIC Part Number: 92814-605 Mobile Phase: 5/95: A/B A= 0.1 M Ammonium formate, pH=3 (adj.) B= Acetonitrile Flow Rate: See chart Pressure: See chart Temperature: 30°C Detection: UV 254 nm, VWD Injection Volume: 2.0 μ L Sample Solvent: 10/90: Water/Acetonitrile Response Time: 0.02 sec. Flow Cell: 2.5 μ L semi-micro LC System: Shimadzu Prominence UFLC XR ECV: ~14 μ L

Similar selectivity is achieved between the 5 µm and 2.7 µm HALO Penta-HILIC particle sizes through a slight flow rate adjustment allowing easy method transfer.

Comparable Selectivity between HALO-5 and HALO 2.7 µm HILIC Phases



TEST CONDITIONS:

Column 1: 4.6 x 100 mm, HALO 5 μ m HILIC Part Number: 95814-601 Column 2: 4.6 x 100 mm, HALO 2.7 μ m HILIC Part Number: 92814-601 Mobile Phase: 11/89: A/B A = 0.1 M Ammonium formate, pH=3 (adj.) B = Acetonitrile Flow Rate: See chart Pressure: See chart Temperature: 30°C Detection: UV 254 nm, VWD Injection Volume: 2.0 µL Sample Solvent: mobile phase Response Time: 0.02 sec. Flow Cell: 2.5 µL semi-micro LC System: Shimadzu Prominence UFLC XR ECV: ~14 µL

Go Ballistic with 5-µm HPLC Columns

- Ballistic gradients
 - Utilize narrow-bore columns (1.0, 2.1 mm ID)
 - High mobile phase linear velocities (fast flow rates for column diameter)
 - Fast gradient times (1 5 minutes) over a wide range of organic modifier (e.g., 5 – 95% or 0 – 100%)
- Useful when high throughput and ruggedness is required
 - Analyzing biological sample extracts (urine, plasma, liposomes, etc.)
 - Assessing identity and purity of compound libraries
 - Screening new product candidates
 - Following reactions
 - Monitoring dissolution experiments

Example Ballistic Gradient Run on HALO-5 Column



Analytes, in elution order: hesperidin, myricetin, quercetin, naringenin & apigenin (coeluted), hesperetin, kaempferol, biochanin

Conclusions

- Superficially porous particles (SPP) of nominally 5 µm diameter demonstrate exceptionally high plate numbers for their particle size.
- Reduced plate height values (*h*) for 5 µm SPP columns are even smaller than those found for columns packed with 2.7 µm SPP, likely because it is easier to pack larger particles into homogeneous beds.
- Column efficiencies of 5 µm SPP are comparable to columns packed with 3 µm totally porous particles with about half the operating pressures.
- Narrow-bore 5 µm SPP columns are well-suited for high speed "ballistic gradient" separations.