New Fused-Core™ Particles for Very Fast HPLC Separations

Joseph J. Kirkland, Timothy J. Langlois & Joseph J. DeStefano

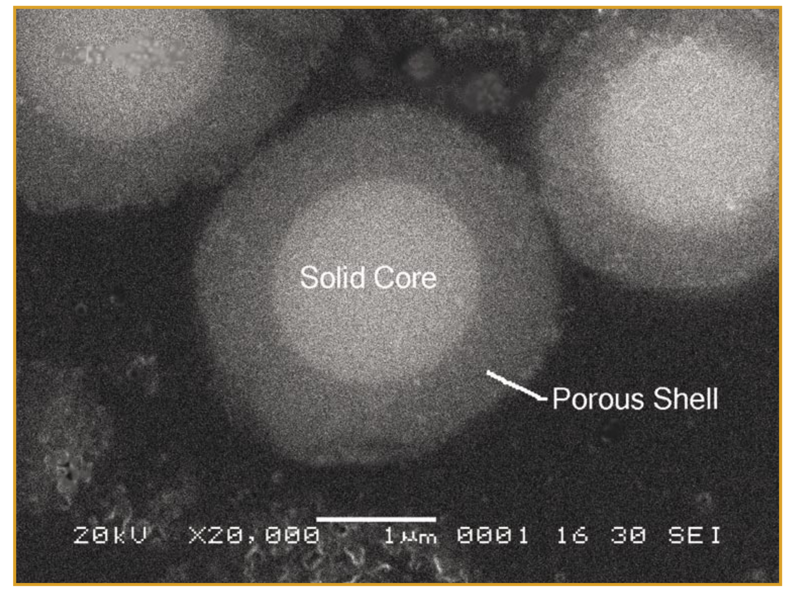
Advanced Materials Technology, 3521 Silverside Road, Suite 1-K, Quillen Bldg. Wilmington, DE 19810

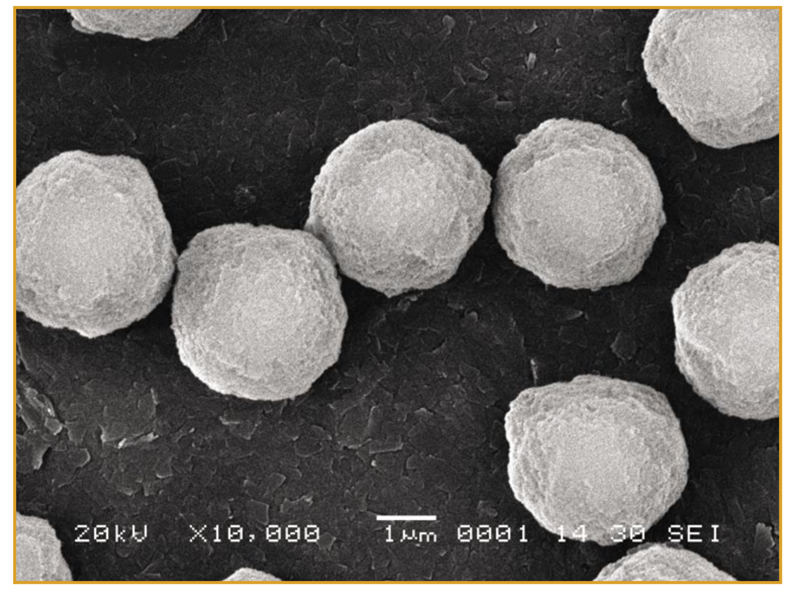
Abstract

There is considerable interest in HPLC columns for performing very fast separations either for increasing sample throughput or for use in multidimensional (2-D) separations. One approach for achieving fast separations is to use very small particles for the column packing. However, columns of sub-2-μm particles must be operated at very high back pressures for expected efficiency. In some cases special HPLC apparatus is required for optimum performance with these very small particles. Unique silica-based particles now have been developed for packed HPLC columns that allow ultra-fast separations at operating pressures available with most existing instruments. These new "fused core" particles are 2.7-μm overall with a 1.7-μm solid core and a 0.5-μm porous outer shell of ~9 nm pores. This configuration provides a fortunate compromise of characteristics that result in very rapid separations with modest operating pressures. Columns of these new particles exhibit unusual efficiency, with reduced plate heights of 1.5 for small molecules under optimum conditions (> 200,000 plates/meter). This high level of efficiency is believed to be a result of the very narrow particles size distribution (5%, 1-sigma from the average) and higher particle density because of the solid core. Relative to totally porous sub-2-μm particles, columns of the new particles generate a competitive number of theoretical plates in the same time frame, but with greatly reduced back pressures. Compared to columns of sub-2-μm particles, the short diffusion paths of the thin porous crust and reduced back pressure of the 2.7-μm particles allow mobile phase velocities at the plate height minimum for highest efficiency per unit time while staying within the 400 bar pressure limit of most HPLC instruments. Different from sub-2-μm particles, the 2.7-μm particles can be packed into stable columns of expected efficiency. These 2.7-μm particles are more easily packed efficiently and require inlet frits or retainers that have larger pores that are less likely to be plugged with use. Allowed longer columns with the new particles also exhibit reduced problems with extra-column band broadening effects. Surface areas of ~150 m2/g for the new fused core particles are suitable for separating small molecules with good sample loading characteristics. The new particles exhibit the strength and stability required for operation at pressures of at least 600 bar.

Study Purpose

Develop unique high-purity silica particles for stable high-efficiency packed columns that are capable of very fast separations with modest back pressures.

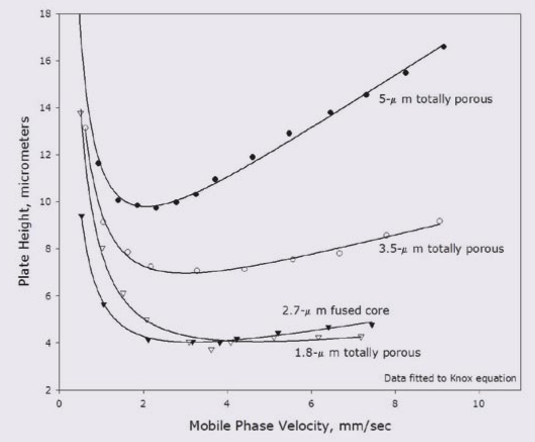




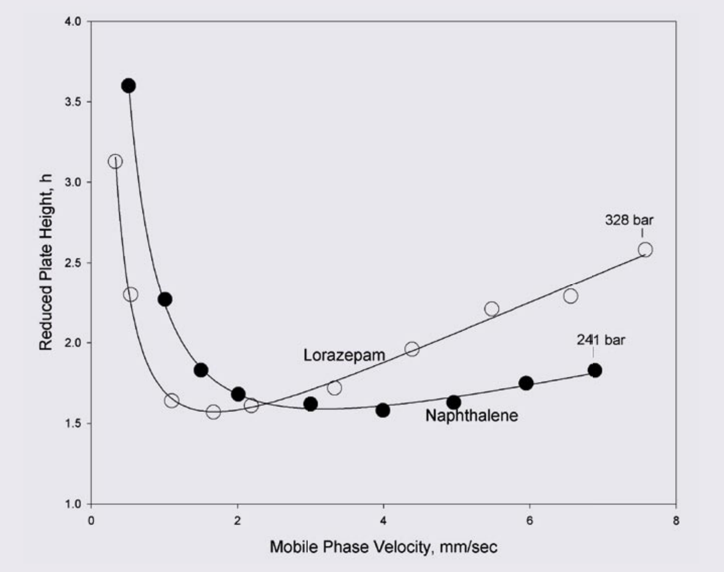
Specifications for Fused-core Particles

- 2.7 μm overall diameter
- 1.7 μm solid core
- 0.5-μm-thick porous outer shell
- 9 nm pores in porous shell
- Highly purified Type B silica
- Surface area ~150 m2/g
- Pressures to at least 600 bar

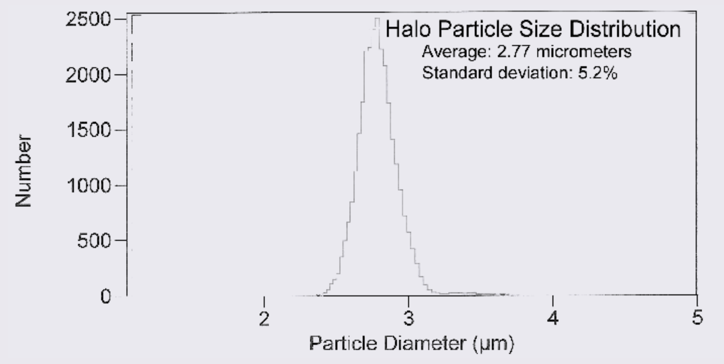
Effect of Particle Size and Type
Columns 50 x 4.6mm, Ace5-C18; Zorbax XDB-C18. 3.5 μm; Zorbax XDB-C18, 1.8 μm; HALO C18, 2.7 μm
Solute: naphthalene; mobile phase: 60% ACN/40% water, 24°C



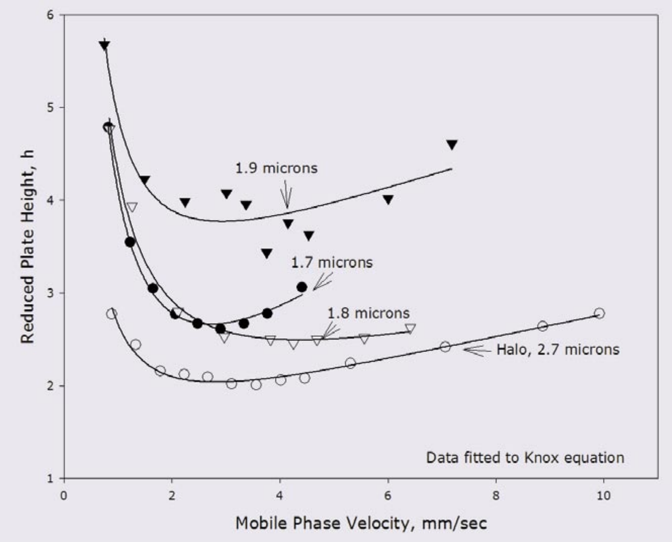
Reduced Plate Height h for Fused-Core Particles
Column: 50 x 4.6 mm, Halo C8; Temperature 24 C
Naphthalene (MW 128); 60% ACN/40% water
Lorazepam (MW 321): 30% ACN/70% 20 mM phosphate buffer, pH 3.5



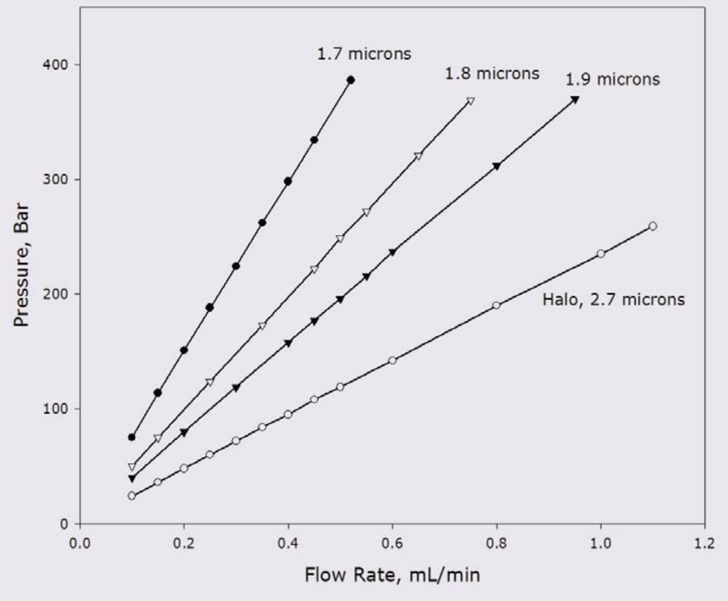
HALO Particle Size Distribution
Average: 2.77 micrometers
Standard deviation: 5.2%



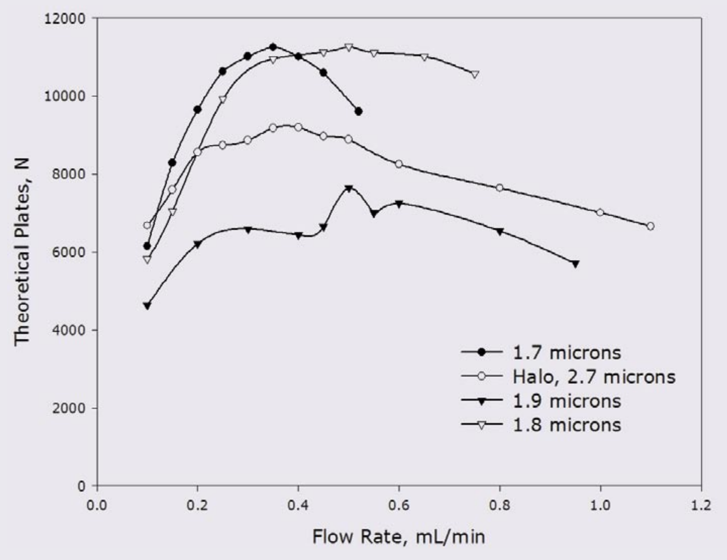
Reduced Plate Height Plots
Columns: 50 x 2.1mm, C18; Mobile phase: 70% ACN/30% water
Temperature: 24°C; Agilent 1100



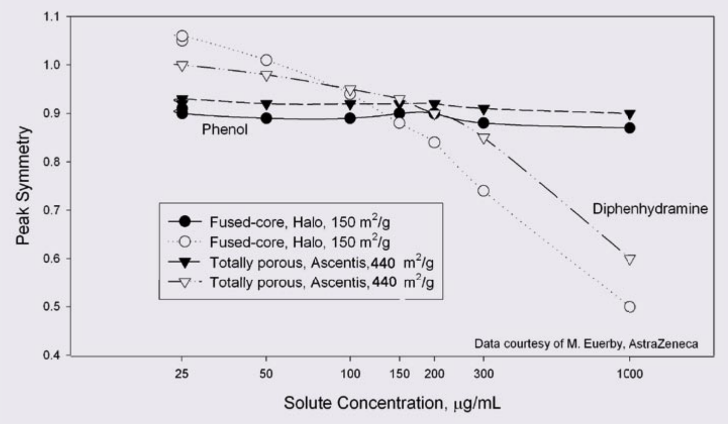
Back Pressure Plots for High-Speed Columns
Columns: 50 x 2.1mm, C18; Mobile phase: 70% ACN/30% water
Temperature: 24°C; Agilent 1100



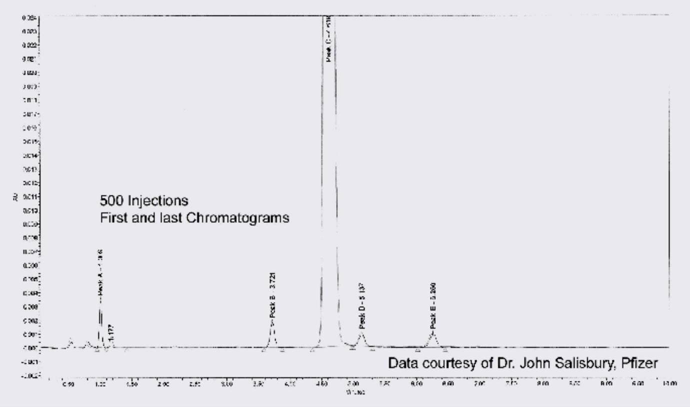
Theoretical Plates for High-speed Columns
Columns: 50 x 2.1mm, C18; Mobile phase: 70% ACN/30% water
Temperature: 24°C; Agilent 1100



Sample Loading Study: Totally Porous vs. Fused-core
Columns: Ascentis C18 and HALO C18, 150 x 4.6mm
Detection: 214 nm; Temperature 60°C; Injection: 5μL
Mobile Phase: A=20 nm KH_2PO_4, pH2.7; B=20nM KH_2PO_4, pH 2.7 in 35:65 v/v water/methanol; A : B = 30:70 v/v

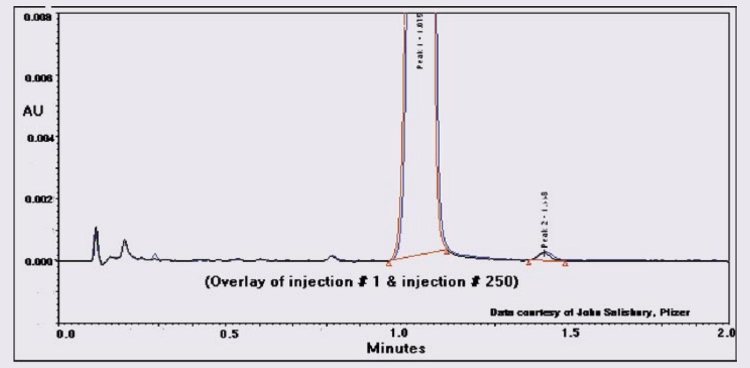


Column Stability Test
Columns: HALO C18, 100 x 4.6mm
70% ACN/30% water; 2.0mL/min; 35 C
Pressure: 218 bar
Retention times: 0.09% RSD
Theoretical plates: 0.70% RSD
Peak tailing: 0.27% RSD
Resolution, Peaks C and D: 0.39% RSD

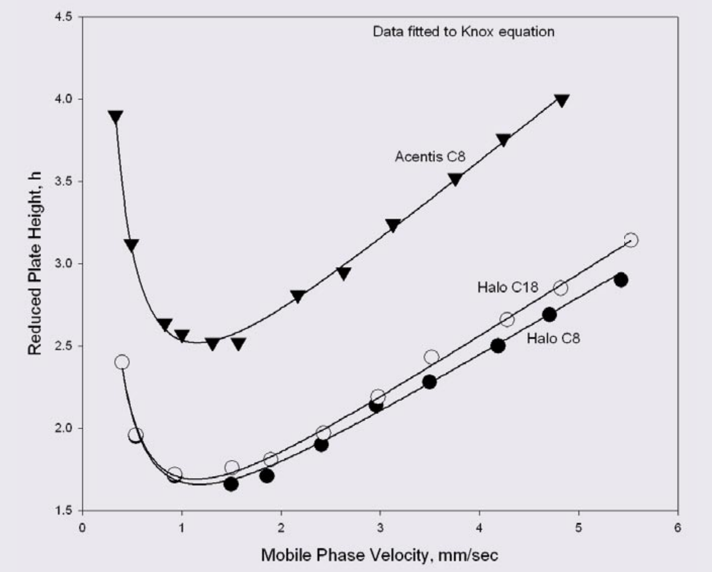


Fused-core Particle Column Stability Test

Columns: 2.1 x 100 mm HALO C18	Results, peak 1, n = 250
Mobile phase: 70% ACN/30% water	
Flow rate: 1.6 mL/min	RSD, retention time: 0.06%
Column temperature: 30°C	RSD, plates: 0.38%
Column back pressure: 12264 psi	RSD, tailing: 0.53%
Number of injections: 250	RSD, Rs, peaks 1,2: 2.7%

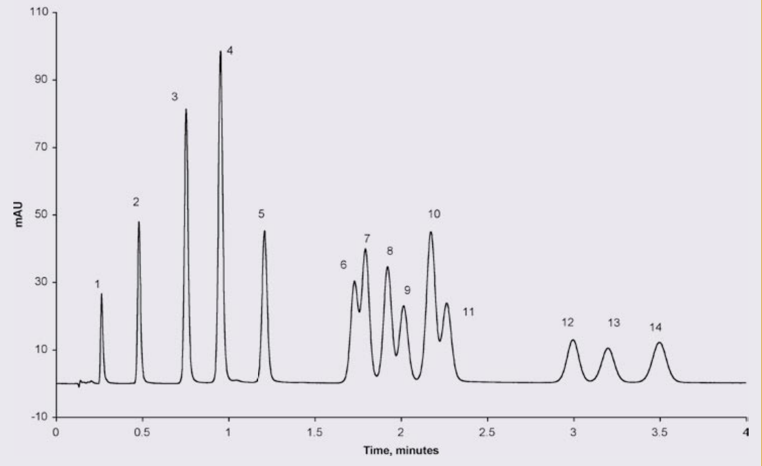


Column Comparison Study with Virginiamycin (MW=574)
Ascentis C8 (3.1μm) ; HALO C8 and HALO C18 (2.7μm)
Columns: 50 x 4.6 mm; Temperature: 30°C; Agilent 1100
Mobile phase: 35% ACN/65% potassium phosphate buffer, pH 3.0



Separation of Explosives
Column: 2.1 x 50 mm Halo C18
Mobile Phase: 27% Methanol / 73% Water **Temperature:** 40°C
Flowrate: 0.85 ml/min **Detector:** UV @ 254 nm
Pressure: 310 bar **Instrument:** Agilent 1100

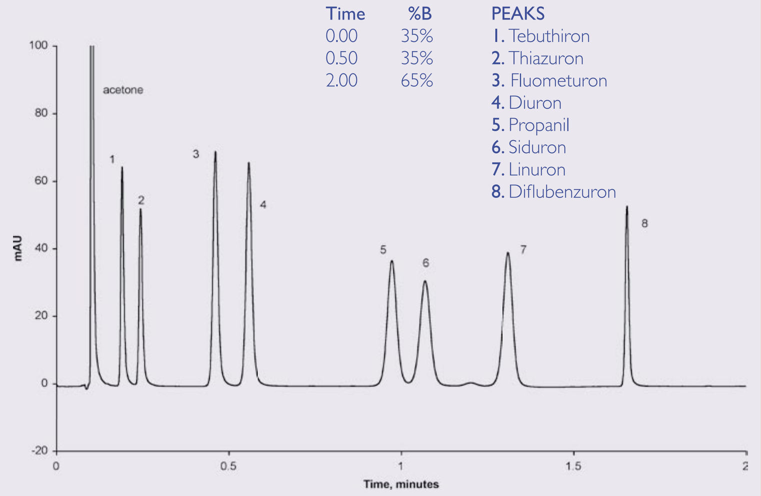
PEAKS
1. HMX
2. RDX
3. 1,3,5-trinitrobenzene
4. 1,3-dinitrobenzene
5. tetryl
6. nitrobenzene
7. 2,4,6-trinitrotoluene
8. 4-amino-2,6-dinitrotoluene
9. 2-amino-4,6-dinitrotoluene
10. 2,6-dinitrotoluene
11. 2,4-dinitrotoluene
12. 2-nitrotoluene
13. 4-nitrotoluene
14. 3-nitrotoluene



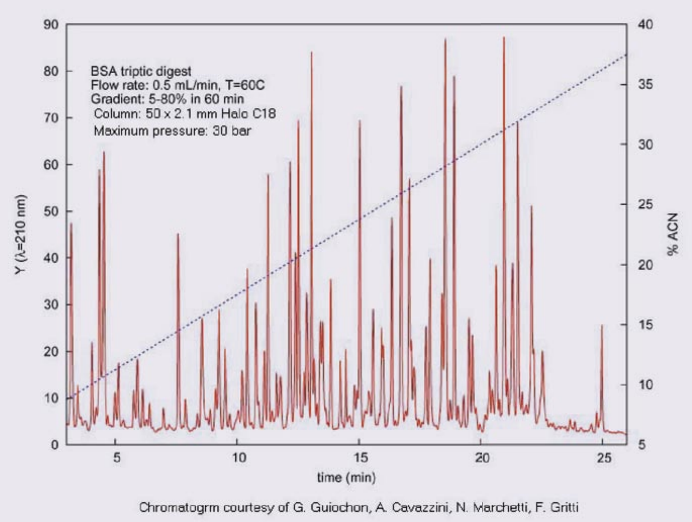
Separation of Herbicides
Column: 2.1 x 50 mm Halo C18 **Flowrate:** 1.0 ml/min
Detector: UV @ 245 nm **Temperature:** Ambient
Instrument: Agilent 1100 **Pressure:** 370 bar (initial), 295 bar (final)
Mobile Phase: gradient (A= H2O, B= ACN)

Time	%B
0.00	35%
0.50	35%
2.00	65%

PEAKS
1. Tebuthiron
2. Thiazuron
3. Fluometuron
4. Diuron
5. Propanil
6. Siduron
7. Linuron
8. Diflubenzuron



BSA triptic digest
Flow rate: 0.5 mL/min, T=60°C **Gradient:** 5-8-% in 60 min
Column: 50 x 2.1 mm HALO C18 **Maximum Pressure:** 30 bar



Chromatogrm courtesy of G. Guiochon, A. Cavazzini, N. Marchetti, F. Gritti

Conclusions Regarding Columns of 2.7-μm Fused-core Particles

- Produce unusual column efficiency; reduced plate heights *h* of ~1.5 for small molecules
- Efficiency and separation speed rivals that of sub-2-μm columns, but with about one-half the pressure drop
- Thin porous outer shell results in superior mass transfer kinetics and better efficiency at high mobile phase velocities, especially for larger molecules
- 2-μm porosity frits allow the convenience and ruggedness of columns with 5 μm particles
- Sample loading competitive with conventional totally porous particles
- Strong particles allow pressures of at least 600 bar