Comparison of Conventional, Sub-2-µm, and Superficially Porous (Fused-Core®) Particles for Nanobore LC/MS

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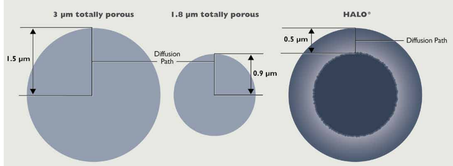
Introduction

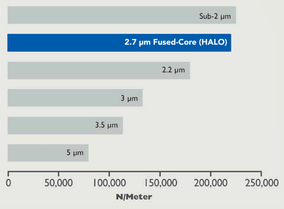
HPLC columns packed with sub-2-µm size particles are readily available from a variety of suppliers and have shown benefits of speed and resolution when packed in conventional columns with internal diameters of ≥1.0 mm. However, questions remain about whether these particles offer any significant advantages, or are even preferable to other types of stationary phase particles for typical nanobore LC/MS applications when optimizing either peak capacity or analysis time, or both. This paper will review three different types of stationary phase particles: 3.5 µm totally porous (conventional), 1.8 µm totally porous (sub-2-µm), and 2.7 µm superficially porous (Fused-Core) and discuss advantages and disadvantages of each for nanobore LC/MS.

FIGURE 1: Schematic comparison of totally porous 3 µm and sub-2 µm particles and superficially porous (Fused-Core) particle.



2.7 µm Fused-Core particles have shorter diffusion paths than either 3 µm or 1.8 µm totally porous particles. Shorter diffusion path reduces axial dispersion of solutes and minimizes peak broadening.

FIGURE 2: Comparison of column efficiency



In general, the efficiency (N, column plate number) of columns packed with totally porous particles varies with the inverse of the particle diameters. An exception to this is the Fused-Core particles. Although 2.7 µm in size, columns packed with these particles generate efficiencies that one would expect from 1.9 µm totally porous particles.

FIGURE 3: Calculation of peak capacity

$$P = 1 + [\sqrt{N}\, m\Delta\Phi / (2.3V_m m(\Delta\Phi/t_G F)+1) \bullet 1.9]$$

Where:
N: Column plate number
m: A solute dependent constant
ΔΦ: Gradient range
V_m: Column dead volume
t_G: Gradient time
F: Flow rate

Researchers have proposed several equations for calculating peak capacity. The one we have chosen to use in this study allows us to more easily explore the effects of column plate number, gradient range, gradient slope, and flow rate on peak capacity.

Materials & Methods:

INSTRUMENTATION:

- Eksigent NanoLC –2D
  - o 2% ACN, 0.1% Formic Acid
  - o Isocratic 1-5 µL/min, 2.5 µL/min typical
  - Elution channel: (low flow)
  - o A=0.1% formic acid; B=0.1% formic acid in ACN
  - o Gradient elution: linear gradient, 2% to 50% B
  - o Flow velocity from 0.12 to 0.42 cm/sec
- Leap HTC autosampler
  - 1 µL full loop injection with 6-port nanovalve (Valco)
  - On-column injection with 5 min wash/desalt
- Thermo LCQ Deca 3-d Ion Trap
  - Full scan MS: 300-1500 m/z, 3 microscans/spectra
  - Fast gradients acquired in Turboscan mode
- Digital PicoView nanospray source (New Objective) model DPV-150

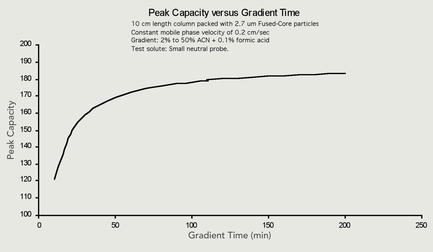
COLUMNS:

- PicoFrit analytical column: 75 um x 100 mm; 15 um integral emitter (New Objective, Inc.) packed with the following particles:
  - 3.5 µm totally porous silica, C18 bonded phase
  - 2.7 µm superficially porous "Fused-Core™ silica, C18 bonded phase (HALO® C18)
  - Sub-2 µm totally porous silica, C18 bonded phase

SAMPLES:

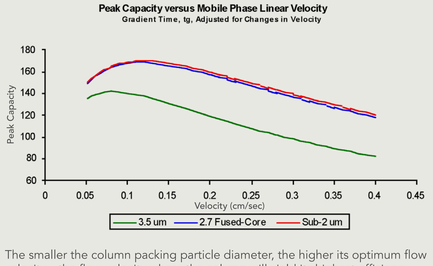
- Tryptic digest standard of Bovine Serum Albumin (Waters Corp) diluted to 100fmol/µL

FIGURE 4: Peak capacity and gradient time



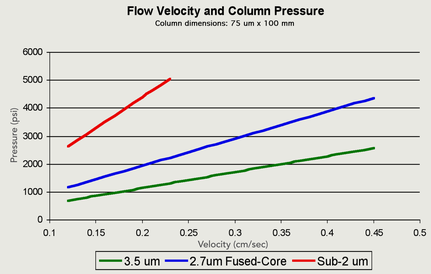
Previous articles have confirmed that peak capacity increase in an asymptotic fashion with gradient time.

FIGURE 5: Peak capacity and mobile phase flow velocity



The smaller the column packing particle diameter, the higher its optimum flow velocity—the flow velocity where the column will yield its highest efficiency. The 2.7 µm Fused-Core particles yield their highest efficiency at a flow velocity similar to that of 1.9 µm totally porous particles.

FIGURE 6: Comparison of column pressure



The excessively high back pressure of nanobore columns packed with sub-2 um particles limit their effective use with typical LC/MS systems that have maximum acceptable pressure limits of 5,000 psi (330 bar). The sub-2 µm column will exceed 5,000 psi at a flow velocity of greater than ~ 2.2 cm/sec. Both the 3.5 µm and 2.7 µm Fused-Core columns can be operated at twice that flow rate and still not exceed the maximum acceptable pressure limit.

Peak Capacity, Pressure and Analysis Time Comparison

Column: 1.8 µm, 75 µm x 100 mm

Flow Velocity (cm/sec.)	Peak Capacity	Analysis Time (min.)	Pressure (psi)
0.10	169	25	2190
0.15	168	17	3290
0.20	160	12	4400
0.25	150	10	5490

Column: 2.7 µm Fused-Core, 75 µm x 100 mm

Flow Velocity (cm/sec.)	Peak Capacity	Analysis Time (min.)	Pressure (psi)
0.10	168	25	1070
0.15	166	17	1460
0.20	157	12	1940
0.25	147	10	2430
0.30	137	8	2910
0.35	127	7	3400
0.40	118	6	3880

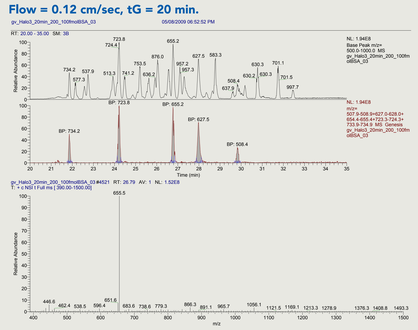
Column: 3.5 µm, 75 µm x 100 mm

Flow Velocity (cm/sec.)	Peak Capacity	Analysis Time (min.)	Pressure (psi)
0.10	140	25	570
0.15	131	17	860
0.20	119	12	1140
0.25	108	10	1430
0.30	98	8	1710
0.35	90	7	1990
0.40	82	6	2280

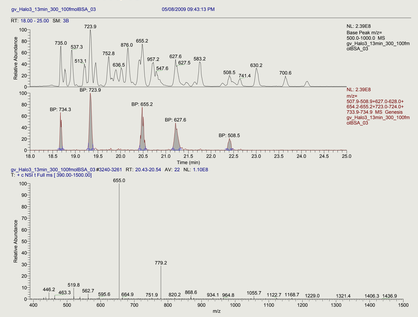
Columns packed with 3.5 µm particles can provide faster separation times because it can be run at higher flow velocities before encountering pressure limitations, however, they will yield much lower peak capacity than either the sub-2 µm or 2.7 µm Fused-Core columns. Columns packed with sub-2 µm particles will likely give the highest peak capacity, but the 5,000 psi pressure maximum of many LC/MS systems severely limits the flow velocity that can be used and, therefore, limits separation speed. Columns packed with 2.7 µm Fused-Core particles can be operated at high mobile phase flow velocities without exceeding the 5,000 psi pressure limit permitting them to run much faster separations than sub-2 µm packed columns when used with typical LC/MS system, although with some reduction in peak capacity.

FIGURE 7: Reducing analysis time by increasing flow velocity and adjusting gradient time

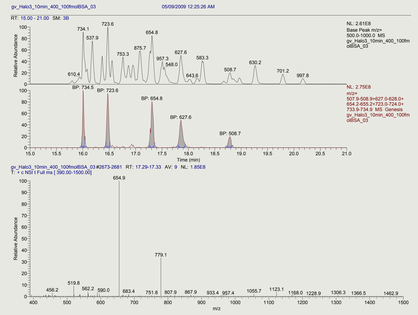
Flow = 0.12 cm/sec, tG = 20 min.



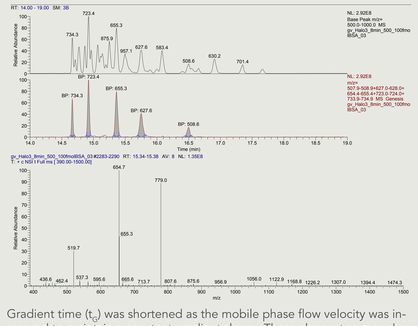
Flow = 0.18 cm/sec, tG = 13 min.



Flow = 0.24 cm/sec, tG = 10 min.



Flow = 0.30 cm/sec, tG = 8 min.



Gradient time (t_G) was shortened as the mobile phase flow velocity was increased to maintain a constant gradient slope. These chromatograms show how analysis time can be significantly reduced and band spacing maintained by increasing flow velocity and reducing t_G accordingly. However, on our system (see Materials and Methods) the peak capacity was over 20% lower than what was expected based on data generated on more conventional (column ID >2.0 mm) columns. We are currently investigating the cause of these lower than expected results and will explore techniques for packing these unique Fused-Core particles in nanobore columns. We will also explore the possibility that some unexpected secondary retention of solutes or pore volume limitations may be playing a role.

Conclusion

Nanobore columns packed with 3.5 µm totally porous stationary phase support particles, provide adequate peak capacity for many LC/MS applications and offer the advantages of low back pressure and reliable operation. 2.7 µm Fused-Core particles (HALO) are favored when both peak capacity and speed of separation are important, particularly when typical LC systems with acceptable pressure limits of less than 5,000 psi are being used. Where UHPLC equipment is available, nanobore columns packed with sub-2 µm particles can offer the speed of Fused-Core columns with 2 to 5% higher peak capacity, although at a cost of 40 to 50% higher pressure.

References

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