Effect of Changing Pore Size and Shell Thickness on Fused-Core® Particles

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INTRODUCTION

Columns of Fused-core® 2.7 µm superficially porous particles with 0.5 µm thick shells have shown high separation efficiencies for small molecules up to about 5000 MW. With fused-core particle technology, one has the advantages of sub-2-µm performance without the corresponding high operating pressures. Recently, fused-core particles with 160 Å pores were introduced for rapid high-resolution separations of polypeptides and small proteins with molecular weights up to about 20,000. The superior mass transfer properties of this technology enables very fast separations of protein digests with good peak resolution. Since the bonded phase is stable at temperatures up to at least 100 °C and at pHs as low as 1, these columns lend themselves to use for high-throughput LC/MS analyses with excellent sensitivity and precision. To further examine the benefits of fused-core technology, particles with varying porous shell thickness and pore size have been developed and studied with regard to column performance, particularly for large molecules.

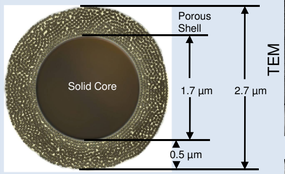
MATERIALS AND METHODS

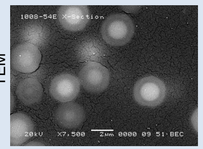
- Columns of HALO C18, HALO Peptide ES-C18, and columns of 2.3 µm size with a 0.3 µm shell were produced at Advanced Materials Technology Inc. (Wilmington, DE).

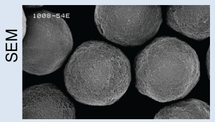
- HPLC analyses used the quaternary Agilent 1100 controlled with ChemStation software. The capillary LC was connected to the ThermoFisher LTQ ion-trap mass spectrometer via the Michrom Bioresource Advance spray source. Samples from the autoinjector were captured on the EXP Stem Trap (2.6 µL) cartridge packed with Halo Peptide ES-C18 (Optimize Technologies), using the LTQ automated valve. Acetonitrile and trifluoroacetic acid were purchased from EMD Chemicals (Gibbstown, NJ). Ribonuclease A and Leucine-enkephalin were purchased from Sigma Aldrich (St. Louis, MO). LH-RH and β-amyloid were purchased from AnaSpec (Freemont, CA).

- Chromatographic peak widths are reported as determined at 50% height. Peak capacities were calculated as $(t_f - t_i)/W_{4\sigma}$, where t_f is the final time of the gradient , t_i is the initial time of the gradient, and $W_{4\sigma}$ is the average width of specific peaks measured at 4σ.

FUSED-CORE PARTICLES



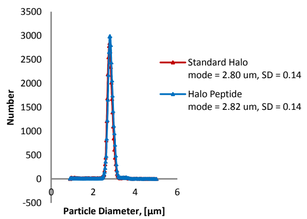




Halo Peptide Particles
Silica ----------High Purity Type B
Ave. pore diameter----------160 Å
Surface area, nitrogen----------80 sq.m/g
Pore volume ----------0.30 mL/g
Particle density ----------1.3 cc/g

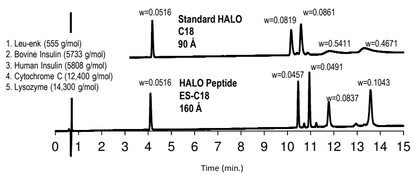
PORE SIZE EFFECTS ON FUSED-CORE PARTICLES

Particle Size Distributions: 90 Å vs. 160 Å

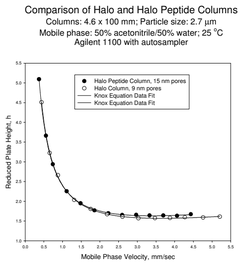


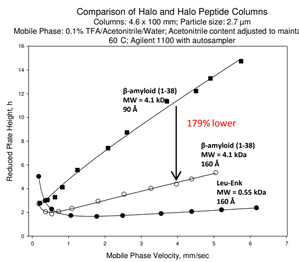
Effect of Pore Size on Protein and Peptide Separations

Columns: 4.6 x 100 mm; **Flow rate:** 1.5 mL/min; **Temp:** 30° C; **A:** 0.1% TFA/10% ACN, **B:** 0.1% TFA/70% ACN; **Gradient:** 15% to 50% B in 15 min.; **Injection volume:** 5 µL



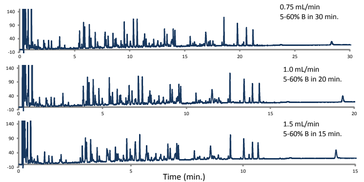
Effect of Pore Size on Efficiency



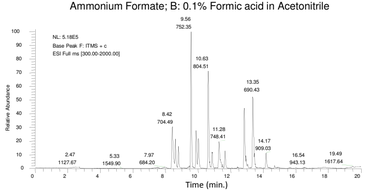


Fast Tryptic Digest Separations Using Halo Peptide ES-C18

Column: 2.1 x 100 mm Halo Peptide ES-C18; A: Water/ 0.1% TFA, B: 80% ACN / 0.1% TFA., Detection: 215 nm, Sample: apotransferrin tryptic digest, Injection of 15 uL (15 µg), Temp: 60 C

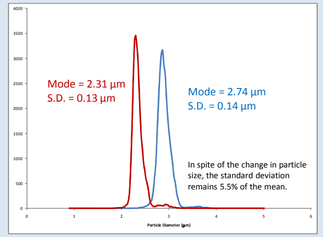


Halo Peptide ES-C18, 0.2 mm ID x 50 mm, Flow Rate 9 µL/min., 2-45% B in 15 minutes, 3 pmol apoMyoglobin digest in 2 µL; A: 0.1 % Formic Acid/10 mM Ammonium Formate; B: 0.1% Formic acid in Acetonitrile



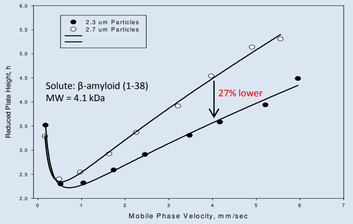
SHELL THICKNESS EFFECTS ON FUSED-CORE PARTICLES

Particle Size Distributions: 2.7 µm vs. 2.3 µm



Effect of Shell Thickness on Efficiency

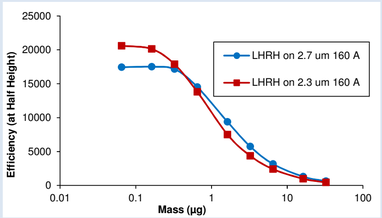
Particle size (µm)	Shell thickness (µm)	Surface Area (m^2/g)	Pore Size (Å)	Naphthalene k'	Efficiency (50% height)	Pressure (bar)
2.7	0.5	124	90	1.90	13579	112
2.3	0.3	76	90	1.43	13326	168
2.7	0.5	77	160	1.19	12861	122
2.3	0.3	49	160	0.90	15440	164



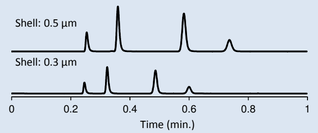
Columns: 4.6 x 50 mm SP-C8, 2.7 µm, 0.5 µm shell, 160 Å and 2.3 µm, 0.3 µm shell, 160 Å
Mobile Phase: 27.4% ACN/72.6% Water/0.1% TFA
Temperature: 60 °C
Detection: 215 nm

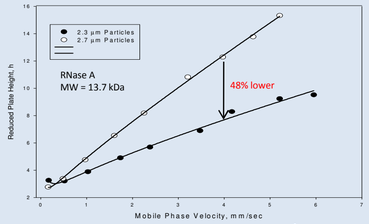
Effect of Shell Thickness on Sample Loading

Column: 4.6 x 100 mm SP-C8; **Mobile Phase:** Isocratic: 2.7 µm: 17% ACN/83% Water/0.1% TFA; 2.3 µm: 16.5% ACN/83.5% Water/0.1% TFA; **Flow rate:** 1.0 mL/min; **Temp:** 60 °C; **Detection:** 220 nm; **LC System:** Agilent 1100; **Sample:** Luteinizing Hormone-Releasing Hormone (LH-RH) MW = 1182



Column: 4.6 x 50 mm SP-C8; **Mobile Phase:** 60% ACN/40% Water; **Flow rate:** 1.8 mL/min **Temp:** 30 °C; **Detection:** 254 nm; **LC System:** Agilent 1100; **Sample:** uracil, phenol, 4-chloronitrobenzene, and naphthalene



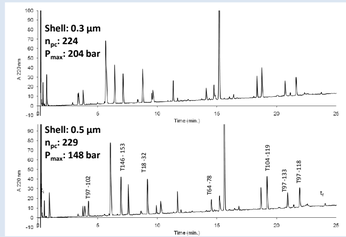


Columns: 4.6 x 50 mm SP-C8, 2.7 µm, 0.5 µm shell, 160 Å and 2.3 µm, 0.3 µm shell, 160 Å
Mobile Phase: 24.5% ACN/75.5% Water/0.1% TFA
Temperature: 60 °C
Detection: 215 nm

Effect of Shell Thickness on Peak Capacity

Peak capacity was calculated by averaging the peak widths of the labeled tryptic digest peaks above and using the following equation: $n_{pc} = \frac{t_f - t_i}{W_{4\sigma}}$

Columns: 4.6 x 50 mm SP-C8 2.7 µm 160 Å and 4.6 x 50 mm SP-C8 2.3 µm 160 Å; **Mobile Phase:** A: Water/0.1% TFA B: 80% ACN/20% Water/0.1% TFA; **Gradient:** 5-60% B in 30 min.; **Flow rate:** 2.4 mL/min; **Temp:** 60 °C; **Detection:** 220 nm; **LC System:** Agilent 1100; **Sample:** Apo-myoglobin tryptic digest



CONCLUSIONS AND FUTURE DIRECTIONS

Increasing the pore size and decreasing the shell thickness could be beneficial for the analysis of large molecules. The performance of the altered particles was investigated by measuring column efficiency, analyte kinetics, and efficiency relative to sample loading. We conclude:

- Fused-core particles can be produced with tight size distributions with different pore sizes and different shell thicknesses.

- Increasing the pore size of the Fused-core particles improves the mass transfer for larger molecular weight solutes.

- Decreasing the shell thickness from 0.5 to 0.3 µm exhibited significant mass transfer improvement for larger molecular weight solutes.

- The 0.3 µm shell particles exhibited higher efficiencies at low sample loads relative to the 0.5 µm shell particles. At intermediate sample loads the advantages were eliminated.

- Peak capacities of protein tryptic digests were similar on the 2.7 and 2.3 µm fused-core particles due to decreased retention balancing peak width improvements with the 2.3 µm particles.

This leads us to believe that there is an optimum shell thickness and pore size for the analysis of large molecules. In our future work, we will investigate the effects of larger pore sizes while varying both core diameter and shell thickness.