Effects of Changing Shell Thickness and Pore Size of Fused-Core Particles

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Objective

The objective is to determine how changes to the shell thickness and pore size affect the performance of Fused-core particles, particularly for large molecules.

Introduction

Columns of fused-core particles of 2.7 µm diameter with 0.5 µm thick shells or porous layers have given efficiencies comparable to columns packed with totally porous sub 2 µm particles. The advantage of using fused-core particles is that they perform like sub 2 µm particles without the high back pressure. Recently, a fused-core particle with 160 Å pores was developed specifically for the separation of peptides and peptide tryptic digests. This study examines the feasibility of enhancing the performance of superficially porous particles by changing the shell thickness and pore size. The potential exists that a thinner shell will benefit separations of larger molecules, such as proteins.

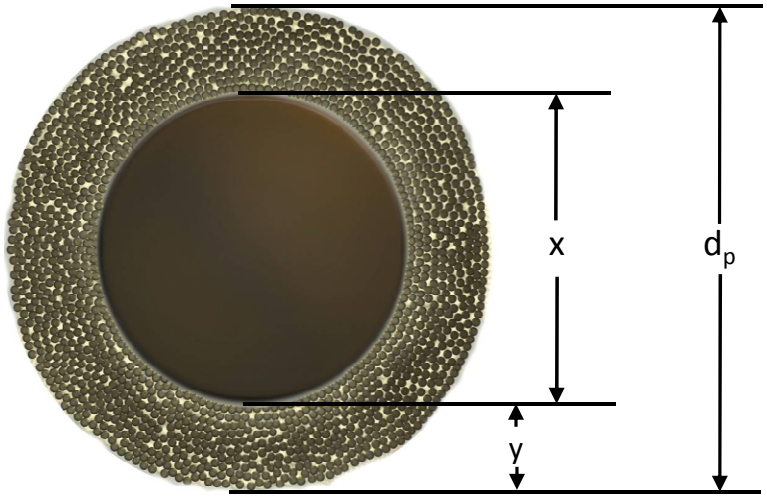
Materials and Methods

- Columns of 2.7 and 2.3 µm fused-core particles with bonded phase SP-C8 were obtained from Advanced Materials Technology, Inc. (Wilmington, DE).
- RP-HPLC separations were conducted on both an Agilent 1100 and Agilent 1200. Stability studies were carried out on a Shimadzu Prominence LC System. Peptides and proteins were obtained from Sigma Aldrich (St. Louis, MO). Acetonitrile and trifluoroacetic acid were purchased from EMD Chemicals (Gibbstown, NJ). LH-RH was purchased from AnaSpec (Freemont, CA).
- Chromatographic peak widths are reported as determined at 50% height, and 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.

Superficially Porous Particles

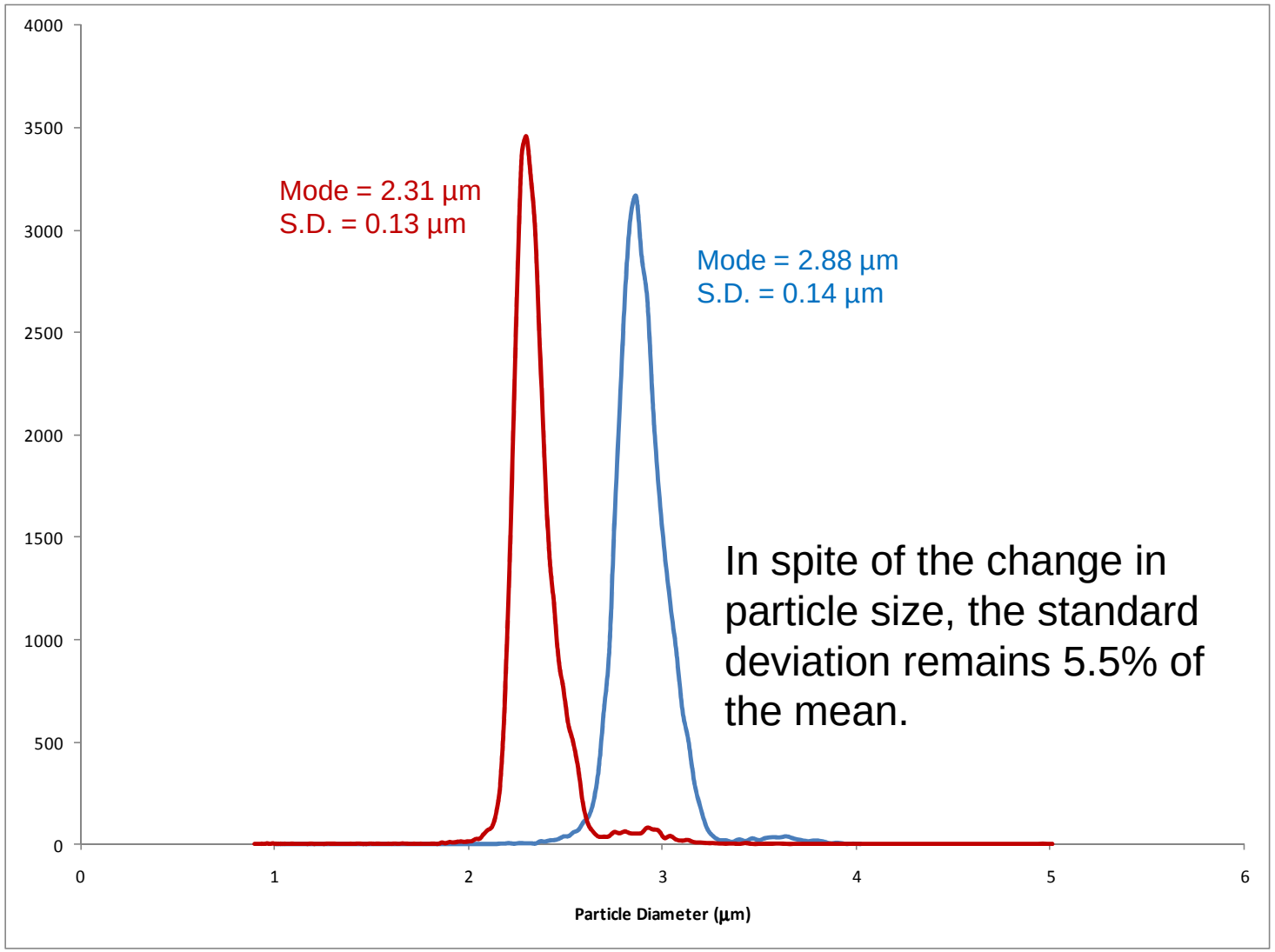
Fully rehydroxylated silica microspheres were prepared as previously described[1], with a fixed solid core of 1.7 µm diameter, while varying the shell thickness from 0.5 to 0.3 µm. The resulting materials were covalently bonded to yield diisopropyl-octyl silane bonded phase.



x (µm)	y (µm)	d_p (µm)	Surface Area (m^2/g)	Pore Volume (mL/g)
1.7	0.5	2.7	124	0.27
1.7	0.3	2.3	76	0.18

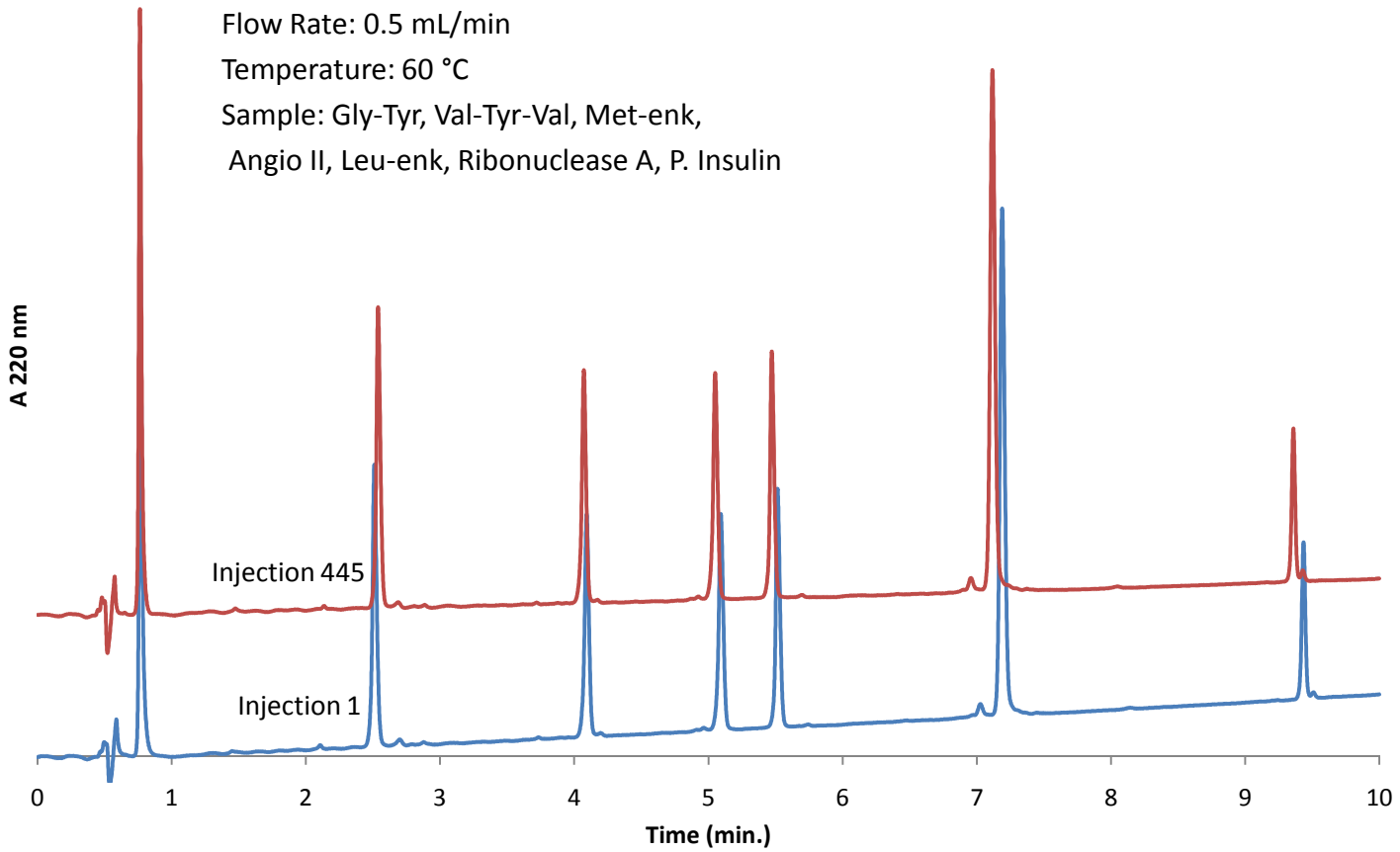
American Laboratory, 39, 2007, 18-21.

Particle Size Distribution



Stability

Column: 2.1 x 100 mm SP-C8 2.7 µm 160 Å
Mobile Phase: A: 0.1% TFA; B: 0.1% TFA/70% ACN;
Gradient: 9-55% B in 10 min
Flow Rate: 0.5 mL/min
Temperature: 60 °C
Sample: Gly-Tyr, Val-Tyr-Val, Met-enk,
Angio II, Leu-enk, Ribonuclease A, P. Insulin



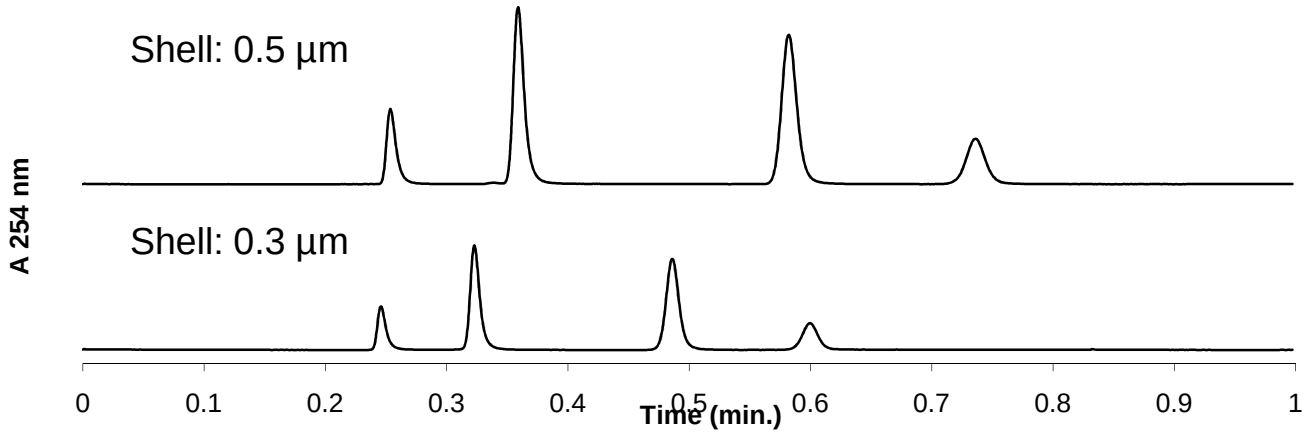
The SP-C8 bonded phase is highly stable at both high temperature and low pH.

Effect of Shell Thickness on Efficiency

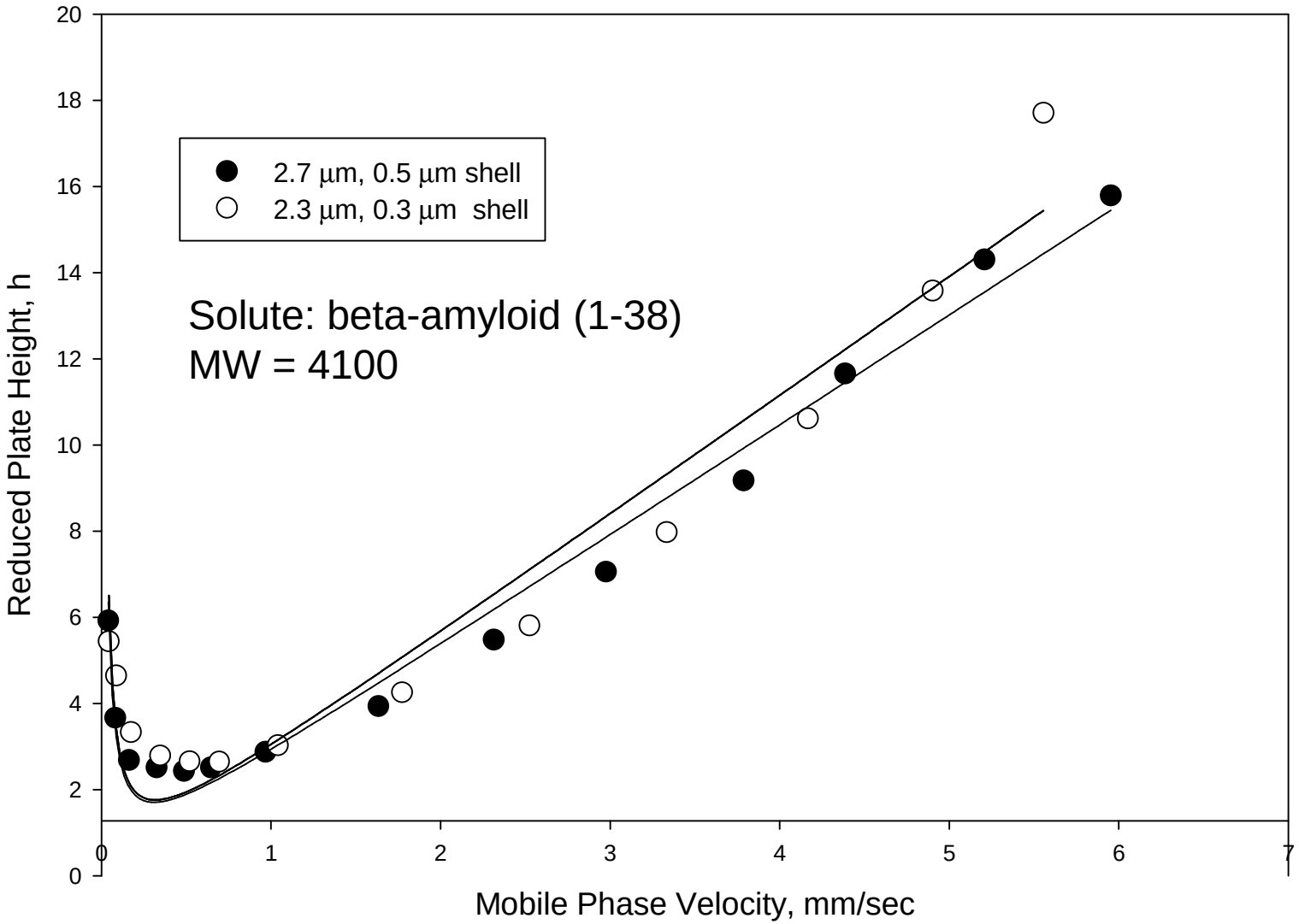
Particle size (µm)	Shell thickness (µm)	Surface Area (m^2/g)	Nominal Pore Size (Å)	Naphthalene k'	Efficiency (50% height)	Pressure (bar)
2.7	0.5	124	90	1.90	13579	112
2.3	0.3	76	90	1.43	13326	168
2.7	0.5	77	160	1.19	12861	122
2.3	0.3	49	160	0.90	15440	164

Column: 4.6 x 50 mm SP-C8 90 Å
Mobile Phase: 60% ACN/40% Water
Flow rate: 1.8 mL/min
Temperature: 30 °C

Detection: 254 nm
LC System: Agilent 1100
Sample: uracil, phenol, 4-chloronitrobenzene, and naphthalene



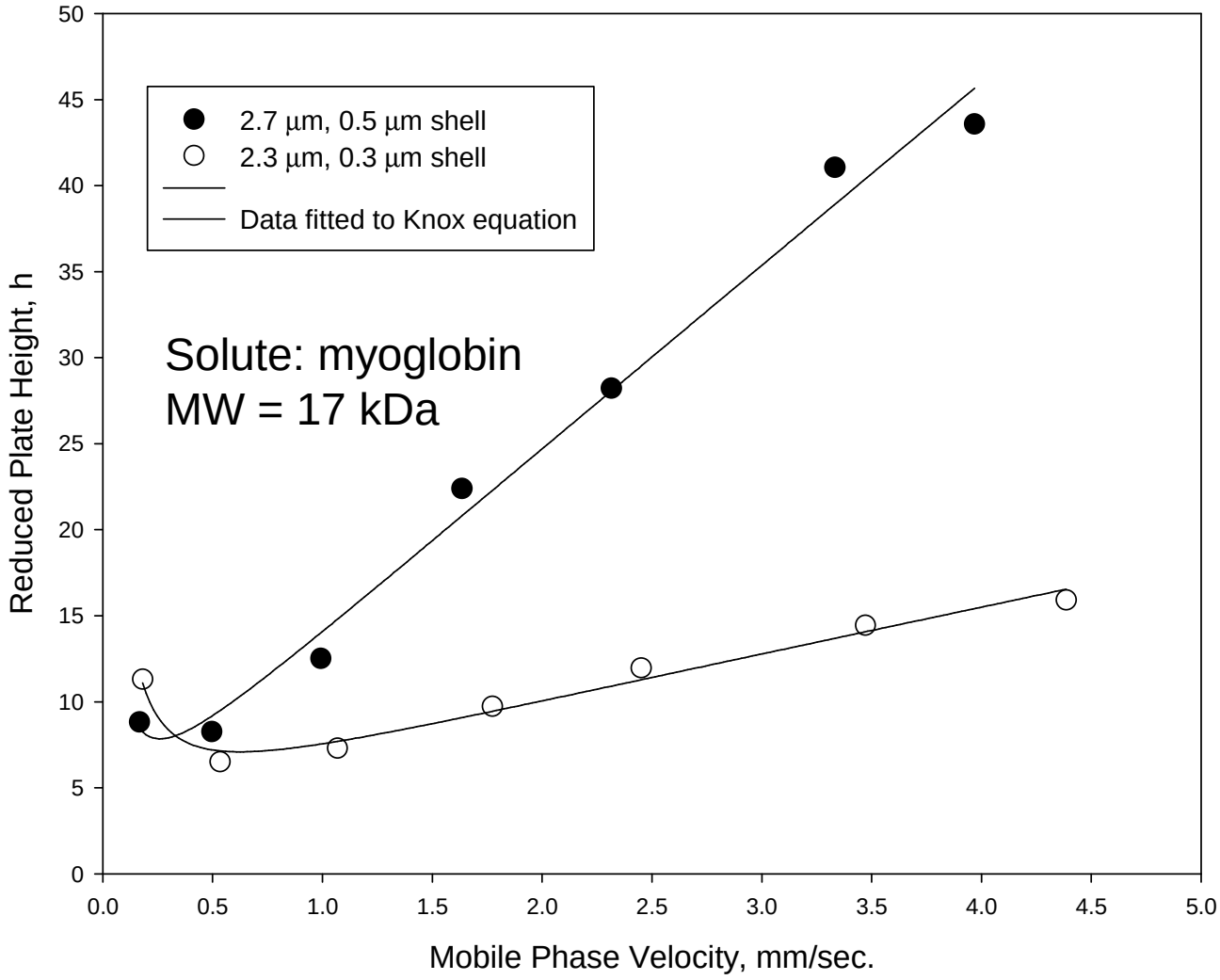
Effect of Shell Thickness on Efficiency



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: 2.7 µm: 27.8% ACN/72.2% Water/0.1% TFA
2.3 µm: 27.4% ACN/72.6% Water/0.1% TFA

Temperature: 60 °C
Detection: 215 nm
LC System: Agilent 1200

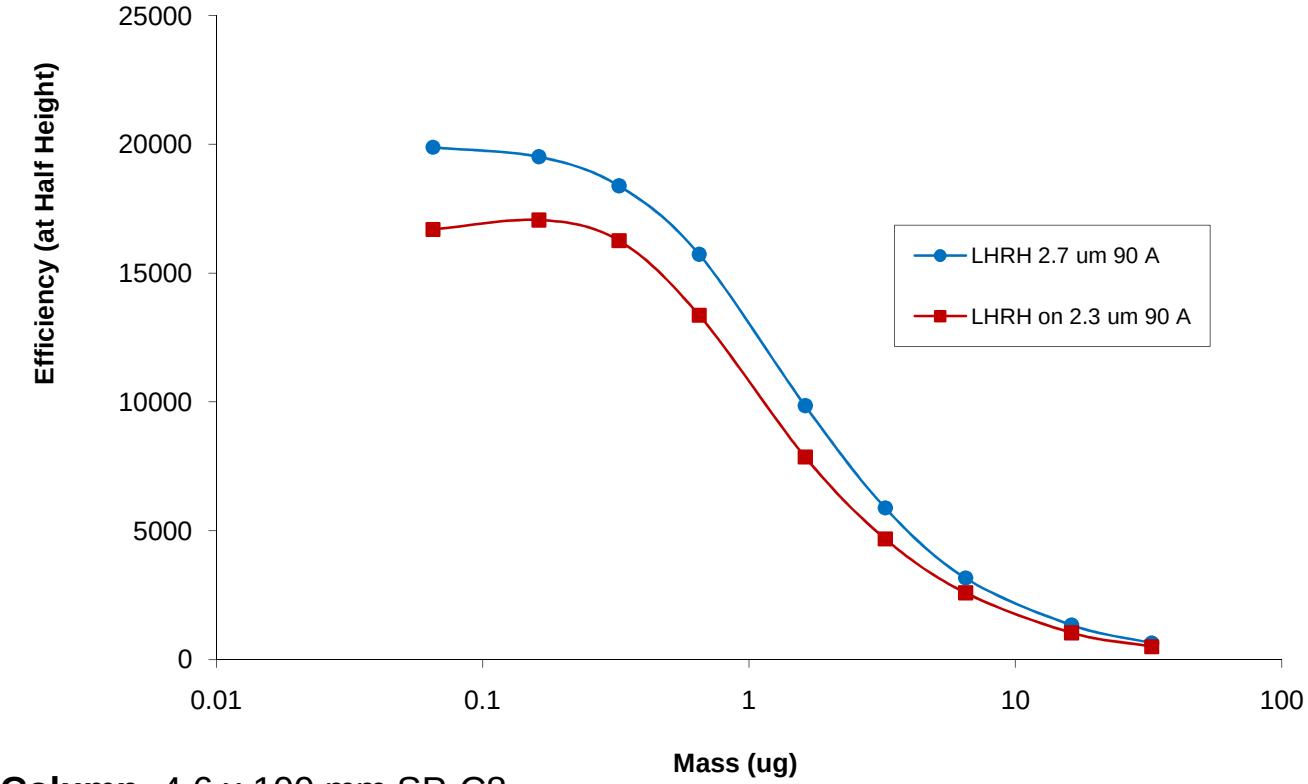
Effect of Shell Thickness on Efficiency



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: 39.7% ACN/60.3% Water/0.1% TFA

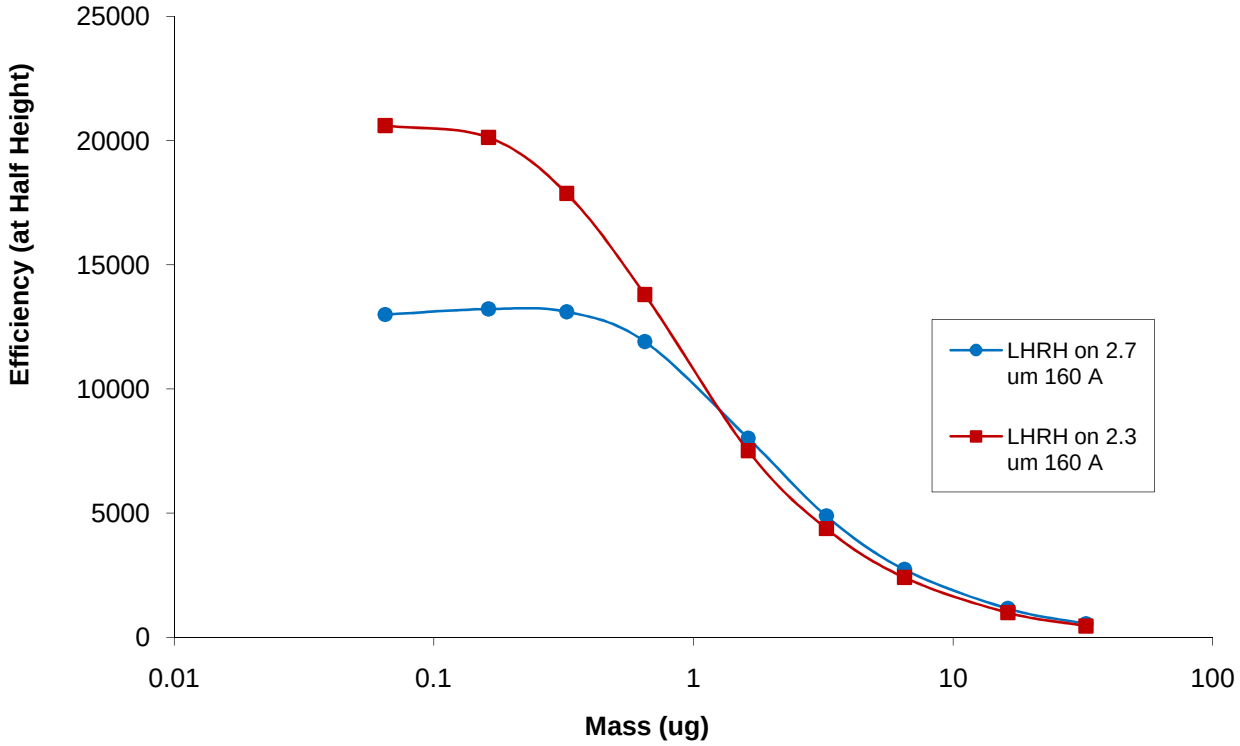
Temperature: 60 °C
Detection: 215 nm
LC System: Agilent 1100

Effect of Shell Thickness on Sample Loading



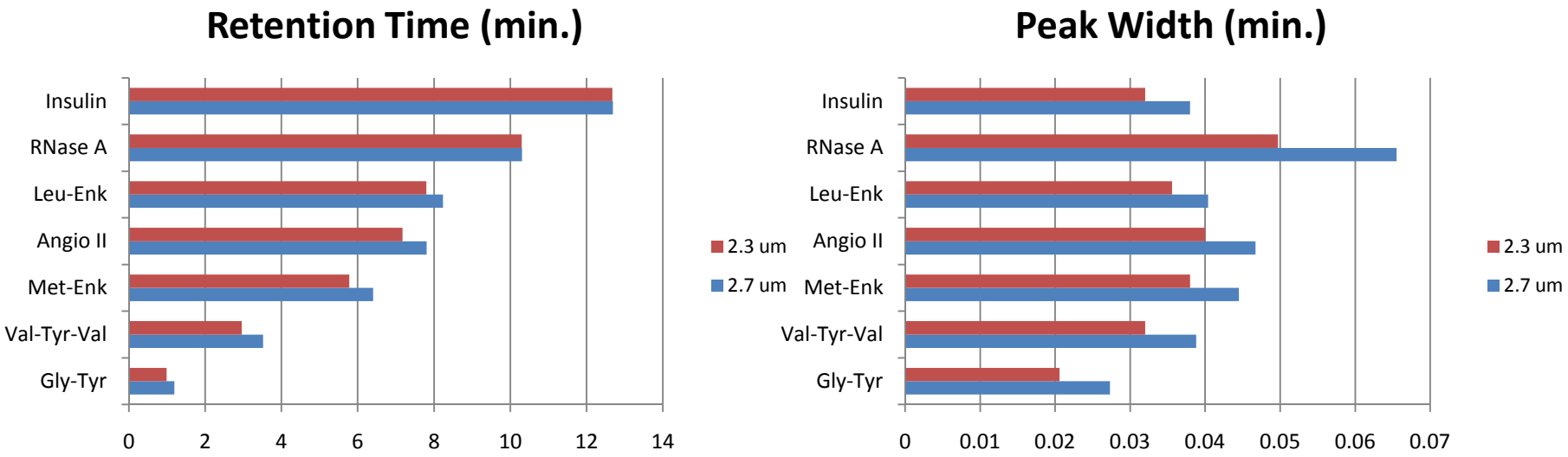
Column: 4.6 x 100 mm SP-C8
Mobile Phase: Isocratic: 2.7 µm: 18% ACN/82% Water 2.3 µm: 17.4% ACN/82.6% Water
Flow rate: 1.0 mL/min
Temperature: 60 °C
Detection: 220 nm
LC System: Agilent 1100
Sample: Luteinizing Hormone-Releasing Hormone (LH-RH) MW = 1182

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 2.3 µm: 16.5% ACN/83.5% Water
Flow rate: 1.0 mL/min
Temperature: 60 °C
Detection: 220 nm
LC System: Agilent 1100
Sample: Luteinizing Hormone-Releasing Hormone (LH-RH) MW = 1182

Effect of Shell Thickness on Retention and Peak Width

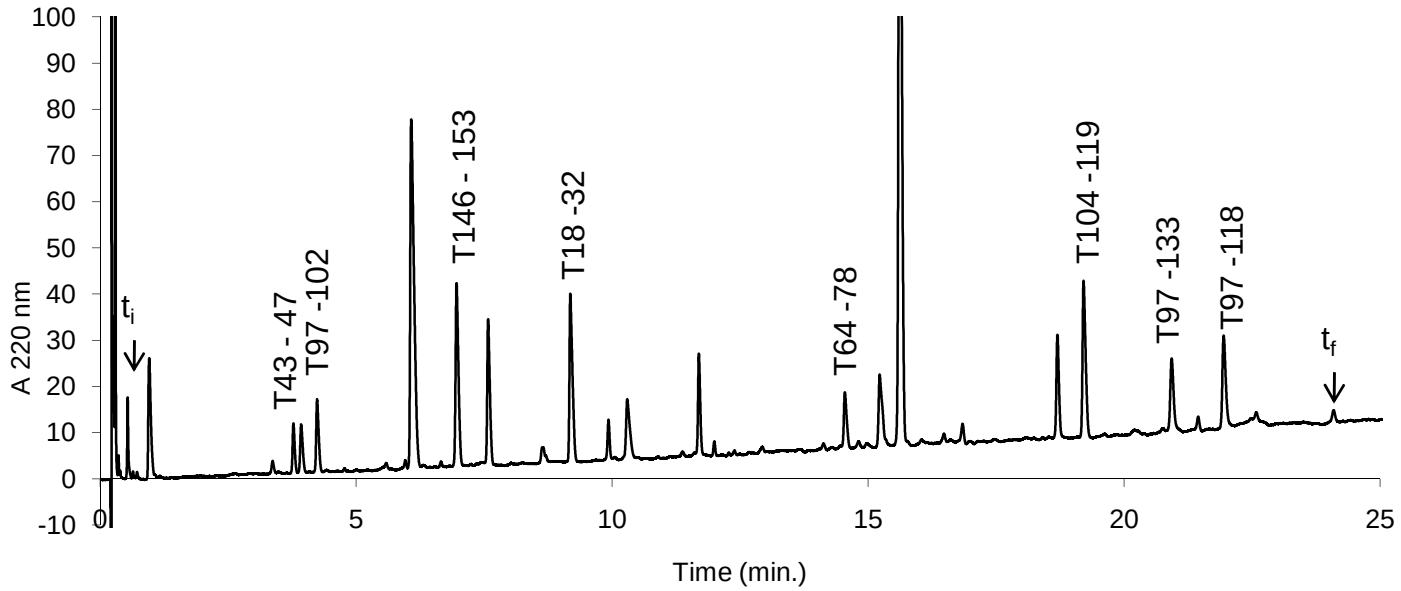


Columns: 4.6 x 100 mm SP-C8, 2.7 µm, 0.5 µm shell, 160 Å and
2.3 µm, 0.3 µm shell, 160 Å
Mobile Phase: A: 10% ACN/90% Water/0.1% TFA; B: 70% ACN/30% Water/0.1% TFA

Gradient: 0-50% B in 15 min.
Flow rate: 1.5 mL/min
Temperature: 30 °C
Detection: 220 nm
LC System: Agilent 1100

The retention times are shorter for the peptides on the 0.3 µm shell particles, but about the same for the proteins compared to the 0.5 µm shell particles. This is expected due to the smaller surface area of the 0.3 µm shell particles. The peak widths are smaller for both the peptides and the proteins using the 0.3 µm shell particles, but the peak width is reduced the most for RNase A, which is the largest analyte in the sample.

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:

$$n_{pc} = \frac{t_f - t_i}{W_{4\sigma}}$$

Particle Size (µm)	Peak Capacity	Pressure$_{max}$ (bar)
2.7	229	148
2.3	224	204

Column: 4.6 x 50 mm SP-C8 2.7 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
Temperature: 60 °C
Detection: 220 nm
LC System: Agilent 1100
Sample: Apo-myoglobin tryptic digest

Summary and Conclusions

Manipulating shell thickness and pore size have a direct impact on the performance of fused-core particles. 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 stability, efficiency, analyte kinetics, and efficiency relative to sample loading. Based on the observations made throughout the course of this work the following conclusions have been drawn:

- The 2.7 and 2.3 µm fused-core particles can both be produced with tight size distributions.
- The SP-C8 bonded phase has been proven to be stable at high temperature and low pH.
- Decreasing the shell thickness from 0.5 to 0.3 µm exhibited significant mass transfer improvement for large molecular weight solutes (MW approximately > 10 kDa).
- In general, the 2.3 µm particles with 0.3 µm shell thickness exhibited higher efficiencies at low sample loads relative to the 2.7 µm particles with 0.5 µm shell thickness. At intermediate sample loads the advantages were eliminated.
- Peak capacities of protein tryptic digests were not significantly different 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 for the analysis of large molecules. In our future work, we will investigate the effects of changing core diameter and shell thickness while maintaining a fixed particle size.