Fused-Core® Particles: Varying Shell Thickness and Pore Size

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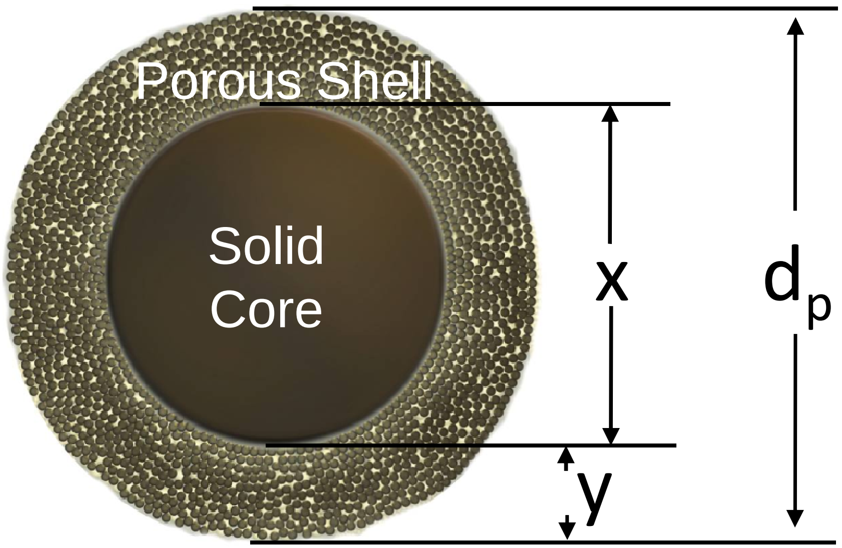
Overview

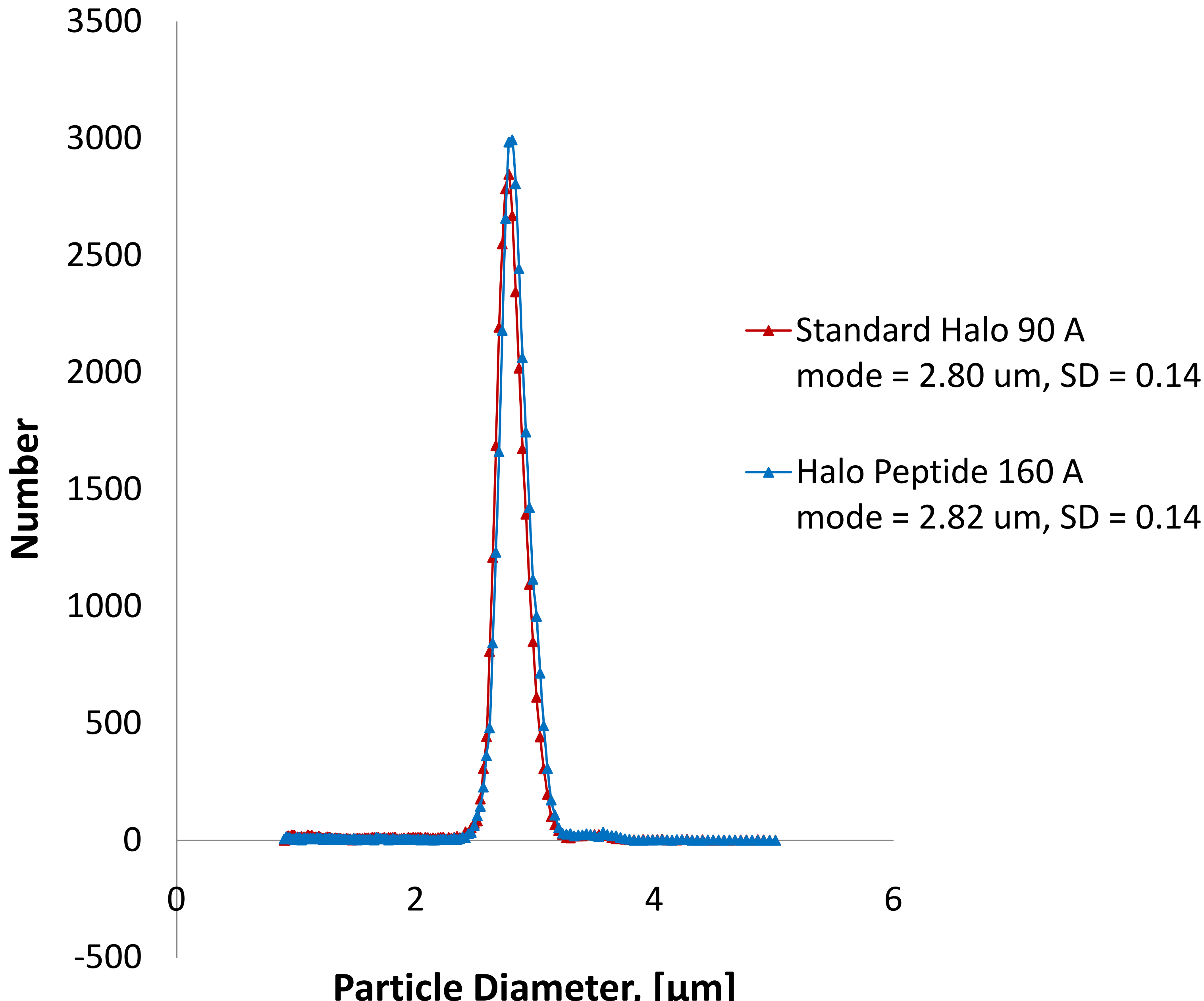
- Fused-core particles
  - Changes to pore size
  - Changes to shell thickness
- Conclusions
- Future Directions

Fused-Core Particles: Varying Pore Size



x (µm)	y (µm)	d_p (µm)	Pore Size (Å)	Surface Area (m^2/g)
1.7	0.5	2.7	90	150
1.7	0.5	2.7	160	80

Particle Size Distributions



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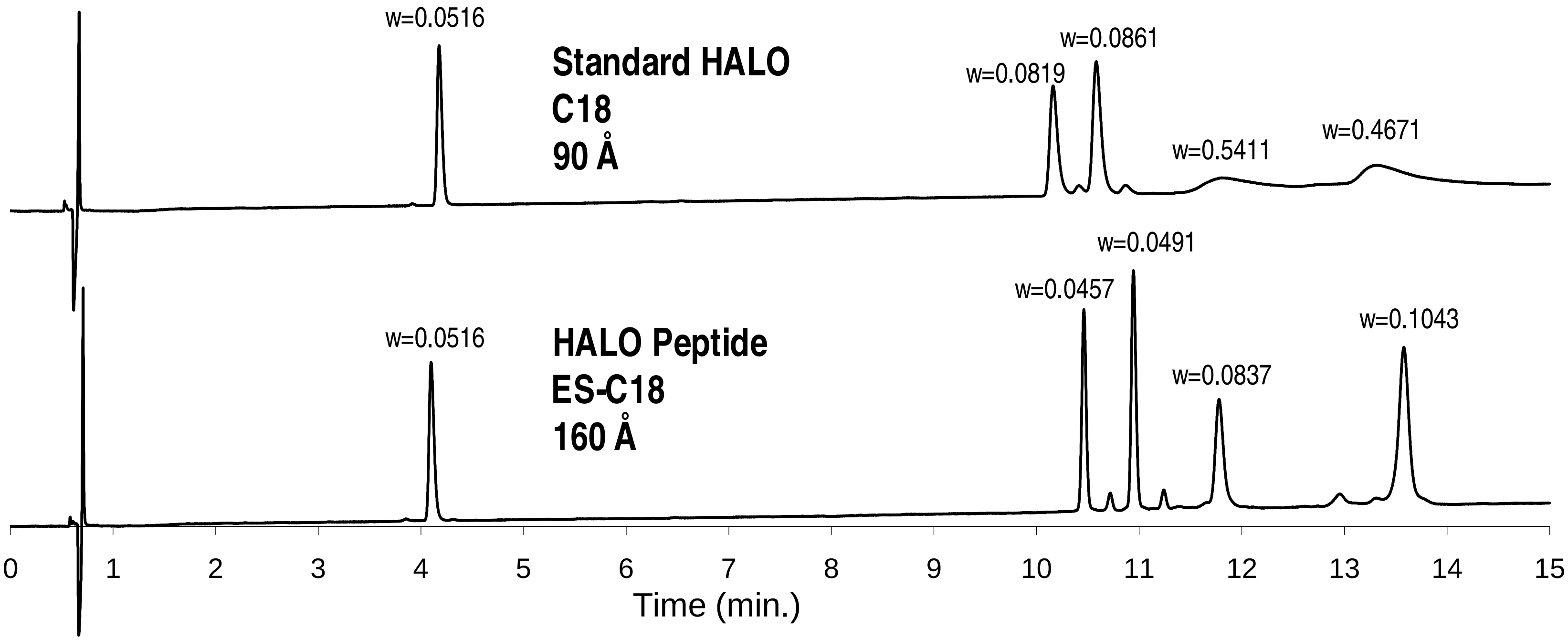
Effect of Pore Size on Peptide and Small Protein Separations



1. Leu-enk (555 g/mol)
2. Bovine Insulin (5733 g/mol)
3. Human Insulin (5808 g/mol)
4. Cytochrome C (12,400 g/mol)
5. Lysozyme (14,300 g/mol)

Columns: 4.6 x 100 mm
Flow rate: 1.5 mL/min
Temperature: 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
Detection: 220 nm

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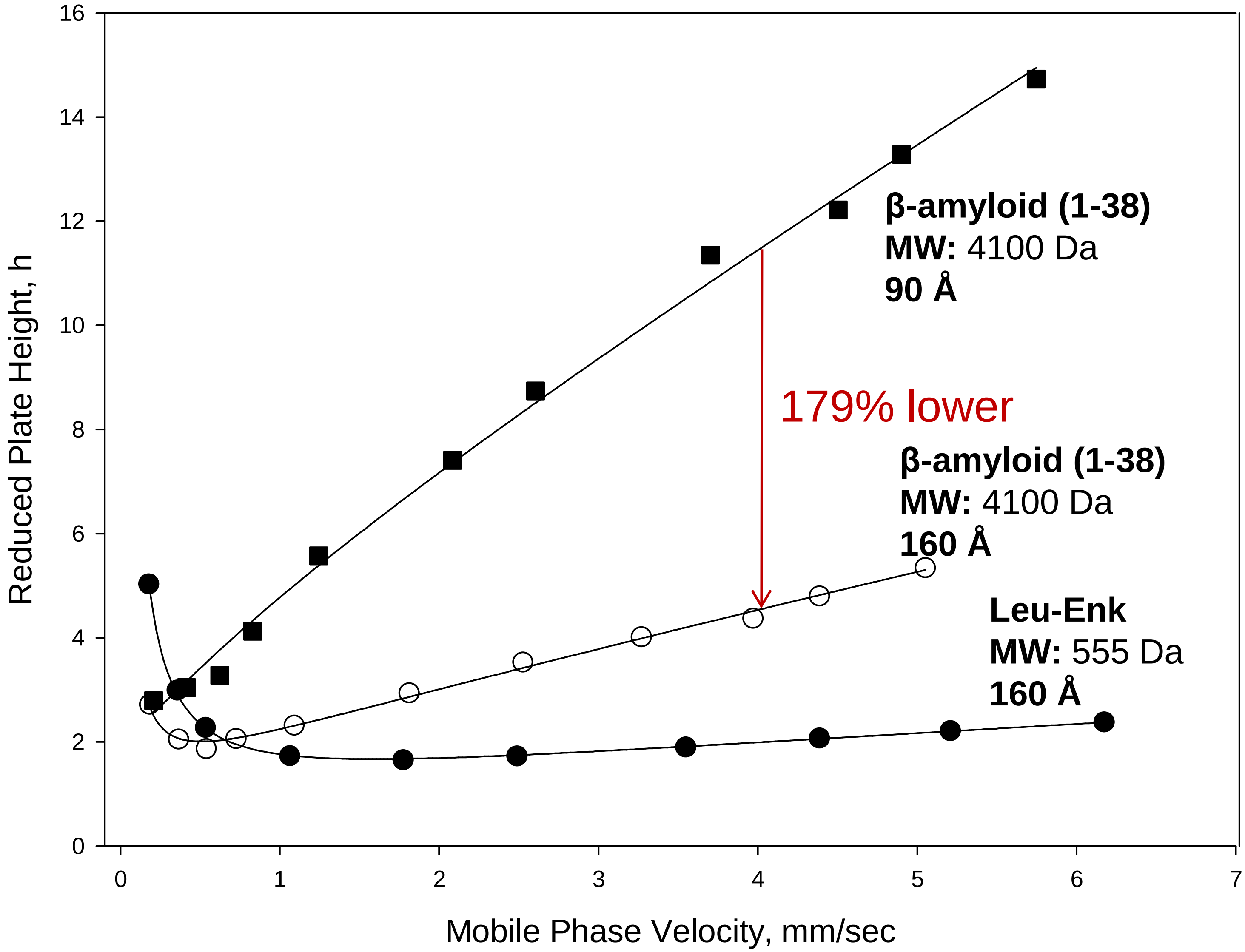
Effect of Pore Size on Efficiency



Columns: 4.6 x 100 mm HALO C18, 2.7 µm, 90 Å
4.6 x 100 mm HALO Peptide ES-C18, 2.7 µm, 160 Å

Mobile Phase: Leu-Enk: 21% ACN/79% Water/0.1% TFA
β-amyloid (1-38) 160 Å : 29% ACN/71% Water/0.1% TFA
β-amyloid (1-38) 90 Å : 27% ACN/73% Water/0.1% TFA

Temperature: 60 °C
Detection: 215 nm

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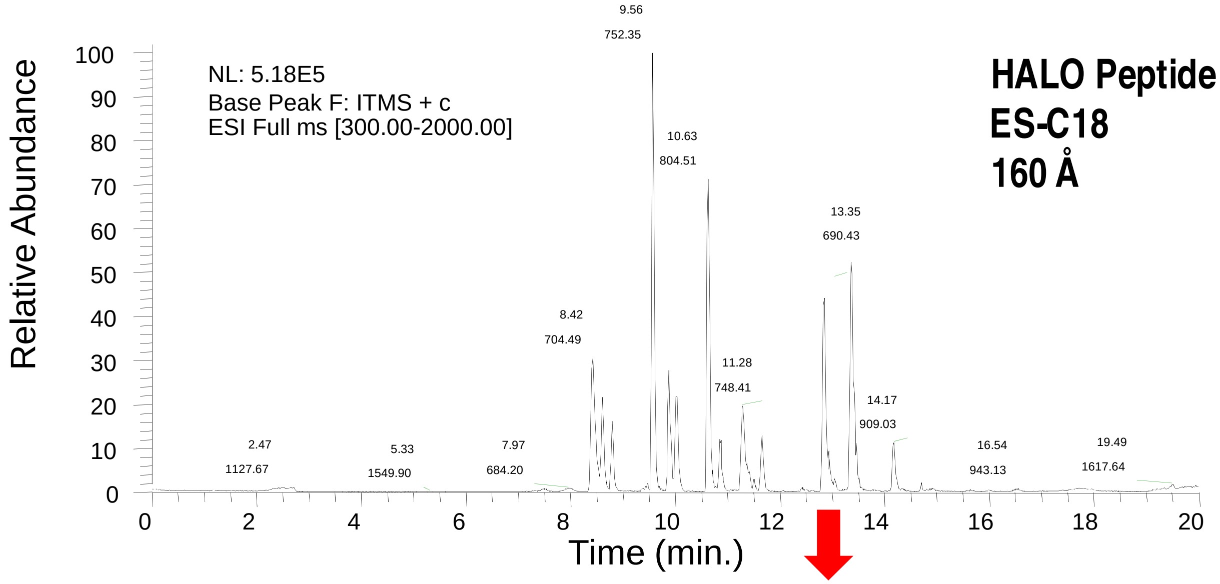
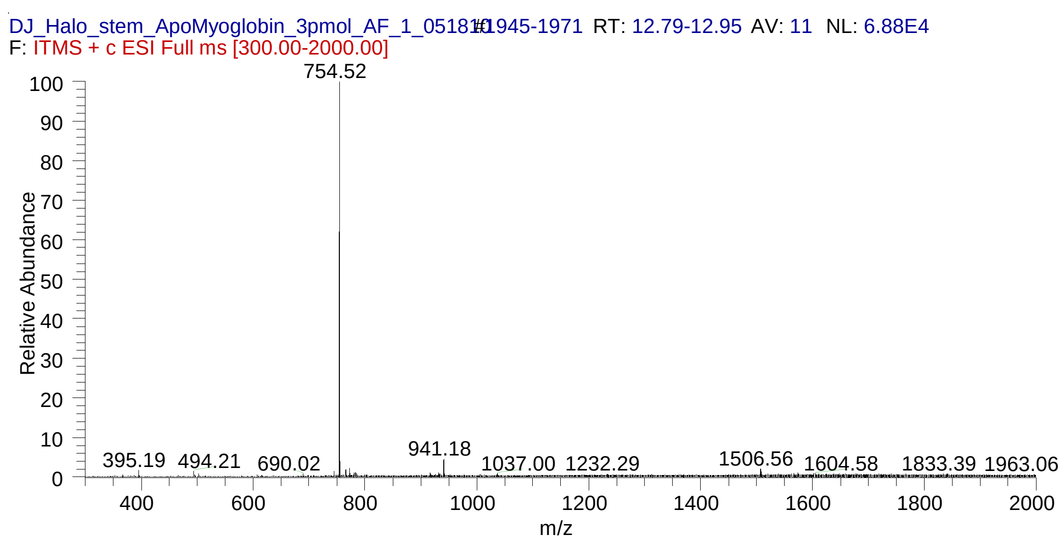
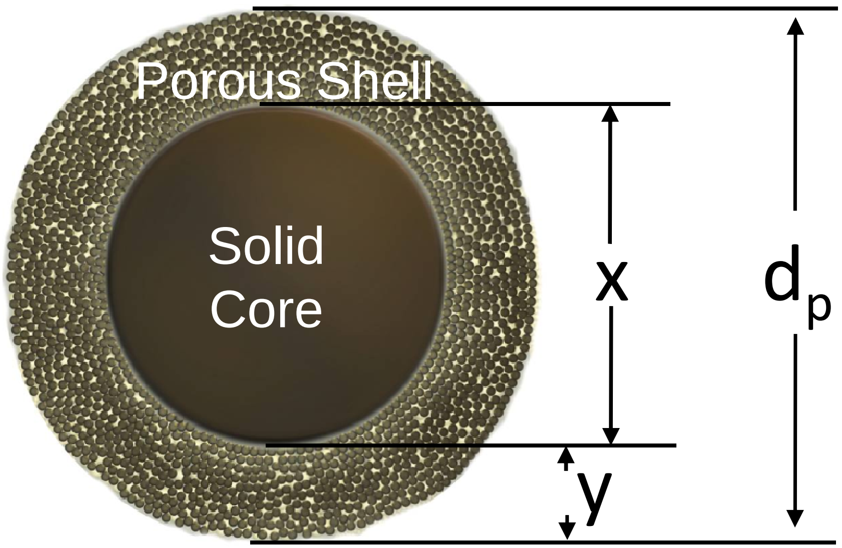
High Mobile Phase Velocity LC/MS Analysis of a Tryptic Digest





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

Fused-Core Particles: Varying Shell Thickness



x (µm)	y (µm)	d_p (µm)	Surface Area (m^2/g)	Pore Size (Å)
1.7	0.5	2.7	80	160
1.7	0.3	2.3	49	160

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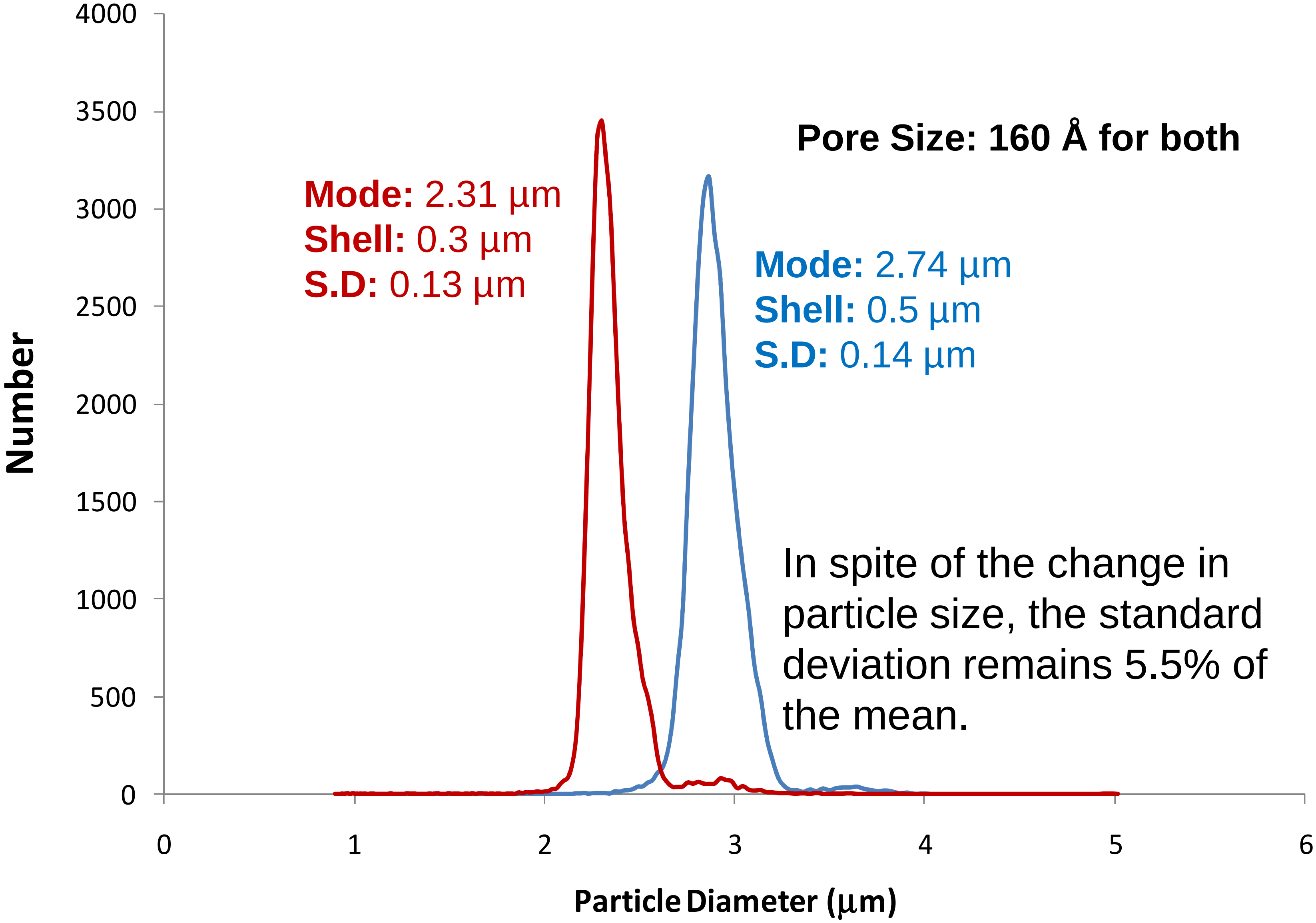
Particle Size Distributions



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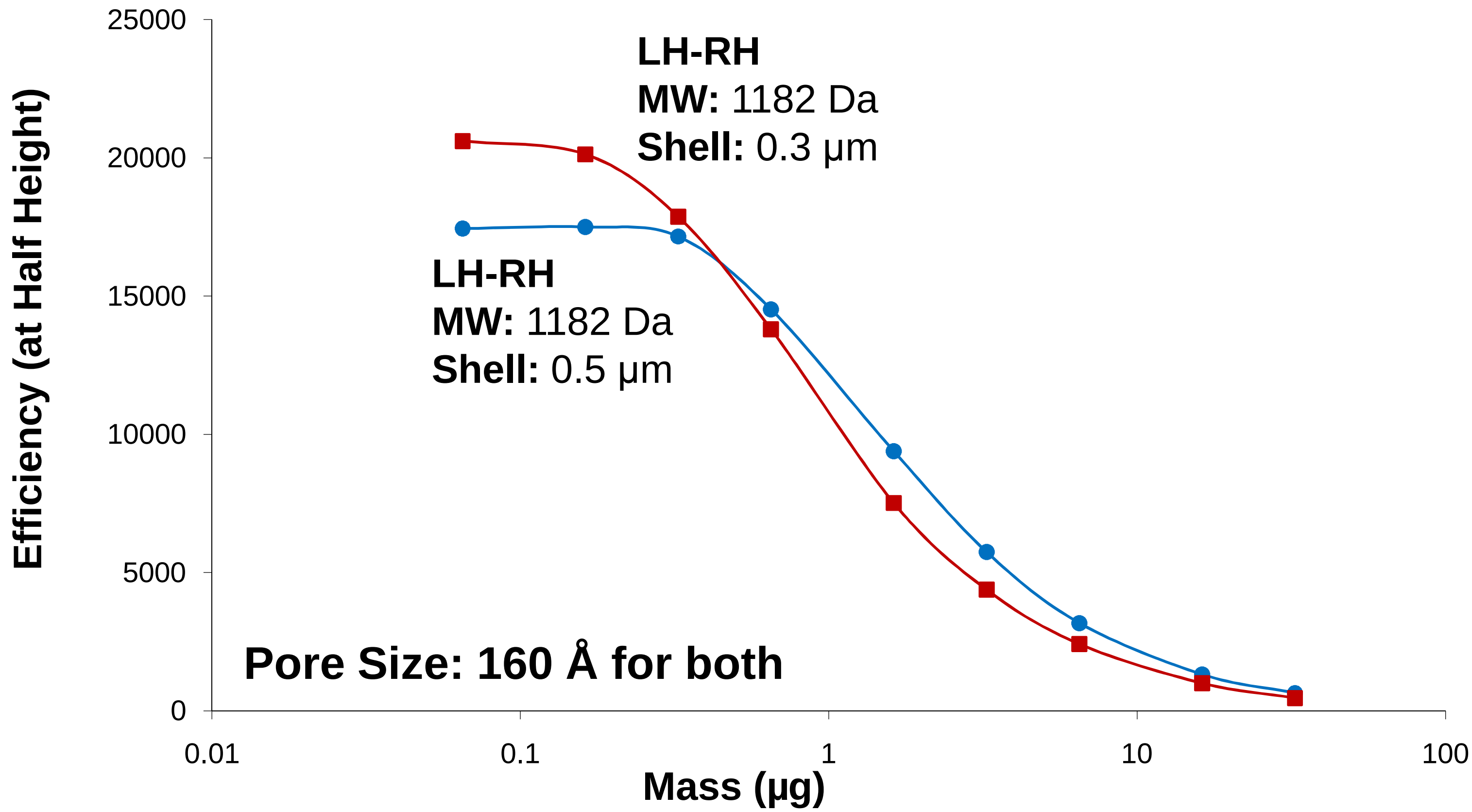
Effect of Shell Thickness on Sample Loading



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
Temperature: 60 °C
Detection: 220 nm
LC System: Agilent 1100
Sample: Luteinizing Hormone-Releasing Hormone (LH-RH) MW = 1182

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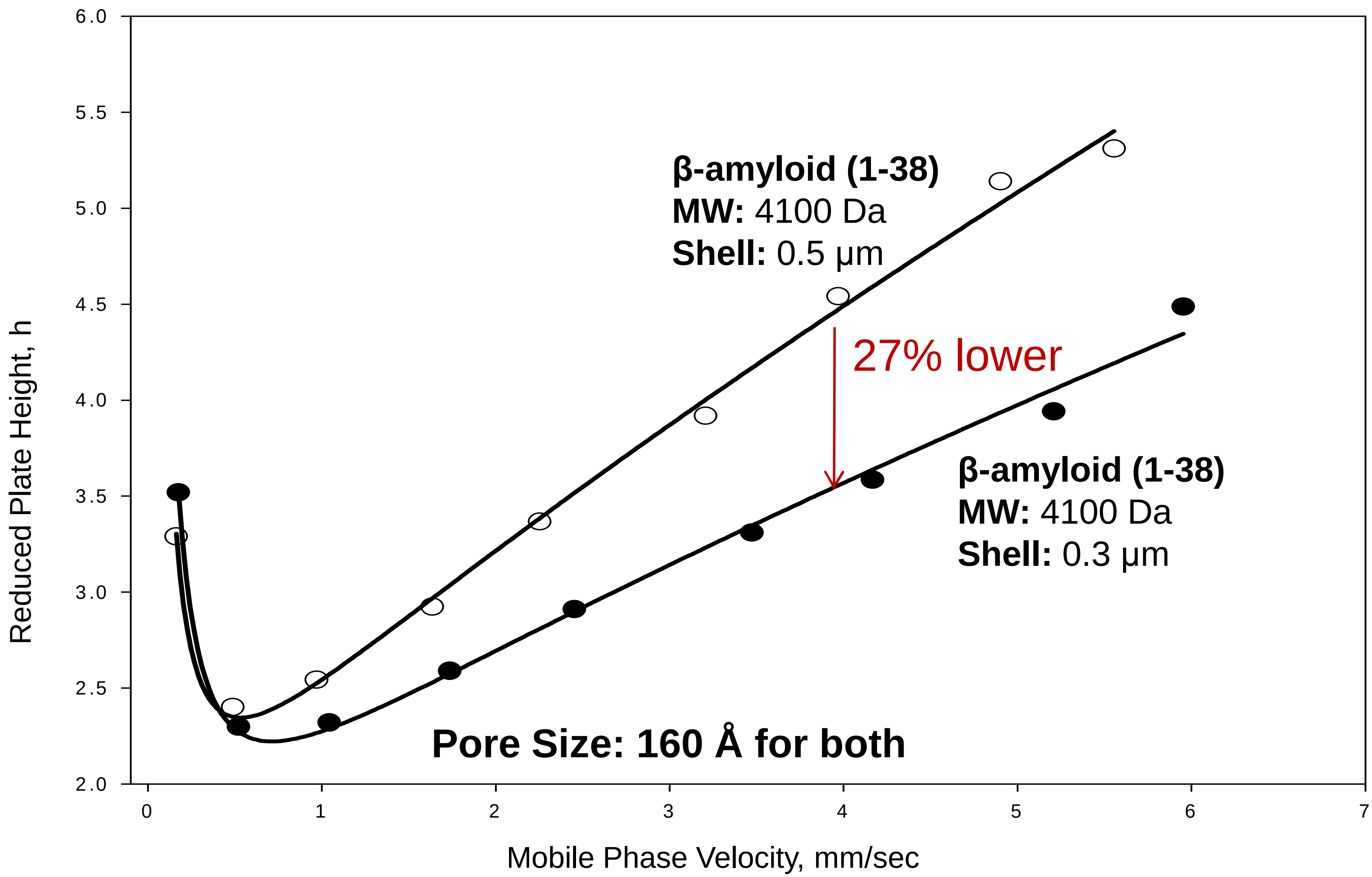
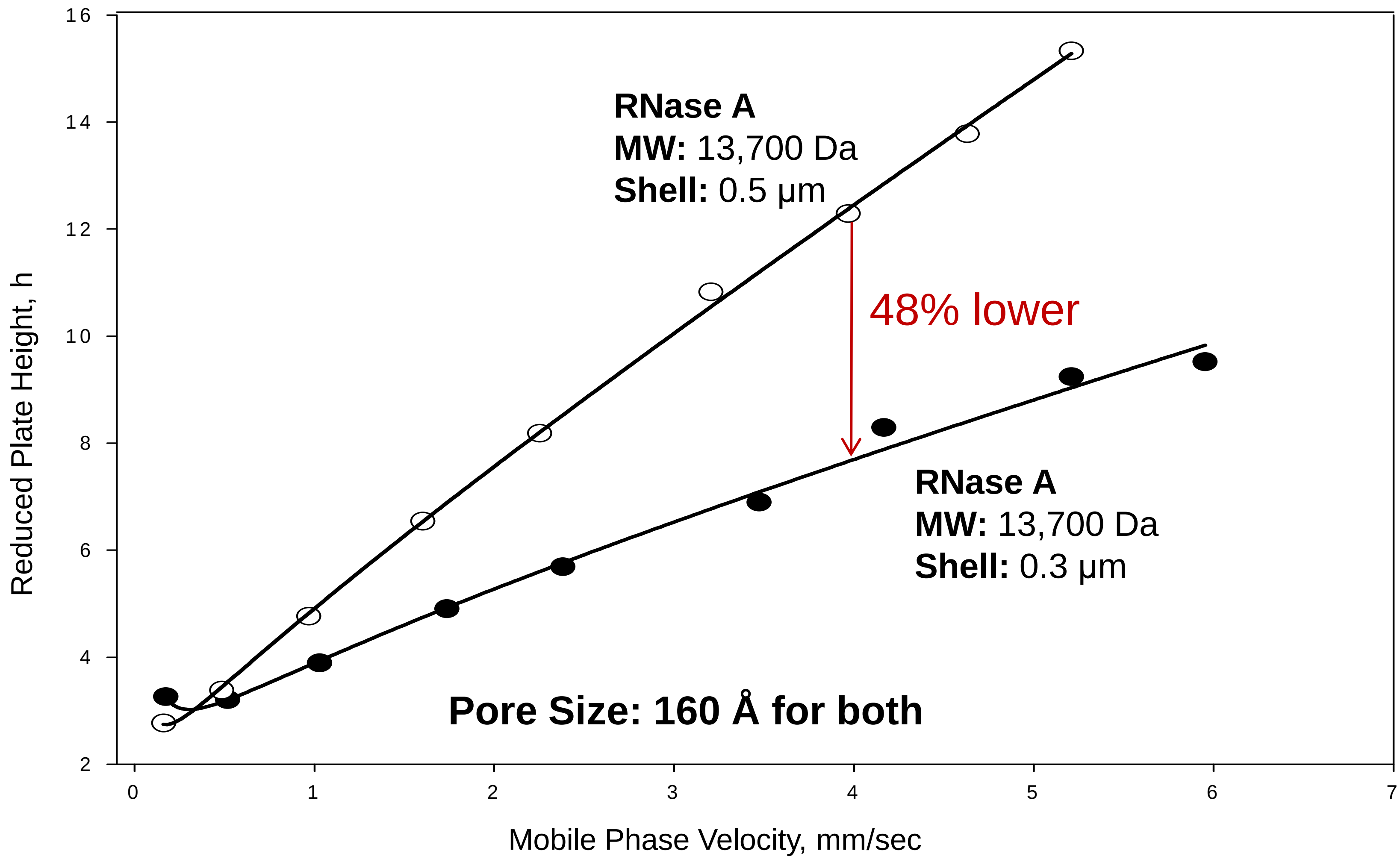
Effect of Shell Thickness on Efficiency



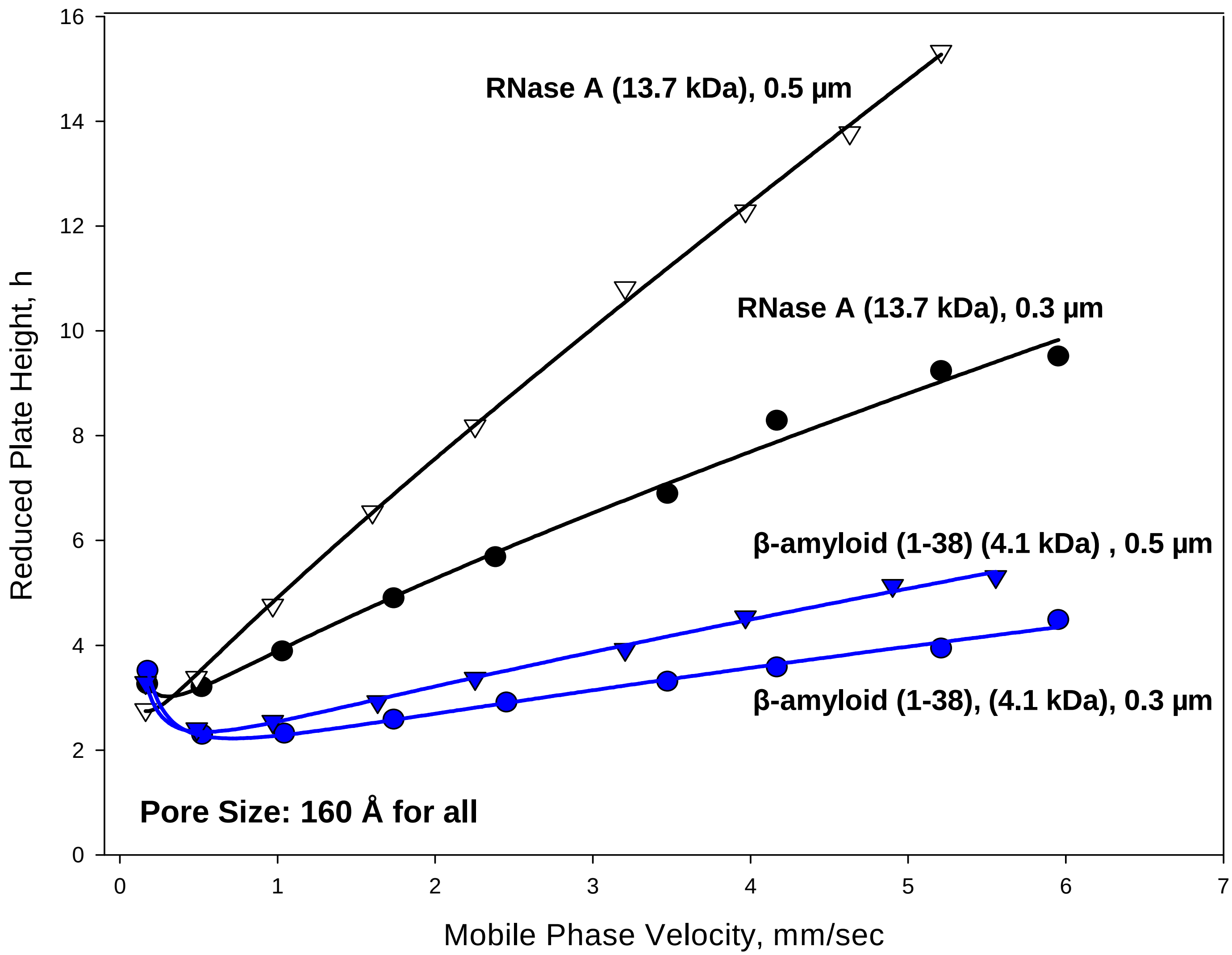
Columns: 4.6 x 50 mm SP-C8, 2.7 µm, 0.5 µm shell, 160 Å and 2.3 µm, 0.3 µm shell, 160 Å
Mobile Phase: 27.4% ACN/72.6% Water/0.1% TFA
Temperature: 60 °C
Detection: 215 nm

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: 24.5% ACN/75.5% Water/0.1% TFA
Temperature: 60 °C
Detection: 215 nm

Effect of Shell Thickness on Efficiency

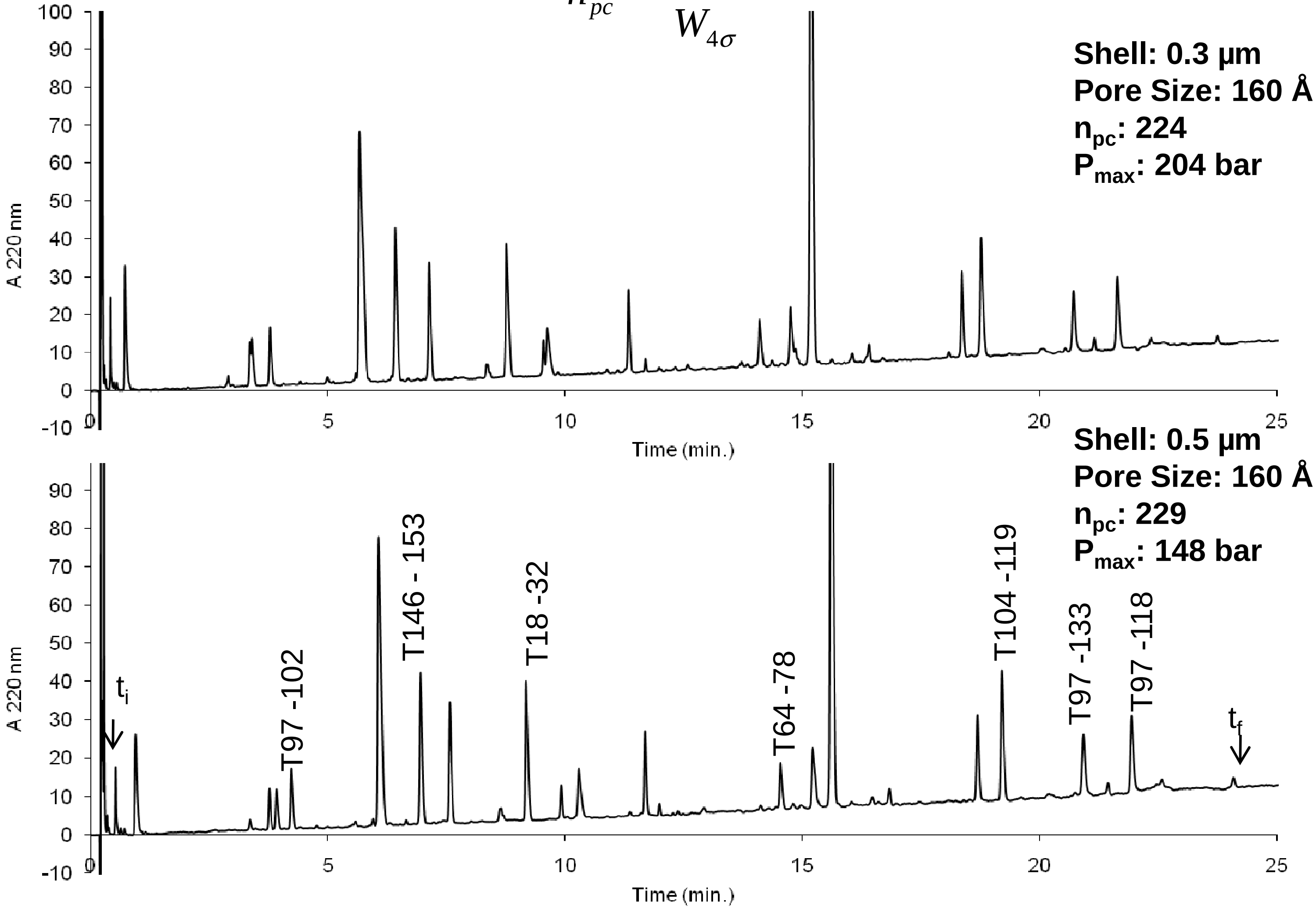


Columns: 4.6 x 50 mm SP-C8, 2.7 µm, 0.5 µm shell, 160 Å and
2.3 µm, 0.3 µm shell, 160 Å
Mobile Phase: RNase A: 24.5% ACN/75.5% Water/0.1% TFA
β-amyloid (1-38): 27.4% ACN/72.6% Water/0.1% TFA
Temperature: 60 °C
Detection: 215 nm

Effect of Shell Thickness on Peak Capacity

Peak capacity was calculated by averaging the peak widths of the labeled tryptic digest peaks below 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
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

Temperature: 60 °C
Detection: 220 nm
LC System: Agilent 1100
Sample: Apo-myoglobin tryptic digest

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Conclusions

- Increasing the pore size of the Fused-core particles improves the mass transfer for larger molecular weight solutes
- Different property Fused-core particles can be produced with extremely narrow size distributions
- Decreased shell thickness improves the mass transfer for larger molecular weight solutes
  - Degree of improvement is a function of molecular size; analytic details under investigation (diffusion dependence)
  - Sample load/retention decreased by lower surface area

Future Studies

1) Investigate the effect of even larger pores on particle characteristics for separating large molecules
2) Explore the advantages and disadvantages of smaller fused-core particles
3) Determine the practical role of shell thickness for (1) and (2)

Acknowledgements

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  - Tim Langlois
  - Brian Wagner
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