Novel Wide-Pore Superficially Porous Particles for Biomacromolecular Separations

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Objective

- The objective of this work is to demonstrate fast, efficient HPLC separations of intact proteins using columns of Fused-core particles that have been optimized in terms of pore size, shell thickness, and particle size.

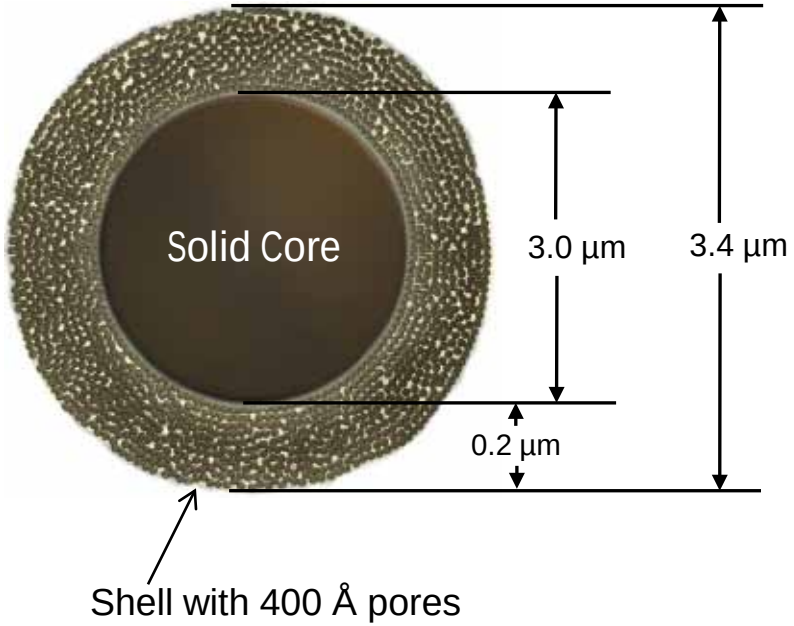
Abstract

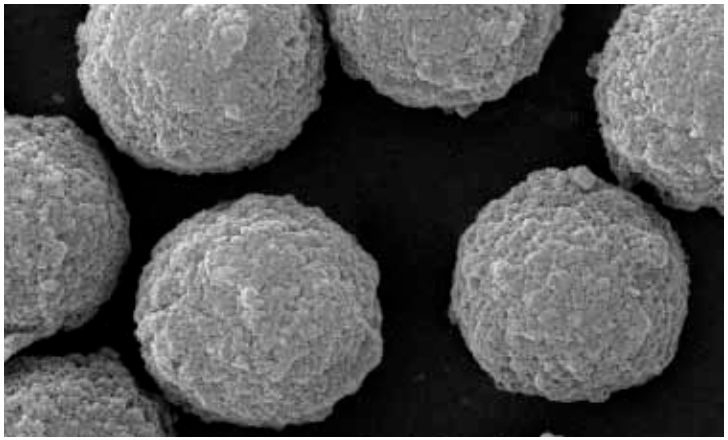
Superficially porous particles designed to separate small molecules now are in wide use because of their superior properties. More recently, such particles with somewhat wider pores have been made available for separating peptides and small proteins. This report describes new superficially porous silica particles with large 400 Å pores that are specifically designed for separating proteins up to at least 400 kDa, depending on molecular conformation. The large pores of these particles allow separations of large proteins without restricted diffusion, which would compromise separating efficiency. These new fused-core particles have thin outer shells that provide excellent mass transfer (kinetics) for large proteins, resulting in superior separations compared to conventional totally porous particles. Optimization of particle size, shell thickness and bonded stationary phase allow fast, efficient separations of macromolecules. Excellent particle strength permits columns of these new particles to be operated at pressures up to at least 600 bar with superior packed bed stability. These new wide-pore particles provide the basis for separating large biomolecules with the known advantages of the superficially porous structure.

Physical Characteristics of Fused-Core Particles

Fused-Core Particle	Particle Size, µm	Pore Size, Å	BET Surface Area, m^2/g	Shell Thickness, µm	% Porosity	Pore volume, cm^3/g
Halo	2.7	90	135	0.5	75	0.26
Halo Peptide	2.7	160	80	0.5	75	0.29
Wide-pore	2.7	400	30	0.35	59	0.23
Wide-pore	3.4	400	15	0.2	31	0.11

HALO® Wide-Pore Fused-Core Particles

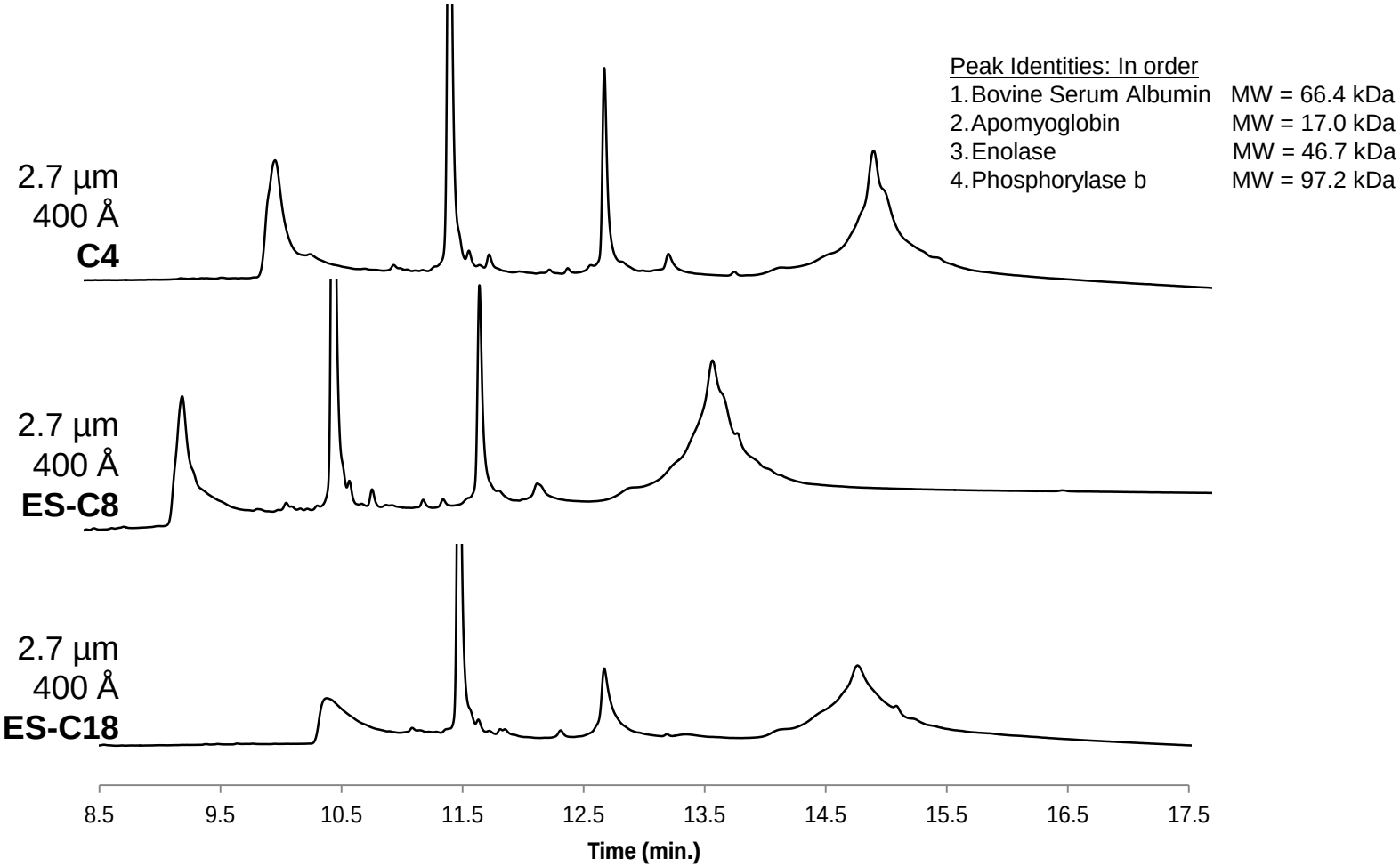




SEM image of the Fused-core particles

Effect of Bonded Phase

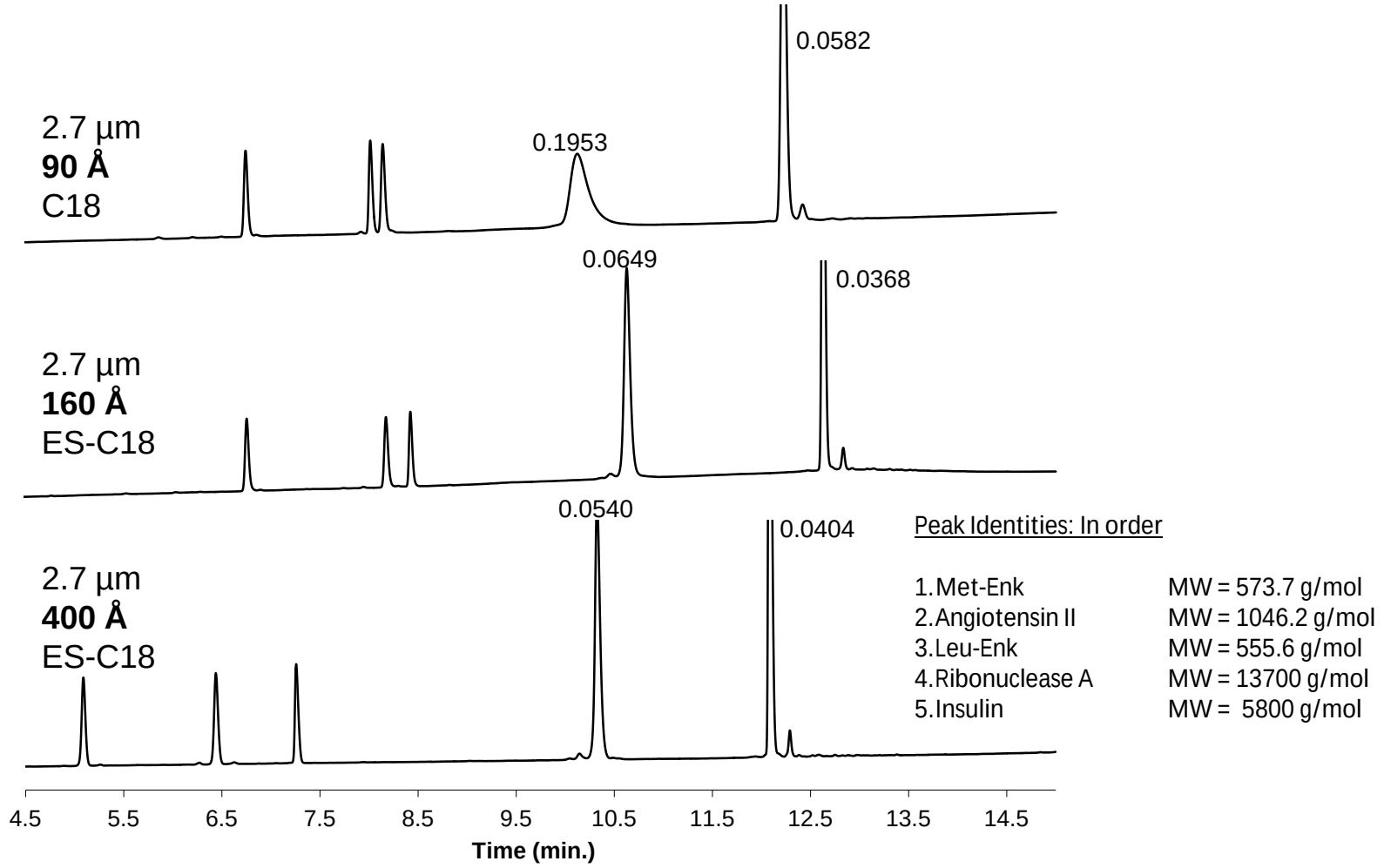
Columns: 2.1 x 100 mm; Instrument: Agilent 1200 SL; Flow rate: 0.3 mL/min; Injection Volume: 1 µL
Detection: 215 nm; Mobile Phase: 20–70% ACN/water w/0.1% TFA in 20 min.,Temperature = 60 ^{o}C



- The C4 bonded phase shows increased retention compared to the ES-C8 phase.
- The prototype C4 and ES-C8 phases show sharper peaks than the prototype ES-C18.

Effect of Pore Size

Columns: 4.6 x 100 mm; Instrument: Agilent 1100; Flow rate: 1.5 mL/min; Injection Volume: 5 µL; Detection: 220 nm;
Mobile Phase: A: 10% ACN / 0.1% TFA; B: 70% ACN / 0.1% TFA; Gradient: 0% B to 50% B in 15 min.; Temperature = 30 ^{o}C

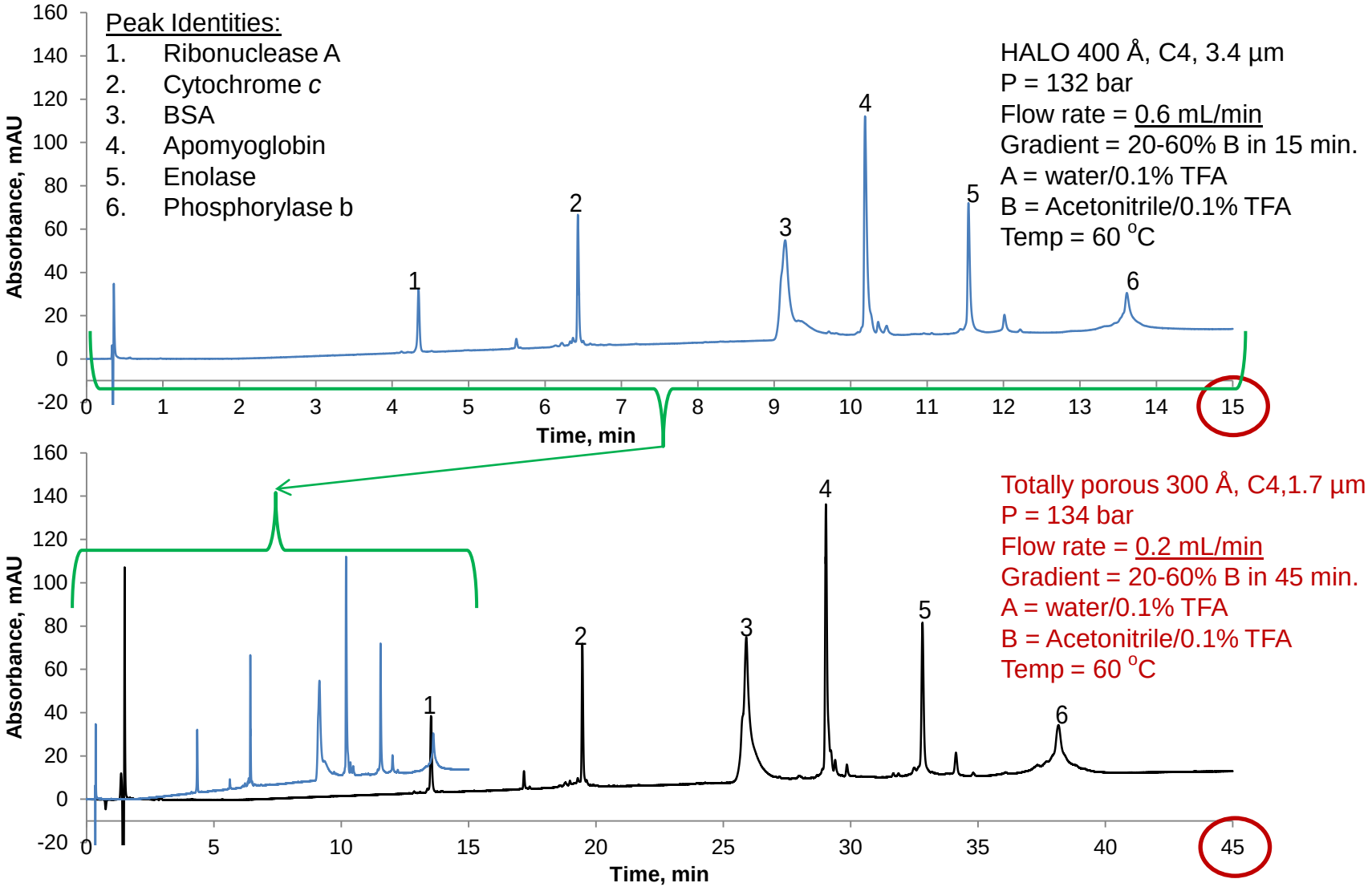


Peak Identities: In order

1. Met-Enk	MW = 573.7 g/mol
2. Angiotensin II	MW = 1046.2 g/mol
3. Leu-Enk	MW = 555.6 g/mol
4. Ribonuclease A	MW = 13700 g/mol
5. Insulin	MW = 5800 g/mol

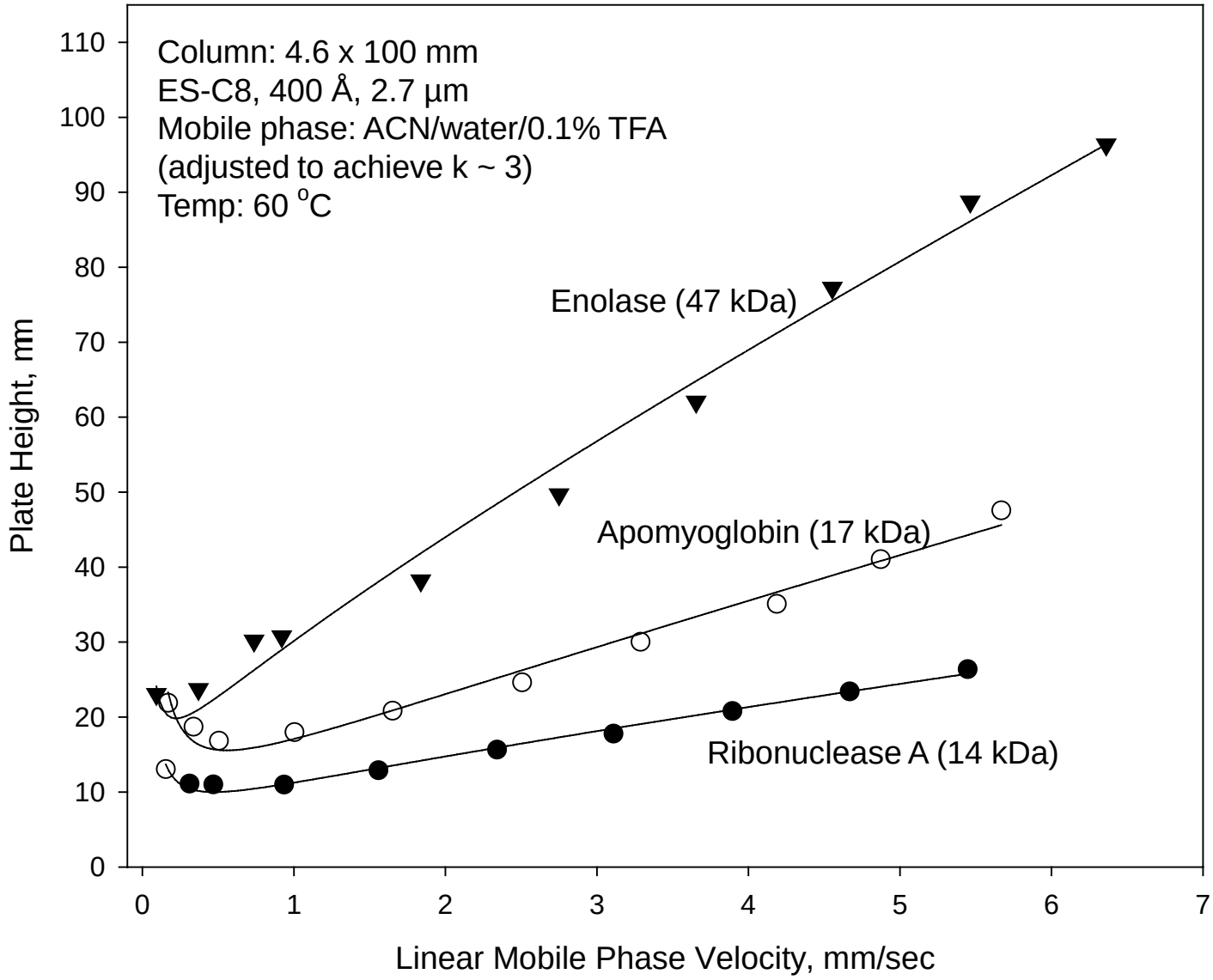
- The peak widths in minutes are shown for ribonuclease A and insulin.
- As the pore size increases, the peak widths decrease, which shows that the proteins do not exhibit restricted diffusion with the 400 Å Fused-Core particles.

Fast Protein Separations using HALO 400

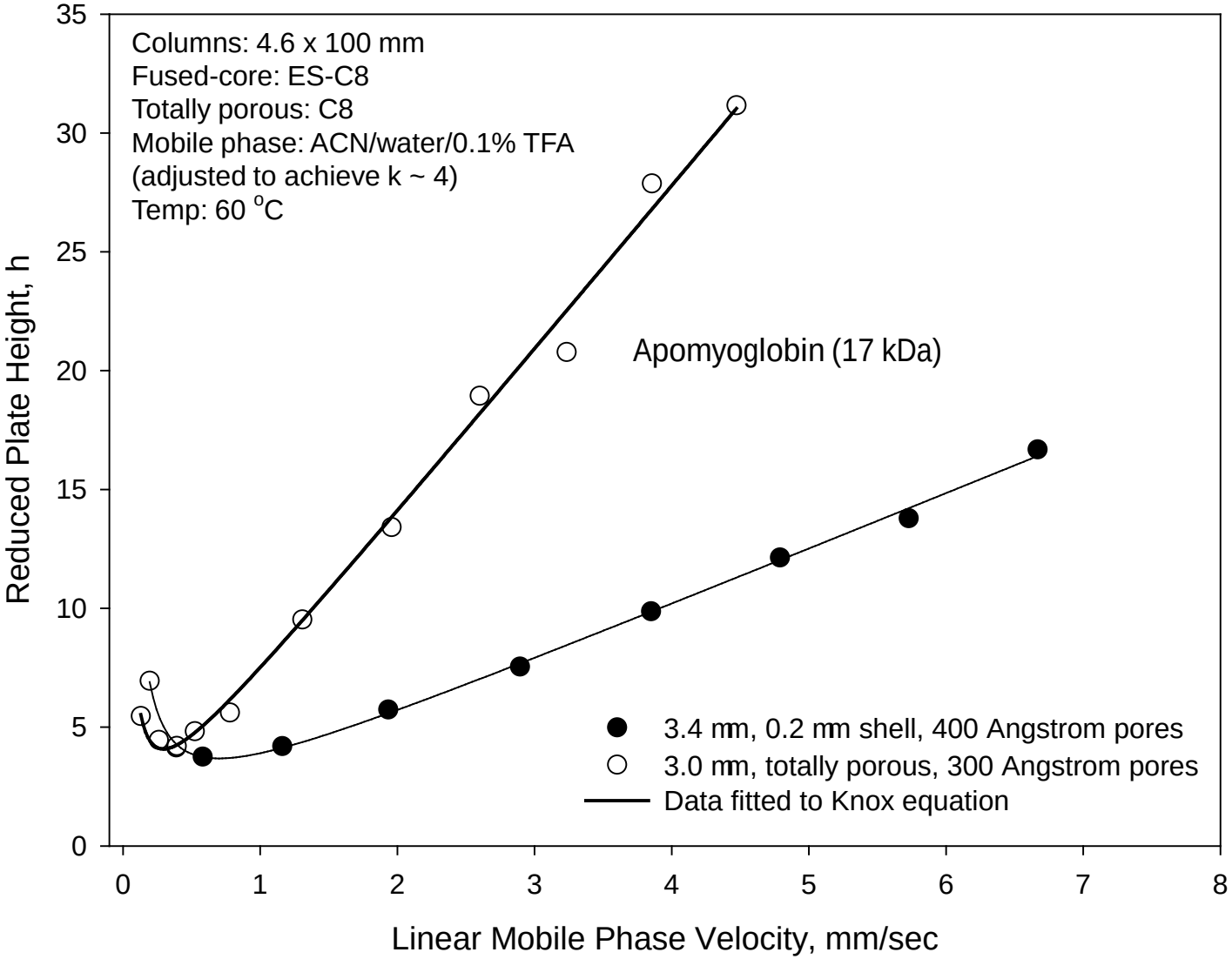


- Separation is 3 times faster on the HALO 400 column compared to the same sample run on a sub-2-µm totally porous particle column

Effect of Protein MW on Efficiency



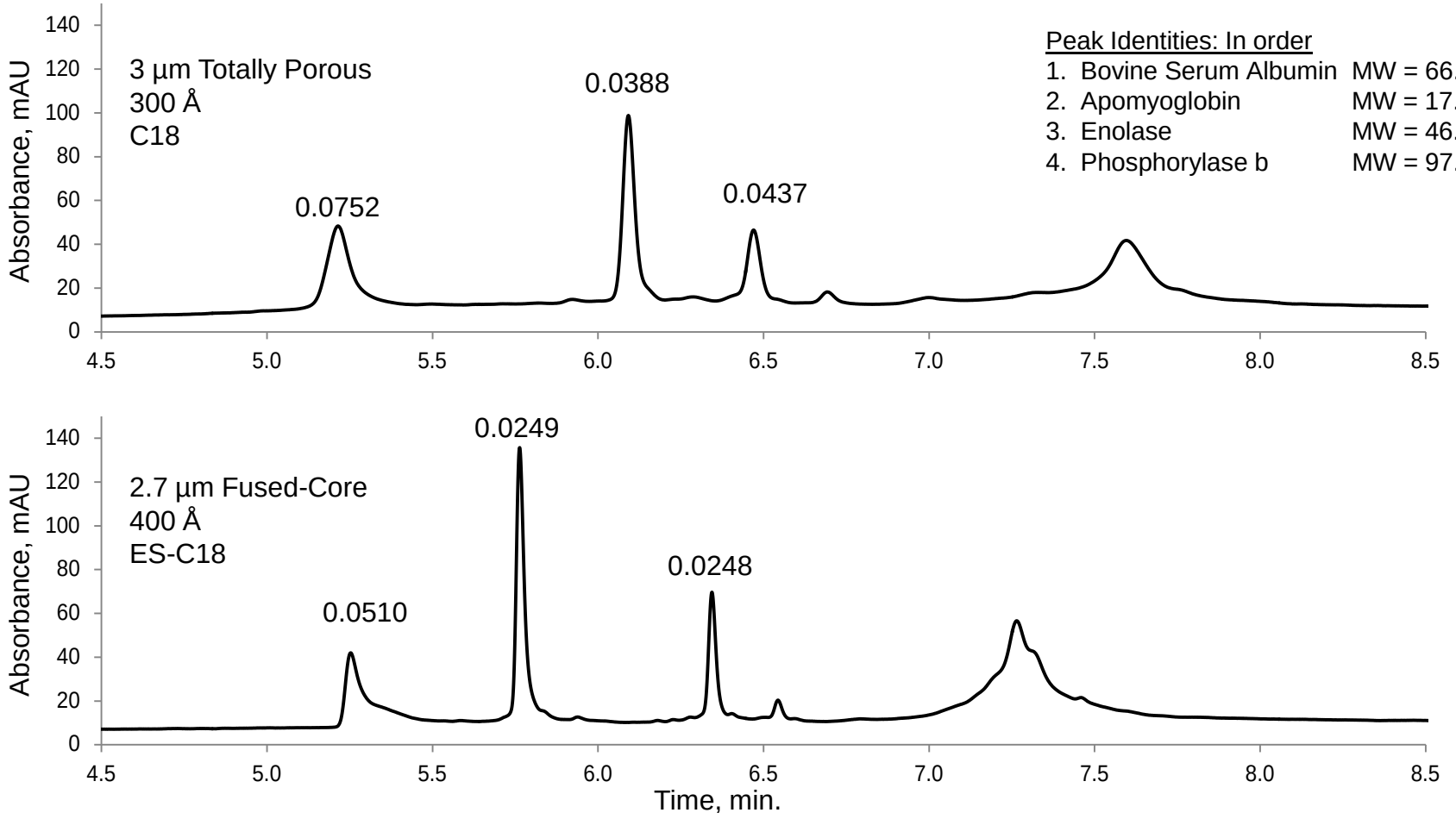
Superior Mass Transfer using HALO 400



- The Fused-Core particle shows a flatter van Deemter curve compared to the totally porous particle, allowing for faster flow rates while maintaining resolution.

Protein Separations: Fused-Core vs. Totally Porous

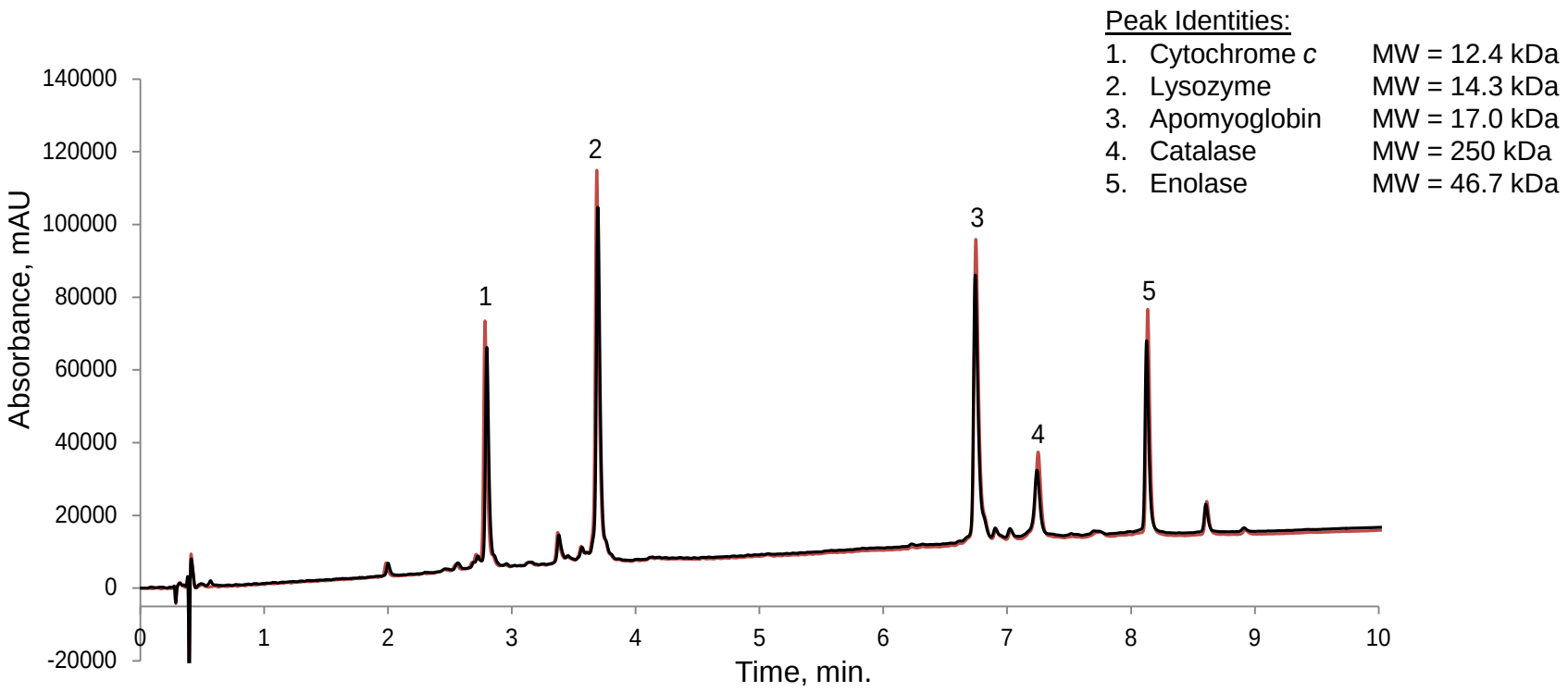
Columns: 4.6 x 100 mm; Temperature: 60 ^{o}C
Mobile phase: A = water/0.1% TFA; B = Acetonitrile/0.1% TFA
Gradient: 20-70% B in 10 min.; Flow rate = 1.5 mL/min; Detection = 215 nm; Injection = 5 µL



- The peak widths in minutes are shown for selected peaks. The peaks are sharper on the 400 Å Fused-Core particle column.
- The phosphorylase b peak is much more clearly defined on the 400 Å Fused-Core particle column.

High Temperature Stability of HALO 400

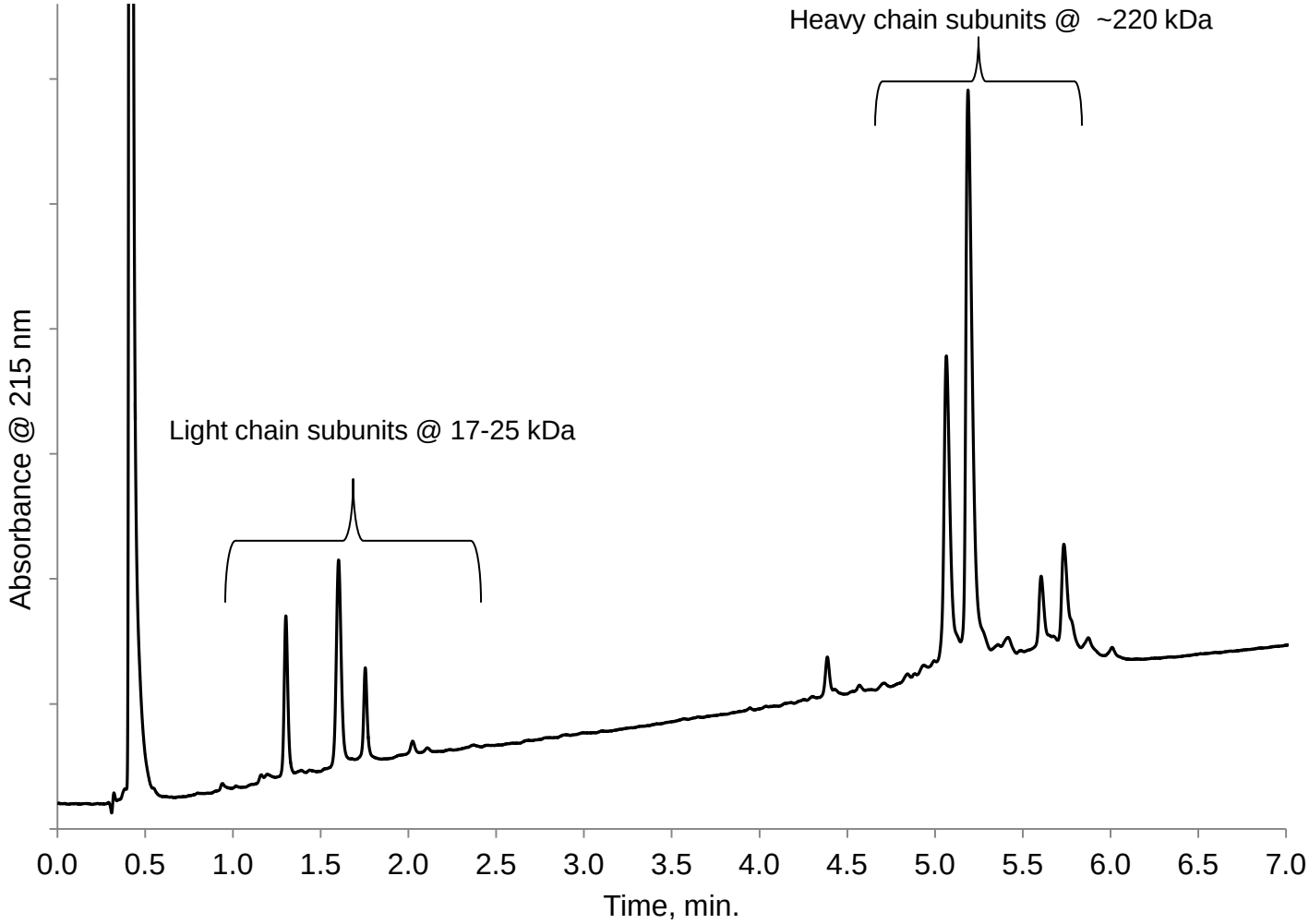
Column: 2.1 x 100 mm 3.4 µm, 400 Å, C4; Mobile phase gradient: 25 – 50% acetonitrile/water/0.1% trifluoroacetic acid in 10 min.
Flow rate: 0.5 mL/min; Temperature: 60 °C; PDA detection: 215 nm; Instrument: Shimadzu Nexera



- The first injection (red trace) and last injection (black trace) display the same separation with no indication of degraded column performance after running for ~ 11,200 column volumes at 60 °C and pH 1.9.

Large Protein Separation with HALO 400

Column: 100 x 2.1 mm, 3.4 µm Fused-core, ES-C8, 400 Å; Mobile phase: A = water/0.1% trifluoroacetic acid
B = acetonitrile/0.1% trifluoroacetic acid, Gradient: 35 – 50% B in 7 min.; Flow rate: 0.45 mL/min
Temperature: 80 ^{o}C; Detection: 215 nm; Solute: myosin (440 kDa)



- Sharp peaks are observed for both the heavy and light chains of myosin

Conclusions

1. Particles with 400 Å pores are effective for efficiently separating proteins without restricted diffusion

2. C4 and C8 may be preferred for separating proteins

3. Protein separations can be run approximately 3 times faster on columns of Fused-core particles compared to columns of sub-2-µm particles

4. Fused-core particles have performance advantages over totally porous particles for separating proteins

5. Columns of 400 Å particles are both efficient and stable

Acknowledgements

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