Superficially Porous Silica Particles with Wide Pores for Biomolecular Separations

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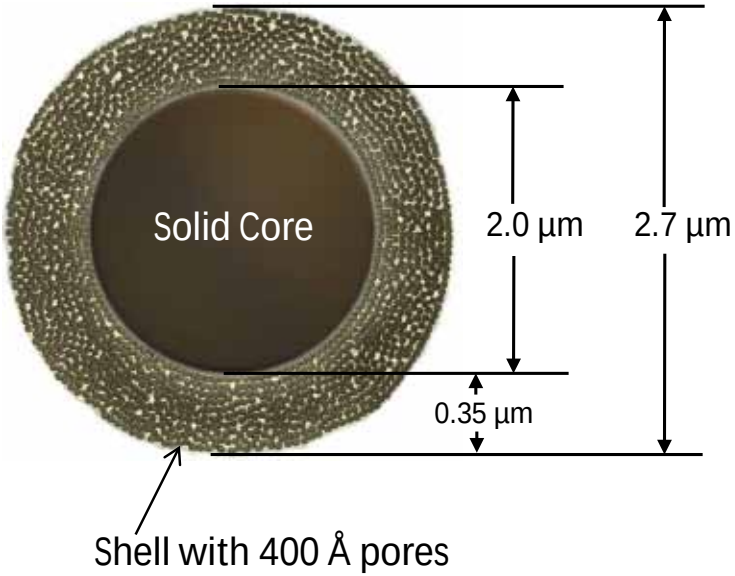
Study of Wide-pore Fused-Core particles developed to show the effect of:

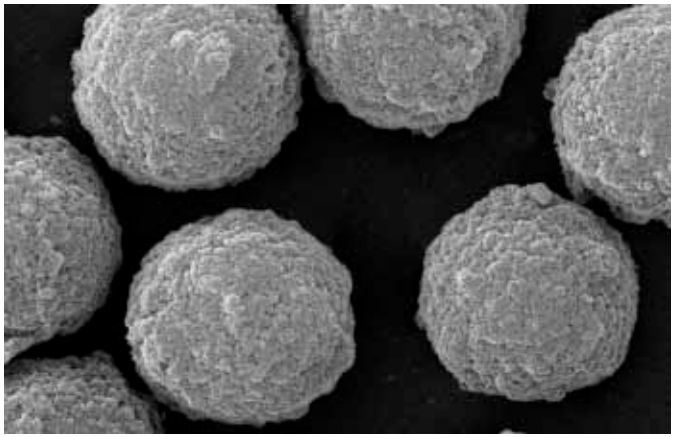
1. Particle size and shell thickness on column efficiency for proteins
2. Stationary phase on protein separation performance
3. Pore size in separating large proteins
4. Shell thickness, particle type, particle size on sample loading
5. Particle type (Fused-core, totally porous) on column efficiency for proteins
6. Stability of columns with wide-pore Fused-core particles

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	2.7	400	14	0.2	46	0.11
Wide-pore	3.4	400	10	0.2	31	0.068

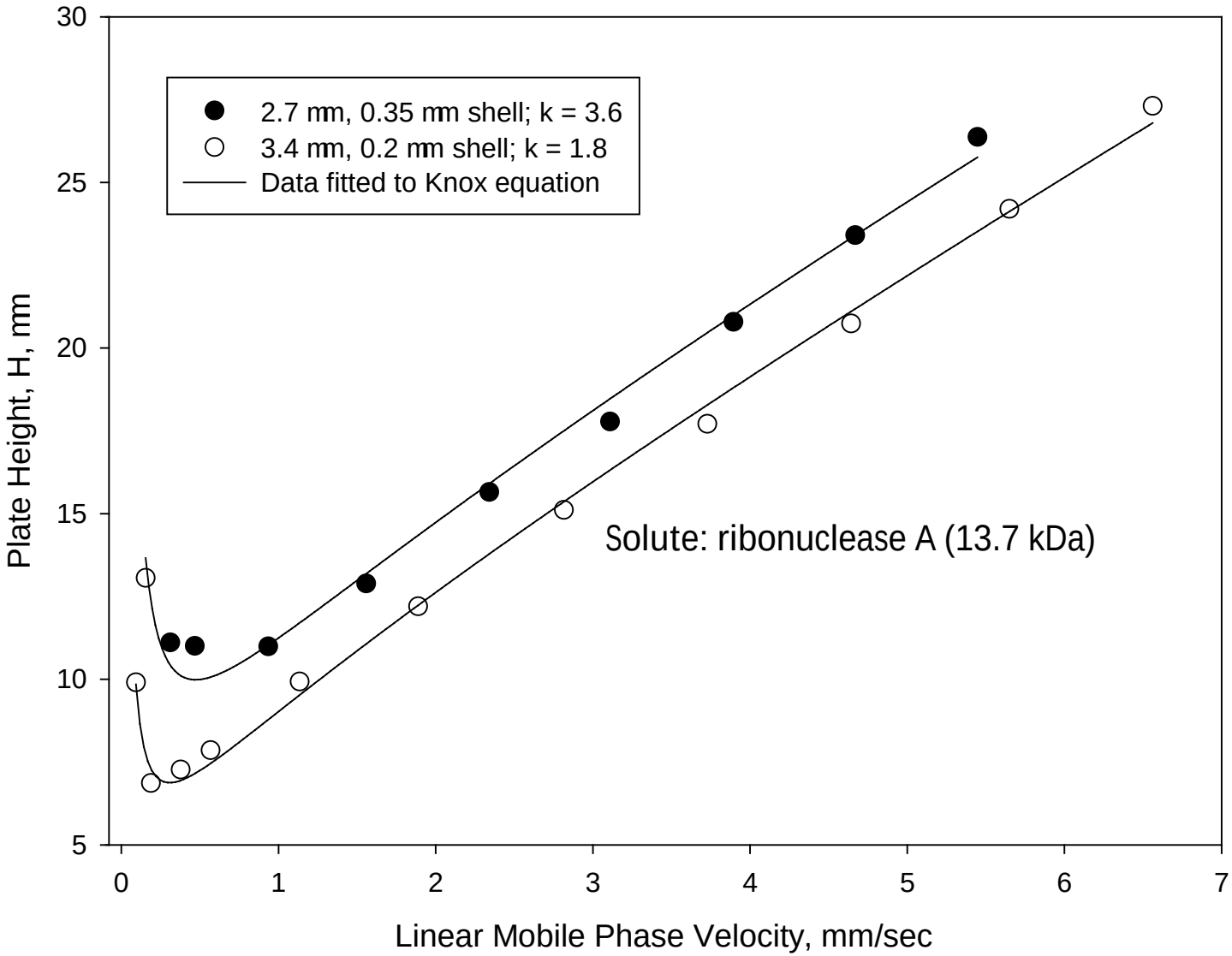
Halo® Wide-pore Fused-core Particles





Effect of Particle on Performance

Columns: 4.6 x 100 mm; Temperature: 60 ^{o}C
Mobile phase: 23.9% acetonitrile/76.1% aqueous trifluoroacetic acid, 0.1%
Agilent 1100 with autosampler



Effect of Particle on Performance

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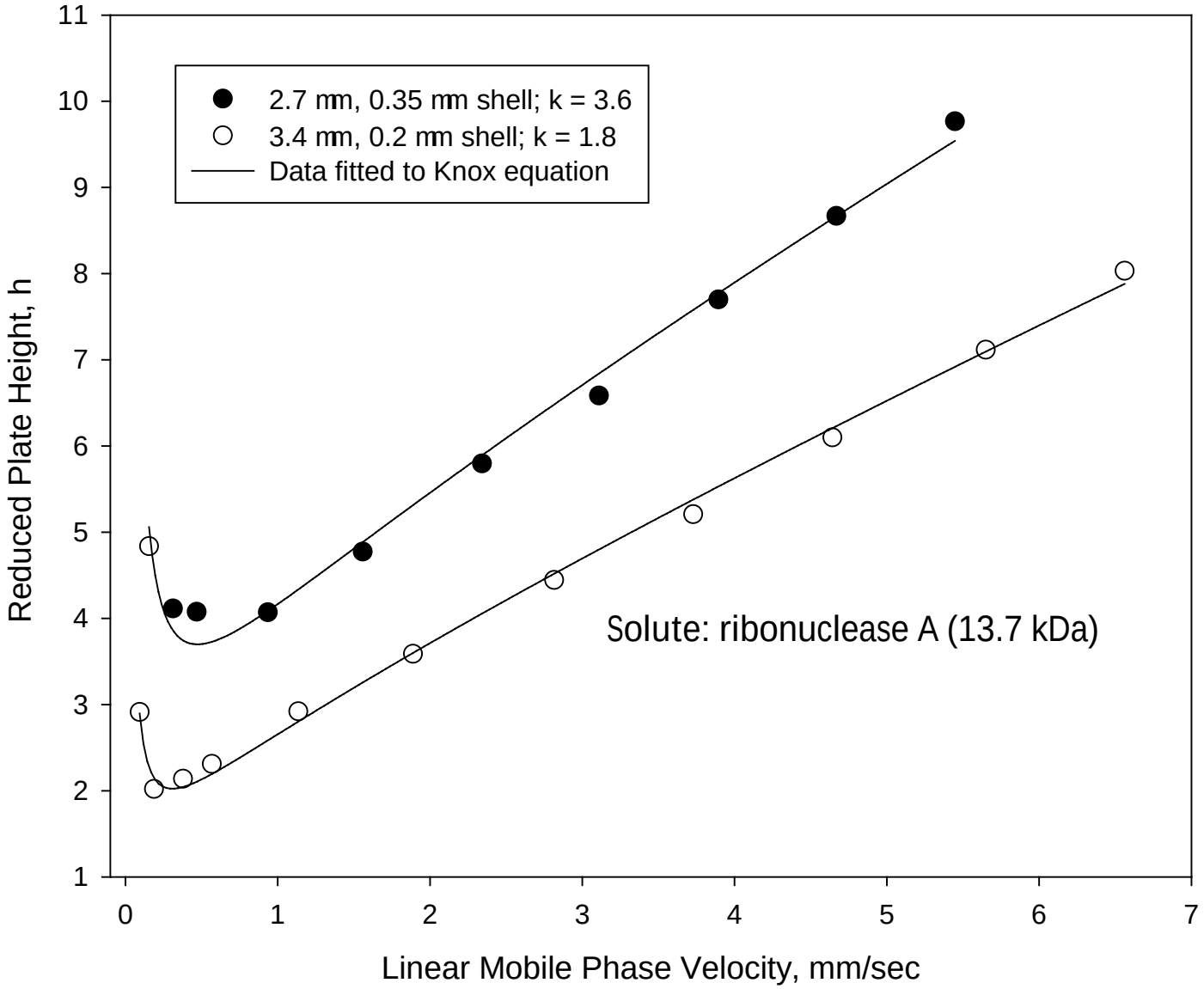
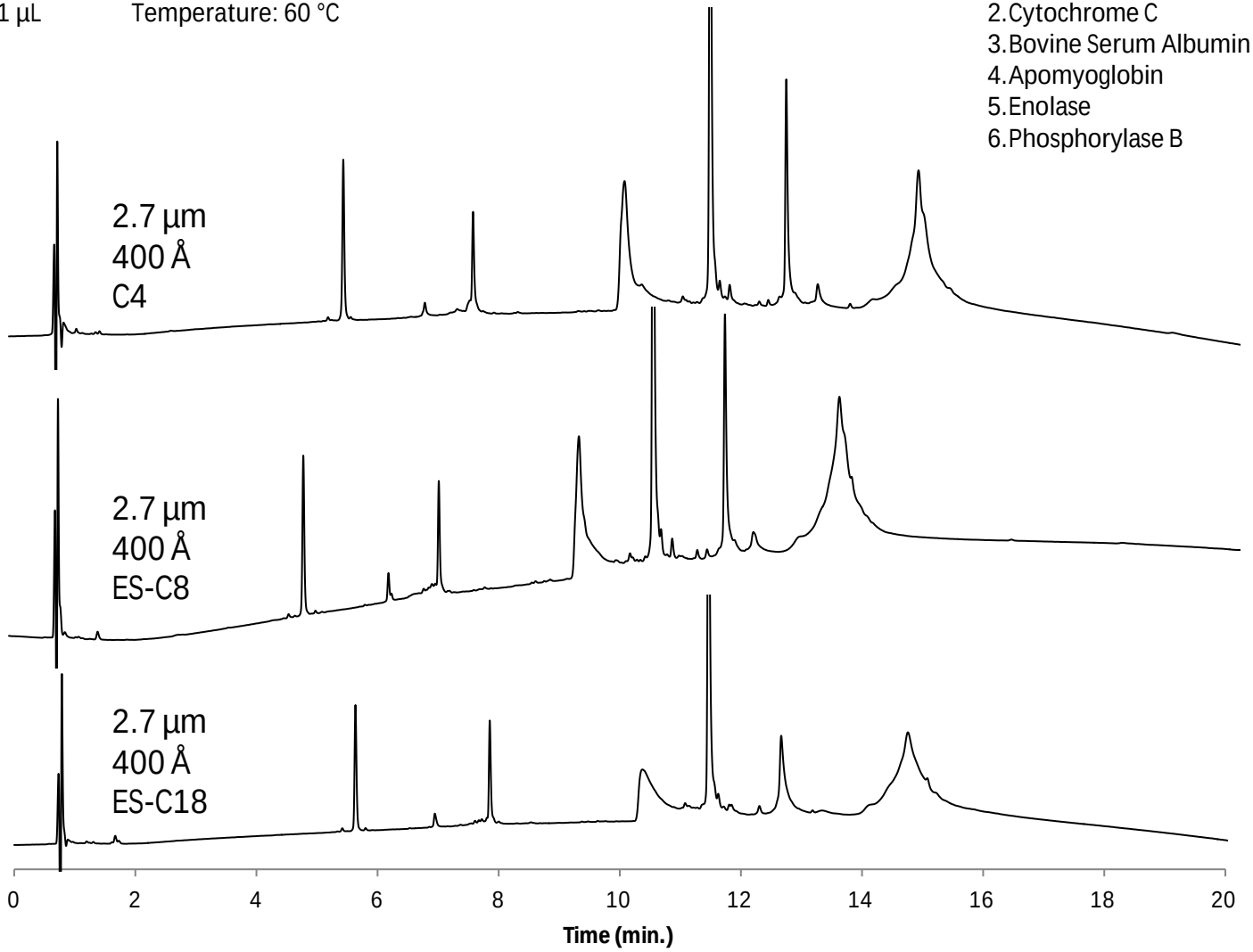


Effect of Bonded Phase

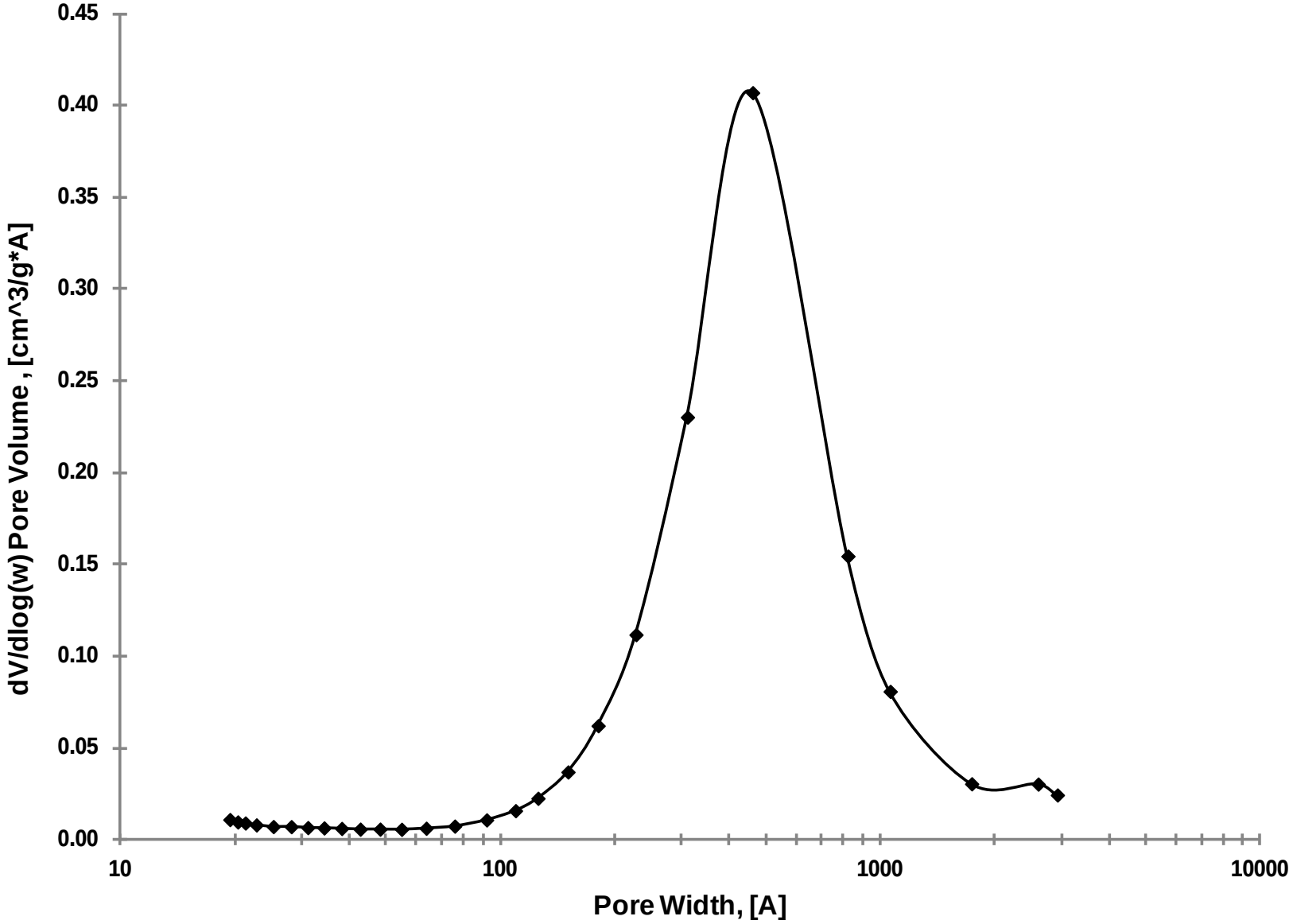
Columns: 2.1 x 100 mm
Instrument: Agilent 1200 SL
Injection Volume: 1 µL
Detection: 215 nm

Mobile Phase: 20–70% ACN/water/0.1% TFA in 20 min.
Flow rate: 0.3 mL/min
Temperature: 60 °C

Sample: In order
1. Ribonuclease A MW = 13.7 kDa
2. Cytochrome C MW = 12.4 kDa
3. Bovine Serum Albumin MW = 66.4 kDa
4. Apomyoglobin MW = 17.0 kDa
5. Enolase MW = 46.7 kDa
6. Phosphorylase B MW = 97.2 kDa

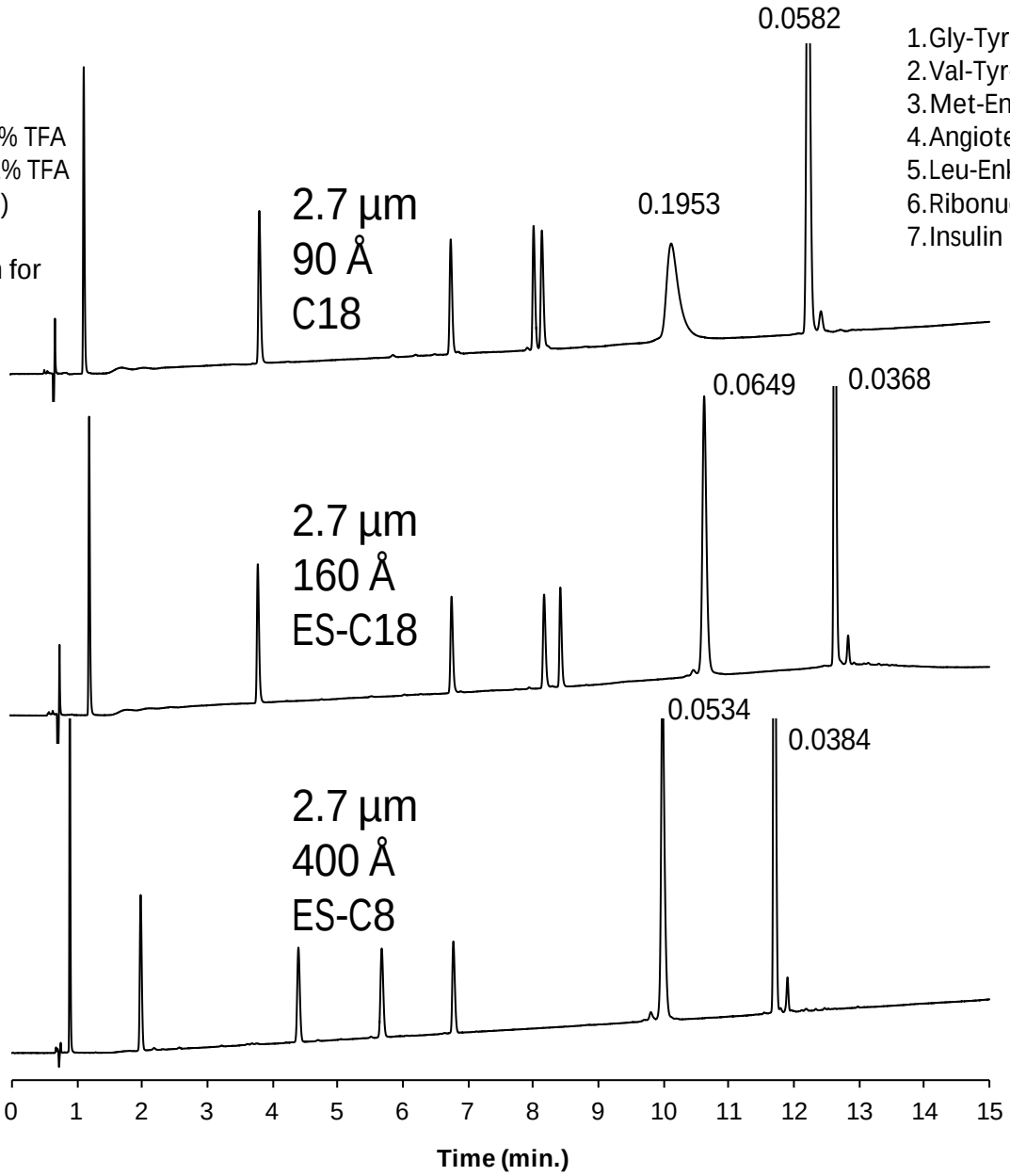


Pore Size Distribution of Fused-Core Particles



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 (15min)
Temperature = 30 °C
Peak widths at 50% height given for ribonuclease A and insulin



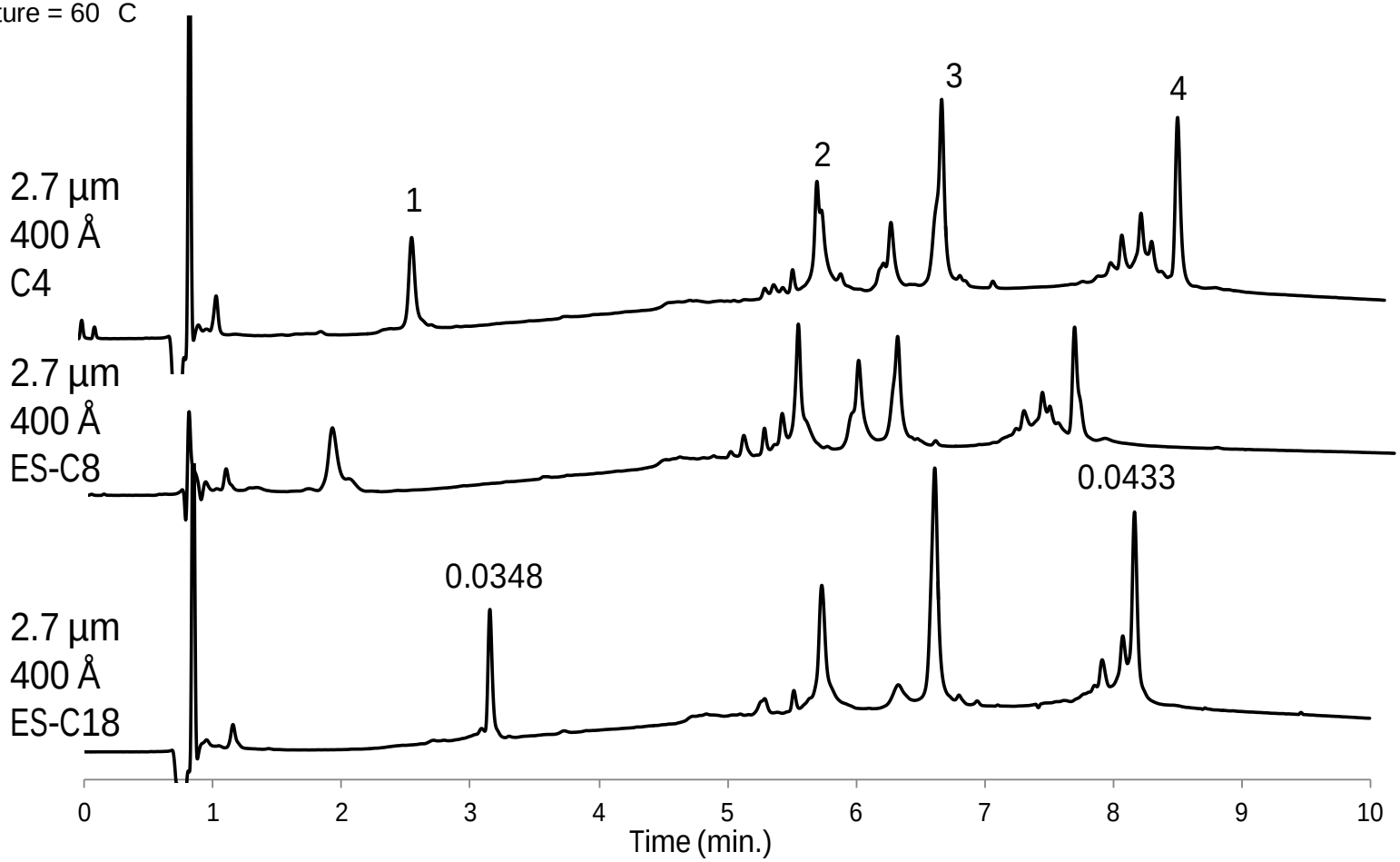
Peak Identities: In order

1. Gly-Tyr	MW = 238.2 g/mol
2. Val-Tyr-Val	MW = 379.5 g/mol
3. Met-Enk	MW = 573.7 g/mol
4. Angiotensin II	MW = 1046.2 g/mol
5. Leu-Enk	MW = 555.6 g/mol
6. Ribonuclease A	MW = 13700 g/mol
7. Insulin	MW = 5800 g/mol

Large Protein Separations

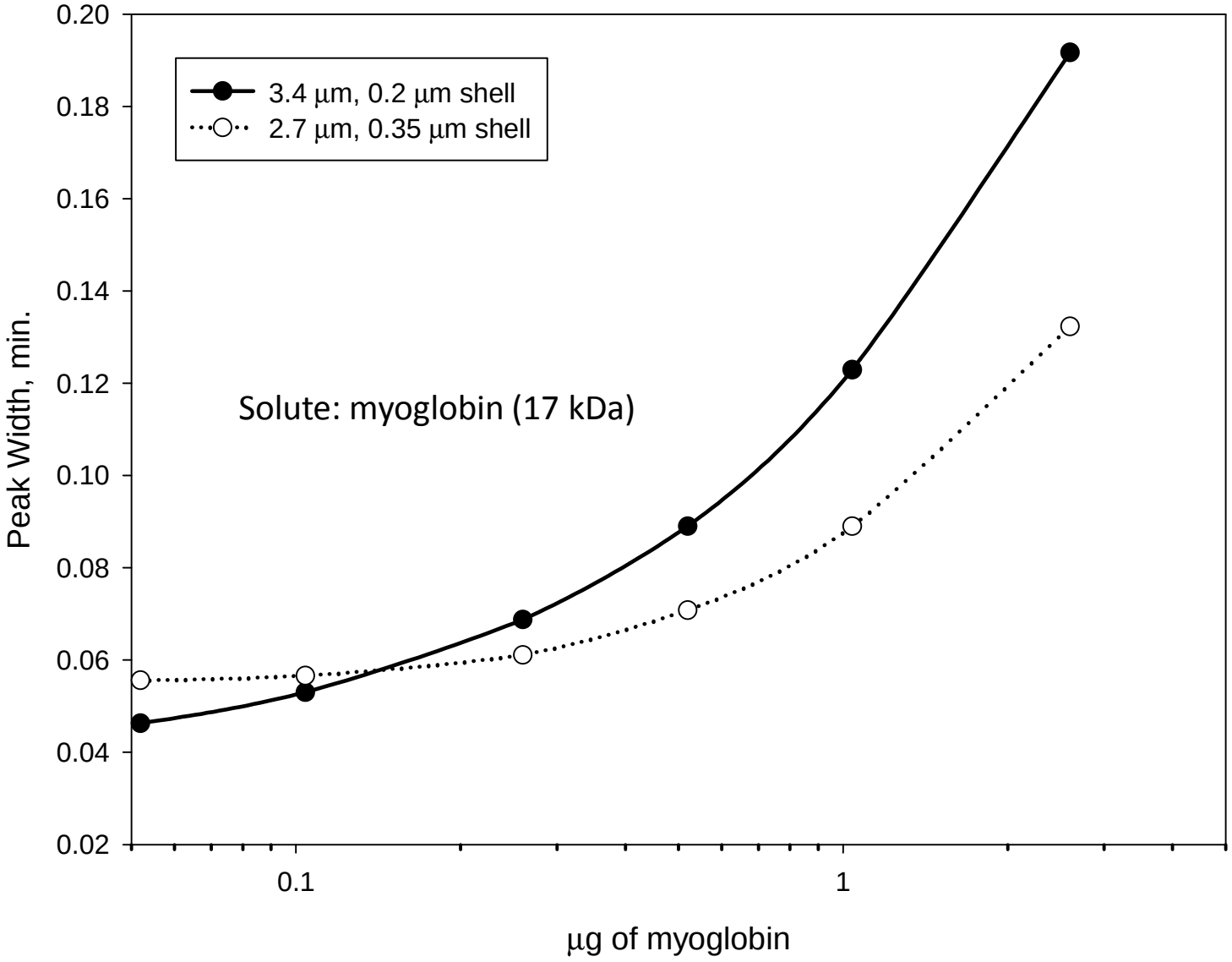
Columns: 2.1 x 100 mm
Instrument: Agilent 1200 SL
Flow rate: 0.3 mL/min
Injection Volume: 1 µL
Detection = 215 nm
Mobile Phase: A: Water/0.1% TFA
B: ACN/ 0.1% TFA
Gradient: 30% B to 70% B in 10 min.
Temperature = 60 C

Sample: In order
1. Cytochrome C MW = 12.4 kDa
2. Ferritin MW = 443 kDa
3. β-Amylase MW = 200 kDa
4. Myosin MW = 500 kDa [220 kDa monomer]



Effect of Particle Type on Sample Loading

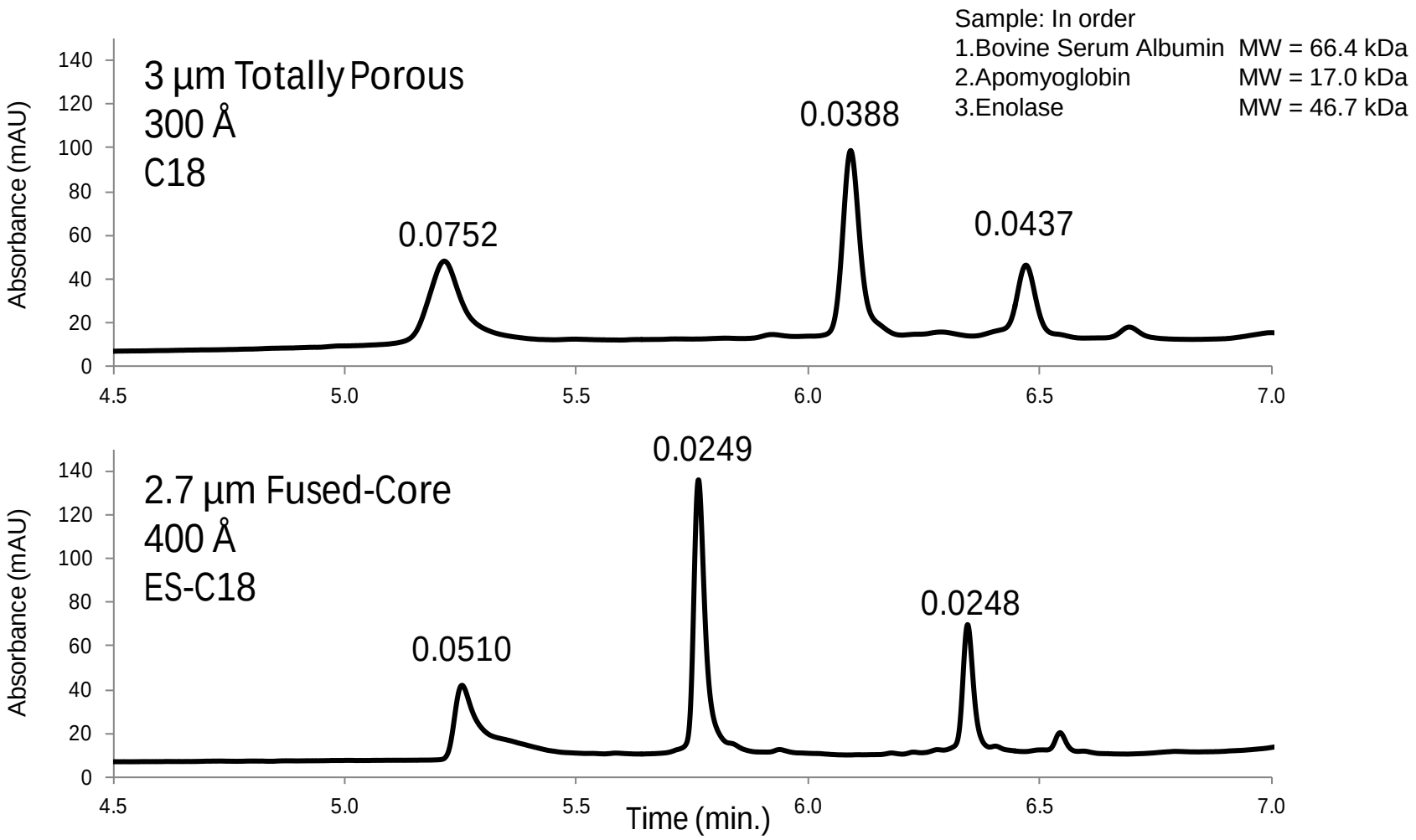
Columns: 4.6 x 100 mm; Temperature: 60 °C; Agilent 1100: Injection: 5 µL
Mobile phase- A: water/0.1% trifluoroacetic acid, B: acetonitrile/0.1% trifluoroacetic acid
Gradient: 37 - 47 % B in 10 min; Flow rate: 0.5 mL/min



Protein Separations

Fused-Core vs. Totally Porous

Columns: 4.6 x 100 mm; Temperature: 60 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



400 Å Fused-Core Particle Stability

Column: 2.1 x 100 mm 2.7 µm 400 Å ES-C8; Temperature: 60 °C
Mobile phase: A = water/0.1% TFA; B = 70% ACN/30% water/0.1% TFA;
Gradient: 9-55% B in 10 min.; Flow rate = 0.5 mL/min; Detection = 220 nm; Injection = 1 µL,
Retention times given for each peak, Peak widths at half height for selected peaks (min.)

Peak Identities: In order

1.Gly-Tyr	MW = 238.2 g/mol
2.Val-Tyr-Val	MW = 379.5 g/mol
3.Met-Enk	MW = 573.7 g/mol
4.Angiotensin II	MW = 1046.2 g/mol
5.Leu-Enk	MW = 555.6 g/mol
6.Ribonuclease A	MW = 13700 g/mol
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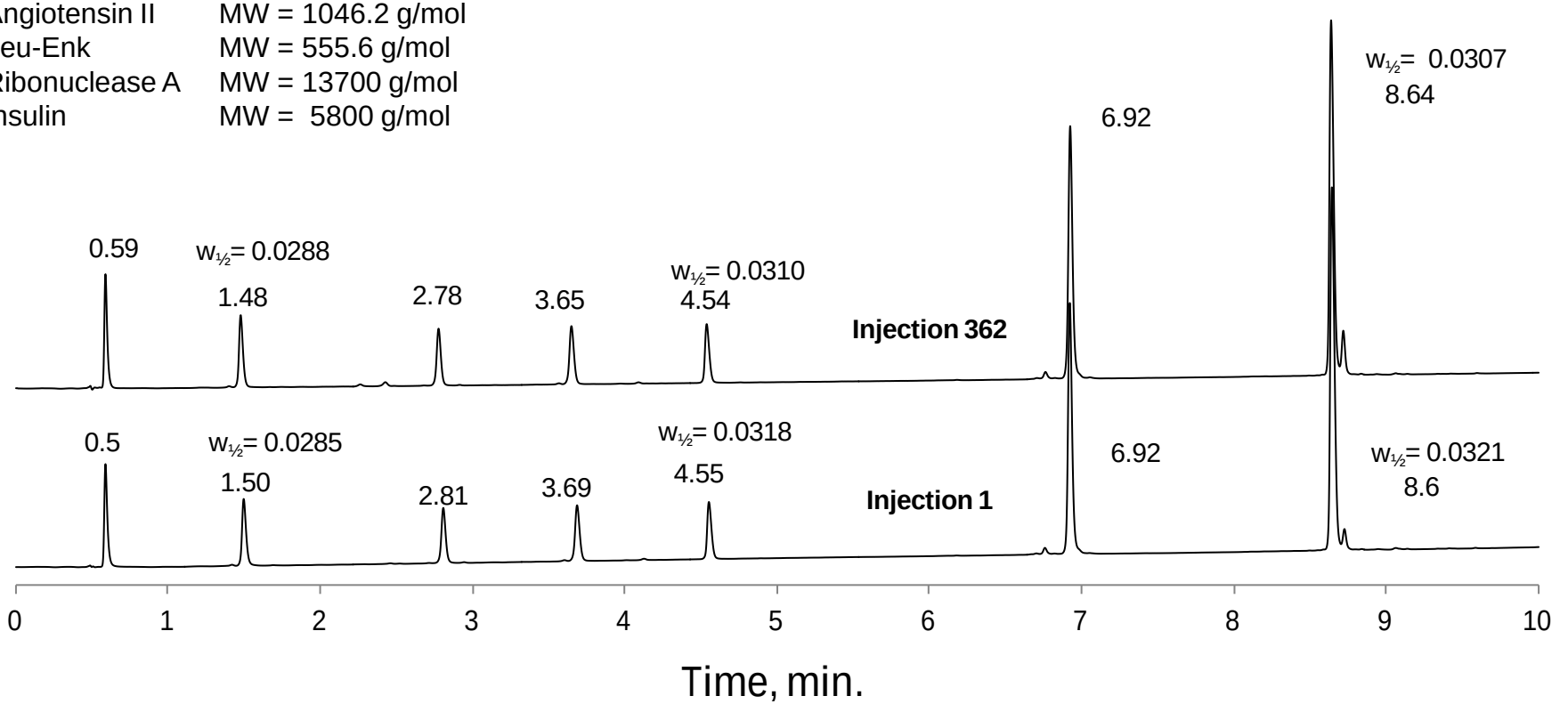
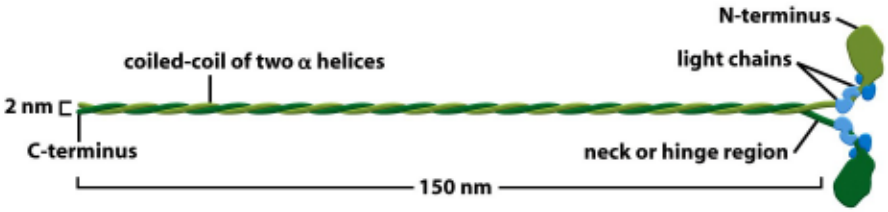


Rabbit Skeletal Myosin

Columns: 2.1 x 100 mm; Temperature: 80 C
Mobile phase: A = water/0.1% TFA; B = Acetonitrile/0.1% TFA
Gradient: 35-65% B in 15 min.; Flow rate = 0.45 mL/min.; Detection = 215 nm; Injection = 1 µL



Structure adapted from Alberts, et al., *Molecular Biology of the Cell* (© Garland Science 2008)

Conclusions from Study

Chromatographic characteristics of wide-pore particles:

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

2. C4 and C8 may be preferred for separating proteins

3. Thicker-shell particles have greater mass loading properties, but somewhat poorer efficiency than thinner-shell 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