

# Superficially Porous Silica Particles with Wide Pores for Biomolecular Separations

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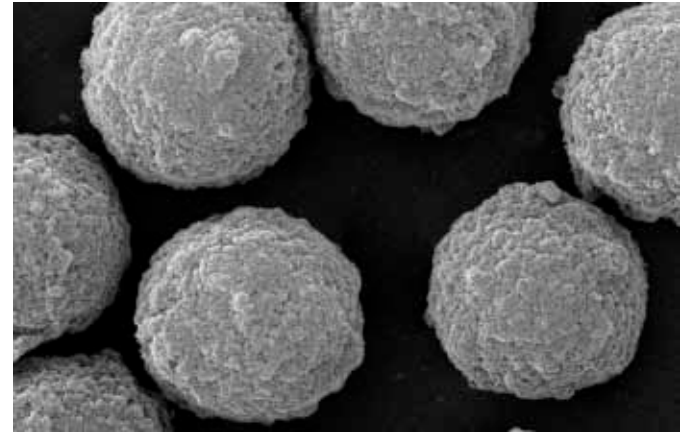
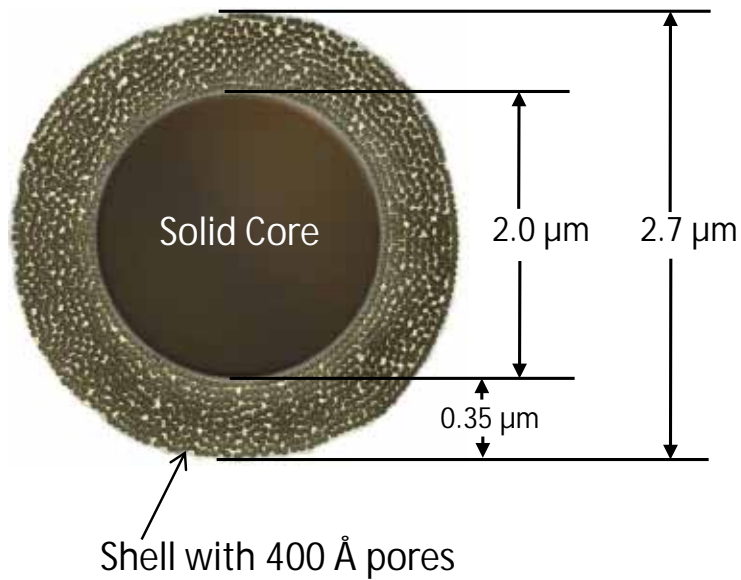
# Study of Wide-pore Fused-Core particles developed to show the effect of:

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, $\mu\text{m}$ | Pore Size, $\text{\AA}$ | BET Surface Area, $\text{m}^2/\text{g}$ | Shell Thickness, $\mu\text{m}$ | % Porosity | Pore volume, $\text{cm}^3/\text{g}$ |
|---------------------|------------------------------|-------------------------|---|--------------------------------|------------|-------------------------------------|
| 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                               |

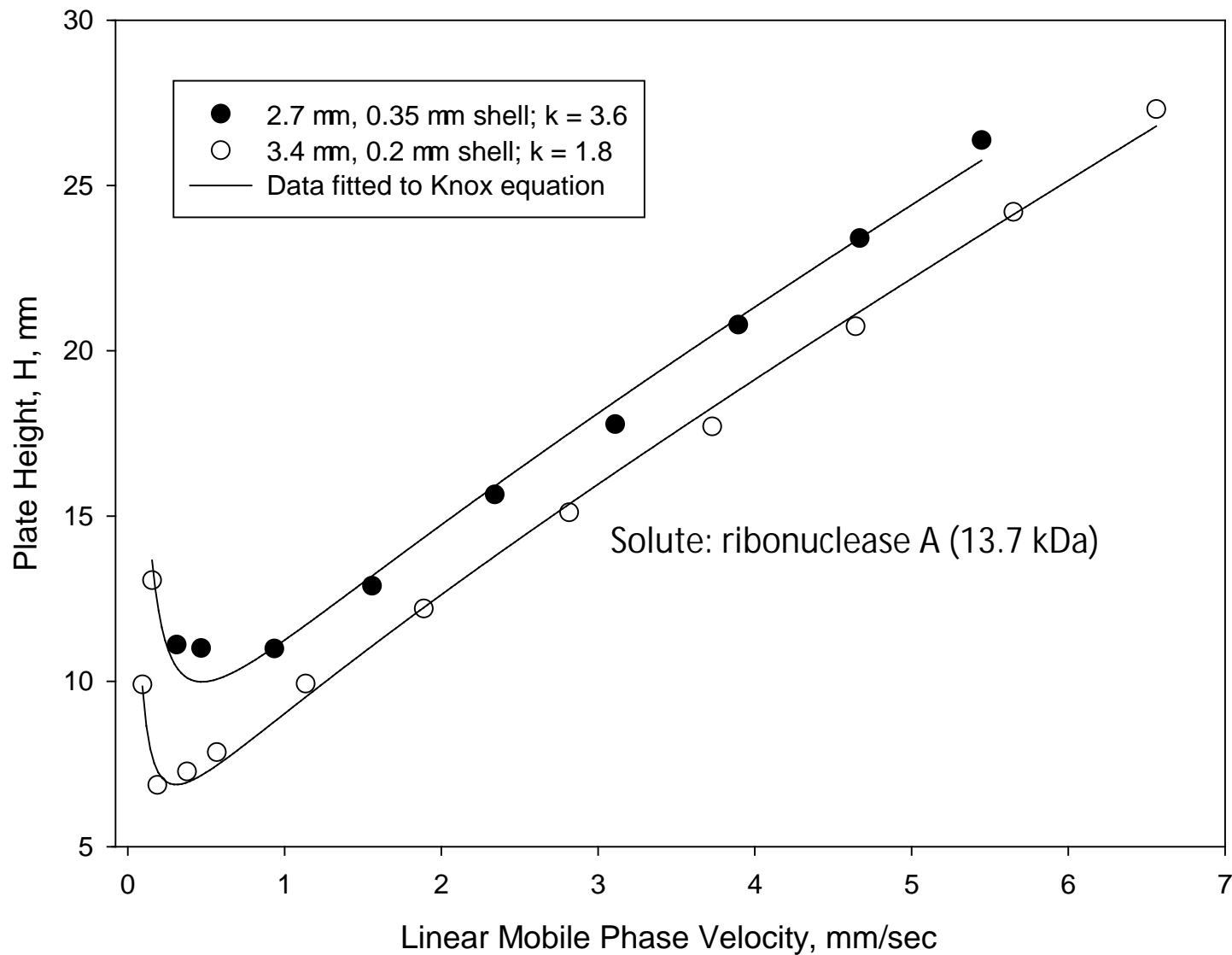
# Halo<sup>®</sup> Wide-pore Fused-core Particles



# Effect of Particle on Performance

Columns: 4.6 x 100 mm; Temperature: 60 °C

Mobile phase: 23.9% acetonitrile/76.1% aqueous trifluoroacetic acid, 0.1%  
Agilent 1100 with autosampler

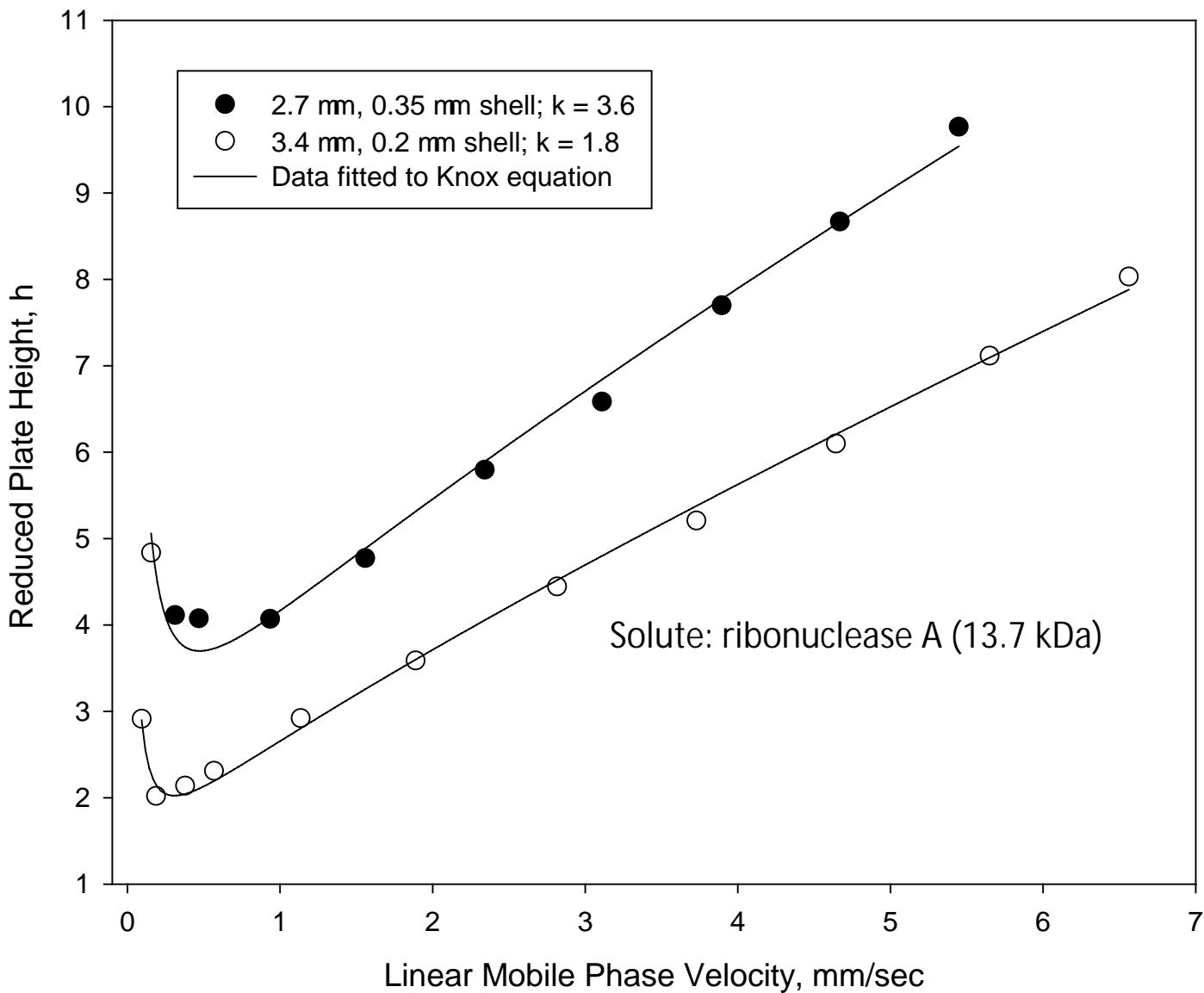


# Effect of Particle on Performance

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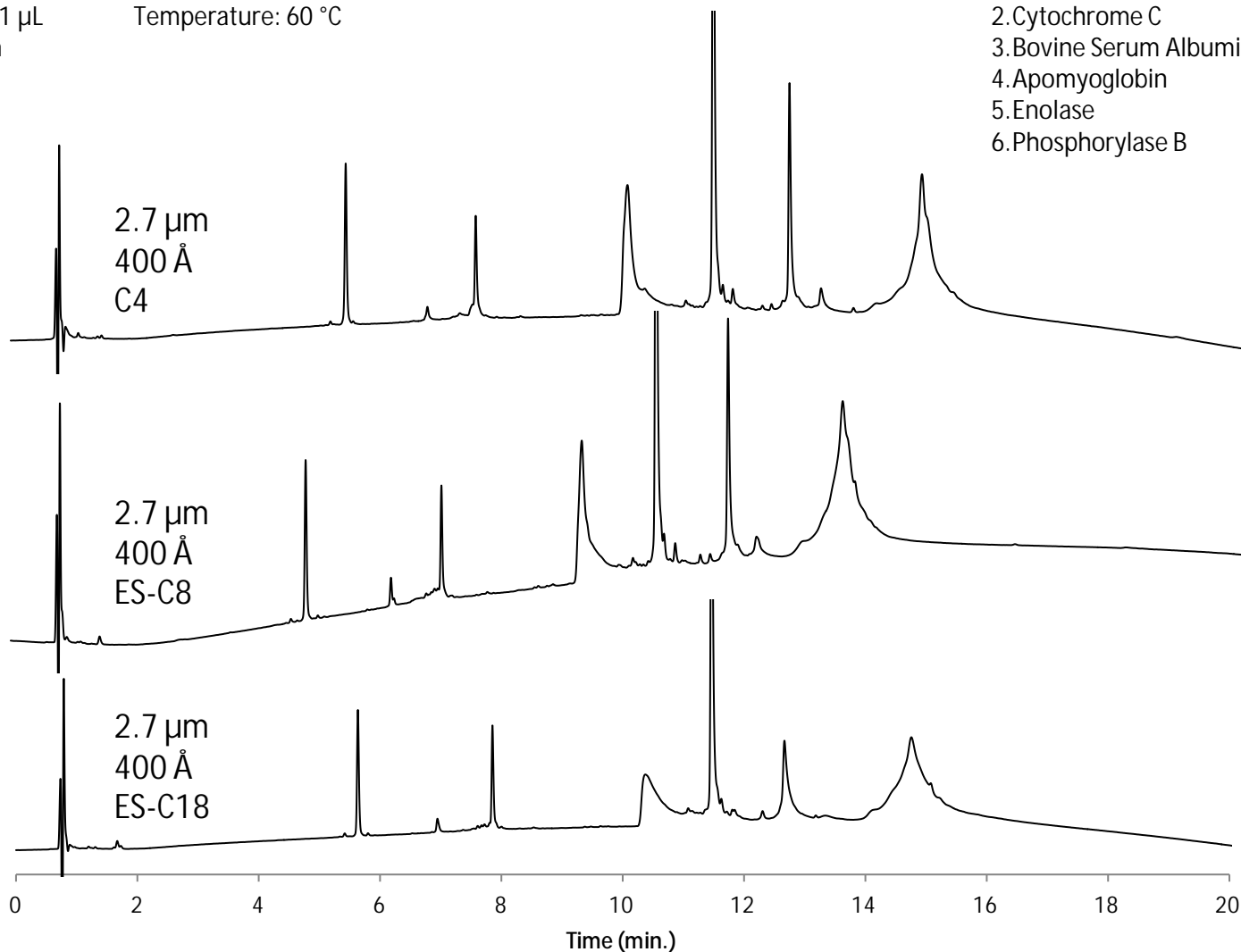


# Effect of Bonded Phase

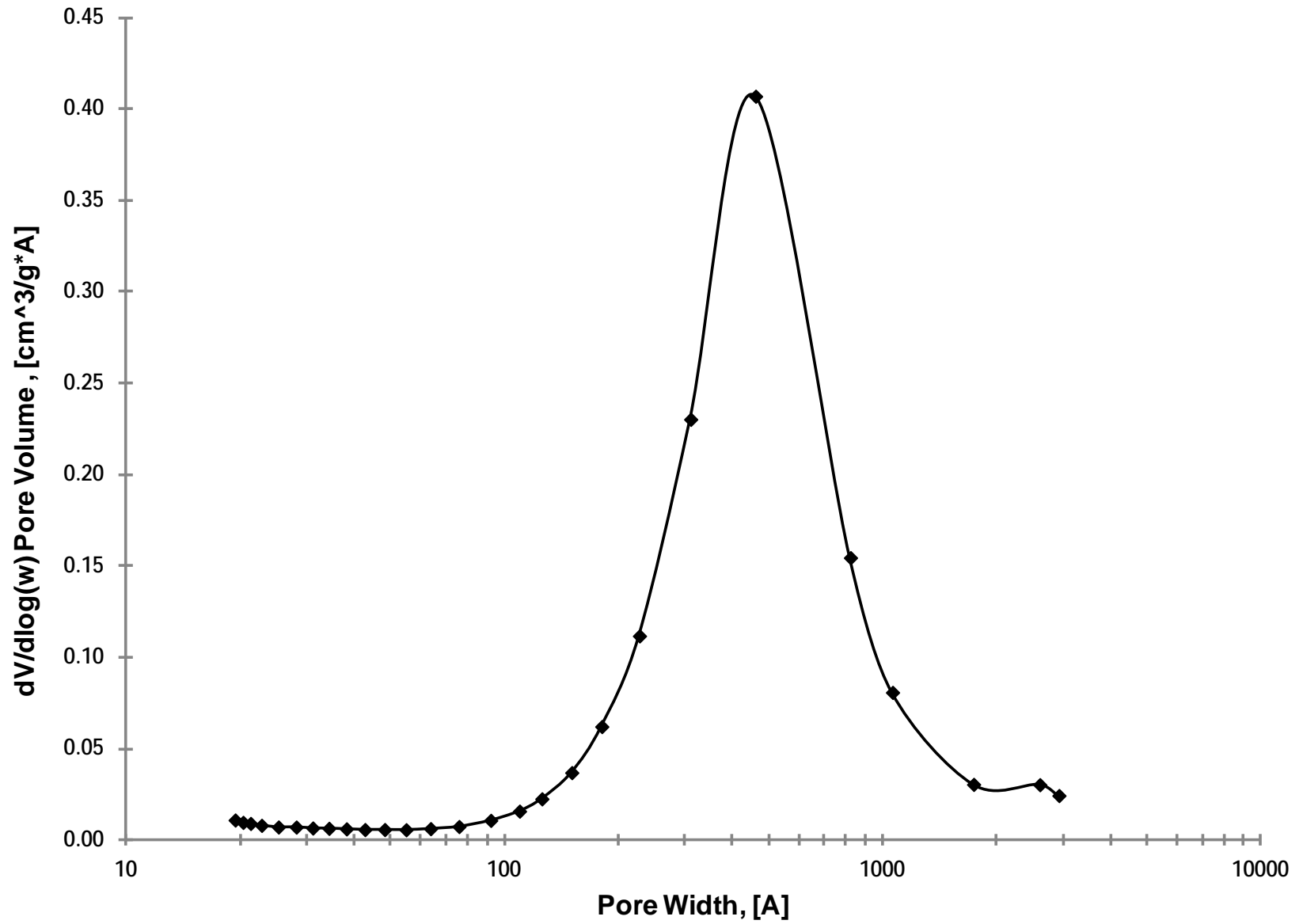
Columns: 2.1 x 100 mm  
Instrument: Agilent 1200 SL  
Injection Volume: 1  $\mu$ L  
Detection: 215 nm

Mobile Phase: 20–70% ACN/water/0.1% TFA in 20 min.  
Flow rate: 0.3 mL/min  
Temperature: 60  $^{\circ}$ 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



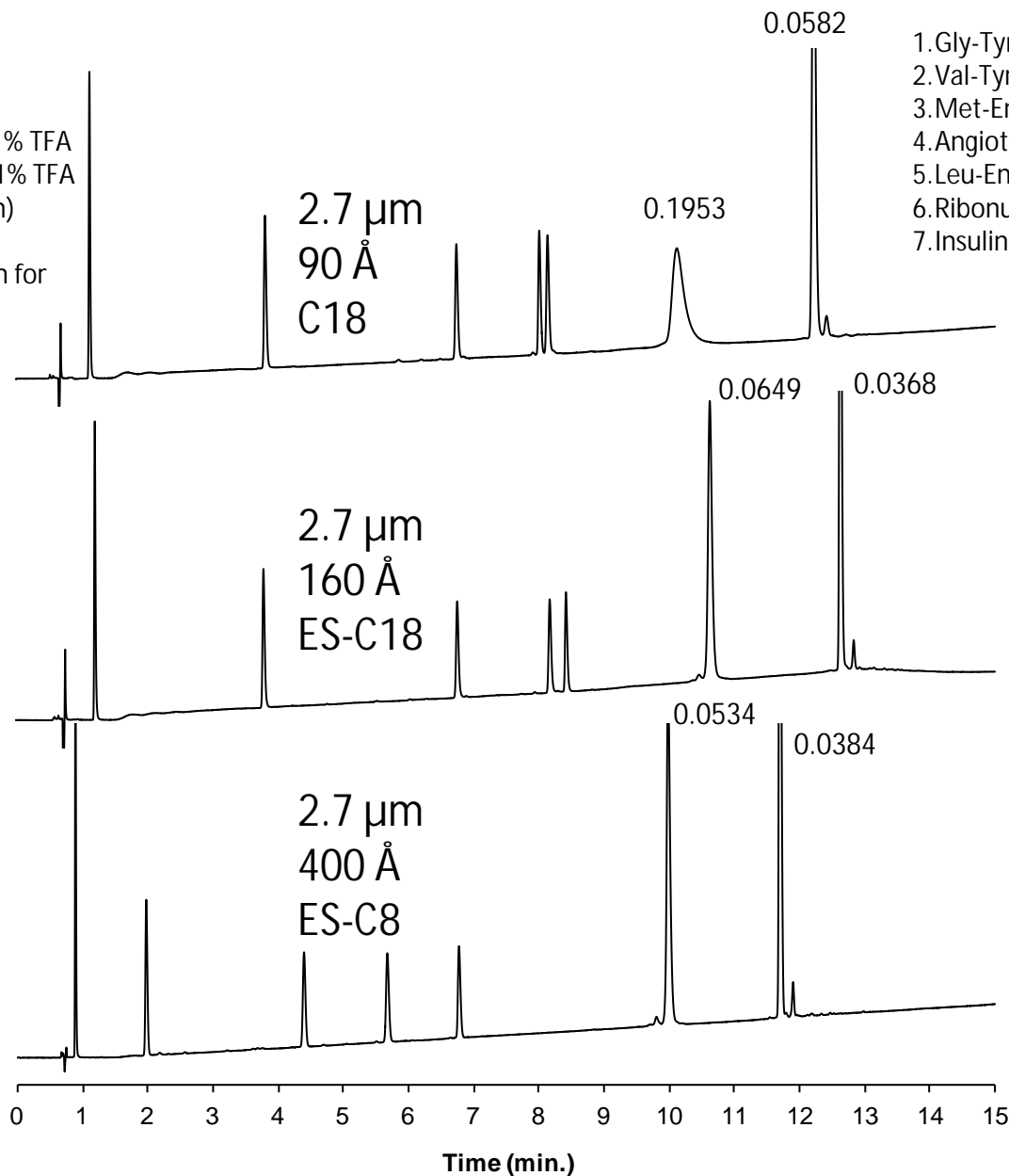


# Effect of Pore Size

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   |

Columns: 4.6 x 100 mm  
Instrument: Agilent 1100  
Flow rate: 1.5 mL/min  
Injection Volume: 5  $\mu$ L  
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  $^{\circ}$ C  
Peak widths at 50% height given for  
ribonuclease A and insulin



# Large Protein Separations

Columns: 2.1 x 100 mm  
Instrument: Agilent 1200 SL  
Flow rate: 0.3 mL/min  
Injection Volume: 1  $\mu$ L  
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

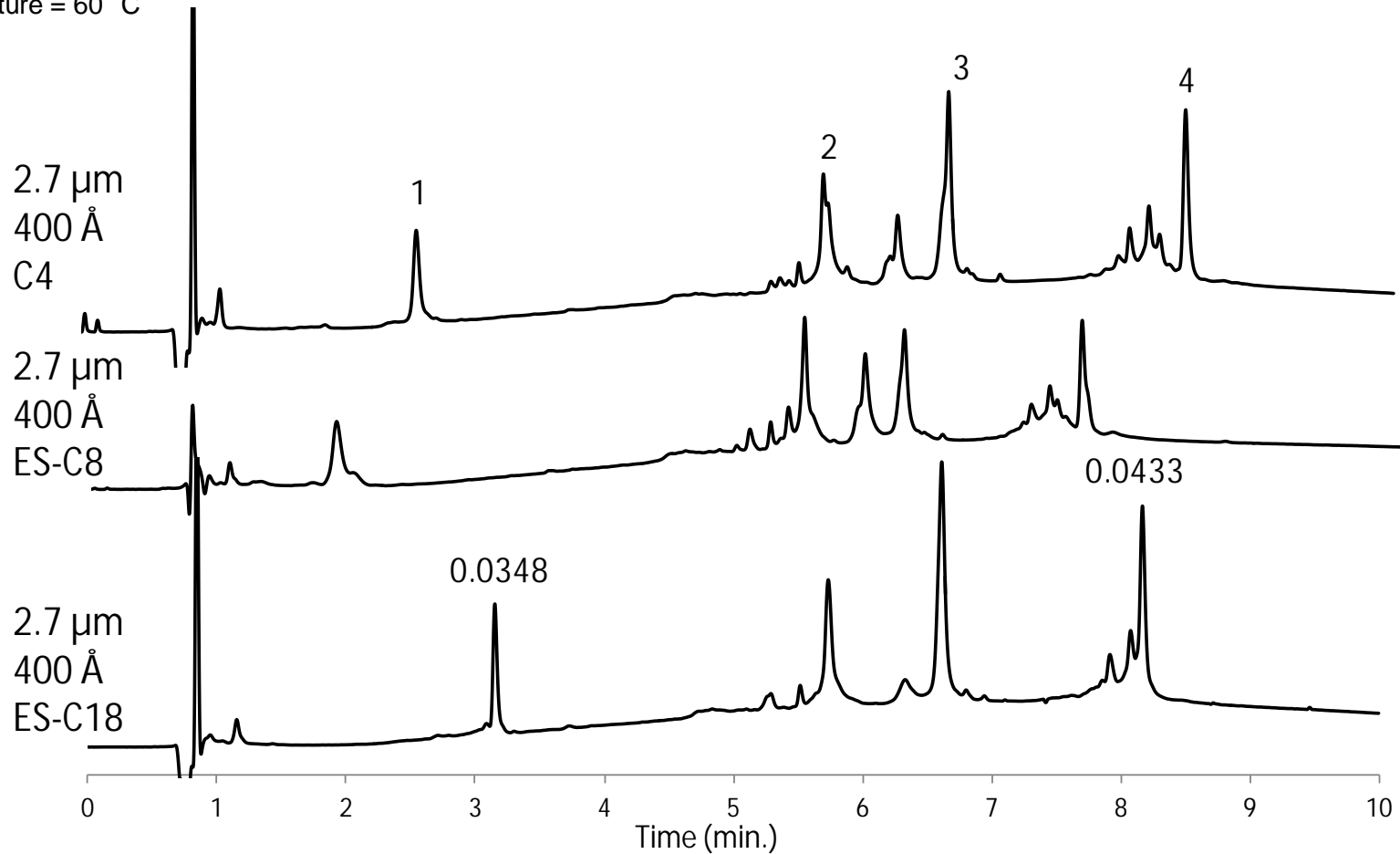
1. Cytochrome C
2. Ferritin
3.  $\beta$ -Amylase
4. Myosin

MW = 12.4 kDa

MW = 443 kDa

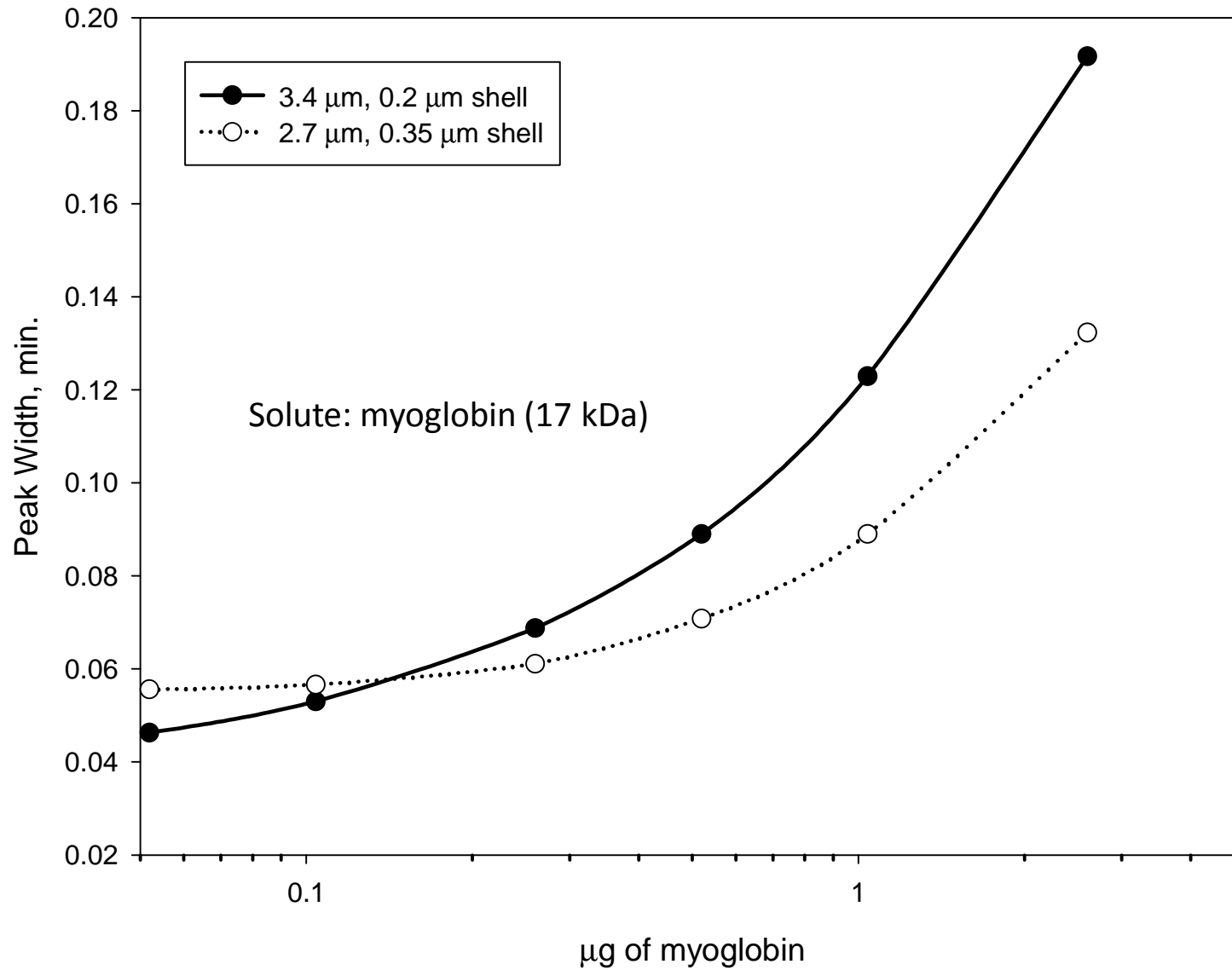
MW = 200 kDa

MW = 500 kDa [220 kDa monomer]



# Effect of Particle Type on Sample Loading

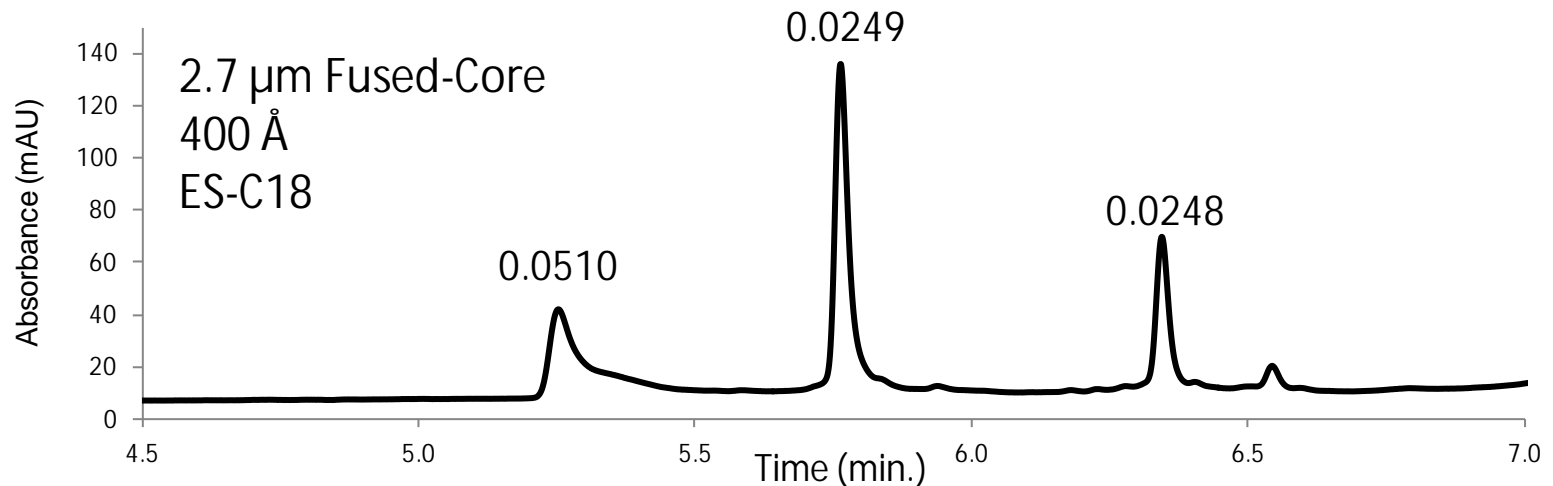
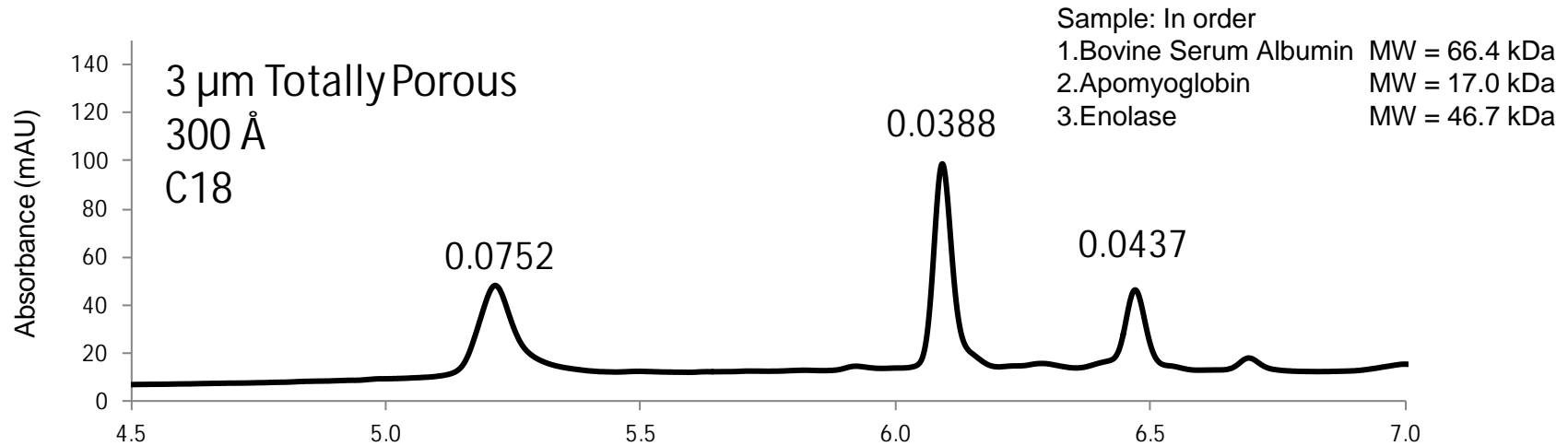
Columns: 4.6 x 100 mm; Temperature: 60 °C; Agilent 1100; Injection: 5  $\mu$ L  
Mobile phase- A: water/0.1% trifluoroacetic acid, B: acetonitrile/0.1% trifluoroacetic acid  
Gradient: 37 - 47 % B in 10 min; Flow rate: 0.5 mL/min



# 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  $\mu$ L



# 400 Å Fused-Core Particle Stability

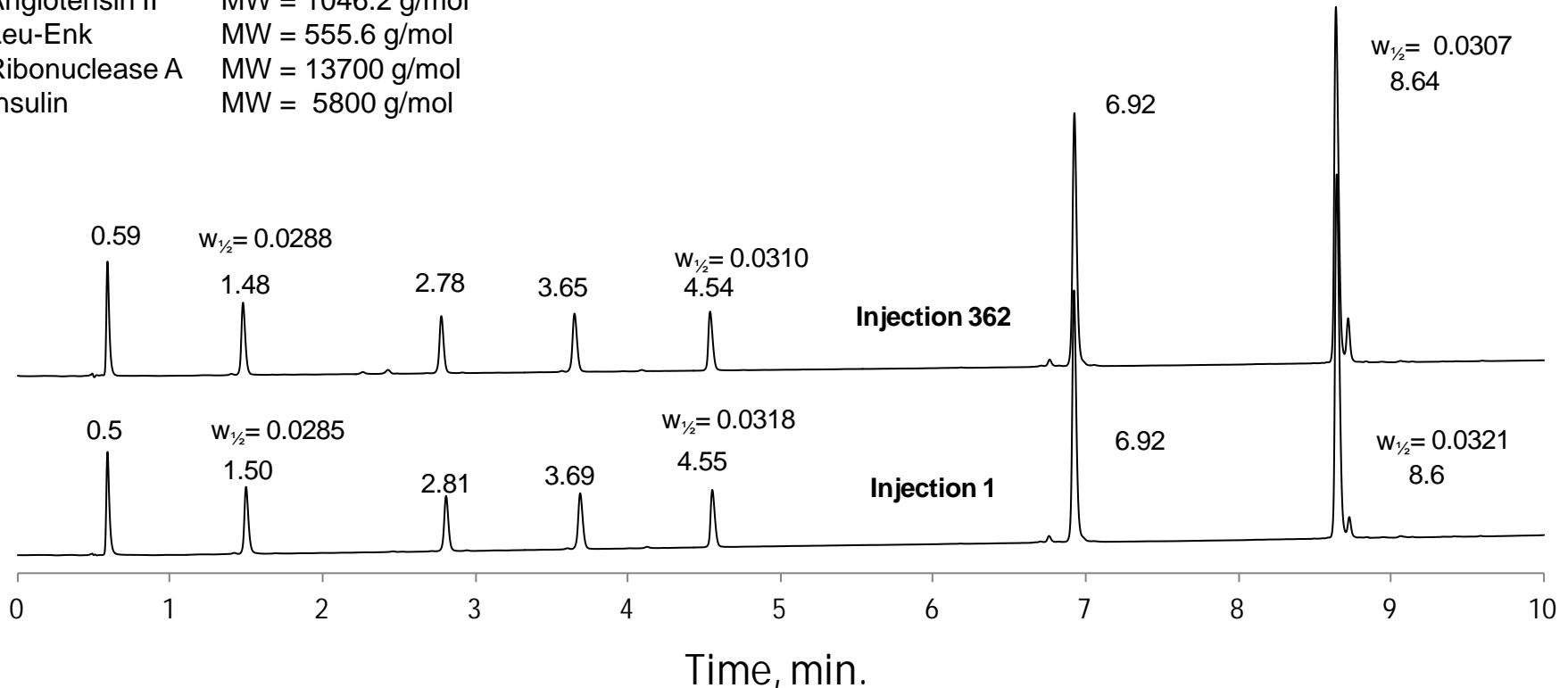
Column: 2.1 x 100 mm 2.7 µm 400 Å ES-C8; Temperature: 60 °C

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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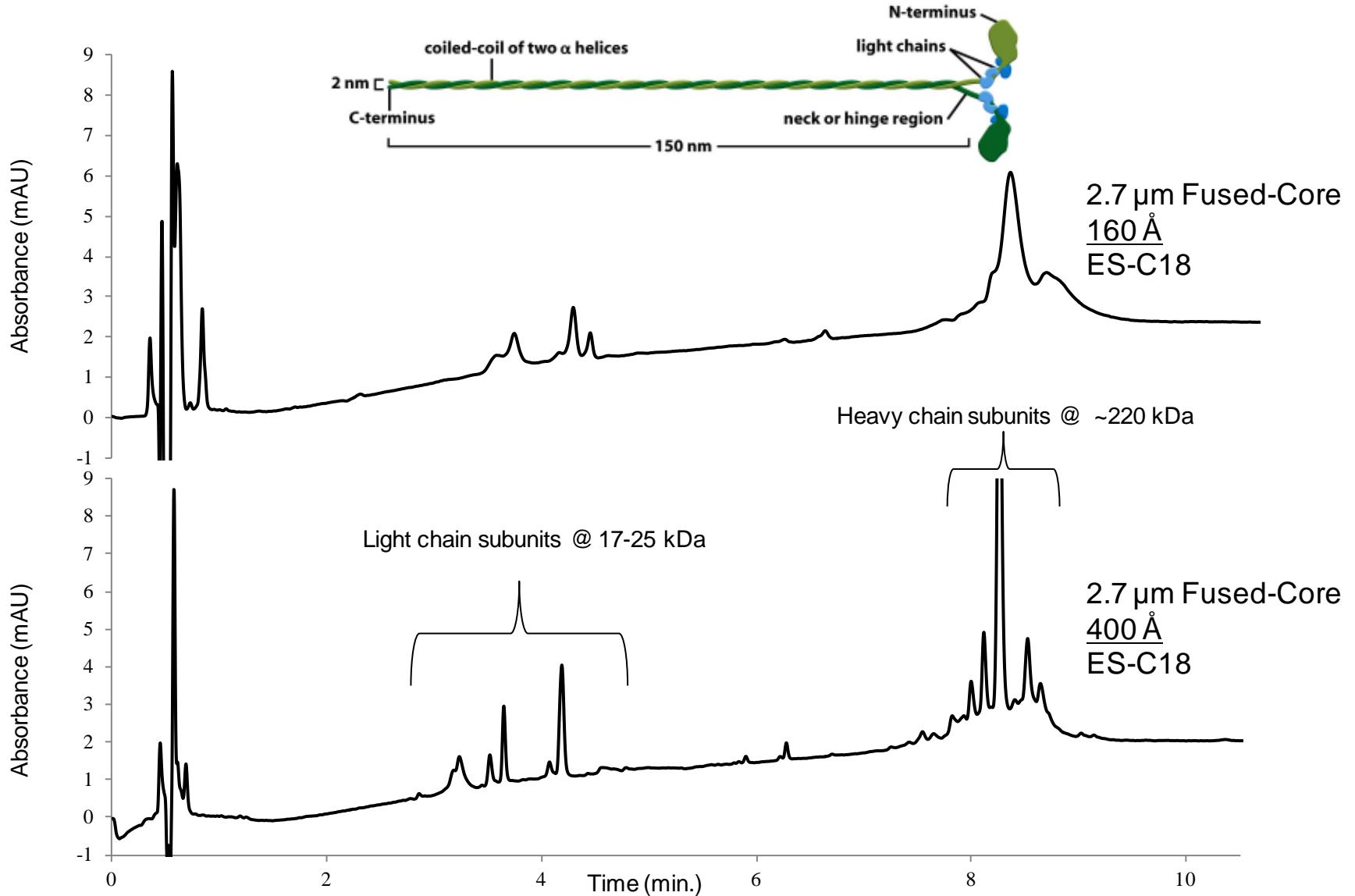


# Rabbit Skeletal Myosin

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



# 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