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

<table>
<thead>
<tr>
<th>Fused-Core Particle</th>
<th>Particle Size, µm</th>
<th>Pore Size, Å</th>
<th>BET Surface Area, m²/g</th>
<th>Shell Thickness, µm</th>
<th>% Porosity</th>
<th>Pore volume, cm³/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halo</td>
<td>2.7</td>
<td>90</td>
<td>135</td>
<td>0.5</td>
<td>75</td>
<td>0.26</td>
</tr>
<tr>
<td>Halo Peptide</td>
<td>2.7</td>
<td>160</td>
<td>80</td>
<td>0.5</td>
<td>75</td>
<td>0.29</td>
</tr>
<tr>
<td>Wide-pore</td>
<td>2.7</td>
<td>400</td>
<td>30</td>
<td>0.35</td>
<td>59</td>
<td>0.23</td>
</tr>
<tr>
<td>Wide-pore</td>
<td>2.7</td>
<td>400</td>
<td>14</td>
<td>0.2</td>
<td>46</td>
<td>0.11</td>
</tr>
<tr>
<td>Wide-pore</td>
<td>3.4</td>
<td>400</td>
<td>10</td>
<td>0.2</td>
<td>31</td>
<td>0.068</td>
</tr>
</tbody>
</table>
Halo® Wide-pore Fused-core Particles

Shell with 400 Å pores

Solid Core

2.0 µm

2.7 µm

0.35 µm
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

Linear Mobile Phase Velocity, mm/sec

Plate Height, $H$, μm

Data fitted to Knox equation

Solute: ribonuclease A (13.7 kDa)
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

Data fitted to Knox equation

Solute: ribonuclease A (13.7 kDa)
Effect of Bonded Phase

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

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

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Flow rate: 0.3 mL/min
Temperature: 60 °C
Pore Size Distribution of Fused-Core Particles

**Graph:**

- **Y-axis:** dV/dlog(w) Pore Volume [cm³/g*A]
- **X-axis:** Pore Width [Å]

The graph shows the distribution of pore widths, indicating a peak at a certain width and decreasing values on either side.
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 µL
Detection: 220 nm
Mobile Phase: A: 10% ACN / 0.1% TFA
B: 70% ACN / 0.1% TFA
Gradient: 0% B to 50% B (15 min)
Temperature = 30 °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 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]

Large Protein Separations

2.7 µm
400 Å
C4

2.7 µm
400 Å
ES-C8

2.7 µm
400 Å
ES-C18

Time (min.)
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

Solute: myoglobin (17 kDa)
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

Sample: In order
1. Bovine Serum Albumin  MW = 66.4 kDa
2. Apomyoglobin                MW = 17.0 kDa
3. Enolase                          MW = 46.7 kDa

3 µm Totally Porous
300 Å
C18

2.7 µm Fused-Core
400 Å
ES-C18

![Graph of protein separations between 3 µm Totally Porous and 2.7 µm Fused-Core columns with sample information and absorbance values.](chart.png)
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
7. Insulin    MW = 5800 g/mol

Time, min.
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