Preparation, Characterization, and Application of New Superficially Porous Silica Particles for Fast HPLC Separation

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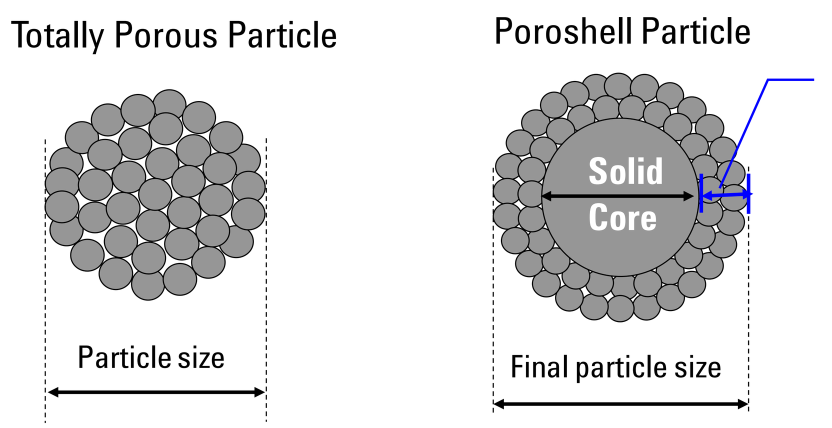
Abstract

The need for higher productivity in the pharmaceutical and other industries drives the trend towards faster and faster HPLC separations. Porous sub 2 µm silica particles are becoming more and more popular as the column choice for ultra fast and high resolution separations. More recently, 2.7 µm superficially porous particles are being seen as an alternative to fully porous sub 2 µm particles for high speed and/or high resolution HPLC separations. of small molecules. There are several publications which experimentally and theoretically studied the unique chromatographic performance of these superficially porous particles . Experimentally and theoretically, these 2.7 µm superficially porous silica particles show comparable efficiency to fully porous sub 2 µm particles, with only about half of the back pressure of sub 2 µm particles. These particles can be used in traditional HPLC instruments for fast separations in a short column format or in high pressure HPLC instruments for extremely high resolution in a longer column format

Introduction

Totally Porous Particle vs. Superficially Porous Particles



- For totally porous particles, the high efficiency is achieved by decreasing particle size.
- For superficially porous particles, the high efficiency is achieved by decreasing the mass transfer distance.

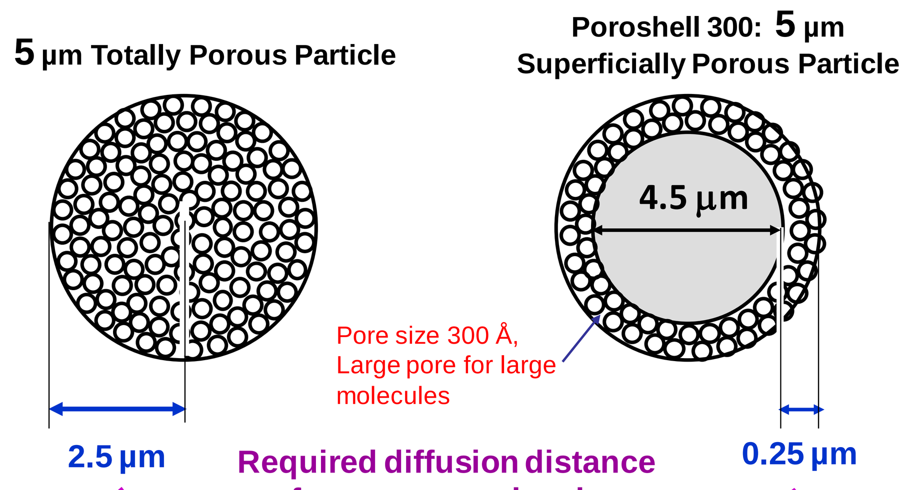
What Improves the Efficiency with Superficially Porous Particles

(van Deemter equation) $H = A + B/u + C \times u$

- A term – eddy diffusion and flow distribution
  - Column particle size and column packing quality impact this
  - **Tight particle size distribution improves the A term**
- B term – longitudinal diffusion
  - Impact in superficially porous particle not yet determined
- C term – mass transfer component
  - **Mass transfer is improved by using shorter diffusion paths, improving the C term**
  - This is improved with a superficially porous particle
  - The C term has more effect on large molecules than on small molecules

Poroshell 300 for large molecules

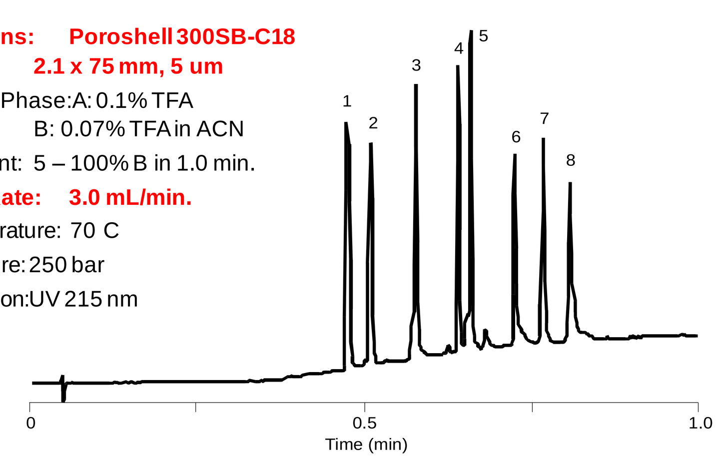
Poroshell 300 Particles for Separation of Large Molecules



Required diffusion distance for a macromolecule reduced 10 fold !
Diffusion time down 10 fold!

High Flow Rates with 2.1 mm ID Poroshell 300 for High Resolution and Fast Separations

Columns: Poroshell 300SB-C18
2.1 x 75 mm, 5 um
Mobile Phase: A: 0.1% TFA
B: 0.07% TFA in ACN
Gradient: 5 – 100% B in 1.0 min.
Flow Rate: 3.0 mL/min.
Temperature: 70 C
Pressure: 250 bar
Detection: UV 215 nm

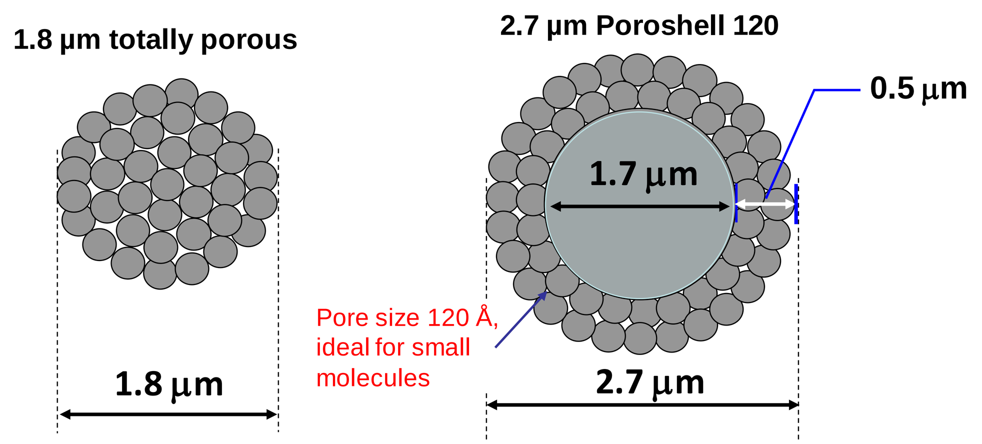


Sample:
1. Angiotensin II
2. Neurotensin
3. Rnase
4. Insulin
5. Lysozyme
6. Myoglobin
7. Carbonic Anhydrase
8. Ovalbumin

- Poroshell can provide high efficiency at higher flow rates for extremely rapid separations of proteins and peptides.
- This is due to more rapid mass transfer of the superficially porous particle

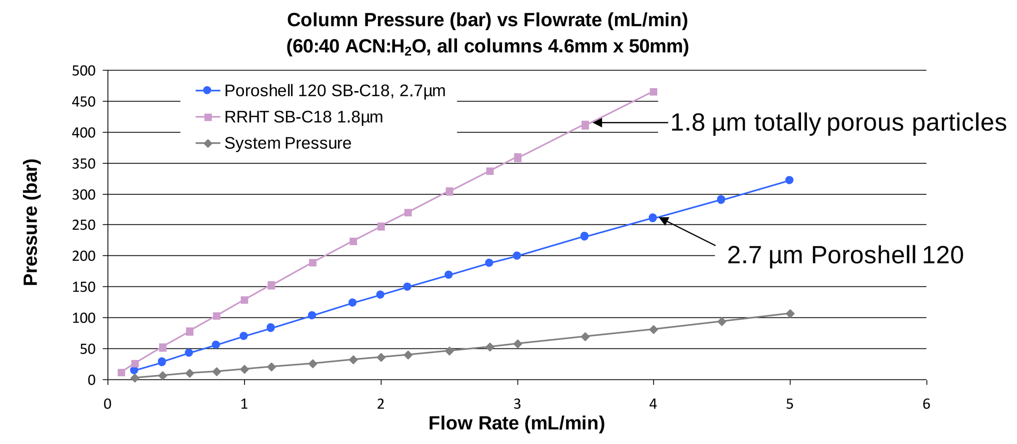
Poroshell 120 for small molecules

Poroshell 120 for Separation of Small Molecules



- The high efficiency of Poroshell 120 particles; similar to sub 2 µm totally porous particles.
- The high efficiency is due to short mass transfer distance and much narrower particle size distribution.
- Very low back pressure, 40-60% of sub 2 µm totally porous particles.

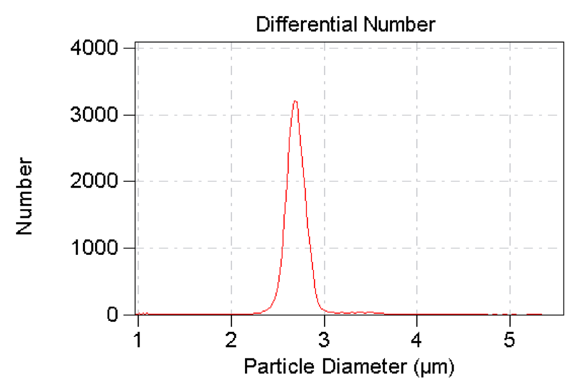
Back Pressure Comparison with 1.8 µm Totally Porous Particles



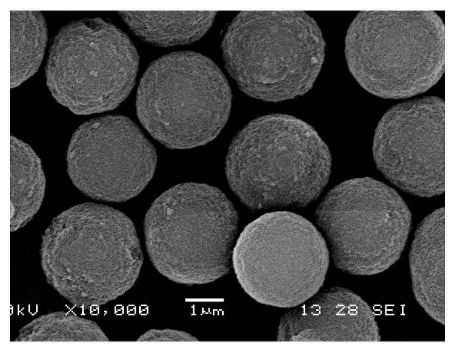
- Superficially porous particles have 40%-50% back pressure of 1.8 µm totally porous particles.

Results

Poroshell 120 Particles Have Very Narrow Particle Size Distribution



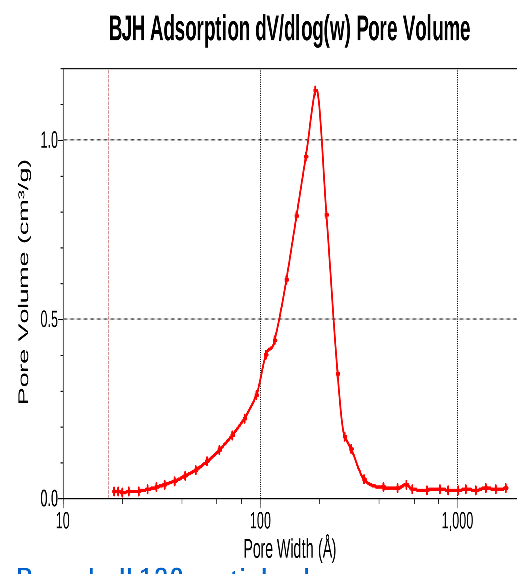
Particle Size Distribution of Poroshell 120



SEM Picture of Poroshell 120

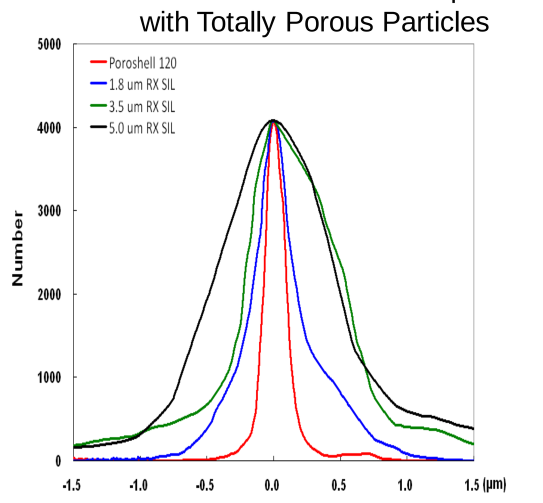
Poroshell 120 particles also show spherical and smooth surface.

Poroshell 120 Pore and Particle Size Distribution



Poroshell 120 particles have an average pore size of 120 Å.

Particle Size Distribution Comparison with Totally Porous Particles



Normalized Particle Size Distribution

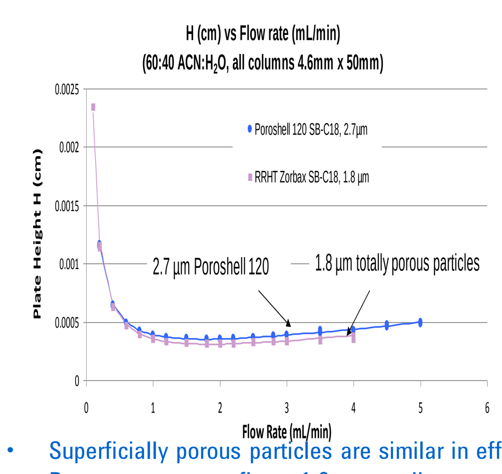
Poroshell 120 Particle Size Distribution is 25% Narrower

	Poroshell 120	1.8 µm	3.5 µm	5.0 µm
10%	2.48 µm	1.67 µm	3.07 µm	4.59 µm
90%	2.75 µm	2.45 µm	4.44 µm	6.21 µm
90%/10% ratio	1.11	1.47	1.45	1.35

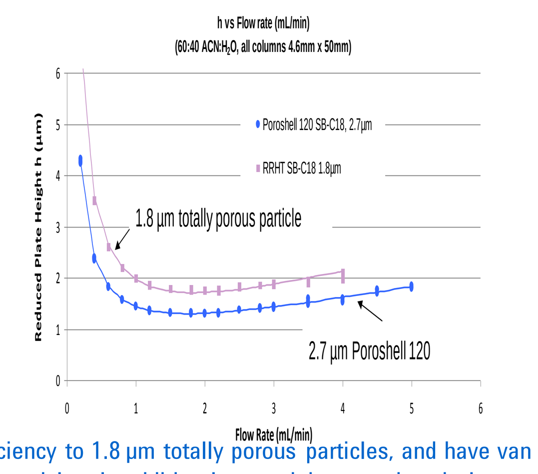
- The 1.8, 3.5 and 5 µm particles all have a normal particle size distribution.
- A number below 1.5 would be expected for the totally porous particles
- This narrower particle size distribution improves column efficiency over a totally porous particle.

van Deemter Comparison

Plate Height Comparison with 1.8 µm Totally Porous Particles



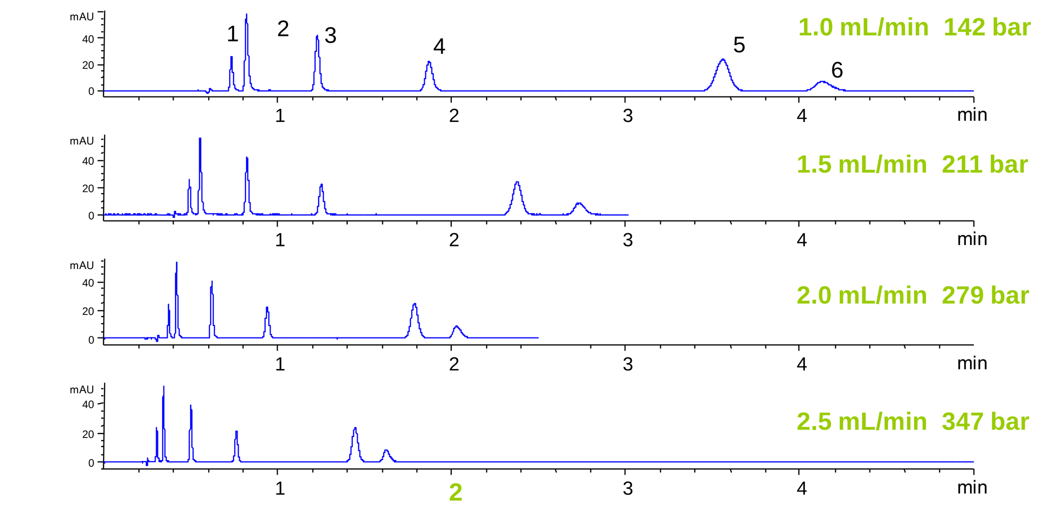
Reduced Plate Height Comparison with 1.8 µm Totally Porous Particles



- Superficially porous particles are similar in efficiency to 1.8 µm totally porous particles, and have van Deemter curves as flat as 1.8 µm totally porous particles. In addition lower minimum reduced plate height is found with superficially porous particles compared to totally porous particles, indicating the column is better packed due to the narrow particle size distribution.

Fast Analysis of Analgesics on Poroshell 120 – High Flow Rates, Low Pressure is Possible

1. Acetaminophen, 2. Caffeine, 3. 2-acetamidophenol, 4. Acetanilide, 5. Phenacetin, 6. Salicylic acid



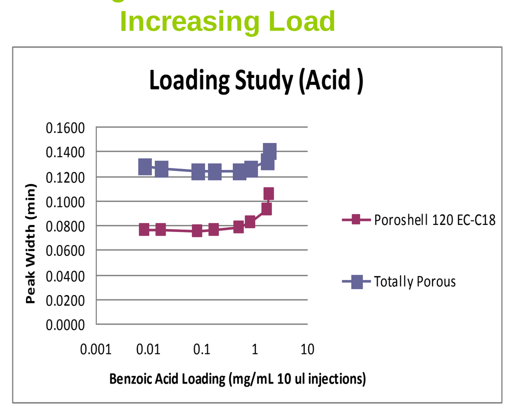
Conditions: Column: Poroshell 120 SB-C18, 4.6 x 50mm, 2.7um Mobile Phase: 0.2% Formic Acid 80% water:20% ACN
Temperature: 25 C, Detection: 275 nm, Sample Injection: 2ul

Comparison of Sample Loading with 2.7 µm Totally Porous Particles

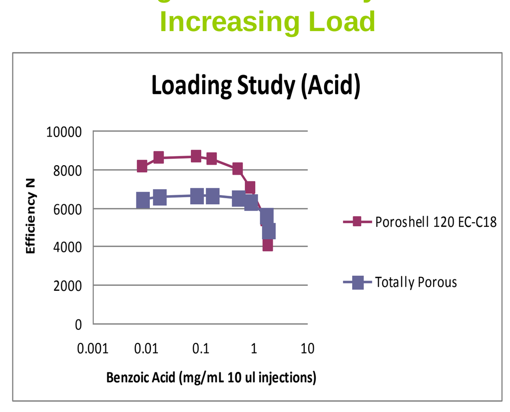
- Columns Compared
  - Superficially Porous 2.7 µm media – Poroshell 120
    - Surface areas of 100-110m²/g
  - Totally Porous 2.7 µm media –Not a commercial product
    - Surface area of 210m²/g
- Columns were compared using benzoic acid as an analyte.
- Two parameters were measured that would change as a result of increasing the load on the column
  - Peak width (typically peak width doubling indicates overload)
  - Efficiency (decreases in efficiency go with band broadening)

Sample Loading of Acids on 2.7 µm Totally Porous and Superficially Porous Columns

Changes in Peak Width with Increasing Load



Changes in Efficiency with Increasing Load



- Mobile phase:85 % Sodium Phosphate Buffer pH 3.0, 25 mM 15 % ACN
- Flow Rate: 1.5 ml/min

- The superficially porous 2.7µm columns have sharper, more efficient peaks.
- This is expected of this technology.
- Both of the columns show very similar loading behavior – based on peak width changes.

Conclusions

- New 2.7 µm superficially porous silica particles, Poroshell 120, were developed for fast separation of small molecules.
- Smaller A-term of the van Deemter plot due to the narrower particle size distribution and smaller C-term due to the short mass transfer distance result in very high efficiency and flat van Deemter curve of the column, comparable to 2 µm totally porous particles, and very low back pressure, about half of sub 2 µm totally porous particles.
- Poroshell 120 particles demonstrate similar sample loadability to totally porous silica particles.
- Fast analysis on Poroshell 120–high flow rates, low pressure is possible.

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Note: All the data of Poroshell 120 in this presentation is from particles in development, not a commercial product.