Evaluation of Porous Layer Thickness of Core Shell Particle for Separation of Proteins

Tomoyasu Tsukamoto, Norikazu Nagae

ChromaNik Technologies Inc., 6-3-1 Namiyoke, Minato-ku, Osaka, Japan

ChromaNik Technologies Inc.

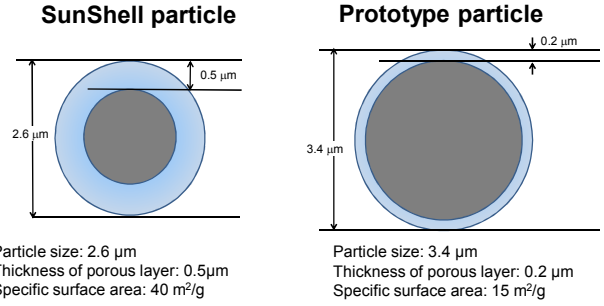
Abstract

The feature of superficially porous (core shell) particle used as a highly efficient material is existence of a core, a thin porous layer and narrow particle size distribution, which lead to higher efficiency than totally porous particle. Recently a core shell particle with wide pore for biomacromolecular separations has developed by a few manufacturers. It has been said that thin porous layer of core shell particle have an advantage for separation of large molecules such proteins because a diffusion coefficient becomes small to proportional to a molecular weight and a mass transfer speed also decreases. In this paper, thickness of porous layer of core shell particle was evaluated to separate proteins.

2 kinds of thickness of porous layer such as 0.2 µm and 0.5 µm thickness were applied for separation of standard protein samples. One was 3.4 µm particle size, 0.2 µm porous layer and 15 m2/g specific surface area and another was 2.6 µm, 0.5 µm and 15 m2/g. Separation was achieved using a gradient elution with 0.1% trifluoroacetic acid and acetonitrile including 0.08% trifluoroacetic acid.

On fast separation, 0.2 µm of porous layer showed sharper peaks than 0.5 µm of porous layer. However at 80 degree Celsius and using 60 min gradient time program, 0.5 µm of porous layer showed much sharper peaks than 0.2 µm of porous layer. It was considered that 0.5 µm of porous layer had the a wider specific surface area than 0.2 µm of porous layer and this wider specific surface area leaded separation efficiency concerning the partition interaction on the stationary phase to be large. Better separation of proteins contributes not only the thin porous layer but also the large surface area.

2 kinds of coreshell particles



Particle size: 2.6 µm
Thickness of porous layer: 0.5µm
Specific surface area: 40 m²/g

Particle size: 3.4 µm
Thickness of porous layer: 0.2 µm
Specific surface area: 15 m²/g

Figure 1. Schematic diagram of a core shell silica particles

Pore size distribution

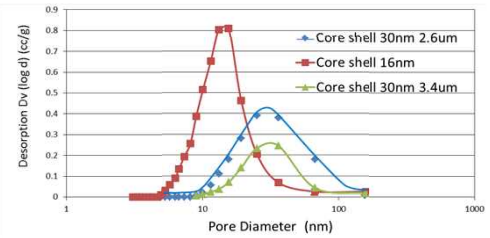
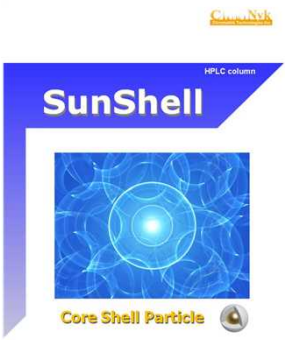


Figure 2. Pore distribution of core shell particles

Measurement : Nitrogen adsorption method



Effect of gradient time

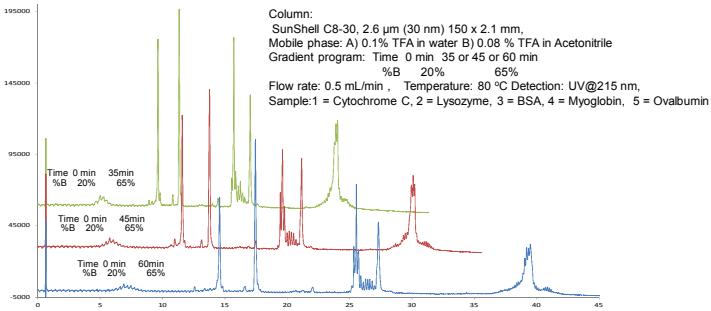


Figure 3. Chromatogram of protein mixture

Influence of temperature

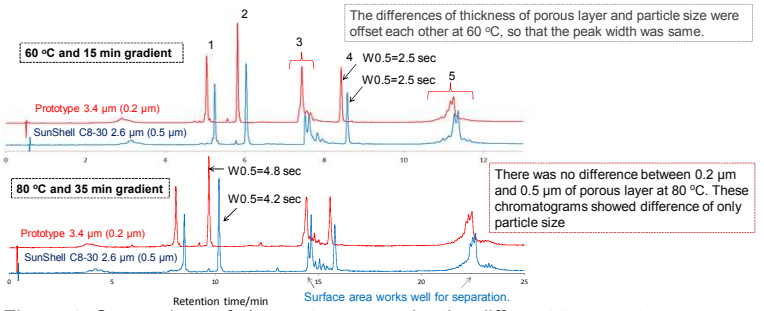


Figure 4. Comparison of chromatogram under the different temperature

Column: SunShell C8-30, 2.6 µm (30 nm, 0.5 µm layer) 100 x 2.1 mm, Sunshell C8-30, 3.4 µm (30 nm, 0.2 µm layer) 100 x 2.1 mm (prototype)
Mobile phase: A) 0.1% TFA in water B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 15 or 35 min
%B 20% 65%
Flow rate: 0.5 mL/min , Temperature: 60 or 80 °C, Detection: UV@215 nm, Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin

Influence of gradient time

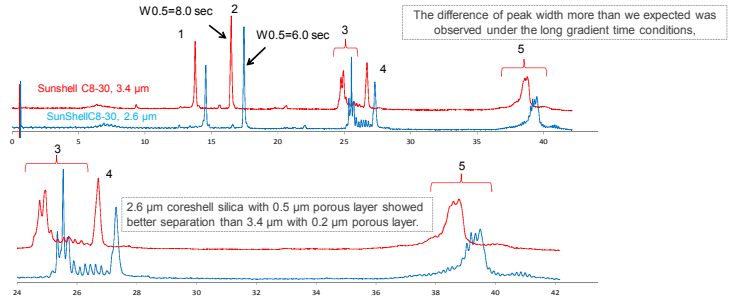


Figure 5. Comparison of chromatogram under long gradient time

Column: SunShell C8-30, 2.6 µm (30 nm, 0.5 µm layer) 150 x 2.1 mm, Sunshell C8-30, 3.4 µm (30 nm, 0.2 µm layer) 150 x 2.1 mm (prototype)
Mobile phase: A) 0.1% TFA in water B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 60 min
%B 20% 65%
Flow rate: 0.5 mL/min , Temperature: 80 °C, Detection: UV@215 nm, Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin

Influence of gradient time 2

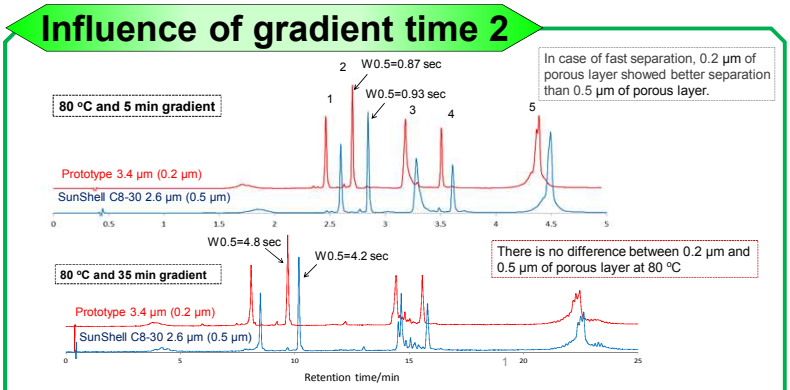


Figure 6. Comparison of chromatogram under short gradient time

Column: SunShell C8-30, 2.6 µm (30 nm, 0.5 µm layer) 100 x 2.1 mm, Sunshell C8-30, 3.4 µm (30 nm, 0.2 µm layer) 100 x 2.1 mm (prototype)
Mobile phase: A) 0.1% TFA in water B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 5 or 35 min
%B 20% 65%
Flow rate: 0.5 mL/min , Temperature: 80 °C, Detection: UV@215 nm, Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin

Which is better ?

Particle size: 2.6 µm
Thickness of porous layer: 0.5 µm
Specific surface area: 40 m²/g

Particle size: 3.4 µm
Thickness of porous layer: 0.2 µm
Specific surface area: 15 m²/g

It is said that thin layer of porous layer is suitable for separation of large biomolecules such as proteins. At more than 60 degree C, however, there is little difference of efficiency between 0.2 µm and 0.5 µm of porous layer.
Separation of proteins using 2.6 µm of particle and 0.5 µm of porous layer is better than one using 3.4 µm of particle and 0.2 µm of porous layer at 80 degree C and 35min gradient time because of a small particle.

Conclusions

- **0.5 µm thickness porous layer core shell silica showed better separation of proteins than 0.2 µm thickness porous layer core shell silica under the long time gradient condition.**
- **In fast separation, the peak shape for 0.2 µm porous layer core shell silica was better than 0.5 µm porous layer core shell**
- **It is important to choice the column which is fit to use for your purpose.**

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