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

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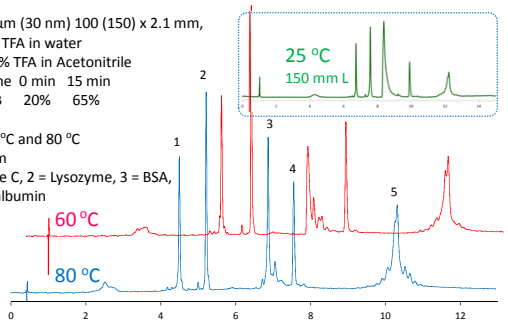
Abstract

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. 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 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.

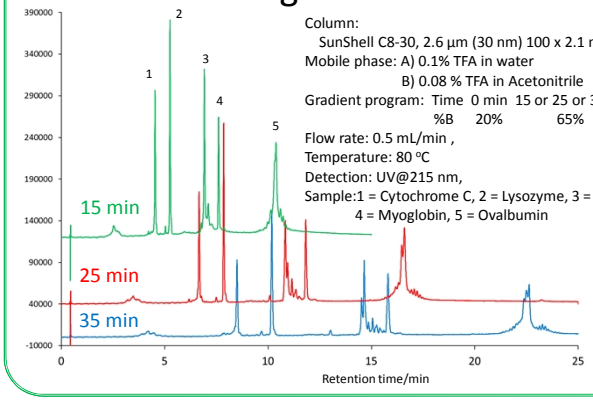
Effect of temperature

Column:
SunShell C8-30, 2.6 µm (30 nm) 100 (150) x 2.1 mm,
Mobile phase: A) 0.1% TFA in water
B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 15 min
%B 20% 65%
Flow rate: 0.5 mL/min
Temperature: 25 °C 60 °C and 80 °C
Detection: UV@215 nm
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin

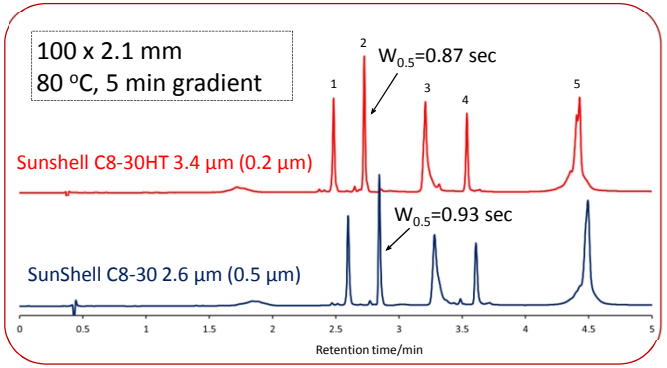


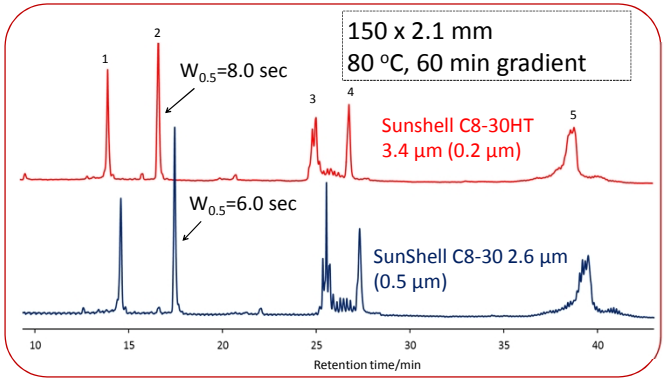
Effect of gradient time

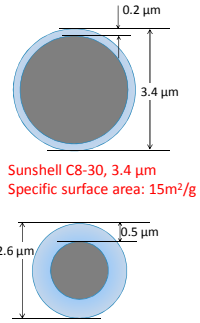
Column:
SunShell C8-30, 2.6 µm (30 nm) 100 x 2.1 mm,
Mobile phase: A) 0.1% TFA in water
B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 15 or 25 or 35 min
%B 20% 65%
Flow rate: 0.5 mL/min ,
Temperature: 80 °C
Detection: UV@215 nm,
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA,
4 = Myoglobin, 5 = Ovalbumin

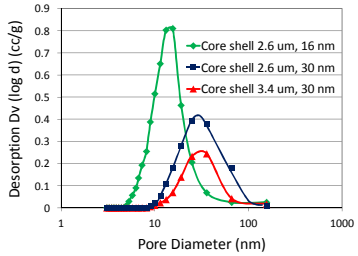


Comparison of thickness of porous layer









Pore size distribution

Column:
Sunshell C8-30HT, 3.4 µm (30 nm, 0.2 µm layer) 100 or 150 x 2.1 mm
SunShell C8-30, 2.6 µm (30 nm, 0.5 µm layer) 100 or 150 x 2.1 mm
Mobile phase: A) 0.1% TFA in water, B) 0.08 % TFA in Acetonitrile
Gradient program: Time 0 min 5 or 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

✓ In case of fast separation using 5 minute gradient program,
is better than

✓ In case of high resolution separation using 60 minute gradient program at 80 °C,
reversely is better than