Improvement of Separation of Monoclonal Antibodies Using Core-Shell Column

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Following low-molecular medicine, the antibody medicine group is attracting attention and recognized as the most important medicine due to its effectiveness. It is well known that size exclusion chromatography (SEC), hydrophobic Interaction chromatography (HIC) and reversed phase chromatography are used as a typical separation analysis method for monoclonal antibodies.
In this study, silica base material, pore diameter and stationary phase were compared in reversed phase chromatography. Although it is known that the core-shell silica column has a higher number of plate and higher efficiency than the totally porous silica column, the same result was obtained for the separation of the monoclonal antibodies. Although a packing material with 30 nm of pores has been frequently and satisfactorily used for a separation of proteins whose molecular weight was 100,000 or less, 100 nm of pore leaded a better separation of monoclonal antibodies whose molecular weight was around 150,000 to compare with 30 nm of pore. As a result, core-shell silica with 100 nm pore bonded with butyl group (C4) showed the best separation for monoclonal antibodies (IgG) which was obtained by cell culture and purified on a protein G affinity column.

Core shell particle for separation of peptides and proteins

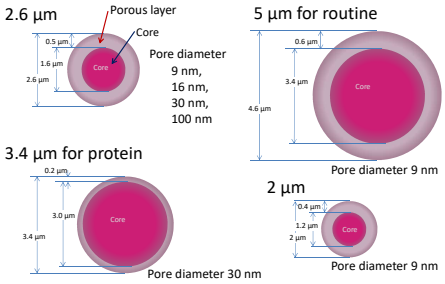


Figure 1. Schematic diagrams of core shell silica

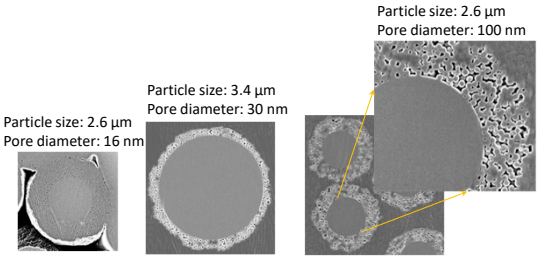


Figure 2. Electron micrograph of core shell silica

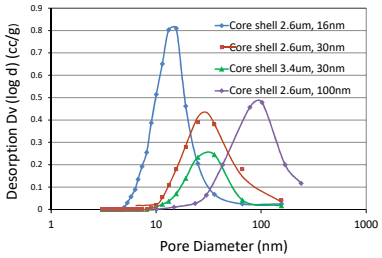


Figure 3. Pore size distribution of core shell silica

Separation of standard proteins

Table 1. Specification of used core shell columns

	Particle size	Core diameter	Thickness of porous layer	Pore diameter	Surface area	Carbon loading	Surface coverage	End-capping
SunShell C4-100 2.1 x 100 mm	2.6 µm	1.6 µm	0.5 µm	100 nm	22 m²/g	0.6%	3 µmol/m²	Yes
SunShell C4-30 2.1 x 100 mm	2.6 µm	1.6 µm	0.5 µm	30 nm	40 m²/g	0.9%	3 µmol/m²	Yes
SunShell C8-100 2.1 x 100 mm	2.6 µm	1.6 µm	0.5 µm	100 nm	22 m²/g	0.9%	2.5 µmol/m²	Yes
SunShell C8-30 2.1 x 100 mm	2.6 µm	1.6 µm	0.5 µm	30 nm	40 m²/g	1.2%	2.5 µmol/m²	Yes
SunShell C8-30HT 2.1 x 100 mm	3.4 µm	3.0 µm	0.2 µm	30 nm	15 m²/g	0.5%	2.5 µmol/m²	Yes

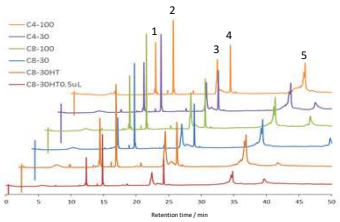


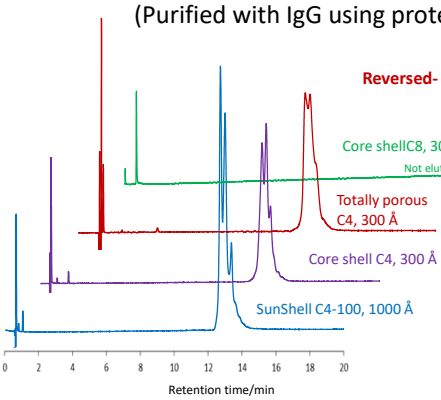
Figure 4. Comparison of C4 and C8 phases

Column: listed on the table 1
Mobile phase: A) 0.1% TFA in water
B) 0.1 % 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
Injection volume: 1.0 µL or 0.5 µL
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin
UHPLC instrument: HITACIHI Chromaster

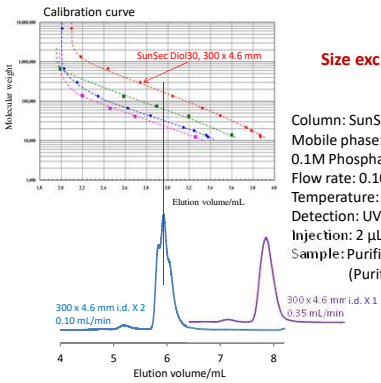
Table 2. comparison of peak width ($W_{0.5}$, min)

	C4-100	C4-30	C8-100	C8-30	C8-30HT	C8-30HT 0.5uL	Sample concentration
Cytochrome C	0.167	0.177	0.160	0.155	0.212	0.144	0.050%
Lysozyme	0.164	0.180	0.153	0.166	0.196	0.145	0.050%
BSA	0.308	0.410	0.276	0.514	0.422	0.330	0.100%
Myoglobin	0.197	0.221	0.180	0.199	0.238	0.176	0.050%
Ovalbumin	0.391	0.889	0.247	0.428	0.184	0.176	0.050%

Separation of purified antibody obtained using cell culture technique (Purified with IgG using protein G affinity column)



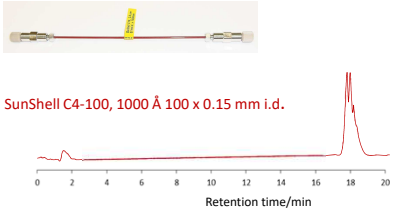
Column dimension: 100 x 2.1 mm i.d.
Mobile phase: A) 0.1% TFA in water
B) 0.085% TFA in acetonitrile
30-45%B (0-30 min)
Flow rate: 0.4 mL/min
Temperature: 80 °C
Detection: UV at 215 nm
Injection: 5 µL
Sample: Purified antibody obtained by cell culture
(Purified with IgG using protein G affinity column)



Size exclusion mode (SEC)

Column: SunSec Diol30, 4 µm 300 x 4.6 mm i.d. X 2
Mobile phase:
0.1M Phosphate buffer + 0.2 M NaCl (pH6.8)
Flow rate: 0.10 mL/min
Temperature: 25 °C
Detection: UV at 220 nm
Injection: 2 µL
Sample: Purified antibody obtained by cell culture
(Purified with IgG using protein G affinity column)

Figure 4. Separation of purified antibody obtained by cell culture using RP column and SEC column



Column dimension: 100 x 0.15 mm i.d.
Mobile phase: A) 0.1% TFA in water
B) 0.085% TFA in acetonitrile
30-45%B (0-15min)
Flow rate: 0.004 mL/min
Temperature: 80 °C
Detection: UV at 215 nm
Injection: 0.1 µL
Sample: Purified antibody obtained by cell culture
(Purified with IgG using protein G affinity column)

Figure 5. Separation of purified antibody obtained by cell culture using nano RP column

Conclusion

- A core shell silica with from 9 nm to 100 nm pore is available.
- C4 and C8 phase with 100 nm pore showed a better separation of standard proteins than those with 30 nm pore.
- In case of small sample injection, C8 with 0.2 µm thickness of porous layer and 30 nm pore showed good separation.
- Regarding reversed phase separation of monoclonal antibody (IgG), not only core shell C4 with 30 nm pore showed the better separation than totally porous C4, but also 100 nm of pore leaded the best separation.
- Nano column showed almost the same separation of IgG as semi-micro column.