# Development of Core Shell Particle with Large Pore for Separation of Peptides and Proteins

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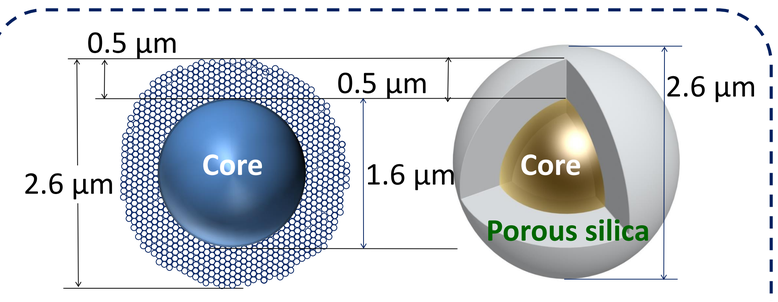
## Abstract

More than 10 kinds of core shell particle column have been available recently because core shell media offers significant improvements such as higher efficiency and lower pressure drop for existing HPLC operations without having to replace existing HPLC systems with UHPLC systems. Moreover, large molecules such peptides or proteins have been watched as medicine in recent years. Silica gel with 30 nm pores has generally been used for separation of proteins.

In this study, a 2.6 µm core-shell silica with a non-porous core approximately 1.6 µm in diameter and a superficially porous layer of 0.5 µm and 30nm pore was developed. As a novel bonding chemistry, hexa-functional C18 reagent with two sets of trichlorosilane was applied. It is considered that this reagent makes acidic stability high because of six positions of siloxane at most. Acidic stability was evaluated under 0.1 % formic acid solution/acetonitrile as a typical LC/MS mobile phase condition at 70 degree Celsius. It was confirmed that the hexa-functional C18 was stable for more than 1000 hours. The developed materials bonded with hexa-functional C18, C8 and C4 were evaluated to separate not only standards of peptides and proteins but also tryptic digest of a protein using UV and Mass spectrometry detectors.




Particle diameter: 2.6 µm, Core diameter: 1.6 µm, Thickness of porous silica: 0.5 µm, Pore volume: 0.30 mL/g, Specific surface area: 90 $m^2/g$ and 40 $m^2/g$, Pore diameter: 16 nm and 30 nm.

Figure 1. Schematic diagram of a core shell silica particle

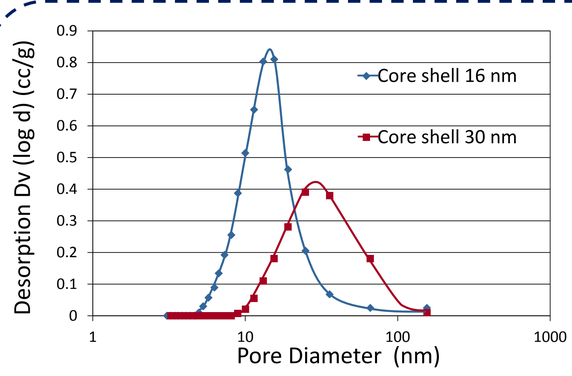



Figure 2. Pore distribution of core shell particle

Measurement : Nitrogen adsorption method

Table 1. Pore distribution of core shell particle

| | Pore diameter | Surface area | Carbon loading | Ligand Surface coverage | End-capping |
|---|---|---|---|---|---|
| SunShell C18-WP | 16 nm | 90 $m^2/g$ | 5% | 2.5 µmol/$m^2$ | Yes |
| SunShell HFC18-16 | 16 nm | 90 $m^2/g$ | 2.5% | 1.2 µmol/$m^2$ | Yes |
| SunShell HFC18-30 | 30 nm | 40 $m^2/g$ | 1.3% | 1.2 µmol/$m^2$ | Yes |
| Sunshell C18-16 | 16 nm | 90 $m^2/g$ | 2.5% | 1.2 µmol/$m^2$ | Yes |
| Sunshell C18-30 | 30 nm | 40 $m^2/g$ | 1.3% | 1.2 µmol/$m^2$ | Yes |
| Sunshell C8-30 | 30 nm | 40 $m^2/g$ | 1.2% | 2.5 µmol/$m^2$ | Yes |
| SunShell C4-30 | 30 nm | 40 $m^2/g$ | 0.9% | 3 µmol/$m^2$ | Yes |

Hexa-Functional C18 has six functional groups.
This HFC18 is much more stable under acidic condition.

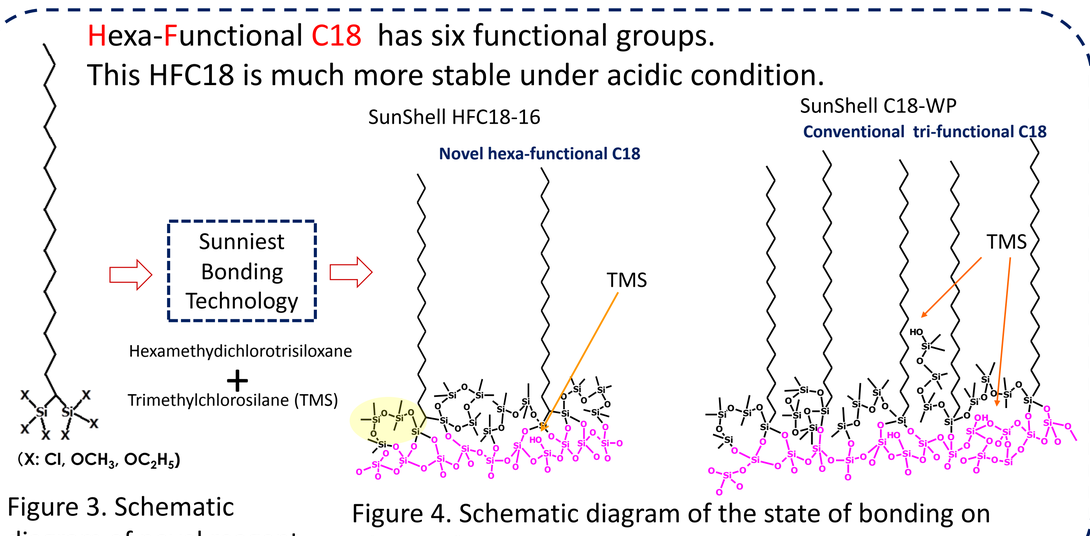



Figure 3. Schematic diagram of novel reagent

Figure 4. Schematic diagram of the state of bonding on silica surface

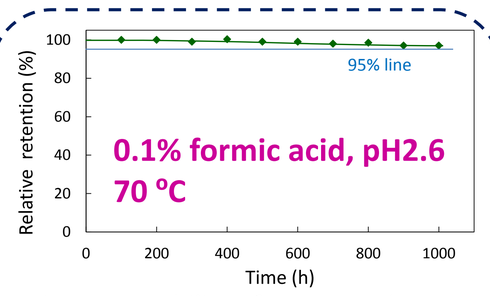



Figure 5. Stability of HFC18 under LC/MS mobile phase condition

Durable test condition
Column : SunShell HFC18-16 2.6µm, 50 x 2.1 mm
Mobile phase: $CH_3CN$/0.1% formic acid, pH2.6=40/60
Flow rate: 0.4 mL/min
Temperature: 70 °C

Measurement condition
Mobile phase: $CH_3CN/H_2O$=60/40
Flow rate: 0.4 mL/min
Temperature: 40 °C
Sample: 1 = Uracil, 2 = Butylbenzene

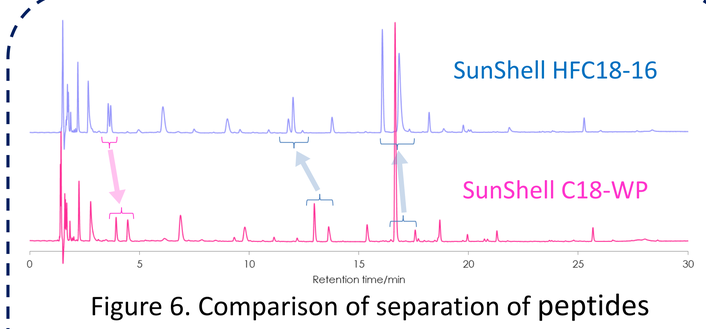



Figure 6. Comparison of separation of peptides

Column: SunShell HFC18-16, 2.6 µm (16 nm) 150 x 4.6 mm
SunShell C18-WP, 2.6 µ m (16 nm) 150 x 4.6 mm
Mobile phase: A) 0.1% TFA in Acetonitrile/water(10:90)
B) 0.1 % TFA in Acetonitrile
Gradient program:

| Time | 0 min | 5 min | 40 min |
|---|---|---|---|
| %B | 5% | 5% | 50% |

Flow rate: 1.0 mL/min , Temperature: 25 °C, Detection: UV@210 nm
Sample: Tryptic digest of cytochrome C

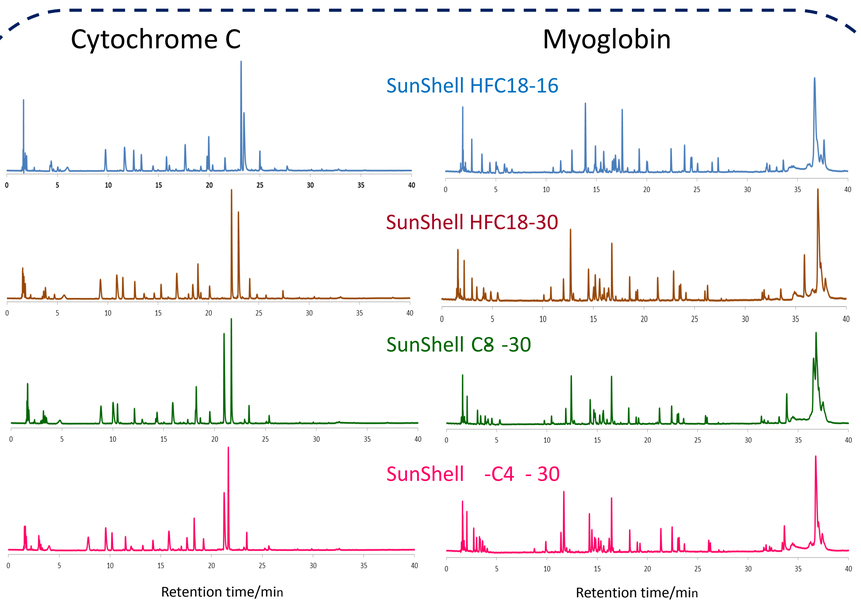



Figure 7. Separation of peptides

Column dimension: 150 x 4.6 mm
Mobile phase: A) 0.1% TFA in water, B) 0.1 % TFA in Acetonitrile
Gradient program: Time 0 min 3 min 40 min
%B 5% 5% 50%
Flow rate: 1.0 mL/min
Temperature: Ambient
Detection: UV@210 nm
Sample: Tryptic digest of cytochrome C and myoglobin

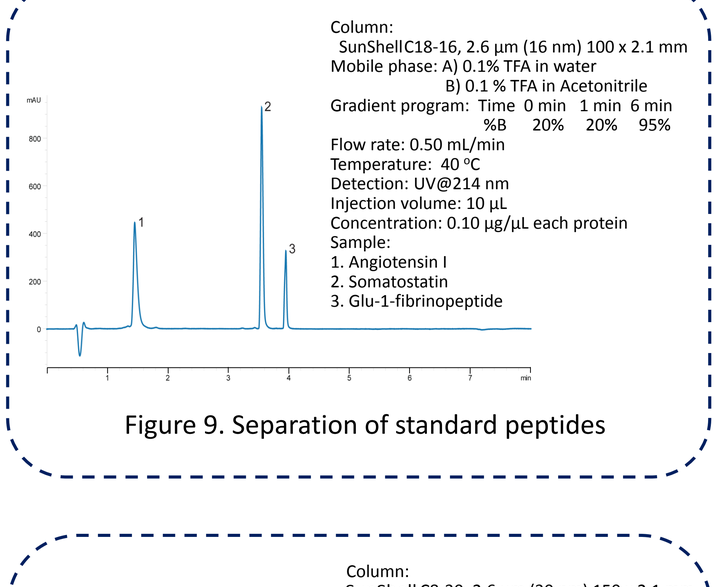



Figure 9. Separation of standard peptides

Column:
SunShell C18-16, 2.6 µm (16 nm) 100 x 2.1 mm
Mobile phase: A) 0.1% TFA in water
B) 0.1 % TFA in Acetonitrile
Gradient program: Time 0 min 1 min 6 min
%B 20% 20% 95%
Flow rate: 0.50 mL/min
Temperature: 40 °C
Detection: UV@214 nm
Injection volume: 10 µL
Concentration: 0.10 µg/µL each protein
Sample:
1. Angiotensin I
2. Somatostatin
3. Glu-1-fibrinopeptide

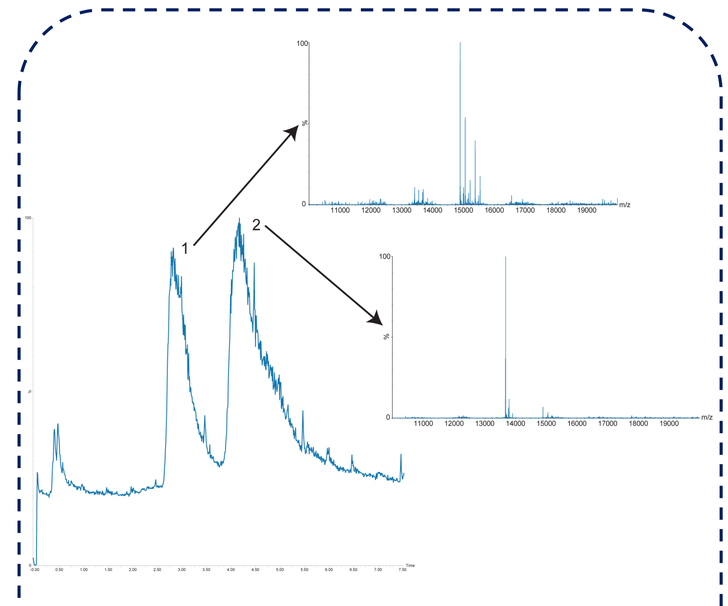



Figure 11. Separation of ribonuclease A/B

Column:
SunShell C8-30, 2.6 µm (30 nm) 100 x 2.1 mm
Mobile phase: A) 0.1% Formic acid in water
B) 0.1 % Formic acid in Acetonitrile
Gradient program: Time 0 min 10 min
%B 17.5% 17.5%
Isocratic separation
Flow rate: 0.50 mL/min
Temperature: 60 °C
Detection: MS,
Injection volume: 10 µL
Concentration: 0.10 µg/µL each protein
Sample:
1. Ribonuclease B
2. Ribonuclease A

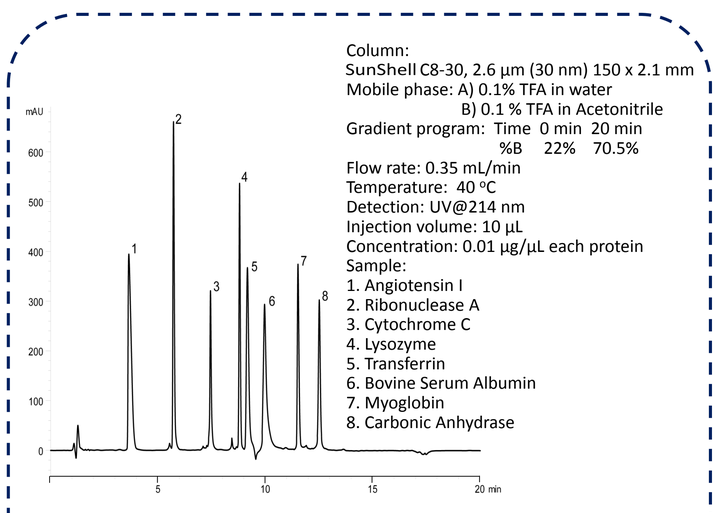



Figure 10. Separation of standard proteins

Column:
SunShell C8-30, 2.6 µm (30 nm) 150 x 2.1 mm
Mobile phase: A) 0.1% TFA in water
B) 0.1 % TFA in Acetonitrile
Gradient program: Time 0 min 20 min
%B 22% 70.5%
Flow rate: 0.35 mL/min
Temperature: 40 °C
Detection: UV@214 nm
Injection volume: 10 µL
Concentration: 0.01 µg/µL each protein
Sample:
1. Angiotensin I
2. Ribonuclease A
3. Cytochrome C
4. Lysozyme
5. Transferrin
6. Bovine Serum Albumin
7. Myoglobin
8. Carbonic Anhydrase

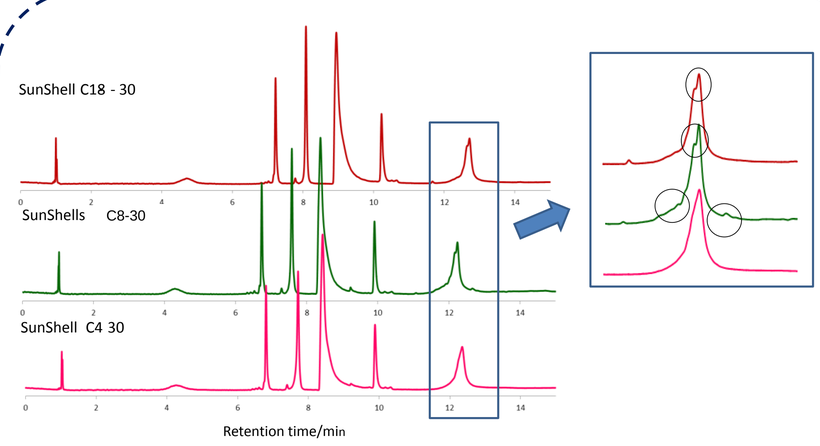
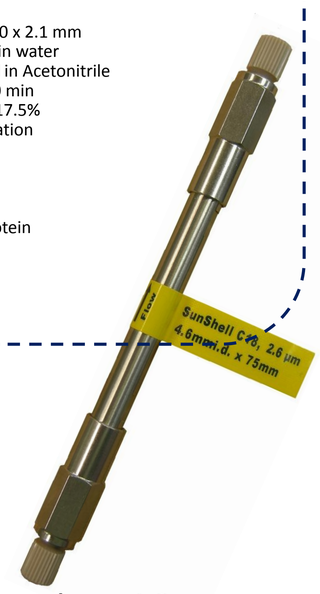



Figure 8. Separation of proteins

Column dimension: 150 x 4.6 mm
Mobile phase: A) 0.1% TFA in water, B) 0.1 % TFA in Acetonitrile
Gradient program: Time 0 min 15 min
%B 20% 65%
Flow rate: 1.5 mL/min
Temperature: Ambient
Detection: UV@210 nm
Sample:1 = Cytochrome C, 2 = Lysozyme, 3 = BSA, 4 = Myoglobin, 5 = Ovalbumin




## Conclusion

**Regarding* the pore size distribution, 30nm core shell particle was wider than 16 nm one.
*A novel Hexa-functional C18 phase showed not only high acidic stability for more than 1000 hours under typical LC/MS mobile phase condition but also different selectivity of peptides from a conventional tri-functional C18.
*30 nm C8 phase showed the best separation of ovalbumin in the three kinds of 30 nm phase.