크로마토그래피의 원리와 분석법

HPLC의 기본원리 -3

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Column Dimensions

Effect on chromatography

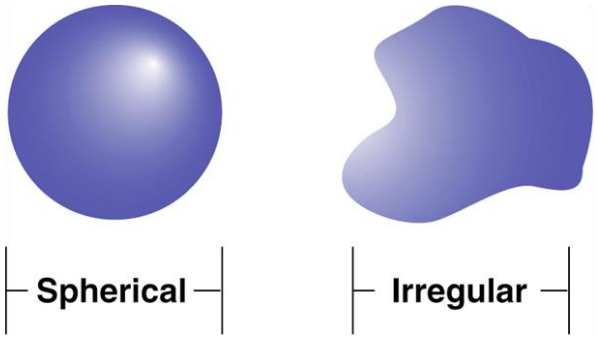
Column Dimension

- **Short** (30-50mm) - short run times, low backpressure
- **Long** (250-300mm) - higher resolution, long run times
- **Narrow** (≤ 2.1mm) - higher detector sensitivity
- **Wide** (10-22mm) - high sample loading

Particle Shape

Effect on chromatography

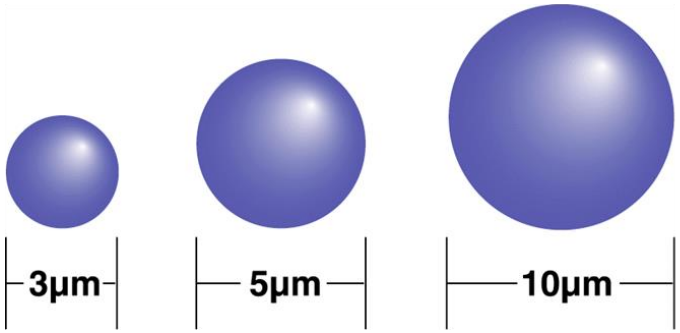
Spherical particles offer reduced back pressures and longer column life when using viscous mobile phases like 50:50 MeOH:H_2O.



Particle Size

Effect on chromatography

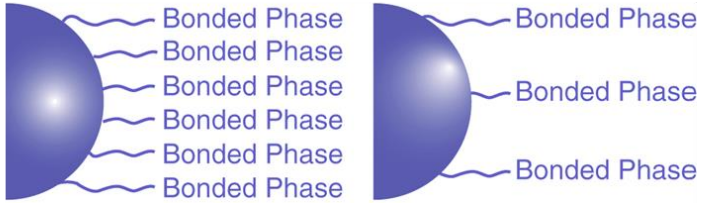
Smaller particles offer higher efficiency, but also cause higher backpressure. Choose 3µm particles for resolving complex, multi-component samples. Otherwise, choose 5 or 10µm packings.



Carbon Load

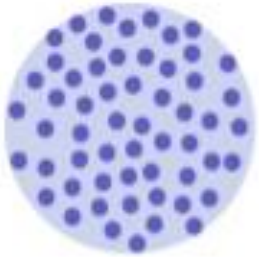
Effect on chromatography

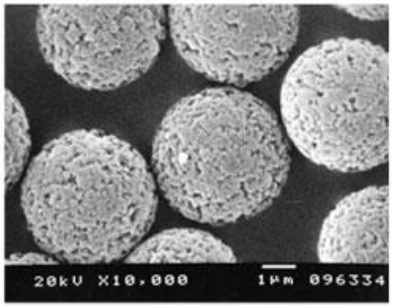
Higher carbon loads generally offer greater resolution and longer run times. Low carbon loads shorten run times and many show a different selectivity.

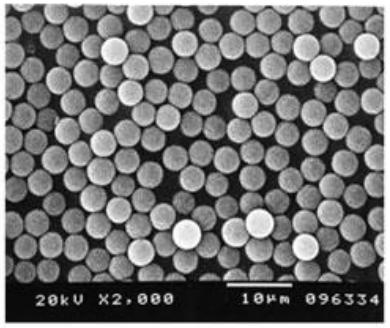


Pore Size-Effect on Chromatography

Larger pores allow larger solute molecules to be retained longer through maximum exposure to the surface area of the particles. Choose a pore size of 150Å or less for sample MW ≤ 2000. Choose a pore size of 300Å or greater for sample MW > 2000.

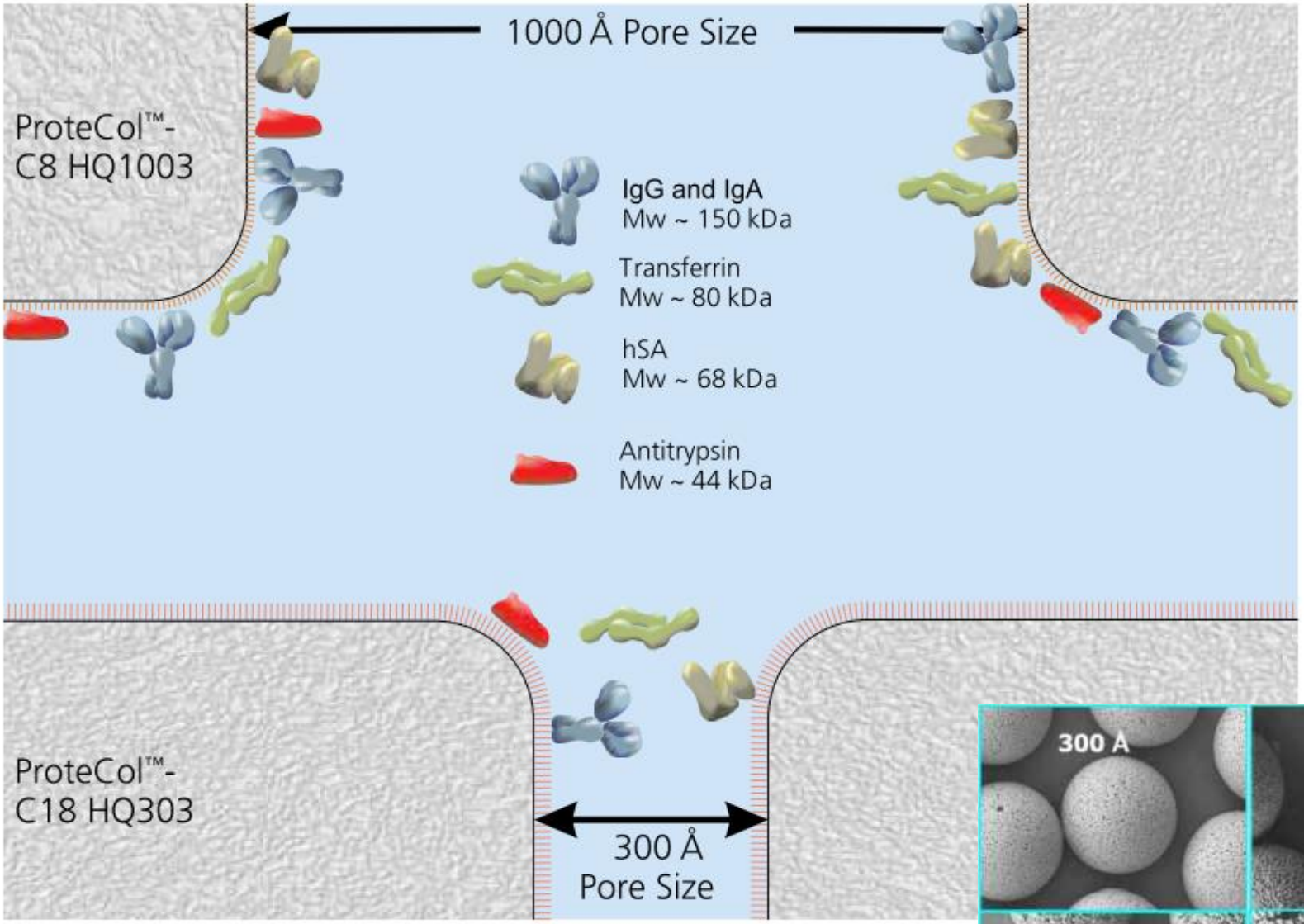






Pore Size	Spherical Analyte		Cylindrical Analyte	
	Lower	Upper	Lower	Upper
100	30	800	13	350
120	50	1,400	23	610
200	240	6,400	100	2,800
300	800	21,400	350	9,500
1000	29,400	800,000	13,000	350,000

Pore Size (Å) Lower and Upper ranges shown in Daltons (Da)



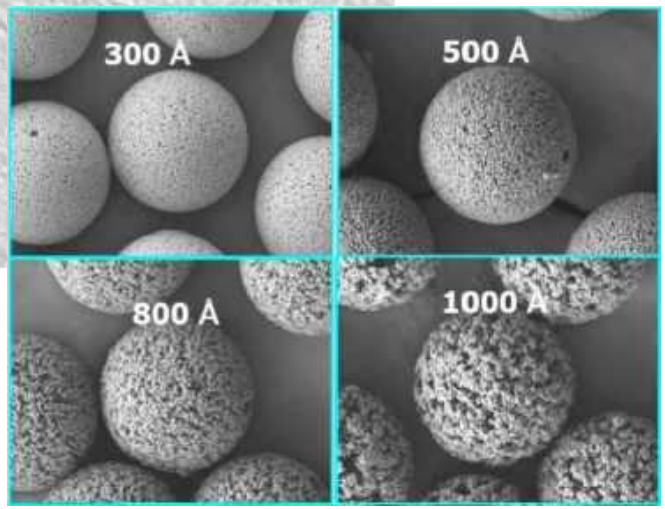
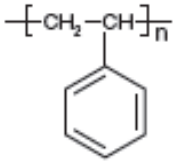


Figure 7: Schematic representation of the relative sizes of the five most abundant plasma proteins.

Correlation between pore size and retention of large molecules

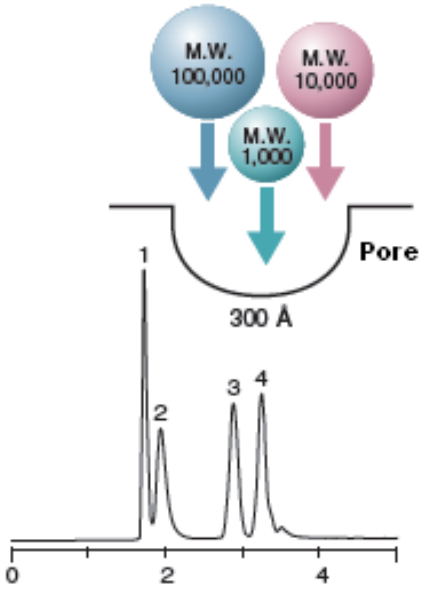
Conditions

Column : Inertsil series
normal phase column
(5 μm, 250 × 4.6 mm I.D.)
Eluent : THF
Flow Rate : 1.0 mL/min
Col. Temp.: Ambient
Detection : UV 254 nm
Analyte : 1. Polystylene Mp = 1,020,000
2. Polystylene Mp = 115,000
3. Polystylene Mp = 9,200
4. Polystylene Mp = 950

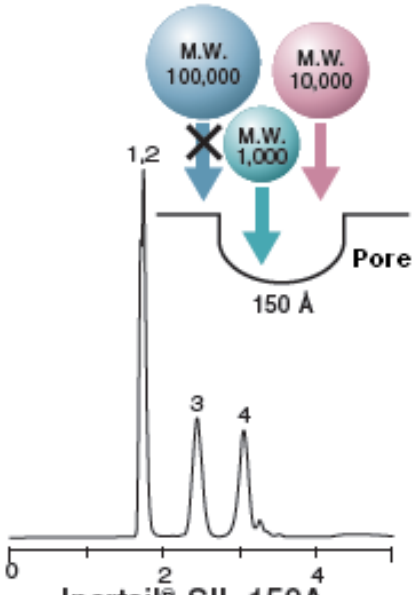


1. ~ 4. Polystylene

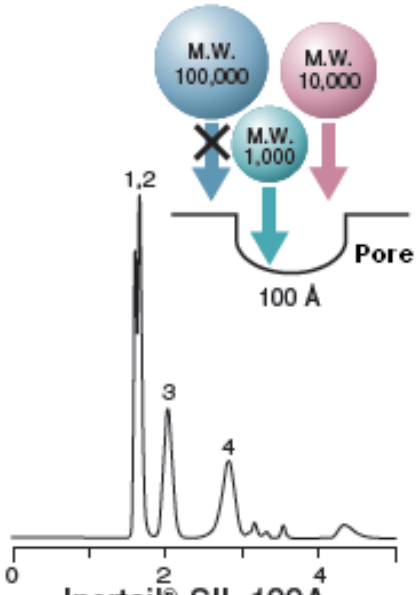
Silica gel's pores hold molecules on its inner surface.
Large molecules cannot enter small pores.



Inertsil WP300 SIL



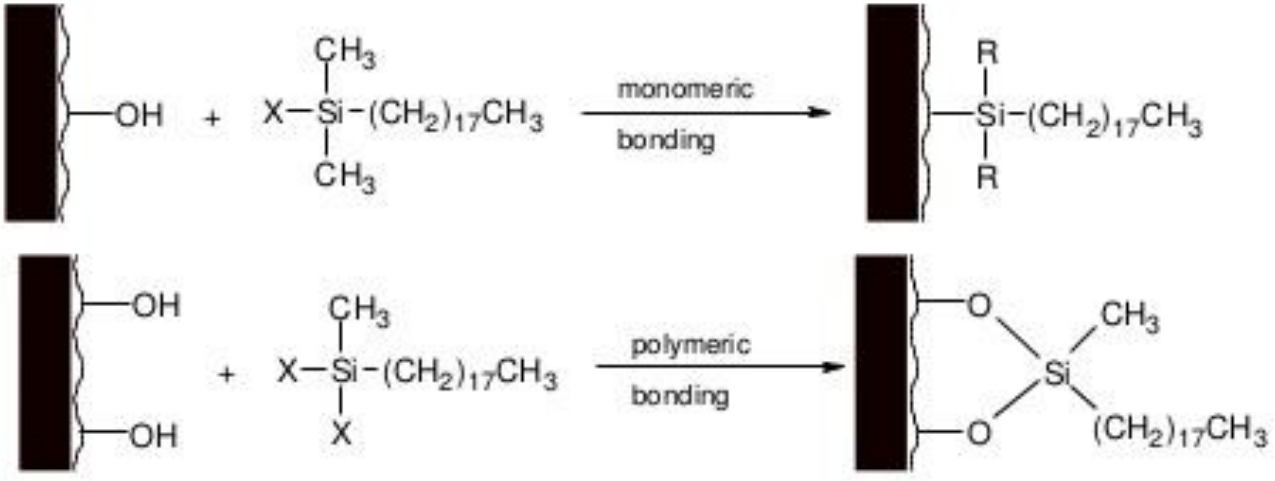
Inertsil® SIL-150A



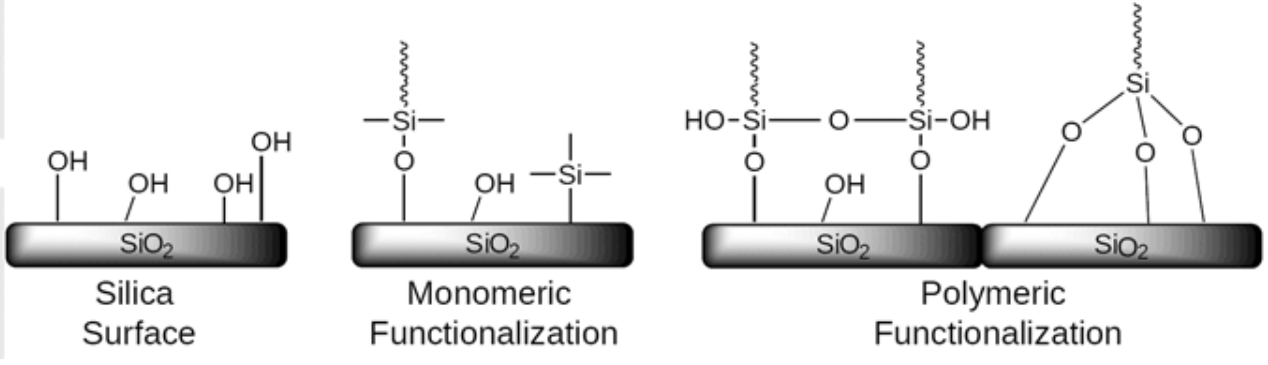
Inertsil® SIL-100A

Bonding Type-Effect on Chromatography

Monomeric bonding offers increased mass transfer rates, higher column efficiency, and faster column equilibration.



Polymeric bonding offers increased column stability, particularly when highly aqueous mobile phases are used. Polymeric bonding also enables the column to accept higher sample loading



Isocratic 과 Gradient 의 차이점

- Isocratic (등 용매이송) : 분석 시간 동안 용매의 조성이 변화 하지 않는 용매 이송 방식.

  (특징: 재 현 성이 매우 좋다)

  Eluent A: 20%(Start) – 20%(end), Eluent B: 80%(start) – 80%(end)

- Gradient(기울기 용매 이송) : 분석 시간 동안 용매의 조성 비율이 변하는 이송 방식.

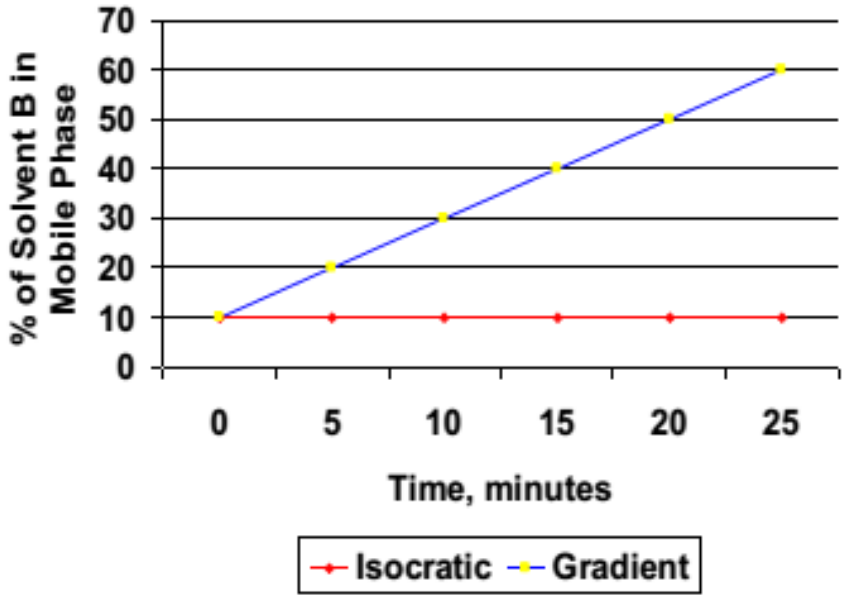
  (특징:분석 방법을 쉽게 찾을 수 있으나, 재현성이 매우 떨어진다)

  Eluent A: 20%(Start) – 80%(end), Eluent B: 80%(start) – 20%(end)

Gradient Mode는 분석 동안 용매의 조성을 바꾸어 줄 수 있을 뿐만 아니라, 시간에 따른 변화 및 그 형태 까지도 변화시킬 수 있다. → 분리도의 개선 효과

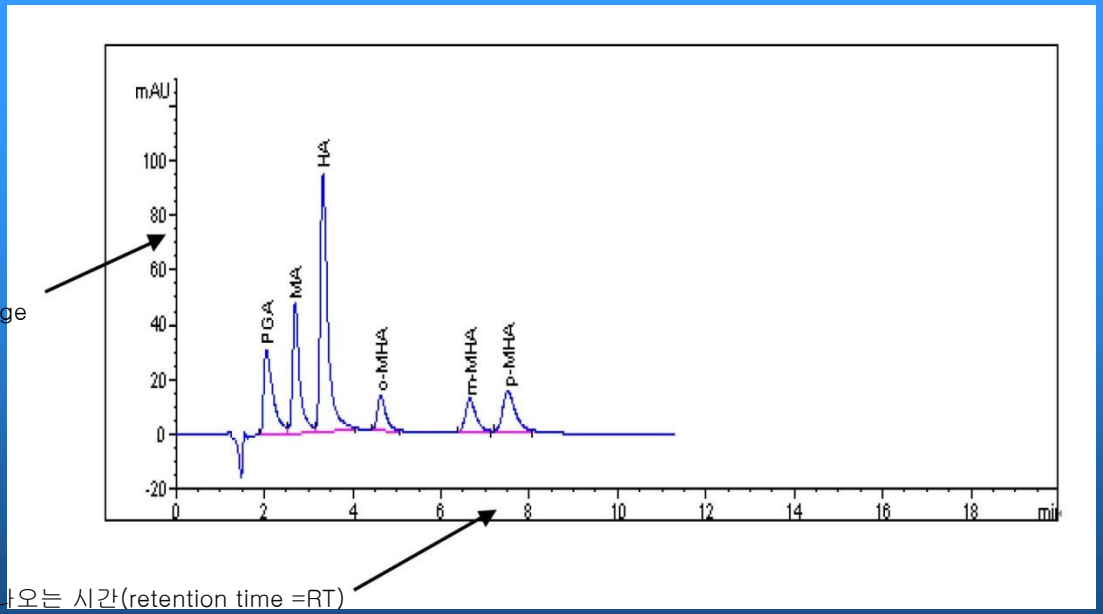
- In isocratic elution, peak width increases with retention time linearly with the number of theoretical plates. This leads to the disadvantage that late-eluting peaks get very flat and broad.
- Best for simple separations

- Gradient elution decreases the retention of the later-eluting components so that they elute faster, giving narrower peaks . This also improves the peak shape and the peak height
- Best for the analysis of complex samples



HPLC로 할 수 있는 일은 무엇인가요?

- 시료의 특성에 따라 정량, 정성 및 분취 가 가능 하다.
  - 정량은 크로마토그램의 Peak 면적을 이용하여 가능.
  - 정성은 크로마토그램의 Peak Retention time을 통하여 가능.
  - 많은 량의 시료를 고 순도의 단일 물질의 분리에도 많이 사용.



The End.