크로마토그래피의 원리와 분석법

HPLC의 기본원리 -2

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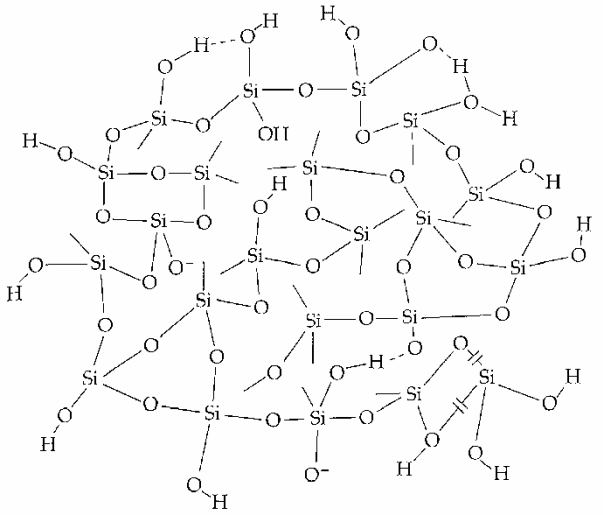
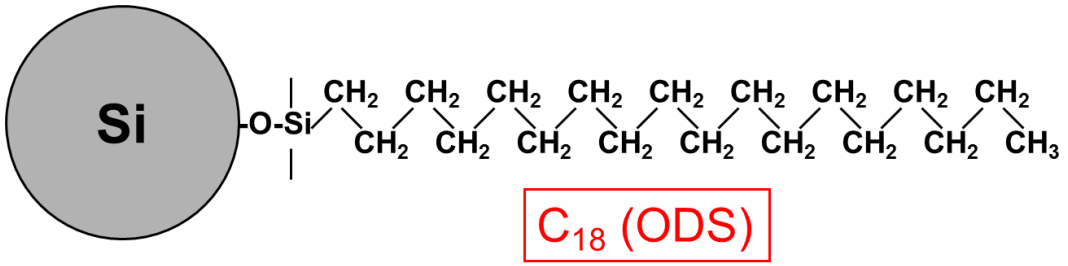
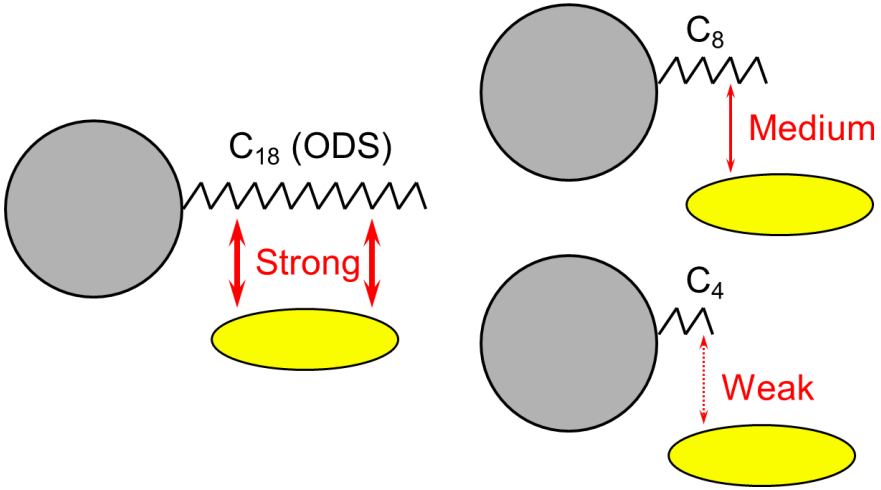
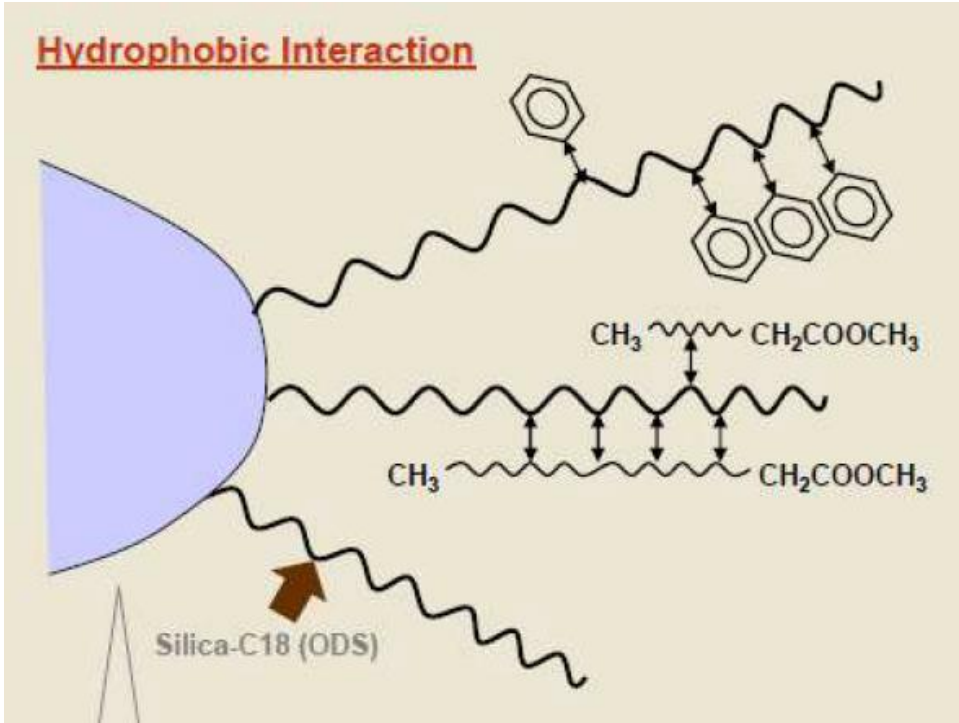
임 정 균 교수




Si
-O-Si
CH2 CH2 CH2 CH2 CH2 CH2 CH2 CH2 CH2
CH2 CH2 CH2 CH2 CH2 CH2 CH2 CH2 CH3
C18 (ODS)


C18 (ODS)
Strong
C8
Medium
C4
Weak


Hydrophobic Interaction
CH3
CH2COOCH3
CH3
CH2COOCH3
Silica-C18 (ODS)

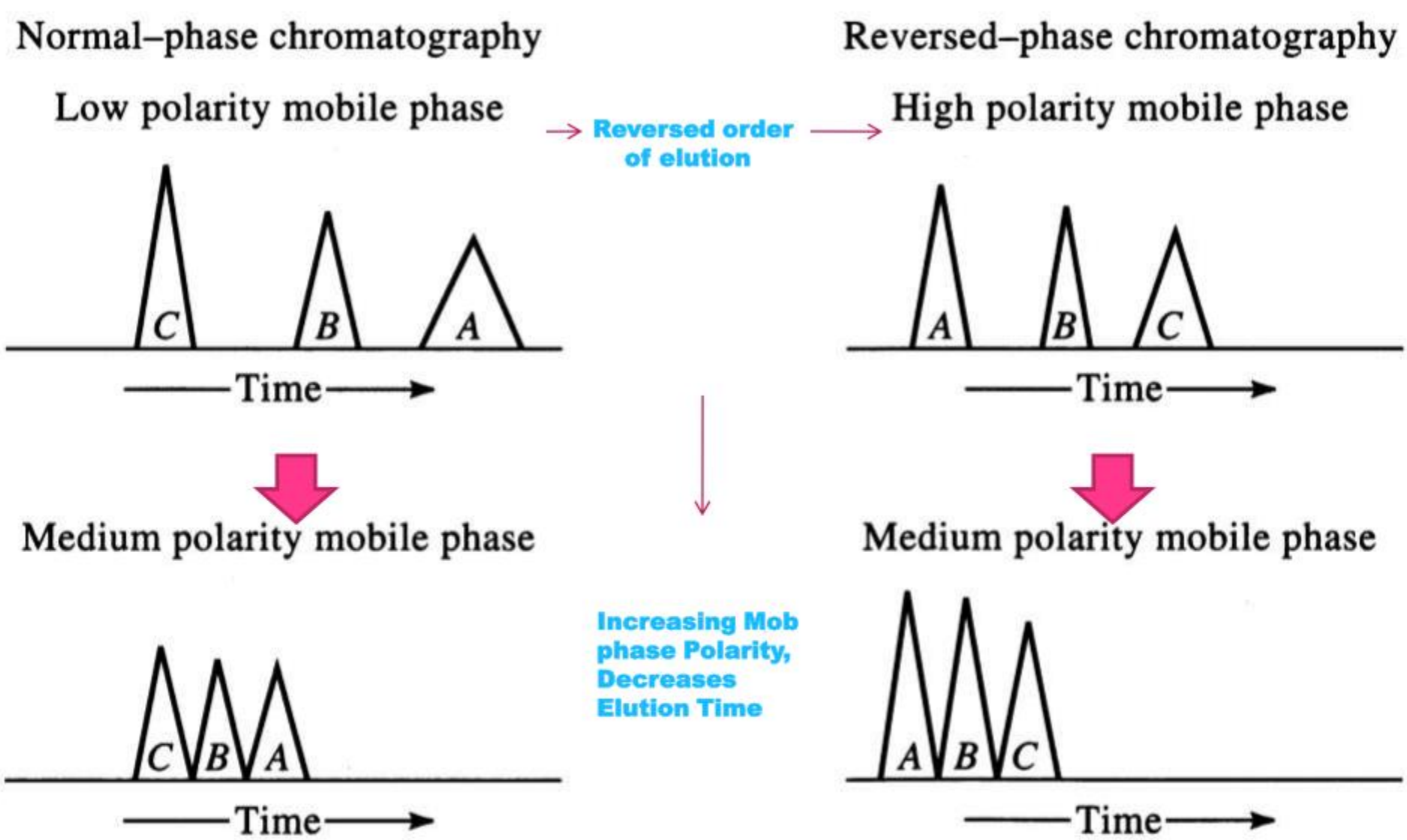


Figure 28-14 The relationship between polarity and elution times for normal-phase and reversed-phase chromatography.

HPLC Columns

Within the Column is where separation occurs.

Proper choice of column is critical for success in HPLC

Column dimensions in HPLC:

- **Analytical** [internal diameter (i.d.) 1.0 - 4.6-mm; lengths 15 – 250 mm]
- **Preparative** (i.d. > 4.6 mm; lengths 50 – 250 mm)
- **Capillary** (i.d. 0.1 – 0.5 mm; various lengths)
- **Nano** (i.d. < 0.1 mm, or sometimes stated as < 100 µm)

LC Columns - analytical

Column Particle Sizes:

- **7, 5, 3.5 (RR), & 1.8 um (RRHT)**

Materials of construction for the tubing

- **Stainless Steel** (the most popular; gives high pressure capabilities)
- **Glass** (mostly for biomolecules)
- **PEEK** polymer (biocompatible and chemically inert to most solvents)

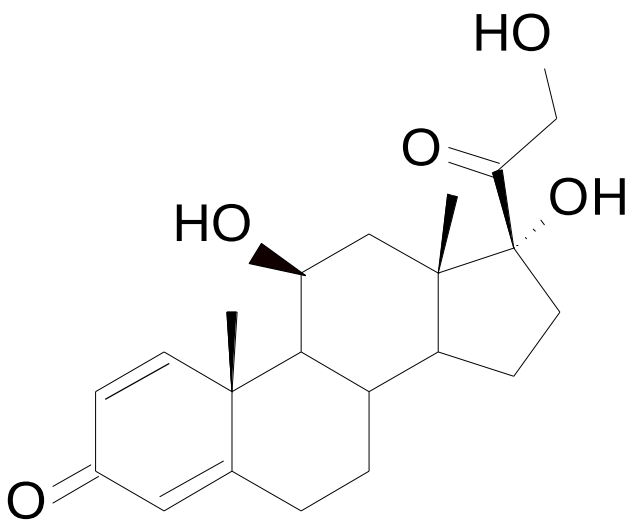
Column Selection Chart

Method Goals	Default Column (Good for most Applications)	High Efficiency	High Capacity	Low Backpressure	High Resolution	High Sample Loadability	Suitable for MW >2000	High Stability	High Sensitivity	Fast Analysis	Low Mobile Phase Consumption	Stability at pH Extremes	Fast Eqilibration
Particle Size													
small (3µm)		•			•								
medium (5µm)	•												
large (10µm)				•									
Column Length													
short (30mm)				•					•	•	•		•
medium (150mm)	•												
long (300mm)					•								
Column ID													
narrow (2.1mm)									•		•		
medium (4.6mm)	•												
wide (22.5mm)						•							
Surface Area													
low (200m²/g)	•									•			•
high (300m²/g)			•		•	•							
Pore Size													
small (60Å)			•		•								
medium (100Å)	•												
large (300Å)							•						
Carbon Load													
low (3%)										•			
medium (10%)	•												
high (20%)			•		•	•							
Bonding Type													
monomeric	•	•											
polymeric			•			•		•				•	
Particle Shape													
spherical	•	•		•				•					
irregular			•										

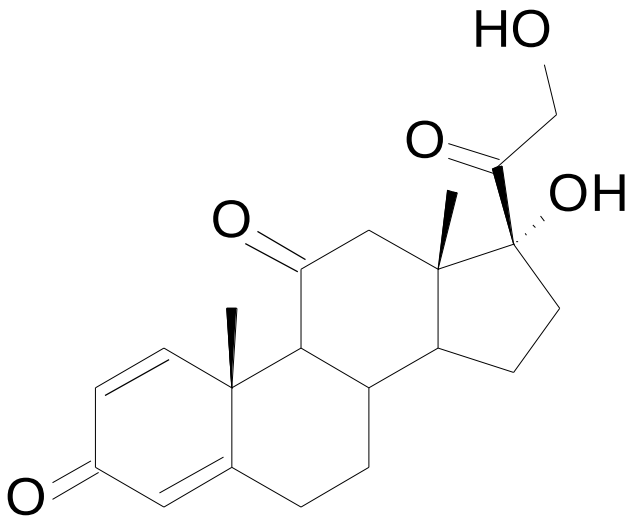
Choosing the Bonded Phase

Draw the molecular structures for all known components of the mixture. Identify the two compounds whose structures are the most similar.

e.g.:



Prednisolone

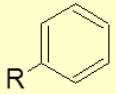
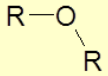
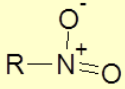
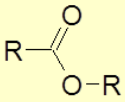
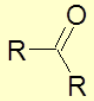
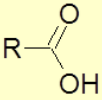


Prednisone

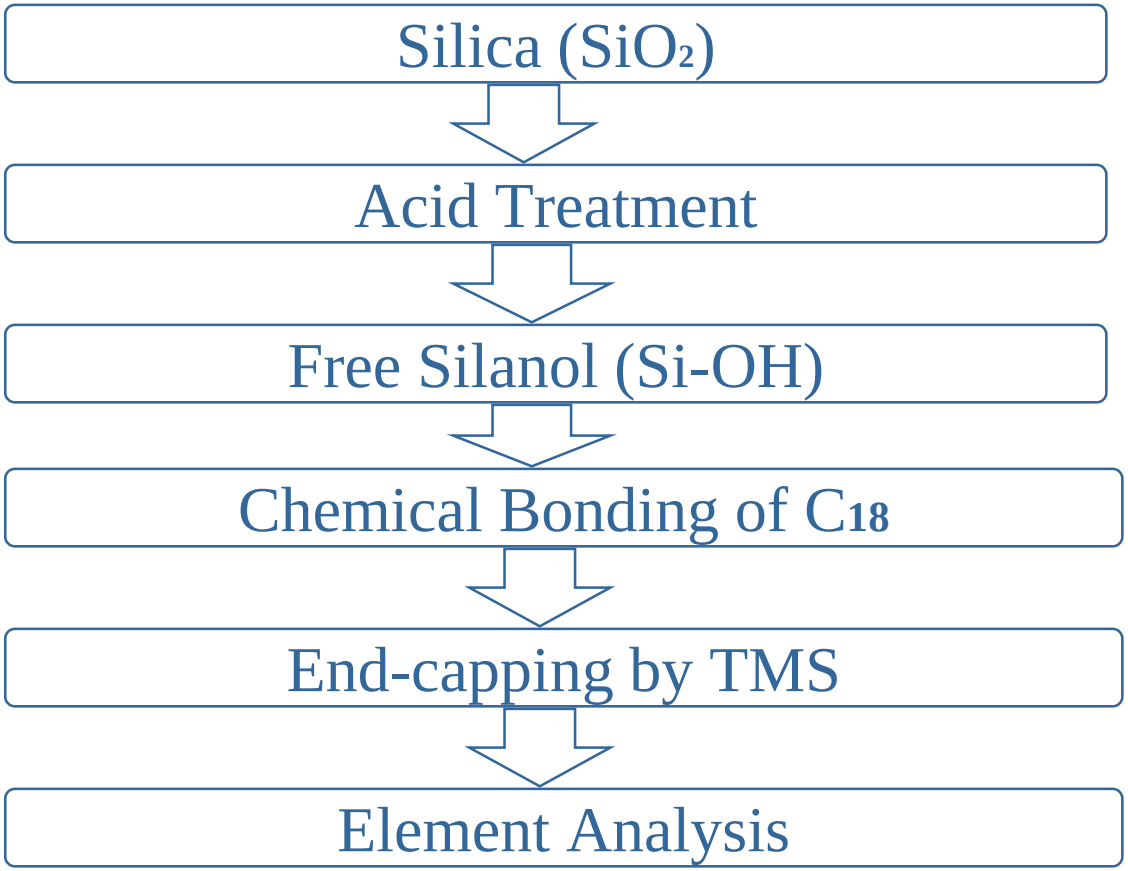
Use the results of the structural comparison to select a bonded phase showing optimal selectivity for these two molecules. In this case consider using a silica column (no bonded phase) for its ability to retain polar solutes through hydrogen bonding.

Quiz: 둘 중에 어느게 더 polar할까?

Functional Group Polarity Comparisons

Polarity	Functional Group	Structure	Bonding Types	Intermolecular Forces Displayed
Low	Methylene	$R-(CH_2)_2-$	σ	London
	Phenyl		σ , π	London
	Halide	R—F, Cl, Br, I	σ	London, Dipole-Dipole
	Ether		σ	London, Dipole-Dipole, H-bonding
	Nitro		σ , π	London, Dipole-Dipole, H-bonding
	Ester		σ , π	London, Dipole-Dipole, H-bonding
	Aldehyde		σ , π	London, Dipole-Dipole, H-bonding
	Ketone		σ , π	London, Dipole-Dipole, H-bonding
	Amino	$R-NH_2$	σ , π	London, Dipole-Dipole, H-bonding, Acid-base chemistry
	Hydroxyl	R—OH	σ	London, Dipole-Dipole, H-bonding
High	Carboxylic Acid		σ , π	London, Dipole-Dipole, H-bonding, Acid-base chemistry

제조방법


Silica (SiO_2)
Acid Treatment
Free Silanol (Si-OH)
Chemical Bonding of C_{18}
End-capping by TMS
Element Analysis

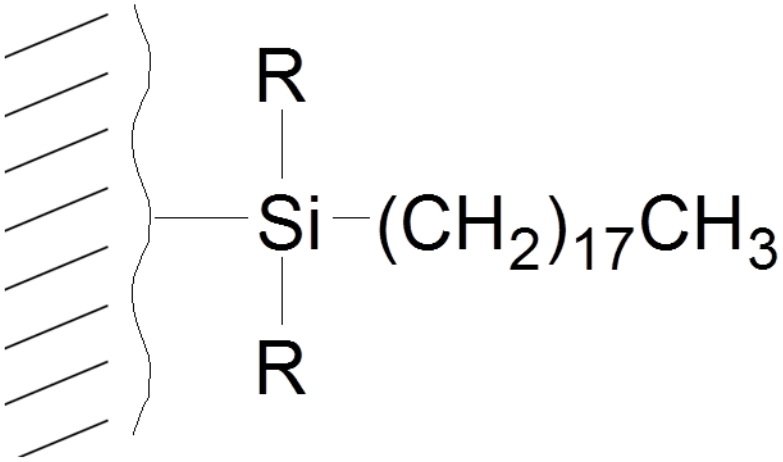
Choosing the Bonded Phase

Examples of bonded phases used for HPLC packing media:

C18 or Octadecylsilane (ODS)

Very nonpolar - Retention is based on London (dispersion) interactions with hydrophobic compounds.

Example Alltech Phase: Alltima™ C18

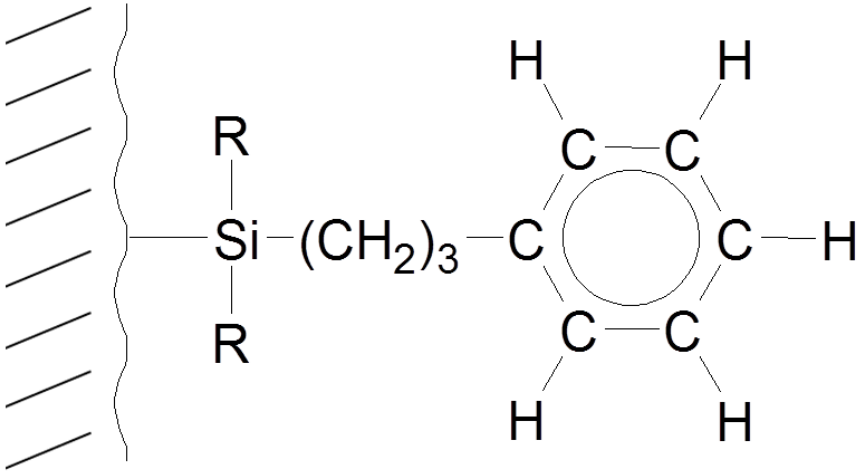


Choosing the Bonded Phase

Phenyl

Nonpolar - Retention is a mixed mechanism of hydrophobic and π - π interactions.

Example Alltech Phase: Platinum™ Phenyl

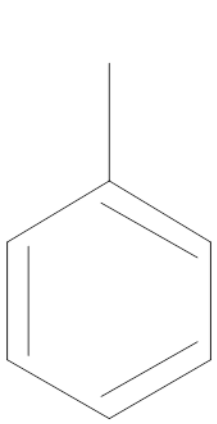


Choosing the Bonded Phase

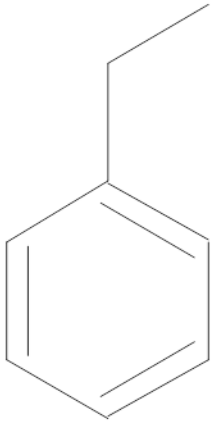
Each bonded phase has unique selectivity for certain sample types.

As a practical example, to separate toluene and ethyl benzene:

- Note a difference of one -CH_2- unit
- Choose a C18 bonded phase for retention by hydrophobicity
- Maximize hydrophobic selectivity with a high silica surface area, high carbon load material like Alltima C18

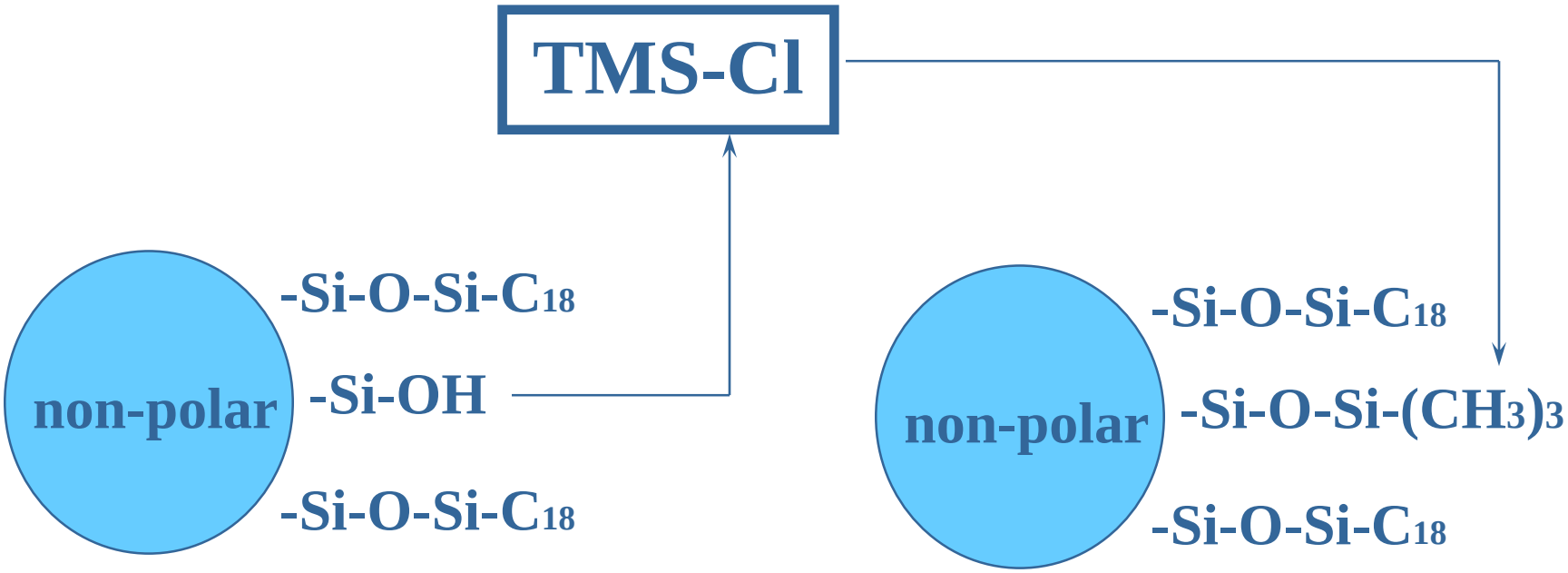


Toluene

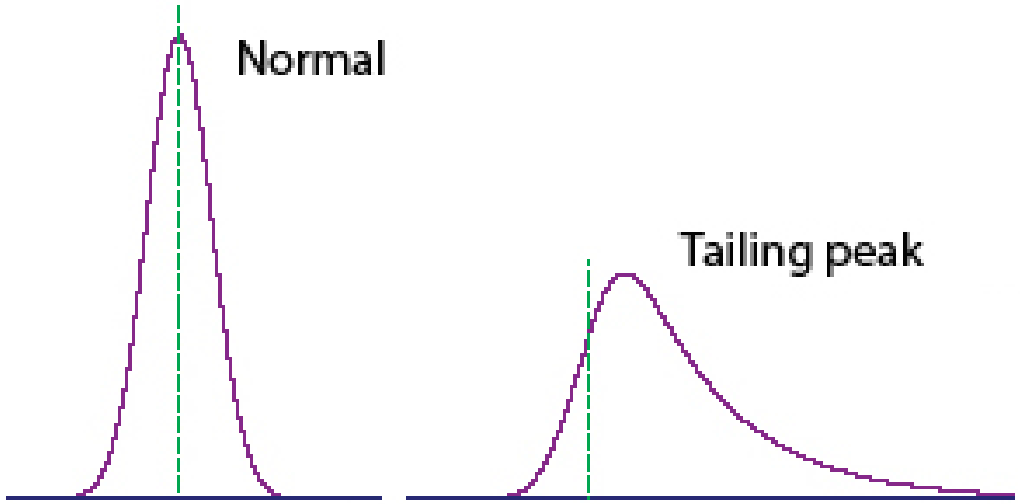


Ethyl Benzene

End-capping이란?



- end-capping을 하면 -NH2와 같은 염기성 시료의 분석시 문제가 되는 peak의 tailing현상을 줄일 수 있다.
- http://webbook.nist.gov/cgi/cbook.cgi?ID=C60275&Mask=400#UV-Vis-Spe
- 왜냐하면 SiOH의 분자구조를 가지고 있으면 산으로 작용하여 SiO-형태의 음이온구조를 형성하게된다. 이 음이온은 중성의 mobile phase를 사용하여 분석을 할때 염기성시료와의 상호작용으로 말미암아 peak broading이나 tailing의 효과를 형성하게 된다.
- 이러한 작용은 염기성시료를 분석할때 많은 제약을 가져오게 되어 현재는 많은 컬럼에서 end-capping을 하고 있다.


Normal
Tailing peak

The End.