



# HPLC

- (data analysis system)
  - (mobile phase reservoir and degasser)
  - (pump)
  - (injector)
  - (column)
  - (detector)
- HPLC
- HPLC가
- HPLC가
- HPLC
- (mobile phase reservoir and degasser)

- (data analysis system)
- 1. (mobile phase reservoir and degasser)
- 1.1 Frit
- (reservoir)
- 1-1 (frit)
- 가
- stainless(sus), hastalloy C, PP (polypropylene), glass
- stainless(sus) : 가 porosity
- 가 3 $\mu$ m
- hastalloy C : ,
- 가 가 .
- PP, glass : 가
- ion chromatography
- glass PP
- bio
- sus
- porosity가 10 $\mu$ m
- 1.2 (degasser)
- gas pump

(RT)  
 가  
 , helium gas purging, degasser 1.3  
 : (0.45 $\mu$ m)  
 gas  
 :  
 helium gas purging : 1  
 helium purging : 10  
 가  
 pH가 polar  
 1 가 vent  
 가 helium  
 purging  
 가 vent line  
 (degasser) :  
 membrane 가  
 option

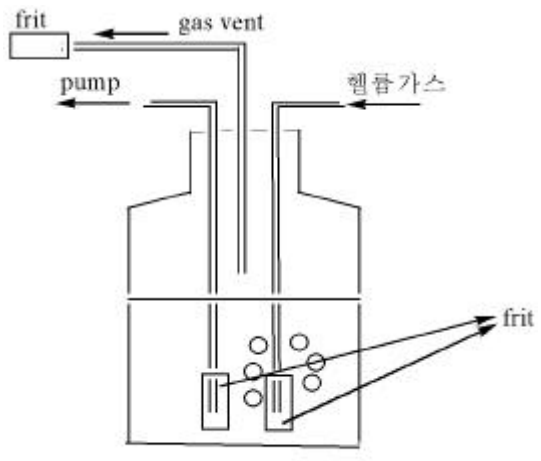


그림1 이동상 저장통의 구조

(volume : volume)  
 : =  
 6:4 600M $\ell$ , 400M $\ell$   
 가  
 organic solvent 5:5  
 pump 가  
 pH 가  
 buffer pH buffer  
 organic solvent pH  
 pH pH  
 pH meter  
 pKa pH  
 pH RT (  
 pH meter  
 1-1 1-4 buffer  
 가  
 pH  
 buffer  
 pH pH  
 meter pH  
 1-1 1-4  
 pH meter  
 1-1 1-4  
 pH가 pH  
 2. Pump  
 Pump HPLC  
 pump

1- 1. pH			
pH	가	(Mℓ)	(Mℓ)
2.0	565		435
2.2	455		545
2.4	345		655
2.6	250		750
2.8	175		825
3.0	110		890
3.2	55		945

가) : 0.1M  
 ) : 0.1M  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  13.6g 1

1-3. Citrate pH			
pH	가	(Mℓ)	(Mℓ)
3.0	930		70
3.2	870		126
3.4	810		190
3.6	750		260
3.8	700		300
4.0	660		340
4.2	610		370
4.4	560		440
4.6	510		490
4.8	460		540
5.0	410		590
5.2	360		640
5.4	320		680
5.6	270		726
5.8	230		764
6.0	190		810
6.2	140		856
6.4	60		940
6.6	40		960
6.8	30		970
7.0	15		985

가) : 0.1M Citric acid(21.0g 1 )  
 ) : 0.1M Sodium citrate  $\text{C}_6\text{H}_5\text{ONa} \cdot \text{H}_2\text{O}$   
 (29.4g 1 )

1- 2. pH			
pH	가	(Mℓ)	(Mℓ)
3.6	926		74
3.8	880		120
4.0	820		180
4.2	736		264
4.4	610		390
4.6	510		490
4.8	400		600
5.0	296		704
5.4	210		790
5.6	176		824

가) : 0.1M (6.0g 1 )  
 ) : 0.1M (8.2g 1 )  
 0.1M 3 (13.6g 1 )

1- 4. pH			
pH	가	(Mℓ)	(Mℓ)
5.6	948		52
5.8	920		80
6.0	877		123
6.2	815		185
6.4	735		265
6.6	685		315
6.8	510		490
7.0	390		610
7.2	280		720
7.4	190		810
7.6	130		870
7.8	85		915
8.0	33		947

가) : 0.1M  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  13.6g 1  
 ) : 0.1M  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  26.8g 1  
 ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  35.9g 1 )

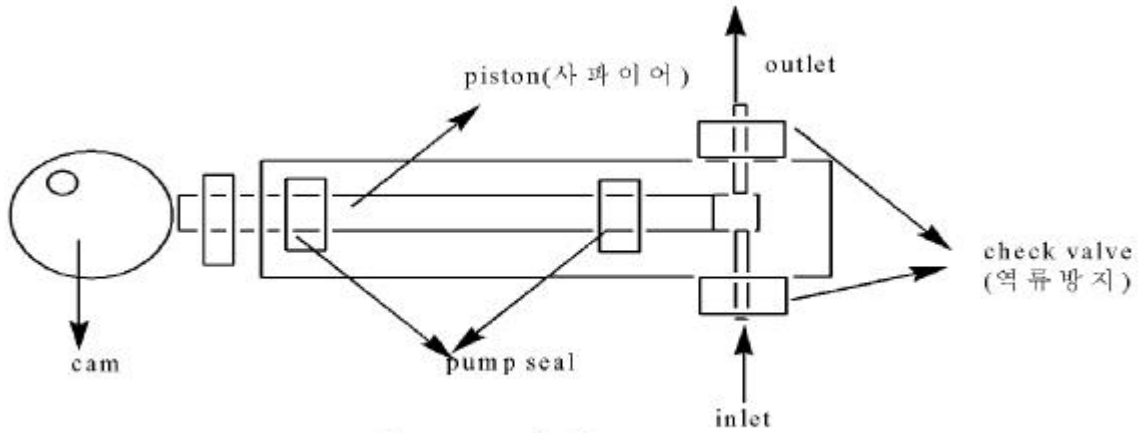


그림 2 pump의 구조

2 pump head, (piston: ), pump head sealing sea-ling , transducer( ), outlet check valve, purging system . pump single , binary , quaternary .

prep type : preparation sample 10Ml/min

analytical type : 0.5Ml- 2Ml/min 가

2.1 pump single : pump 가 (isocratic) binary : pump gradient . quaternary : pump 4 binary gradient 가 .

$\mu$ -LC type, nano-LC type : capillary pump 200nl- 30 $\mu$ l/min MS proteomics

pump sample 가 MS 가 analytical binary pump semi narrow bore 0.2Ml/min quate- rinary pump 0.4-0.5Ml/min 가 semi (0.2Ml/min)

2.2 Pump prep type, analytical type,  $\mu$ -LC type, nano-LC type .

3. (Injector autosampler)

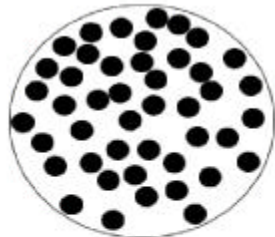
switching System

HPLC 가

Sample loading : sample port loop( ) loading (

switching, system  
sample

3a)  
Sample injection : port valve  
on/off loop  
( 3b)



1) Silica sol

injector sample loading  
loop loop  
sample injection  
3 injector sample



2) 세척과정



3) 최종

● :  $Na_2SO_4$  (분자)

4 switching system  
pump injection port  
sample system

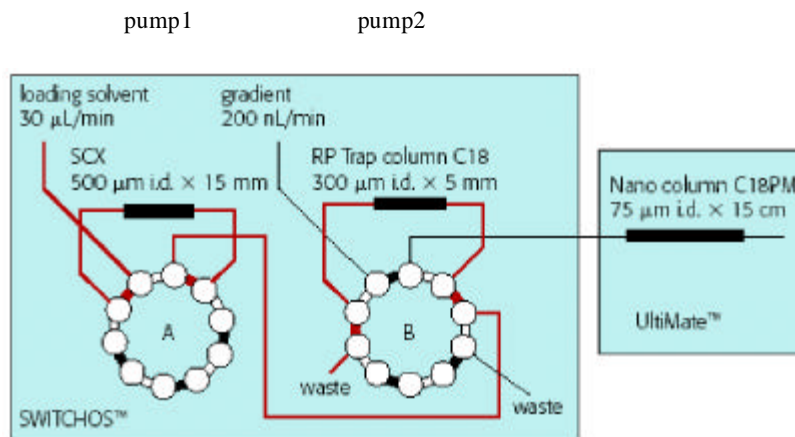
(SCX)

(RP trap column  $C_{18}$ )

data 가 sample  
, 가

4 2-D (2-dimensional) LC system

Fig.3 Silica gel의 pore 생성과정



4. proteomics switching valve 2  
column switching system

pump muticolumn swi-  
 tching multidimensional LC  
 SPE(solid phase extraction) sys- tem,  
 method development system system  
 Autosampler injector  
 system dilution  
 system, autosam-  
 per  
 4. (Column)  
 analytical  
 4.1  
 1)  
 2  
 pump sem-  
 analytical analytical  
 tube, detector cell  
 ( 4.6- 3.0mm)  
 semianalytical(2.0- 1.0mm)  
 peak가  
 injection  
 pump  
 가  

$$= \left( \frac{\text{가}}{4.6\text{mm}} \right) \times \left( \frac{\text{가}}{3.0\text{mm}} \right)^2$$

$$/ \left( \frac{\text{가}}{2\text{mm}} \right)^2$$
 50%  
 2 가  
 75% 가  
 2)  
 3  
 stainless PEEK  
 Pt  
 peak  
 2.  
 (type)

analytical	3mm- 4.6mm	3- 25cm	0.5- 2Mℓ min	
preparative	2cm	10cm	10Mℓ min	
semianalytical	1- 2mm	15cm,25cm	0.05- 0.25Mℓ min	1) mass
μ -LC	0.2- 0.5mm	50- 250mm	1- 25μℓ min	mass proteomics
nano-LC	75μm	10- 100mm	200 n /min	mass proteomics

\* 가 injection  
 가 가 sample 가 mass

3.

stainless	가	HCl
PEEK	poly ether ether ketone tography	5000psi
glass		(100psi)

4.

	reverse, normal phase ( , reverse phase )
	Ion ( , )
GFC (gel filtration column)	(size exclusion column : SEC )
Chiral	
Biological	biochromatography protein, peptide biomolecules

3)

4	GFC (size exclusion column) pore size
	(reverse phase :
RP) (normal phase) hydrophobic( ) (hydrophilic)	Chiral
(hydrophilic), hydrophobic( )	Biological proteomics pore size
	functional
	4) (stationary phase)
(IC) ion	(stationary phase)
(cation : SCX)	(anion : SAX) 가 .( 5)

5.

silica base	pore size 가		pH가	silica	functional group pH
polymer base	polystyrene pH	divinylbenzene	cross link		polymer
	silica				
	pH silanol 가	가	3-8	1) Silica base	(stationary phase)
tailing 가 1-13			pH		
polymer pH가				silica	2 silica sol silica sol silica
graphite carbon	, Alumina	, zirconium		(fig.3)	mass
5) Guard	guard cartridge			transfer가	
	guard			silica	functional
guard cartridge guard : 가					polysiloxane func-
guard cartridge : kit					silica base
cartridge		ca-			
tridge		dead volume		6 silica	37가
peak				silanol	free silanol, geminal silanol, vicinal free silanol
fitting	guard cartridge			functional	silanol silica chlo- ride C <sub>18</sub> silicon- oxygen-carbon chloro- silane functional (stationary phase)
4.2 Silica base	(stationary phase)				



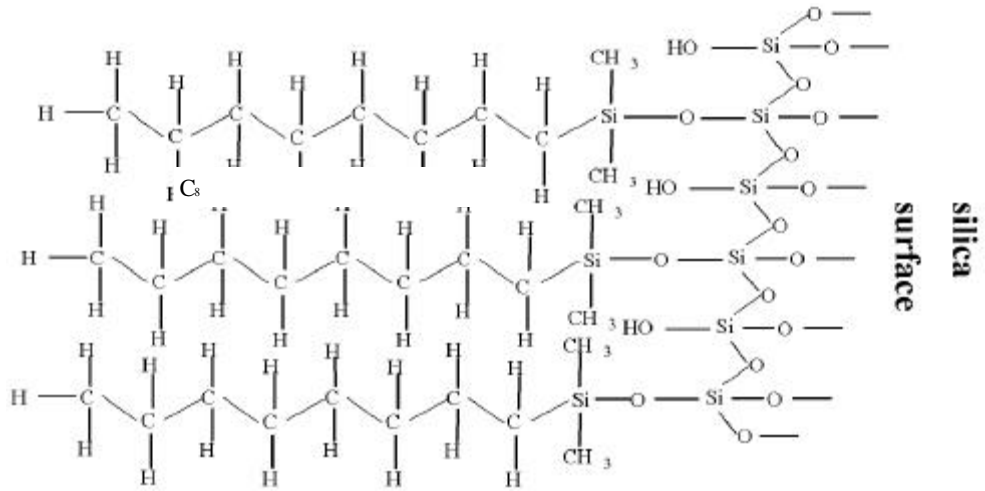
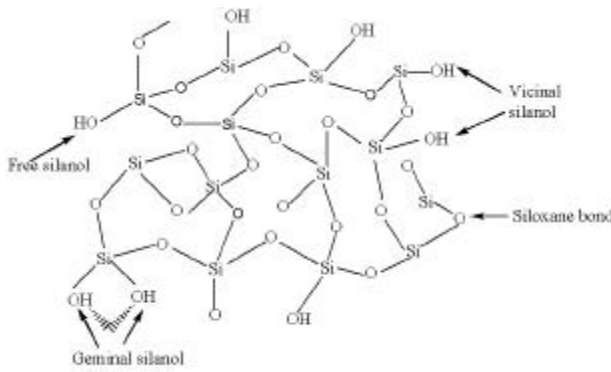


Fig. 4 실리카겔에 C8을 bonding하여 생긴 솔모양의 표면



6. Silica gel

organosilane stationary phase 4  
 dimethyloctylsilyl-chlorosilane  
 brush phase  
 fluidized bed( )  
 functional  
 monofunctional trifunctional  
 7,8

trifunctional polymer  
 Trifunctional polymer base

s, C<sub>8</sub>, C<sub>6</sub>, CN, NH<sub>2</sub>  
 silanol 가  
 C<sub>18</sub> 가  
 가 silanol 가  
 silanol pH가  
 peak tailing  
 silanol  
 free silanol가 가 , silanol  
 가 가 silanol pH  
 silica base  
 pH 가 3-8 pH가 8  
 silica sol  
 silanol  
 pH 가  
 method development peak tailing  
 free silanol  
 endcapping , silanol  
 functional  
 silanol  
 Endcapping  
 peak tailing peak  
 (width) 가  
 buffer

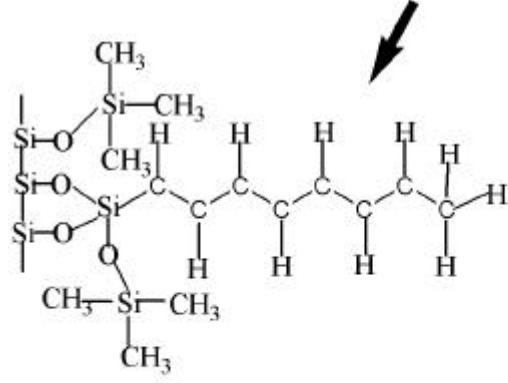
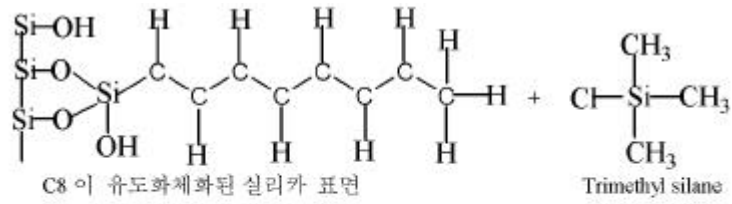


그림6 Endcapping된 실리카표면

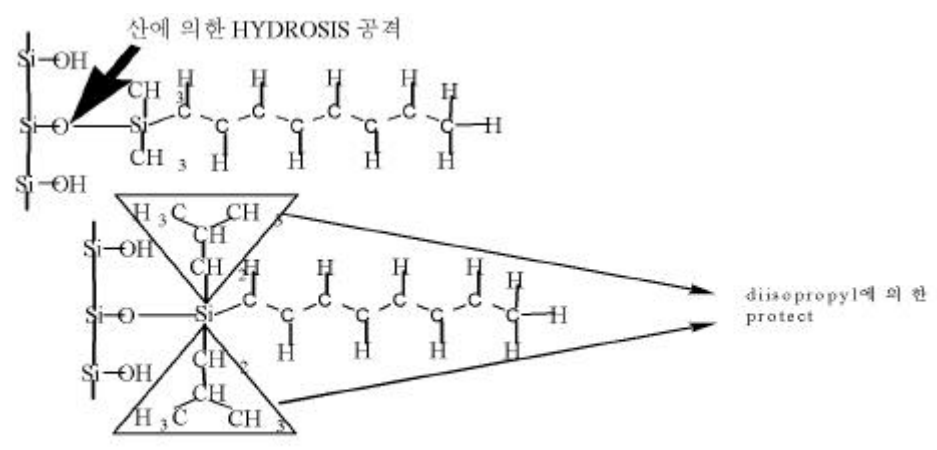


그림 7 protect된 실리카 표면

가      . Silica base      pH가 3  
 ,      functional 가 hydrolysis  
 Endcapping      7      silane      ,      protect  
 , double, triple endcapping      .      Protect  
 ,      Si-O-Si(CH<sub>3</sub>)<sub>2</sub>-(CH<sub>2</sub>)<sub>7</sub>CH<sub>3</sub>  
 endcapping      .      free silanol      8      hydrolysis가  
 pH      functional 가      가      ,

isobutyl Si 10 $\mu$ m, 5 $\mu$ m, 3 $\mu$ m . 가  
 hydrosis functional 가 가 5 $\mu$ m . , back-  
 . Functional 가 hydrosis가 pressure, 가  
 , C<sub>18</sub> C<sub>2</sub> C<sub>4</sub> C<sub>6</sub> C<sub>8</sub> C<sub>18</sub> 3 $\mu$ m .  
 , C<sub>18</sub> endcapping protect backpressure 가  
 pH 가 . Silica pump backpressure 4000psi .  
 base pH 가 HPLC pump preparation 1/2  
 hydrophilic (polar ) . 10 $\mu$ m preparation  
 pKa pH .  
 가 backpressure가  
 가 가 가  
 가 가 preparation  
 가 batch tion cleanup( )  
 batch 가 SPE(solid phase extraction)  
 silanol . 3 pore  
 가 silanol 가 10nm bio pore  
 가 가 , 가 pore 가 pre-  
 100% preparation, SPE(60 , 40 $\mu$ m) .  
 9 silica support  
 . C<sub>2</sub>, C<sub>4</sub>, C<sub>6</sub>, C<sub>8</sub>, C<sub>18</sub>, phenyl  
 endcapping ( ), endcapping C<sub>18</sub> reverse cyanopropyl, aminopropyl  
 ( ) silica normal ion  
 exchange . Cyanopropyl, aminopropyl  
 peak tailng reverse, normal .  
 가 peak tailng ,  
 . benzoic acid , pro-  
 가 broa- teomics  
 den , .  
 . C 4.3  
 . C<sub>18</sub> C<sub>8</sub>  
 ion  
 100% . C<sub>18</sub> 100%  
 buffer 90% , RT(  
 )가 .  
 dewetting phenmenon 10.a

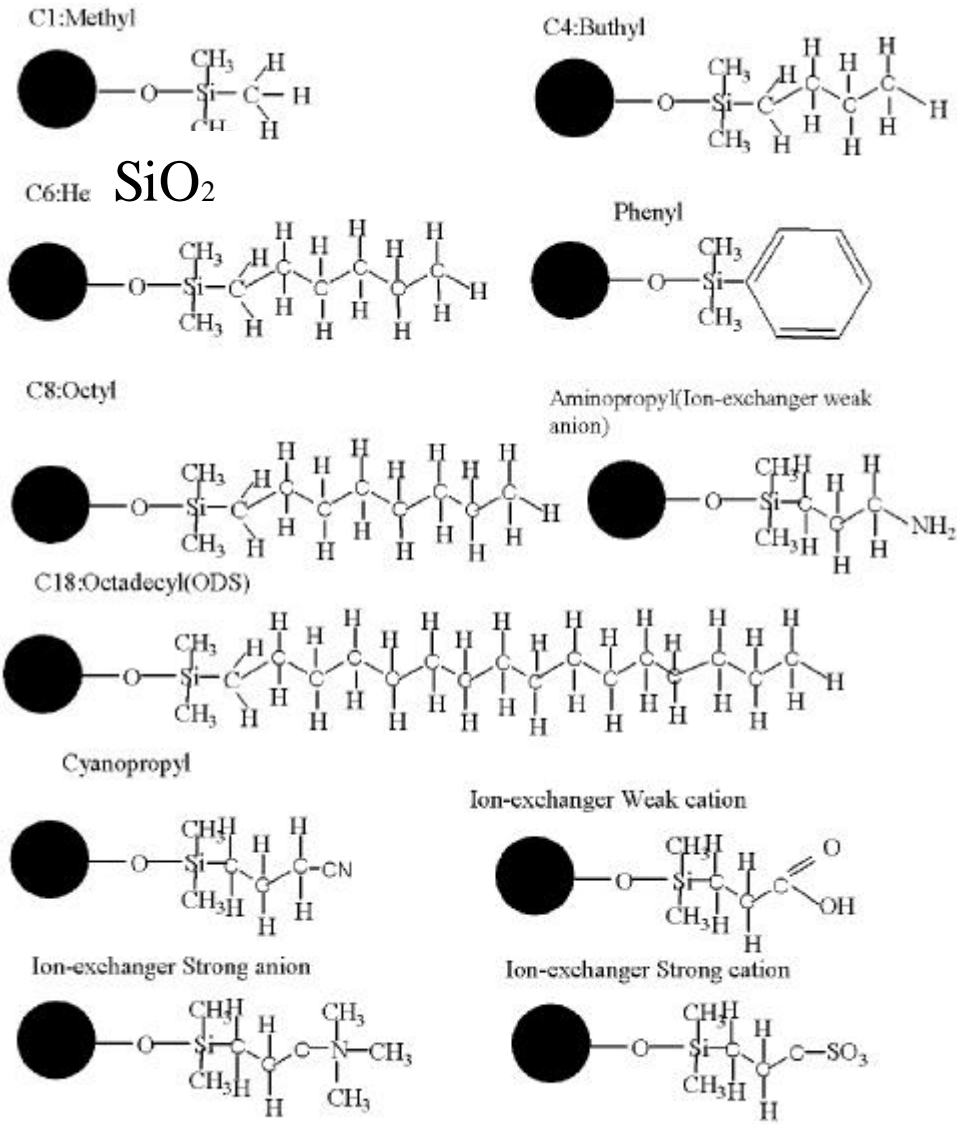
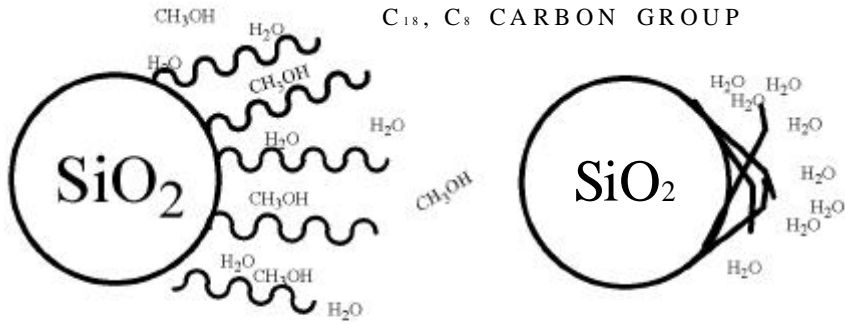


그림8 silica support 결합

functional chain  
 10.b 100% buffer chain hydrophilic  
 RT가 , C<sub>18</sub> C<sub>1</sub>, C<sub>2</sub> C<sub>18</sub>  
 11.a 가 pore hydrophobic  
 11.b carbon chain endcapping  
 100% buffer pore hy- drophobic carbon chain  
 hydrophilic pore RT가  
 RT가



C<sub>18</sub>, C<sub>8</sub> CARBON GROUP  
 그림9.a 이동상 물과메탄올      그림9.b 이동상 100%물

그림9 이동상에따른 carbon chain의 모습

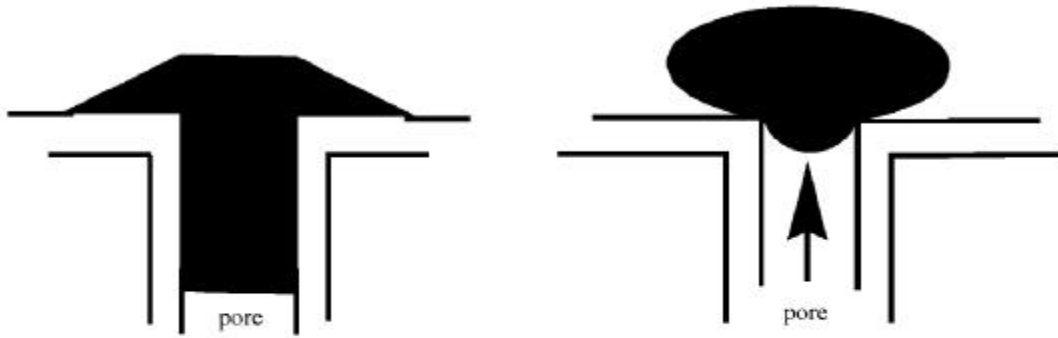


그림10.a 분석물질이 retained된상태      그림10.b 분석물질이 약간 retained된 상태      polar  
 organic solvent가 함유된 이동상      이동상이 100%buffer

그림10 실리카 pore에서wetting과 dewetting되는현상  
 RT가      ( 12)

hydrophilic	acid	가      propionic
가	C <sub>18</sub> 가	
polar	endcapping      100% bu- ffer      가      C <sub>30</sub>	
	C <sub>18</sub> bonding	
polar	bonding      C <sub>30</sub> hydrophobicity가      RT	
hydrophilic	가      가      melting temperature가      C <sub>18</sub>	
hydrophilic endcapping	29-30      , C <sub>30</sub> 68-69	
polar-embed ded	10	
polar ether amide	C <sub>30</sub> melting      가	
polar	polar      dewetting	
	Si      carbon      가      가      polar	

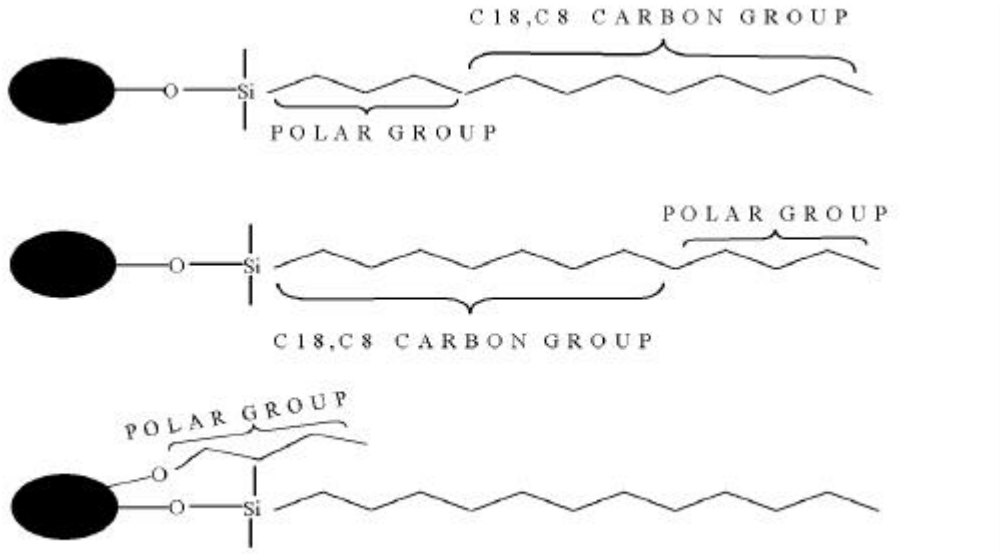


그림 11 C18, C8 에 polar group을 갖게 한 여러 형태의 컬럼

가 polar scanning 가 가 , DAD PDA

5. (Detector)

HPLC UV( 가 ), FL( ), RI( ), ECD( ), UV scanning spectrum spectrum ELSD (Evaporative Laser scatterer Detector), MSD( ) 가 UV spectrum

5.1 UV(UltraViolet)

UV UV spectrum spectrum spectrum UV spectrum lamp UV visible , paraben spectrum 1 HPLC lamp D<sub>2</sub>, Tungsten lamp UV Paraben spectrum paraben spectrum spectrum UV absorbance 가 MS scanning spectrum 가 DAD (Diode Array Detector) PDA(Photo Diode Array)

scanning system sample  
 scan- ning matrix  
 system upgrade UV  
 upgrade UV spec- trometry  
 D<sub>2</sub> lamp 가 scanning UV  
 가 spectrometer가 excitation  
 Tun- gsten lamp 가 absorbance

5.2 FL(Fluorescence)

excitation emission  
 UV  
 UV  
 가  
 UV

5.3 RI(Reflex index)

RI  
 reference cell measuring cell  
 cell 가  
 가 가  
 gradient

5.4 ELSD(Evaporative Laser Scatter Detector)

UV absorbance , b.p.가  
 sample matrix가 nebulizer nebulization  
 UV 가 laser  
 가 carbaryl, carbofuran UV 가 RI 가  
 가 UV 가 system gradient  
 benzo(a)pyrene  
 UV 가  
 UV 296nm 가 lizer 가 ( , )  
 10ppb 가 bombe generator가

buffer 가 가  
 30 ppm 가 LC μ-LC, nano-LC  
 가 LC  
 LC/MS interface laser 가 LC/MS  
 가 가

5.5 ECD(Electrochemical Detector)

ion 가  
 conductivity  
 amperometry , condu-  
 ctivity ( ,  
 ), , alkyl sulfonate  
 amperometry

5.6 MSD(Mass spectrum Detector)

가  
 가  
 fragment ion pattern 가  
 interface( ), ion-  
 ization, mass filtering, analyser, ion detection  
 . Interface nebulizer가  
 droplet ionization  
 EI API API online  
 API APCI ESI , solvent ICOS DIAM-  
 가 가 hobicity pKa hydrop-  
 가 LC/MS 가 ELUEX  
 가 fragment ion library가 pH buffer  
 MSD organic solvent org-  
 . MSD anic solvent pKa  
 가 computer simulation  
 LC

6. Data system

LC system  
 soft-  
 ware . S/N , resolu-  
 tion software data  
 method development  
 software computer simulation  
 software가 offline  
 data offline  
 software online  
 . computer simulation  
 가 Drylab  
 2-3 data  
 . Drylab  
 online  
 solvent ICOS DIAM-  
 OND, hydrop-  
 hobicity pKa  
 ELUEX  
 pH buffer  
 organic solvent org-  
 pH  
 anic solvent pKa  
 computer simulation

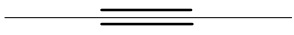


development  
computer simulation

method  
가

GC/MS NCI PCI  
가  
upgrade가

computer simulation



가  
가  
LC/MS APCI ESI