Column Characteristics

Compact, uniform packing, of the chromatographic column with the stationary phase is important since it affects the column plate number. When using high pressures with a specific column, the stationary phase should not collapse mechanically (and should not change its volume).

Most commonly, the stationary phase is packed in the column as a slurry in a specially selected liquid that allows the formation of a homogeneous suspension of the stationary phase and hinders the particle aggregation by a good solvent.

The particle concentration in the slurry is typically between 7 and 15%. For the case of rigid particles, either axial compression or radial compression can be applied to generate a dense bed of particles.

Slurry packing involves the use of high pressure (usually 50% higher than the maximum pressure at which the column will be used) to push a dilute slurry of stationary phase through the column.

The maximum pressure at which a column can be used depends on the particle strength (resistance to crushing) and may range between 9000 and 15,000 psi (600 to 1000 bar) for silica-based columns.

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Some physical properties of stationary phases.

Property	Range
Nature of phase support	Silica, organic/inorganic silica (ethylene bridged), polymer-coated silica, hydride silica, graphitic carbon, zirconia, organic polymers
Phase structure	Particles, monolithic
Particle shape	Irregular or spherical
Particle size	1 μm to 20 μm (Particles below 2.5 μm are typically used in UPLC)
Particle type	Porous, core-shell, pellicular
Particle size distribution	Narrow (1–2% size variation) to larger distributions used e.g in SEC
Porosity type	One type or multiple type of channels
Porosity (for silica)	50 Å to 4000 Å see Table 6.1.1. (Type A, Type B, Type C porosity)
Purity (for silica)	High purity (Type B purity), medium (Type A purity)
Surface area (for silica)	50 m^2/g to 500 m^2/g

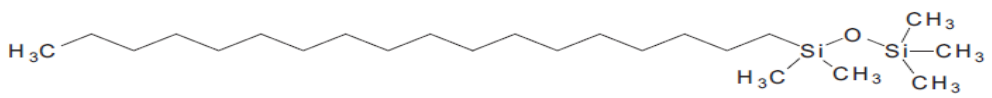
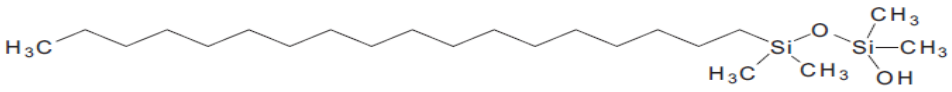
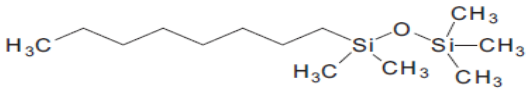
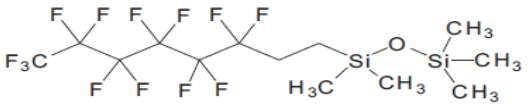
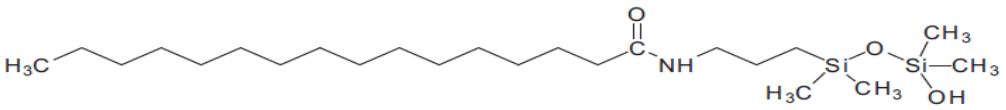
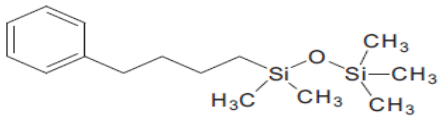
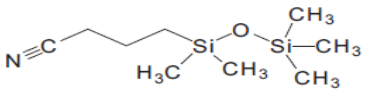
Classification of HPLC columns based on dimensions and purpose

Type	Inner diameter (mm)	Length (mm)	Typical flow rate (mL/min)	Void volume (mL)	Sample loading
Preparative	>25	300, larger	>20	>50	>25 mg
Semi-preparative	10	250, larger	5–10	>5	10–20 mg
Conventional	3, 4.6	50, 100, 150, 250	0.5–2	>1	50–200 µg
Narrowbore	2, 2.1	50, 100, 150, 250	0.2–0.5	0.2	20–100 µg
Microbore	1, 1.7	50, 100	0.05–0.1	<0.1	<5 µg
Micro LC-capillary	<0.5	50,100	1–10 µL/min	10–20 µL	1 µg
Nano LC-capillary	<0.1	50	<1 µL/min	0.1–1 µL	<0.1 µg

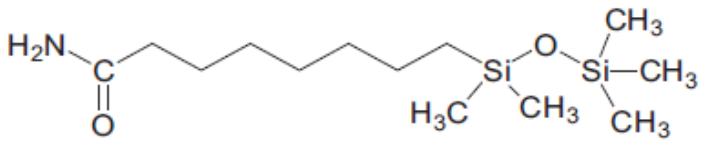
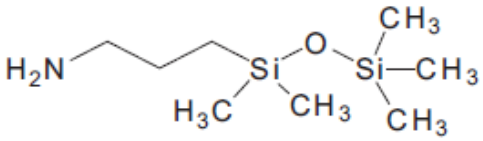
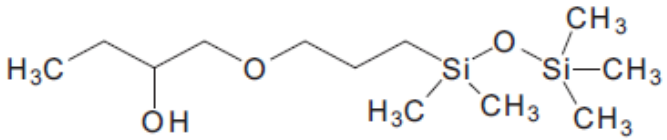
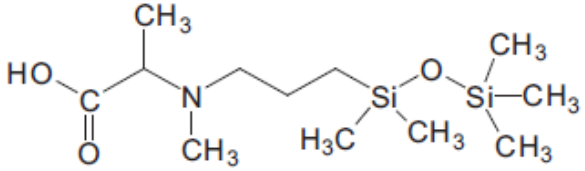
USP classification of HPLC chromatographic columns.

Code	Description	Examples
L1	Octadecyl silane (C18) chemically bonded to porous silica or ceramic microparticles, 1.5 to 10 µm in diameter, or a monolithic rod.	Over 250 columns such as: Luna C18(2), Luna C18(2)-HST, Gemini C18, Synergi Hydro-RP, C C18 (M), Aquity UPLC BEH C18, Aquity UPL Shield RP 18, Atlantis T3, µBondapack C18, Nova-Pak C18, Symmetry C18, ABridge C18, XTerra MS C18
L2	Octadecyl silane (C18) chemically bonded to silica gel of a controlled surface porosity that has been bonded to a solid spherical core, 30 to 50 µm in diameter.	Bondapak Prep C18, etc.
L3	Porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	Luna Silica(2), Aquity UPLC BEH HILIC, Atla HILIC Silica, Onyx Si (M), SunFire Silica, XBri HILIC, Zorbax SIL, etc.
L4	Silica gel of controlled surface porosity bonded to a solid spherical core, 30 to 50 µm in diameter	Porasil Prep Silica
L5	Alumina of controlled surface porosity bonded to a solid spherical core, 30 to 50 µm in diameter.	
L6	Strong cation-exchange packing: sulfonated fluorocarbon polymer-coated on a solid spherical core, 30 to 50 µm in diameter.	
L7	Octyl silane (C8) chemically bonded to totally porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	Luna C8(2), Aquity UPLC BEH C8, Nova-Pak Resolve C8, SunFire C8, Symmetry C8, XBridge XTerra MS C8, XTerra RP8, Onyx C8 (M), Nucl C8, Zorbax C8, etc.
L8	An essentially monomolecular layer of	Luna 10 µm NH2, µBondapak NH2, Waters

this polarity is difficult to assess from column to column because it refers to a solid phase that is not even uniform, containing groups belonging to both the support and the bonded moieties.

Column	Model structure	log K_{ow}*
C18 end-capped		9.75
C18 not end-capped		7.75
C8 end-capped		5.97
Fluorinated		8.38
Amide embedded		7.86
Butyl-phenyl		5.81
Cyanopropyl		2.80

(Continued)

Column	Model structure	log K_{ow}*
HILIC amide		3.69
HILIC amino		2.25
HILIC diol		1.46
Zwitterionic		0.43

Core-shell

The use of core-shell particles significantly increases the number of theoretical plates as compared to porous particles of the same dimensions. For example, in a comparison to a traditional 3 mm particle C18 column (150 mm length and 4.6 mm i.d.) that had N=166,502 theoretical plate per m, a core-shell column with the same dimensions (Kinetex ™ from Phenomenex) had N=295,343 per m.

A traditional 1.7 µm particle C18 column (50 mm length and 2.1 mm i.d.) had N=272,080 per m, while a core-shell column with the same dimensions (Kinetex™ from Phenomenex) had N=318,680 per m.

On the other hand, core-shell columns may have lower k.

The use of monolith columns may also reduce peak broadening.

Peak shape

The decrease in the column resolution due to tailing or other effects seen in a column with high skew, the precision of the peak area integration.

Peak tailing is frequently caused by hydrophobic interactions and polar interactions, which may take place together, for example, in a column with a hydrophobic phase

on silica that also has numerous free silanol groups.

A variety of phases were designed to reduce this problem, for example, those using end-capping.

The very high purity of the silica base (B type than A type) contributes to the reduction of tailing.

The pH range

Conventional silica stable at pH 2 to 8.

Mobile Phases

Most separations on hydrophobic stationary phases take place using a water-organic solvent with a solvent ratio between 20 and 80%. Phases that can be used at higher water content (even in 100% water) have been developed and are preferable in numerous applications.

Stability of stationary phases

The stability in time of the stationary phase is another important characteristic. This characteristic can be estimated by the number of injections n that can be made on a column, with unaltered results for the chromatography.

The efficiency of the pre-column and the frequency with which this pre-column is changed also influence the number of injections n, as does the nature of the mobile phase.

pH is another factor influence on stability.

In practice, it can also be noted that columns with a larger amount of stationary phase appear to be more resilient in time and lead to larger n.

Backpressure

$$\Delta p = \frac{\eta U L}{\pi K^0 r^2 d_p^2}$$

Δp is the pressure necessary for a liquid to flow through the column with the volumetric flow rate U. η is the mobile phase viscosity (typically given in mPa s), K^0 is specific permeability for the phase (adimensional), r is the column radius, L is its length, and dp is the diameter of the particles in the bed (in μm) (Darcy equation).

Columns with core-shell particles and monolithic columns characterized by the same number of theoretical plates N compared to traditional columns have lower backpressure for similar flow conditions.

For example, for a traditional C18 column, 1.7 mm particles, 50 mm long, 2.1 mm i.d, a typical working backpressure in CH3CN/H2O 50/50 is higher than 400 bar at 0.6 mL/min flow rate (N=270,000 per m). A core-shell C18 column with 2.6 mm particles that has a similar N, and identical length and i.d., develops pressures below 300 bar.

Column Protection, Cleaning, Regeneration, and Storing

The columns for:

- reversed phase are usually received filled with acetonitrile/water 65:/35 v/v;
- silica based ion-exchange columns are received filled with methanol or methanol/water;
- normal phase columns are received filled with isopropanol or hexane.
- Ion exclusion can filled with water.
- HILIC columns that can be received in acetonitrile/water (e.g., 90:10 v/v) that contains 100 mM of HCOONH4.
- Columns used for protein separations may be washed with 0.1% trifluoroacetic acid (TFA) in water followed by 0.1% TFA in acetonitrile/ isopropanol ½ v/v, followed by rinsing with a common mobile phase (columns should not be stored in THF).
- For RP columns this temperature is typically not higher than 60˚C.

Columns that are not used for a certain period of time must be stored appropriately.

For RP-type columns, a mixture of 65% acetonitrile and 35% water is recommended.

For normal-phase columns, isopropanol is typically recommended as storing solvent.

Ion-exchange columns are usually stored in methanol or methanol/water.

For size-exclusion columns, water with 0.05% NaN_3 (to avoid bacteria growth) or with 10% methanol is typically recommended.

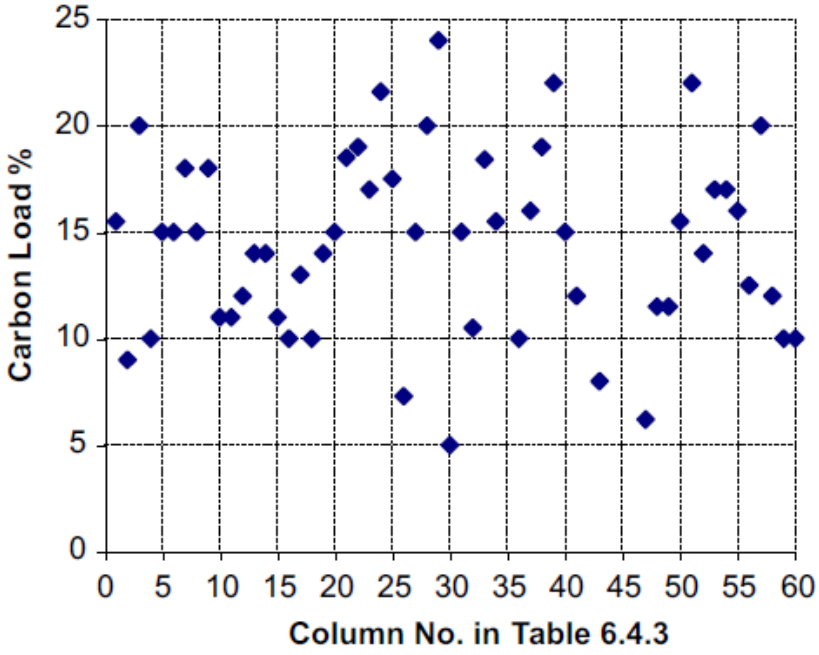
High-resolution ^{29}Si-NMR has proved to be a powerful tool for structural elucidation of organic and inorganic silicon compounds. Because of the sensitivity of ^{29}Si chemical shifts, detailed information can be obtained by ^{29}Si-NMR on the structural surroundings of a given Si atom in a complex molecular framework. This information can be used to study microstructural details of Si-O-Si linkages connected in chains or branched and crosslinked structures.

Capacity factor ln k for toluene on C18 stationary phases (5 mm particles).

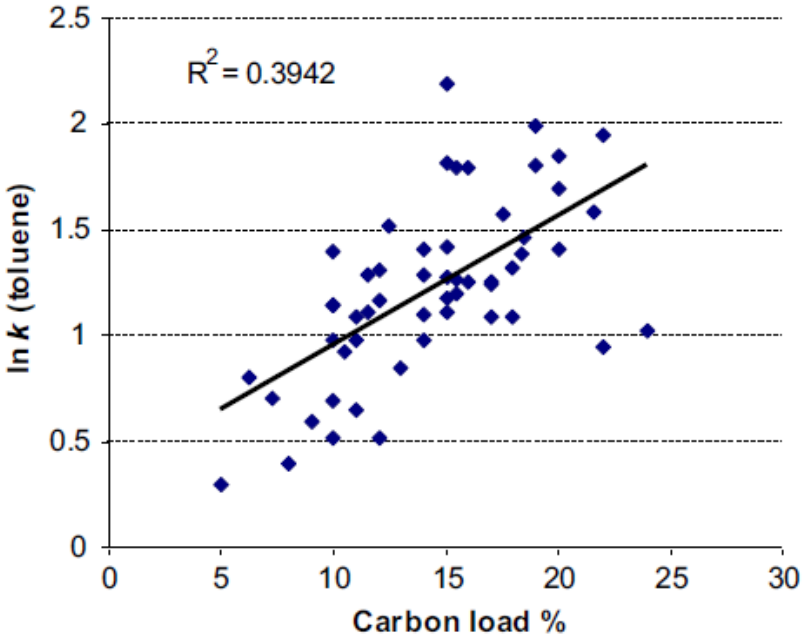
No.	Column	ln *k*	No.	Column	ln *k*
1	ACE C18	**1.27**	23*	LiChrosorb RP-18	**1.095**
2	ACE C18-300	**0.59**	24	LiChrospher RP-18	**1.59**
3	ACE C18-HL	**1.85**	25	Luna 5 C18(2)	**1.58**
4*	µBondapak C18	**0.52**	26**	Novopak C18	**0.7**
5	Capcell Pak AG C18	**1.18**	27	Nucleosil C18	**1.42**
6	Capcell Pak UG C18	**1.26**	28	Nucleosil C18 HD	**1.405**
7	Develosil ODS-HG	**1.09**	29	Nucleosil C18AB	**1.02**
8	Develosil ODS-MG	**1.82**	30*	Partisil ODS	**0.3**
9	Develosil ODS-UG	**1.32**	31*	Partisil ODS2	**1.28**
10	Exsil ODS	**1.09**	32*	Partisil ODS3	**0.92**
11	Exsil ODS1	**0.65**	33	Prodigy ODS2	**1.39**
12	Exsil ODSB	**0.52**	34	Prodigy ODS3	**1.8**
13	Gemini C18	**1.41**	35	Purospher RP18-e	**2.05**
14	Hichrom RPB	**1.1**	36	Resolve C18	**1.15**
15	Hypersil BDS C18	**0.98**	37	SunFire C18	**1.8**
16	Hypersil GOLD	**0.69**	38	Symmetry C18	**1.81**
17	Hypersil HyPurity C18	**0.85**	39	TSK ODS-120T	**0.95**
18	Hypersil ODS	**0.98**	40	TSK ODS-80TM	**1.11**
19	Inertsil ODS	**1.29**	41	Ultrasphere ODS	**1.17**
20	Inertsil ODS3	**2.19**	42	Vydac 218MS	**0.4**
21	Inertsil ODS2	**1.47**	43	Vydac 218TP	**0.4**
22	Kromasil C18	**1.99**	44	Vydac Selectapore 300M	**0.33**
45	Vydac Selectapore 300P	**0.4**	53	YMC ODS A	**1.26**
46	Vydac Selectapore 90M	**0.77**	54	YMC ODS AM	**1.25**
47	Waters Spherisorb ODS1	**0.8**	55	YMC Pro C18	**1.26**
48	Waters Spherisorb ODS2	**1.29**	56	Zorbax Extend C18	**1.52**
49	Waters Spherisorb ODSB	**1.11**	57	Zorbax ODS	**1.7**
50	XTerra MS C18	**1.2**	58	Zorbax Rx-C18	**1.31**
51**	YMC J'Sphere ODS H80	**1.95**	59	Zorbax SB-C18	**1.15**
52**	YMC J'Sphere ODS M80	**0.98**	60	Zorbax XDB-C18	**1.4**

* *Note: Indicates 10 µm particle size*
** *Note: Indicates 4 µm particle size*

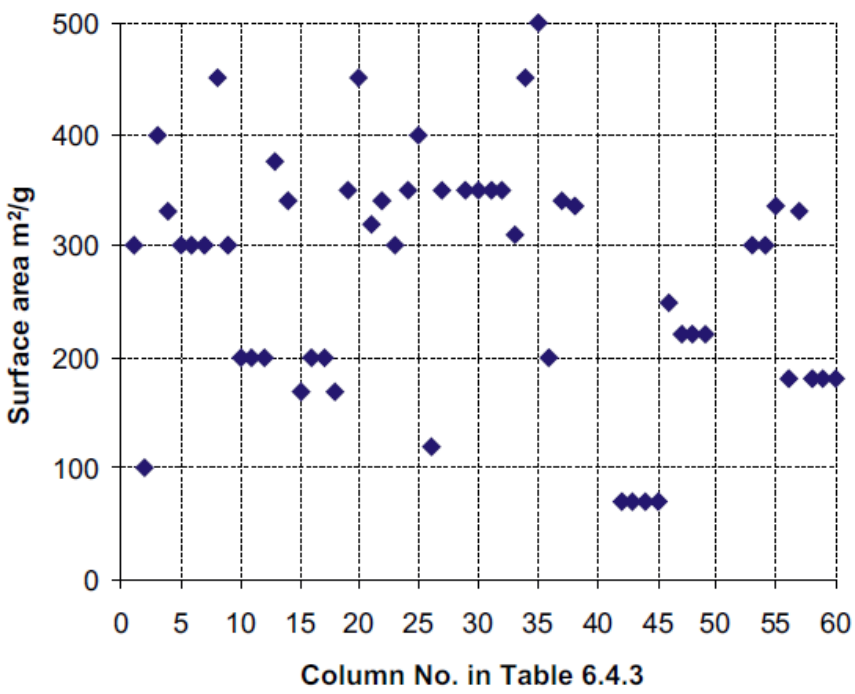
Values for carbon load % for the columns listed in Table 6.4.3.



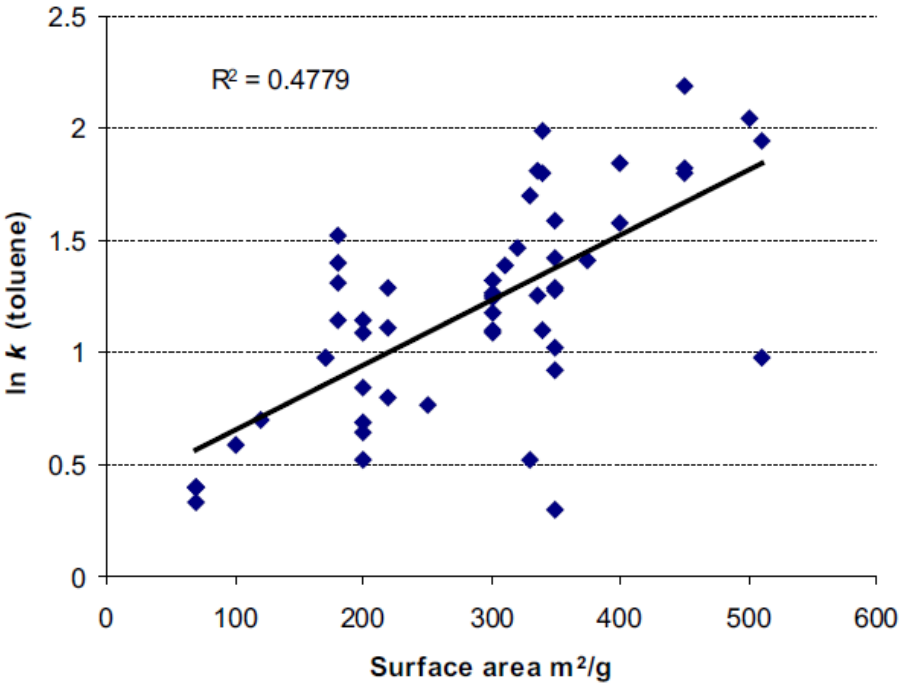
Variation of capacity factor ln k as a function of carbon load.

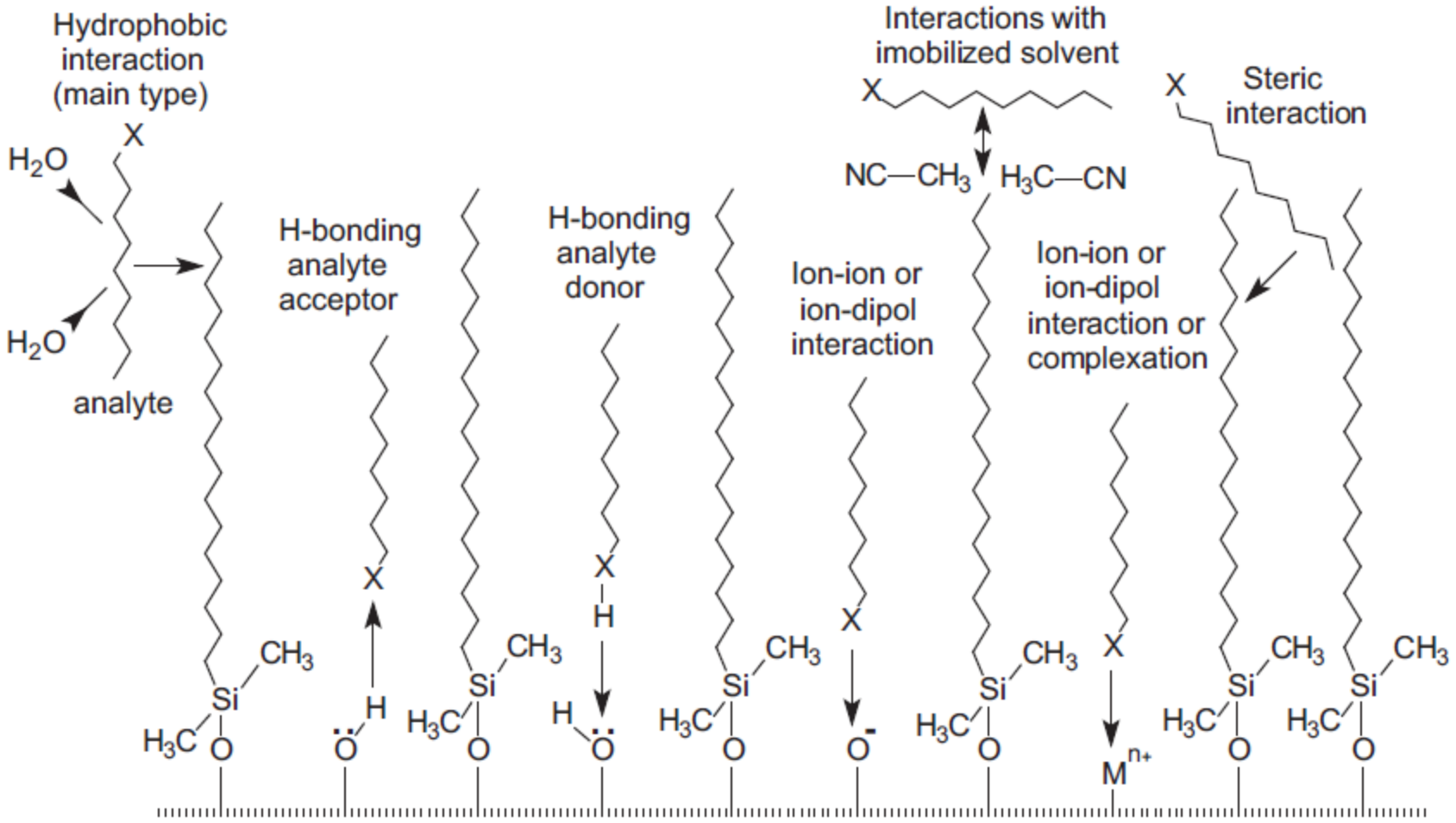


Values for surface area m2/g for the columns listed in Table 6.4.3.



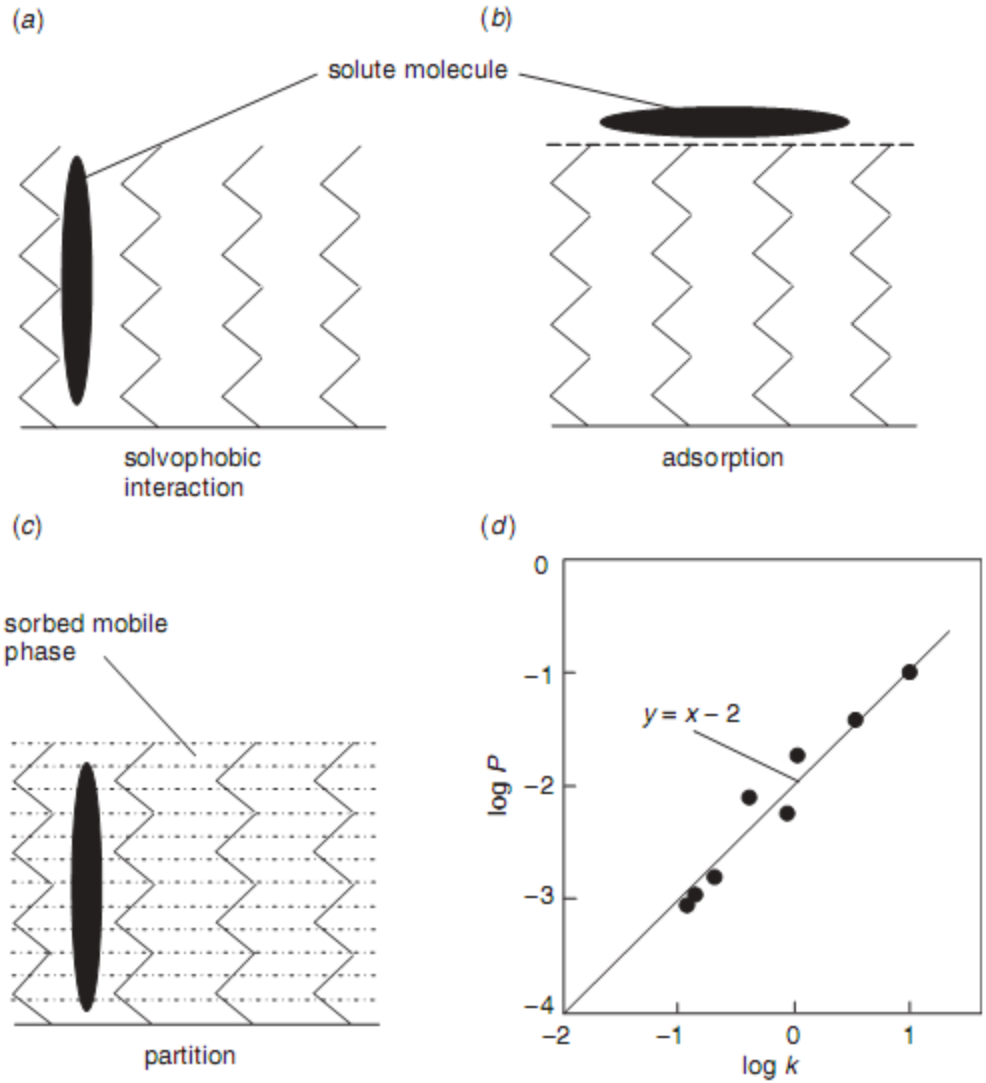
Variation of capacity factor ln k as a function of surface area of the particles.





Schematic representation of various interactions in RP-HPLC.

Different possibilities for the retention of a solute molecule in reversed-phase chromatography. (a) Solvophobic interaction; (b) adsorption ; (c) partition; (d) comparison of RPC retention (k) with octanol-water partition P; sample; eight amino acids; column: C8; mobile phase: aqueous buffer (pH-6.7); 70 °C.



it was observed that RPC retention (values of k) correlates with partition coefficients P for the distribution of the solute between octanol and water.