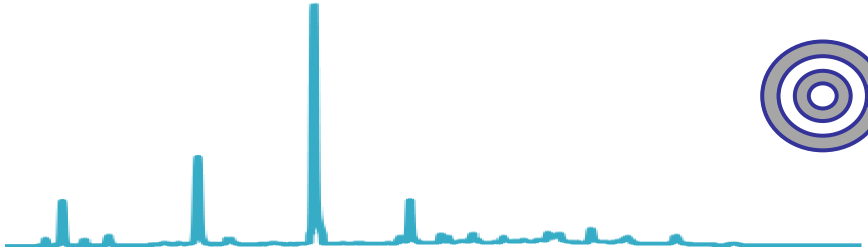
بسم الله الرحمن الرحيم

Chem 651
Advanced Studies in Instrumental Analysis
Advances in Chromatography Stationary Phases



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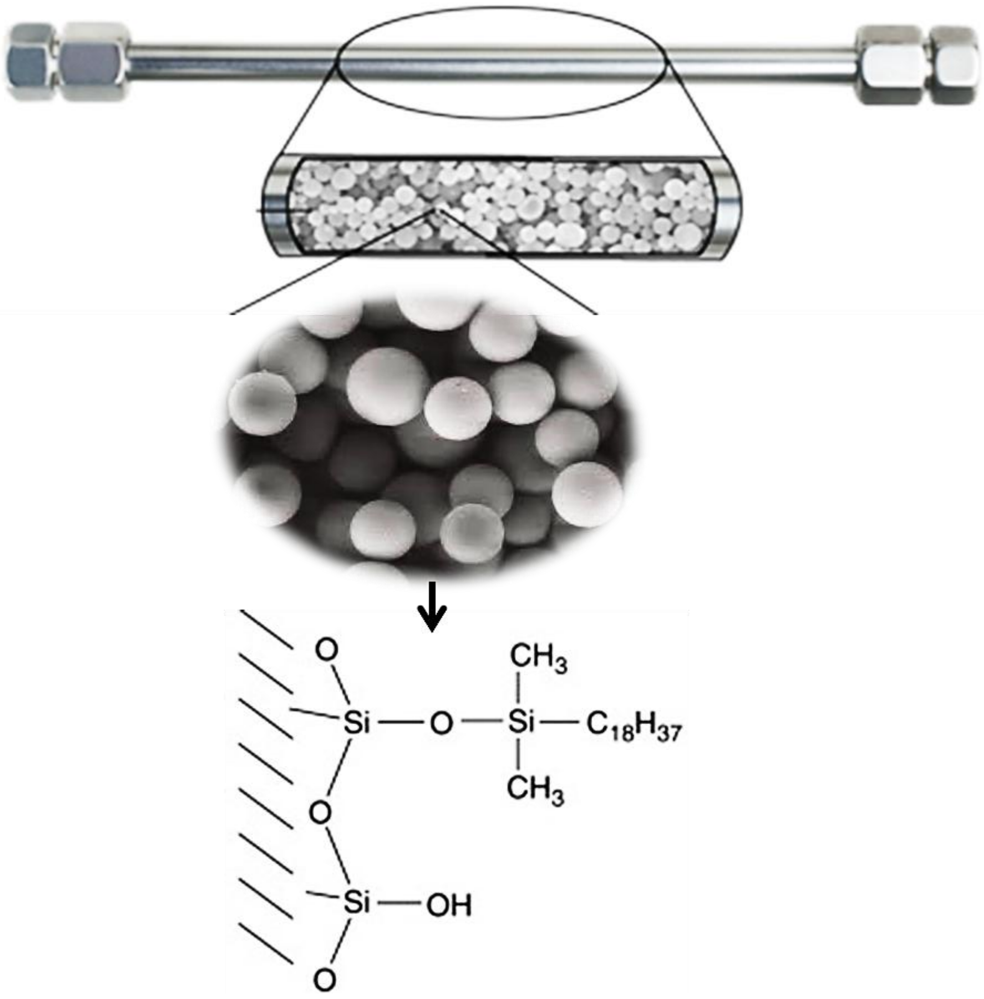
Stationary Phase

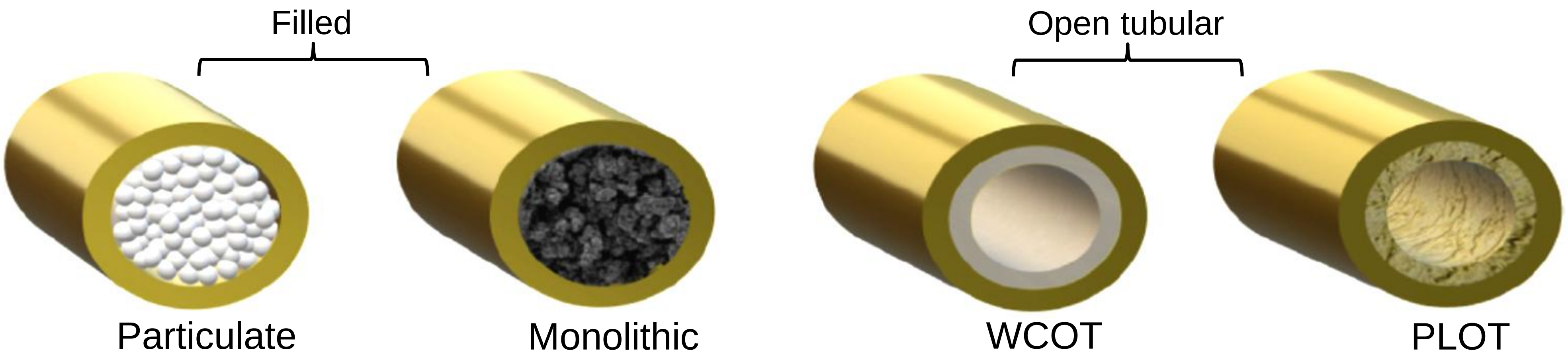
Although it is usually the smallest part, the column is the most important part in any chromatographic system. The column is the only device in the separation system which actually separates an injected mixture.

Column packing materials are the media producing the separation, and properties of this media are of primary importance for successful separations. The selectivity, capacity and efficiency of the column are all affected by the nature of the packing material or the materials of construction.

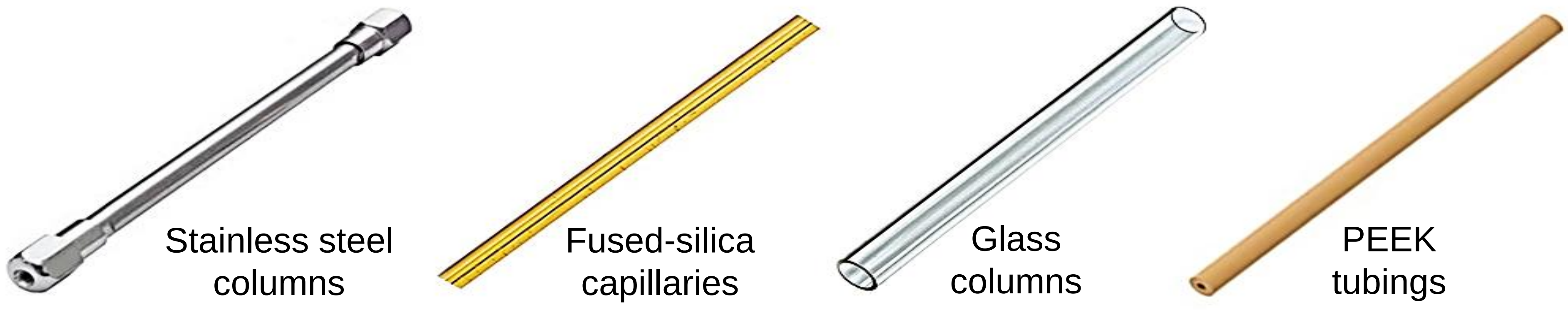
Materials chemistry and polymer science are highly rich in options, reactions and modifications. Therefore, stationary phases faced various developments and can be still improved.



Basic types of columns used in chromatography



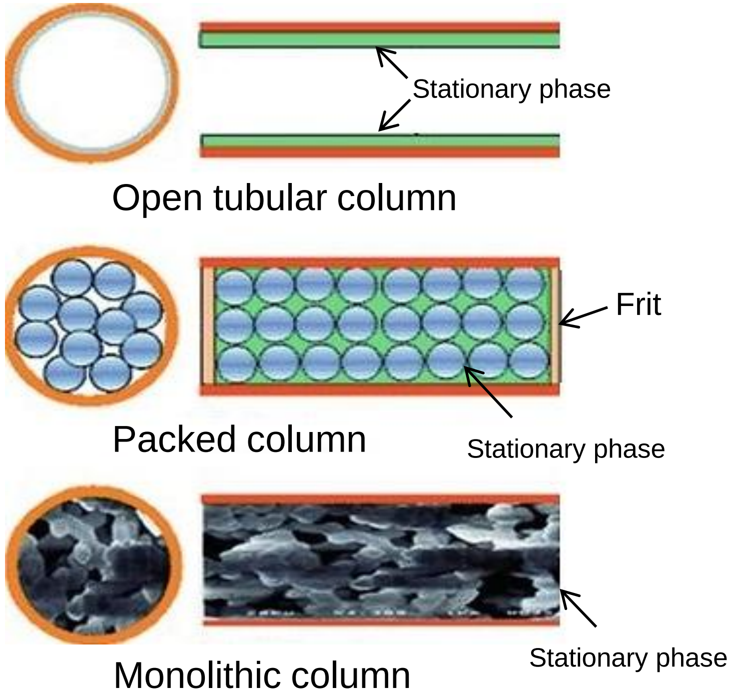
Columns commonly used, which are typically prepared inside

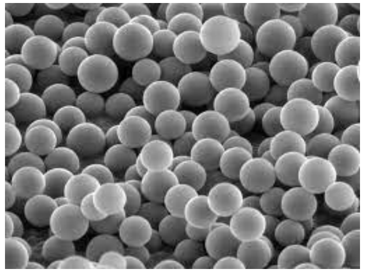


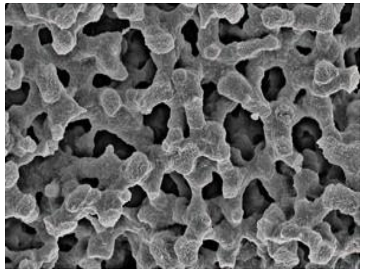
Great varieties of different columns are currently available on the market.
Five distinct characteristics could be used for column classification:

(1) Type of packing materials

particulate; non-particulate (interconnected, one-piece or continuous phase); porous; nonporous; core-shell; packed; open-tubular; monolith; etc.








Nonporous particle


Porous layer


Completely porous

(2) Type of base material

silica SiO_2; polymeric; alumina Al_2O_3; zirconia ZrO_2; carbon-based; etc.

(3) Stationary phase geometry

surface area; interaction sites, pore size or diameter; pore volume; pore size distribution; permeability; particle size; particle shape; particle size distribution; porosity; etc.

(4) Surface chemistry

type of bonded ligands; functional groups; bonding density; carbon content; etc.

(5) Stability and rigidity

surface reactivity; chemical stability, physical stability; mechanical stability; stability under pressure; stability in common LC solvents; pH stability; stability to hydrolysis in acidic and basic media; stability at elevated mobile phase flow rate; column temperature; structural rigidity; re-usability; etc.

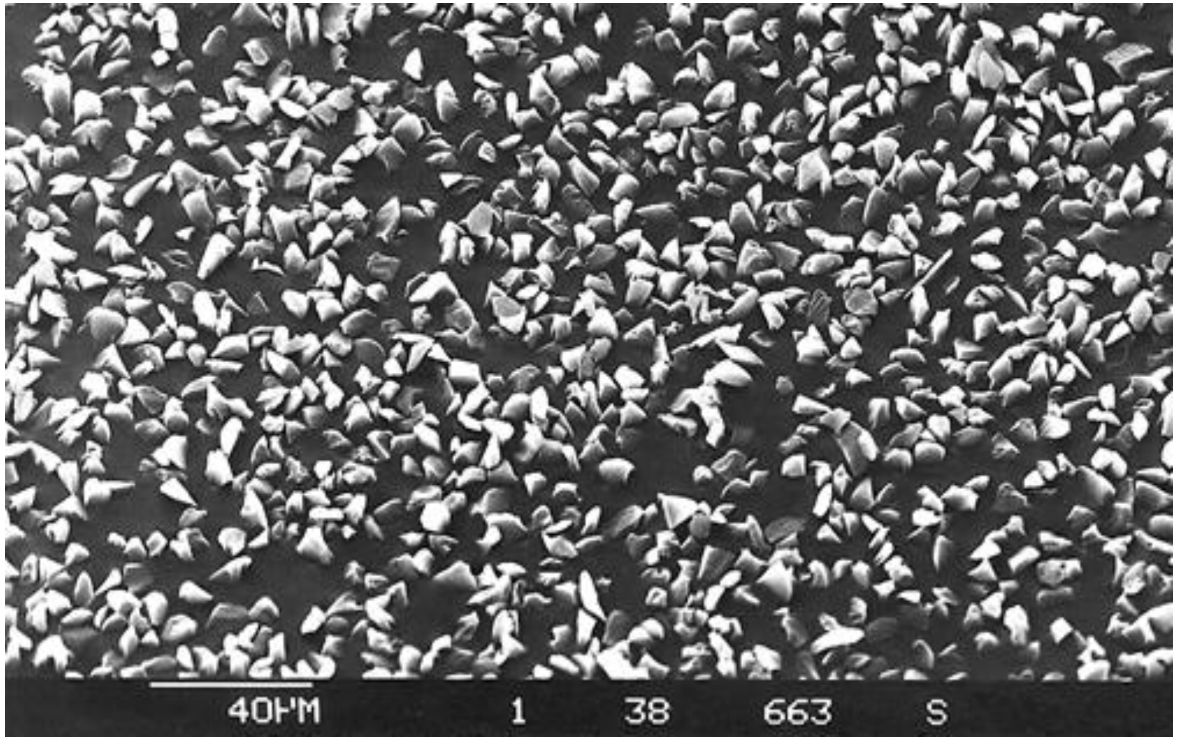
All these parameters are interrelated in their influence on the chromatographic performance of the column. The quality of a separation column is a subjective factor, which is dependent on the types of analytes and even on the chromatographic conditions used for the evaluation of the overall quality.

In conclusion, all these characteristics could be classified by either **physical** or **chemical** properties of the stationary phases.

Irregularly shaped silica

1st generation

-Synthesis via *SIL-GEL* condensation; grinded and sieved.
-Irregular material.
-Contaminated with metal ions ($Fe^{2+/3+}$, Na^{+}, Ca^{2+}, Al^{3+}, ~ 25-75 ppm).
-In use for preparative LC (FLASH, large scale).



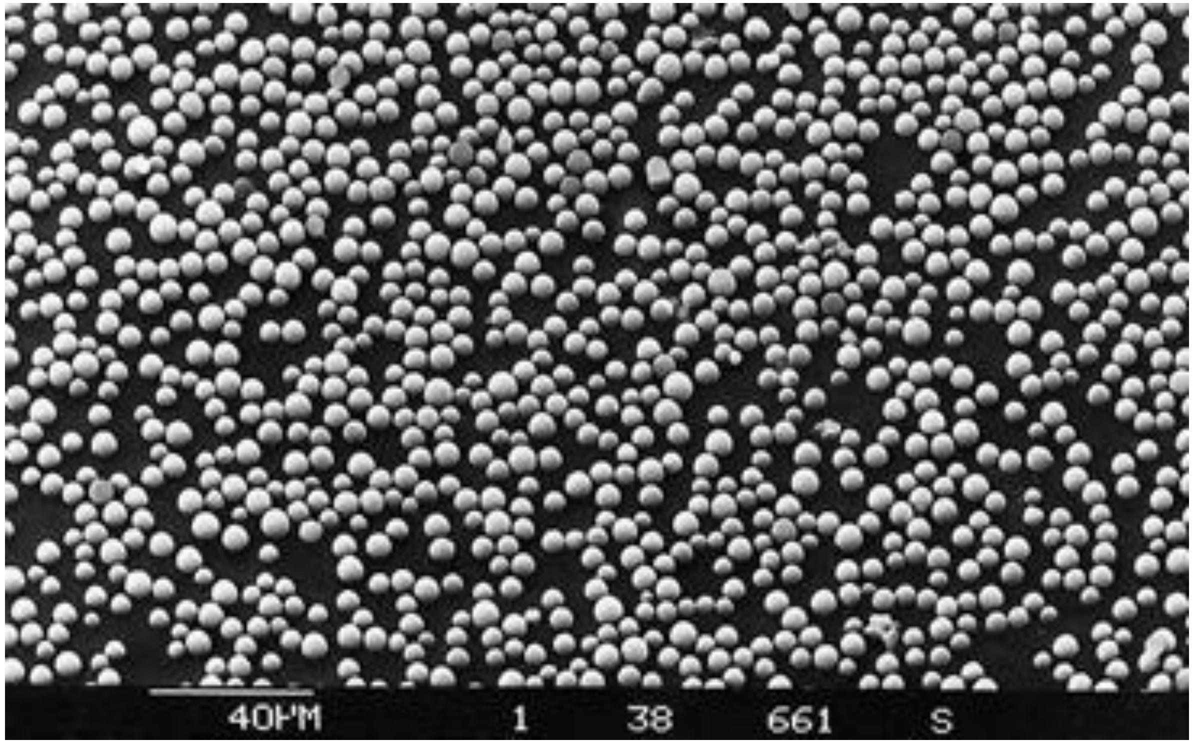
POLYGOSIL® Particle size: 7 µm, SEM micrograph

Spherical silica

<u>2nd generation</u>

-Synthesis via *SIL-GEL* condensation.
-Spherical material.
-Contaminated with metal ions ($Fe^{2+/3+}$, Na^{+}, Ca^{2+}, Al^{3+}, ~ 25-75 ppm).
-Higher efficiency than irregular silicas in packed HPLC columns.

e.g. colloidal silica solution is sprayed into fine droplets and subsequently dried in a hot air stream.



NUCLEOSIL® particle size: 7 µm, SEM micrograph

Spherical silica

3rd generation

-Synthesis via *Sol-Gel* condensation of alkoxysilanes.
-Spherical material, very homogeneous surface, high mechanical stability.
-Very low concentration of metal ions, ultra-pure (< 10 ppm).

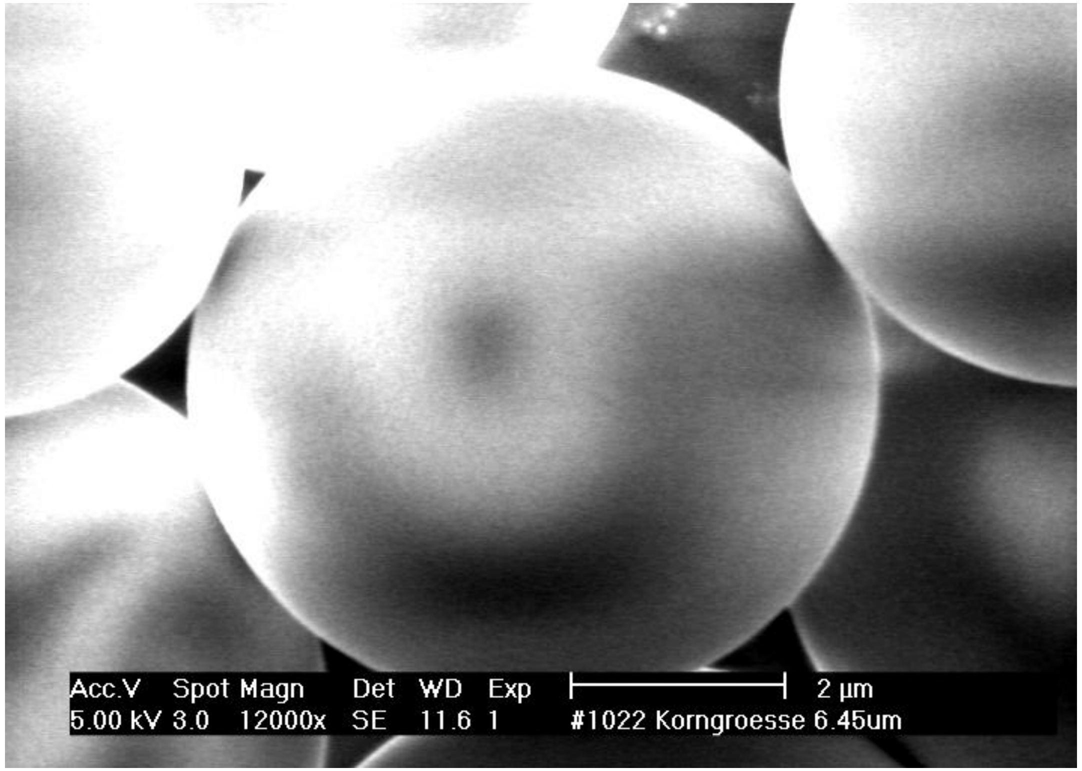
$$Si\,(OC_2H_5)\;+\;4\,H_2O$$

$$\downarrow$$

$$Si(OH)_4\;+\;4\,C_2H_5OH$$

$$\downarrow$$

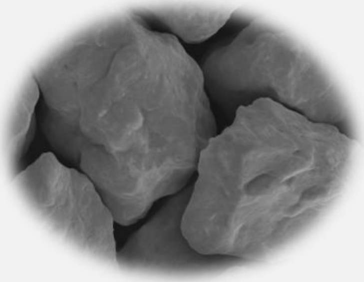
$$n\,Si(OH)_4$$



NUCLEODUR® particle size: 5 µm, SEM micrograph

Trend toward smaller stationary phases particle sizes

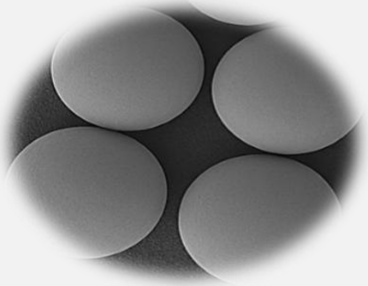
1950’s



Nominal size
~ 100 µm

Plates / 15 cm
~ 200

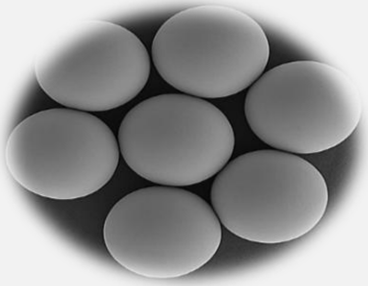
1960’s



Nominal size
~ 50 µm

Plates / 15 cm
~ 1000

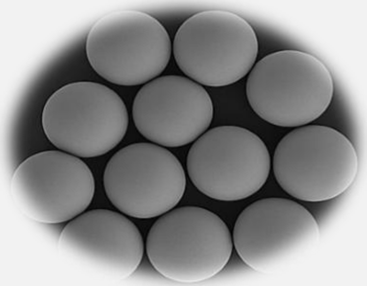
1970’s



Nominal size
10 µm

Plates / 15 cm
~ 6000

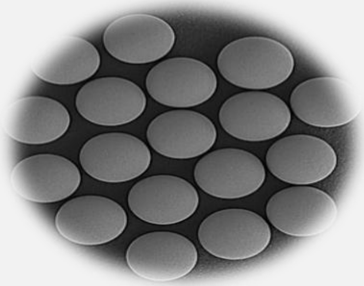
1980’s



Nominal size
5 µm

Plates / 15 cm
~ 12000

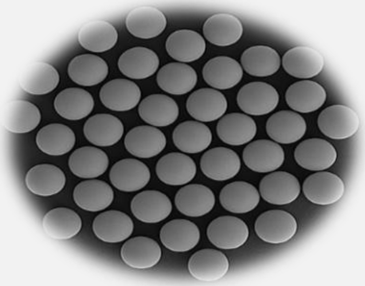
1990’s



Nominal size
3 µm

Plates / 15 cm
~ 22000

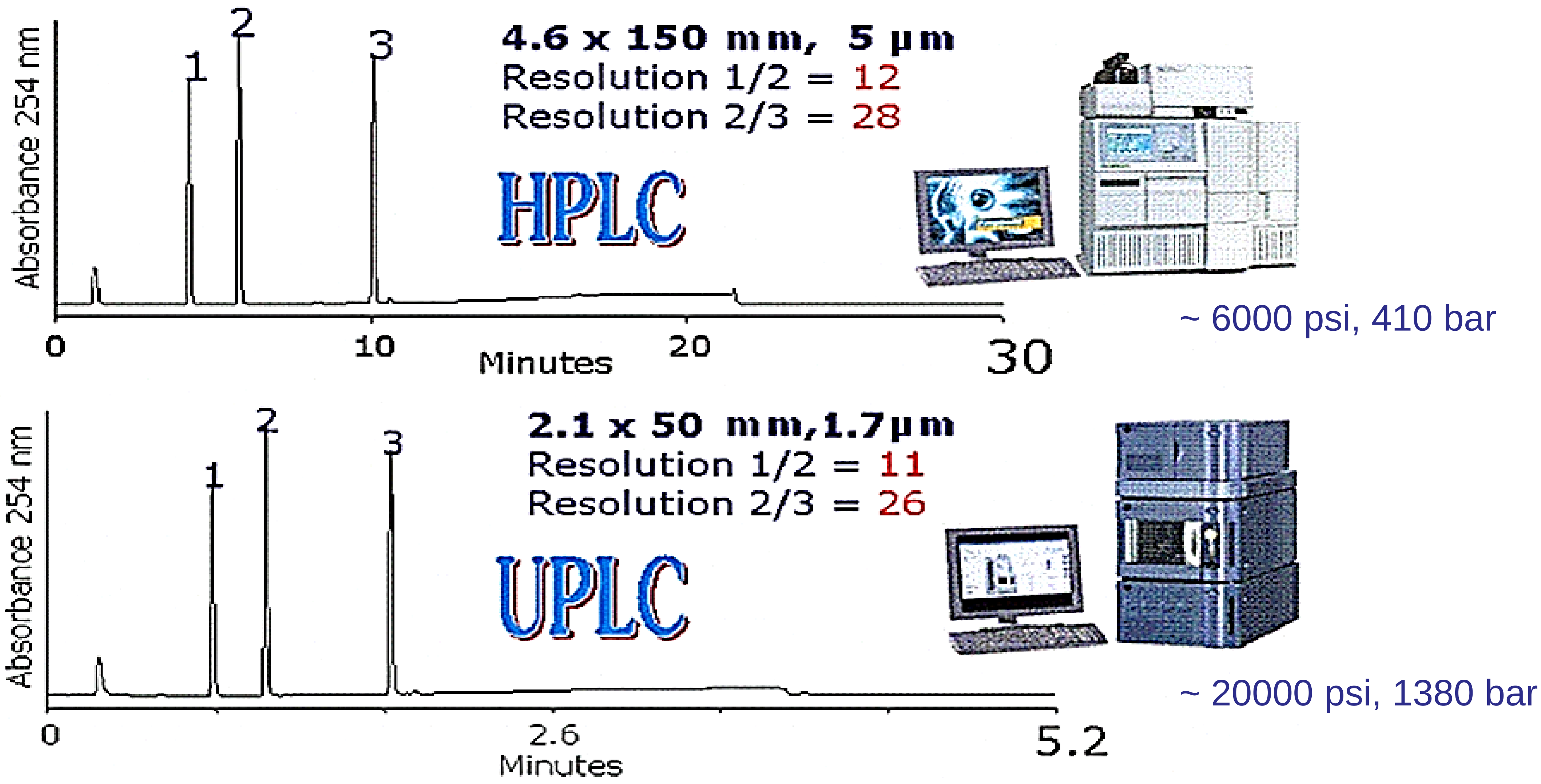
2000’s



Nominal size
Sub-2 µm

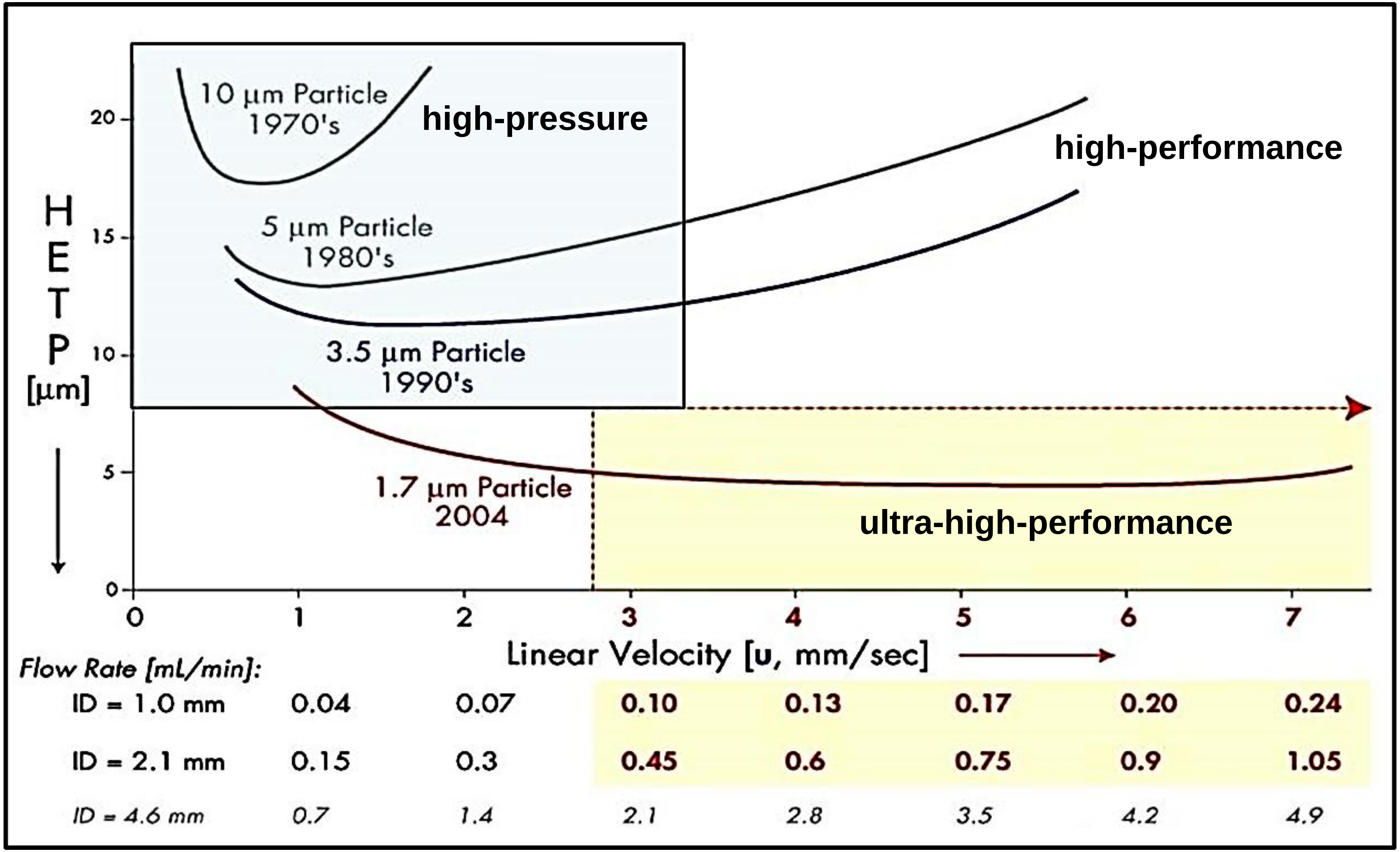
Plates / 15 cm
~ 32000

Modern **HPLC** systems have been improved to work at much higher pressures, and therefore be able to use much smaller particle sizes in the columns (< 2µm). These are ultra performance liquid chromatography systems or **UPLC's (UHPLC)**.



Evolution of particle technology

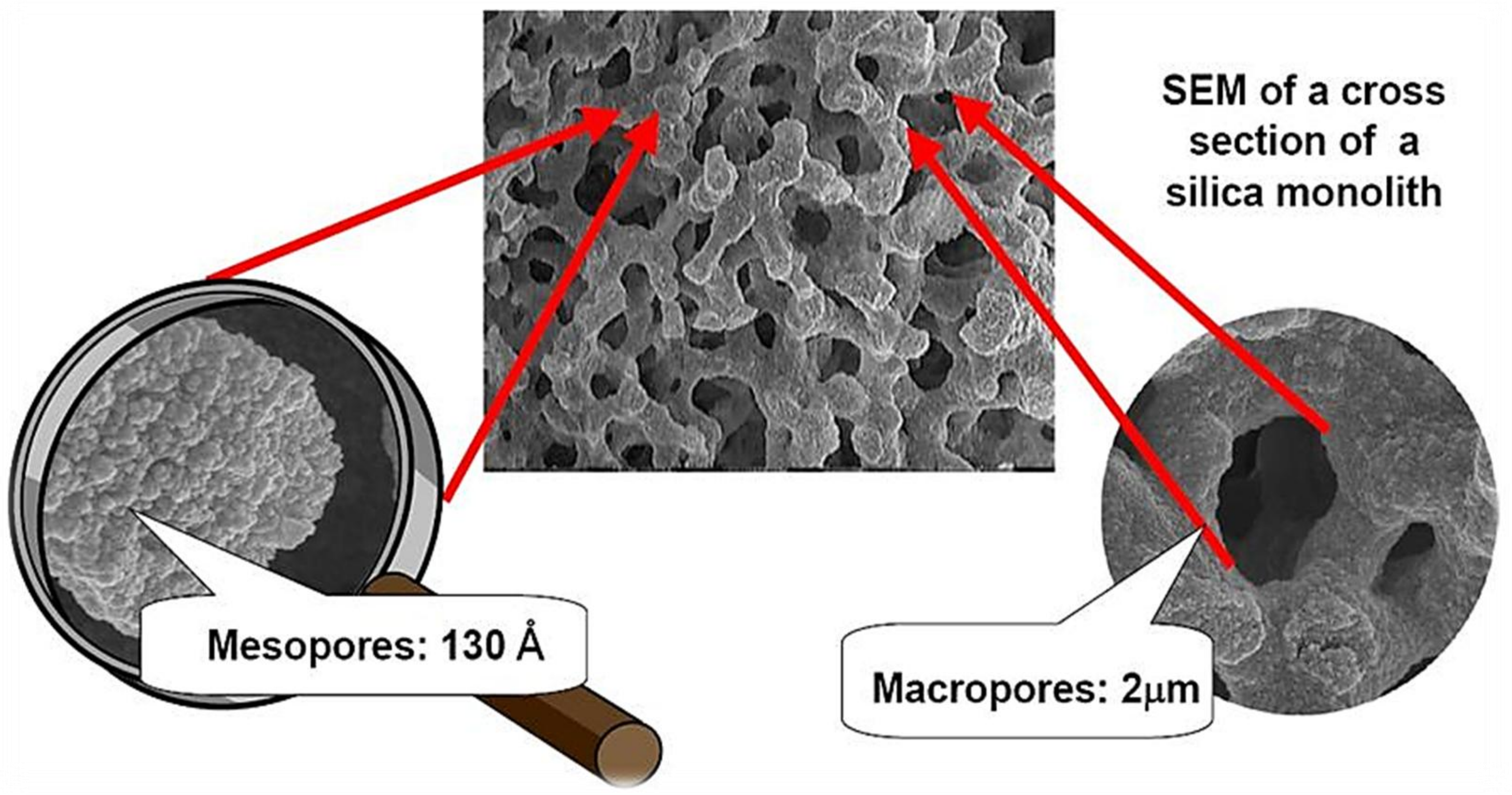
van Deemter plot, illustrating the evolution of particle sizes



Monolith material

4th generation

Monoliths are a single block piece of continuous materials made of highly porous rods with two types of bimodal pore structure distribution (macropores and mesopores).

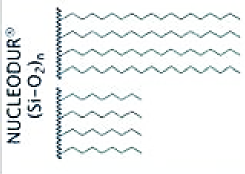
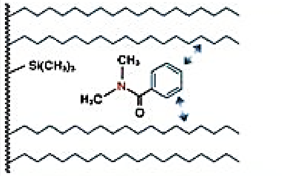
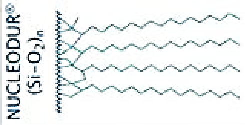
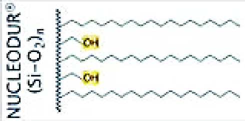
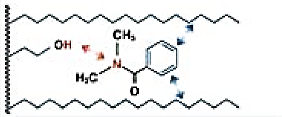
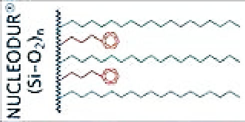
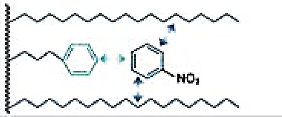
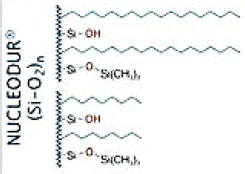
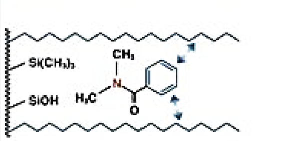
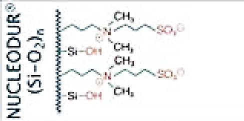
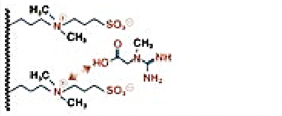
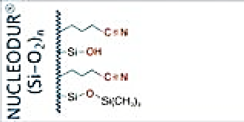
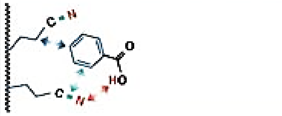
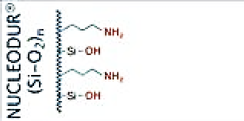
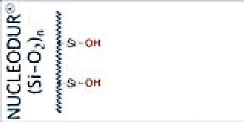


Stationary Phase Chemistry

Common packings and bonded phases in liquid chromatography

Stationary phase	Typical modes and applications
Silica (unmodified)	Polar compounds in general (NP)
Alumina (unmodified)	Similar to silica; can be adjusted for acidic, basic, or neutral analytes (NP)
Polymeric	Used at very high or very low pH, where the silica-based phases might degrade (RP)
Amino: Si-(R)-NH_2	Can be used as a weak ion-exchange phase; ionizable compounds; phenols, petroleum fractionation, sugar, saccharides, drugs, aromatics (NP or RP; depending on R)
Cyano: Si-(R)-CN	Low hydrophobicity, alternative to silica, broad spectrum of mixtures with different polarities; polar organics, peptides, protiens, drugs, metabolites and pesticides (NP or RP; depending on R)
Diol: Si-(R)-CHOH-CH_2-OH	Less acidic than silica, complex mixtures, antibiotics, proteins, peptides (NP or RP; depending on R)
Phenyl: Si-(R)-C_6H_5	Aromatic and moderately polar compounds (RP)
C_{18} (or RP-18): -Si-$(CH_2)_{17}$-CH_3	General purpose; hydrocarbons, drugs, metabolites, pesticides, peptides, organics specially acids (RP)
C_8 (or RP-8): -Si-$(CH_2)_7$-CH_3	Similar to C_{18}; generally, less hydrophobic (RP)
C_2 (or RP-2): -Si-CH_2-CH_3	Less retention than C_8 or C_{18}; applications are similar, also used for purification and preparative (RP)

A longer carbon chain means a less polar stationary phase and a higher retention of non-polar solutes.

Phase	Specification	Characteristics*			Stability	Structure	Application	Similar phases**	Separation principle · Retention mechanism	
C_{18} / C_8 Gravity	octadecyl phase, high density coating multi-endcapping C_{18} Gravity: 18% C · USP **L1** C_8 Gravity: 11% C · USP **L7**	A	C_{18}	⬡⬡⬡⬡⬡	pH stability 1 – 11, suitable for LC/MS		in general compounds with ionizable functional groups such as basic pharmaceuticals and pesticides for C8 Gravity generally shorter retention times for nonpolar compounds	**NUCLEOSIL® C_{18} HD** Waters Xterra® RP_{18} / MS C_{18}; Phenomenex Luna C18 (2), Synergi™ and Max RP; Zorbax Extend C18; Inertsil ODS III; Purospher RP-18; Star RP-18 **NUCLEOSIL® C_8 HD**; Waters Xterra® RP_8 / MS C_8; Phenomenex Luna C8; Zorbax Eclipse; XDB-C8	**only hydrophobic interactions** (van der Waals interactions)	
			C_8	⬡⬡◖						
		B	C_{18}	◖						
			C_8	⬡						
		C	C_{18}	⬡⬡◖						
			C_8	⬡◖						
C_{18} Isis	octadecyl phase with specially crosslinked surface modification endcapping 20% C · USP **L1**	A		⬡⬡⬡⬡◖	pH stability 1 – 10, suitable for LC/MS		high steric selectivity, thus suited for separation of positional and structural isomers, planar/nonplanar molecules	**NUCLEOSIL® C_{18} AB** Inertsil ODS-P; YMC Pro C18RS	steric interactions and **hydrophobic interactions**	
		B		⬡⬡						
		C		⬡⬡⬡⬡⬡⬡						
C_{18} Pyramid	C_{18} modification with polar endcapping 14% C · USP **L1**	A		⬡⬡⬡⬡	stable against 100% aqueous eluents, pH stability 1 – 9, suitable for LC/MS		basic pharmaceutical ingredients, very polar compounds, organic acids	Phenomenex Aqua; YMC AQ; Waters Atlantis® dC18	**hydrophobic interactions** and **polar interactions** (H bonds)	
		B		⬡⬡◖						
		C		⬡⬡						
Sphinx RP	bifunctional RP phase, propylphenyl and C_{18} ligands; endcapping 15% C; USP **L1** and **L11**	A		⬡⬡⬡	pH stability 1 – 10, suitable for LC/MS		compounds with aromatic and multiple bond systems	no similar phases	**π-π interactions** and **hydrophobic interactions**	
		B		⬡⬡◖						
		C		⬡						
C_{18} ec C_8 ec	octadecyl / octyl phase, medium density coating endcapping C_{18} ec: 17.5% C · USP **L1** C_8 ec: 10.5% C · USP **L7**	A	C_{18}	⬡⬡⬡⬡	pH stability 1 - 9		robust C18 / C8 phase for routine analyses	**NUCLEOSIL® C_{18}** Spherisorb® ODS II; Hypersil ODS; Waters Symmetry® C18; Inertsil ODS II; Kromasil C18; LiChrospher RP 18 **NUCLEOSIL® C_8 ec / C_8** Spherisorb® C8; Hypersil MOS; Waters Symmetry® C8; Kromasil C8; LiChrospher RP 8	**only hydrophobic interactions** (van der Waals interactions) some residual silanol interactions	
			C_8	⬡⬡						
		B	C_{18}	⬡						
			C_8	⬡◖						
		C	C_{18}	⬡⬡⬡◖						
			C_8	⬡⬡◖						
HILIC	zwitterionic ammonium sulfonic acid modification 7% C	A		⬡	pH stability 2 - 8.5, suitable for LC/MS		hydrophilic compounds such as organic polar acids and bases, polar natural compounds	Merck Sequant ZIC®-HILIC; Sielc Obelisc™	**ionic / hydrophilic interactions, electrostatic interactions**	
		B		⬡⬡⬡⬡⬡						
		C		-						
CN / CN-RP	cyano (nitrile) phase for NP and RP separations 7% C · USP **L10**	A		⬡	pH stability 1 - 8, stable towards highly aqueous mobile phases		polar organic compounds (basic drugs), molecules containing π electron systems	**NUCLEOSIL® CN / CN-RP**	**π-π interactions, polar interactions (H bonds), hydrophobic interactions**	
		B		⬡⬡⬡⬡						
		C		-						
NH_2 / NH_2-RP	amino phase for NP and RP separations 2.5% C · USP **L8**	A		⬡	pH stability 1 - 8, stable towards highly aqueous mobile phases		sugars, sugar alcohols and other hydroxy compounds, DNA bases, polar compounds in general	**NUCLEOSIL® NH_2 / NH_2-RP**	**polar /ionic interactions, hydrophobic interactions**	
		B		⬡⬡⬡⬡						
		C		-						
SiOH	unmodified high purity silica USP **L3**	A		-	pH stability 2 - 8		polar compounds in general	**unmodified NUCLEOSIL®**	**polar /ionic interactions**	
		B		n.a.						
		C		-						

* A = ⬡ hydrophobic selectivity, B = ⬡ polar/ionic selectivity, C = ⬡ steric selectivity

** phases which provide a similar selectivity based on chemical and physical properties


Thank You!