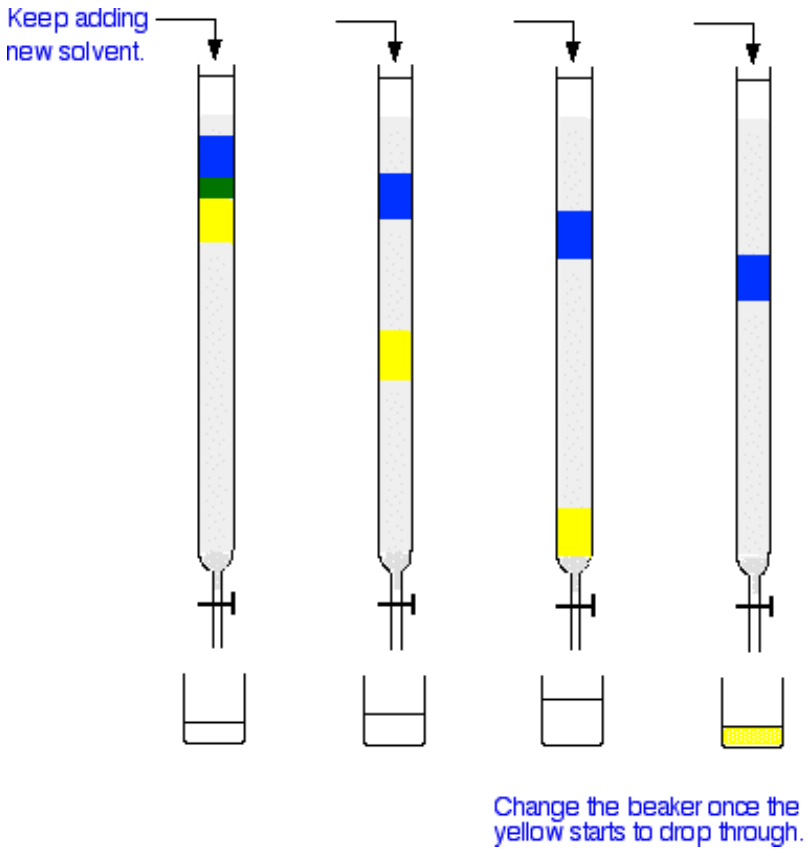
➢ SOLID PHASE EXTRACTION

ADSORPTION CHROMATOGRAPHY (LSC)

Separation of mixture of analytes and coextractives (crude extract) using sorbent column in glass column equipped with frit (approx. 50 cm x 1 cm)

Usually selective irreversible adsorption of unwanted compounds (lipids, pigments) and elution of analytes using suitable solvent

Extract applied in starting solvent (used for column preparation and stabilisation)



➢ SOLID PHASE EXTRACTION

ADSORPTION CHROMATOGRAPHY (LSC)

SORBENTS:

- *activation* (water removal by the annealing in the furnace)
- *deactivation* (standardisation of water content – reduction of sorption)

Silica gel (silica): SiO_2 x H_2O, usually deactivated, weakly acidic – unsuitable for separation of strongly alkaline analytes (strong sorptions); possible impregnation with $AgNO_3$

Florisil: magnesium silicate, lipids removal and other less polar compounds, necessary to activate by heating immediately before application (130°C, already annealed in the furnace)

➢ SOLID PHASE EXTRACTION

ADSORPTION CHROMATOGRAPHY (LSC)

SORBENTS:

Aluminium oxide (alumina): separation of not too polar compounds (pigments trapping), usually weakly alkaline (pH10) – can decompose esters, rinsing by HCl and H_2O neutral reaction is obtained (pH7.5) – weaker sorption, acidic (pH 4.5); possible impregnation with $AgNO_3$

Magnesium oxide: weak affinity to double bonds, mostly in mixtures of sorbents (with diatomaceous earth)

Activated carbon, charcoal: nonpolar sorbent, often irreversible sorption, pigments removal

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

Interaction of 3 components: SOLID PHASE / ANALYTE / SOLVENT

FREQUENT GOAL = "DIGITAL CHROMATOGRAPHY"

a) retention of analyte, elution (breakthrough) of interferences

b) retention of interferences, elution of analytes

S P E columns:

Body material: polypropylene, glass
Frit: 20 μm, polyethylene, steel
Sorbent: predominantly silica gel, particle size 40 μm, pores size 60 Å
Volume: various – e.g. 1, 3, 6 ml
Sorbent amount: various – e.g. 100, 500 mg, 1, 2, 5, 10 g
Column capacity:10 - 20 mg of analyte / g of sorbent (coextractives!)
Breakthrough volume: sorbent saturation - not retaining analytes

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

SPE realisation - extraction modes

A) S e l e c t i v e e x t r a c t i o n
retention of analytes, other components of sample - no retention, elution of analytes

B) S e l e c t i v e e l u t i o n
retention of analytes and other components of sample, selective elution of analytes

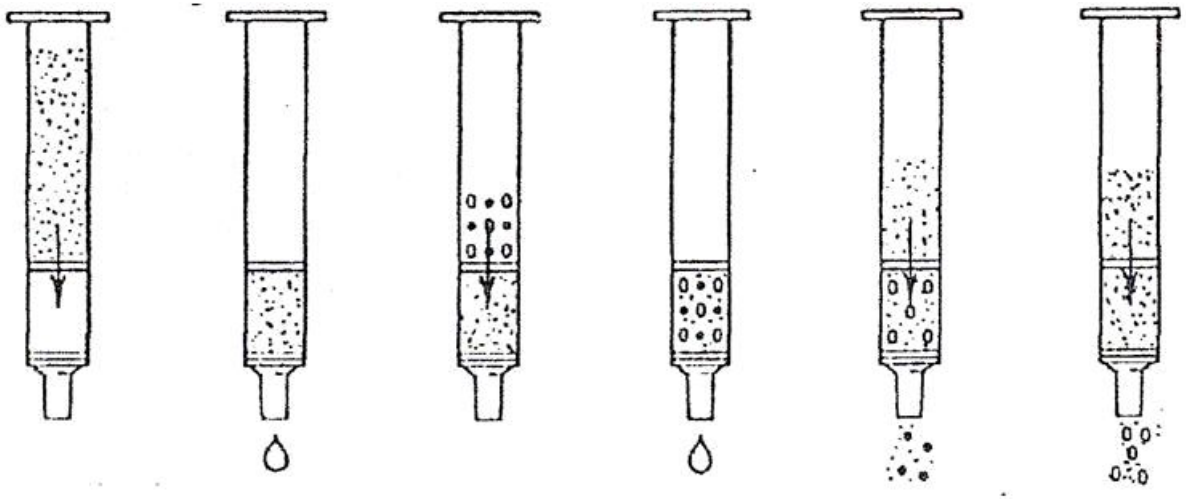
C) S e l e c t i v e r i n s i n g
retention of analytes and other components of sample, rinse of interfering compounds (analytes sorbed), elution of analytes

D) R e m o v a l o f s a m p l e m a t r i x
retention of sample components, analytes eluted with no retention

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

GENERAL SPE SCHEME



Coextractives
Analytes

Conditioning Sample introduction Rinsing Elution

Driving force: suction – vacuum, overpressure, centrifugation

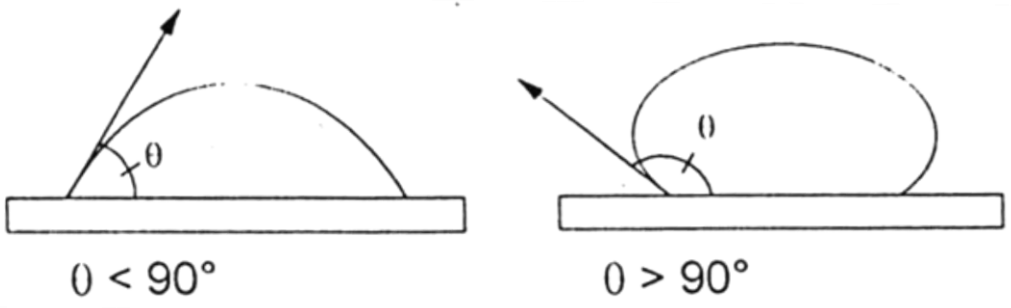
➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

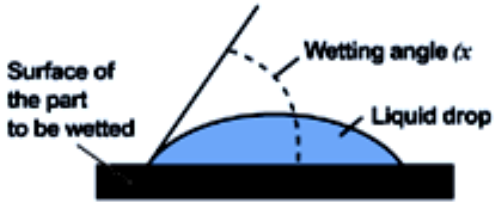
GENERAL SPE SCHEME

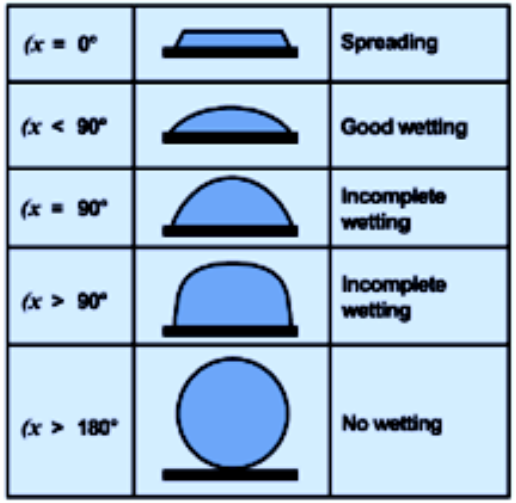
1. **C o n d i t i o n i n g** (1 - 2 ml / 100 mg of sorbent)
   washing of impurities (the same strength as for sample)
   changing to solvent of sample (lesser or the same strength)

WETTING OF SURFACE WITH LIQUID:
contact angle less than 90°



WETTED UNWETTED





➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

GENERAL SPE SCHEME

2. Sample introduction

treatment (pH, ionic strength, dilution,
decreasing of analyte solubility in sample)

volume (↑ volume → removal of conditioning solvent → ↓ efficiency, yield)

flow rate (max. 5 ml/min, sufficient contact sample - phase)

Volume affects:

- size and type of column
- concentration of analyte
- amount of interferences
- analyte retention in sorbent

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

GENERAL SPE SCHEME

3. **R i n s i n g** (0.5 ml / 100 mg of sorbent)
   washing of impurities (the same or greater strength as for sample)

4. **E l u t i o n** (0.5 ml / 100 mg of sorbent)
   solvent type (like dissolves like, easy to evaporate)
   solvent volume (2x dead volume, approx. 2x 100 µl / 100 mg of sorbent

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

REASONS FOR SPE APPLICATION

- Removal of interfering compounds
- Concentration of analyte
- Group fractionation
- Change of solvent for sample
- Desalination

The goal:
removal of interferences or maximum concentration – decreasing of LODs

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

SELECTION OF SOLVENTS

„STRENGTH" of solvent pro given system „weak“ solvent = analyte sorption

„strong“ solvent = analyte elution

NORMAL PHASE	WEAK	REVERSE PHASE
Hexane		Water
Isooctane		Methanol
Toluene		Isopropyl alcohol
Chloroform		Acetonitrile
Methylene chloride		Acetone
Tetrahydrofuran		Ethyl acetate
Ethyl ether		Ethyl ether
Ethyl acetate		Tetrahydrofuran
Acetone		Methylene chloride
Acetonitrile		Chloroform
Isopropyl alcohol		Toluene
Methanol	STRONG	Isooctane
		Hexane

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

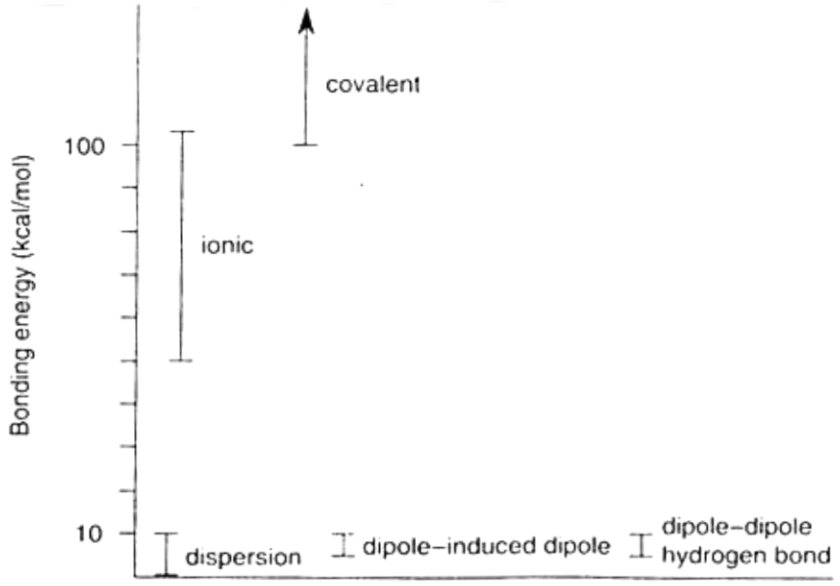
BONDING INTERACTION: ANALYTE - SORBENT

A) Hydrophobic interaction: dispersion forces (van der Waals)
nonpolar phase, energy 1-10 kcal/mol

B) Polar interaction: hydrogen bond, dipole-dipole,
polar phase, energy 5 - 10 kcal/mol

C) Ionic (electrostatic) interaction: 50 - 200 kcal/mol

BONDING ENERGY



➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

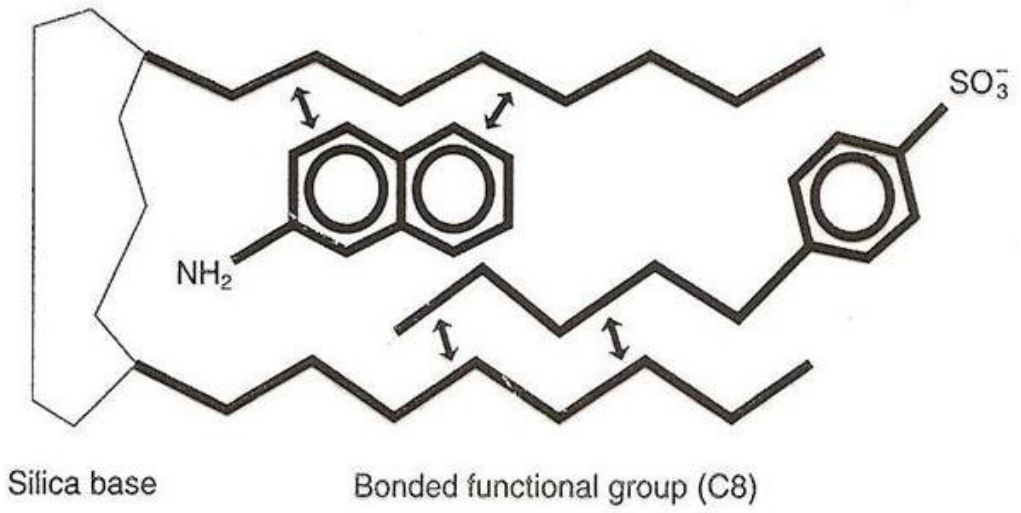
BONDING INTERACTION: ANALYTE - SORBENT

A) Hydrophobic interactions - dispersion forces (van der Waals)

nonpolar phase, energy 1-10 kcal/mol

interaction between nonpolar molecules as a result of induced dipoles formation

- attractive and repulsive
- weaker than hydrogen bond or dipole-dipole interaction



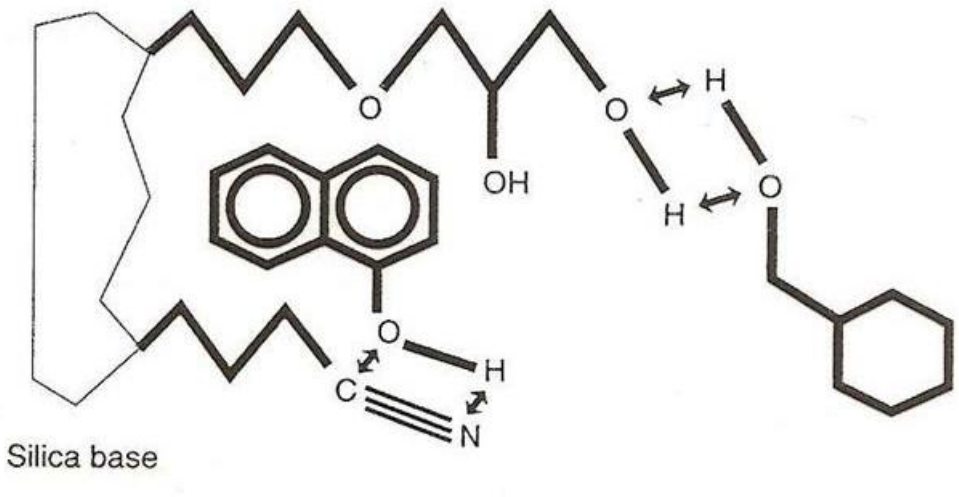
➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

BONDING INTERACTION: ANALYTE - SORBENT

B) Polar interactions – polar phase, energy 5 - 10 kcal/mol

- **hydrogen bond -** *between molecules containing covalently bonded hydrogen to strongly electronegative element (O, N, F)*
- **dipole-dipole -** between *polar molecules with permanent dipole moment*



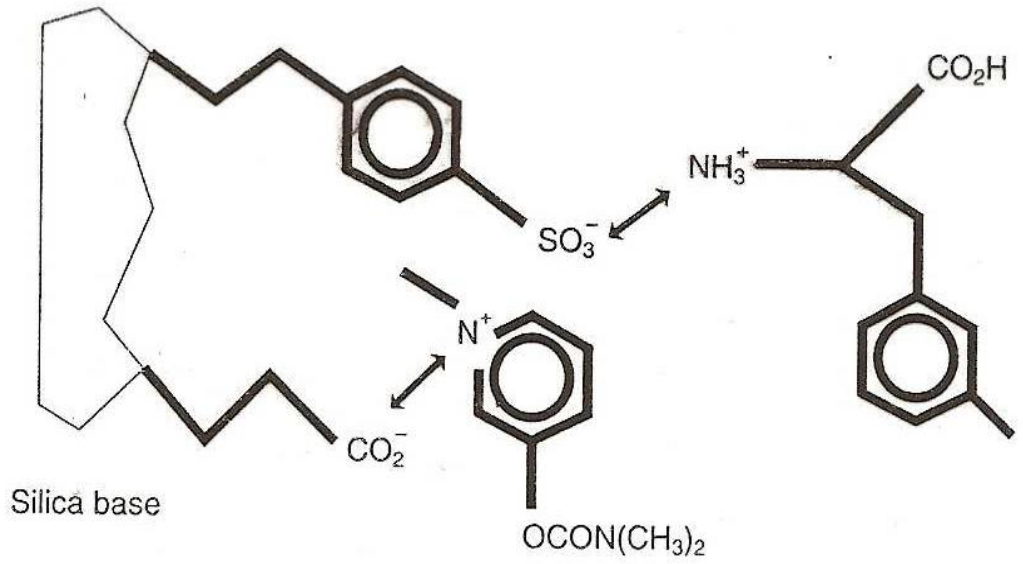
➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

BONDING INTERACTION: ANALYTE - SORBENT

C) Ionic (electrostatic) interaction: 50 - 200 kcal/mol

- *between oppositely charged groups of ion exchanger and analyte*



➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

PHASES USED FOR SPE

Phase selection:

- analyte character
- solvent of sample
- type of interferences

High retention of analytes: **a similar polarity of analyte and phase**

Interferences: less polar than analyte - normal phase
more polar than analyte - reverse phase

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

PHASES USED FOR SPE

A) N o r m a l p h a s e - polar

- silica gel, aluminium oxide, Florisil, polar modified silica gel (CN, diol, NH_2)

B) R e v e r s e p h a s e - nonpolar

- nonpolar modified silica gel (C_{18}, C_8, C_4, CH, PH, CN)

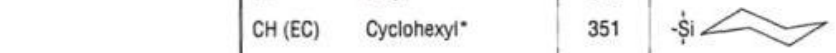
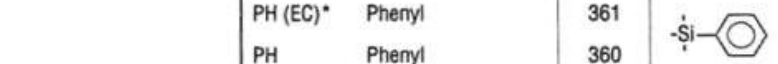
C) I o n e x c h a n g e r

- ANEX – silica gel with chemically bonded positively charged modifier (quaternary amine, secondary amine)
- CATEX – silica gel with chemically bonded negatively charged modifier (benzene or propylsulfonic acid)

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

PHASES USED FOR SPE

Description		Sorbent Ref No.	Sorbent structure	Available retention mechanism: Non-polar	Polar	Ion-exchange
Non-polar sorbents (with available retention mechanisms in aqueous samples)						
C18 (EC)*	Octadecyl	221		❶	③	③
C18	Octadecyl	220	-Si-$C_{18}H_{37}$	❶	②	②
MF C18	Octadecyl[1]	240		❶	②	②
C8 (EC)*	Octyl	291		❶	③	③
C8	Octyl	290	-Si-C_8H_{17}	❶	②	②
C2 (EC)*	Ethyl	321		❶	③	③
C2	Ethyl	320	-Si-C_2H_5	❶	②	②
CH (EC)	Cyclohexyl*	351		❶	③	③
PH (EC)*	Phenyl	361		❶	③	③
PH	Phenyl	360		❶	②	②
CN (EC)*	Cyanopropyl	421	-Si-$CH_2CH_2CH_2CN$	❶	③	③
Polar sorbents (with available retention mechanisms in non-aqueous samples)						
Silica		460	-Si-OH		❶	③
NH_2	Aminopropyl	470	-Si-$CH_2CH_2CH_2NH_2$		❶	②
DIOL	2,3-Dihydroxypropoxypropyl	430	-Si-$CH_2CH_2CH_2OCH_2$CH(OH)-CH_2(OH)		❶	
CN	Cyanopropyl	420	-Si-$CH_2CH_2CH_2CN$		❶	
Ion-exchange sorbents (with available retention mechanisms in aqueous samples)						
NH2	Aminopropyl	470	-Si-$CH_2CH_2CH_2NH_2$ (pKa = 9.8)	③		❶
SAX	Trimethylaminopropyl (Quaternary amine)	500	-Si-$CH_2CH_2CH_2\overset{\oplus}{N}$-$(CH_3)_3$ $Cl^{\ominus}$	③		❶
CBA	Carboxypropyl	520	-Si-$CH_2CH_2CH_2COOH$ (pKa = 4.8)	③		❶
SCX	Benzenesulphonic acid	530		②		❶
PRS	Propylsulphonic acid	540	-Si-$CH_2CH_2CH_2SO_3^{\ominus}H^{\oplus}$	③		❶

* (EC) = End-capped [1] MF C18: manufactured from a monofunctional silane

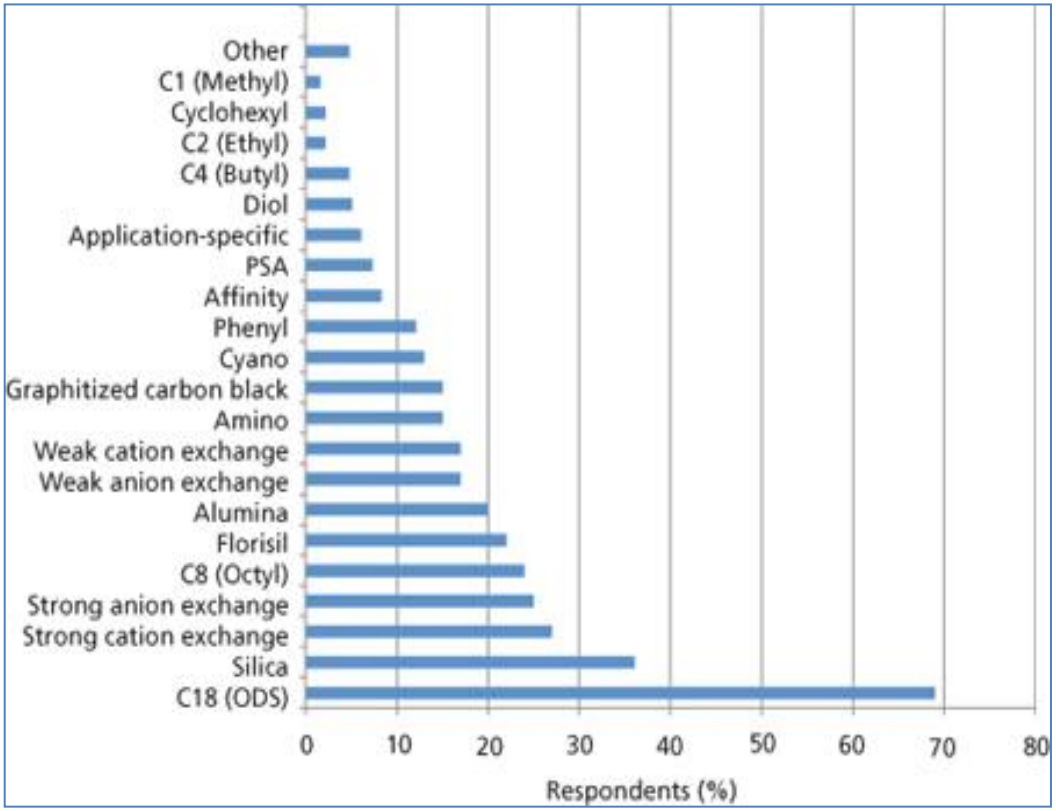
Key to available retention mechanisms

❶ = Primary ◯ = Secondary ◯ = Silanol cation-exchange **2** = Strong **3** = Weak

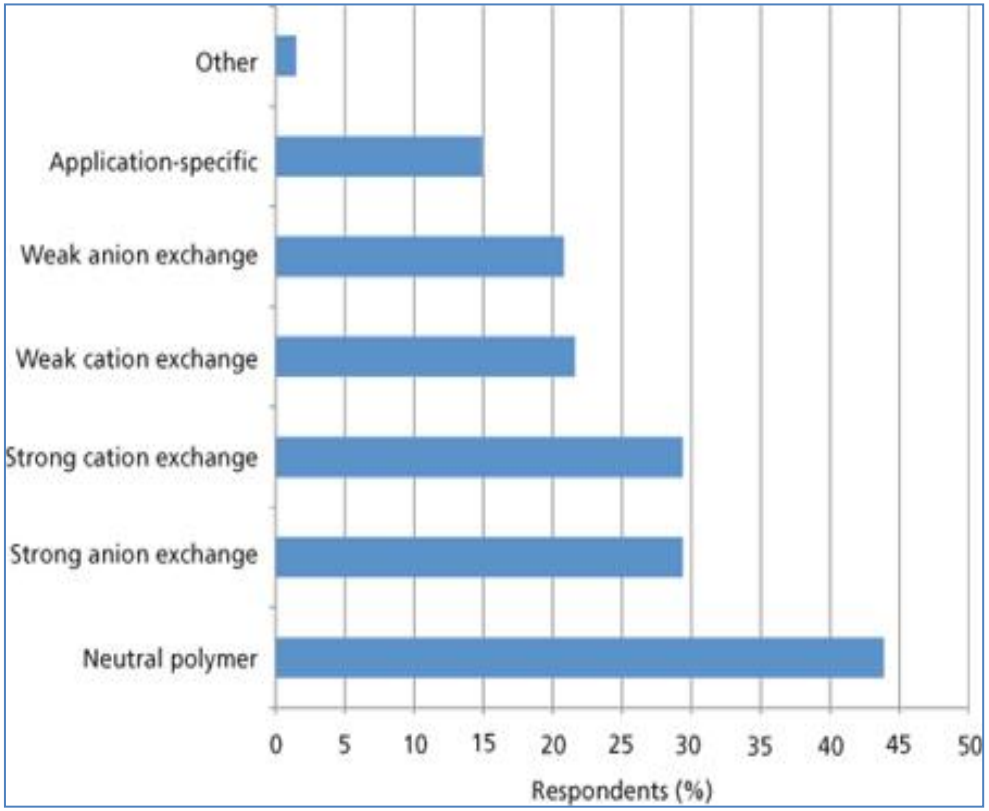
➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

PHASES USED FOR SPE



Silica-based phases use.



Polymer-based SPE phases used.

R.E. Majors: Trends in Sample Preparation , LCGC North America, Vol. 31, Issue 3, pp. 190-203, Mar 1, 2013

➢ SOLID PHASE EXTRACTION

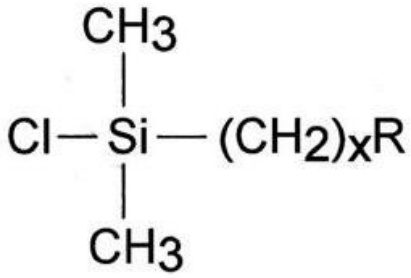
COLUMNS, CARTRIDGES

SYNTHETHIS OF CHEMICALLY BONDED SORBENTS

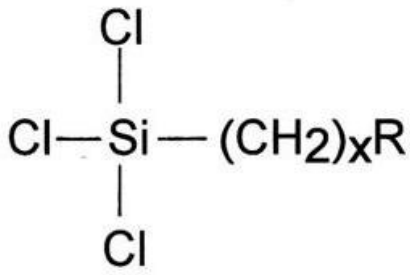
SILICA GEL + DERIVATISATION AGENT → SILOXANES

Derivatisation agents: mono- up to tri- halo or alkoxy silyl derivatives
alkylchlorsilanes

a) Monofunctional (M)



b) Trifunctional (T)



➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

SYNTHETHIS OF CHEMICALLY BONDED SORBENTS

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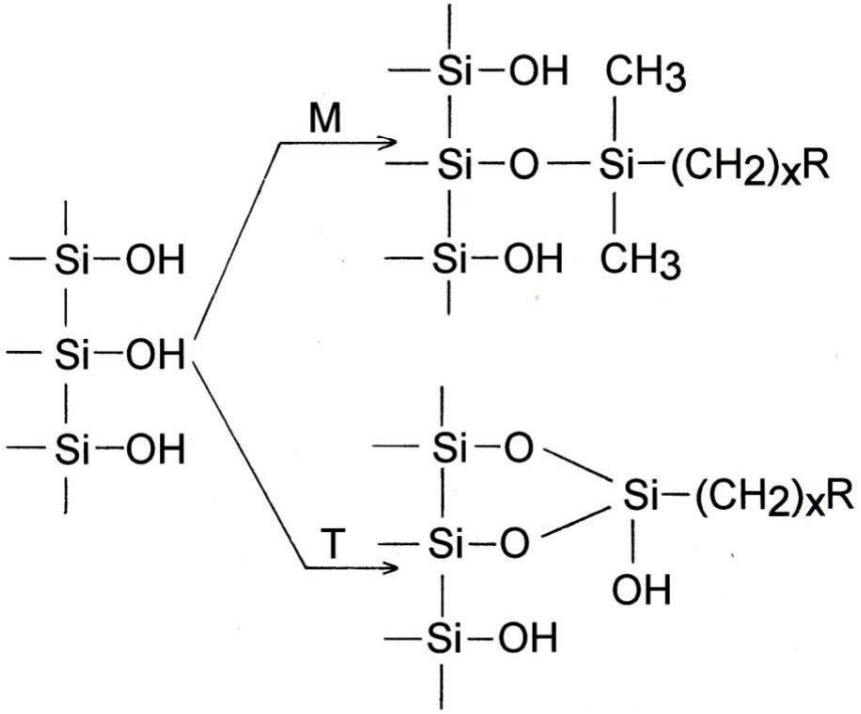


➢ SOLID PHASE EXTRACTION

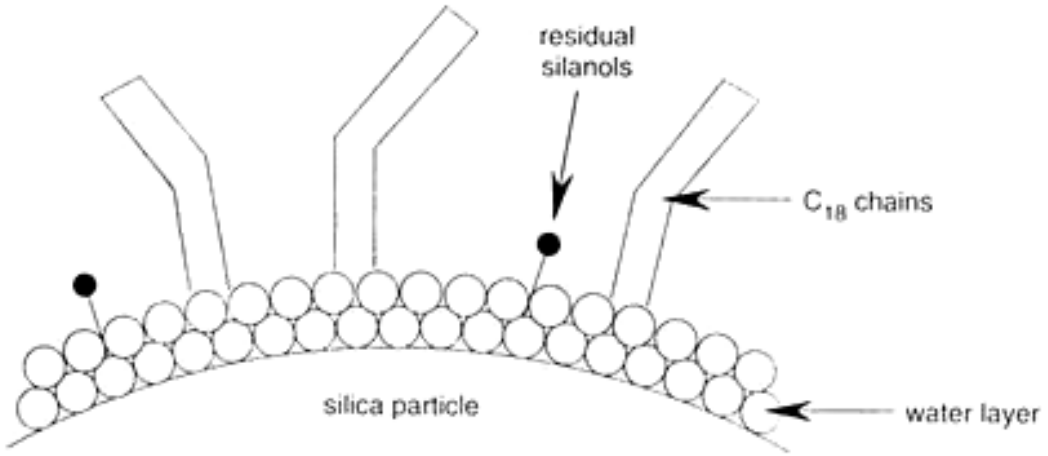
COLUMNS, CARTRIDGES

LIMITATIONS OF SILICA-BASED SORBENTS

Possible drawbacks:

- necessary conditioning - must not dry out X automation
- residual silanol groups - retention of alkaline compounds
- limited stability depends on pH (2-8)
- ↓ pH ⇒ hydrolysis of bonded phase
- ↑ pH ⇒ dissolution of silica gel
- insufficient retention of more polar analytes
- nonselective (cleanness of extract!)
- too strong (irreversible) sorptions of nonpolar analytes

SURFACE OF C18-silica SPE particle



➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

LIMITATIONS OF SILICA-BASED SORBENTS

SILICA GEL (normal phase)
- **strong sorption of very polar compounds** (glycerol)
  → application of modified silica gels (weaker retention)

C18 (reverse phase):
- **nonselective, sometimes too strong sorptions**
  → application of phases with more polar modifiers (C_8, C_4)
    - part of coextractives passes without retention

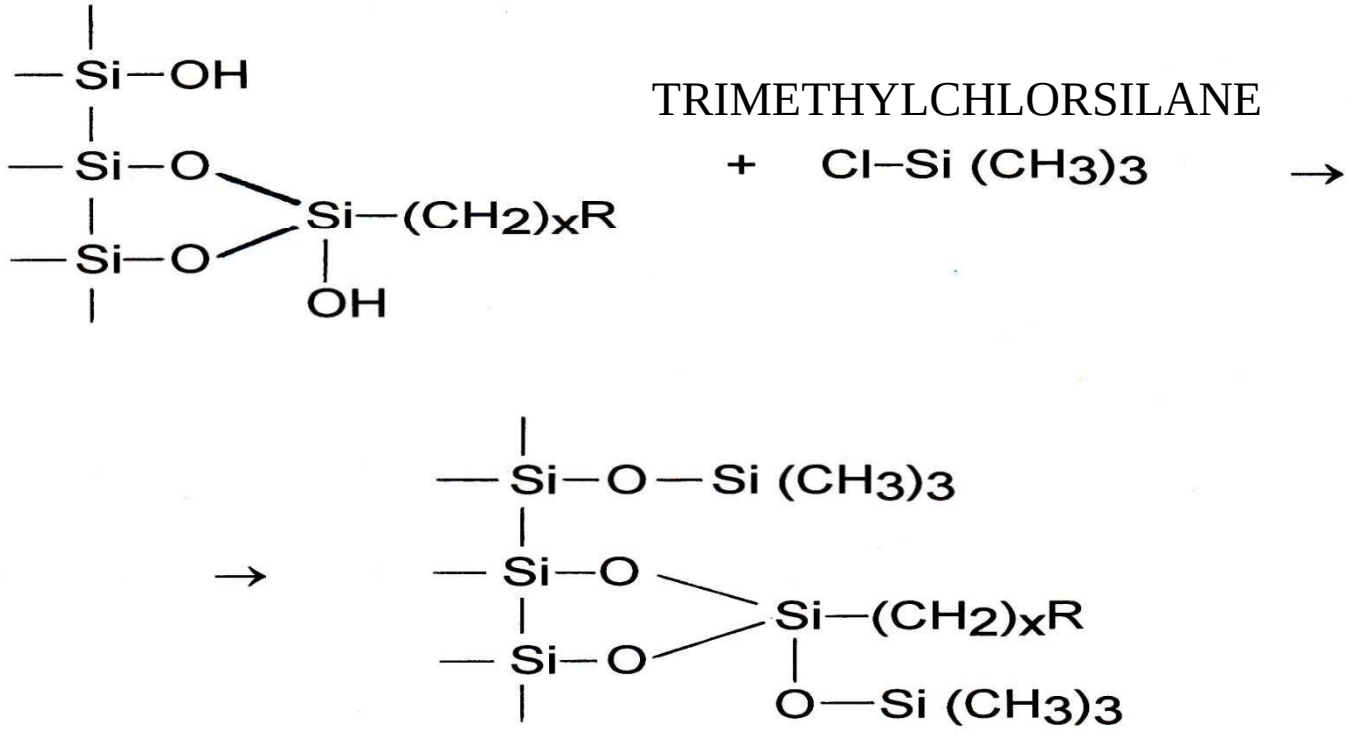
APPLICATION OF PHASES BASED ON PS-DVB: without silanols
- stable in wide range of pH – better retention of polar analytes

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

ACTIVITY OF RESIDUAL SILANOL GROUPS

Suppression: endcapping – 70% in maximum (steric reasons)



➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

ACTIVITY OF RESIDUAL SILANOL GROUPS

Suppression - endcapping – 70% in maximum (steric reasons)

- pH adjustement (at pH2 silanol is undissociated,
  at pH>2 silanol is dissociated
  → negative charge
  → electrostatic interactions)
- masking of silanols – using bases (triethylamine)
- increasing of the ionic strength of sample solvent
  (prevention of analyte bonding)

Support - pH adjustment ($\geq$4 – ionisation of silanols)

- nonendcapped phases

➢ SOLID PHASE EXTRACTION

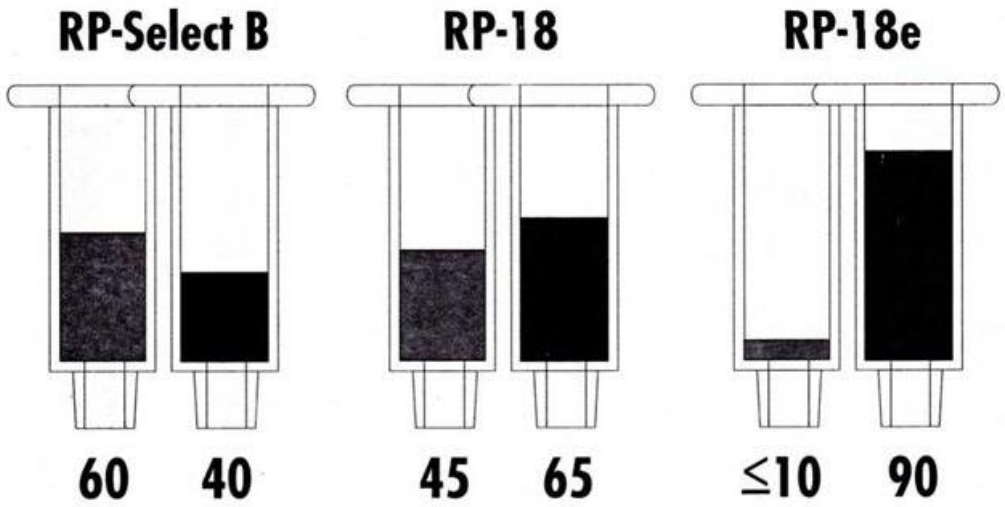
COLUMNS, CARTRIDGES

TRENDS IN DEVELOPMENT OF SPE SORBENTS

Merck:

3 types of sorbents based on C18-silica differing in modification of silica gel basis

Comparison using capacity of hydrophilic (caffeine) and lipophilic (diisodecylphthalate)



Caffeine - L **DIDP - R**

Capacity = mg of analyte / g sorbent)

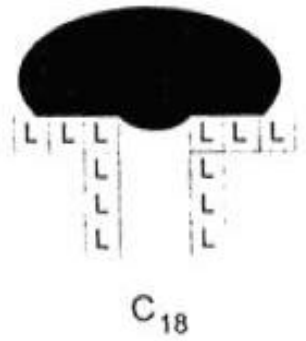
➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

TRENDS IN DEVELOPMENT OF SPE SORBENTS

Waters: **OASIS™ HLB – hydrophilic-lipophilic sorbent**
copolymer of N-vinylpyrrolidone (increasing water wettability)
and divinylbenzene (RP)

- drying out do not decrease yield (automation)
- higher stability in wider range of pH (x C18)
- higher retention mainly for polar compounds (x C18)
- universal (also for polar and alkaline analytes)



Sorbents wetting:
C18 and OASIS™ HLB
H-hydrophilic part
L-lipophilic part

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

TRENDS IN DEVELOPMENT OF SPE SORBENTS - MIXED SORBENTS

Supelclean™ ENVI-Carb II / PSA SPE - retention RP + ANEX
„chemical filter“ – removal of key interferences in pesticides analysis

Upper layer - Supelclean™ ENVI-Carb II:
- nonporous *graphitized carbon*
- surface area 100 m^2/g
- affinity to planar molecules
- high retention of pigments (chlorophyll, carotenoids) and sterols

Lower layer - Supelclean™ PSA:
- *N-propylethylendiamin* (primary-secondary amine)
- high retention of fatty acids, organic acids and some polar pigments and sugars

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

TRENDS IN DEVELOPMENT OF SPE SORBENTS

Carbograph – *graphitized carbon*
(heating of soot to 2700 – 3000°C in inert atmosphere)

- retention RP + ANEX
- nonporous, nonpolar
- surface area up to 100 m^2/g
- surface contains oxygen compounds interacting with acidic compounds (separation from bases and neutrals without pH adjustment)
- quantitative extraction of very polar compounds from large volumes

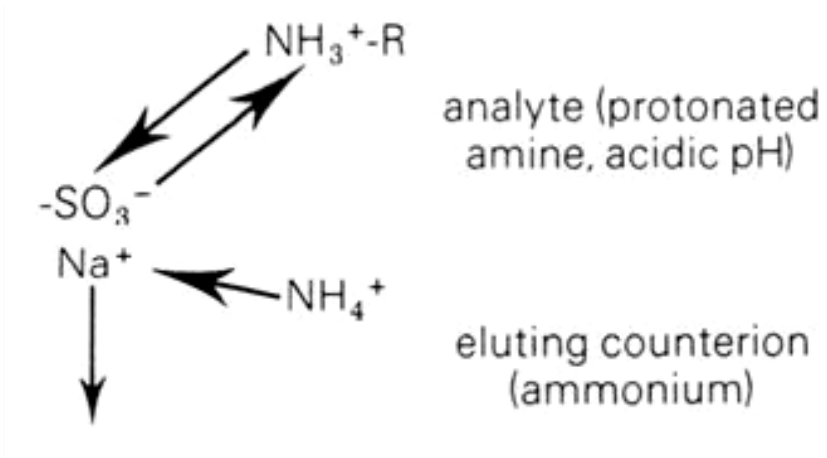
➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

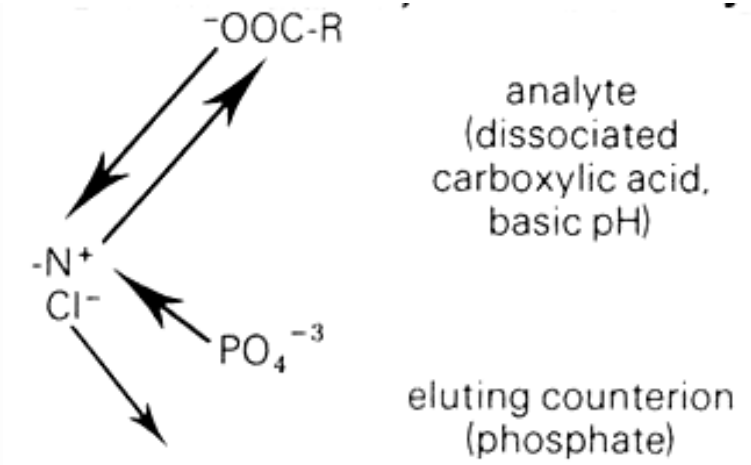
ION EXCHANGERS

Extraction of acids and bases from aqueous solutions according to ion exchange rules (interaction of charged analyte and oppositely charged ionex)

Strong CATEX - benzenesulfonic acid
Extraction of bases (positive ions)



Strong ANEX - quaternary amine
Extraction of acids (negative ions)



Results of extraction depends on pH, ionic strength and counterion type.

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

ION EXCHANGERS

pH – analytes must be ionised – according to pK_a value → pH adjustment 2 units above or below pK_a (acetic acid 4.75, cyclohexylamine 10.66)

Ionic strength – total concentration of ions in sample
- competition with analyte for binding sites on ionex

Counterion type

CATEX: Li^+, H^+, Na^+, NH_4^+ - easy changeable
Cu^{2+}, Ca^{2+}, Ba^{2+} - difficulty changeable

ANEX: OH^-, F^- - easy changeable
HSO^{3-}, NO^{3-}, CN^-, Cl^- - difficulty changeable

➢ SOLID PHASE EXTRACTION

COLUMNS, CARTRIDGES

ION EXCHANGERS

CATEX – extraction of bases (positive ions)

	pH*	Ionic strength **	Counterion type
RETENTION	< pK_a of analyte > pK_a of sorbent	↓ ↓	↓ ↓
ELUTION	> pK_a of analyte < pK_a of sorbent	↑ ↑	↑ ↑

ANEX – extraction of acids (negative ions)

	pH*	Ionic strength **	Counterion type
RETENTION	> pK_a of analyte < pK_a of sorbent	↓ ↓	↓ ↓
ELUTION	< pK_a of analyte > pK_a of sorbent	↑ ↑	↑ ↑

* … at least for 2 pH units; ** ↓ …< 0.1 M; ↑ …< 0.1 M

➢ SOLID PHASE EXTRACTION

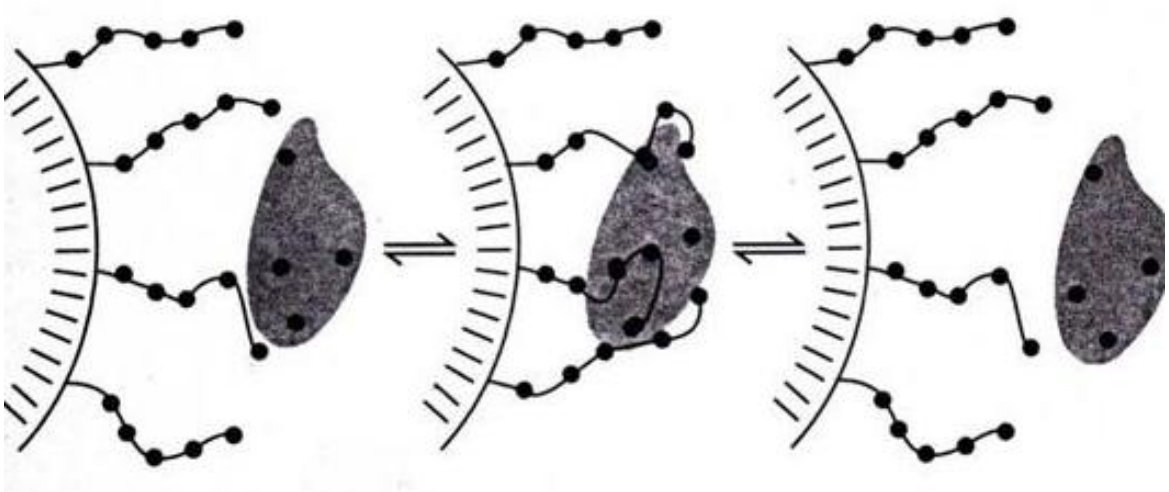
COLUMNS, CARTRIDGES

TENTACLE ION EXCHANGERS

- functional groups located along the moving chain
  - covalently bonded, composed of 5-20 monomers

- (classic ion exchanger – functional groups bonded on the support in the rigid position)

- good accessibility of analytes to functional groups = fast mass transport, high capacity, small elution volumes



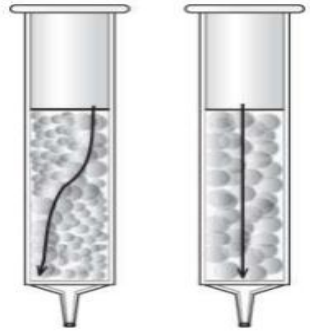
➢ SOLID PHASE EXTRACTION

EXTRACTION DISKS

DRAWBACKS of SPE columns:

- limited flow rate (small ratio of flow area and sorbent column)
- formation of channels (inhomogeneity of sorbent, space between particles)

→**non-uniform flow rate** → ↓ **sorption capacity and reproducibility**



SPE DISKS characterisation:

Flat disks: similar to membrane filters
Thickness: ≤ 1 mm
Diameter: 4 - 96 mm
↑diameter →↑ surface →↑flow rate

A) R i g i d d i s k s
B) M e m b r a n e d i s k s

➢ SOLID PHASE EXTRACTION

EXTRACTION DISKS

ADVANTAGES of SPE disks:

- **larger flow area** – thin layer, small pressure difference →↑ flow rates
- **reduced mass of sorbent** → ↓ dead volume → ↓ sample volume
  ↓ elution solvent volume
  ↓ interferences (less retention)
- **without channels formation** → ↑ retention efficiency, capacity and yield
- **faster drying**
- **time saving** (1 l of water: 10 min for 45 mm disk, 2 h for column)

XXX limited number of phases

➢ SOLID PHASE EXTRACTION

EXTRACTION DISKS

A) R I G I D D I S K S

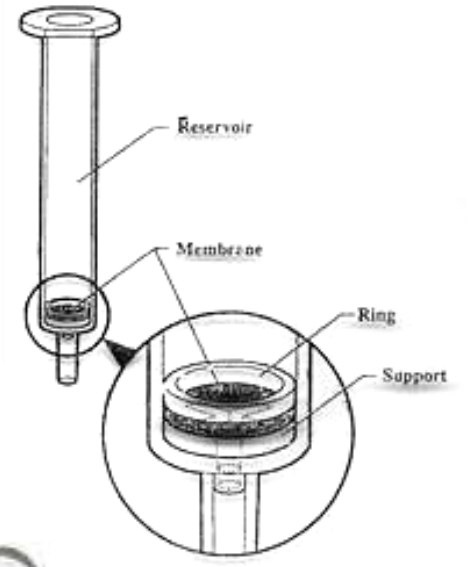
- rigid glass fibers with embedded phase (silica gel, modified silica gel)
- compare to membrane disks: cheaper, quicker, less risk of blockage

SPEC m i c r o c o l u m n s (Ansys)

- polypropylene body, no frits
- silica gel, NH_2, CN, PH, C2, C8, C18, catex, anex

ENVI ™ d i s k s (Supelco, Inc.)

- diameter 47 and 90 mm
- C8, C18



➢ SOLID PHASE EXTRACTION

EXTRACTION DISKS

Change of sorbent mass – change of diameter or thickness of membrane
change of sorbent porosity

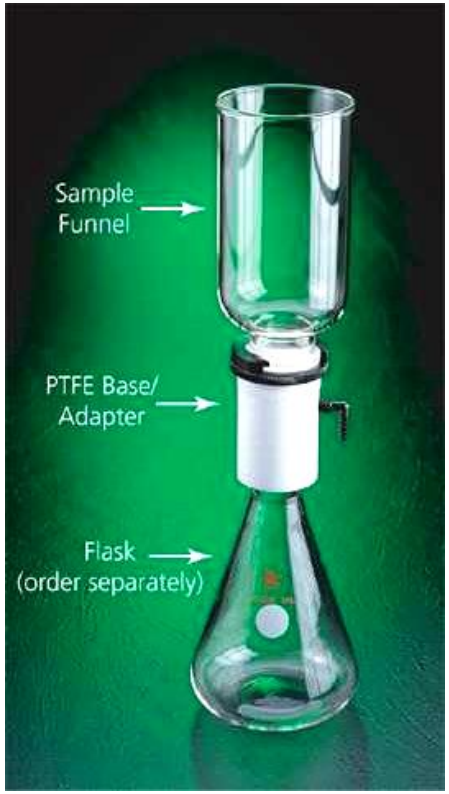
Diameter (mm)	7.5		12.1		47
Mass (mg)	1.5	15	3.5	35	580
Thickness (mm)	0.4	1	0.4	1	0.6
Dead volume (µl)	10	50	25	115	600
Particle size (µm)	5	7	5	7	5
Pore size (Å)	85	70	85	80	80

➢ SOLID PHASE EXTRACTION

EXTRACTION DISKS

B) M E M B R A N E D I S K S

- elastic net based on PVC or PTFE with chemically bonded stationary phase
- compare to rigid disks: smaller flow rates and greater risk of blockage → prefiltration



EMPORE ™ d i s k s (3M Corp. + Varian)

10 % PTFE + 90% silica gel phase

C8, C18, PS-DVB, anex, catex

diameter:

standard - 25, 47, 90 mm

reduced in columns - 4, 7, 11 mm

➢ SOLID PHASE EXTRACTION

EXTRACTION DISKS

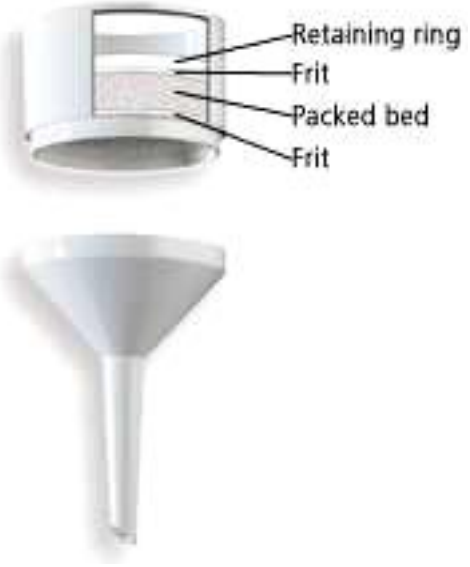
- **VersaPure™ Büchner funnel** (prefilled - disposable)

Polypropylene body

Polyethylene frits (20 μm)

0.7 μm membrane from glass fibers below bottom frit

12.5 – 100 g of sorbent



- **Discovery SPE 96-Well Plates** (prefilled - disposable)

2ml polypropylene oblong plates with 96 positions

25 – 100 mg of sorbent / position