QFL 5736-2023
Extração em Fase Sólida: Princípios, Aplicações e Tendências

Programação

Dia ou Semana		Aula
21/08	1	Apresentação/Início
28/08	2	Desenvolvimento de métodos – Questões artigo 1
11/09	3	Mecanismos de Fase Normal e Reversa - Questões artigo 2
18/09	4	Experimento 1
25/09	5	Mecanismos de troca iônica e modos mistos - Questões artigo 3
02/10	6	Experimento 2
09/10	7	Reconhecimento molecular - Questões artigo 4
16/10	8	Experimento 3
23/10	9	Seminários 1, 2, 3
30/10	10	Seminários 4, 5, 6
06/11	11	Prova

Critério de Avaliação

$$MF = \frac{Q + S + 2T}{4}$$

Q = média aritmética das questões sobre artigos
S = nota do seminário
T = nota da prova teórica

Referências:

E. M. Thurman and M.S. Mills, Solid-Phase Extraction. Principles and Practices. John Wiley & Sons, Inc., New York, 1998

Marie-Clare Hennion, Solid Phase Extraction: Method Development sorbents and coupling with liquid chromatographs. J. Chromatogr. A 856 (1999) 3-54

S. Moldeveanu, V. David, Modern Sample Preparation for Chromatography, Elsevier 2015, Chapter 7 (Solid Phase Extraction)
ISBN 978-0-444-54319-6 (Livro disponível pelo VPN USP)

Recent Trends in Solid-Phase Extraction for Environmental, Food and Biological Sample Preparation, Chromatographia, Volume 82, Issue 8, August 2019

Outros

Artigos para leitura:

Artigo 1: M. Rigobello-Masini, E.A.O. Pereira, G. Abate, J.C. Masini, Solid-Phase Extraction of Glyphosate in the Analyses of Environmental, Plant, and Food Samples, Chromatographia 2019, 82: 1121 - 1138

Artigo 2: I. Hanke, H. Singer, J. Hollender, Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography–tandem mass spectrometry: performance tuning of derivatization, enrichment and detection, Anal. Bioanal. Chem. Anal. Bioanal. Chem. 2008, 391: 2265 - 2276

Artigo 3: N. Fontanals, R.M. Marcé, F. Borrull, Solid-phase extraction followed by liquid chromatography-high resolution mass spectrometry to determine synthetic cathinones in different types of environmental water samples, J. Chromatogr. A, 2017, 1524: 66-73.

Artigo 4: H. Shao, H. Zhou, T. Zhang, X. Zhao, Z. Jiang, Q. Wang, Preparation of molecularly imprinted hybrid monoliths for the selective detection of fluoroquinolones in infant formula powders, J. Chromatogr. A, 2019, 1588: 33-40

Temas dos Seminários

- Interação hidrofílica
- Polímeros de impressão molecular (MIPs)
- Redes Metalorgânicas (MOFs)
- Nanomateriais
- Microextração em fase sólida (CG)
- Análises de traços em ambientes aquáticos
- Materiais monolíticos
- Análise de amostras biológicas
- Barra de agitação adsortiva (SBSE – Stir bar sorptive extraction)

Solid Phase Extraction

SPE is a method of sample preparation that concentrates and purifies analytes from solution by sorption onto a properly conditioned disposable solid sorbent, followed by washing and elution of the analyte with a solvent appropriate for the instrumental analysis

Solid Phase Extraction - History

SPE developed from classical chromatography:
Use of an adsorbing medium to separate analytes according to their differing equilibrium distribution affinities between the sorbent and the mobile phase

1906 – Tswett separated plant pigments by using $CaCO_3$ as stationary phase and petroleum ether as mobile phase – The term "chromatography" was coined – Normal phase chromatography in today's nomenclature

1930's – Silica, alumina, florisil ($MgO{:}XSiO_2{\bullet}H_2O$), diatomaceous earth used for analyte concentration (today's normal phase SPE)

1941 – Marin and Synge created the partition chromatography. They adsorbed water onto silica gel and moved the sample with a solvent (chloroform modified with ethanol) to isolate and separate acetyl derivatives of amino acids (Martin, A.J.P. and Synge, R.L.M. 1941. A new form of chromatogram employing two liquid phases. J. Biochem. **35**, 1358 – 1368)

Solid Phase Extraction - History

1950 – Howard and Martin – the first use of what was to become reversed phase liquid chromatography
They treated silica with dichlorodimethylsilane alternating the nature of silica from polar to nonpolar.
Long-chain fatty acids were separated from aqueous-methanol (80:20) mobile phase by partitioning the solutes in an n-octane saturated stationary phase.
(Howard, G.A., Martin, A.J.P., 1950. The separation of C12-C18 fatty acids by reversed-phase partition chromatography. J. Biochem. **46**: 532 – 538)

1966 – Abel et al synthesized a solid reversed phase material by reacting silica with trichlorosilane to produce chemically modified bonded silica surfaces – a revolution in chromatography - Production of a host of different modified-silica phases by varying the chemistry of the organosilanes.
(Abel, E.W., Pollard, F.H., Uden, P.C., Nickless, G. 1966. A new gas-liquid chromatographic phase. J. Chromatogr. **22**, 23-28

Solid Phase Extraction - History

1990's – The polymer monolithic columns are invented

2000's
- The silica based monoliths were marketed by Merck
- Sub 2-μm totally porous silica particles developed and marketed by Waters – UPLC
- Core-shell particles

All these new materials are then used in SPE devices

Solid Phase Extraction - History

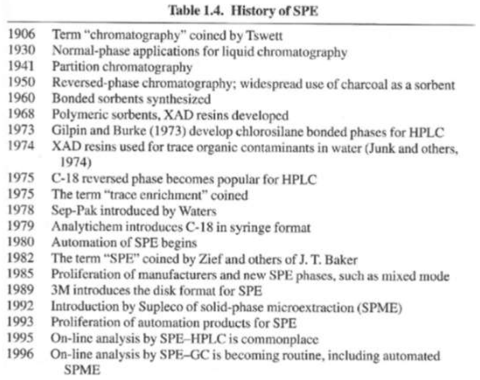


Table 1.4. History of SPE

1906	Term "chromatography" coined by Tswett
1930	Normal-phase applications for liquid chromatography
1941	Partition chromatography
1950	Reversed-phase chromatography; widespread use of charcoal as a sorbent
1960	Bonded sorbents synthesized
1968	Polymeric sorbents, XAD resins developed
1973	Gilpin and Burke (1973) develop chlorosilane bonded phases for HPLC
1974	XAD resins used for trace organic contaminants in water (Junk and others, 1974)
1975	C-18 reversed phase becomes popular for HPLC
1975	The term "trace enrichment" coined
1978	Sep-Pak introduced by Waters
1979	Analytichem introduces C-18 in syringe format
1980	Automation of SPE begins
1982	The term "SPE" coined by Zief and others of J. T. Baker
1985	Proliferation of manufacturers and new SPE phases, such as mixed mode
1989	3M introduces the disk format for SPE
1992	Introduction by Supleco of solid-phase microextraction (SPME)
1993	Proliferation of automation products for SPE
1995	On-line analysis by SPE–HPLC is commonplace
1996	On-line analysis by SPE–GC is becoming routine, including automated SPME

Solid Phase Extraction - History

Current trends: Molecular recognition mechanisms

Immunosorbents

Molecularly imprinted polymers

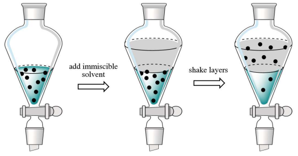
Aptamers

All based on affinity chromatography

Solid Phase Extraction

Traditional sample preparation involves:

- Sampling
- Sample dissolution
- Purification
- Extraction (conventionally made by liquid-liquid extraction, LLE)



LLE Advantages:

- Efficient
- Low cost

LLE Disadvantages:

- Large volumes of organic solvents (expensive and toxics)
- Cumbersome glassware
- Analyst exposure
- Formation of emulsion with aqueous samples
- Difficult to automate

Solid Phase Extraction

Difficulties of LLE are mostly overcome by SPE

SPE, invented in the mid-1970s, is an alternative to LLE

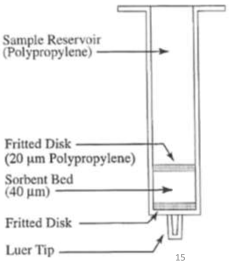
Retention Mechanisms:

- Normal phase
- Reversed phase
- Ion exchange
- Molecular affinity (molecular recognition)
- Others

Solid Phase Extraction

Currently off-line SPE columns are made with:

- Polypropylene housing (syringes)
- 40-µm packing material
- 20-µm polypropylene frits to contain 50 mg to 10 g of packing material

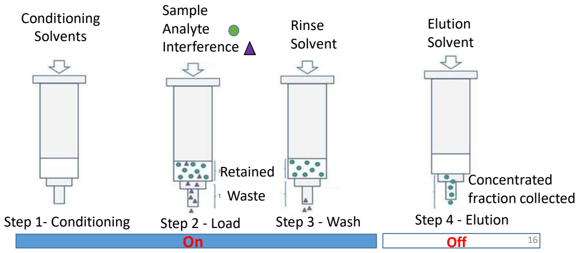


Solid Phase Extraction

Typically, load 1 mL to over 1L of sample solution by positive pressure or by a vacuum manifold

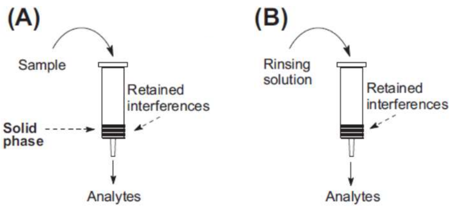
After quantitative sorption, remove the analyte with a suitable solvent → **Digital liquid Chromatography**

On/Off mode



Solid Phase Extraction

Retaining Interferences – Just Cleanup



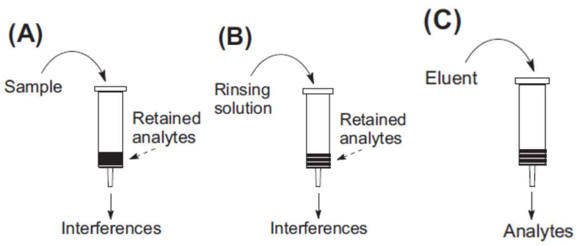
Sample Loading

Sample elution

Minimum 4 bed volumes of solvent

Solid Phase Extraction

Retaining the analytes – Concentration + Cleanup



SPE with analyte retention followed by elution

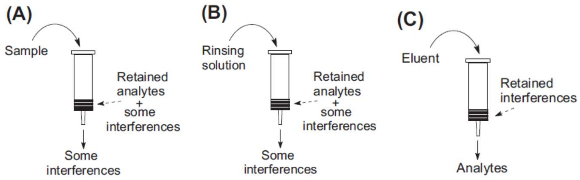
A – Sample loading

B – Sample wash with minimum 4 bed volumes of solvent

C- Sample elution with 4 – 8 bed volumes of solvent

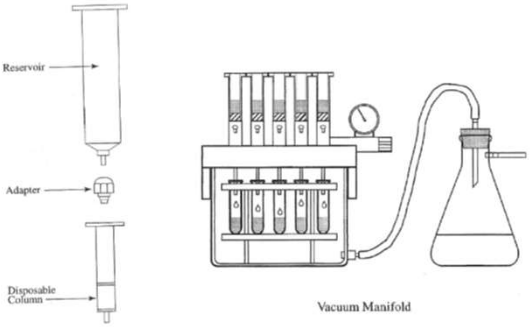
Solid Phase Extraction

Retaining the analytes – Concentration + Selective Elution

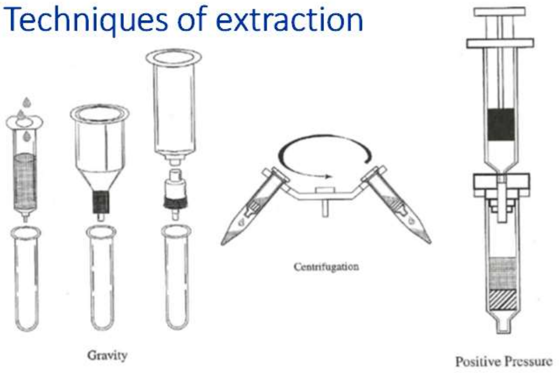


SPE with analyte retention followed by selective elution
A – Sample loading
B – Sample wash with minimum 4 bed volumes of solvent
C- Sample elution with 4 – 8 bed volumes of solvent

Solid Phase Extraction
Techniques of extraction



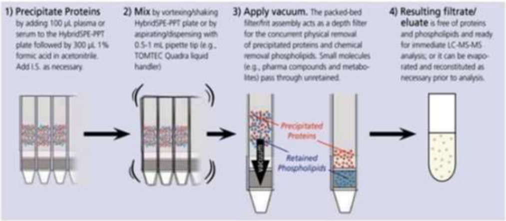
Solid Phase Extraction – Techniques of extraction



Techniques – 96 well plate SPE

Removal of phospholipids from human plasma prior to LC/MS-MS analysis

50 mg
2 mL



1) Precipitate Proteins by adding 100 µL plasma or serum to the HybridSPE-PPT plate followed by 300 µL 1% formic acid in acetonitrile. Add I.S. as necessary.

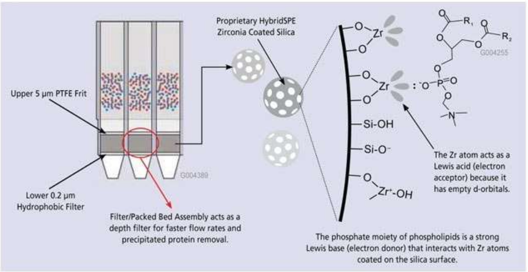
2) Mix by vortexing/shaking HybridSPE-PPT plate or by aspirating/dispensing with 0.5-1 mL pipette tip (e.g., TOMTEC Quadra liquid handler)

3) Apply vacuum. The packed-bed filter/frit assembly acts as a depth filter for the concurrent physical removal of precipitated proteins and chemical removal phospholipids. Small molecules (e.g., pharma compounds and metabolites) pass through unretained.

4) Resulting filtrate/eluate is free of proteins and phospholipids and ready for immediate LC-MS-MS analysis; or it can be evaporated and reconstituted as necessary prior to analysis.

Techniques – 96 well plate SPE

Removal of phospholipids from human plasma prior to LC/MS-MS analysis

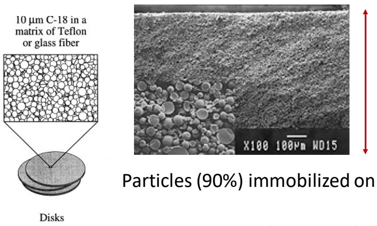


Filter/Packed Bed Assembly acts as a depth filter for faster flow rates and precipitated protein removal.

The Zr atom acts as a Lewis acid (electron acceptor) because it has empty d-orbitals.

The phosphate moiety of phospholipids is a strong Lewis base (electron donor) that interacts with Zr atoms coated on the silica surface.

Solid Phase Extraction –Techniques of extraction – Extraction disks



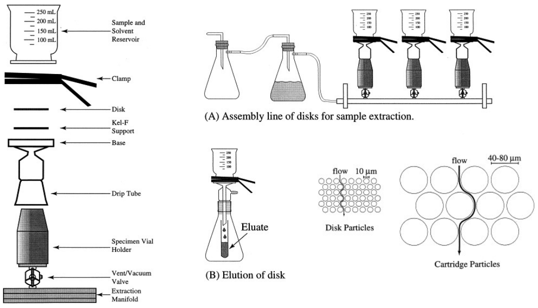
Thickness
0.50 ± 0.05 mm

Diameter:
47 – 90 mm

Particles (90%) immobilized on PTFE (10%)

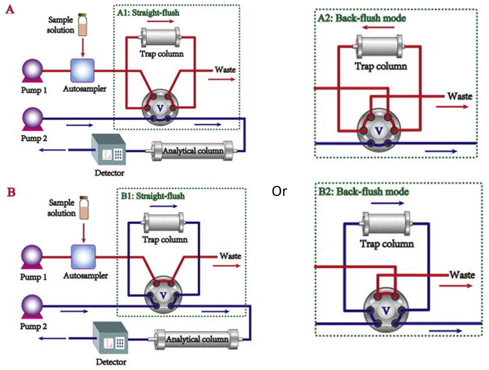
- Enable high flow rates for fast extraction of large volumes of
- Environmental applications – water analyses

Solid Phase Extraction –Techniques of extraction – Extraction disks



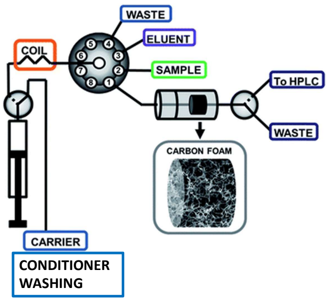
E.M. Thurman, K. Snavely, Advances in solid-phase extraction disks for environmental chemistry. TrAC Trends in Analytical Chemistry 19 (2000) 18 - 26

Automated Online Solid Phase Extraction



J. Pan, C. Zhang, Z. Zhang, G. Li, Review of online coupling of sample preparation techniques with liquid chromatography. Anal. Chim. Acta 815 (2014) 1 – 15

Automated Online Solid Phase Extraction



M. Ghani, F. Maya, V. Cerdá, Automated solid phase extraction of organic pollutants using melamine-formaldehyde polymer derived carbon foams. RSC Advances 6 (2016) 48558 - 48565

Automated Solid Phase Extraction

Advantages of Automated SPE

- Minimizes sample handling
- Minimizes the risks of sample losses by evaporation and sample contamination

In the Online approach:

- All the extracted analyte is injected in the analyzer
- Processes smaller volumes of samples than in the off-line approach

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Solid Phase Extraction – Sorbents and modes of interaction

Similar to those used in liquid chromatography

Normal phase

Stationary phase more polar than the solvent or sample matrix – water is not a solvent (too polar)

The sample is an organic solvent containing the analyte

Main Interactions: hydrogen bonding and dipole-dipole

Solid Phase Extraction – Sorbents and modes of interaction

Reversed phase

Stationary phase more hydrophobic than the sample

Used mostly for aqueous samples

Main Interactions: van der Waals forces (also called nonpolar, hydrophobic or reversed phase interactions). Secondary Interactions: hydrogen bonding and dipole-dipole

Solid Phase Extraction – Sorbents and modes of interaction

Size exclusion

Based on molecular size of the analytes
Large molecules excluded

Small molecules retained

Associated with reversed phase and/or ion exchange

Ion exchange

Stationary phases containing ion exchangers

Retention by electrostatic interaction

Elution by exchanging charged species

Solid Phase Extraction – Sorbents and modes of interaction

Mixed Mode

Retention by both reversed phase and ion exchange

Enhanced Selectivity

Different washing solutions may be used to remove interferences

Molecular recognition

Principles of affinity chromatography

Immunoaffinity, Molecularly Imprinted Polymers (MIP)

Solid Phase Extraction – Common Sorbents

Table 1.1. Common Sorbents Available for SPE

Sorbent	Structure	Typical Loading
	Reversed Phase	
Octadecyl (C-18)	—$(CH_2)_{17}CH_3$	17%C
Octyl (C-8)	—$(CH_2)_7CH_3$	14%C
Ethyl (C-2)	—CH_2-CH_3	4.8%C
Cyclohexyl	—CH_2CH_2-cyclohexyl	12%C
Phenyl	—$CH_2CH_2CH_2$-Phenyl	10.6%
Graphitized carbon	Aromatic carbon throughout	
Copolymers	Styrene–divinylbenzene	
	Normal Phase	
Cyano (CN)	—$(CH_2)_3CN$	10.5%C, 2.4%N
Amino (NH_2)	—$(CH_2)_3NH_2$	6.4%C, 2.2%N
Diol (COHCOH)	—$(CH_2)_3OCH_2CH(OH)CH_2(OH)$	8.6%C
Silica gel	—SiOH	—
Florisil	Mg_2SiO_3	—
Alumina	Al_2O_3	—
	Ion Exchangers	
Amino (NH_2)	—$(CH_2)_3NH_2$	1.6 meq/g
Quaternary amine	—$(CH_2)_3N^+(CH_3)_3$	0.7 meq/g
Carboxylic acid	—$(CH_2)_3COOH$	0.4 meq/g
Aromatic sulfonic acid	—$(CH_2)_3$-Phenyl-SO_3H	1.0 meq/g
	Size Exclusion	
Wide pore hydrophobic (Butyl)	—$(CH_2)_3CH_3$	5.9%C
Wide pore ion exchangers	—COOH	12.2%C

Solid Phase Extraction – Sorbents

The secret of successful SPE relies on the ability to turn the retention on and off

Isolate the analyte from the sample solution during the sorption step

Elute the analyte more concentrated and in a purified form

What are the sorbents to achieve these goals?

Silica Particles

- 40 – 60 µm particle onto which a liquid phase is bonded
- Water deactivates silica gel
- 1950's – 1970's attempts e progresses – explored Si – O – C bonds

Solid Phase Extraction – Sorbents

Silica Particles

- - Gilpin and Burke described a process involving chlorosilanes (*Gilpin, R.K. and Burke, M.F. 1973. Role of trimethysilanes in tailoring chromatographic adsorbents, Anal. Chem. 45: 1383 – 1389*).
- The Si – O – Si is stronger than the Si – O – C explored in the earlier works
- The base of the modern bonded phases used in LC and SPE

Properties of Silica particles used in SPE:
~ 40 µm, surface area = 200 – 600 $m^2 g^{-1}$, mean pore size = 60 nm

Solid Phase Extraction – Sorbents

Silica Particles

Pores

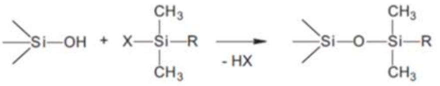
< 2 nm – micropores

2 – 50 nm – mesopores

> 50 nm – macropores

Where the main part of retention occurs

Suitable for size exclusion retention for 2000 D
Larger molecules: 300 nm pores required



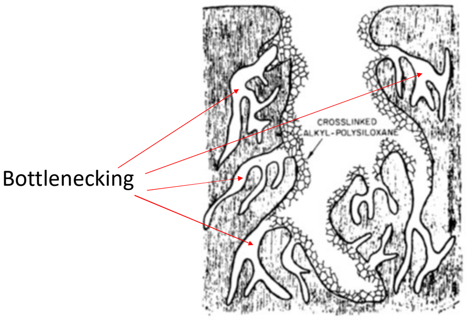
$$\geq Si{-}OH + X{-}\overset{CH_3}{\underset{CH_3}{Si}}{-}R \xrightarrow[-\,HX]{} \geq Si{-}O{-}\overset{CH_3}{\underset{CH_3}{Si}}{-}R$$

Pore < 10 nm cannot be easily accessed by the chlorosilane – Bottlenecking effect

Part of the silanols are not derivatized

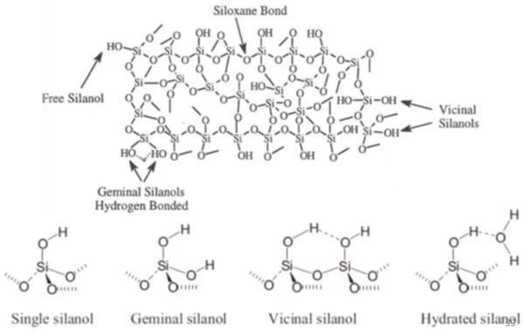
Solid Phase Extraction – Sorbents

Silica Particles



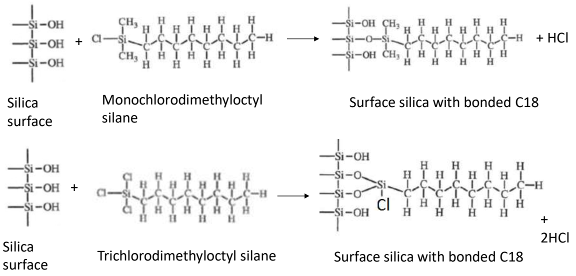
Solid Phase Extraction – Sorbents

Silica Particles – Silica structure



Solid Phase Extraction – Sorbents

Silica Particles – Derivatization with organo - chlorosilanes



Silica surface

Monochlorodimethyloctyl silane

Surface silica with bonded C18

Silica surface

Trichlorodimethyloctyl silane

Surface silica with bonded C18

Solid Phase Extraction – Sorbents

Silica Particles residual silanols

Unreacted silanols – they are sorbents, but they are not RP sorbents

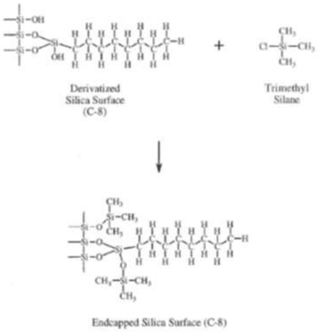
Unreacted silanols (residual silanols) Interact with analytes via hydrogen bonding or weak cation exchange

Example – Interaction with weakly basic compounds I.E.– amino containing drugs

Hydrogen bonding with –Si-O-H and hydrophobic interaction with C8 or C18 groups

Solid Phase Extraction – Sorbents

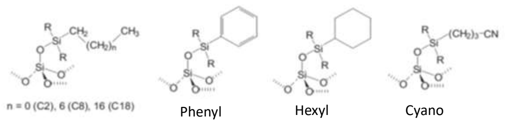
Silica Particles endcaping of residual silanols



Solid Phase Extraction – Sorbents

Silica particles - The functionalization defines the retention mode

Nonpolar – Reversed phase

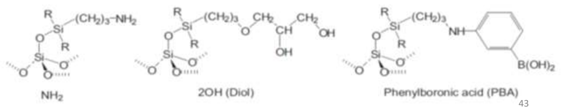


Phenyl

Hexyl

Cyano

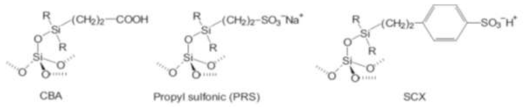
Polar – Normal phase, affinity, etc



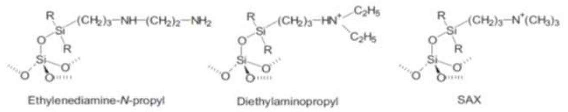
Solid Phase Extraction – Sorbents

Silica particles - The functionalization defines the retention mode

Cation exchangers



Anion exchangers



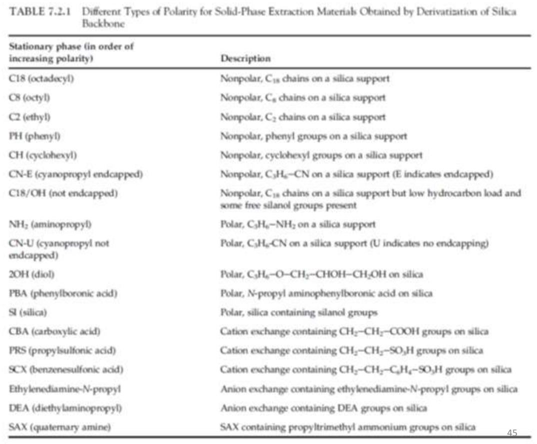


TABLE 7.2.1 Different Types of Polarity for Solid-Phase Extraction Materials Obtained by Derivatization of Silica Backbone

Stationary phase (in order of increasing polarity)	Description
C18 (octadecyl)	Nonpolar, C_{18} chains on a silica support
C8 (octyl)	Nonpolar, C_8 chains on a silica support
C2 (ethyl)	Nonpolar, C_2 chains on a silica support
PH (phenyl)	Nonpolar, phenyl groups on a silica support
CH (cyclohexyl)	Nonpolar, cyclohexyl groups on a silica support
CN-E (cyanopropyl endcapped)	Nonpolar, C_3H_6–CN on a silica support (E indicates endcapped)
C18/OH (not endcapped)	Nonpolar, C_{18} chains on a silica support but low hydrocarbon load and some free silanol groups present
NH_2 (aminopropyl)	Polar, C_3H_6–NH_2 on a silica support
CN-U (cyanopropyl not endcapped)	Polar, C_3H_6-CN on a silica support (U indicates no endcapping)
2OH (diol)	Polar, C_3H_6–O–CH_2–CHOH–CH_2OH on silica
PBA (phenylboronic acid)	Polar, N-propyl aminophenylboronic acid on silica
SI (silica)	Polar, silica containing silanol groups
CBA (carboxylic acid)	Cation exchange containing CH_2–CH_2–COOH groups on silica
PRS (propylsulfonic acid)	Cation exchange containing CH_2–CH_2–SO_3H groups on silica
SCX (benzenesulfonic acid)	Cation exchange containing CH_2–CH_2–C_6H_4–SO_3H groups on silica
Ethylenediamine-N-propyl	Anion exchange containing ethylenediamine-N-propyl groups on silica
DEA (diethylaminopropyl)	Anion exchange containing DEA groups on silica
SAX (quaternary amine)	SAX containing propyltrimethyl ammonium groups on silica

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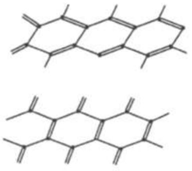
Solid Phase Extraction – Sorbents

Polymeric

Activated carbon and Styrene-co-divinylbenzene – the most used

Activated Carbon

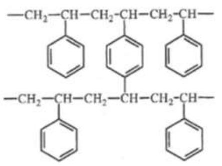


- 600 – 1200 m^2 g^{-1}
- pH stability in a range between 2 – 12
- Larger capacity compared with C18 bonded phases on silica

Hennion, M.C., Pichon, V. 1994. Solid-phase extraction of polar organic pollutants from waters, Envrion. Sci. Technol. 28: 576A – 583 A.

Solid Phase Extraction – Sorbents

Polymeric Styrene-co-divinylbenzene - Base of XAD resins



Aromatic rings enable the electron-donnor interactions between the sorbents and the π bonds of the solute

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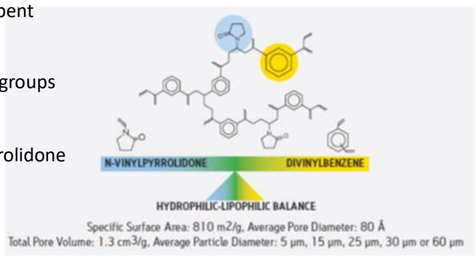
Solid Phase Extraction – Sorbents

Polymeric

Doped with small amounts of hydrophilic groups for more effective mass transfer from bulk sample solution to the surface of the sorbent

Sulfonate groups (IST)

N-vinylpirrolidone (Waters)



Specific Surface Area: 810 m2/g, Average Pore Diameter: 80 Å
Total Pore Volume: 1.3 cm3/g, Average Particle Diameter: 5 µm, 15 µm, 25 µm, 30 µm or 60 µm

Also used for ion exchange

Solid Phase Extraction
Sorption Mechanisms

Reversed Phase

Sorption of analytes on a nonpolar stationary phase

Energy:
~ 20 kJ/mol – analogous to liquid-liquid extraction from water

Organic phase is chemically bonded to the silica

Other common phases for RP: C_2, C_4, C_8, cyclohexyl and phenyl groups

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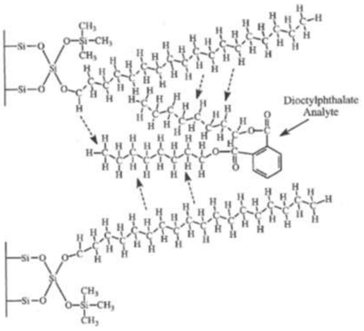
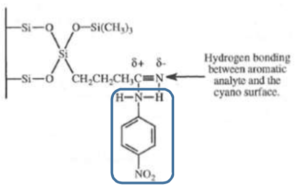
Solid Phase Extraction
Sorption Mechanisms – Reversed Phase



Solid Phase Extraction – Sorption Mechanism – Normal Phase

Analyte is adsorbed on a polar stationary phase

Cyanopropyl Sorbent



Polar interactions:
- Hydrogen bonding Dipole-dipole
- Induced dipole-dipole
- π-π interactions

Nonbonded phases: silica, alumina, magnesium silicate (Florisil)
Bonded phases: aminopropyl, cyanopropyl, propyldiol

Solid Phase Extraction – Sorption Mechanism – Normal Phase

Water never used – it is adsorbed by the sites of the stationary phase, competing with the analyte

Used for clean-up of organic extracts of water, soil, food, etc.

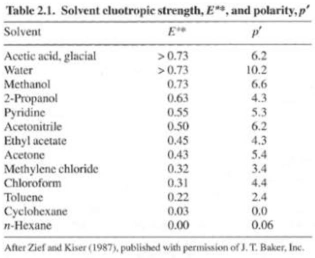


Table 2.1. Solvent eluotropic strength, $E^{\circ *}$, and polarity, p'

Solvent	$E^{\circ *}$	p'
Acetic acid, glacial	> 0.73	6.2
Water	> 0.73	10.2
Methanol	0.73	6.6
2-Propanol	0.63	4.3
Pyridine	0.55	5.3
Acetonitrile	0.50	6.2
Ethyl acetate	0.45	4.3
Acetone	0.43	5.4
Methylene chloride	0.32	3.4
Chloroform	0.31	4.4
Toluene	0.22	2.4
Cyclohexane	0.03	0.0
n-Hexane	0.00	0.06

After Zief and Kiser (1987), published with permission of J. T. Baker, Inc.

Elution is a function of eluotropic strength and polarity of the solvent

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Solid Phase Extraction – Sorption Mechanism – Ion Exchange

Charged resins (ion-exchangers) retain oppositely charged analytes (~ 335 ~kJ/mol)

High energy ionic interaction

↓

Polar ionic solutes may be effectively removed from polar solvents – water, for instance

Ionic strength of the sample affects the retention – charge competition

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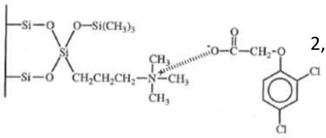
Solid Phase Extraction – Sorption Mechanism – Ion Exchange

Ion exchangers are classified as Strong or Weak

Strong exchangers are permanently charged resins (independent of the pH)

Strong Anion Exchanger: Positively charged – quaternary ammonium salt based resins:

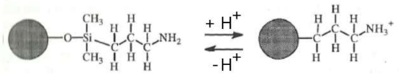


Strong Cation Exchanger: Negatively charged – sulfonic groups

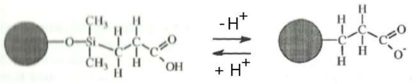
Solid Phase Extraction – Sorption Mechanism – Ion Exchange

Weak ion exchangers: The charge of the resin depends on the solution pH

A weak anion exchanger based on amino group:



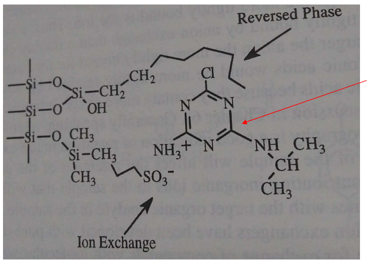
A weak cation exchanger based on carboxylic group:



Solid Phase Extraction – Sorption Mechanism – Mixed Mode

Two diferent groups in the same sorbent

Usually cation exchange and reversed phase

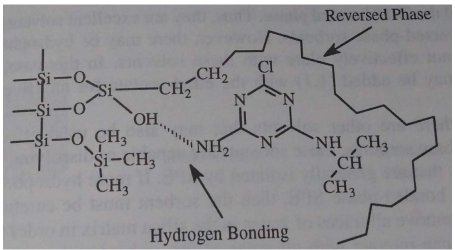


Metabolite of atrazine

The retention mechanism is both high and low energy

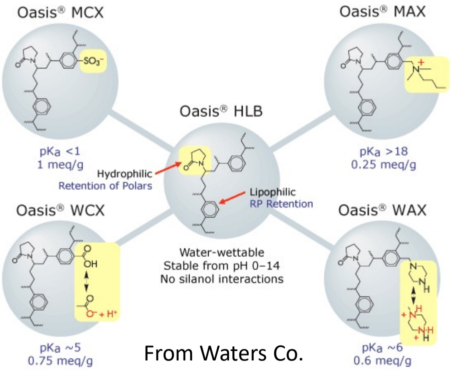
Solid Phase Extraction – Sorption Mechanism – Mixed Mode

Mixed mode in a residual silanol of a non endcaped C18 based silica sorbent:



The phenomenon affects both the retention and recovery of the SPE

Solid Phase Extraction – Sorption Mechanism – Mixed Mode



From Waters Co.

Solid Phase Extractions

A video of very basic manual operations of SPE:

https://www.youtube.com/watch?v=EuG9bXlaPF4&t=173s

A video of an automated SPE:

https://www.youtube.com/watch?v=x9WSOc1vtns&feature=youtu.be

Summary

- The concept of SPE
- Techniques for using SPE
- Modes of Interactions
- Most common sorbents
- Functional groups and retention
- Retention/recovery mechanism