

Polare SPE

(veröffentlicht auf www.analytik-news.de am 3. November 2009)

Nachdem wir uns in den ersten Folgen dieser Reihe ausführlich mit der unpolaren SPE beschäftigt haben – dort kommen C18- und Polymerphasen für die Probenvorbereitung wässriger Proben und Extrakte zum Einsatz - wollen wir uns im Folgenden mit der **polaren SPE** auseinandersetzen.

Wann kann polare SPE angewendet werden?

- ✓ Wenn die **Analyten** eine oder mehrere **polare Gruppen** tragen, z.B. Alkohol, Amin, Aldehyd, Keton, Ester etc.

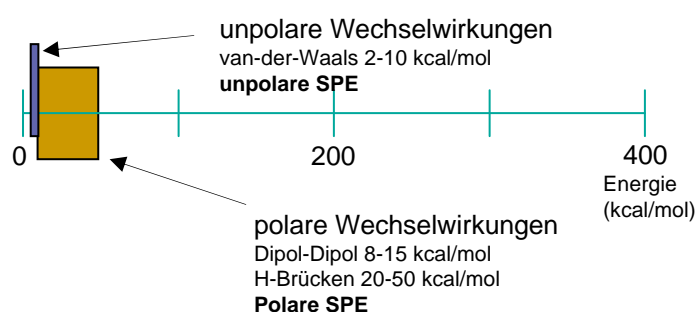
UND

- ✓ die **Matrix unpolar** ist, z.B. ein Extrakt in Hexan oder Dichlormethan, ein Öl etc.

Welche Wechselwirkungen finden statt?

Bei der polaren SPE finden hauptsächlich **Dipol-Dipol-Wechselwirkungen** und **Wasserstoffbrückenbindungen** statt.

Es handelt sich dabei um viel stärkere Wechselwirkungen als bei unpolarer SPE,



weshalb die **polare SPE** hervorragend zur **Fraktionierung von ähnlichen Substanzen** geeignet ist, was mit unpolarer SPE nicht möglich wäre.

Welche Sorbentien werden bei der polaren SPE verwendet?

extrem polare Sorbentien
(für sehr starke Wechselwirkungen):

- ✓ Kieselgel (Si)
- ✓ Aluminiumoxid (Al_2O_3)
- ✓ Florisil (MgO_3Si)

moderat polare Sorbentien
(einfacher zu handhaben, wenn polare Lösemittel oder Feuchtigkeit mit im Spiel sind):

- ✓ Diol (2OH)
- ✓ Aminopropyl (NH_2)
- ✓ Cyanopropyl (CN)
- ✓ Ethylendiamin-N-Propyl (PSA)
- ✓ Diethylaminopropyl (DEA)

Auf den letzten Seiten finden Sie einen Auszug aus dem Varian-Katalog mit der **Beschreibung dieser Sorbentien**.

Wie sieht grob eine Standardmethode aus?

Konditionierung

- ✓ mit 1 - 2 Säulenvolumina Hexan, Dichlormethan oder dem unpolaren Lösemittel, in dem die Probe gelöst ist

Probenaufgabe

- ✓ Probe langsam durchlaufen lassen (2 – 5 mL/min)

Waschen

- ✓ mit 1 – 2 Säulenvolumina Hexan, Dichlormethan oder dem unpolaren Lösemittel, in dem die Probe gelöst ist

Trocknen

- ✓ je nach Elutionsmittel kurz oder vollständig trocknen
- ✓ auch hier gilt:
wenn ein Lösemittel zur Elution verwendet wird, das nicht mit der Probe bzw. dem Waschlösemittel mischbar ist, muss das Sorbens vollständig getrocknet werden

Elution

- ✓ mit einem Lösemittel ansteigender Polarität
- ✓ in mehreren kleinen Portionen

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Welche Elutionsmittel stehen zur Verfügung?

Die **eluotrope Reihe** sieht etwa wie folgt aus: (kann leicht unterschiedlich sein für verschiedene Sorbentien, hier für Kieselgel)

- ✓ Hexan
- ✓ Cyclohexan
- ✓ Toluol
- ✓ Diethylether
- ✓ Chloroform
- ✓ Dichlormethan
- ✓ THF
- ✓ Aceton
- ✓ Dioxan
- ✓ Ethylacetat
- ✓ Acetonitril
- ✓ Isopropanol
- ✓ Ethanol
- ✓ Methanol
- ✓ Wasser

Zunehmende Elutionsstärke



Häufig werden auch **Gemische** verwendet, zum Beispiel **Hexan/Isopropanol**. (Je polarer die Analyten, desto höher der Isopropanol-Anteil.)

Das Elutionsmittel muss folgende Zwecke erfüllen:

- ✓ Die Analyten müssen darin gut löslich sein.
- ✓ Es muss die Analyten vom Sorbens verdrängen, also Wechselwirkungen mit dem Sorbens eingehen können.
- ✓ Es sollte möglichst Begleitsubstanzen aus der Matrix, die ebenfalls am Sorbens adsorbiert sind, nicht oder nur wenig lösen/eluieren.

D.h. wenn mehrere Lösemittel als Elutionsmittel zur Verfügung stehen, sollte man **nicht von vorne herein das mit der stärksten Elutionskraft nehmen**, sondern **eines, das zwar gut genug ist**, um die Analyten zu eluieren, aber nicht alles polare vom Sorbens holen kann. So kann man **reinere Extrakte** erhalten, als wenn man mit dem stärksten möglichen Elutionsmittel eluiert.

Beachten sollte man aber, dass die **Elutionsstärke** des Lösemittels das **Elutionsvolumen** beeinflusst. Ein schwächeres Elutionsmittel kann zur vollständigen Desorption der Analyten ein größeres Elutionsvolumen erfordern.

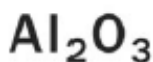
Anwendungsbeispiele zur polaren SPE in der nächsten Folge.

Beschreibung der Sorbentien

(Auszug aus dem Varian Katalog)

Alumina-A (AL-A)

- Acidic Alumina (Al₂O₃)
- pH: 4.5
- Particle size: 25 µm
- Primary retention mechanism: Lewis acid/base, polar, and ion exchange



Alumina is a classic Lewis acid, lacking two electrons in the aluminum center. The Lewis acid properties are enhanced in Alumina-A, making the sorbent more retentive towards electron-rich compounds. This sorbent has a slightly cationic nature through pretreatment with acidic solutions. The result is a surface that favors neutral and anionic species (e.g. neutral acid or acid anion), while discouraging retention of cationic species. Weak ion exchange is also possible with anionic compounds.

Alumina-B (AL-B)

- Basic Alumina (Al₂O₃)
- pH: 10.0
- Particle size: 25 µm
- Primary retention mechanism: Lewis acid/base, polar, and ion-exchange

Alumina-B has a surface that favors retention of cationic or hydrogen-bonding species. This sorbent has an anionic nature through the pretreatment of the alumina with basic solutions. The result is a surface that favors cation exchange, though the surface also exhibits some Lewis base properties and therefore can also retain electron-donors like neutral amines. However, this capacity is much lower than for the neutral and acidic aluminas. Strong hydrogen bonding is possible on Alumina-B, making it particularly effective for polar cations.

Alumina-N (AL-N)

- Neutral Alumina (Al₂O₃)
- pH: 7.5
- Particle size: 25 µm
- Primary retention mechanism: Lewis acid/base, polar, and ion exchange

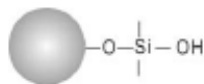
Alumina, like silica, is an extremely polar sorbent. The alumina surface tends to be slightly more stable under high pH conditions than unfunctionalized silica. The smaller particle size ensures high extraction efficiency and small (50 mg) sorbent beds are therefore commonly used. This sorbent has an electrically neutral surface that favors the retention of electron-rich compounds like aromatic species and aliphatic amines. It also favors retention of compounds containing electronegative groups (for example, functional groups with oxygen, phosphorus and sulfur atoms). It may be used in extracting either nonpolar or polar species from aqueous or nonaqueous samples respectively.

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Silica (SI)

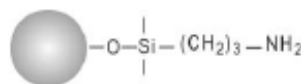
- Sorbent description: native silica, unfunctionalized
- Primary retention mechanism: strongly polar
- Typical sample types: nonpolar organic solvents, oils, and lipids



Silica is generally regarded as the most polar sorbent available. Unbonded, activated silica is also fairly acidic. SI is one of the best sorbents available for selectively separating compounds of very similar structure. Applying the analytes in a nonpolar solvent, then gradually increasing the solvent polarity in successive wash steps by adding increasing concentrations of polar modifiers, such as THF or ethyl acetate, can accomplish this separation.

Aminopropyl (NH₂)

- Ideal for structural isomer fractionation
- Anion exchange and polar retention mechanisms
- Weak nonpolar retention



Aminopropyl (NH₂), a polar sorbent, utilizes both hydrogen bonding and anion exchange. With a pK_a of 9.8, NH₂ is a weaker anion exchanger than sorbents such as SAX (a quaternary amine sorbent that is always charged) and is therefore a better sorbent choice for retention of very strong anions, such as sulfonic acids, which may retain irreversibly on SAX. Because a propyl tether supports the NH₂ functionality, it can be used for nonpolar isolations from polar samples, but its strong polarity is its primary characteristic. NH₂ is excellent for separation of structural isomers.

Florisil

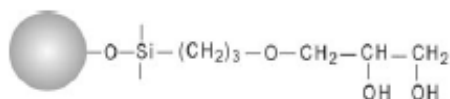
- Sorbent description: MgO₃Si
- Primary retention mechanism: polar
- Typical sample types: environmental, organic extracts

MgO₃Si

Florisil is a magnesia-loaded silica gel. Like silica, it is extremely polar in nature and ideal for the isolation of polar compounds from nonpolar matrices. The larger particle size – 200 μm – of the Florisil material enables fast flow of large-volume samples and thus is a potential alternative to silica when extracting viscous samples. Florisil is ideal for the separation of chlorinated pesticides, and is used in numerous ADAC- and EPA- regulated methods. Florisil has also been used as a replacement for Alumina in cases where the Lewis-acidity of alumina interferes with the extraction.

Diol (2OH)

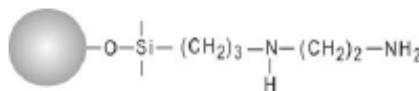
- Polar sorbent with better reproducibility than silica
- Ideal for prostaglandin separation and other fractionations
- Typical sample types: nonpolar organic extracts, oils, and lipids



Diol (2OH) is typically used for polar extractions from nonpolar solvents. Diol resembles unbonded silica in its tendency for strong hydrogen bonding. It also shares silica's important ability to discriminate between similar compounds such as structural isomers. Prostaglandins, for example, can be separated into classes using Diol by slightly varying the solvent polarity. Diol is a more predictable sorbent than silica for extraction methods requiring a polar sorbent conditioned with a polar solvent. Diol can also be used for nonpolar retention by virtue of its hydrocarbon spacer.

PSA

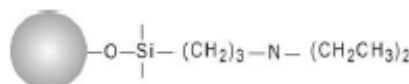
- Bonded functional group: ethylenediamine-N-propyl (primary and secondary amines)
- Primary retention mechanism: weak anion exchange (aqueous matrix) or polar (nonpolar organic matrix), chelation
- Typical sample types: water, biological fluids, and organic extracts



Bond Elut PSA is an anion exchange sorbent that is similar to NH₂. PSA has two amine groups that offer slightly greater ionic capacity (1.4 meq/g) than NH₂ (1.1 meq/g), and the pK_as of its primary and secondary amine groups are higher (10.1 and 10.9, respectively). PSA is therefore a stronger anion exchanger than NH₂. The PSA functional group is also a very good bidentate ligand, making PSA an excellent sorbent for chelation applications (useful for metal ion extractions). Its higher carbon loading makes it a less polar sorbent than NH₂, and thus a better choice for very polar compounds that retain too strongly on NH₂ sorbent. SPEC PSA offers similar selectivity to Bond Elut PSA.

Diethylaminopropyl (DEA)

- Bonded functional group: diethylaminopropyl
- Primary retention mechanism: weak anion exchange aqueous matrix) or polar (nonpolar organic matrix)
- Typical sample types: water, biological fluids, nonpolar extracts



Bond Elut DEA bears some resemblance to PSA and NH₂ in its properties. It has slightly lower capacity as an anion exchange sorbent (1.0 meq/g), and a more nonpolar character due to a longer alkyl tether. DEA is somewhat more polar than C₈, but less polar than C₂ or CN.