SiliaBond®

Chromatographic and Ion Exchange Phases

SILICYCLE Inc.
UltraPure SILICA GELS

Importance of Quality Control

The Quality Control Department's objective is to provide default-free products. In light of this goal, we have determined the critical points that need to be addressed for each product line. These points are based on customer's and Account Managers' recommendations as well as on our employees' scientific knowledge.

Each product family has its own quality control procedures, which are strictly adhered to. QC test results are checked and confirmed by the person in charge of them before being cleared for shipping. Complete procedures for each product line are available upon request.

Thus, SiliCycle is committed to high quality standards. In doing so, every product meets the quality specifications our customers demand. All products are shipped with a Certificate of Analysis (*CofA*) and a sample from every batch is kept for subsequent analysis. If you feel that the product you have received does not meet these specifications, please contact us and we will make sure you are satisfied.

Bare Silica Gel

The backbone of most of SiliCycle's products is Silia*Flash* F60 (*40-63 µm, 60 Å*) silica gel. It provides superior performance for chromatographic applications due to its narrow particle size distribution and high purity.

Before functionalization, every silica is rigorously characterized and analyzed by the procedures below to ensure lot-to-lot reproducibility.

Functionalized Silica Gel

The process for functionalizing the silica is highly dependent on the group being attached. However, it is still possible to functionalize 90% of the surface, verified by ^{29}Si MAS NMR. The remaining 10% of the surface may be endcapped to provide a completely inert support. After being functionalized, the product is submitted to further analysis and quality control as outlined below.

Quality Control

Type of Analysis	Performed by:
Bare Silica Gel	
Carbon, nitrogen & sulfur content	Elemental analyzer
Total trace metal	ICP-OES
Surface area & porosity	Nitrogen adsorption analyzer
Particle size distribution	Laser light diffraction
Tapped density analysis	Density measurement
Water content	Moisture balance
pH	pH-meter
Functionalized Silica Gel	
Residual solvent content	Moisture balance
Specific reactivity analysis	GC-FID, GC-MS, LC-MS/MS, ICP-OES
Organic function signature	Infrared spectroscopy
Purity analysis	GC-MS

Analysis Descriptions

Elemental Analysis of Organic Compounds

Silia*Flash* silica gel has a very low organic content. All lots are subjected to elemental analysis to determine the carbon, nitrogen and sulfur levels.

Total Trace Metal Analysis

To improve the quality of the separation, SiliCycle manufactures silica gels with very low traces of metal content. All silica gels are analyzed for more than 45 metals by ICP-OES down to ppm, and reach up to 99.4% silica purity. This removes any issues from metal oxides that may act as Lewis acids and prevents «Tailing» of most polar compounds (*frequently ionizable*) that can be caused by silica with a high metal content.

Surface Area and Porosity Analysis

The efficiency and reliability of silica gel depend on its surface condition. We use the Brunauer, Emmet, and, Teller analysis to determine the surface area, and the Barret-Joyner-Hatenda method to determine the pore diameter and pore volume. A larger surface area results in more contact or interaction with the analyte, thereby increasing the segregation of different products. Pore diameter and pore volume permit semi-exclusion chromatography where smaller molecules fit into pores more easily than larger ones. This justifies the use of several types of silica to achieve better discrimination in chromatographic separations.

Particle Size Distribution Analysis

Particle size distribution is determined by laser diffraction. Usually, more than 90% of the silica gel is kept within the appropriate range.

Water Content Analysis (*silica gel activity*)

The amount of water on the silica's surface affects chromatographic performance. An anhydrous silica gel will be extremely polar, while a wetted one will be considerably less polar. Every batch is carefully adjusted to a specific percentage of water content.

pH Analysis

The pH can increase the retention of some ionizable compounds. However, some products can become hydrolyzed or rearranged when in contact with acidic silica. A neutral pH, with a range between 6.5 and 7.5, is the most important factor in determining the reliability and inert behavior of the silica. This pH test involves suspending the silica gel in pure water (*5% w/w*).

Silia*Bond* Chromatographic and Ion Exchange Phases

SiliCycle offers a large range of silica-based chromatographic and ion exchange phases:

- Non Polar Silia*Bond* Phases: C1 to C18
- Polar Silia*Bond* Phases: Amine, Cyano and Diol
- Ion Exchange Silia*Bond* Phases: SCX, SCX-2, WCX, SAX, SAX-2 and WAX

Silia*Bond* Chromatographic Phases

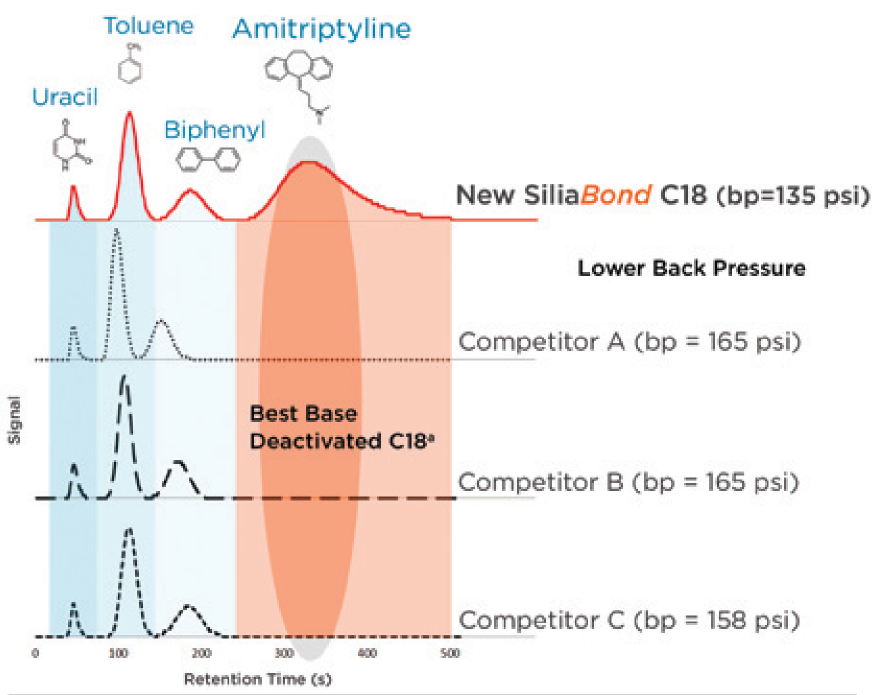
Silica is the most widely used matrix in chromatography. These bare and grafted supports possess great properties for use as stationary phases and are particularly appreciated for their high mechanical resistance. In chromatography, there are two phases: the stationary phase that is packed in a column and the mobile phase that will be eluted through the stationary phase. If the analyte is strongly soluble in the mobile phase, there will be no retention. If the analyte interacts strongly with the stationary phase, there will be no or low migration. In a mixture, the interactions between the two phases will generate the separation. So, depending on the analyte's polarity, the apropriate stationary phase has to be chosen, and the mobile phase's polarity has to be tuned.

Silia*Bond* Reversed-Phases

In reversed-phase chromatography, the packing material is always non-polar (*hydrophobic*) while the mobile phase is polar to non-polar. An important parameter affecting chromatographic efficiency is the hydrophobicity of the sorbent. As a general rule, stationary phase hydrophobicity increases with the alkyl chain length.

Last year, SiliCycle developed a new and innovative C18 chromatographic phase characterized by a homogeneous coverage of the alkyl chains on the surface. Consequently, the endcapping step is more controlled, which leads to much improved separations and also to inhibition of the non-specific interactions with silanol groups (*highly deactivated silanol phase*). This chromatographic phase is available on irregular (*R332-*) and spherical (*S032-*) high quality supports. This grafting process will be available soon for all other reversed phases.

Compared to competitive products, this endcapped 17% C18 exhibits high hydrophobicity and base deactivated properties. We have compared this new chromatographic phase to comparable 20% C18 phases on the market. The comparison was done on a mixture of compounds to evaluate the dead volume (*uracil*), the hydrophobicity (*toluene and biphenyl*) and the silanol activity (*amitriptyline*). The test was done in isocratic conditions, with a mobile phase composed of 8/20 methanol/buffer (*20 nM potassium phosphate pH = 7*). The results are presented below:



The basic product, amitriptyline, interacts with residual silanol groups and stays immobilized on all the competitor phases, but not on the new Silia*Bond* C18. This new C18 phase presents a better separation property with a better endcapped surface. Also, the Silia*Bond* C18 presents lower back pressure compared to the competition.

Silia*Bond* Reversed-Phases Portfolio

The table below presents all the reversed phases available from SiliCycle:

Silia*Bond* Reversed-Phases

Sorbent Phase	Functional Group	Endcapping	%C Loading[a]	Density (g/mL)	SiliCycle P/N
C18	Monofunctional C18	Yes	17.0	0.639	R33230B
C18 *nec*	Monofunctional C18	No	15.5	0.640	R33330B
C18 Low Loading	Monofunctional C18	Yes	11.0	0.619	R33530B
C18 High Loading	Trifunctional C18	Yes	23.0	0.864	R00030B
C18 High Loading *nec*	Trifunctional C18	No	23.0	0.867	R00130B
C18 Moderate Loading	Trifunctional C18	Yes	17.0	0.735	R02130B
C18 Low Loading	Trifunctional C18	Yes	11.0	0.705	R00430B
C12	Trifunctional Adamantyl	Yes	16.0	0.705	R53030B
C8	Monofunctional C8	Yes	11.0	N/A	R30830B
C8	Trifunctional C8	Yes	12.0	0.759	R31030B
C8 *nec*	Trifunctional C8	No	11.0	0.703	R31130B
C6	Trifunctional Cyclohexyl	Yes	10.0	0.662	R61530B
C4	Monofunctional C4	Yes	7-8.0	N/A	R32730B
C4	Trifunctional C4	Yes	8.0	0.656	R32030B
C4 *nec*	Trifunctional C4	No	8.0	0.692	R32130B
C1	Methyl	Yes	5.0	0.599	R33030B
CN	Trifunctional Cyano	Yes	7.0	0.703	R38030B
PHE	Monofunctional Phenyl	Yes	9.0	N/A	R33830B
PHE	Trifunctional Phenyl	Yes	9.0	0.637	R34030B
PHE *nec*	Trifunctional Phenyl	No	9.0	0.607	R34130B
PFP	Pentafluorophenyl	Yes	9.0	N/A	R67530B

Also available on all irregular Silia*Flash* Silica. Example: the 300 Å, 40-63 µm (*Rxxx30M*) [a]Based on our Standard Silia*Flash* Silica matrix R10030B, 40-63 µm, 60 Å

Typical applications using Silia*Bond* Reversed-Phases

Sorbent Phase	Typical Applications
C18	Peptides, pesticides, PCBs, PAHs, toxins, drugs & their metabolites in physiological fluids
C8	Highly hydrophobic pesticides, peptides, heavy drugs and their metabolites in physiological fluids
C6 (*cyclohexyl*)	Phenols, chloroanilines and anthelmintics from tissues and water
C4	Molecules with large hydrophilic regions such as peptides, proteins and zwitterions (*300 Å*)
C1	Polar and non-polar pharmaceutical natural products, highly hydrophobic molecules and biomolecules
CN	Cyclosporine and carbohydrates
PHE	Aflatoxins, caffeine, and phenols from water
PFP	Conjugated compounds or for a new selectivity approach

Silia*Bond* Normal Phases

Normal-phase chromatography is used to separate polar compounds through polar interactions with the support. The interactions take place on the highly polar silanols of the silica gel surface, but there are also moderately polar interactions with the hydrogen bonds on amino or diol functions. The non-endcapped cyano phase can be used in applications in normal-phase chromatography as a less polar alternative to silica. The $AgNO_3$ phase is particularly useful to separate isomers that present unsaturated groups.

Silia*Bond* Normal Phases

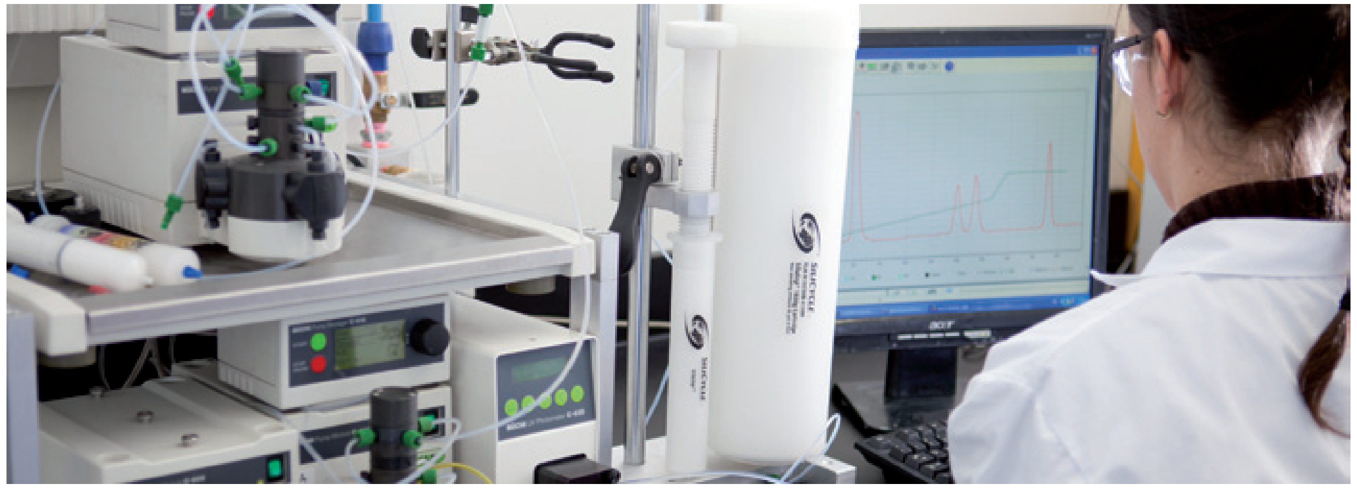
Sorbent Phase	Functional Group	Endcapping	Loading[a]	Density (g/mL)	SiliCycle P/N
SiO_2	Bare silica gel	No	N/A		R10030B
NH_2 *nec*	Amine	No	1.6	0.687	R52130B
CN *nec*	Cyano	No	1.0		R38130B
Diol *nec*	Diol	No	1.0	0.687	R35030B
$AgNO_3$	Silver Nitrate	No	10% w/w	0.604	R23530B

Also available on all irregular Silia*Flash* Silica. Example: the 300 Å, 40-63 µm (*Rxxx30M*)

[a] Based on our Standard Silia*Flash* Silica matrix R10030B, 40-63 µm, 60 Å

Typical applications using Silia*Bond* Normal Phases

Sorbent Phase	Typical Applications
NH_2 *nec*	Sugars, nucleotides and water-soluble vitamins
CN *nec*	Polar organic compounds such as basic drugs and molecules containing π electron systems
Diol *nec*	Peptides, proteins and malto-oligosaccharides
$AgNO_3$	Cis/trans isomers of unsaturated compounds such as alkenes, lipids, steroids and terpenes



Silia*Bond* Ion Exchange Phases

In an ion exchange process, the silica support is modified by a function carrying a charge with its counter ion. This counter ion is exchangeable with other ions in solution. If the immobilized phase is carrying an anion, the exchangeable species is a cation. Inversely, if the immobilized phase carries a cation, the ion exchangeable species will be an anion. Ion exchange phases are widely used in separation, purification and decontamination.

The stationary phase can be a cation exchanger of varying strength:

- Strong cation exchanger such as our Silia*Bond* Tosic Acid (*SCX*) and Silia*Bond* Propylsulfonic Acid (*SCX-2*)
- Weak cation exchanger such as our Silia*Bond* Carboxylic Acid (*WCX*)

The stationary phase can also be an anion exchanger of varying strength:

- Strong anion exchanger such as our Silia*Bond* TMA Chloride *nec* (*SAX*), Silia*Bond* TMA Acetate *nec* (*SAX-2*) and Silia*Bond* TBA Chloride
- Weak Anion exchanger such as our Silia*Bond* Amine *nec* (*WAX*) and Silia*Bond* Diethylamine *nec* (*WAX-2*)

SiliCycle has recently developed Silia*Bond* TMA Acetate, which has been particularly effective in customers' anionic exchange applications.

Silia*Bond* Ion Exchange Phases

Sorbent Phase	Functional Group	Endcapping	Loading (mmol/g)[a]	Density (g/mL)	SiliCycle P/N
WAX	Amine	No	1.60	0.687	R52130B
WAX-2	Diethylamine	No	1.20	0.761	R76630B
SAX	Trimethylammonium Chloride	No	1.10	-	R66230B
SAX-2	Trimethylammonium Acetate	No	0.70	0.707	R66430B
TBA Chloride	Tributylammonium Chloride	No	0.50	0.656	R65530B
SCX	Tosic Acid	No	0.80	-	R60430B
SCX-2	Propylsulfonic Acid	No	1.00	0.642	R51430B
WCX	Carboxylic Acid	No	1.40	6.682	R70130B

Also available on all irregular Silia*Flash* Silica. Example: the 300 Å, 40-63 µm (*Rxxx30M*)

[a] Based on our Standard Silia*Flash* Silica matrix R10030B, 40-63 µm, 60 Å

Silia*Bond* Ion Exchange Phases (*con't*)

Typical applications for using Silia*Bond* Ion Exchange Phases

Sorbent Phase	Typical Applications
Silia*Bond* Amine (WAX)	A weak anion exchanger with pKa of 9.8. At pH 7.8 or below, the functional groups are positively charged. It facilitates the rapid release of very strong anions such as sulfonic acids that may be retained irreversibly on SAX.
Silia*Bond* Diethylamine (*WAX-2*)	With a pKa of 10.5, this phase is prefered over the Silia*Bond* TMA Chloride (*SAX*) when performing catch and release purification of compounds bearing a permanent negative charge such as salts of sulfonic acids. Using SAX in this case could make the release of the compounds of interest difficult (*but not necessarily impossible*). not to say irreversible, due to the strong interaction between the two strong ions.
Silia*Bond* TMA Chloride (*SAX*)	The quaternary amine is permanently charged (*pH independant*). It is commonly used for the extraction of weak cations (*such as carboxylic acids*) that may not bind strongly enough to weaker anion exchangers.
Silia*Bond* TMA Acetate (*SAX-2*)	The acetate counter ion is easily exchangeable (*so than the chloride ion*) for compounds with pKa < 5, such as carboxylic acids. This phase can be used in organic chemistry applications to selectively purify acidic compounds or remove acidic impurities from reaction mixtures.
Silia*Bond* TBA Chloride	Silia*Bond* TBA Chloride may be used in the same applications as Silia*Bond* TMA Chloride. This phase is more sterically hindered, which offers a different selectivity than other anion exchangers.
Silia*Bond* Tosic Acid (*SCX*)	Due to the very low pKa (*< 1*) these functions are strong cation exchangers since they maintain a negative charge throughout the pH scale. The most common use is likely for catch and release purification.
Silia*Bond* Propylsulfonic Acid (*SCX-2*)	
Silia*Bond* Carboxylic Acid (*WCX*)	At a pH of 6.8 or above, this weak cation exchanger carries a negative charge. A pH of 2.8 or below is needed for easier elution of strong cationic analytes that are neutralized only at extreme basic conditions. This phase is commonly used for the extraction of strong cationic species, which would be irreversibly retained on strong cation exchangers.