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# HILIC Stationary Phases

**A common and useful classification of the HILIC stationary phases is on the basis of the presence of functional groups on the surface and their charged state: they can be divided in unbounded and bonded phases, and these ones are classified in neutral, charged, and zwitterionic phases.**

The number of HILIC stationary phases available on the market is growing every day, so that the selection of the appropriate column can be, in some cases, not straightforward. A general description of HILIC phases is "*hydrophilic materials that can hold water on their surface*", but they are not all equivalent. First of all, the specific hydrophilicity of the material influences the thickness of the water layer in which the partition of the analyte can take place, with direct consequences on the retention. In addition, the presence of polar or charged functional groups contribute to electrostatic and hydrogen bond interactions. Therefore, it is extremely important to select the HILIC material on the basis of the chemical nature of the analytes to be separated.

A helpful approach to choose the most suitable column is to consider the type of phase material disclosed beyond the trade name.

HILIC phases are silica or polymer-based and they can be divided into two large groups: unmodified bare silica gels and polar chemically-bonded phases. A scheme of the classification of HILIC stationary phases is presented in Figure 1.

The first HILIC applications have been developed with unmodified bare silica gels (Type A and B), and such kind of phases are still among the most popular materials, in particular for carbohydrate separations. Silica gels present silanol groups that at mobile phase pH above 4–5 are deprotonated and they can work also as cation-exchangers, so that positively charged basic analytes are strongly retained.

Almost all column manufacturers and several research groups have spent increasing efforts in the last years in the development of polar chemically-bonded phases tailored for specific applications. These phases are prepared by derivatization of the support surface (silica or polymer) with polar functional groups and they are conveniently classified in neutral, charged and zwitterionic phases on the basis of the charged state of the groups.

Neutral stationary phases contain polar functional groups that are in neutral form in the range of pH 3–8, usually used for the mobile phase in HILIC, and, thus, the retention is mainly supported by hydrophilic interactions. Many HILIC stationary phases belong to this category, which comprises a large variety

Figure 1

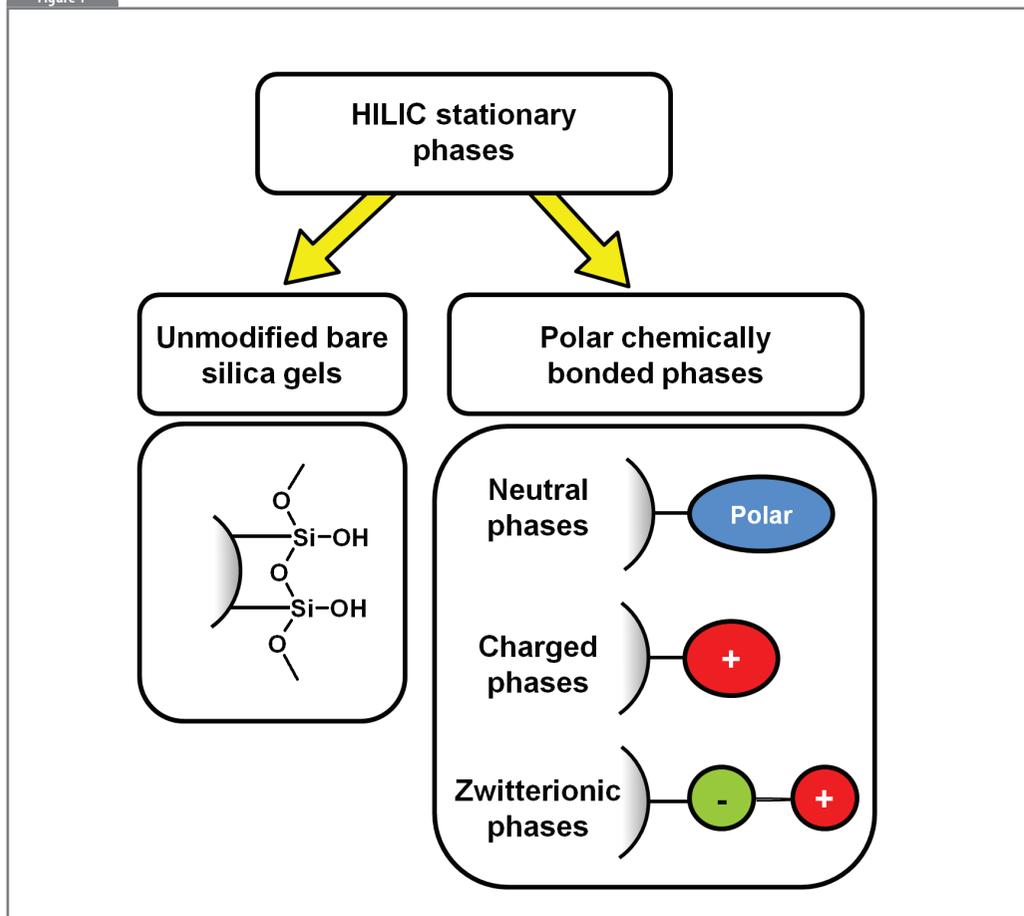


Figure 1: Classification of HILIC stationary phases.

of functional groups, such as the amide (TSK gel Amide-80), aspartamide (PolyHYDROXYETHYL A), diol (YMC-pack Diol), cross-linked diol (Luna HILIC), cyano (Alltima Cyano) and cyclodextrin (Nucleodex  $\beta$ -OH) groups. They have found application for the separation of oligosaccharides, peptides, proteins, and oligonucleotides. Cyclodextrin phases are also used for HILIC chiral separations.

Amino phases are among the principal HILIC charged phases. The functional group is an aminopropyl ligand with a primary amino group that is positively charged and shows high affinity for anionic acid compounds that can be occasionally irreversibly adsorbed. In this case, the separation of charged analytes is largely based on an anion-exchange mechanism. Amino phases can also be successfully employed for the separation of neutral polar compounds

that are strongly retained owing to the high hydrophilicity of these phases.

Zwitterionic HILIC stationary phases are particularly versatile and they can be considered the HILIC all purpose phases. Some of the zwitterionic phases are commercialized under the trademarks ZIC-HILIC, ZIC-cHILIC, KS-polyMPC, Obelisc R and Obelisc N.

Zwitterionic ligands comprise a permanent positive charged group and a permanent negative charged group. These phases are particularly hydrophilic but at the same time they have modest ion-exchange properties. For that reason, they can be employed for the separation of neutral, acid and basic analytes, as well as for the separation of inorganic ions.

A good rule for selecting the most appropriate HILIC phase is to keep in mind that neutral analytes are in general less hydrophilic than charged ones, and high hydrophilic phases are required for their retention, as charged, zwitterionic and amide phases. In contrast, charged compounds are too strongly retained on the charged columns due to electrostatic attractions, so that neutral and zwitterionic phases give better results.

After the selection of the stationary phase one has to choose the elution solvents, thus in the next instalments we will discuss the selection and preparation of the mobile phase.

**Next Tip:** *HILIC mobile phase: solvents*

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Thomas Letzel, Associate Professor, is head of the Analytical Research Group at the Technische Universität München, Germany. He received his PhD in Chemistry with Aerosol Analysis, worked as Post-Doc performing pharmaceutical analysis, built up his research group in bioanalysis with Habilitation in 2009 and extended his analytical experience from then in food and water analysis. In all areas he developed novel analytical platforms based on LC-MS for the characterization of organic molecules in complex matrices. Thereby techniques (such as HILIC or RP-UHPLC) are applied for new analytical solutions often in direct flow-coupling with (bio)functional assays. He is the author of more than 50 publications and two books.

Thomas Letzel wants to share his experience in liquid chromatography, especially in HILIC, with the community to accelerate the dissemination about HILIC theory and practical handling.