SILICA HYDRIDE COLUMNS: A RUNNING SUCCESS FOR SEPARATING POLAR AND NONPOLAR COMPOUNDS

Sometimes scientific progress is deliberate, other times it's serendipity. Joe Pesek, professor emeritus at San Jose State University, has spent over 50 years in academia researching and developing novel chromatographic separation materials. To date, the most successful technology to come out of his lab is a silica hydride (Si–H) stationary phase for high-performance liquid chromatography (HPLC). While Pesek created these particles with an eye toward stability, it turns out they have an added bonus: superior separations for many applications, especially of polar compounds.

Most HPLC columns are packed with silica. Many have ligands bonded to the silica to tweak its surface chemistry and therefore its separation capabilities. Pesek developed his alternative version with a Si–H surface (Figure 1) during a quest early in his career to make a rugged silica-based stationary phase with octadecyl and cholesterol-like ligands. At that time and still today, all the silica columns on the market used siloxane (Si–O–Si) linkages to attach ligands to the surface of silica particles. Pesek wanted to use a silicon-carbon bond linkage instead. "A silicon-carbon bond is very stable in comparison to the siloxane linkages," he explains.

Pesek devised a two-step process to modify silica particles with organic molecules via Si–C bonds. First, effectively convert silanol (Si-O-H) end groups on the surface of silica

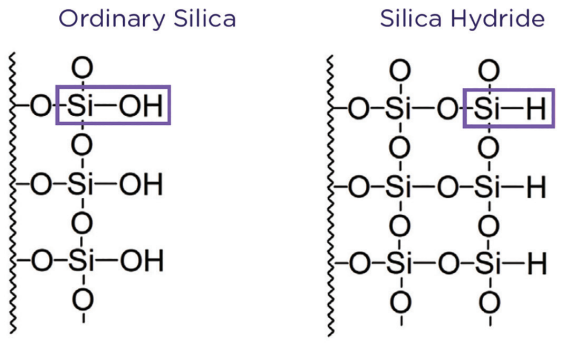

Figure 1: Silica hydride has the same basic structure as ordinary silica, but with different surface chemistries.

Source: MicroSolv Technology

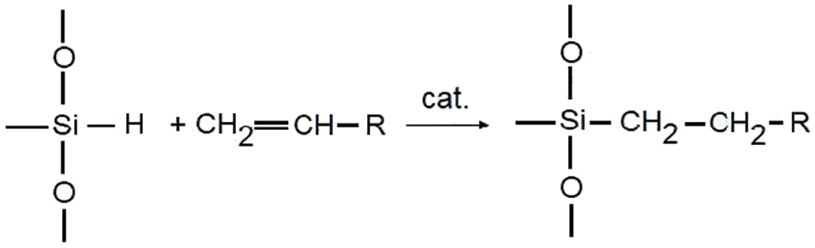

Figure 2: Silica hydride can be modified just as easily as ordinary silica through the use of a well-characterized reaction referred to hydrosilation, shown here.

Source: MicroSolv Technology

particles to silica hydrides. Second, react these groups with the organic molecule using the hydrosilation reaction (Figure 2).[1] "The only way to efficiently bond organic molecules to silica was this process of creating a hydride surface," says Bill Ciccone, the president of MicroSolv Technology, which commercialized the technology in the early 2000s.

It worked. The material was indeed exceptionally robust, and the separation achieved was even better than expected, Pesek says, especially for polar molecules. He optimized the addition of the Si–H layer in his academic lab and established that the layer can form silicon-carbon bonds with molecules other than octadecyl and cholesterol. Any molecule possessing a terminal alkene or alkyne can be added to the surface.

Over the decades, Pesek and MicroSolv Technology have attached many different ligands to the silica hydride–coated particles and, working with collaborators, Pesek has demonstrated the prowess of these novel stationary phases for separating polar and nonpolar compounds using various HPLC separation modes.

REVERSED-PHASE CHROMATOGRAPHY

Reversed-phase HPLC, especially when coupled to mass spectrometers and ultraviolet detectors, is widely used by multiple industry sectors, including food, pharmaceuticals, clinical, environmental, and forensics.

Chromatography separates mixtures of compounds in accordance with how their components adsorb and desorb from the surface of the stationary phase. In reversed-phase chromatography, a nonpolar stationary phase and a polar solvent are used. As like attracts like, the most polar compounds elute first and the least polar last.

The explanation for the superior separation aptitude for silica hydride–based columns in this mode lies with the significant polarity differences between the two particle types.[1,2] Unmodified silica particles are inherently extremely polar, whereas particles with the Si–H layer are slightly nonpolar.

In traditional silica-based stationary phases, the particles can be modified to make them suitable for use in reversed phase, either by capping some of

the Si-O-H with bulky end groups or modifying them with ligands that tame their polarity. But the effects of the polarity of the underlying surfaces are still observed, with the Si-O-H end groups that remain on the silica particles creating stickiness issues. Polar compounds can permanently adhere to silica particles or elute very slowly, which results in peaks with long tails in the chromatogram.

"The hydride surface, being mildly hydrophobic, does not allow polar molecules to easily adsorb on the surface," Pesek says. Polar molecules are therefore more efficiently removed from Si–H columns, giving consistent symmetrical peaks.

Silica hydride columns are commercially available with a number of different ligands attached to the particle surfaces under the Cogent™ brand. Scientists can therefore optimize separation and purification strategies by selecting columns that match up with the properties of the molecule of interest.

For the separation of aromatic molecules, Si–H particles modified with phenyl groups may be an appropriate choice. These particles have a four-carbon linker with a terminal phenyl group attached to the Si–H surface by the silica carbon bond. π-π stacking interactions between the phenyl groups on the column and the aromatic molecules assist the separation process. Researchers recently demonstrated this Phenyl Hydride™ column's utility in the analysis of synthetic cathinones in hair.[3] The polar cathinones are stimulants sold illicitly as "bath salts" and are banned in the US, UK, and other countries.

Silica hydride–based columns also perform well in the separation of nonpolar compounds in reversed-phase mode. For example, the cholesterol column discussed at the beginning of this article can separate compounds based on small structural differences, even if they are similar in polarity. In the case of the cis and trans isomers elaidic and oleic acid, the liquid crystal properties of the cholesterol-like ligand facilitate separation, Pesek explains. "With the ordered structure on the surface, analytes that are more linear than bulky tend to retain better," he says. "Therefore, you have a way to discriminate between molecules of similar molecular composition but have a different structural shape."

NORMAL-PHASE CHROMATOGRAPHY

Normal-phase HPLC also is used in multiple industry sectors and is extensively applied to separate polar molecules such as amines, acids, metal complexes, and fats.

In normal-phase chromatography, a polar stationary phase and nonpolar solvent are used. This means nonpolar compounds elute from the column first, with the polar compounds coming off later. The mechanism explaining the performance boost for silica hydride–based columns compared with silica particles in normal-phase mode is different for that of reversed phase. Here, the different-sized hydration shells around the particles are responsible.[1]
In traditional silica stationary phases, the silanol end groups on a silica particle surface strongly absorb any water molecules present in the solvent, forming

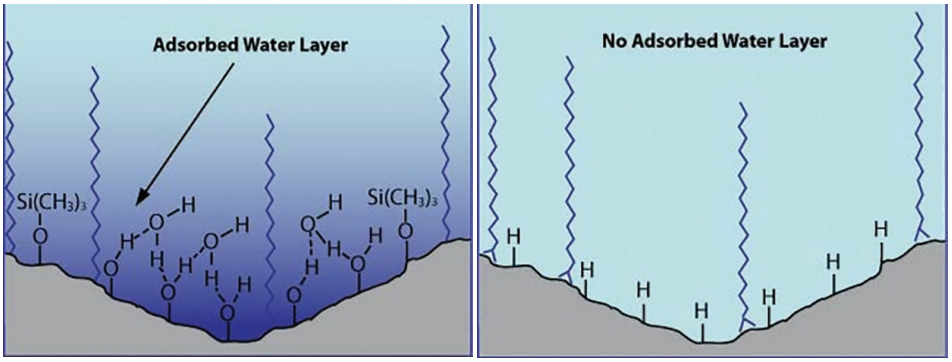

Figure 3: The silanol end groups on a silica particle surface (left) strongly adsorb any water molecules present in the solvent. By contrast, silica hydride particles (right) only weakly attract water.

Source: MicroSolv Technology

a thick hydration shell up to 10 water molecules deep. (The organic solvent used for chromatography is never completely dry and contains more than enough water for silica particles to form these hydration shells.) "Silica is very adsorbent," Pesek says. "Those little packets that come with electronic components—they're all filled with silica because it adsorbs water very well."

By contrast, nonpolar silica hydride–coated particles are much less hydroscopic (Figure 3). They only weakly attract water and as a result host less than half a monolayer of water on their surface. As hydration shells can hinder the absorption and desorption processes needed for the normal phase chromatographic separation, a thin hydration shell is an obvious advantage.

AQUEOUS NORMAL-PHASE CHROMATOGRAPHY

To further boost the separation of polar compounds in normal phase chromatography with silica hydride–based columns, 10% or more water can be added to the organic solvent mobile phase. Adding water completely changes the separation mechanism, according to Pesek. He and his lab and life partner, Maria Matyska-Pesek, along with collaborators in Australia have investigated the science behind this separation improvement.[2]

The water component causes an electrical charge to form on the column that influences the separation, Ciccone says. "The mechanism is the same as that of oil spilt in the ocean." Hydrophobic oil goblets don't sit around in easy-to-clean-up slicks; they break down into microparticles that dissipate. "The microparticles absorb hydroxyl groups, formed by the dissociation of water, gaining a negative charge which makes the oil disperse in water," Ciccone explains. On the column, the silica hydride–coated particles gain negative charges in water due to the absorption of hydroxyl groups. "This gives [the particle] a negative charge that makes it retain polar compounds by charge attraction," Ciccone says. Polar compounds are retained on the column longer than nonpolar ones.

Because it has a different separation mechanism and separation performance characteristics than normal-phase chromatography, and because of the addition

of water, this type of chromatography has been named aqueous normal-phase chromatography.

Users tend to confuse aqueous normal-phase chromatography with another popular variant of chromatography that uses predominantly organic solvent with small amounts of water: mobile-phase hydrophilic interaction chromatography (HILIC). Compounding the confusion is that this mode is also marketed for separating polar compounds. But the HILIC mechanism is not based on electrical charge and therefore doesn't have any of the advantages offered by the silica hydride–based stationary phase, Ciccone says. As well as having a unique mechanism, aqueous normal phase chromatography also has very different performance characteristics compared to HILIC. It offers faster equilibration times after gradients allowing for a shorter gap between sample injections, improved method precision and often selectivity benefits, he adds.

Aqueous normal-phase chromatography is highly popular with those working in metabolomics and bioanalysis, according to Ciccone. Many metabolites and bioanalytes can be highly polar molecules, meaning retention is a regular issue during their separations. A Si–H column with three-carbon ligands with a total of 2% carbon load has been devised specifically for this market. This is known as the Diamond Hydride™ column. "The very small amount of carbon gives the surface a little more hydrophobicity," Pesek says. "This causes minor differences in retention between the unmodified Si–H and the Diamond Hydride™ column and tends to diminish strong polar interactions and thus improve peak shape." Separations that this column has been used for under aqueous normal-phase conditions include the four polar vitamins: ascorbic acid, riboflavin, pyridoxine, and thiamine (Figure 4). If reversed-phase chromatography with a silica column was attempted for this analysis, additives called ion pair agents would likely be needed to achieve an acceptable separation and would complicate the method.

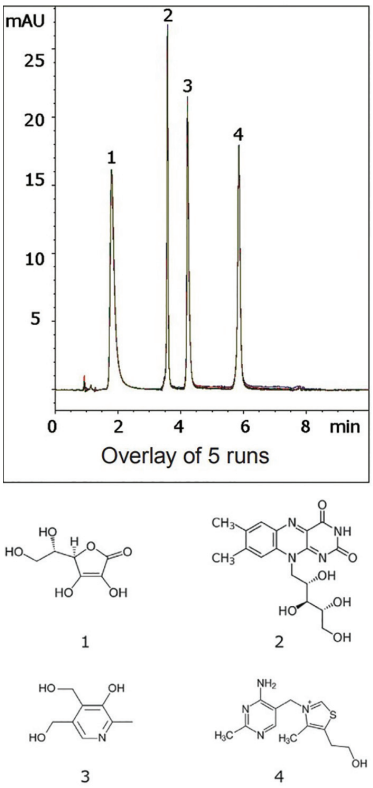

Figure 4: A chromatogram showing the separation of four polar vitamins ascorbic acid (1), riboflavin (2), pyridoxine (3), and thiamine (4) using aqueous normal phase chromatography and a silica hydride column.
Source: MicroSolv Technology

A more challenging demonstration of the Diamond Hydride™ column, under aqueous normal-phase conditions, was the simultaneous separation of a mixture of very polar and nonpolar peptides in a single run.[4] The proportions of the two solvents used for the separation was varied during the run. "For proteomics, two columns are typically

needed—one very often is ion exchange, the other is reversed phase—to be able to sort out all the peptides," Pesek says.

Aqueous normal-phase chromatography has also proved its worth in the food industry as a solution in separating polar sugars. The Si–H column designed for this purpose has ligands with amide functional groups bonded to the Si–H surface. Pesek's demonstrations of this column's effectiveness include the separation of the structural isomers fructose and glucose in cola. "Separations depend on polar interactions with the surface and the different structural isomers interact to different degrees with the bonded amide," Pesek says.

CONCLUSION

Silica hydride-based stationary phases offer robust, long-lasting columns suitable for reversed-phase, normal-phase, and aqueous normal-phase chromatography. The latter is a mode unique to silica hydride columns that tends to be confused with the better known HILIC. However, aqueous normal phase chromatography has both a distinct separation mechanism and distinctive performance characteristics compared to HILIC.

As an entirely different type of stationary phase to their silica counterparts, silica hydride-based columns often need some quick method development or optimization to get the best out of them. "It's a learning curve," Pesek says, but the superior separation results make the effort worth it. He, Matyska-Pesek, and MicroSolv Technology's technical support department frequently support separation scientists with this process. Ciccone says it's time for chromatographers to invest a little more time in finding a more robust and better separation method, rather than going with the first one that sort of works.

REFERENCES

1. Maria Matyska and Joseph Pesek, "The Development of Silica Hydride Stationary Phases for High-Performance Liquid Chromatography from Conception to Commercialization," *Separations* 6, no. 2 (June 2019): 27–41, https://doi.org/10.3390/separations6020027.
2. Chadin Kulsing et al., "Insights into the Origin of the Separation Selectivity with Silica Hydride Adsorbents," *J. Phys. Chem Part B* 119, no. 7 (Feb. 19, 2015): 3063–69, https://doi.org/10.1021/jp5103753.
3. Joseph Pesek at al., "Evaluation of Silica Hydride Materials for the LC–MS Analysis of Cathinones and Benzylpiperazines," *Forensic Chem*. 8 (May 2018): 90–94, https://doi.org/10.1016/j.forc.2018.03.002.
4. Reinhard I. Boysen et al., "Simultaneous Separation of Hydrophobic and Hydrophilic Peptides with a Silica Hydride Stationary Phase Using Aqueous Normal Phase Conditions," *J. Chromatogr. A* 1218, no. 44 (Nov. 4, 2011): 8021–26, https://doi.org/10.1016/j.chroma.2011.09.009.

AMERICAN CHEMICAL SOCIETY
1155 Sixteenth Street, NW,
Washington, DC 20036