Chromatography Focus

ONE STATIONARY PHASE, THREE MODES OF SEPARATION: REVERSED PHASE AND NORMAL PHASE SEPARATIONS ON THE SAME COLUMN

Joseph J. Pesek, Maria T. Matyska

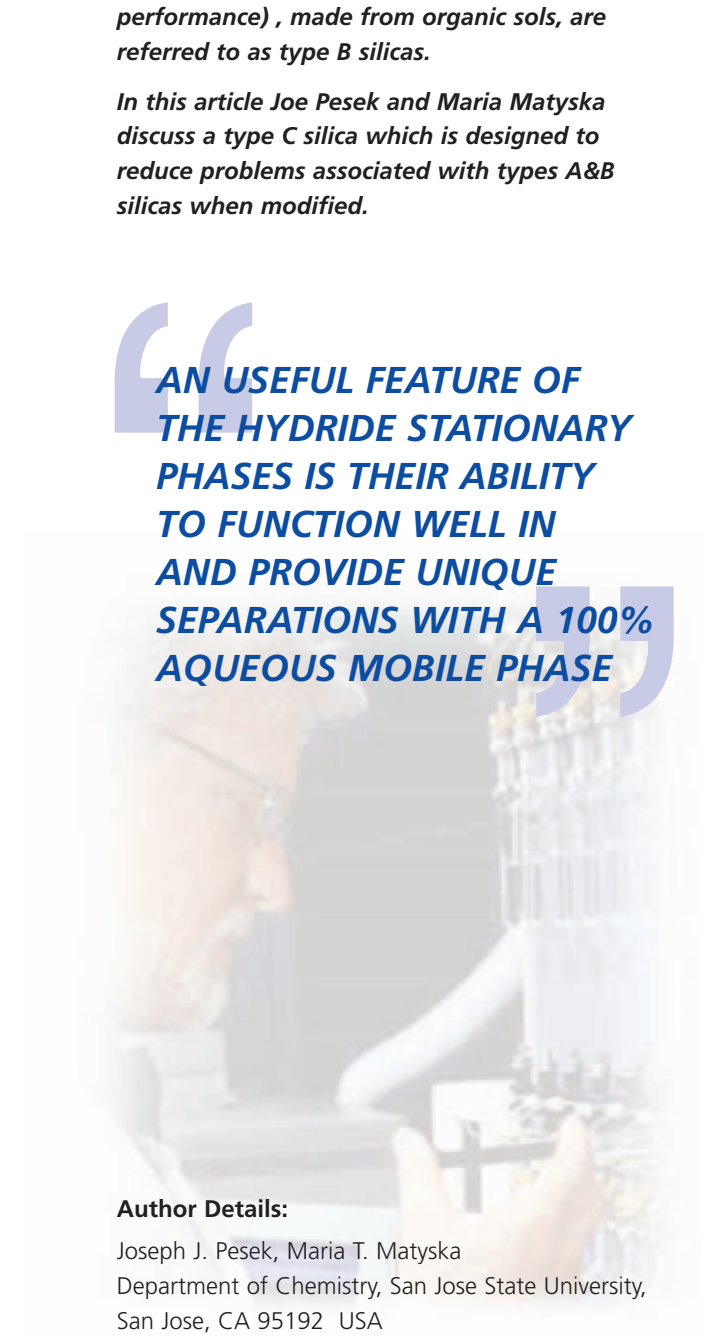
The most commonly used support material for HPLC packings are spherical, micro porous particles of silica and these are amorphous (i.e. non-crystalline) in nature. The surface of the silica particles shows poor homogeneity, being a mixture of silanols (Si-OH) of varying degrees of activity, and siloxane groups (Si-O-Si).

This makes subsequent surface modifications to produce bonded phases greatly reliant on the nature of the base silica.

Early silicas, made from sodium silica sols , were referred to as type A silicas. The more recent 'ultra pure' silicas (exhibiting much lower levels of transition metal ions and better surface homogeneity - resulting in more reproducible and better chromatographic performance) , made from organic sols, are referred to as type B silicas.

In this article Joe Pesek and Maria Matyska discuss a type C silica which is designed to reduce problems associated with types A&B silicas when modified.

"AN USEFUL FEATURE OF THE HYDRIDE STATIONARY PHASES IS THEIR ABILITY TO FUNCTION WELL IN AND PROVIDE UNIQUE SEPARATIONS WITH A 100% AQUEOUS MOBILE PHASE"



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No single stationary phase can solve all separation problems but a single material with versatile properties, i.e. one capable of retaining both polar and nonpolar analytes, is highly desirable considering that many analyses involve a broad range of compounds. However, the vast majority of all commercial stationary phases have a dominant physical characteristic, hydrophobic, hydrophilic, chiral, ion-exchange, etc., and are designed for retaining certain types of solutes. Complex samples for biological, clinical, pharmaceutical, food and environmental analyses often contain analytes with a range of properties, the most common being polarity. Thus typical reversed phase materials such as C18 or C8 retain hydrophobic compounds but not hydrophilic species and more polar stationary phases such as bare silica, amino and cyano will behave in the opposite manner with a preference for hydrophilic analytes while polar solutes elute at or near the void volume. The separation material described in this article is based on silica hydride rather than ordinary silica. The difference between these two stationary phase supports is shown in *Figure 1*.

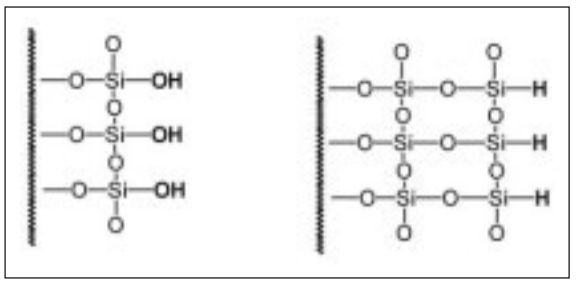


Figure 1. Surface composition of ordinary silica (left) and hydride silica (right).

The hydride surface can be chemically modified like ordinary silica but the highly adsorptive silanols, particularly for basic solutes and water in the mobile phase, are not present. The hydride surface in combination with a bonded group provides the unique capability of retention for both polar and nonpolar species, in many cases simultaneously. Thus complex samples having both hydrophilic and hydrophobic compounds can be separated in a single chromatographic run, in many cases under isocratic conditions. In addition, these bonded materials can also be used for organic normal phase separations. Each of these features will be reviewed in the sections below.

REVERSED PHASE RETENTION

The most common type of stationary phase used in HPLC is based on reversed phase properties; a nonpolar material used in conjunction with an aqueous/organic mobile phase. Retention/separation occurs mainly through differences in hydrophobicities between the various solutes. Hydride based stationary phases are available with C18, C8 and cholesterol (Microsolv Technology, Eatontown, NJ, USA) bonded to the surface and each can function in the reversed phase mode. Reversed phase is characterised by a decrease in retention time as the amount of organic modifier is increased in the mobile phase. *Figure 2* shows a plot of retention time for the hydrophobic solute glyburide (log of the octanol/water partition coefficient (Log P) = 4.79) vs. the percentage of organic constituent in the mobile phase on a hydride-based C8 column. This plot is characteristic of reversed phase behavior and as expected there is less retention for acetontrile (the stronger organic solvent) at a particular mobile phase composition.

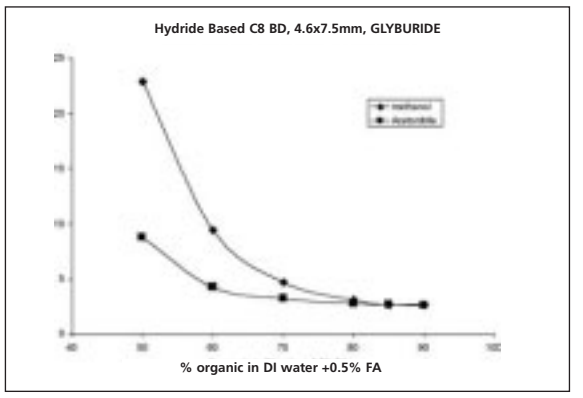


Figure 2. Retention map for glyburide on a hydride-based C8 stationary phase with methanol (●) and acetonitrile (■) as organic modifiers.

Another usual and useful feature of the hydride stationary phases is their ability to function well in and provide unique separations with a 100% aqueous mobile phase. Various structural isomers of carbohydrates (compounds with the same molecular weight in different geometrical arrangements) as well as mixtures of organic acids have been separated using pure aqueous mobile phases.

All hydrophobic molecules, those that generally have positive Log P values, even some possessing ionic or strong polar functional groups, exhibit reversed phase behavior on hydride-based stationary phases. For these compounds, the reversed phase behavior of the hydride columns is similar to that obtained on other high quality commercial endcapped C18 or C8 stationary phases.

AQUEOUS NORMAL PHASE RETENTION

The aqueous normal phase (ANP) is defined as retention of hydrophilic solutes in water/organic mobile phases that increases as the amount of the least polar component is increased.

Thus for an acetonitrile/water eluent, retention would increase as the amount of acetonitrile is increased. This behavior parallels classical normal phase retention but using a mobile phase with an aqueous component. Generally polar stationary phases (silica, cyano, amino, etc.) are utilised to retain hydrophilic compounds in this mode.

This type of behavior is illustrated in *Figure 3* showing the retention map for the basic drug tobramycin. For acetonitrile ANP retention is readily apparent but only a small increase occurs for methanol. This is expected since acetonitrile is less polar than methanol so it should induce greater normal phase retention.

This same pattern is observed for other hydride based phases using polar molecules such as tobramycin with the general trend in ANP retention being cholesterol > C18 - C8. For many solutes, there is considerable ANP retention with methanol on the cholesterol phase.

Most ionic/strongly polar compounds display some degree of ANP behavior on the hydride-based columns. These compounds can even have a positive Log P value, but the presence of these polar groups leads to ANP behavior. Some examples of molecules that exhibit ANP behavior, although not all inclusive, include most compounds with primary amines, some peptides, carbohydrates and many carboxylic acids.

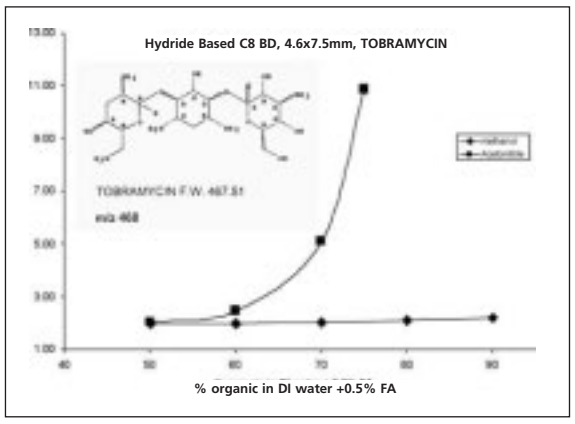


Figure 3. Retention map for tobramycin on a hydride-based C8 stationary phase with methanol (●) and acetonitrile (■) as organic modifiers.

ANP can be considered in some respects to be similar to hydrophilic interaction liquid chromatography (HILIC). However, HILIC stationary phases are primarily able to retain only those compounds with considerable ionic/polar character. Most hydrophobic compounds exhibit little or no retention on a HILIC phase. In contrast, hydride based stationary phases will retain both type of molecules, polar and nonpolar, sometimes even simultaneously. Thus the term ANP provides a useful means for distinguishing between the hydride materials which have two modes of retention and a HILIC phase which has only one.

ORGANIC NORMAL PHASE RETENTION

Another interesting capability of the hydride-based stationary phase is organic normal phase (ONP) retention. In ONP only organic solvents are used and retention increases as the amount of the least polar component in the mobile phase increases. This mode is often used for compounds containing moderately polar but not ionic functional groups. An example of an ONP separation using a hydride-based C18 phase is shown in Figure 4 for phenol compounds.

The mobile phase is hexane:ethyl acetate and retention increases as the amount of hexane in the mobile phase increases. Both peak shape and the reproducibility of this separation are excellent, a result that is often difficult to achieve in ONP. In addition to the bonded phases mentioned above, the unmodified silica hydride can often be used effectively in the ONP mode. Because hydride surfaces do not absorb water to any extent, no special precautions to exclude moisture from the organic solvents are needed in ONP operation.

This characteristic of the hydride phases is probably responsible for the excellent chromatographic data cited above. Gradient separations can also be obtained in the ONP mode on the hydride phases. Reproducible gradient results are often very difficult on traditional normal phase separation materials such as silica, amino, cyano and diol columns. In addition to phenols, many aldehydes, ketones, esters and halogenated compounds have been shown to display ONP behavior on the hydride phases.

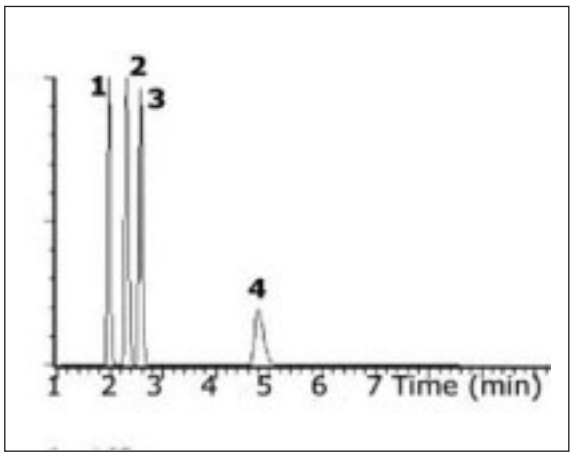


Figure 4. Separation of phenols on a hydride-based C18 stationary phase in the normal phase mode using 95:5 hexane/ethyl acetate. Peaks: 1 = phenol with aldehyde; 2 = parent phenol; 3 = phenol with ketone; 4 = phenol with acid.

ANP can be considered in some respects to be similar to hydrophilic interaction liquid chromatography (HILIC). However, HILIC stationary phases are primarily able to retain only those compounds with considerable ionic/polar character.

Table 1. Reproducibility of Retention Times for Gradient Analysis

Retention Time (min)			
Solute/Equilibration Time	25 min	10 min	1 min
Benzene	7.30	7.35	7.25
Naphthalene	11.10	11.07	11.01
Phenanthrene	14.39	14.37	14.37
Anthracene	14.81	14.80	14.80
Pyrene	16.52	16.51	16.56

Gradient program: 0-3 min ACN/water (50:50); 3-18 min to 100% ACN; 18-23 min 100% ACN; Equilibration to 50:50 ACN/water.

OTHER FEATURES OF HYDRIDE-BASED STATIONARY PHASES

The lack of water adsorption on the hydride surface leads to another significant feature of these stationary phases. For gradient analyses on ordinary silica based stationary phases, it is often necessary to allow a considerable period of time (equilibration) between runs when the mobile phase returns to the starting conditions. This is not the case for the hydride phases as shown in *Table 1*. In this case, retention times for subsequent runs were reproducible after only a one minute equilibration following the completion of the analysis. These results are typical of all hydride-based stationary phases. Because these separation materials have both reversed phase and ANP capabilities, reverse gradients (from higher to lower organic content) are also possible and are reproducible for the separation of hydrophilic analytes. The same rapid equilibration of the stationary phase for subsequent analyses that occurs after standard gradients in the reversed phase mode is present in the ANP mode when using reverse gradients.

Perhaps the most significant property then of the hydride-based materials involves the ability to separate both polar and nonpolar compounds at the same time. This capability is the result of having both RP and ANP retention on hydride based stationary phases. These mechanisms involve using aqueous containing mobile phases so it is a matter of adjusting the composition of the eluent to determine which mode will be dominant. At higher percentages of water in the mobile phase, the RP mechanism will be predominant while at higher percentages of organic the ANP mechanism prevails. The exact point of the crossover depends on both the compounds and the organic component in the mobile phase. Figure 5 shows the separation of a mixture containing both polar and nonpolar species. The molecular properties of the compounds are shown

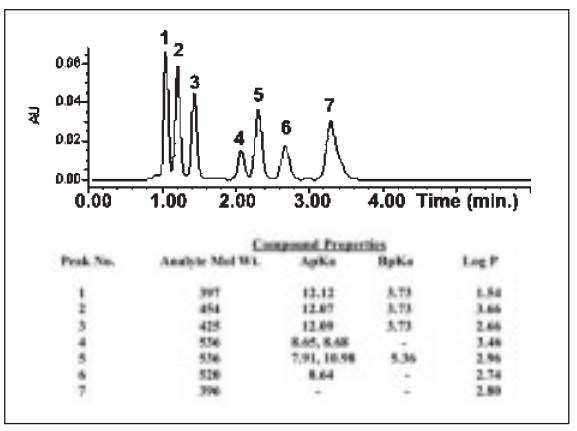


Figure 5. Separation of a mixture containing both polar and nonpolar compounds on a hydride-based cholesterol stationary phase.

in the figure. In this example using a cholesterol bonded hydride phase and a 60:40 acetonitrile/water mobile phase, the nonpolar species are retained more than the polar compounds. In this example Compounds 1-3 are the most polar with strongly basic functional groups while Compounds 4-7 have only weakly basic and weakly acid groups.

Under these circumstances the RP mechanism is stronger that the ANP. However, increasing the amount of acetonitrile in the mobile will lead to ANP becoming stronger than RP and in this case Compounds 4-7 will elute before Compounds 1-3.

Another facet of the hydride-based stationary phases is their ability to operate at high temperatures. Because of the bonding reaction used on the silica hydride material, some stationary phases have a double attachment to the surface, i.e. are usually referred to as bidentate phases. In many cases operation at elevated temperatures can lead to improved mass transfer and thus higher effiencies. In the reversed phased mode, the use of high temperature results in increased solubility for some analytes and using a 100% aqueous mobile phase will extend the upper temperature even more than most mixed eluents, especially if the system is maintained at high pressure.

CONCLUSIONS

Hydride-based stationary phases are a unique and still evolving separation material. The broad range of operating conditions possible on these columns leads to a complete range of retention mechanisms that includes reversed phase and all forms of normal phase.

New formats and bonded moieties may yield improved performance over the current commercially available materials or provide unique selectivities for solving the most demanding separation problems.

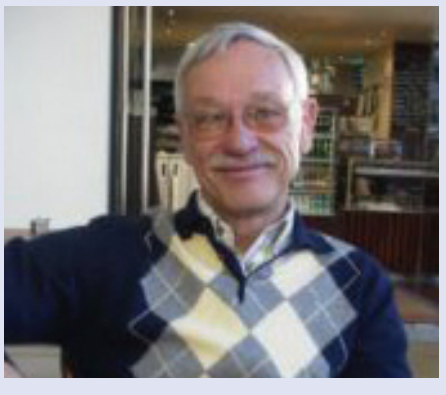
References

1. J. J. Pesek, M.T. Matyska, LC/GC, 24 (2006) 296-303.

2. J.J. Pesek, M.T. Matyska, J. Liq. Chromatogr & Rel. Technol., 29 (2006) 1105-1124.

3. http://www.microsolvtech.com/ where hydride phases are referred to as Type-C silica

BIOGRAPHY – JOSEPH J. PESEK



Joseph Pesek received his B.S. degree in Chemistry from the University of Illinois and his Ph.D. in Analytical Chemistry from the University of California, Los Angeles. He did a one-year postdoctoral fellowship at UCLA before becoming Assistant Professor of Chemistry at Northern Illinois University. He moved to San Jose State University becoming Professor of Chemistry, Department Chair, and Dean for Graduate Studies and Research. He was selected the President's Scholar at San Jose State for his research productivity and contributions to the development of graduate students. He was named a Camille and Henry Dreyfus Foundation Scholar in 1993 and 2001. He has over 160 publications, 3 books, 3 patents and over 200 presentations at a variety of symposia and meetings. He is an editor for the Journal of Separation Science. His research interests are in the development, characterisation and applications of separation materials for chromatographic and electrophoretic processes.