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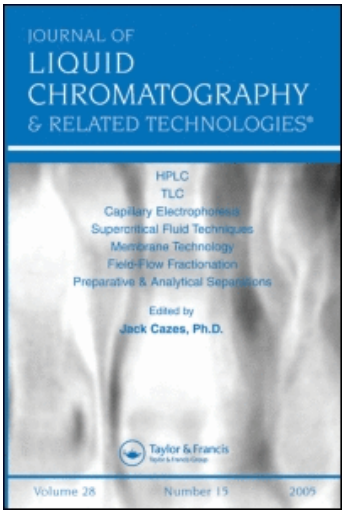

Journal of Liquid Chromatography & Related Technologies
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To cite this Article Pesek, Joseph J. and Matyska, Maria T.(2006) 'Silica Hydride Surfaces: Versatile Separation Media for Chromatographic and Electrophoretic Analyses', Journal of Liquid Chromatography & Related Technologies, 29: 7, 1105 — 1124
To link to this Article: DOI: 10.1080/10826070600574770
URL: http://dx.doi.org/10.1080/10826070600574770

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Journal of Liquid Chromatography & Related Technologies®, 29: 1105–1124, 2006
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ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600574770

Silica Hydride Surfaces: Versatile Separation Media for Chromatographic and Electrophoretic Analyses

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Abstract: Silica hydride, where Si-H groups replace 95% of the silanols on the surface, is rapidly being developed as a chromatographic support material for HPLC. This chemical structure results in changes of fundamental properties compared to ordinary silica, as well as the bonded stationary phases produced by the further modification of the hydride surface. Several unique chromatographic capabilities of hydride based phases are described, as well as some general application areas where these bonded materials may be used in preference to, or have advantages not available from current commercial stationary phases. The fabrication, properties and applications of etched chemically modified capillaries for electrophoretic analysis are also reviewed. The etching process creates a surface that is fundamentally different in comparison to a bare fused silica capillary. The new surface matrix produces altered electroosmotic flow properties and is more compatible with basic and biological compounds. After chemical modification of the surface, the bonded organic moiety (stationary phase) contributes to the control of migration of solutes in the capillary. Both electrophoretic and chromatographic processes take place in the etched, chemically modified capillaries leading to a variety of experimental variables that can be used to optimize separations. Representative examples of separations on these capillaries are described.

Keywords: Silanization/hydrosilation, Aqueous normal phase, Etched capillaries

INTRODUCTION

Separation media with novel or improved analytical capabilities are a continuing source of investigations due to the increasingly complex problems

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encountered in biological, environmental, and forensic samples. Methods for proteomics, metabolomics, water quality, and crime investigations are just a few examples where improved separation techniques are required. In some cases, the analytical capabilities needed in these areas were not even envisioned a few years ago. Chromatographic, as well as in some instances, electrophoretic methods play an important role in solving these challenging analytical problems. For chromatographic methods, HPLC and HPLC-MS provide a broad range of options for complex separations. The key component for HPLC methods is the column and the separation media within it. For electrophoretic methods, capillary electrophoresis and related technology are rapidly evolving and new applications are continuingly being developed. In HPCE, the capillary and how it is configured determines the separation capabilities of the method.

In both methods, silica is a central feature of the separation medium as modified particles for the stationary phase in liquid chromatography and some forms of capillary electrochromatography (CEC), and as the material of choice for the capillary tubing in HPCE. Silica is usually defined as a polymer of silicic acid containing siloxane linkages (-O-Si-O-) and OH groups (silanols), where crosslinking is incomplete or at the terminal position of the chain. A variation of this structure is also possible where the surface of the polymeric structure contains siloxane linkages and hydride groups (Si-H) as the primary chemical features. This new material is usually referred to as silica hydride. While some facets of silica hydride have been know for many years, its use as a separation medium did not begin until around 1990.[1,2] The chemical difference between ordinary silica and its hydride counterpart is illustrated in Figure 1. The essential feature is that the silanol groups (Si-OH) on ordinary silica have been replaced by Si-H moieties on the hydride material. This leads to a number of interesting properties that can be exploited as part of the solid support of stationary phases for HPLC, as well as on the inner walls of capillaries for electrophoretic separation media.

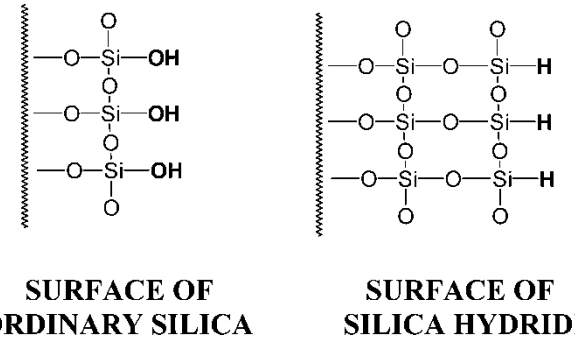

Figure 1. Chemical surface features of ordinary silica and silica hydride.

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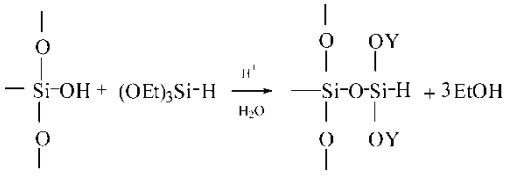
Silica Hydride-Based Stationary Phases

Silica hydride HPLC stationary phases utilize high purity, low metal content commercial silica. However, the fabrication process for silica hydride involves a silanization reaction that produces a surface populated with silicon-hydride groups (Si-H), which are stable even in most aqueous environments and impart a less polar character to the silica surface. The basic chemical reaction is as follows:[3,4]

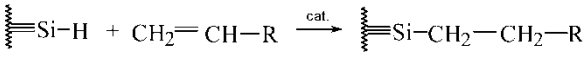
Silanization



Y = Si or H depending on the extent of cross-linking

As illustrated in Figure 1, the surface of the hydride material is predominantly populated (>95% as determined by ^{29}Si CP-MAS NMR[4]) with the non polar, silicon-hydride (Si-H) groups instead of the polar silanol groups (Si-OH) that dominate the surface of ordinary silica. Further modification of the hydride surface occurs through hydrosilation[2] that produces a bonded stationary phase with additional specific properties as a separation medium (hydrophobic, hydrophilic, ion-exchange, chiral, etc.):

Hydrosilation



cat = catalyst, typically hexachloroplatinic acid or free radical initiator

However, since all of the hydrides cannot be reacted in the hydrosilation reaction (typically 10–50%) due to steric restrictions, the underlying surface can still influence the chromatographic behavior of solutes. As shown above, the organic moiety is attached to the surface by a stable Si-C bond. This bonding configuration leads to the high stability reported in certain chromatographic experiments.[5,6] While the most common approach for attaching an organic species to silica hydride utilizes a compound with a terminal olefin, it is also possible to bond molecules with the olefin in a nonterminal position,[7] alkynes[8] and compounds with cyano groups.[9] This versatility in the attachment of organic moieties to the surface leads to the possibility of producing stationary phases not feasible by other bonding methods. One such example is the

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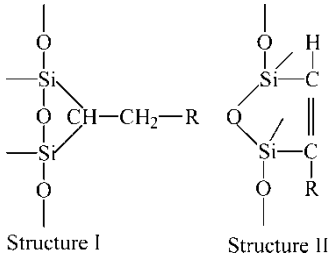
double attachment of the organic group that occurs when an alkyne is used in the hydrosilation reaction.[8] The two structures thought to be most likely from NMR studies are as follows:



Structure I Structure II

The long-term stability of the hydride surface is a crucial aspect of this material because of the limited stability of silanes in aqueous solutions. However, the Si-H moiety on a silica surface is in a chemical environment with different properties, so it cannot be compared to free silane molecules in solution. The improved stability of the Si-H group on a silica surface has been demonstrated in a number of ways. One simple test involved obtaining diffuse reflectance infrared Fourier transform (DRIFT) spectra of several silica hydride samples prepared on various commercial silicas over a period of more than ten years. No special precautions were taken for the preservation of these silica hydride materials (most were stored in simple screw top containers). Therefore, the samples were kept in neither dry nor inert conditions. The key feature in the DRIFT spectra is the Si-H stretching band at 2250 cm^{-1}.[10] For one eight year old sample, the intensity of this band was identical to the intensity obtained at the time of synthesis, proving that these materials have excellent stability when stored as an unprotected solid. A more direct test of the hydrolytic stability of the hydride surface was obtained by packing this material in a chromatographic column and pumping degassed DI water through it for several hours. The column was then unpacked and the DRIFT spectra before and after the water test were compared. The two spectra show intensities of the Si-H stretching peaks at 2250 cm^{-1}, which are essentially the same, indicating little or no decomposition of the hydride layer under these conditions. Further testing of the stability of the Si-H moiety on the silica surface involved a material that was endcapped for slightly more hydrophobic character, and stored in columns for eight months in 0.05% phosphoric acid. From previous investigations, it was determined that the residual silanols were about 5% of the original amount on the surface. The relatively few remaining silanols on the hydride surface is corroborated by elemental analysis of the endcapped material (0.3% carbon) and the weak C-H stretching bands in the DRIFT spectrum. Figure 2 shows some chromatograms run on this column over the eight month test period. No noticeable change in retention of the three test solutes is observed, indicating good hydrolytic stability of the hydride under these aqueous conditions.

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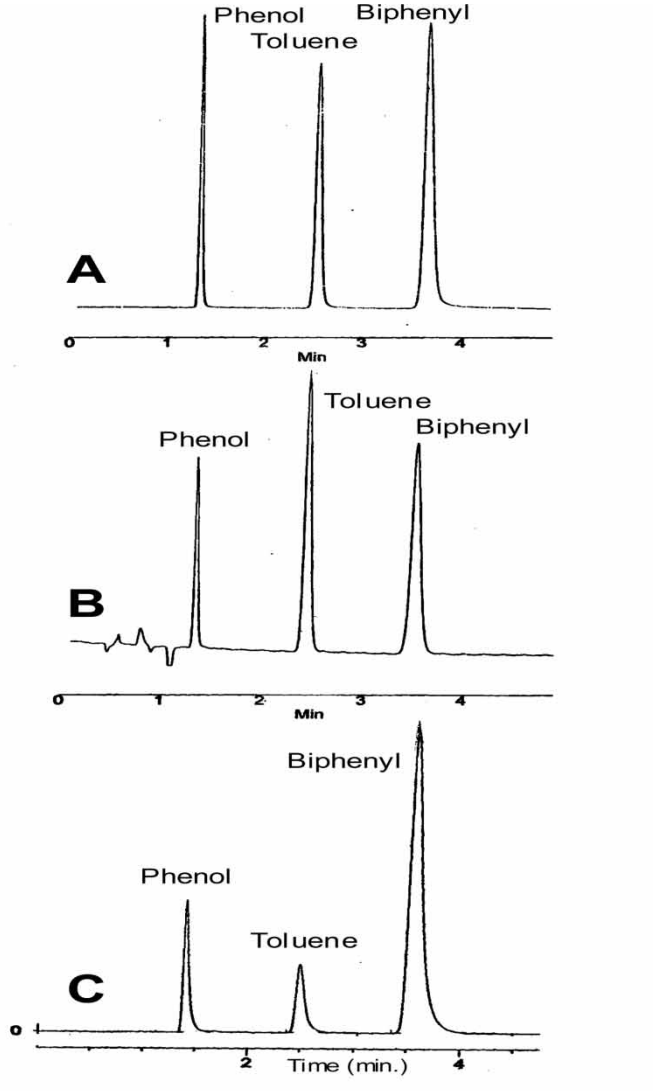

Name /t_R	Day 1	Day 2	Day 5	6 mos.	**8 mos.**	%RSD*
Phenol	1.34	1.32	1.32	1.34	**1.38**	0.87
Toluene	2.52	2.49	2.48	2.49	**2.49**	0.69
Biphenol	3.57	3.55	3.53	3.52	**3.55**	0.63

* Data for 8 months obtained in a different lab and on different equipment are excluded from the calculations

Figure 2. Reversed-phase HPLC chromatograms of a hydrocarbon mixture on end-capped silica hydride: A) initial chromatogram; B) after six months; and C) after eight months (obtained at another lab with a different sample). Mobile phase: 50 : 50 acetonitrile/water (0.05% H_3PO_4). Column: 15 cm × 4.6 mm. Detection at 254 nm.

The above results are confirmed by chromatographic studies on hydride based stationary phases, where columns have been used for thousands of column volumes at low pH (~2) and high pH (9–10) with little evidence of deterioration.[5,6] One example is a test on a bidentate C_{18} column (fabricated by hydrosilation of silica hydride with 1-octadecyne), which was used for more than one thousand column volumes, with various samples and several organo/aqueous mobile phases. The next step involved subjecting the column to a 90 : 10 ammonium acetate-ammonia (pH 10)/acetonitrile mobile phase for more than 1,000 additional column volumes. While the basic mobile phase was eluted a mixture of uracil/prydine/phenol was periodically injected. The k′ of pyridine was measured as 0.3 ± 0.05 and the k′ of phenol was 8.9 ± 0.1 during the 1,000 column volumes of basic mobile phase. The DRIFT spectrum of the material from this column after the chromatographic testing was compared to the spectrum obtained immediately after synthesis. Both the carbon-hydrogen stretching bands between 2,800–3,000 cm^{-1} and the Si-H band at 2,250 cm^{-1} are nearly identical in the two spectra, confirming the results of the chromatographic tests that no significant decomposition of the bonded phase and the underlying surface occurred as a result of exposure to the various mobile phases.

An example of the fundamental difference between silica hydride and ordinary silica (where silanols are the predominant functional group on the surface) is demonstrated in Figure 3. The chromatograms shown involve the measurement of the retention of uracil, pyridine, and phenol under reverse phase conditions. The polarity of the ordinary silica surface results in no retention of uracil and phenol, while pyridine is retained because of its basic properties (Figure 3A). However, the much less polar silica hydride surface has only minimal retention of pyridine (slightly separated from uracil) but more significant retention of phenol (Figure 3B). The elution order corresponds to typical reversed phase behavior with the most polar compound eluting first and the least polar species being the most retained. This silica hydride retention can also be contrasted to a commercial C_{18} phase that is not endcapped (Figure 3C). The presence of a significant number of silanols leads to the elution of pyridine after phenol. The nonpolar nature of the silica hydride surface results in less adsorption of polar solvents (particularly water) on the surface of the separation media. The consequence of this property is that aggressive components in the mobile phase (such as trifluoroacetic acid, phosphate, or bases) are less likely to attack the surface and/or the bonded moiety, and changes in the mobile phase composition can be accomplished efficiently so that the separation system rapidly reaches equilibrium. The latter feature is of particular advantage when doing gradient separations because repeated analyses can be done with a minimum of re-equilibration time between runs. For aromatic and polyaromatic hydrocarbons on both C_8 and C_{18} columns made from the respective alkynes, it was possible to get reproducible retention using equilibration times after the end of the gradient of less than 5 min.

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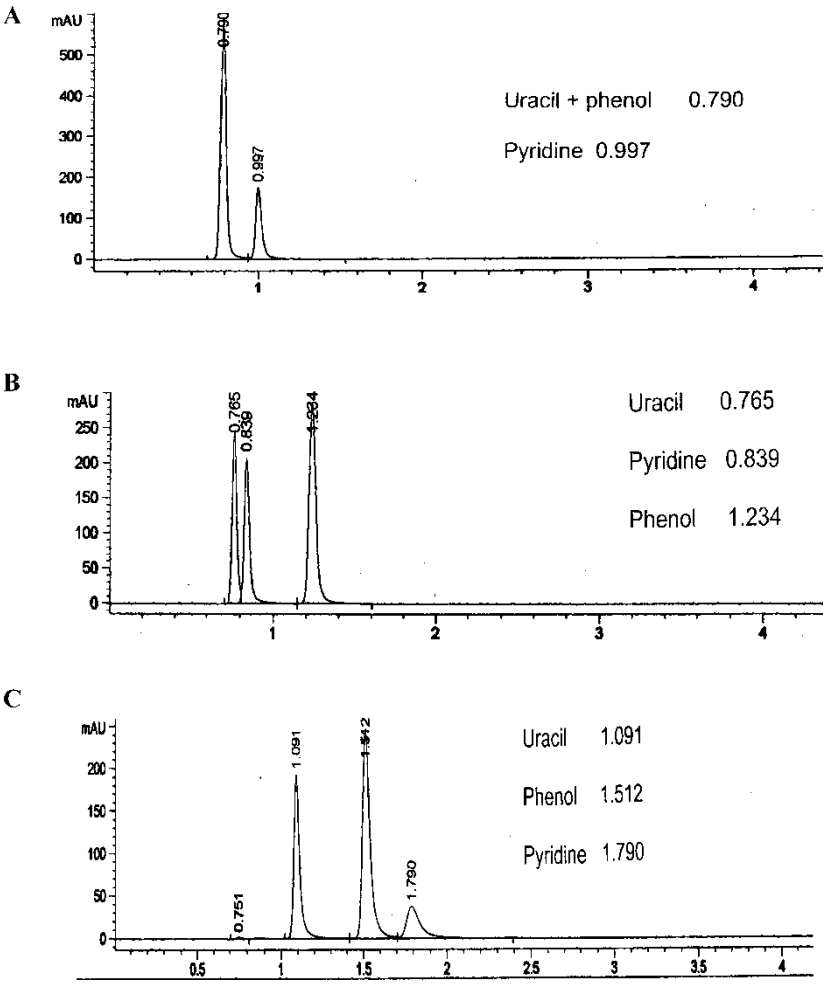

Figure 3. Chromatograms of a simple hydrocarbon test mixture on columns utilizing ordinary silica and silica hydride materials. A) unmodified ordinary silica; B) silica hydride: and C) C_{18} on ordinary silica (nonendcapped). Mobile Phase: A) and B) 90 : 10 water (0.05% H_3PO_4)/acetonitrile and C) 70 : 30 water (0.05% H_3PO_4)/acetonitrile.

The silica hydride-based columns are unique in that they can be used in any of the following three modes: aqueous reverse phase (ARP); aqueous normal phase (ANP) defined below; and organic normal phase (ONP). Silica hydride stationary phases can function simultaneously in both the aqueous reverse phase and the aqueous normal phase modes. Methods that utilize a mobile phase consisting of water/acetonitrile that varies in the concentration of acetonitrile from 0% to between 50–70% will result in

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decreasing retention of the analytes as the less polar solvent is increased. With 100% aqueous mobile phases retention is the greatest for neutral compounds. These retention characteristics are typical reversed phase behavior. What is unique is that when analyzing ionizable (acids or bases) compounds in a charged state (usually controlled by the mobile phase pH) and the acetonitrile concentration is above 50–70%, then increasing retention is observed up to 100% acetonitrile. From this maximum retention that occurs at 100% acetonitrile, the retention time will thus decrease as the more polar solvent (water) increases. This normal phase separation behavior involves the use of an aqueous mobile phase and, hence, is given the designation aqueous normal phase. Therefore, for the same compounds, one can change elution order and retention times by changing either pH (removing the charged state) or by changing the organic concentration of the mobile phase. In both the "aqueous" (using water) and "organic" (using non polar solvents) normal phase modes, as the more polar solvent is increased, the retention time of the analyte decreases. The elution order is based on the functionality/ionic state of the solutes. The maximum retention of the analytes is at 100% concentration of the least polar solvent and fits the definition of normal phase.

Two compounds with vastly different properties provide a means of illustrating the dual retention mechanism properties of silica hydride bonded phases. The elution characteristics of the highly polar pharmaceutical molecule metformin (Log P -2.64) and the relatively nonpolar drug molecule glyburide (Log P 4.79) shown in Figure 4, are used to illustrate this phenomenon. For glyburide (● and ▲), retention decreases as the amount of either methanol or acetonitrile increases in the mobile phase; typical reversed phase behavior. For metformin (◆ and ■), retention increases as the amount of acetonitrile is added to the mobile phase; typical normal phase behavior. For methanol, only a slight increase is observed above 80% organic in the mobile phase. An interesting feature of this plot is, that for acetonitrile, it is possible for the highly polar compound metformin and the non polar compound glyburide to coelute with both having reasonably good retention. This coelution would occur at 70% acetonitrile with a retention time of over four minutes. Thus, it is possible to retain both polar and non polar compounds on the same stationary phase by utilizing the unique properties of the silica-hydride stationary phase. The polar compounds are retained by the ANP mechanism, while the non polar solutes undergo typical reversed phase behavior. It can be said, that under these circumstances, the use of a silica-hydride-based stationary phase allows for separation in two dimensions on a single column.

Further confirmation of the reversed phase properties is shown for the cholesterol bonded material in Figure 5. In this case, several steroids are analyzed on a miniaturized column (2.1 mm × 20 mm) that allows for direct injection to a mass spectrometer. The general elution order in the separation is based on the hydrophobicity of the analyte, i.e., typical reversed phase

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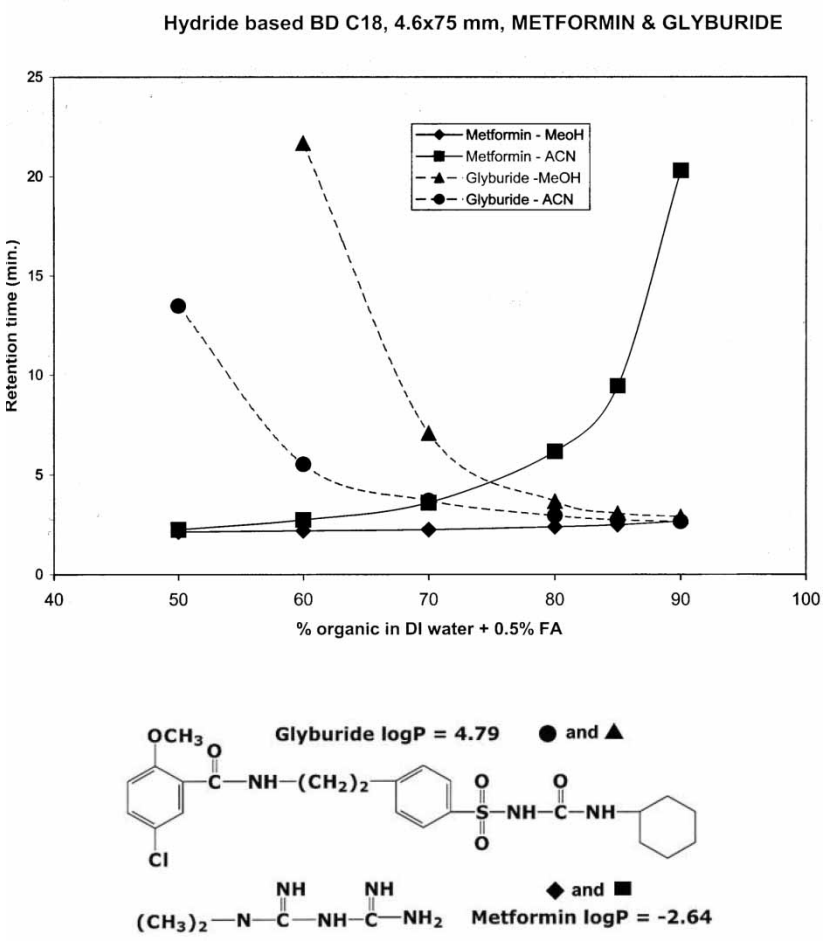

Figure 4. Retention time vs. % organic in mobile phase (retention map) for metformin and glyburide on silica-hydride based Cogent Bidentate C_{18} Column (MicroSolv Technology Corporation, Eatontown, NJ) using methanol and acetonitrile.

behavior. However, with steroids and other classes of compounds with varying molecular geometries such as polycyclic aromatic hydrocarbons (PAHs), the hydride based cholesterol phase separates molecules on both their hydrophobic properties as well as planarity.[11]

The organic normal phase properties of a hydride based material are illustrated by the retention behavior of two brominated phenols on the bidentate C_{18} column shown in Figure 6. The mobile phase is composed of 95 : 5 hexane/ethyl acetate. The two compounds are baseline resolved with good efficiency and peak symmetry in a total analysis time of less than two minutes. In this case, the two compounds were identified from their mass

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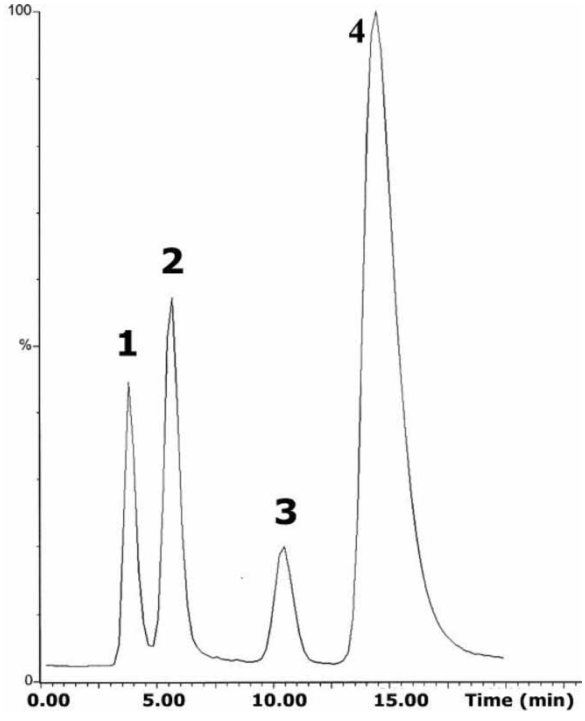

Figure 5. Reversed phase separation of steroids on hydride-based cholesterol column. APCI+, Single Ion Monitoring. Column dimensions: 2.1 × 20 mm. Injection: 1 μL Flow rate: 0.3 mL/min. Mobile phase: 35 : 65 methanol/DI water + 0.5% formic acid. Sample: 1 = Adrenosterone (m/z 301); 2 = Corticosterone (m/z 347); 3 = Estrone (m/z 253); 4 = 4-Adrostene-3,17-dione (m/z 287).

spectrum. The retention of the two compounds follows typical normal phase behavior, i.e., as the amount of hexane in the mobile phase is increased, the retention time of both compounds becomes longer. Similar results are obtained with methylene chloride as the modifier. This data indicates that the hydride surface has a profound effect on the chromatographic properties of the stationary phase, since in every case when such a hydrophobic group is attached to ordinary silica with a comparable bonding density, only reversed phase retention is observed.

Many of the properties of stationary phases based on silica hydride in comparison to conventional bonded materials have not yet been determined. Two tests using procedures developed by Neue, et al.[12] for HPLC materials were run, in order to understand some of the retention and surface characteristics of the hydride stationary phases. One test comparing the retention of naphthalene and acenaphthene determined the hydrophobicity parameter (ν) for two of the hydride phases: bidentate $C_{18} = 0.0397$ and cholesterol = 0.0339. The same determination made on a Phenomenex Luna (2) C_{18} column gave a ν of

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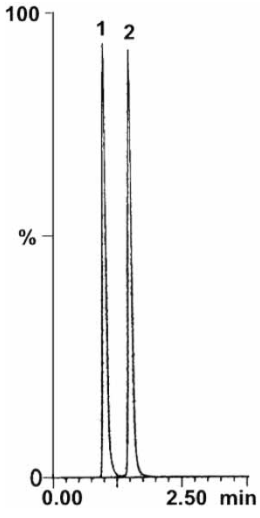

Figure 6. Separation of two brominated phenols on a bidentate C_{18} hydride based stationary phase. Mobile phase: 95 : 5 hexane/ethyl acetate. Flow rate: 1.0 mL/min. Peak 1 m/z = 324 and Peak 2 m/z = 228.

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0.0400 in comparison to the reported value of 0.0397.[12] The hydrophobicity of the bidentate C_{18} column, with respect to the more than 100 commercial packings tested,[12] is similar to the Luna (2) C_{18} and the Zorbax Eclipse XDB C_{18}. These stationary phases are in the upper one third of the hydrophobicity values of the phases tested. The cholesterol has lower hydrophobicity as expected and is similar to many of the C_8 phases.[12] This result is consistent with previous studies of retention for polycyclic aromatic hydrocarbons on the cholesterol phase.[11] The other property tested was the silanophilic interaction parameter (S). The values determined were as follows: Bidentate $C_{18} = 1.429$ and cholesterol = 0.949. In comparison to the more than 100 columns tested,[12] the bidentate C_{18} is at the low end of classical C_{18} packings, particularly with respect to its hydrophobicity. The cholesterol has an even lower S parameter and is similar to many phases with polar embedded groups, as well as some phenyl and cyano phases. The S test is based on the retention of the polar compound amitriptyline. For both hydride phases, the peak symmetry was excellent indicating that relatively few silanol groups were interacting with this basic solute: $A_S = 1.02$ on bidentate C_{18} and 0.95 on cholesterol.

Etched Hydride Based Capillaries

Another unique application for the use of hydride surfaces has been developed in a new capillary column configuration to improve the performance of open

tubular capillary electrochromatography (OTCEC). The fabrication of this separation medium is based on first etching the inner wall of a fused silica capillary by heating it at a temperature of 300 or 400°C in the presence of ammonium hydrogen difluoride (NH_4HF_2) for three to four hours. Under these conditions, the surface area of the inner wall will be increased by a factor of 1000 or more and radial extensions of up to 5 μm in length can also be created.[13,14] This process can overcome, to some degree, the low capacity of the bare capillary and shorten the distance solutes must travel to interact with a stationary phase attached to the etched surface. In addition, some elements from the etching reagent, nitrogen from the ammonium moiety and fluoride ion, are also incorporated into the new surface matrix.[15] These additional ionic species decrease the strong adsorptive properties of the silanols, thus, making the new surface more biocompatible. Further improvements in capillary performance can be achieved by creating a hydride layer on the etched surface. This additional modification essentially eliminates the effects of residual silanols as described above for particulate silica. The selectivity of the capillary is achieved by attaching an organic moiety to the etched hydride surface via hydrosilation in a manner similar to that used to create HPLC stationary phases. The complete fabrication process is shown in Figure 7.

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The etching process transforms the capillary wall surface matrix so that it is composed of not only silicon and oxygen but fluoride and nitrogen. The existence of these four elements near the etched surface has been verified by ESCA and EXAFS spectroscopy.[15] The presence of nitrogen significantly affects the electrophoretic behavior of the capillary. With low pH buffers the nitrogen species (exact chemical nature is not known) near the surface become protonated, giving an overall positive charge to the inner wall of the capillary. The positive charge leads to a reverse electroosmotic flow (EOF) under these conditions. As the buffer pH is raised to less acidic conditions, the EOF passes through zero and eventually becomes cathodic due to each of the following three conditions: the deprotonation of the nitrogen species; ionization of the remaining silanols; and the presence of negatively charged fluoride species.[15,16] These additional surface matrix elements do not interfere with the subsequent chemical modification (silanization/hydrosilation). The

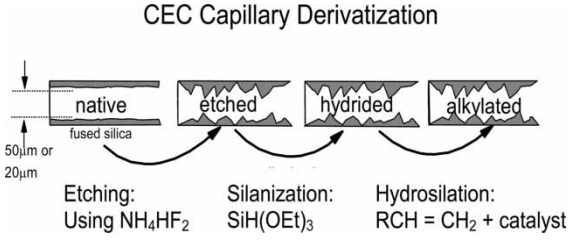

Figure 7. Process for producing etched chemically modified capillaries for OTCEC.

typical EOF behavior of an etched chemically modified capillary is shown in Figure 8.

The biocompatibility and adsorptive nature of the etched chemically modified surface can be evaluated by obtaining the electrochromatogram of a sample containing basic proteins that could readily interact with silanols on the surface. Figure 9 shows an OTCEC separation of five basic proteins on an etched capillary subsequently modified by silanization/hydrosilation. The moiety bonded to the surface is a linear eight carbon molecule with hydroxy groups in positions 7 and 8 (diol). All of the constituents are well resolved and the peaks have excellent symmetry indicating a minimum of surface adsorptive effects. Similar electrochromatograms with good peak shapes have been obtained for samples of a variety of peptides and small basic compounds.[14,17–23]

A comparison of the resolving power of OTCEC with respect to gradient HPLC for a synthetic peptide is shown in Figure 10. Gradient HPLC has been the method of choice to determine the purity of synthetic peptides since it was believed that a wide range of species could be identified by this approach. However, it can be seen in Figure 10, that the resolving power of OTCEC is considerably better when compared to an optimized gradient HPLC method. As a control to the OTCEC and gradient HPLC experiments, the sample was also analyzed by MALDI-TOF. Mass spectrometric analysis confirmed that the minor components were due to the presence of deletion peptides, side chain reaction products, and Group I ion adducts produced

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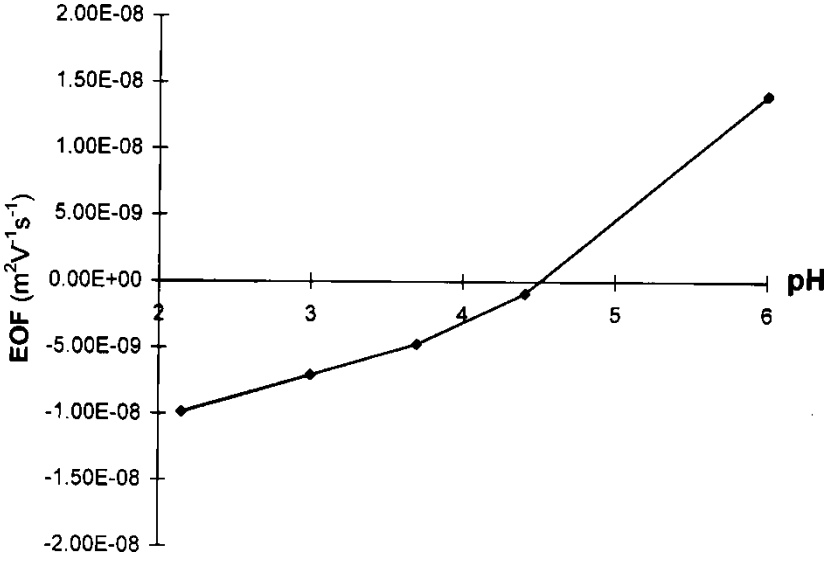

Figure 8. Plot of electroosmotic flow as a function of pH for an etched octadecyl (C_{18}) modified capillary.[21]

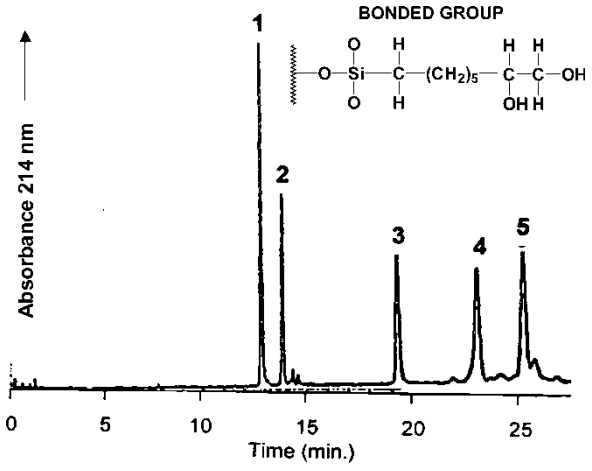

Figure 9. Separation of a mixture of five basic proteins on an etched capillary modified by silanization and subsequent hydrosilation with 7-octene-1,2-diol. Column: 50 μm id; L = 50 cm; l = 25 cm. Pressure injection at 50 mbar. Mobile phase pH 3.7 buffer. V = 22 kV. Detection at 214 nm. Solutes: 1 = turkey lysozyme; 2 = bovine cytochrome c; 3 = ribonuclease A; 4 = trypsinogen; 5 = α-chymotrypsinogen.

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during the synthesis, purification, or upon storage.[24] The number of species detected by MALDI-TOF was identical to the number of peaks in the OTCEC electrochromatogram. This example provides further evidence of the advantage gained in OTCEC with etched chemically modified capillaries by having two separation mechanisms; electrophoretic migration and chromatographic interactions.

Since the migration behavior of solutes on the etched chemically modified capillaries is a function of both chromatographic and electrophoretic effects, a number of experimental variables can be used to optimize the separation capabilities of the capillaries. These variables include the bonded phase moiety, pH, buffer components, amount and type of organic modifier in the buffer, and temperature. In many instances, the choice of stationary phase, like in HPLC, has a profound effect on elution behavior and resolution. An example is the separation of a synthetic peptide sample containing some minor components in addition to the main product. Using an etched C_{18} modified capillary, four components, in addition to the main product, are well resolved under the experimental conditions used. In comparison, a cholesterol column or a butylphenyl column provide complete resolution of only one minor component and partial resolution of the others under the same experimental conditions. Another variable that is crucial to either chromatographic or electrophoretic separations is the choice of an organic modifier in the mobile phase. For another sample of a synthetic peptide with methanol in the mobile phase only one minor constituent was observed, while with

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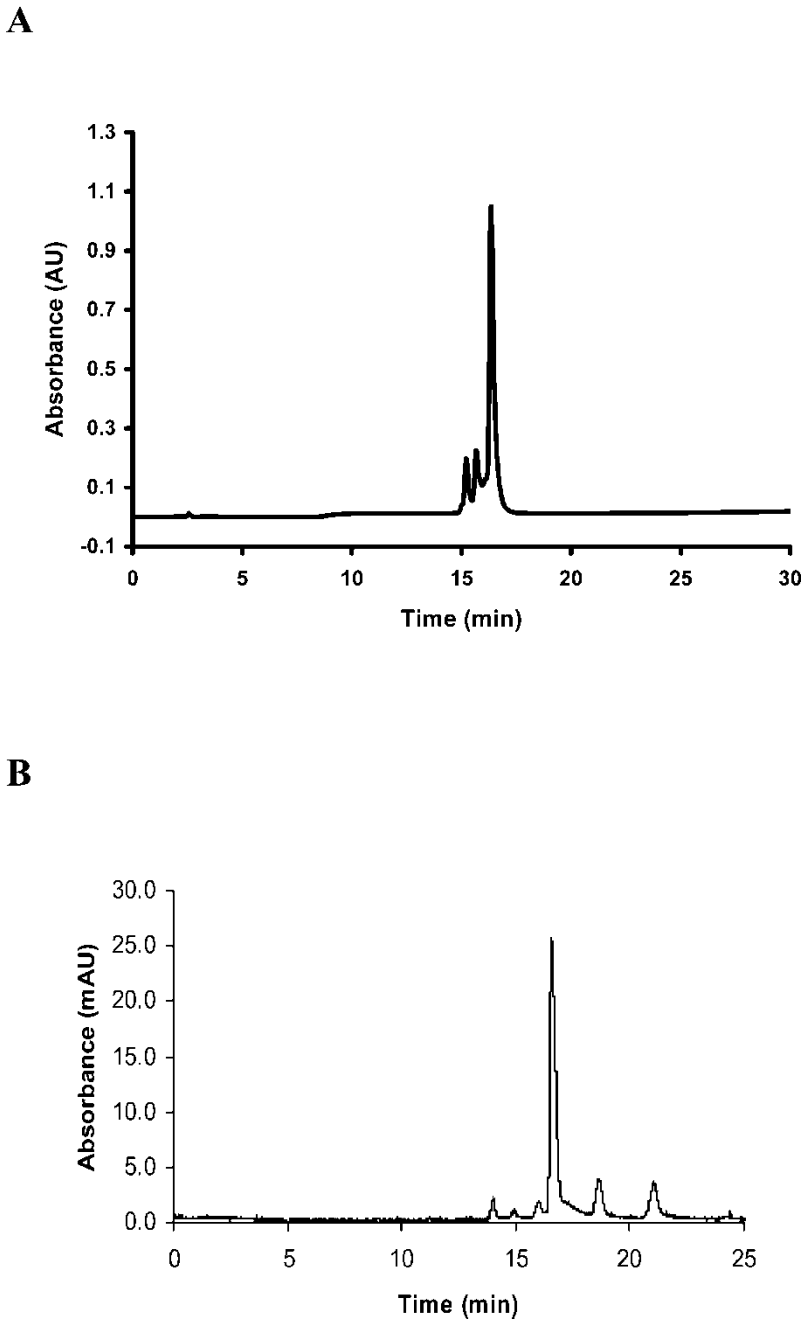

Figure 10. Separation of a synthetic peptide sample by A) gradient elution HPLC on a RP C_{18} stationary phase and B) OTCEC on a 50 μm i.d. etched butylphenyl modified capillary using a 70 : 30 pH 2.14 buffer/methanol mobile phase.

ethanol as the organic modifier at least five well resolved minor components can be detected. Similar to other electrophoretic methods, the pH of the running buffer can be used to control the migration of various components in the sample. In Figure 11, the elution order of the two components in the sample is reversed by changing the pH from 2.14 (Figure 11A) to 3.00 (Figure 11B). Thus, pH can be one of the most effective variables for optimizing OTCEC separations. One more variable that in some cases can have a substantial effect on solute migration is temperature. Using van't Hoff plots the migration of two peptides on both cholesterol and C_{18} modified capillaries were compared. Over the temperature range (20–70°) studied, the elution order of the two peptides is reversed at two different points on the cholesterol capillary, while only minor changes in the migration are observed on the octadecyl capillary. The substantial effect of temperature on the etched cholesterol capillary has been documented in a number of studies, and has been

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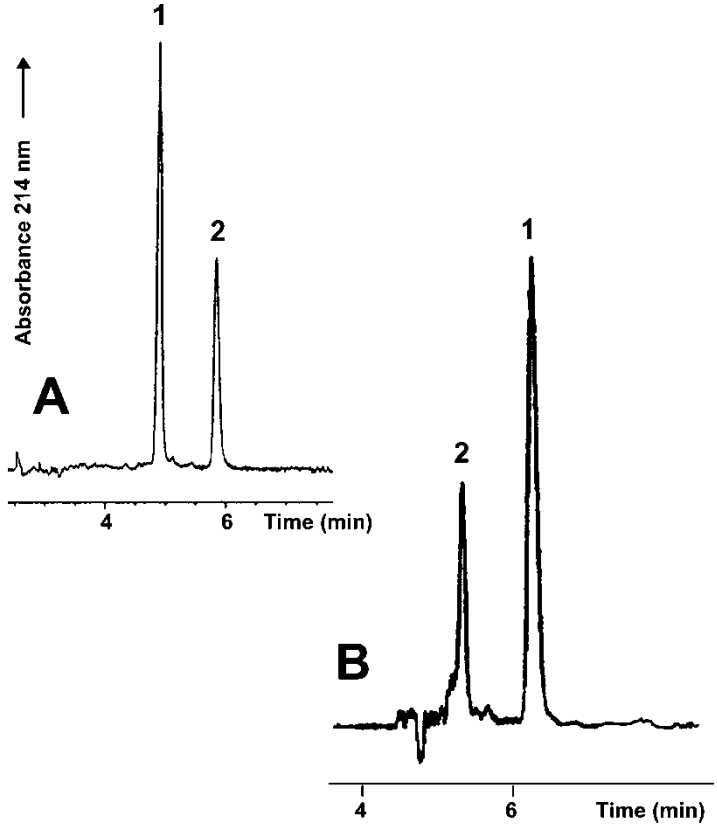

Figure 11. Effect of pH on the migration of synthetic peptide sample (sequence: Gln Asp Gln His Asn His Phe His Arg) using an etched butylphenyl modified capillary: A) pH = 2.14 and B) pH = 3.00. Mobile phase 90 : 10 buffer/methanol.[24]

attributed to the liquid crystal properties of this particularly organic moiety.[12] The degree of ordering and the exact structure of the liquid crystal moiety on a silica surface can be affected by temperature and the solvent composition around it.

A number of small molecules, including many basic compounds like tricyclic antidepressants and tetracyclines have been analyzed by OTCEC with etched chemically modified capillaries.[17] An example of both the resolving capability of these capillaries and the effect of the stationary phase for small molecule analysis was demonstrated with a mixture containing purine/pyrimidine bases and a nucleoside that was separated on two etched capillaries with different bonded moieties but under the same buffer, pH, and applied voltage conditions.[15] The sample is analyzed successfully on both columns with high efficiency and good peak shape. However, the retention order is different on the two capillaries, which directly illustrates the importance of the bonded group attached to the etched surface.

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Analysis of polyethylene glycol modified (PEG) proteins and peptides for clinical and quality assurance purposes is complicated by the heterogeneity that results from the distribution of the PEG among a number of different sites on the biopolymer.[25] The attachment of PEG usually occurs at lysine and N-terminal amino groups. The potential number of species created and, hence, the number that must be identified in any analysis is given by the following formula:[26]

$$P = \frac{N!}{(N - k)!\,(k!)}$$

where N = is the number of possible sites and k is the number of sites actually modified. This equation assumes that all of the proteins are modified with the same number of PEG chains which may not always be true. HPLC analysis has not always proved satisfactory in determining the actual number of PEGylated species present in a particular sample. Figure 12 shows the OTCEC analysis of two PEGylated proteins using etched chemically modified capillaries.[27] In both cases, there are a substantial number of components in these samples, indicating extensive PEGylation of these proteins. The resolution, time of analysis, peak shape (A_S is 1.1 or less), and efficiencies (N/m generally in the range of 100,000–200,000) found in these columns is excellent compared to the other separation methods currently available. With respect to other types of analytical methods such as MALDI-TOF, the analysis is simpler and less costly.

Finally, these capillaries exhibit long durability and excellent reproducibility. Typical column lifetimes are at least several hundred injections with many capillaries performing well after 300–400 analyses.[20,24,27] Reproducibility studies have demonstrated that consecutive runs of a particular analyte show %RSD values less than one, and less than two when an initial injection is compared to a result taken after one hundred or more subsequent analyses.

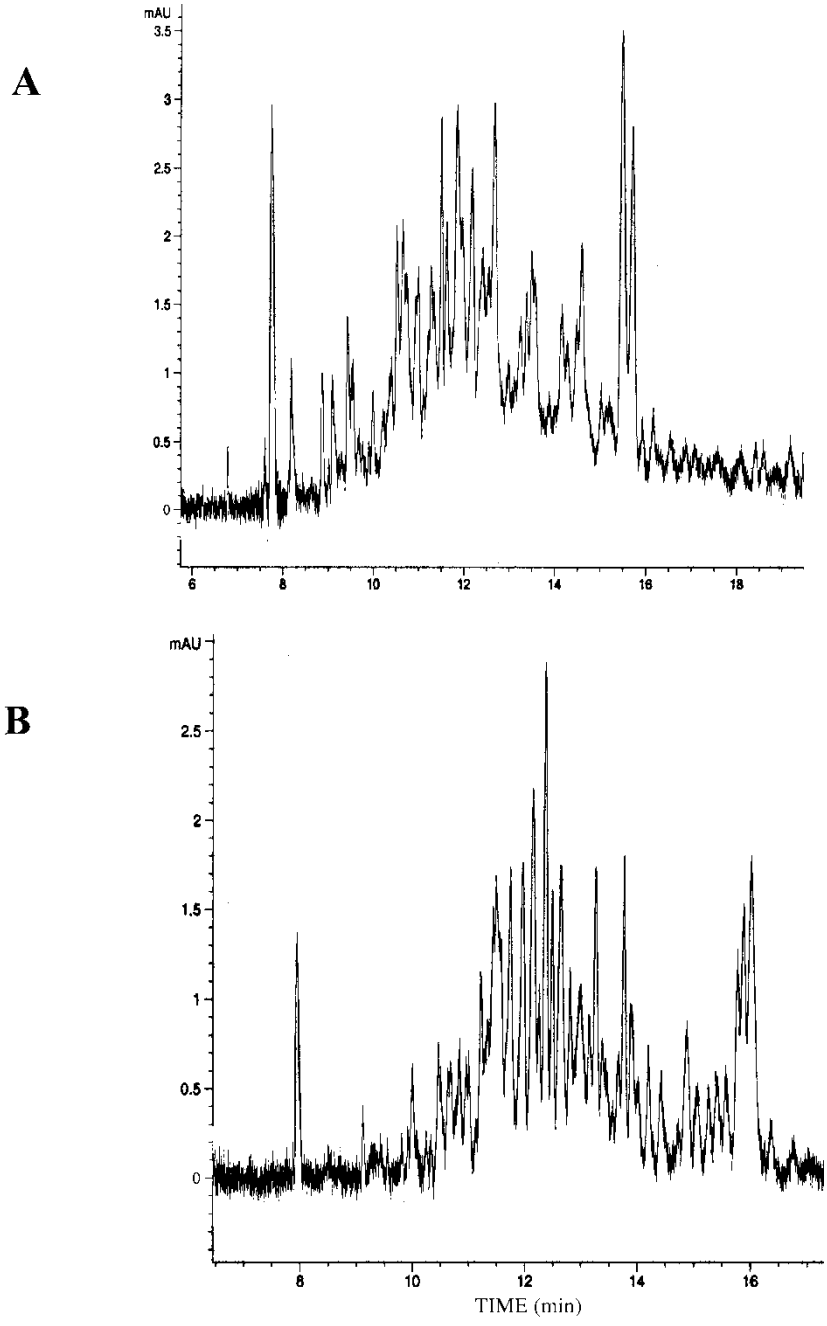

Figure 12. Separation of PEGylated proteins on an etched cholesterol modified capillary: A) PEG-catalase and B) PEG-protease. Mobile phase: aqueous pH 2.14 buffer. $V = 20\,kV$.[27]

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Capillary to capillary reproducibility is also good with variations in the relative migration of two analytes on the order of one percent. These data suggest that all of the factors involved in the fabrication of the capillaries are reproducible, and the organic moiety bonded to the surface by the silanization/hydrosilation process is also stable.

CONCLUSIONS

Silica hydride-based materials represent a new separation technology that provide unique stationary phases for HPLC. The properties of hydride silica and its bonded phases result in a broad range of chromatographic capabilities that are not normally available from the ordinary silica widely used for current commercial products. The hydride stationary phases have been studied and developed for more than a decade and are now in the process of becoming viable commercial products. Hydride-based materials have some properties, such as the complete range of retention mechanisms (ARP, ANP and ONP) in a single stationary phase, as one example that are not available from current commercial products.

Etched chemically modified capillaries have been shown to possess capabilities that make them suitable for a variety of electrophoretic analyses. The EOF characteristics and biocompatible surface are particularly useful for the analysis of small basic compounds, peptides, and proteins. Numerous examples of analyses of these types of compounds have been identified and more applications are currently being investigated. In addition, both the durability and reproducibility of this electrophoretic separation medium are excellent.

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ACKNOWLEDGMENTS

The financial support of the National Institutes of Health (NIH MBRS Grant 0819210-21) and the National Science Foundation (CHE 0094983) is gratefully acknowledged. One of the authors (JJP) would like to acknowledge the support of the Camille and Henry Dreyfus Foundation through a Scholar Award.

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Received September 27, 2005
Accepted December 19, 2005
Manuscript 6770E

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