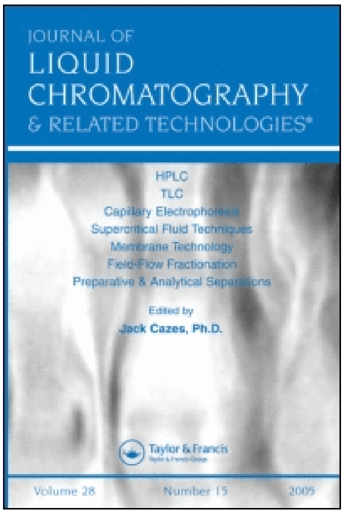

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Synthesis, Characterization and Applications of Hydride-Based Surface Materials for HPLC, HPCE and Electrochromatography

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SYNTHESIS, CHARACTERIZATION AND APPLICATIONS OF HYDRIDE-BASED SURFACE MATERIALS FOR HPLC, HPCE AND ELECTROCHROMATOGRAPHY

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ABSTRACT

The chemical modification method based on the silanization of an oxide surface to a hydride intermediate followed by hydrosilation with an organic molecule containing a terminal olefin is reviewed. The resulting bonded organic moiety is attached to the surface via a direct Si-C bond which leads to high stability. The method has been more extensively applied to silica surfaces for the production of stationary phases in HPLC but it can also be used on other oxides such as alumina, zirconia, titania and thoria. More recent applications have been in the modification of the inner wall of fused silica capillaries for HPCE. The bonded moieties possess high stability and useful applications have been developed for the separation of proteins and peptides under a variety of buffer conditions. The same procedure for modifying the inner wall of a fused silica capillary has also been extended to etched surfaces for use in electrochromatography (CEC). This type of CEC has been shown to be applicable to the separation of macromolecules as well as small solutes.

INTRODUCTION

Chemically bonded stationary phases for high performance liquid chromatography have been an essential component of this method for nearly twenty-five years. Since the majority of phases for HPLC consist of silica as the support material, the bonding of various organic moieties to the substrate is based on the chemistry of the silanols at the surface.[1] A number of possible reactions can and have been used for the attachment of organic ligands to silica, which ultimately lead to a range of surface properties varying from hydrophobic to hydrophilic and ionic. A summary of these reaction schemes is given in Figure 1.

The first reaction, generally referred to as esterification, involves bonding of an alcohol to the silanol. Because of its simplicity, it was the first method used for making chemically bonded stationary phases for HPLC.[2] However, the Si-O-C linkage which is formed in this reaction is hydrolytically unstable and therefore not suitable for any aqueous mobile phases. This bonding method was quickly supplanted by the second reaction type usually referred to as organosilanization. It is still today the method of choice for producing a wide variety of silica-based chemically bonded stationary phases for HPLC.

As shown in Figure 1 there are two general approaches to organosilanization. The first (a) involves the use of an organosilane reagent with a single reactive group (X). X is usually chlorine, methoxy or ethoxy. This process results in a monomeric stationary phase, i.e. one where each organic moiety is bonded to a single silanol on the surface. The second approach (b) involves the use of an organosilane with three reactive groups. In this case, bonding not only occurs to the surface of silica but there is extensive cross-linking between adjacent organosilane moieties resulting in what is usually referred to as a polymeric phase. It should be noted that the so-called "self-assembled monolayer phases" also are based on the principle of organosilanization.[3]

The third approach is a two-step process which involves chlorination of the silica surface with thionyl chloride followed by attachment of the organic moiety through either a Grignard reagent or organolithium compound.[4] The main advantage to this method is that the product is bonded to the surface

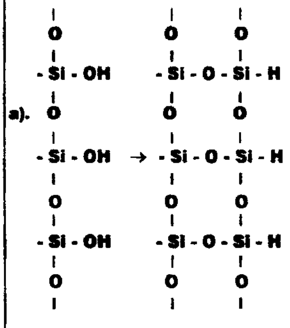
REACTION TYPE	REACTION	SURFACE LINKAGES
ESTERIFICATION	$Si-OH + R-OH \rightarrow Si-OR + H_2O$	Si - O - C
ORGANO-SILANIZATION	a). $Si-OH + X-SiR'_2R \rightarrow Si-O-SiR'_2R + HX$ b). $Si-OH + X_3-Si-R \rightarrow Si-O-\underset{OY}{\overset{OY}{Si}}-R + 3HX$ Y = Si or H	Si - O - Si - C
CHLORINATION FOLLOWED BY REACTION OF GRIGNARD REAGENTS AND ORGANOLITHIUM COMPOUNDS	$Si-OH + SOCl_2 \xrightarrow{Toluene} Si-Cl + SO_2 + HCl$ a). $Si-Cl + BrMgR \rightarrow Si-R + MgClBr$ or b). $Si-Cl + Li-R \rightarrow Si-R + LiCl$	Si - C
a). TES SILANIZATION		a). Si - H monolayer
b). HYDROSILATION	b). $Si-H + CH_2=CH-R \xrightarrow{Catalyst} Si-CH_2-CH_2-R$	b). Si - C

Figure 1. Chemical reactions used to modify silica surfaces

through a direct silicon-carbon linkage which is hydrolytically more stable than the Si-O-Si-C structure which is formed during organosilanization. The principal drawback to this approach is the scrupulously dry conditions which must be maintained during the synthesis. The chlorinated surface can be easily hydrolyzed back to silanols.

An alternative procedure for achieving the same or similar material to that produced by the chlorination/organometallic process is shown by the last reaction type cited in Figure 1. In this method, the silanols on the surface are

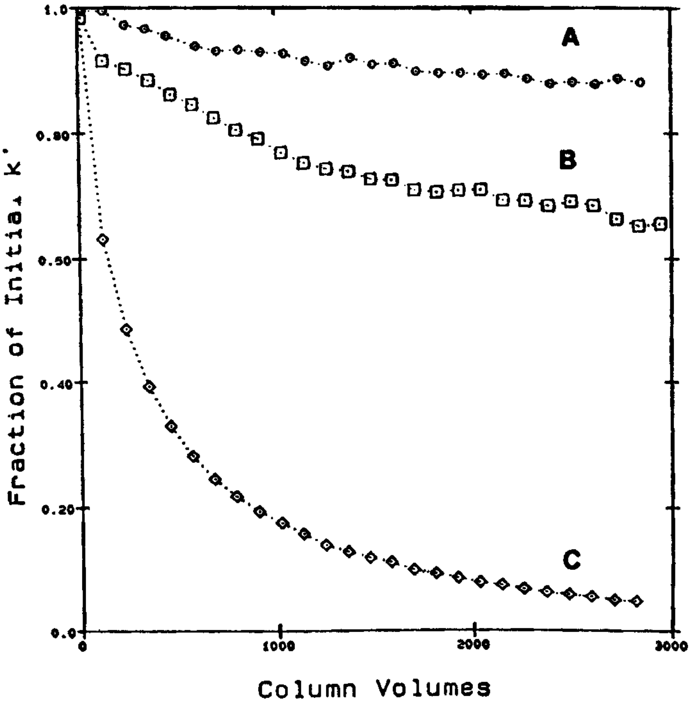


Figure 2. Relative loss of retention for C_8 columns as a function of column volumes of mobile phase. A = hydrosilation product; B = high coverage conventional organosilanization product; C = low coverage conventional organosilanization product. (Reprinted with permission from ref. 9)

first converted to hydrides and these sites are then reacted with a terminal olefin in the presence of a suitable catalyst to produce the final bonded material. There are two ways in which the silanols can be replaced by hydrides. One approach involves reducing the silanols with lithium aluminum hydride.[5] This process can be visualized as a direct replacement for surface silanols since each hydride that is formed requires an Si-OH group. The second approach involves silanization of the surface[6] with an appropriate reagent such as triethoxysilane (TES) as shown in Figure 1.

Under carefully controlled conditions a monolayer is formed on the surface so that the final result should be complete replacement of hydroxide groups by hydrides. This appears to be the case since the physical properties such as pore size and surface area of the hydride intermediate are the same as the starting silica.[6] After the hydride surface is formed, the organic moiety is

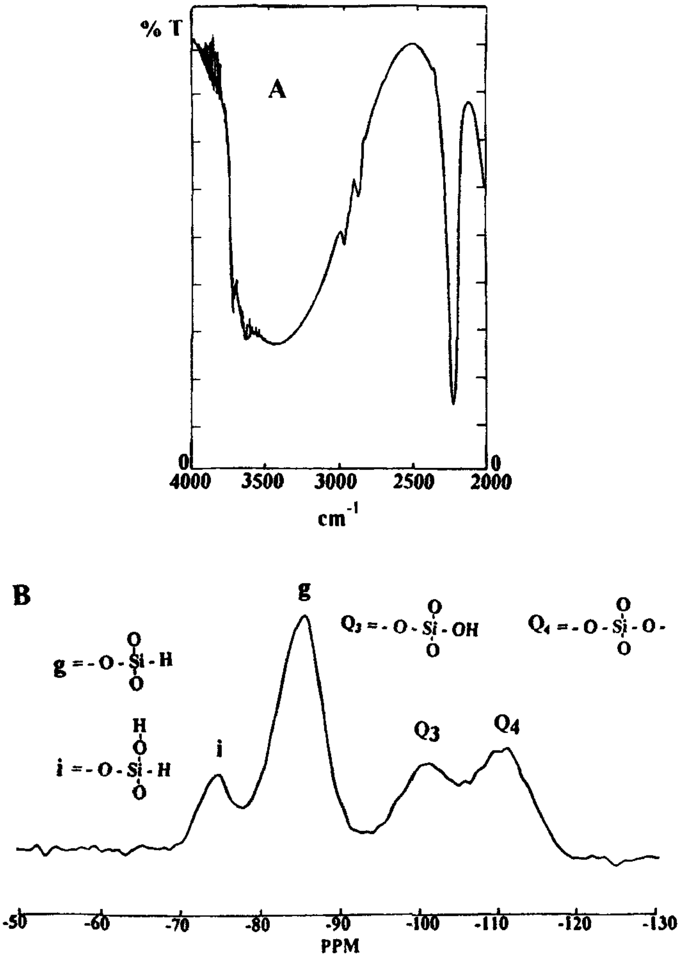
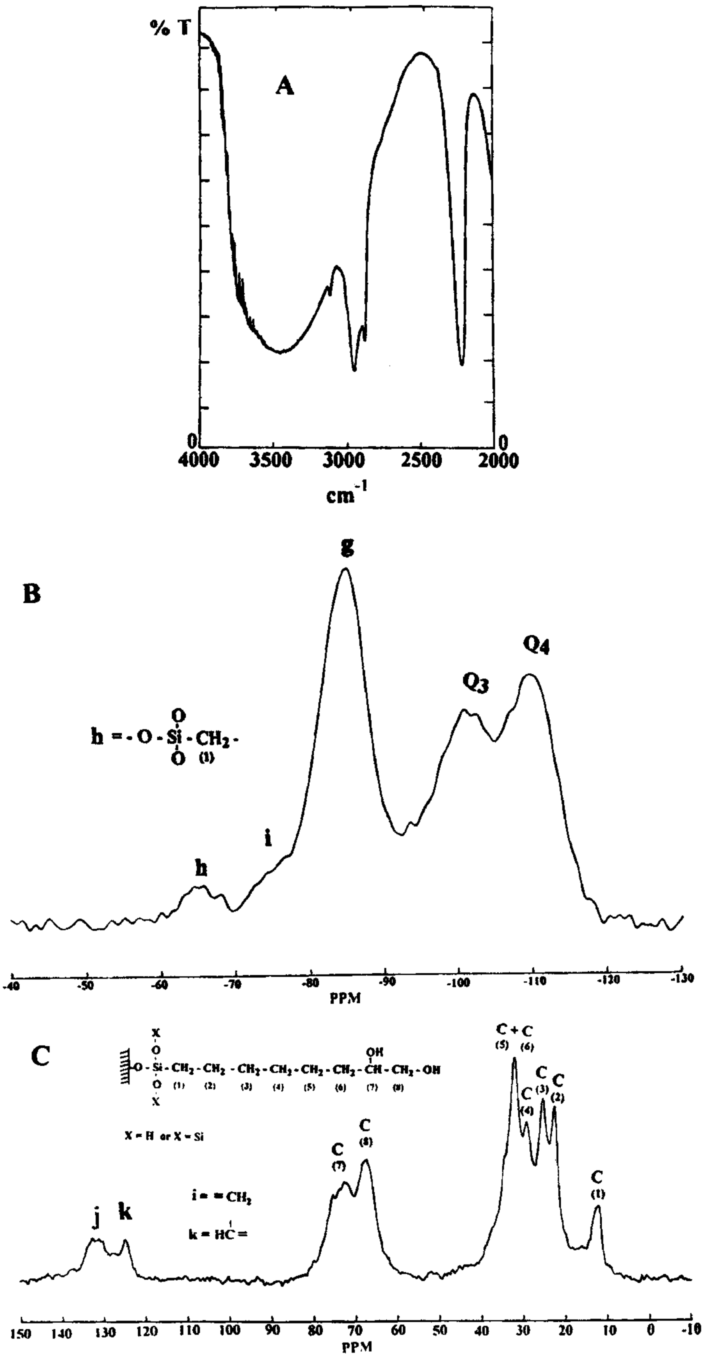


Figure 3. Spectral characterization of hydride intermediate on silica. A = DRIFT spectrum; B = ^{29}Si CP-MAS NMR spectrum.

attached via hydrosilation[7,8] as shown in the second step of this sequence in Figure 1. Because there is a single point of attachment for the organic moiety, it should more closely resemble the monomeric phases produced in organosilanization reaction "a)", or those produced by the two-step chlorination/organometallic reaction sequence. In fact, chromatographic tests with a standard polycyclic aromatic hydrocarbon mixture show that these phases behave like densely coated monomeric materials.[9] The stability of the material produced by this process is significantly greater than similar phases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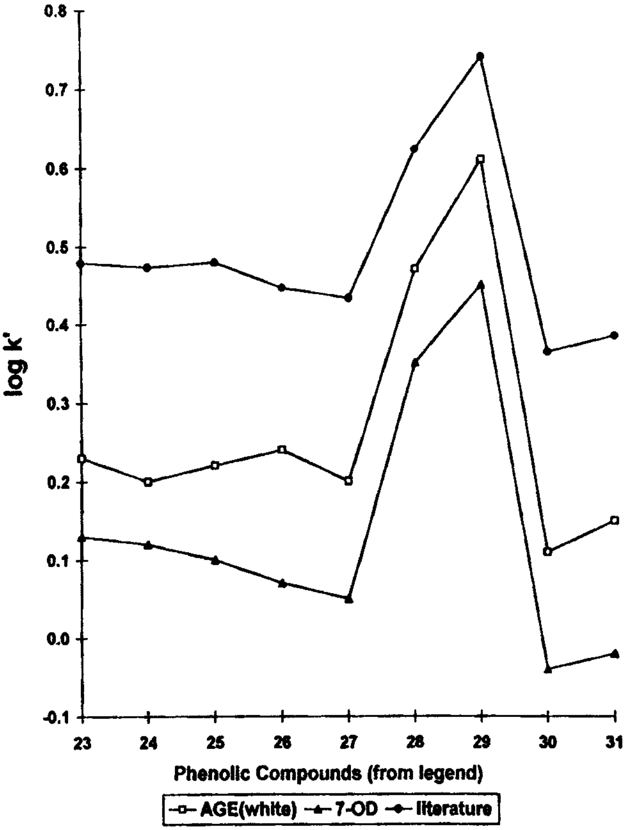

Figure 5. Comparison of retention data for phenols on various diol columns. AGE = diol from hydrosilation of allyl glycidyl ether on hydride intermediate; 7-OD = diol from hydrosilation of 7-octene-1,2-diol on hydride intermediate; literature = Merck diol. Solutes: **23**; 4-bromophenol, **24**; 3-chlorophenol, **25**; 4-chlorophenol, **26**; 4-fluorophenol, **27**; 2-napthol, **28**; 3-nitrophenol, **29**; 4-nitrophenol, **30**; phenol, **31**; 4-phenylphenol. (Reprinted with permission from ref. 19)

which are made by conventional organosilanization.[9] Figure 2 shows the log k' value of a test probe as a function of columns volumes of an aggressive mobile phase containing trifluoroacetic acid for the hydride-based material (A) and

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Figure 4 (left). Spectral characterization of diol product made by hydrosilation of 7-octene-1,2-diol on hydride intermediate. A = DRIFT spectrum; B = ^{29}Si CP-MAS NMR spectrum; C = ^{13}C CP-MAS NMR spectrum.

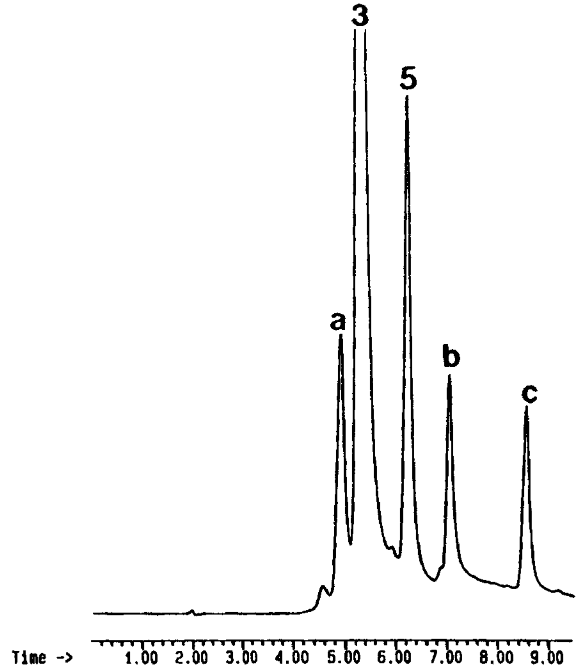


Figure 6. Gradient separation of typical commercial tetracycline mixture on mono-ol column made from hydrosilation of 1-octene-8-ol on hydride intermediate. Solvent A - 10:90 MeCN/0.02M $NaClO_4$ (pH=2.5) and solvent B 50:50 MeCN/0.02M $NaClO_4$ (pH=2.5). Gradient: 0-1 min, 100% A; 1-5 min, linear increase to 100% B. Solutes: a) 4-epitetracycline; b) 4-epianhydrotetracycline; c) anhydrotetracycline; 3) tetracycline; 5) chlorotetracycline.

conventional phases with high (B) and low (C) surface coverage. Clearly, the hydride-based material exhibits less of a decrease in k' than either of the conventional phases. Similar results are obtained at high pH so that it can be concluded the silicon-carbon linkage leads to the higher stability observed.

POROUS OXIDE MATERIALS

Silica

The synthetic scheme for the silanization/hydrosilation process described in Figure 1 can be readily monitored by spectroscopic means. Two of the most useful methods for the evaluation of chemically bonded stationary phases have

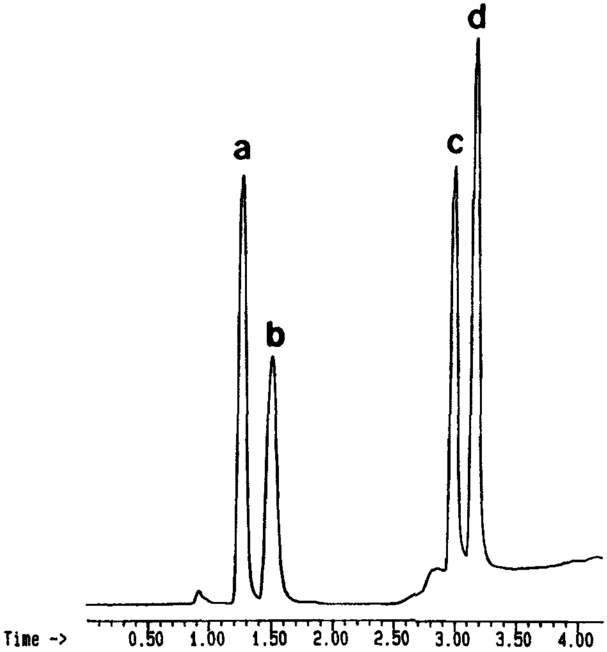


Figure 7. Gradient separation of steroid mixture on diol column made from hydrosilation of allyl glycidyl ether on hydride intermediate. Gradient: 2-propanol in methylene chloride from 2.5% to 15% between 1 and 2.5 min in the chromatographic run. Solutes: a) 4-androstene-3,17-dione; b) adrenosterone; c) corticosterone; d) prednisone. (Reprinted with permission from ref. 19)

been diffuse reflectance infrared Fourier transform (DRIFT) and solid-state cross-polarization magic-angle spinning (CP-MAS) NMR spectroscopy.[10] Their utility in the silanization step with TES is documented in Figure 3. The partial DRIFT spectrum for a typical hydride intermediate is shown in Figure 3A. The most essential feature is the strong Si-H stretching frequency which is observed near 2250 cm^{-1}. The appearance of this band is accompanied by a significant decrease in the free silanol stretching near 3750 cm^{-1}. In addition, the ^{29}Si CP-MAS NMR spectrum (Figure 3B) confirms the presence of the Si-H moiety on the surface. Two peaks in the spectrum (Q_3 and Q_4) are the result of the base silica material, with Q_4 representing the silicon atoms in the siloxane backbone and Q_3 representing silicon atoms that have a surface silanol attached to them. The other two peaks are the result of the silanization process. Peak g at -85 ppm is due to silicon atoms with three siloxane linkages and a hydride, while Peak i at -75 ppm represents silicon atoms with two siloxane linkages, a hydroxide and a hydride. These observations which confirm the formation of

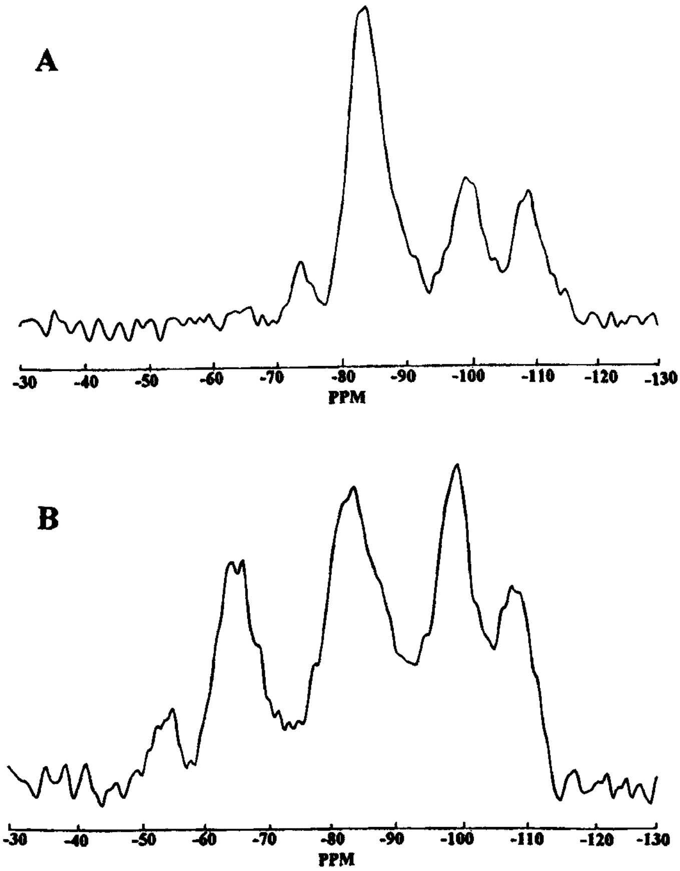


Figure 8. ^{29}Si CP-MAS NMR spectra of zirconia support material. A. Zirconia after reaction with TES. B. Zirconia after material in A is reacted with 1-octene by hydrosilation. (Reprinted with permission from ref. 25)

the hydride intermediate are independent of the commercial source (Vydac, Partisil, Kromasil, Nucleosil, YMC, Porasil) of the silica. In addition, ^{29}Si CP-MAS NMR data indicate that greater than 90% efficiency is achieved in the silanization reaction.[6]

Similar spectroscopic characterization of the final product can be done after the hydrosilation step as shown in Figure 4. In this step any compound containing a terminal olefin should react with the hydride intermediate in the presence of a suitable catalyst such as hexachloroplatinic acid.[11] The specific

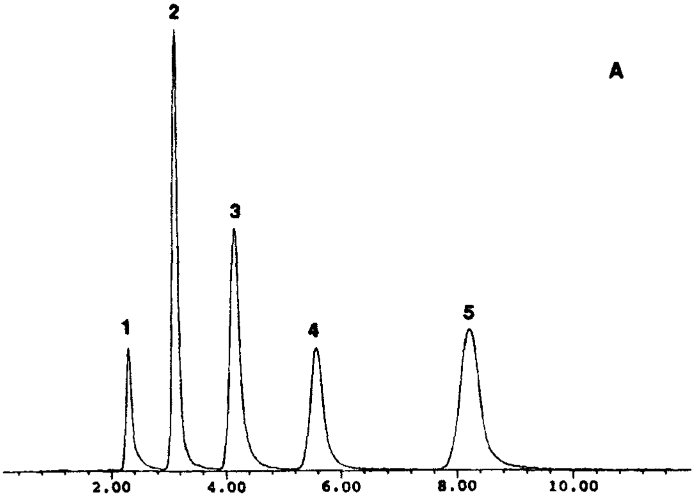
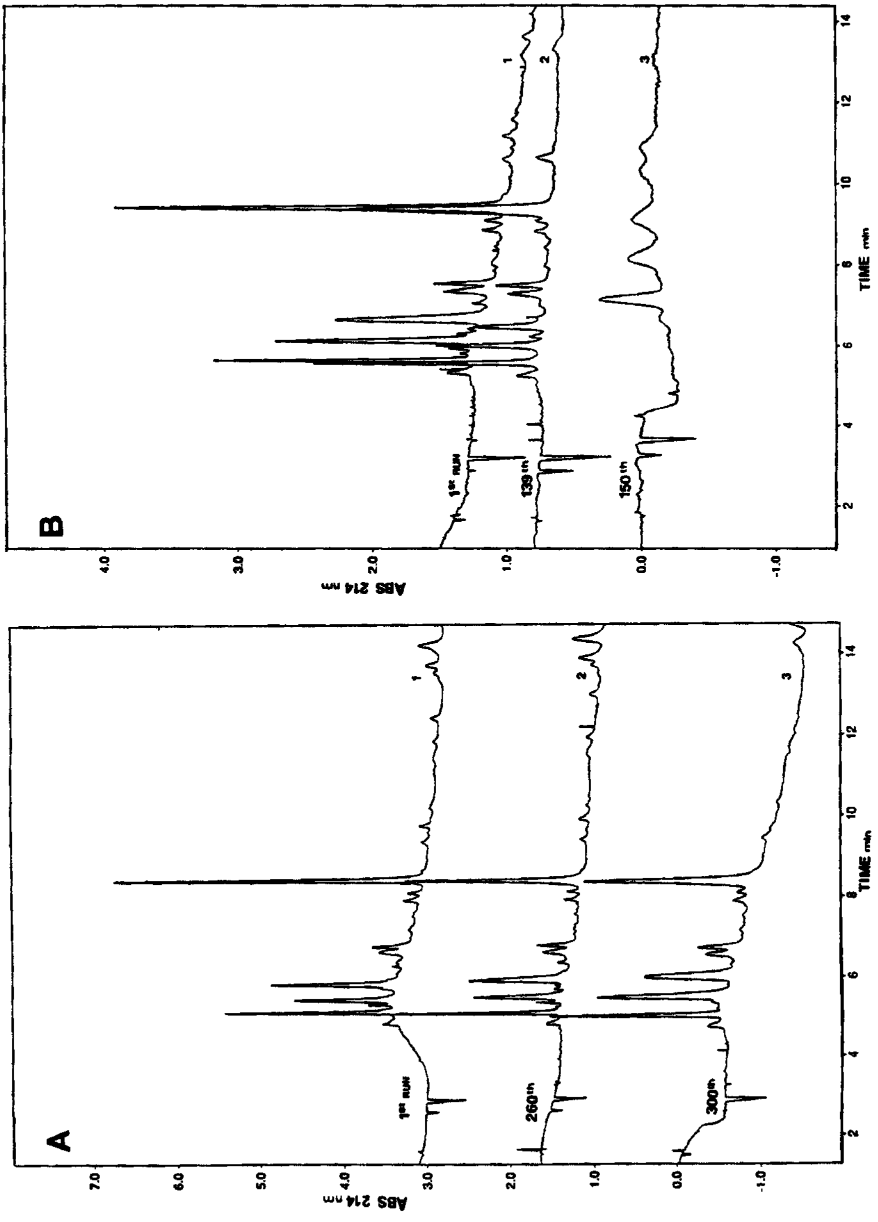


Figure 9. Chromatogram of test mixture for reversed-phase behavior on alumina modified by hydrosilation of 1-octadecene on a hydride intermediate. Mobile phase: MeCN-water (50:50). Solutes: 1) theophylline; 2) *p*-nitroaniline; 3) methylbenzoate; 4) phenetol; 5) *o*-xylene. (Reprinted with permission from ref. 24)

olefin used for the material which is spectroscopically characterized in Figure 4 is 7-octene-1,2-diol (7-OD). The partial DRIFT spectrum (A) reveals two important features of the surface. First, the presence of the organic moiety is confirmed by the strong C-H stretching bands between 2800 and 3000 cm^{-1}. Second, the surface below the bonded organic ligand consist mainly of hydride groups as seen by the strong Si-H band at 2250 cm^{-1} and the absence of any isolated silanol groups with no evidence of a peak at 3750 cm^{-1}. A more definitive answer concerning bonding can be obtained from the ^{29}Si CP-MAS NMR spectrum (B). In addition to the peaks observed for the hydride intermediate described in Figure 3B, a new peak is observed near -65 ppm. This is due to a silicon atom with three siloxane linkages and a carbon atom. This spectrum is used to prove the success of the hydrosilation reaction since the presence of the peak at -65 ppm confirms reaction of the hydride to form an Si-C bond at the surface. The presence of the Q_3 peak indicates that there are still silanols as part of the overall composition of the material. However, the absence of the 3750 cm^{-1} peak in the DRIFT suggests that most of the silanols are associated and not isolated. Because of the mesopores that exist in all particulate silica material, it is not surprising that some silanols remain since they are probably inaccessible to the TES silanization reagent. Further confirmation of the surface structure can be obtained from the ^{13}C CP-MAS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NMR spectrum which is shown in Figure 4C. Peaks for each of the carbons in the bonded moiety can be easily assigned according to the expected structure. Of particular interest is the peak at 12 ppm, which is due to the methylene carbon attached to the silica surface. In addition, for this molecule with two hydroxide groups, it appears that some bonding of 7-OD has occurred through one of the OH groups because two peaks in the olefinic portion of the spectrum can be seen between 120 and 140 ppm. In combination, the three spectra shown in Figure 4 give a relatively complete picture of the surface and the bonded moiety.

One of the advantages of the silanization/hydrosilation bonding scheme is the lack of interferences in the reaction from other functional groups which might be present on the organic ligand being attached to the hydride.[11] It would be expected that normal hydrocarbons with a terminal olefin should present the fewest problems in bonding. This has been observed for the bonding of 1-octene and 1-octadecene with surface coverages that are comparable to or better than those obtained by conventional organosilanization to form monomeric phases.[7,9] Since the majority of separations done by HPLC are done in the reverse phase mode, this result along with the high stability cited earlier[9] make this process a viable alternative for producing bonded phases suitable to a wide range of applications.

However the versatility advantages of this method facilitate the synthesis of a wide variety of specialty phases including some that might be difficult or impossible to make by other reaction schemes. One such possibility has been cited above which is a diol phase based on the bonding of 7-OD.[12] This material is different than the usual diol phases which are based on a propyl glycidyl ether moiety. The 7-OD phase has an alkyl chain of six methylene groups in addition to the diol functionality, while the current commercial phases consist of two methylene groups, an ether linkage (-CH_2-O-CH_2-) and the diol group. However, a phase identical to those now available can be made by bonding allyl glycidyl ether (AGE) to the hydride intermediate.[12] These two phases, as well as others that might be synthesized with different alkyl chain lengths and the diol group, lead to a range of hydrophobic/hydrophilic

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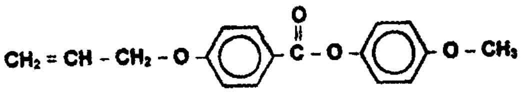
Figure 10 (left). Protein separations on chemically modified capillaries. A) poly(AAEE) capillary modified by silanization/hydrosilation method after 1st, 260th and 300th runs and B) polyacrylamide capillary modified by organosilanization after 1st, 139th and 150th runs. Conditions: V = 20kV, buffer is 25 mM bicine-Tris (pH=8.5). Four largest peaks in order of increasing migration time: trypsin inhibitor, β-lactoglobulin A, β-lactoglobulin B, and α-lactalbumin. (Reprinted with permission from ref. 26) properties which can be utilized in either reverse phase or normal phase chromatography. Some other examples of bonded materials synthesized by the silanization/ hydrosilation method with a terminal olefin, include a mono-ol phase based on a C_8 moiety, a butyl phenyl phase and a perfluorinated C_8 phase.[13] Current testing of these bonded materials involve normal phase, hydrophobic mode and reverse phase applications.

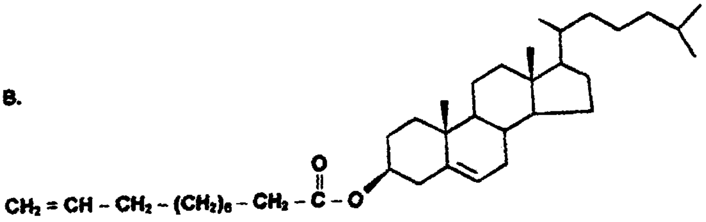
Perhaps one of the more unique moieties bonded to the hydride surface for use in HPLC are two liquid crystals, which also contain terminal olefins. Their structures are shown below. Bonded liquid crystals have been shown to possess properties which are different from other organic groups attached to silica surfaces and in particular, they exhibit a high selectivity based on molecular shape.[14-16] It appears that there is a high degree of association between adjacent ligands. This has been shown through variable temperature solid-state NMR studies. The spin-spin relaxation time measured from the line width (T_2*) can be used to determine the molecular motion of the bonded moiety.[17] In a typical C_{18} bonded phase, T_2* decreases regularly as the temperature is lowered indicating a restriction of the molecular motion.[17] However, for liquid crystal phases an increase in T_2* over a restricted range is observed as the temperature is lowered.[18] This observation has been attributed to

A.



4-Methoxyphenyl-4-allyloxy benzoate

B.



Cholesteryl 10-undecenoate

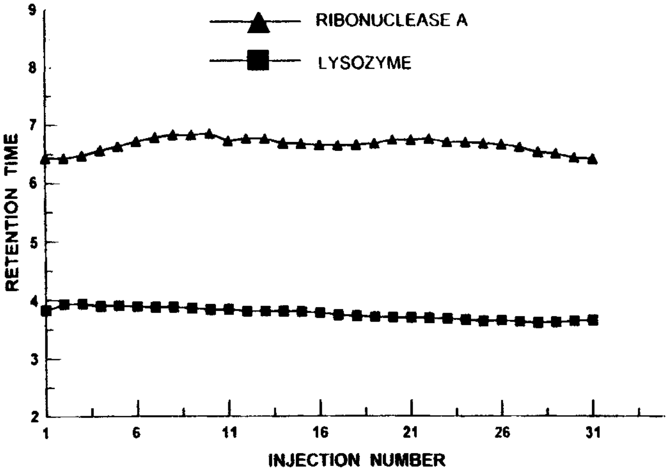


Figure 11. Retention time as a function of injection number for two proteins on an etched C_{18} modified capillary.

phase transitions where the bonded moieties change their degree of association similar to the molecular reorientations which occur for pure liquid crystalline materials. In the pure state, liquid crystals exhibit physical properties intermediate between those of a solid and an isotropic liquid over a well-defined temperature range. A similar phenomena in the bonded state could be responsible for the high shape selectivity exhibited in the chromatographic data.

Chromatographic characterization and testing potential applications provides further information about the nature of the hydride-based materials. For example, the effects of the hydride surface and the differences in diol phases have been tested in the normal phase with a heptane-chloroform mobile phase using a series of substituted phenols as solutes.[19] The results are shown in Figure 5 where the log k' values are plotted for each compound. The relative order of retention is the same for the three columns: commercial diol > diol from AGE > diol from 7-OD. In this case, the degree of retention changes as a function of the hydrophilic nature of the bonded material. The highest retention is for the commercial phase since it has a shorter alkyl chain, an ether linkage and residual silanols on the surface. When the residual silanols are mostly removed in the hydride material, retention decreases for the same bonded organic moiety as shown by the AGE phase. Retention decreases further as the alkyl chain length increases, the ether linkage is removed and the hydride surface is present in the case of the 7-OD phase.

With respect to practical applications, the hydride-based material has been tested in both normal and reverse phase. An example in reverse phase involves the separation of various tetracycline mixtures at low pH on the mono-ol column.[20] The analysis of a typical commercial tetracycline sample is shown in Figure 6. The separation shown here is considerably better than previous reports involving polymeric stationary phases, which cannot separate the first two components well (a & 3) and retention of the last two components (b & c) is too long to be practical. In addition, the low pH conditions (2.0-2.5) did not result in any deterioration of the column after long exposure to the mobile phase. An example in normal phase is the separation of a steroid mixture shown in Figure 7.[19] The diol column with the hydride surface, is well-suited to the broad range of polarities present in this mixture. The use of a gradient is still necessary to resolve the low polarity components which elute first, as well as the more polar species which are more strongly retained. However, the excellent selectivity observed in these examples and for other hydride-based bonded materials in both normal and reverse phase modes, as well as their stability, indicates their potential for a wide variety of practical applications.

While terminal olefins have been almost exclusively used for the attachment of organic moieties to hydride-based supports, hydrosilation is also possible for double bonds at other points on an alkyl chain.[11] An interesting example is the attachment of squalene to a silica hydride intermediate.[21] Squalene contains six double bonds, none being in a terminal position. The ^{13}C CP-MAS NMR spectrum of the bonded materials contains olefinic peaks indicating that not every double bond has reacted.

However, the ^{29}Si CP-MAS NMR spectrum has a peak at -65 ppm which confirms the formation of an Si-C bond, proving that at least one olefin per squalene molecule has reacted with the hydride surface to provide attachment of the organic moiety. This result opens up the possibility of synthesizing other useful bonded phases from molecules with a double bond in other than the terminal position. The squalene phase was prepared for use in gas chromatographic analysis of low molecular weight hydrocarbons.

Another example of a gas chromatographic phase[22,23] utilized a hydride intermediate prepared from a cyclic siloxane (pent-amethylcyclopentasiloxane). Subsequent attachment of 1-octadecene or 1-octene indicates that this approach can also provide a hydride surface suitable for hydrosilation. HPLC testing of bonded phases, based on the cyclic siloxane and TES intermediates, would be useful in determining whether one of the approaches produces a material with any specific chromatographic advantages.

Other Oxides

In principle, the silanization/hydrosilation reaction scheme can apply to any oxide surface where hydroxides are present. While silica is the most common support material due to its availability with a wide range of physical properties such as particle size, pore size and surface area, it is limited mainly by its hydrolytic stability, generally from pH 2-8. Other oxides which have the necessary hydroxide groups and are stable over a broader pH range include alumina, zirconia, titania and thoria. It has already been demonstrated that formation of hydride surfaces on each of these four oxides is possible.[24,25]

Once the hydride intermediate is formed, then hydrosilation should proceed in the same manner as on silica. These processes can be readily monitored spectroscopically by DRIFT and solid-state CP-MAS NMR. An example of the spectroscopic evaluation by ^{29}Si CP-MAS NMR of a modified zirconia material is shown in Figure 8. The spectrum of the hydride intermediate (A) has a large peak at -85 ppm which represents the Si-H moiety on the surface. In addition, there also is a peak near -75 ppm indicating there are some silicon atoms with both a hydride and a hydroxide.

An interesting feature are the peaks at -110 and -100 ppm. These peaks indicate that some polymerization of the TES has occurred during formation of the hydride layer. This is the only explanation for the presence of a silicon with four siloxane bonds. The spectrum of the octyl bonded phase (B) contains the same four peaks which are found in the hydride intermediate (the peak at -75 ppm is not resolved) as well as two additional peaks which are characteristic of attached organic moiety. The peak at -65 ppm represents a silicon atom with a carbon attached to it, while the peak at -55 ppm is due to a silicon atom with a carbon and a hydroxyl group. The presence of these two peaks are confirmation that bonding of the 1-octene to the hydride intermediate was successful. Similar spectroscopic evidence has been used to confirm both the formation of the hydride intermediate and the bonded product on alumina, titania and thoria.

For other supports to be competitive with silica, they must also possess good chromatographic properties in addition to their potential for use under conditions which are not compatible with current stationary phases. Figure 9 shows the separation of a reverse phase test mixture run on a column with an octadecyl moiety bonded via a hydride intermediate on an alumina support.[24] The chromatogram has good peak shape with the most polar component eluting first and the least polar component eluting last.

FUSED SILICA CAPILLARIES

The same reactions which are used to modify porous silica surfaces can be applied to the modification of fused silica capillary walls. As a consequence, organosilanization has been the most popular method for modifying capillaries in high performance capillary electrophoresis (HPCE). However, the same advantages that the silanization/hydrosilation reaction scheme has for porous silica should be present for fused silica capillaries because the reactive site is the same, i.e. silanols.

The first reported application of this method to HPCE involved the attachment of a linear polymer, poly(acryloylaminoethoxyethanol), to a capillary wall for the separation of proteins.[26] This particular polymer, poly(AAEE), was selected because it is hydrolytically more stable than polyacrylamide. Therefore, the combined effect of having a more stable attachment of the organic moiety to the capillary wall via an Si-C linkage, and the resistance to hydrolysis of the substituted amide, results in a separation media that can function under buffer conditions which cause the rapid deterioration of conventionally bonded polymers such as polyacrylamide. This improvement in performance is documented in a series of electropherograms shown in Figure 10. The comparison was run on a series of acidic proteins so that a basic pH buffer was selected with the separation taking place using reverse polarity and cathodic injection. Panel A shows selected runs of these four proteins on the poly(AAEE) capillary. It is clear that no significant change in the electropherogram has occurred between the 1st and the 300th injection. However, for polyacrylamide (Panel B), little if any analytical information is available from the electropherogram by the 150th injection. Similar high efficiencies and stability were achieved when basic proteins were separated under acidic or neutral conditions. Electroosmosis was low and stable with the poly(AAEE) capillary at pH 8.5, while the polyacrylamide capillary showed a rapid increase in a relatively short time under identical buffer conditions. Since the stability observed in HPCE parallels that obtained in HPLC, the attachment of other organic moieties to capillary surfaces should also result in continued high performance after many injections.

While additional examples of capillaries modified by the silanization/hydrosilation reaction method for HPCE are not yet available, there is further confirmation of the utility of this bonding scheme in capillary electrochromatography (CEC). However, the approach to CEC is somewhat different than the normal one where the capillary is packed with particulate silica identical to material used in HPLC. In this method the capillary is first extensively etched with an aggressive reagent, ammonium hydrogen difluoride,[27] which increases the surface area by up to a factor of 1000. Then

Table 1

Separation Factors for Various Pairs of Proteins and Peptides

Solute Pair	Bare Capillary	Etched C_{18} Capillary
Angiotensin III/lysozyme	1.12	1.15
Bradykinin/angiotensin III	1.02	1.28
Ribonuclease A/bradykinin	0.97	1.16
Angiotensin I/ribonuclease A	1.11	1.15
Angiotensin I/lysozyme	1.22	1.97

the surface is modified by the silanization/hydrosilation method in a manner similar to that reported for HPCE.[26] The increase in surface area and the presence of radial extensions from the inner capillary wall leads to an increase in solute/bonded phase interactions (k'). Therefore, electromigration and/or electroosmosis is the driving force which moves the solute through the column, while separation is achieved by differences in electrophoretic mobility and/or k' interactions, depending on whether the solute is charged or neutral. The presence of solute/bonded phase interactions can be verified in several ways. For several charged solutes, the peak width in a bare, etched or hydride-modified capillary is narrow, with efficiencies between 300,000 and 500,000. For a C-18 modified etched capillary, peaks become noticeably broader leading to efficiencies between 30,000 and 70,000[27] due to resistance to mass transfer effects. This result is certainly a strong indication of solute/bonded phase interactions. Measurable differences in retention are also found for small neutral molecules such as 1- and 2-napthol. Finally, a comparison of migration times for various proteins and polypeptides leads to significant differences in separation factors between bare and etched C-18 modified capillaries, as shown in Table 1. These results indicate that k' interactions are present in the etched modified capillary since the separation factors clearly change from the bare capillary where migration is determined only by electrophoretic mobility. There are increases in separation factors, a reversal of the order of migration for one pair and a much greater range in the difference of migration times, which can be seen by comparing the α values for the first and last eluted compounds on both columns. This system requires no packing or frits and there is no bubble formation as a consequence of these two factors.

The question of stability is also important in CEC just as it is in HPLC and HPCE. Because the modification is based on the silanization/hydrosilation reaction scheme, the bronded moiety should possess the same high stability that was demonstrated for porous silica and ordinary modified capillaries for CE.

Table 2

Separation Factors for Component Pairs in Commercial Tetracycline*

Solute Pair	α
4-epi-anjydrotetracycline/4-epitetracycline	1.08
Tetracycline/4-epi-anhydrotetracycline	1.05
Chlorotetracycline/tetracycline	1.20
anhydroetracycline/chlorotetracycline	1.10

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*Conditions: electrolyte, 30mM citric acid + 24.5 mM β-alanine (pH=3.0), V=30 kV, 1=25 cm.

Table 3

Separation Factors for Component Pairs in Commercial Oxytetracyline*

Solute Pair	α
Oxytetracycline/tetracycline	1.10
4-epioxytetracycline/oxytetracycline	1.08
α-apo-oxytetracycline/4-epioxytetracycline	1.25

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* Conditions: electrolyte, 30 mM phosphate and 19 mM Tris (pH=2.14). 60:40 buffer/MeOH, V=30 kV, 1=35 cm.

Figure 11 shows the results of 31 consecutive injections of lysozyme followed by 31 consecutive injections of ribonuclease A. No discernible increase or decrease in retention times is observed for either protein. The reproducibility for each series of 31 injections was ±1.5%. The test was not conducted on a new capillary, but one that had been mounted and demounted several times, washed several times with methanol followed by dry storage and had more than 100 injections of other samples under a variety of buffer conditions.

A more practical example of the usefulness that this mode of CEC might develop involves the separation of various tetracycline mixtures.[28] Table 2 shows the separation factors obtained for a typical commercial tetracycline mixture with the etched C_{18} modified capillary. Resolution of the various components and analysis time are comparable to the best separations reported in HPLC.[20] An even better result can be obtained for the analysis of commercial oxytetracycline as shown in Table 3. In particular, oxytetracycline and 4-epioxytetracycline were easily separated on the etched C_{18} modified capillary but are not resolved on a polymeric column and only partially resolved on the mono-ol column cited above.[20] In addition, the analysis time for the CEC method is less than 6 min.

CONCLUSIONS

The silanization/hydrosilation method has proven to be a viable method for the modification of oxide supports for HPLC and capillary surfaces for HPLC and CEC. Further development to expand the range of applications in each of these techniques is still necessary. It is likely that other functional groups and other modes of catalysis can be used to attach the organic moiety to the hydride surface during the hydrosilation reaction. Further improvement in the formation of the hydride intermediate and the final bonded product will lead to a wide variety of bonded surfaces and increased stability.

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