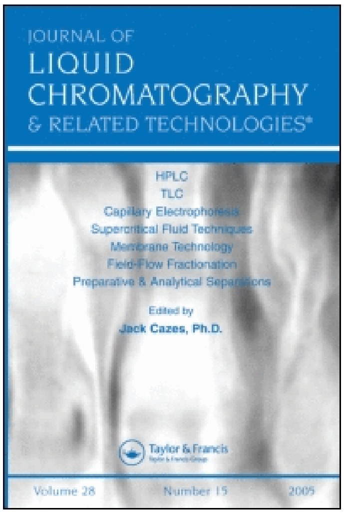

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High Performance Liquid Chromatographic Characterization of Diol Bonded Phases Synthesized via a Hydride Intermediate

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC CHARACTERIZATION OF DIOL BONDED PHASES SYNTHESIZED VIA A HYDRIDE INTERMEDIATE

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ABSTRACT

The chromatographic characterization of two diol stationary phases for HPLC prepared via a hydride intermediate followed by hydrosilation is described. The bonded materials are the result of the reaction of 7-octene-1,2-diol or allyl glycidyl ether with silica hydride. Characterization in the reverse phase mode was done via a standard mixture of three polycyclic aromatic hydrocarbons (PAHs). In the normal phase mode, retention data of model compounds in several different eluents were compared to each other and with data available from the literature. ESCA analysis is used to monitor for the presence of reduced Pt catalyst and when it is detected its effects can be seen in the chromatographic data.

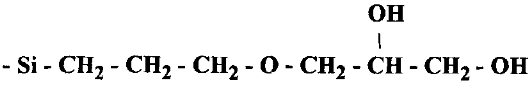
INTRODUCTION

In recent years diol columns have found growing acceptance and application among modern stationary phases. The most common use of diol materials is in normal phase chromatography where extensive characterization has already been reported (1). Applications include: sugars (2), xanathates (3), chalcones (4), aromatic, polyaromatic and phenol compounds (5). In the case of phenols, it appears that retention is the result of donor-acceptor interactions. Another study measured retention on the diol stationary phase of more than 300 aromatic compounds extracted from plants and foodstuffs (6). Despite the obvious advantages of the diol columns in normal phase chromatography, a number of interesting reverse phase applications have been reported as well. A comparison of phospholipid separations has been made between diol, amino and silica columns (7). Sugars can also be separated in the reverse phase mode but a post column reaction with copper-amine is necessary for detection (8). An interesting reverse phase application involves the separation and quantitation of the drug verapamil, used for the treatment of heart disease, and its major metabolite norverapamil (9).

Among the other applications for diol bonded materials, size exclusion chromatography has been among the most important. It has been used to separate water-soluble polymers (10), proteins (11) with molecular weights ranging from 17,000-440,000, hemoglobin subunits (12) and carrageenans (13). Another approach involves immobilizing an active species through the diol moiety which can then be used in high-performance affinity chromatography (14). Cyclodextran has also been immobilized on a diol column for direct serum assays of drug enantiomers (15). The diol bonded phase has also found application in thin-layer chromatography where its performance is often compared to amino and cyano plates (16). A variety of mixtures containing vitamins, pesticides, steroids as well as other natural substances have been compared on these three types of TLC plates(17). Other comparisons among the cyano, amino and diol plates include nucleosides (18), benzodiazepine derivatives (19) and a two-dimensional separation of polyaromatic hydrocarbons involving both a reverse phase and a normal phase development has been reported (20).

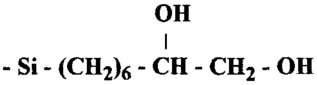
Until recently, the only synthetic route to producing a diol phase involved the bonding of 3-glycidyloxypropyltrimethoxysilane to silica followed by acid hydrolysis of the epoxide group to the diol. However, this method can result in a variety of products instead of the epoxide intermediate and diol product (21). Therefore it is not unusual that similar separations reported in the literature are not identical due to the variability of the bonded material. We have reported another approach to the synthesis of a new phase using a hydride intermediate followed by hydrosilation with the olefin 7-octene-1,2-diol which results in a "true" diol bonded material (22). The goal of this study is to chromatographically characterize this new diol bonded material and compare it to phases which are synthesized from allyl glycidyl ether as well as typical results from the literature. The structures of the two types of diol phases are shown below:

a) Diol via allyl glycidyl ether :



$$-\mathrm{Si}-\mathrm{CH_2}-\mathrm{CH_2}-\mathrm{CH_2}-\mathrm{O}-\mathrm{CH_2}-\overset{\overset{\mathrm{OH}}{|}}{\mathrm{CH}}-\mathrm{CH_2}-\mathrm{OH}$$

b) Diol via 7-octene-1,2-diol :



$$-\mathrm{Si}-(\mathrm{CH_2})_6-\overset{\overset{\mathrm{OH}}{|}}{\mathrm{CH}}-\mathrm{CH_2}-\mathrm{OH}$$

The effects of hydride on the surface in comparison to hydroxide as well as evaluations of similar phases made by conventional organolsilanization have been addressed in another study (23).

EXPERIMENTAL

Materials

The silica gel used was Vydac 101 TP lot # 900201 - The Separations Group (Hesperia, CA, US) - (BET surface area, S_{BET}= 106 m^2/g ; particle diameter, dp = 6.6 μm - all data supplied by manufacturer). The solvents were HPLC grade from Fisher Scientific (Pittsburgh, PA, US) and J.T.Baker (Phillipsburg, NJ, US). Solutes were from Aldrich (Milwaukee, WI, US), Sigma (St. Louis, MO, US), Eastman Organic Chemicals (Rochester, NY, US) and Matheson Coleman & Bell (Norwood, OH, US).

The column Selectivity Test Mixture for Liquid Chromatography (Polycyclic Aromatic Hydrocarbons, SRM 869) was from the National Institute of Standards & Technology (Gaithersburg, MD, US). Samples were diluted in the mobile phases (weights were always less than 10 μg per injection volume - 5 μL - to avoid overloading of the column). Solvent flow was 2 mL/min. The determination of t_0 was carried out using n-pentane as an inert solute by measuring the maximum negative deflection when it was injected into a heptane mobile phase.

Columns

Three types of columns were used: diol via allyl glycidyl ether (AGE - white), diol via allyl glycidyl ether with reduction of the catalyst (AGE - gray) and diol via 7-octene-1,2 - diol (7-OD). The carbon content and surface coverages of the three columns are shown in Table I. The synthesis and spectroscopic characterization of diol-bonded silicas were described earlier (22).

Instrumentation

All chromatographic measurements were made with a Hewlett-Packard (Avondale, PA, US), model 1050 liquid chromatographic system equipped with quaternary gradient pump, automatic injector, variable wavelength UV detector and computer data station. Columns were slurry-packed (10% w/v bonded silica in 9:1 v/v CCl_4/methanol) into 15-cm x 0.46-cm I.D. stainless steel tubes (Altech Co., Deerfield, Il, US) using a Haskel (Burbank, CA, US) pneumatic pump at 40 MP. Methanol was the driving solvent. The ESCA data were obtained with a Perkin-Elmer Model 5600 Multitechnique Spectrometer (Lockheed, CA, US).

Procedures

For ESCA analysis, the silica-powder samples were coated onto double-side-sticky tape, mounted on a sample stage and evacuated to a base pressure of about $1x10^{-9}$ Torr. The H_2PtCl_6 reference catalyst was tested similarly on an inert gold-plated substrate. To enhance the magnitude of the spectral peaks, the sample stage was tilted to a take-off angle of 70^0 with respect to the normal surface plane. A monochromatic Al Kα X-ray source (1486.6 eV) with a 7-mm filament operating at 400 W irradiated the samples. A hemispherical-electrostatic-field analyzer detected the kinetic energies of the emitted photoelectrons. The test-spot sizes for the silica-powder samples and the catalyst were 800x2000 μm and 400 μm, respectively. ESCA-survey spectra between 0 and 1100 eV were first collected with a 187.85 eV pass energy and a 0.40 eV resolution to identify the primary elements present. To enhance spectral resolution for various elements, ESCA-multiplex spectra were then obtained with a 58.70 eV pass energy and a 0.25 eV resolution. The binding energies were normalized to the C 1s line at 284.6 eV. All ESCA data were collected at ambient temperature.

TABLE 1.
Carbon content and surface coverages of diol silica samples

No	Sample	Corrected % C *	α_R μmol/m^2
1.	Vydac-diol (AGE)-gray	4.58	4.25
2.	Vydac-diol (AGE)-white	4.15	3.63
3.	Vydac-diol (7-OD)	5.05	3.66

*** - % C after subtracting the amount before hydrosilation reaction.**

RESULTS AND DISCUSSION

Reverse Phase

SRM 869 has proven to be a useful tool for the classification of bonded phase type. The relative retention of two PAHs, tetrabenzonaphthalene (TBN) to benzo[a]pyrene (BaP), is used to determine if a phase is polymeric, monomeric or intermediate (24). According to the classification scheme developed, α TBN/BaP is <1.0 if the phase is polymeric and >1.7 if it is monomeric. Between 1.0 to 1.7 the phase is classified as intermediate. The method specifies a mobile phase composition of 85% acetonitrile (MeCN)/water. However, under these conditions incomplete separation of the mixture was obtained on the AGE (white) and 7-OD columns. It was found that an eluent composition of 60% acetonitrile/water gave adequate separations in order for the relative retentions to be determined on the two phases. Using this mobile phase and according to classification for SRM 869, the 7-OD column behaves like a monomeric phase ($\alpha_{\text{TBN/BaP}}$ = 1.74), while AGE behaves like an intermediate material ($\alpha_{\text{TBN/BaP}}$ = 1.34).

Figure 1 shows the retention of the three components of SRM 869 on the two diol columns at 50% acetonitrile/water. For the component which shows highest retention on two columns, TBN, retention is in the order 7-OD>AGE(white). The result for 7-OD is expected since it should be more hydrophobic than AGE with the bonded moiety consisting of two hydroxyl groups and an alkyl chain of six methylene groups. AGE has an additional oxygen (ether linkage) and a shorter alkyl chain (four methylene groups). Figure 1 also shows that on the two columns for PAHs with the same F number (25)

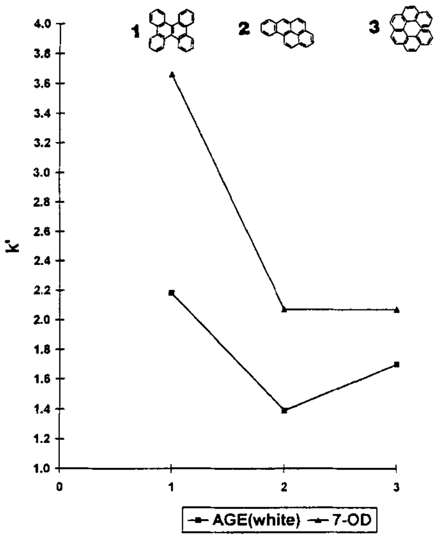


FIGURE 1. k' values of test mixture for liquid chromatography (SRM 869) on diol bonded phases: **AGE (white)** - diol via allyl glycidyl ether and **7-OD** - diol via 7-octene-1,2-diol. Mobile phase: 50 % acetonitrile in water (v/v), solutes: 1 - TBN (Tetrabenzonaphthalene), 2 - BaP (Benzo[a]pyrene), 3 - PhPh (Phenanthro[3,4-c]phenanthrene). Flow rate 2 mL/min.

(TBN and PhPh, F =13, F = number of double bonds + number of of primary and secondary carbons - 0.5 x number of non aromatic rings) longer retention is observed for the more planar of the two compounds (TBN). Recently we have tested the behavior of various tetracyclines in the reverse phase mode using high percentages of water and low pH (26). Excellent separations were obtained for many tetracycline mixtures and their degradation products with no evidence of column deterioration under aggressive mobile phase conditions.

Normal Phase

Aromatic Compounds-

In order to characterize the new materials and compare them to other diol phases aromatic compounds were chosen as test solutes despite their low k' values, because abundant data exists in the literature. Table II gives log k' values for a series of aromatic

TABLE 2
Retention data of aromatic compounds on diol bonded phases

No	SOLUTES	k'		
		1	2	3
1	Biphenyl	0.24	0.11	0.24
2	Naphtalene	0.23	0.11	0.24
3	Fluorene	0.32	0.12	0.33
4	p-Xylene	0.12	0.12	
5	Toluene	0.13	0.12	
6	m-Xylene	0.12	0.12	
7	Pyrene	0.49	0.12	0.64
8	p-Dibromobenzene	0.15	0.12	
9	Styrene	0.15	0.11	
10	1-Methylnaphthalene	0.22	0.12	
11	2-Methylnaphthalene	0.22	0.12	
12	Chlorobenzene	0.15	0.12	
13	Benzene	0.14	0.13	0.09
14	Phenanthrene	0.39	0.15	0.47
15	Anthracene	0.40	0.15	
16	Fluoranthene	0.34	0.20	0.67
17	Durene	0.13	0.26	
18	Chrysene	0.76	0.27	
19	Nitrobenzene	1.34	0.30	
20	Benzo(k)fluoranthene	0.59	0.32	
21	Acetophenone	0.91	0.36	
22	Perylene	0.66	0.37	

(1); Age(white) column, mobile phase-heptane
(2); 7-OD column, mobile phase-heptane
(3); LiChrosorb 100 from Merck (data from literature[5]), mobile phase-heptane

compounds on the two materials synthesized for this study as well as data obtained from the literature (5) for a commercial diol column (Merck LiChrosorb). These results indicate that the 7-OD column is less hydrophilic than either the AGE (white) or the Merck diol based on data from identical solutes. For virtually all of the aromatic solutes studied, 7-OD had the lowest k' with heptane as the mobile phase. A much different order of retention is observed for the same compounds on the AGE (white) and the commercial diol. In almost all cases

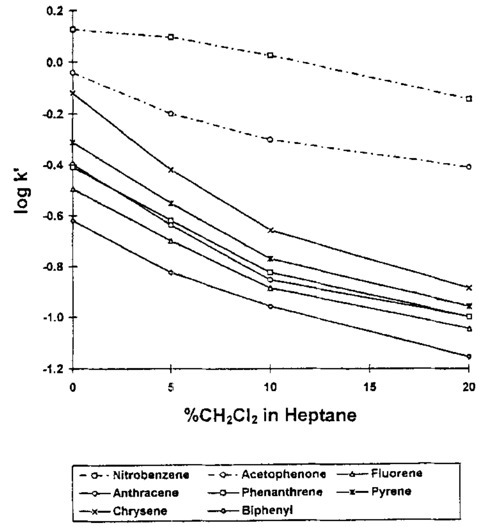


FIGURE 2. Retention data of some aromatic compounds on AGE (white) column with methylene chloride-heptane as the mobile phase. For notation of column see FIGURE 1. Flow rate 2 mL/min.

where literature data is available, the Merck material has a higher k' value. As indicated above, incomplete hydrolysis or the presence of other species which can form during the conventional bonding process may account for this difference. In direct comparisons using heptane as the mobile phase, only benzene has lower retention on the commercial diol. However, the k' is small and no information is given in the literature on the determination of t_0 so that the difference can be easily due to this discrepancy.

Further documentation of the strong polar characteristics of the AGE (white) phase is given in Figure 2. Retention for the unsubstituted aromatic solutes generally increases as the number of aromatic rings increases: biphenyl (2) < fluorene, phenanthrene, anthracene (3) < pyrene, chrysene (4). The shape of the curve is consistent with the donor-acceptor retention mechanism. As the amount of modifier in the mobile phase is decreased, retention (log k') increases faster at lower modifier concentrations. Lower amounts of modifier enhance the donor-acceptor interaction taking place between the aromatic rings of the solutes and the diol moiety of the bonded phase. In contrast the two solutes with a functional group (nitrobenzene and acetophenone) that can hydrogen bond with the diol species display higher k' values and are less affected as the amount of modifier is increased.

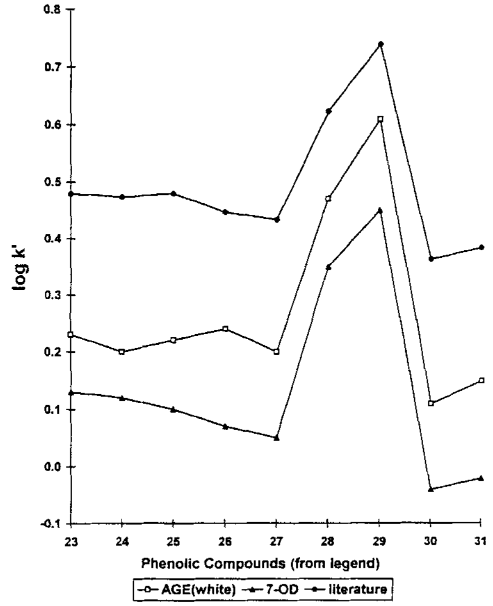


FIGURE 3. Comparison of the retention data of phenols on diol columns with data from the literature (5). Mobile phase heptane-chloroform (50:50 v/v) for AGE (white) and 7-OD columns (For notation of columns see FIGURE 1). Data from literature (5)(for Merck diol column) are with the mobile phase isooctane-chloroform (50:50 v/v). The solutes: **23** ; 4-Bromophenol, **24** ; 3-Chlorophenol, **25** ; 4-Chlorophenol, **26** ; 4-Fluorophenol, **27** ; Naphtol-2, **28** ; 3-Nitrophenol, **29** ; 4-Nitrophenol, **30** ; Phenol, **31** ; 4-Phenylphenol. Flow rate 2 mL/min.

The behavior of another class of aromatic compounds, phenols, is shown in Figure 3 for the two diol columns synthesized for this study as well as for the Merck diol. For the nine compounds studied, the same relative order for each compound on the three columns is observed: 7-OD < AGE (white) < Merck diol (literature), consistent with the hydrophilic/hydrophobic nature of the materials as explained above. At the lowest amounts of modifier in the mobile phase, the AGE (white) column displays lower retention than the commercial diol for all of the phenol solutes. While some differences in retention may be due to the effects of heptane instead of isooctane as the primary mobile phase constituent, the major trends observed are probably too great to be accounted for by this change alone.

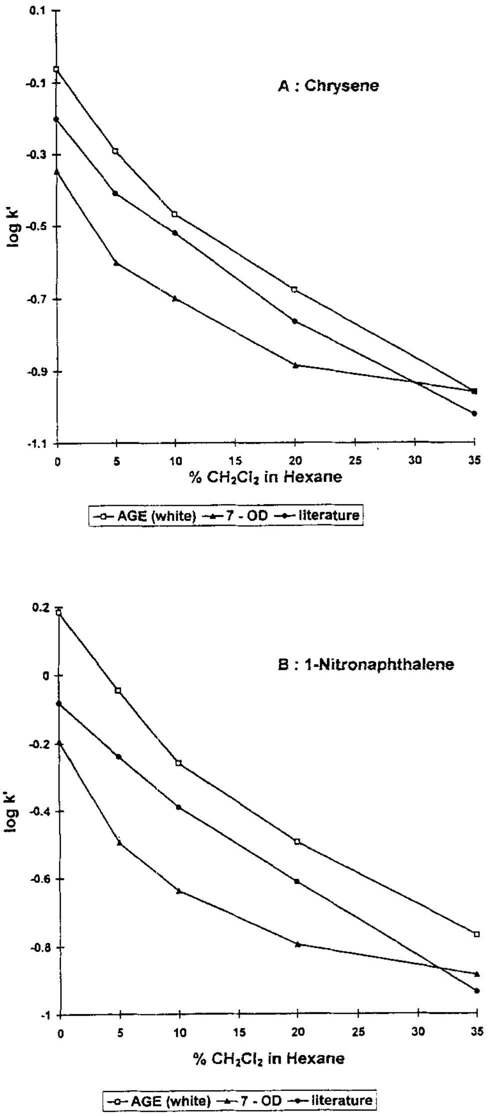
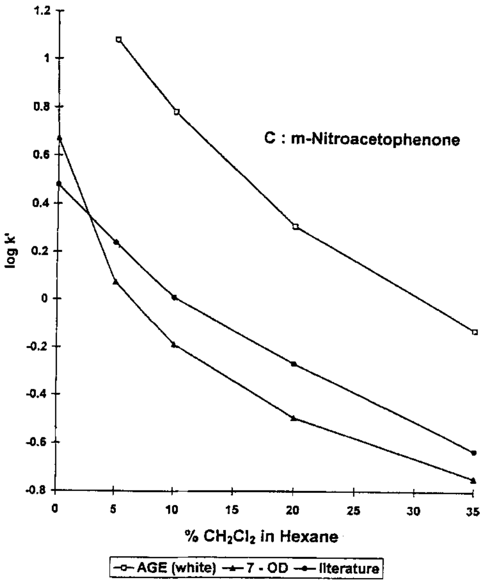


FIGURE 4. Retention data of model compounds on diol columns with methylene chloride-hexane as eluent (for notation of columns see FIGURE 1). Flow rate 2 mL/min. The literature data are from reference number 1 (for DuPont diol column). A ; data for chrysene, B ; data for 1-Nitronaphthalene, C ; data for m-Nitroacetophenone, D ; data for Benzyl alcohol.



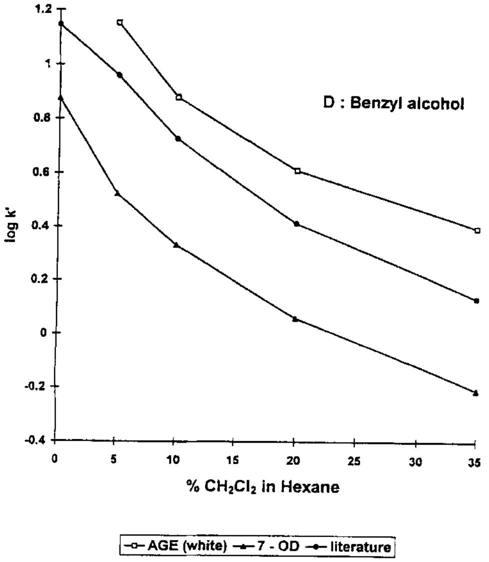


FIGURE 4 (continued)

An illustration of the variability between diol columns is shown in Figure 4 where the two materials in this study are compared to another commercial source (DuPont) using the same solutes in the same mobile phase as in a previous report (1). In the case of AGE (white), retention is consistently longer for all compounds at all modifier concentrations than for the DuPont - diol column. In the case of the least hydrophilic material, 7-OD, only nitroacetophenone in the absence of modifier has greater retention than the commercial column.

Steroids-

A clearer example of the trend observed above can be seen for very highly retained solutes such as steroids. Data from the previous study (1) on the commercial diol column cited above were based on varying amounts of methylene chloride in hexane as the mobile phase to elute five test steroids. Three of the same steroids and a fourth similar in structure to the other two test compounds were used in evaluating the two new diol materials. For AGE (white) only the two least retained steroids could be easily eluted (Figure 5A) in 100% methylene chloride. In order to obtain k' values < 10 for corticosterone and prednisone on the AGE (white) material, it was necessary to modify methylene chloride with 2-propanol. For highly retained solutes the AGE (white) column synthesized for this study is more polar than the DuPont diol column (1). On the 7-OD column the steroids could be eluted in hexane modified with methylene chloride (Figure 5B). In this case, retention for all the test compounds was less than that observed on the DuPont diol column. This result confirms all of the previous observations that 7-OD material is less hydrophilic than any of the diol columns based on hydrolyzed propyl glycidyl ether moieties.

In terms of a practical separation involving the four steroids tested above, the k' values are sufficiently different that it is necessary to use a gradient in order to do the analysis in a reasonable time frame. The AGE (white) column has the strongest retention for the steroids and the greatest range of k' values under a given set of isocratic conditions. Therefore, the separation of the four solutes was optimized on the AGE (white) column and the results are shown in Figure 6A. Good separation in just over three minutes is achieved using 2-propanol as the modifier in methylene chloride with a gradient from 2.5% to 15% between 1 and 2.5 min in the chromatographic run. The same gradient was applied to the 7-OD column (Figures 6B). The very short retention and incomplete separation of the four steroids on the 7-OD column for this same gradient is consistent with its properties as less hydrophilic than the AGE (white) phase.

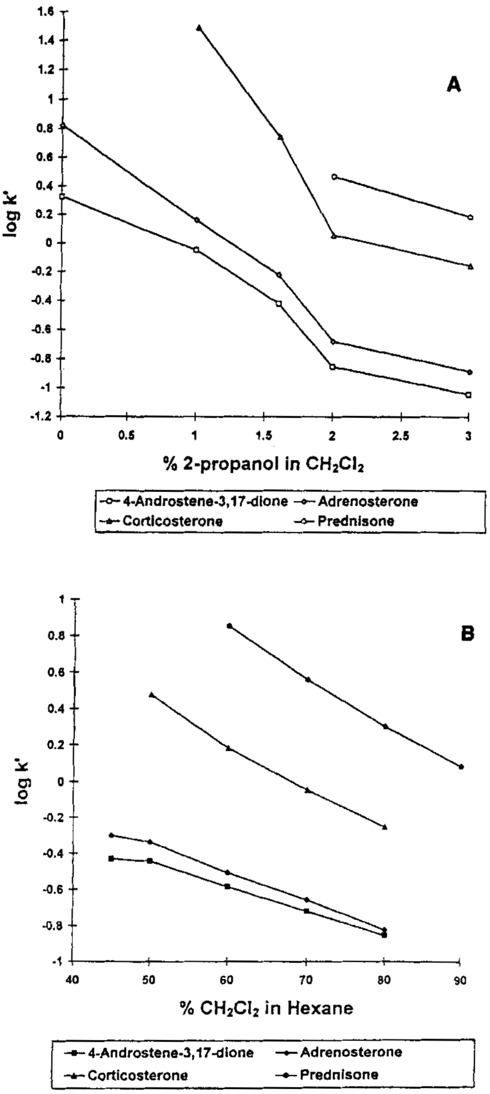


FIGURE 5. Retention data of steroids on: A ; AGE (white) column with 2-propanol-methylene chloride as eluent and B ; on 7-OD column with methylene chloride-hexane as the mobile phase. Flow rate 2 mL/min. For notation of columns see FIGURE. 1.

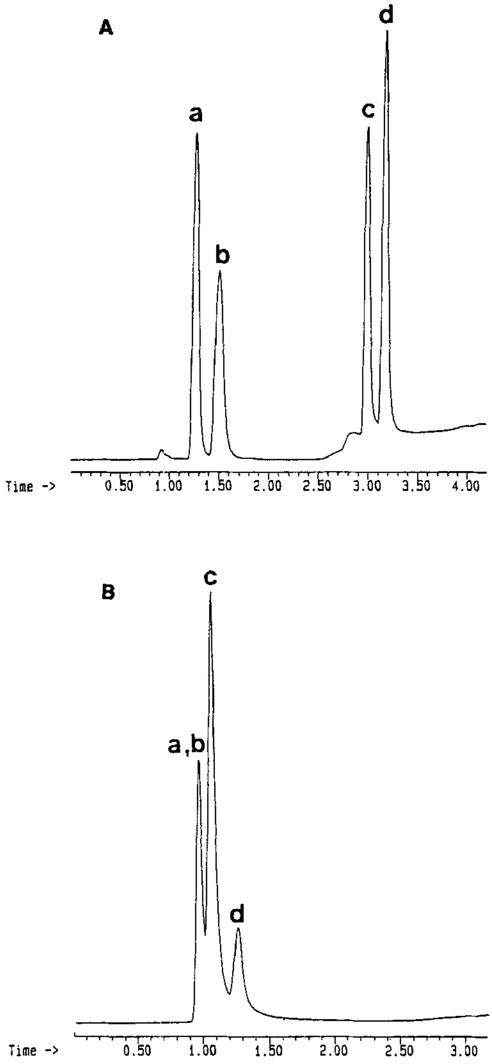


FIGURE 6. Gradient separation of steroids (a ; 4-Androstene-3,17-dione, b ; Adrenosterone, c ; Corticosterone, d ; Prednisone). Gradient conditions: 2-propanol as the modifier in methylene chloride from 2.5% to 15% between 1 and 2.5 min. in the chromatographic run.

A ; AGE (white), B ; 7 - OD and C ; AGE (gray) columns (for notation of columns see Experimental).

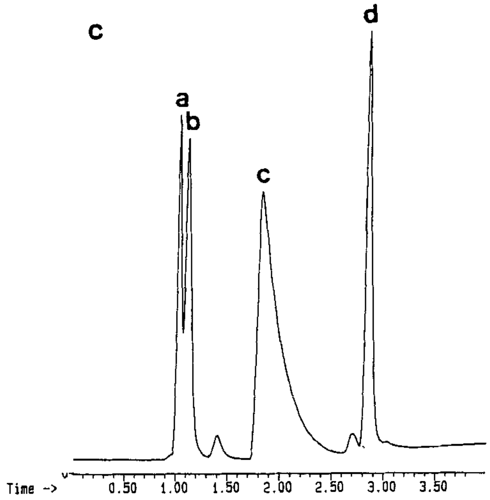


FIGURE 6 (continued)

Pt Reduction

When a large amount of catalyst was used (10 fold increse) it was noted that the final product was gray. This material was packed into a column for testing. The result of the steroids separation is shown in Figure 6C. The interesting feature of this chromatogram is the broad unsymmetrical peak (c) observed for the third component (corticosterone). By making the gradient shallower, the fourth peak becomes broader as well. With a very shallow gradient the first two peaks (a,b) have significant retention and become broader and unsymmetrical. This effect is most likely due to the presence of Pt on the surface (see discussion below) which apparently has a strong interaction with the steroid solutes. The chromatographic behavior can be explained by both the presence of Pt on the surface and the use of 2-propanol as the modifier. At low k' values, interaction between the solute and the stationary phase is relatively small so that there is no significant effect of the Pt. When retention is longer but there is still no significant amount of modifier in the mobile phase, the solute can display strong adsorption at the Pt sites on the stationary phase. Such an effect is observed for the third peak (c) in Figure 6C. Once there is an appreciable quantity of 2-propanol in the mobile phase it will mask the Pt so that the solute does not interact with these adsorption sites but just the organic moiety. The effect of 2-propanol is not suprising since it is the solvent used to dissolve the

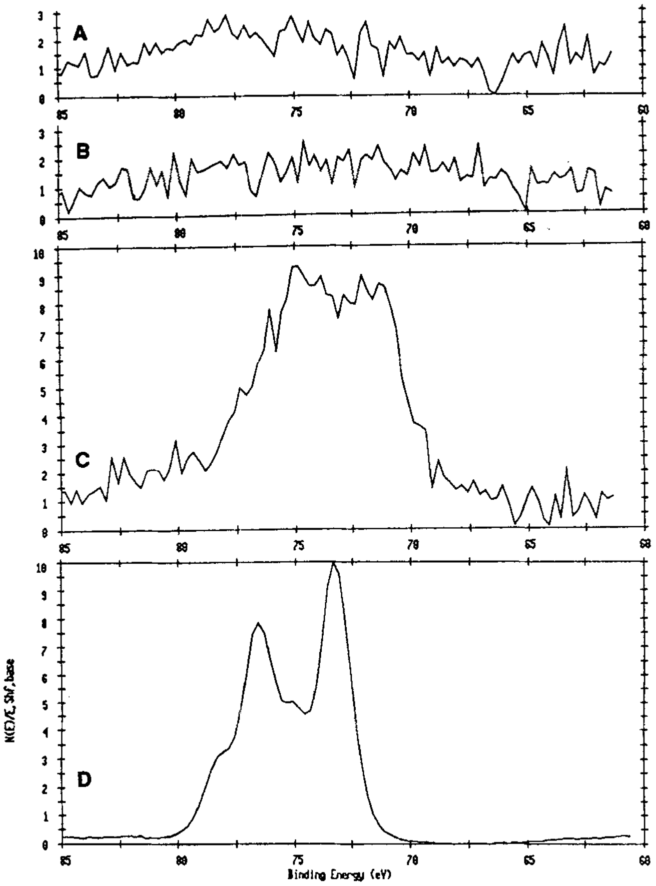


FIGURE 7. Pt 4f region of the ESCA spectra of: A ; bare Vydac TP silica, B ; Vydac-H TP (hydride intermediate) silica, C ; AGE (gray) column material (diol via allyl glycidyl ether with reduction of the catalyst) and D ; catalyst - hexachloroplatinic acid.

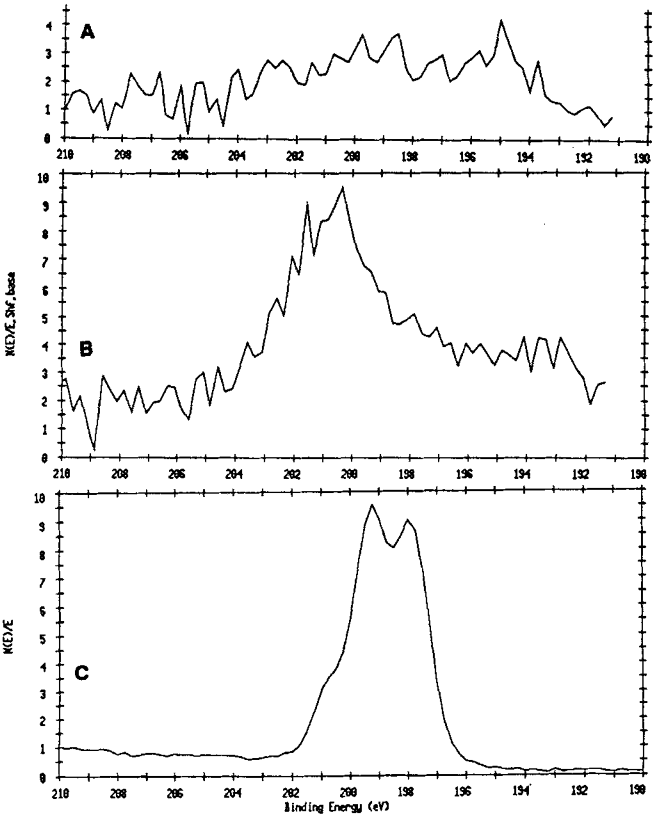


FIGURE 8. Cl 2p region of the ESCA spectra of: A ; Vydac-H TP (hydride intermediate) silica, B ; AGE (gray) column material (for notation of column see FIGURE 7) and C ; catalyst - hexachloroplatinic acid.

hexachloroplatinic acid (Spier's catalyst) for the bonding reaction (hydrosilation). It is interesting to note that such a dramatic effect on chromatographic behavior is caused by a very small amount (.03 atom %) of Pt on the surface. This results illustrates the importance of preventing catalyst deposition during the bonding reaction.

Both the chromatographic data reported above and the color of the AGE (gray) material suggest that some reduction and deposition of the catalyst has occurred. This can be conclusively proven through ESCA which determines the elemental composition of only the surface of a material (27). Figures 7A and 7B show the high resolution ESCA spectra of the platinum 4f region for bare Vydac TP silica and the same hydride intermediate used to make the AGE columns. No Pt is detectable (<0.01 atom %) on the surface of either material. In comparison, Figure 7C shows the same region for the AGE (gray) column material. The Pt 4f doublet is visible above the noise at a level estimated to be around 0.03 atom %. Additionally, the same high resolution scan was obtained for the catalyst, hexachloroplatinic acid, used to bond the AGE via the hydride intermediate (Figure 7D). From the position of the peaks in the two spectra, it can be concluded that the majority of the platinum which is on the AGE (gray) material is in the elemental state. Since the catalyst is soluble under the reaction conditions, the Pt(0) detected must be the result of reduction through interaction with the hydride surface. This observation is also supported by DRIFT spectra which show a measurable decrease in the Si-H stretching band for the gray material in comparison to the white product (22). Further confirmation of the change in oxidation state of the Pt containing material is shown in Figure 8, the high resolution ESCA spectra of the Cl 2p region. The bare silica (Figure 8A) shows no evidence of Cl on the surface. The 2p doublet is visible in the spectrum of the AGE (gray) material near 200 eV. However, for the catalyst the lower energy peak appears near 197 eV. Therefore, it can be concluded that the Cl on the bonded material is not coordinated to Pt(IV) as is the case for the catalyst but is probably free chloride ion which is adsorbed on the surface after the platinum is reduced. It is clear that a relatively small amount of Pt on the surface can lead to a darkening of the product and have a considerable effect on the chromatographic properties of some solutes.

CONCLUSIONS

This study presents chromatographic data on two types of diol phases synthesized via a hydride intermediate. One phase, similar to commercially available products, is made by bonding allyl glycidyl ether while the other contains only a hydrocarbon chain and the diol functional group. In both the reverse phase and normal phase experiments, the 7-OD (diol via 7-octene-1,2 - diol) material is clearly more hydrophobic than the AGE (diol via allyl glycidyl ether) column. The AGE column behaves similarly to those obtained via conventional organosilanization. The potential advantages of the new materials include their pH stability proven in previous studies (23,28) for the same bonding scheme, variable hydrophillicity for normal phase applications depending on whether the AGE or 7-OD phase is selected and the existence of a single product diol phase with no chance of by-products for the 7-OD material.

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