THE INFLUENCE OF THE TREATMENT OF THE SUPPORT UPON PROPERTIES OF THIN-FILM GAS CHROMATOGRAPHY COLUMN PACKINGS

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The intended function of the support in a gas chromatographic column packing is to provide the mechanical or structural elements necessary for the process; an ideal support would be one that did not influence either the qualitative or quantitative aspects of separations for all types of compounds, and a support of this kind for work above about 200° (required for steroids) has not yet been found. An acid wash is normally employed to reduce the adsorptive properties of diatomaceous earth supports, and further deactivation of the support is usually necessary if the packing is to be used for the separation of microgram quantities of steroids with hydroxyl groups. "Silanization" of supports with dichlorodimethylsilane or hexamethyldisilazane is widely practiced[1–3]; the effect of this treatment is to convert the hydrophilic surface of the support to a hydrophobic surface which will accept and retain a thin-film coating of an organic liquid phase, and at the same time alter the "active sites" to inactive ones. A silanizing procedure is particularly satisfactory when non-selective polysiloxane liquid phases are to be applied to the support; the reduction in irreversible adsorption of hydroxy- and keto-substituted steroids that occurs when a silanized support is employed has been documented by HORNING *et al.*[1].

While silanized supports may be used for the preparation of column packings containing polyester liquid phases, it is also possible to obtain highly satisfactory column packings containing polyester phases by employing a "two-coat" technique in which polyvinylpyrrolidone (PVP) is used as a first or deactivating coat, and the polyester is applied as a second coat[4]. The technique is described briefly in the Experimental section; it is not useful for the preparation of column packings with polysiloxanes of the SE-30 or QF-1 type. Its major area of usefulness, as far as present knowledge is concerned, is for work with polyester phases including NGS, NGA, CHDMS and CHDMA. The polyvinylpyrrolidone does not itself act as a liquid phase, but the properties of these packings are substantially modified with respect to both qualitative and quantitative effects, in comparison with packings prepared with silanized supports.

EXPERIMENTAL

All data were obtained with a Barber-Colman Model 10 gas chromatograph equipped with an argon ionization detection system. The columns were glass U-tubes,

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6 ft. × 4 mm I. D. Column packings were prepared according to published procedures[1,2]; silanizing was carried out with dichlorodimethylsilane in toluene[2]. A polyvinylpyrrolidone (PVP; supplied by Matheson, Coleman and Bell under the name "Polyvinylpyrrolidinone") coat was applied in methanol to the acid-washed support (Gas-Chrom P, 80–100 and 100–120 mesh) as described previously[4]. The liquid phases, applied by the slurry filtration technique[2,5], were F-60 (a methylsiloxane polymer containing a low mole per cent of *p*-chlorophenyl groups; Dow Corning Corp.), NGS (neopentyl glycol succinate polyester; Applied Science Laboratories, Inc.), and CHDMS (1,4-cyclohexane-dimethanol succinate polyester; Applied Science Laboratories, Inc.). The operating conditions were as follows: for F-60, 214°, 15 p.s.i.; for NGS, 216°, 18 p.s.i.; for CHDMS, 223°, 20 p.s.i.

RESULTS AND DISCUSSION

Since the introduction of thin-film techniques in 1960[6], it has been observed in many laboratories that relative retention times, observed with both selective and non-selective phases, are affected by the treatment of the support. If commercially available supports of the unsilanized variety are coated directly with non-selective or selective phases both qualitative and quantitative information relating to steroid separations may be without meaning because of participation of the support in the separation process[1]. Acid-washed, but unsilanized, supports can be used for the preparation of thin-film column packings; the active sites will not be entirely suppressed, however, and differences in the quantitative aspects of steroid separations can be demonstrated readily when columns of this kind are compared with those prepared from silanized supports[1]. It is also known that differences in relative retention times are observed when comparisons are made between columns prepared with acid-washed, and with acid-washed and silanized, supports. Tables I–III contain data indicating the magnitude of this effect. Saturated hydrocarbons and methyl ethers show about the same behavior under both circumstances, but with these exceptions most steroids show small to large differences in relative retention times when comparisons are made for these two types of treatment of the support. As might be expected, the smallest differences are observed for columns containing a non-selective phase (F-60). When selective phases such as NGS and CHDMS[7] are employed, substantial differences in retention times may be observed for alcohols and ketones, depending upon the treatment of the support; retention times for an acetyl derivative are affected to a lesser extent. In general, the effect of the silanizing process is to increase the apparent polarity of the polyester phase, as indicated by the increased relative retention times recorded for many of the steroids listed in Tables II and III. The relative (to cholestane) retention time for the TMSi ether of cholestanol is reduced, however, when a silanizing process is used. Fig. 1 shows the effect of the treatment of the support on the separation pattern of the TMSi ethers of four steroids (androsterone, etiocholanolone, dehydroisoandrosterone and pregnanediol). These steroids occur in the "17-ketosteroid" fraction of human urinary steroids (a Girard separation is not necessary when a gas chromatographic analysis is employed); NGS columns are frequently used for the separation of these ethers, and a silanizing process for the support should be used in order to provide the usual separation conditions[8–10].

Polyester columns prepared with PVP-coated supports give retention time

TABLE I

COMPARISON OF RELATIVE RETENTION TIMES OBSERVED AT 214° FOR 1% F-60 COLUMNS WITH ACID-WASHED, AND ACID-WASHED AND SILANIZED SUPPORT

Compound	*Relative retention times*	
	Acid-washed	*Silanized*
Cholestane	1.00[a]	1.00[b]
Coprostane	0.90	0.90
5-Cholestene	1.02	1.01
3,5-Cholestadiene	1.18	1.14
Cholestan-3β-ol	2.42	2.45
Cholestan-3α-ol	2.33	2.30
Cholesterol	2.35	2.43
Cholestan-3-one	2.75	2.65
Cholestanyl methyl ether	2.07	2.02
Cholestanyl trimethylsilyl ether	2.81	2.82
Isocholesteryl methyl ether	1.12	1.11
Cholesteryl acetate	3.68	3.52
Androstane-3,17-dione	0.52	0.48
Androstane-3β, 17β-diol	0.45	0.43
4-Androsten-17β-ol-3-one	0.69	0.62
Pregnane-3,20-dione	0.77	0.72
5α-Pregnane-3,20-dione	0.87	0.82
Pregnane-3α,20α-diol	0.73	0.70

[a] 12.8 min.
[b] 12.0 min.

TABLE II

COMPARISON OF RELATIVE RETENTION TIMES OBSERVED AT 216° FOR 1% NGS COLUMNS WITH ACID-WASHED, ACID-WASHED AND SILANIZED, AND PVP-COATED SUPPORT

Compound	*Relative retention times*			
	Acid-washed	*Silanized*	*1% PVP*	*2% PVP*
Cholestane	1.00[a]	1.00[b]	1.00[c]	1.00[d]
Coprostane	0.92	0.91	0.91	0.91
5-Cholestene	1.09	1.10	1.09	1.12
3,5-Cholestadiene	1.49	1.53	1.53	1.54
Cholestan-3β-ol	6.33	6.59	7.87	8.71
Cholestan-3α-ol	5.67	5.88	6.95	7.65
Cholesterol	6.72	7.11	8.42	9.62
Cholestan-3-one	6.73	7.16	7.22	7.36
Cholestanyl methyl ether	2.71	2.75	2.78	2.78
Cholestanyl trimethylsilyl ether	2.47	2.16	2.39	2.38
Isocholesteryl methyl ether	1.27	1.27	1.30	1.29
Cholesteryl acetate	6.23	6.37	6.32	6.25
Androstane-3,17-dione	4.75	5.43	6.08	6.85
Androstane-3β,17β-diol	4.54	5.24	9.51	13.7
4-Androsten-17β-ol-3-one	8.12	9.32	13.4	17.5
Pregnane-3,20-dione	5.88	6.70	7.27	8.04
5α-Pregnane-3,20-dione	6.42	7.37	8.04	8.86
Pregnane-3α,20α-diol	6.22	7.19	11.3	15.4

[a] 4.2 min.
[b] 4.4 min.
[c] 4.6 min.
[d] 4.2 min.

TABLE III

COMPARISON OF RELATIVE RETENTION TIMES OBSERVED AT 223° FOR 1 % CHDMS COLUMNS WITH ACID-WASHED, ACID-WASHED AND SILANIZED, AND PVP-COATED SUPPORT

Compound	*Relative retention times*			
	Acid-washed	*Silanized*	*1 % PVP*	*2 % PVP*
Cholestane	1.00[a]	1.00[b]	1.00[c]	1.00[d]
Coprostane	0.91	0.91	0.90	0.90
5-Cholestene	1.09	1.09	1.08	1.09
3,5-Cholestadiene	1.50	1.54	1.47	1.49
Cholestan-3β-ol	5.78	6.05	6.17	6.42
Cholestan-3α-ol	5.22	5.54	5.50	5.79
Cholesterol	6.16	6.42	6.64	7.10
Cholestan-3-one	5.94	6.17	5.91	6.07
Cholestanyl methyl ether	2.69	2.67	2.67	2.68
Cholestanyl trimethylsilyl ether	2.41	2.07	2.71	2.53
Isocholesteryl methyl ether	1.20	1.18	1.22	1.21
Cholesteryl acetate	5.73	5.65	5.76	5.71
Androstane-3,17-dione	3.43	3.82	3.45	3.85
Androstane-3β,17β-diol	3.65	3.97	4.66	5.72
4-Androsten-17β-ol-3-one	6.09	6.78	6.96	8.19
Pregnane-3,20-dione	4.49	5.03	4.37	4.91
5α-Pregnane-3,20-dione	4.96	5.50	4.82	5.42
Pregnane-3α,20α-diol	5.19	5.75	5.86	7.23

[a] 8.7 min. [c] 7.8 min.
[b] 7.2 min. [d] 7.3 min.

relationships that are often quite different from those observed when acid-washed and silanized supports are used. The most noticeable effect is the increase in selective retention seen for hydroxyl-substituted steroids. The effect is evident in Fig. 2, and the data in Tables II and III for both monohydroxy- and dihydroxysteroids indicate that the magnitude of the change can be increased by doubling the amount of PVP used for the initial coating of the support. It is not known if this effect is due to partial solution of PVP in the polyester phase.

A considerable decrease in tailing (due to decreased adsorption) for hydroxyl-substituted steroids may be observed when comparisons are made of PVP-treated packings with conventional columns. This was noted for cholesterol in the first description of the method[4]. The same effect has also been noted in other studies with sterols in which column packings prepared in this laboratory[11] or independently were employed. Fig. 2 of this paper shows a striking example of the effect, seen when a dihydroxysteroid (androstane-3β,17β-diol) is employed as a test substance. An estimate of the magnitude of the difference in behavior can be made from Table IV. Assuming that the hydrocarbon reference substance is not adsorbed, there is no evidence of marked adsorption (which would be reflected by a decrease in the peak area ratio with a decrease in sample size) for amounts of a trial sterol (cholestanol) down to about 1 μg.

A third effect of the mode of treatment of the support is a change in the behavior of an argon ionization detection system. This effect is evident from the data in Table IV. Columns prepared with PVP-polyester packings show a higher response ratio for the steroid when a cholestanol–tetratriacontane mixture is used for test purposes over that found for acid-washed and silanized polyester packings. A possible explanation

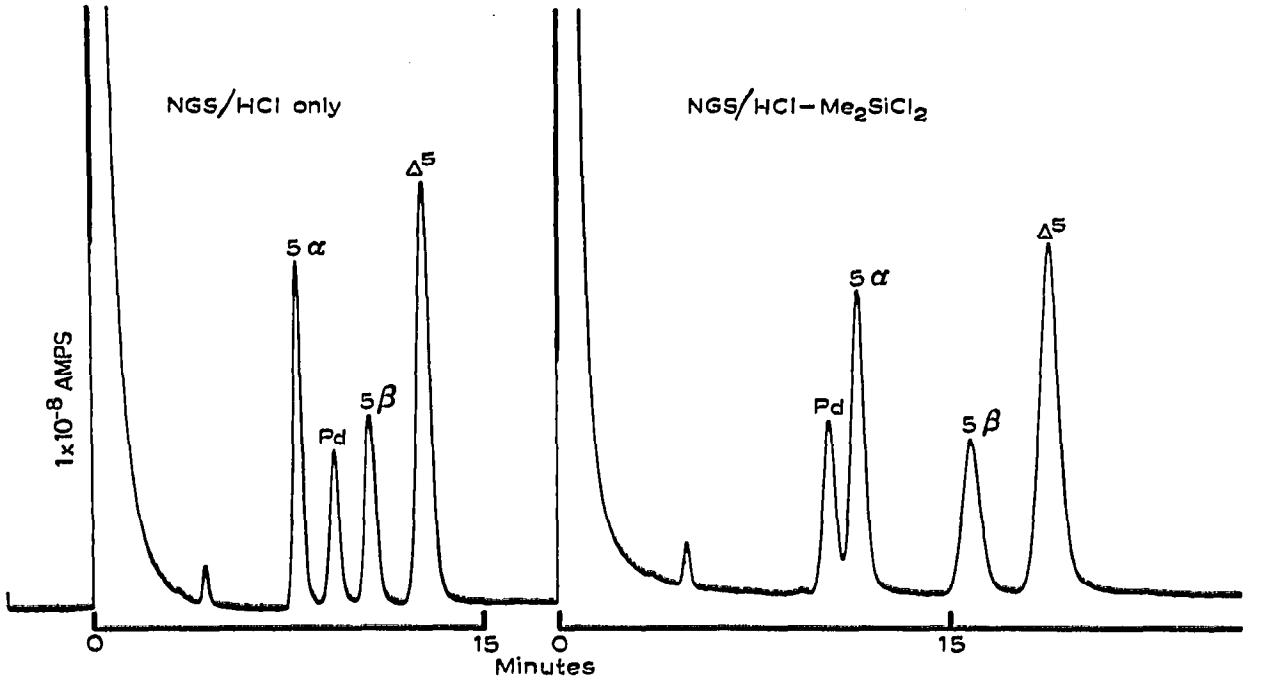

Fig. 1. Gas chromatographic separation of a mixture of the trimethylsilyl ethers of androsterone (5α), etiocholanolone (5β), dehydroisoandrosterone (Δ^5) and pregnanediol (Pd); two different types of support treatment were employed with a 1 % NGS liquid phase. For the left panel, the support was acid-washed; for the right panel, the support was acid-washed and silanized with dichlorodimethylsilane. Conditions: 208°; 15 p.s.i.

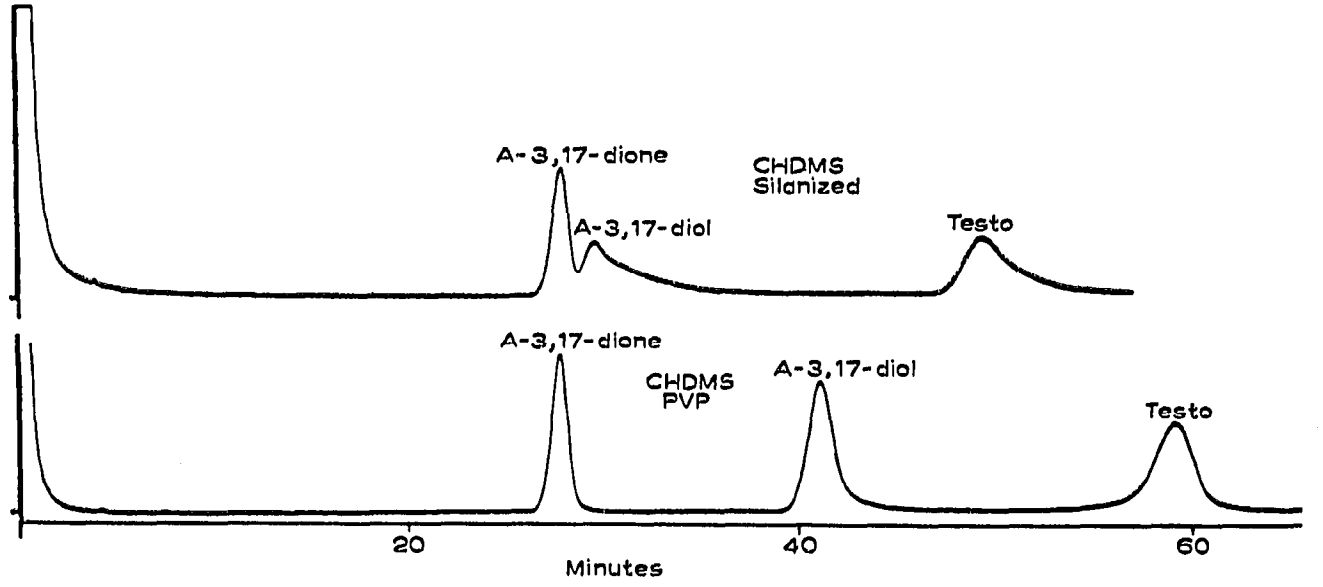

Fig. 2. Gas chromatographic separation of a mixture of androstane-3,17-dione (A-3,17-dione), androstane-3β,17β-diol (A-3,17-diol) and testosterone (4-androsten-17β-ol-3-one) (testo) with 1 % CHDMS on differently inactivated acid-washed supports (upper chromatogram, silanized; lower chromatogram, 2 % PVP). Column conditions are given in the Experimental section.

TABLE IV

AREA RATIO FOR CHOLESTANOL–TETRATRIACONTANE MIXTURE

Sample size[a]	*r % NGS (silanized)*	*r % NGS (r % PVP)*
6 μg	5.82 ± 0.03[b]	6.47 ± 0.02
1 μg	5.16 ± 0.02	6.35 ± 0.02

[a] Of cholestanol.
[b] Experimental values are mean of five determinations.

for this effect is that responses observed with an argon ionization detection system are dependent upon the nature and amount of column bleed passing through the detector, as well as upon the structure of the compounds under study[1,12].

It is therefore clear that the properties observed for gas chromatography columns may be greatly dependent upon the mode of treatment of the support. It is likely that specific uses may be found for silanized and PVP column packings in work with steroids and other types of compounds*.

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SUMMARY

The effect of a variety of procedures for the deactivation of the support surface upon the partitioning and adsorptive properties of thin-film gas chromatography column packings has been investigated. Studies with a silicone stationary phase and two polyesters coated upon acid-washed, acid-washed and silanized, and acid-washed and polyvinylpyrrolidone-treated supports have demonstrated that selectivity of retention of functional group-substituted steroids and quantitative aspects of analysis are influenced by the mode of treatment of the support.

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* The effect of PVP treatment upon the gas chromatography of alkaloids has recently been reported by BROCHMANN-HANSSEN AND FONTAN[13].