

CHROM. 18 577

## VARIABLE REACTIVITY IN THE CHEMICAL MODIFICATION OF SILICA EFFECTS OF INITIAL DEACTIVATION ON HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PERFORMANCE

DAVID B. MARSHALL\*, C. L. COLE and DAVID E. CONNOLLY

*Department of Chemistry, University of Idaho, Moscow, ID 83843 (U.S.A.)*

(First received December 16th, 1985; revised manuscript received February 18th, 1986)

---

### SUMMARY

Microporous silicas have been treated with various amounts of trimethylsilylating reagents prior to octadecylsilylation. The resulting reversed phases are more efficient than phases made without pretreatment. The results show that treatment of about 5% of the total silanols with trimethylmethoxysilane yields the most efficient phase. The effect cannot be attributed to simple dilution of octadecyl groups by trimethyl groups. Fourier transform infrared spectroscopical evidence indicates that this initial reaction occurs with a small population of associated silanols that are chemically distinct from the bulk of the hydrogen-bonded silanols observed in the infrared spectrum.

---

### INTRODUCTION

The surface of microporous silicas that are typically used as stationary support materials in reversed-phase high-performance liquid chromatography (RP-HPLC) is heterogeneous with respect to the type and distribution of silanol groups. These groups ("free", hydrogen-bound, geminal, etc.) are expected to vary in chemical reactivity and physical accessibility<sup>1</sup>. This results in a heterogeneity in the energetics of interaction of solutes with the surface, giving rise to a dispersion in the elution band (if the stationary phase contribution to band broadening is a significant factor). One reason why bonded phases have become popular is that chemical modification of this surface reduces this heterogeneity and improves the chromatographic performance of the resulting phase<sup>2</sup>. However, the bonded phase is still not a perfectly homogeneous material and a stationary phase contribution to band broadening from this heterogeneity may still be important.

Fluorescence spectroscopic measurements have indicated that the organization and distribution of surface-bound molecules on silica is in fact heterogeneous, with evidence for significant clustering of groups at much shorter inter-group distances than those expected on the basis of a model of random distribution of molecules<sup>3,4</sup>. This factor should also give rise to a dispersion in solute interaction energetics, which

will be manifested as a solute sorption-desorption kinetic contribution to the elution band width. Kinetic contributions to band broadening are expected on theoretical grounds to be significant for energetically heterogeneous stationary phases<sup>5</sup>. Chromatographic experiments<sup>6</sup> and direct kinetic measurements<sup>7</sup> have indicated that the kinetic contribution to band broadening is indeed an important factor under modern RP-HPLC "regular bore" conditions.

In an earlier report<sup>8</sup>, the synthesis of octadecyl stationary phases of higher efficiency by initial partial deactivation of the silica surface was described. Initial treatment of silica with small amounts of trimethylsilylating agents, followed by exhaustive octadecylsilylation, yielded phases with improved efficiency relative to octadecyl phases made without such pretreatment. This improvement was attributed to initial reaction of the trimethylsilylating agent with clustered silanols. Partial deactivation of these clusters would then provide a surface with a more homogeneous distribution of surface silanols, leading to a more homogeneous distribution of octadecyl groups and a more efficient stationary phase. Uncertainty remained concerning the chemical nature of the silanol groups involved in this initial reaction, the levels of initial deactivation that yield the best resulting phase, and whether the effect noted is a true initial reaction effect as opposed to a simple octadecyl dilution effect. The work described here represents further efforts to resolve these questions.

## EXPERIMENTAL

### *Materials*

Dimethyloctadecylchlorosilane (DMODCS), trimethylchlorosilane (TMCS) and trimethylmethoxysilane (TMMS) were used as received (Petrarch Systems, Bristol, PA, U.S.A.). Pyridine (Aldrich) was fractionally distilled and stored over barium oxide. Reagent-grade toluene was distilled and the water azeotrope discarded. The remainder was distilled from calcium hydride and stored over sodium metal. All other solvents used were HPLC-grade and were degassed before use. The silicas used were 10  $\mu\text{m}$  particle diameter Lichrosorb Si 100 and Si 60 from EM Reagents, with surface areas of 300  $\text{m}^2/\text{g}$  and 500  $\text{m}^2/\text{g}$ , and mean pore diameters of 10 nm and 6 nm, respectively. The silica was kept in an oven at 130°C for at least one week prior to use. The silicas were then evacuated to millitorr pressure in a dessicator heated to 60°C for at least 24 h, then transferred under dry nitrogen to reaction vessels or spectroscopic cells.

### *Synthesis of bonded phases*

All reactions were performed under dry nitrogen, using glassware that had been previously treated with TMCS, oven-dried at 130°C, and assembled under dry nitrogen. For the pretreated octadecyl phases, the appropriate silica was reacted with a small amount of TMMS or TMCS before exhaustive octadecylation with DMODCS and final end-capping with TMCS. This procedure is described in greater detail elsewhere<sup>8</sup>. "Regular" octadecyl phases were made by reaction of silica with DMODCS followed by end-capping with TMCS. An additional phase was synthesized by reacting a mixture of DMODCS-TMCS (95:5, mol/mol) with Si 60 silica, followed by end-capping with TMCS, to make a "5% dilution" octadecyl phase (see below). All octadecylsilylations were carried out by reaction of silica with DMODS

in refluxing toluene and pyridine for 24 h. All final end-capping reactions were carried out by reaction of the octadecyl silica with TMCS in toluene and pyridine at 50°C, with stirring, for at least 3 h. The phases were washed with dichloromethane, acetone, methanol, methanol-water (50:50) and methanol, then Soxhlet extracted overnight with methanol. All phases were packed into 25 cm × 3.2 mm I.D. stainless-steel columns, using the downward slurry method with isopropanol as the slurry solvent and methanol followed by methanol-water (50:50) as the driving solvents.

#### *Chromatographic measurements*

Chromatographic data were obtained with an HPLC system consisting of Beckman 112 pumps, Altex injector (5  $\mu$ l loop size), a Kratos 520 UV-VIS variable-wavelength detector, and a strip chart recorder. All data were obtained with solvents and phases thermostated ( $\pm$  0.05°C) in a water bath at various temperatures. Peaks were recorded and analyzed as exponentially modified Gaussians according to the procedures recommended by Foley and Dorsey<sup>9</sup>. Plate numbers in this procedure are based on the definition of  $N$  as  $= t_R^2/m_2$ , where  $t_R$  is retention time and  $m_2$  the second statistical moment, equal to  $\sigma^2 + \tau^2$ , where  $\sigma$  and  $\tau$  are the peak width and peak tailing parameters of an exponentially modified Gaussian. Thus, unlike the formulas used more typically to calculate  $n$  (based on an assumption of a purely Gaussian peak shape), the plate numbers reported here are a measure of *both* peak broadening and tailing effects. The numbers are therefore lower than those that are typically reported for these types of materials, but are a more realistic representation of column performance<sup>10</sup>. All plate numbers represent the average of at least three injections for each of the test solutes.

#### *Fourier transform infrared using diffuse reflectance (DRIFT) measurements*

DRIFT spectra in the mid-IR region were obtained with a Digilab FTS-80 FT-IR spectrophotometer with dry air purge, using the Barnes Analytical diffuse reflectance accessory and a narrow-band MCT detector. The Barnes "blocker" device was used to eliminate any specular reflectance component present in the spectra. Data were obtained as absorbance spectra with anhydrous potassium bromide powder as a reference. Care was taken to obtain reproducibly level sample cup loadings. All spectra were acquired as the average of at least 256 interferograms, collected at 16  $\text{cm}^{-1}$  resolution. (Comparison with spectra collected at 2  $\text{cm}^{-1}$  resolution showed no discernable differences in peak width in the 4000–2500  $\text{cm}^{-1}$  region.)

## RESULTS AND DISCUSSION

#### *TMCS-treated series*

The reactivity of different trimethylsilylating reagents with microporous silica varies. Treatment of silica with different reagents under similar conditions of solvent and temperature can give wide variations in the extent of surface coverage<sup>11</sup>, and use of different reagents for "end-capping" can have significant effects on the chromatographic performance of the final octadecyl phase<sup>12–14</sup>. Under the reaction conditions used here, trimethylmethoxysilane (TMMS) is sluggishly reactive compared to trimethylchlorosilane (TMCS)<sup>11</sup>. Treatment of aliquots of Si 100 silica with varying amounts of TMCS, followed by exhaustive octadecylsilylation and then end-capping,

TABLE I

PLATE NUMBERS FOR TEST SOLUTES ON THE TMCS-DEACTIVATED, Si 100 OCTADECYL SILICA SERIES PERCENT TM/ODS/TM

Mobile phase: methanol-water (70:30, v/v). Flow-rate: 0.5 ml/min. Temperature: 25.00  $\pm$  0.05°C. Precision limits (90%) range from 5-90.

Solute	Initial deactivation (%)			
	0	5	10	15
Aniline	1790	1900	975	1020
Benzene	3130	2360	1950	1690
Anthracene	3080	2250	2240	1650

yielded a series of pretreated octadecyl silicas. These silicas are labeled 5, 10 and 15% TM/ODS/TM to denote the various amounts of pretreatment. The octadecyl silica synthesized without any pretreatment is denoted "0% TM/ODS/TM". (The reported pretreatment percentages for this and all subsequent series represent a percentage of total surface silanols, calculated on the basis of the silica surface area and an assumption of seven silanols per 100 Å<sup>2</sup>. Given the uncertainties in these figures, the percentages should be interpreted as relative levels of surface initial deactivation, rather than absolute numbers.)

Study of the chromatographic efficiency of this series of phases using a variety of test solutes showed a general degradation in efficiency with increasing levels of initial surface pre-treatment. Representative plate numbers, calculated by the method of Foley and Dorsey<sup>9</sup> for an exponentially modified Gaussian peak (see Experimental), are given in Table I. Flow rate-dependence studies and Van Deemter plots confirmed that the differences were due to stationary phase contributions rather than *e.g.* packing efficiency differences. The syntheses were repeated several times with both Si 100 and Si 60 silicas with similar results. If the initial trimethylsilylation reaction with the surface is carried out with a highly reactive reagent, there should

TABLE II

CARBON COVERAGES BY ELEMENTAL ANALYSIS AND DRIFT BAND RATIO (3742/2858 cm<sup>-1</sup>), FOR TMMS PRETREATED ONLY (PERCENT TM) AND PRETREATED, OCTADECYL SILICAS (PERCENT TM/ODS/TM)

Material	%C	Band ratio
2.5% TM	0.47 $\pm$ 0.15	0.067
5% TM	0.47 $\pm$ 0.16	0.094
7.5% TM	0.51 $\pm$ 0.43	0.109
10% TM	0.16 $\pm$ 0.5	0.130
0% TM/ODS/TM	17.13 $\pm$ 2	—
2.5% TM/ODS/TM	19.75 $\pm$ 2	—
5% TM/ODS/TM	18.91 $\pm$ 2	—
7.5% TM/ODS/TM	14.17 $\pm$ 2	—
10% TM/ODS/TM	15.78 $\pm$ 2	—
5% TM/ODS dilution	17.13 $\pm$ 2	—

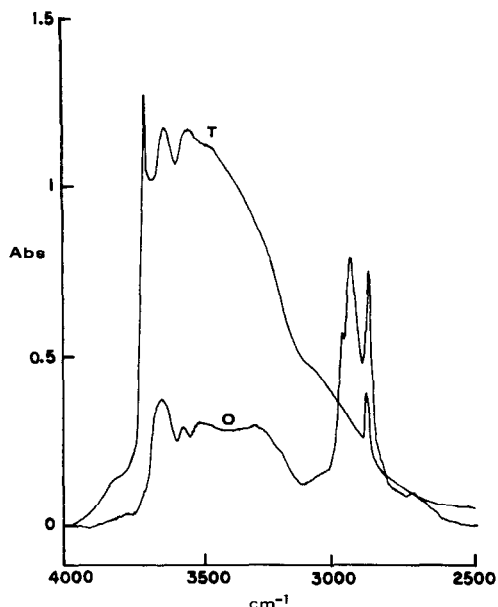


Fig. 1. DRIFT spectra of 5% TM only (T) (partially trimethylsilylated silica) and 5% TM/ODS/TM (O) (trimethyl and octadecyl silica).

be less selectivity of the reagent for the various silanol types. The resulting partially deactivated surface, upon octadecylsilylation, would not yield a surface with a significantly different group distribution. Treatment with a less reactive reagent, in contrast, should selectively remove the more reactive silanols.

#### TMMS-treated series

Aliquots of the Si 60 silica were treated with varying amounts of trimethylmethoxysilane (TMMS) before exhaustive octadecylation and end-capping with

TABLE III

#### CAPACITY FACTORS FOR TEST SOLUTES ON THE TMMS PRETREATED OCTADECYL PHASES

Mobile phase: methanol-water (70:30, v/v). Flow-rate: 0.50 ml/min. Temperature:  $25.00 \pm 0.05^\circ\text{C}$ . Sodium nitrate used to determine  $t_0$ . 3-P-1-P = 3-Phenyl-1-propanol.

Solute	Initial deactivation (%)					
	0	2.5	5	7.5	10	5 dlh.
Phenol	0.98	0.90	0.98	0.94	0.83	0.76
Aniline	0.97	0.77	0.83	0.88	0.73	0.70
3-P-1-P	1.98	1.81	1.95	1.88	1.63	1.58
Benzene	3.51	3.65	3.68	2.72	2.69	3.02
Naphthalene	9.55	10.53	10.58	6.88	6.77	8.24
Anthracene	31.58	35.11	37.25	20.60	20.52	27.25

TMCS. Small amounts of silica from each pretreatment were set aside before octadecylsilylation for analysis (the percent TM materials). Percent carbon data are presented in Table II. These values for the pretreatment only (low carbon) materials, are considered unreliable: upon re-analysis, qualitatively different results were often obtained, with *e.g.* the 2.5% TM material showing first less then more carbon than the 5% material. For this reason, DRIFT spectra were acquired. Representative spectra of exhaustively octadecylated and partially trimethylsilylated materials are shown in Fig. 1. The bands at 2963, 2932 and 2855  $\text{cm}^{-1}$  are due to the hydrocarbon moieties. The sharp band at 3742  $\text{cm}^{-1}$  has been attributed to unassociated or geminal (non-hydrogen-bonded) silanols<sup>15,16</sup>. This band nearly disappears upon complete surface derivatization. The broader bands at 3665–3673, 3580–3588 and 3450–3520  $\text{cm}^{-1}$  are similar to bands reported for various forms of hydrogen bond-associated silanols<sup>15,16</sup>. The ratio of the 3742 band to the 2858 band reported in Table II provides a measure of the extent of surface reaction for the initial step, and shows that percent carbon increases in the order  $0 < 2.5 < 5 < 7.5 < 10\%$  TM only. As the extent of coverage by trimethyl groups increase, the *total* percent carbon for the octadecyl materials should decrease, as silanols that could be reacted with octadecyl groups are deactivated by trimethyl groups instead. This trend is also seen in the DRIFT data, with the C–H band intensities decreasing in the order  $0 > 2.5 > 5 > 7.5 > 10\%$  TM/ODS/TM. Additional features of these spectra are discussed below.

Despite the *decrease* in percent carbon for the TM/ODS/TM materials with increasing initial trimethylsilylation, retention of test solutes in the RP-HPLC experiment for the non-polar solutes first increases then decreases, as shown in Table III. Retention of the more polar solutes is less regular but shows a general decreasing trend with increasing deactivation (decreasing percent carbon). Plate numbers were measured for these test solutes as a function of flow rate. Reduced plate heights were calculated as  $h = L/N \cdot d_p$ , where  $N$  is the plate number,  $L$  the column length (25.0 cm) and  $d_p$  the particle diameter (0.0010 cm). Reduced velocities were calculated as  $v = u(d_p/D_m)$ , where  $u$  is the linear velocity of the mobile phase and  $D_m$  the diffusion coefficient of the solute in the mobile phase, taken here as an average value of  $7 \cdot 10^{-6} \text{ cm}^2/\text{s}$ . (ref. 17). The data were fit, using a non-linear least squares computer program,

TABLE IV  
VALUES OF THE  $C$  TERM FOR PERCENT TM/ODS/TM PHASES

Mobile phase: methanol–water (70:30, v/v). Flow-rates: 0.5–1.5 ml/min. Temperature:  $25.00 \pm 0.05^\circ\text{C}$ . 95% Precision limits.

Deactivation (%)	Solute			
	3-P-1-P	Benzene	Naphthalene	Anthracene
0	0.190 $\pm$ 0.020	0.123 $\pm$ 0.008	0.125 $\pm$ 0.008	0.135 $\pm$ 0.004
2.5	0.163 $\pm$ 0.004	0.095 $\pm$ 0.006	0.065 $\pm$ 0.011	0.230 $\pm$ 0.030
5.0	0.144 $\pm$ 0.002	0.084 $\pm$ 0.004	0.067 $\pm$ 0.006	—
7.5	0.170 $\pm$ 0.090	0.105 $\pm$ 0.045	0.083 $\pm$ 0.007	0.078 $\pm$ 0.002
10.0	0.180 $\pm$ 0.020	0.098 $\pm$ 0.017	0.085 $\pm$ 0.014	0.085 $\pm$ 0.016
5 dln.	0.580 $\pm$ 0.020	0.310 $\pm$ 0.010	0.210 $\pm$ 0.020	0.180 $\pm$ 0.030

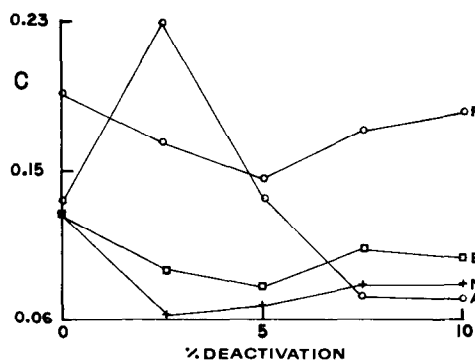


Fig. 2. Plot of the C term versus percent initial deactivation for the percent TM/ODS/TM phases. P = 3-Phenyl-1-propanol; B = benzene; N = naphthalene; A = anthracene.

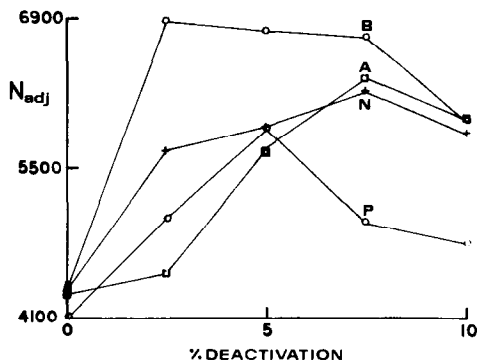


Fig. 3. Plot of adjusted plate number versus percent initial deactivation for the percent TM/ODS/TM phases. For key to abbreviations see Fig. 2.

to the Knox equation<sup>17</sup>:  $h = Av^{1/3} + B/v + Cv$ . Hawkes<sup>18</sup> has advocated the use of a simpler form:  $h = B/v + Cv$ , arguing that this is the only experimentally and theoretically justifiable form. In both forms, stationary phase contributions to the overall value of  $h$  are included in the  $C$  term. It is not the purpose of this work to contribute to this argument: many more experimental data are needed for a proper evaluation. However, fits of these two equations to the data of this work showed that in no case was the fit significantly improved (as measured by the variance of the fit and the standard deviations in the parameter values) by including the extra  $Av^{1/3}$  term, and in many cases inclusion of this term gave spuriously negative values of  $C$ . On the other hand, data for this work were taken for only four flow-rates, and over a limited range that in some cases did not include the increase in  $h$  at very low flow-rates. The values of the  $C$  term obtained in this way do in any case provide a measure of the stationary phase contribution to efficiency, and are shown in Table

TABLE V

ADJUSTED PLATE NUMBERS FOR TEST SOLUTES ON PERCENT TM/ODS/TM PHASES

Mobile phase: methanol-water (70:30, v/v). Flow-rate: 0.50 ml/min. Temperature: 25.00 ± 0.05°C. Precision limits (95%) range from 20 to 100.

Solute	Initial deactivation (%)					5 dlh.
	0	2.5	5	7.5	10	
Phenol	1855	3570	5520	3700	3220	2000
Aniline	1530	*	*	3400	2360	2100
3-P-1-P	1880	3570	5075	3500	3130	1440
Benzene	2400	6890	6730	6600	5200	1870
Naphthalene	2370	4720	5130	5700	4970	1620
Anthracene	2305	2680	4770	5930	5220	1790

\* Values of  $m_2$  were too close to  $m_2$  for sodium nitrate: spuriously high or negative values of  $N_{adj}$  were obtained.

IV for test solutes. The results for very weakly retained solutes (phenol and aniline) gave statistically unreliable results. Data for the other solutes are plotted in Fig. 2 and show, with the exception of anthracene for the 2.5% TM/ODS/TM phase, a general decrease in the stationary phase contribution to band broadening with increasing initial trimethylsilylation up to levels of 5–7.5%, followed by an increase at 7.5–10%. The efficiency of this series thus passes through a maximum at about 5% initial deactivation levels.

Another picture of the differences in the stationary phase contributions to overall efficiency may be obtained by subtracting the effects due to packing and instrumental contributions<sup>19</sup>. If the second moment  $m_2$  of an unretained solute (taken as sodium nitrate in this study) is obtained as the moment of an exponentially-modified Gaussian (as outlined above), then this moment represents the band broadening contribution of extra-phase factors. Assuming the phase contribution to be statistically independent, this unretained solute moment can be subtracted from the second moment of each retained solute band. The plate number that is then calculated from this adjusted moment ( $N = t_r^2/m_2$ ) represents a plate number due to the phase contribution alone. This method does of course assume that the diffusional behavior of sodium nitrate in the mobile phase is identical to that of the other test solutes. While this assumption is not strictly correct, the method should provide a reasonable "first-order" correction to the plate numbers. Values for adjusted plate numbers ( $N_{adj}$ ) obtained in this way are reported in Table V. Plots of  $N_{adj}$  versus percent deactivation are shown in Fig. 3. Again, a maximum in efficiency near 5–7.5% deactivation is indicated, for anthracene as well as the other solutes. This suggests that the  $C$  term results for anthracene on the 2.5% TM/ODS/TM phase (Fig. 2) are in error and should be disregarded.

#### *Dilution study*

It has been suggested<sup>20</sup> that the improvements noted here are due not to any change in the local ordering of octadecyl groups, induced by partial deactivation of clustered silanols, but are instead due to a simple dilution effect of the octadecyl chains by trimethyl groups. The increase in retention and efficiency noted here could also be attributed to a greater surface area of interaction of solutes with the more widely spaced chains. To test this possibility, a mixed reagent of DMODCS–TMCS (95:5, mol/mol) was reacted with Si 60 silica and then end-capped with TMCS. The DRIFT spectrum was nearly identical to that of the 5% TM/ODS/TM material. The chromatographic performance of the resulting phase was then tested. The results for  $k'$ , the  $C$  term and  $N_{adj}$  are shown in the last columns of Tables III–V, respectively. The performance of this material, containing 17.13% carbon (Table II), is seen to be comparable or slightly worse than that of the 0% (no initial deactivation) phase. This result indicates that a simple dilution effect, although perhaps important at higher dilution levels, is not adequate to explain the data reported here.

#### *Temperature-dependent studies*

If the initial deactivation step yields a more homogeneous surface, then the dispersion in the enthalpy of interaction should be less. Plots of  $\ln k'$  versus  $1/T$  should be linear if a single interaction mechanism is operating on the surface<sup>21</sup>. From the Van 't Hoff relationship  $\ln k' = -\Delta H/RT + \ln \phi$ , where  $\phi$  is the phase ratio,



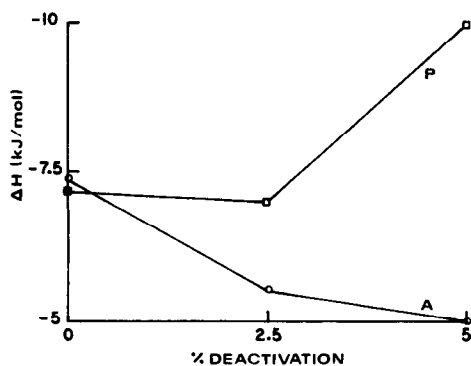
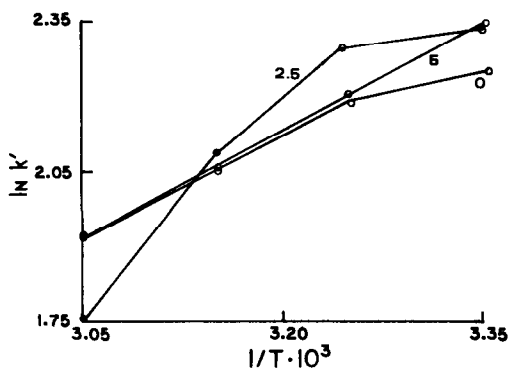


Fig. 4. Van 't Hoff plot of  $\ln k'$  versus  $1/T$  for naphthalene on the 0, 2.5 and 5% TM/ODS/TM phases. Temperatures, 25, 35, 45 and  $55 \pm 0.05^\circ\text{C}$ .

Fig. 5. Enthalpy values for phenol (P) and aniline (A) on the 0, 2.5 and 5% TM/ODS/TM phases.

plots of  $\ln k'$  versus  $1/T$  should yield a straight line of slope  $-\Delta H/R$ , assuming a constant phase ratio. Temperature-dependence studies of  $\ln k'$  for the 0, 2.5 and 5% TM/ODS/TM phases were conducted. A representative Van 't Hoff plot is shown in Fig. 4. Plots for all other test solutes gave qualitatively similar results. The results do show an improvement in linearity in going from 0 to 5% deactivation, but the results for the 2.5% TM/ODS/TM material are worse than either of these two. The reasons for this are presently unclear, but a general trend to a more energetically homogeneous surface with initial deactivation is at the least not counter-indicated by these data. Enthalpy data for all solutes is given in Table VI. The values are consistent with those expected for this weakly interacting RP-HPLC system, where enthalpies of 2–3 kcal/mol (8–12 kJ/mol) are typically observed<sup>21</sup>. A plot of the enthalpy values for phenol and aniline is shown in Fig. 5. These two solutes have similar retention, but are expected to interact with the stationary phase surface in different ways. Phenol should interact primarily with the hydrocarbonaceous mass, while aniline as a weak base should also interact with residual silanols. The decrease in sorption enthalpy for

TABLE VI

ENTHALPY VALUES (kJ/mol) OF TEST SOLUTES FOR THE 0, 2.5 AND 5% TM/ODS/TM PHASES

Precision Limits of 95%.

Solute	Initial deactivation (%)		
	0	2.5	5
Phenol	$-7.32 \pm 0.96$	$-7.14 \pm 0.76$	$-10.35 \pm 2.93$
Aniline	$-7.53 \pm 0.95$	$-5.55 \pm 1.89$	$-4.97 \pm 0.50$
3-P-1-P	$-8.00 \pm 0.80$	$-12.20 \pm 3.00$	$-8.76 \pm 0.30$
Benzene	$-6.40 \pm 1.10$	$-10.97 \pm 3.36$	$-7.87 \pm 0.29$
Naphthalene	$-9.55 \pm 1.26$	$-16.66 \pm 4.12$	$-12.18 \pm 0.24$
Anthracene	$-14.15 \pm 1.91$	$-22.21 \pm 5.60$	$-18.43 \pm 0.50$

TABLE VII

DRIFT ABSORBANCE BAND POSITIONS FOR BARE Si 60 SILICA, TM ONLY AND TM/ODS/TM MATERIALS

Only the largest C-H band (2900  $\text{cm}^{-1}$  region) is listed. Uncertainty in band positions  $\approx 1 \text{ cm}^{-1}$ .

Material	Band positions ( $\text{cm}^{-1}$ )			
	3742	3665	3580	—
Si 60 Silica	3742	3665	3580	—
2.5% TM	3742	3657	3580	2858
5% TM	3742	3665	3580	2858
7.5% TM	3742	3657	3580	2855
10% TM	3742	3670	3581	2858
0% TM/ODS/TM	—	3672	3588	2924
2.5% TM/ODS/TM	—	3672	3588	2924
5% TM/ODS/TM	—	3672	3588	2924
7.5% TM/ODS/TM	—	3672	3588	2924
10% TM/ODS/TM	—	3672	3588	2924
5% dilution	—	3672	3580	2924

aniline with increasing percent deactivation indicates a reduction in the number of accessible silanols with increasing initial deactivation.

#### DRIFT spectra

FT-IR diffuse reflectance spectra were obtained for all materials, as described above. Several features of these spectra may be noted: upon exhaustive octadecylation, the band at 3742  $\text{cm}^{-1}$  disappears, indicating that *most* of the reaction with the silica surface occurs with unassociated or geminal silanols (the IR spectrum cannot be used to distinguish between these two possibilities: all that is indicated is that the reacting groups are not hydrogen bonded, as inferred from the sharpness of the band<sup>16</sup>). This result has been noted by others for these types of reactions<sup>15,16</sup>. However, the results for the *initial* stages of reaction with the surface, as represented by the FT-IR spectra for the 2.5, 5 and 7.5% TM only materials are not as clear. Band positions for these materials and for the bare Si 60 silica precursor are reported in Table VII. Band absorbance ratios are reported in Table VIII. The ratios do not show a monotonic decrease in unassociated silanols relative to associated silanols,

TABLE VIII

DRIFT ABSORBANCE BAND RATIOS FOR THE PERCENT TM ONLY MATERIALS AND FOR BARE Si 60 SILICA

% TM	Absorbance band ratio					
	3742/3665	3665/3665	3580/3665	3742/3742	3665/3742	3580/3742
0	1.227	1	0.922	1	0.815	0.740
2.5	1.044	1	0.961	1	0.958	0.921
5	1.105	1	1.103	1	0.905	0.902
7.5	0.802	1	1.034	1	1.247	1.289
10	1.035	1	0.964	1	0.966	0.932

but show more complicated changes that are more consistent with the interpretation that both types of silanols undergo significant reaction in the early stages of surface derivatization. However, since upon complete reaction the unassociated band has essentially disappeared while a substantial hydrogen-bound band remains, this initial reaction must proceed with some chemically and/or physically distinct subset of the hydrogen-bound silanols. The remaining hydrogen-bound silanols may be spectroscopically accessible but not physically accessible to silylating reagent, or associated in a different way that reduces their reactivity. Reaction with the highly reactive trimethylsilylating reagent trimethylsilylphosphine was noted to provide levels of silanol reaction beyond those obtainable with chlorosilanes<sup>14</sup>. This indicates that some of these remaining associated silanols are still physically accessible, provided a reagent of sufficiently high reactivity is used.

Hair and Hertl<sup>22</sup> have noted an initial rapid reaction (representing about 15% of the total reaction) in the gas phase of chlorosilanes with pressed disks of non-porous silicas. This reaction is attributed to geminal silanols with a band at 3751  $\text{cm}^{-1}$ . However, this reaction was found to be a direct replacement of the OH group by Cl, and was found *not* to occur with methoxysilanes (used for the initial reactions in this work). Extrapolation from gas-phase to solution-phase reactions is difficult, but it is highly unlikely that the initial reactions observed here are also with geminal groups. The broadness of the bands involved indicate some type of hydrogen bond association. An *ab initio* calculation of silanol model groups has shown that silanols with the highest absolute acidity are those that are associated with a Lewis acid<sup>23</sup>. Thus, this distinct population of associated silanols that seems responsible for the earliest stages of reaction may be those associated in this way, perhaps with Lewis acid impurities or in association with the siloxane matrix. Silanols thus associated would naturally occur in clusters rather than as isolated groups. These points are under further investigation, to elucidate how much and in what order various silanol populations undergo reaction, under solution reaction conditions such as those typically used in HPLC bonded-phase synthesis. The advantage of DRIFT studies is of course the ability to study the reaction products without resorting to pressed disks, where the local heating may anneal the surface and distort the silanol distributions of the silica.

## CONCLUSIONS

The spectroscopic and chromatographic results presented here are consistent with the earlier model of the effects of initial trimethylsilylation on octadecyl stationary phases<sup>8</sup>. The silica surface is viewed as heterogeneous with respect to the distribution of silanols, as fluorescence studies have shown<sup>3,4</sup>. Although the majority of surface reaction occurs with unassociated silanols, and there remains a significant population of associated silanols after complete reaction<sup>16</sup>, the earliest stage of reaction appears to also occur with a chemically distinct group of associated silanols of high reactivity. These silanols most likely occur in clusters on the surface. Initial deactivation of these clusters yields a surface with a more homogeneous distribution of remaining silanols. Subsequent reaction to attach octadecyl groups yields a reversed phase with a more homogeneous distribution of bound groups, with fewer solute-accessible residual silanols. This yields a phase with higher chromatographic efficiency.

## ACKNOWLEDGEMENTS

Grateful acknowledgement is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and to NSF (Grant No. CHE 83-06881) for financial support of this research. Thanks are due to J. J. Kirkland for supplying preprints and giving permission to quote work in press.

## REFERENCES

- 1 K. K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979, Chs. 1 and 3.
- 2 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
- 3 C. H. Lochmüller, A. S. Colborn, M. L. Hunnicutt and J. M. Harris, *Anal. Chem.*, 55 (1983) 1344.
- 4 C. H. Lochmüller, A. S. Colborn, M. L. Hunnicutt and J. M. Harris, *J. Am. Chem. Soc.*, 106 (1984) 4077.
- 5 J. C. Giddings, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965, section 6.5.
- 6 Cs. Horváth and H.-J. Lin, *J. Chromatogr.*, 149 (1978) 43.
- 7 D. B. Marshall, J. W. Burns and D. E. Connolly, *J. Chromatogr.*, 360 (1986) 13.
- 8 D. B. Marshall, K. A. Stutler and C. H. Lochmüller, *J. Chromatogr. Sci.*, 22 (1984) 217.
- 9 J. Foley and J. W. Dorsey, *Anal. Chem.*, 55 (1983) 730.
- 10 B. A. Bidlingmeyer and F. V. Warren, Jr., *Anal. Chem.*, 56 (1984) 1583A.
- 11 D. B. Marshall and D. E. Connolly, *Anal. Lett.*, 18 (A8) (1985) 995.
- 12 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549.
- 13 C. H. Lochmüller and D. B. Marshall, *Anal. Chim. Acta*, 142 (1982) 63.
- 14 D. B. Marshall, C. L. Cole and A. D. Norman, *J. Chromatogr. Sci.*, in press.
- 15 M. L. Hair, in A. T. Bell and M. L. Hair (Editors), *Vibrational Spectroscopies for Adsorbed Species*, American Chemical Society, Washington, DC, 1980.
- 16 J. Kohler, D. B. Chase, R. D. Farlee, A. J. Vega and J. J. Kirkland, *J. Chromatogr.*, in press.
- 17 J. H. Knox, in C. F. Simpson (Editor), *Techniques in Liquid Chromatography*, Wiley-Heyden, Chichester, 1982, Ch. 2.
- 18 S. J. Hawkes, *J. Chem. Educ.*, 60 (1983) 393.
- 19 R. W. Stout, J. J. DeStefano and L. R. Snyder, *J. Chromatogr.*, 261 (1983) 189.
- 20 B. Feibusch, personal communication.
- 21 W. R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography: Advances and Perspectives*, Vol. 2, Academic Press, New York, 1980, pp. 192-200.
- 22 M. L. Hair and W. Hertl, *J. Phys. Chem.*, 73 (1969) 2372.
- 23 D. Heidrich, D. Volkmann and B. Zurawski, *Chem. Phys. Lett.*, 80 (1981) 60.