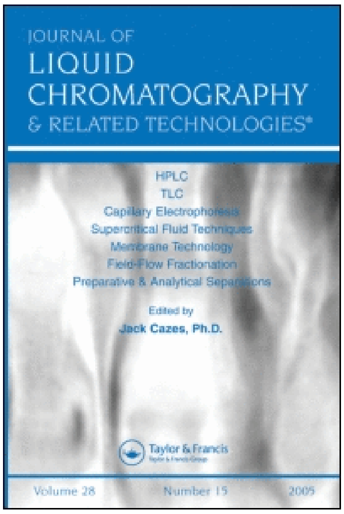

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PREPARATION AND CHROMATOGRAPHIC PROPERTIES OF SOME CHEMICALLY BONDED PHASES FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

Four different monomeric chemically bonded phases suitable for reversed-phase liquid chromatography have been prepared. Duplicate preparations, elemental analysis, post-treatment with TMS and chromatographic properties all indicate that maximum coverage has been obtained. The chromatographic activity of the silica surface is discussed on the basis of TMS-phases with various surface coverages. It is observed that logarithmic retention data for homologous series and different RP-phases —partially taken from the literature— form a set of straight lines with a common intersection point.

INTRODUCTION

The growing interest in chemically bonded phases for liquid chromatography is demonstrated by the publication of one monograph [1] and numerous reviews [2 - 9] in the past five years. Most attention has been devoted to the preparation of phases suitable for reversed-phase chromatography by bonding non-polar molecules to an amorphous silica support. In comparison with the Si-O-C esterbond and the Si-N aminebond the Si-C bond is much more stable. It is obtained by a Grignard reaction, but more frequently through a Si-O-Si-C linkage using chlorosilanes or alkoxysilanes.

The major objective in preparing RP-phases is the modification of as many surface hydroxyl groups as possible, because it is well known that remaining silanol groups contribute to the chromatographic retention [2, 10 - 18] even in the case of long-chain bonded phases [12]. For this reason di- or trichloro and di- or trialkoxysilanes have been favoured over monochloro and monoalkoxysilanes because of their greater reactivity and their possibility to react with two or three hydroxyl groups simultaneously [19, 20]. However, this latter point is debatable, because non-reacted chlorine or alkoxygroups will be quickly converted to hydroxyl groups when treated with water, e.g. from the mobile phase. Consequently, when using a trichloro or trialkoxysilane the number of hydroxyl groups will only be reduced if the average number of reacting groups is larger than 1.5. For steric reasons a simultaneous reaction of a trichlorosilane with three surface hydroxyl groups is highly improbable, but Karch et al. [10] and Hemetsberger et al. [11, 12] all suggest that di- and trichlorosilanes show a bifunctional reaction with the surface silanols. However, Davydov et al. [21] find a monofunctional reaction of dichlorosilanes more probable. In an extensive review of the literature Unger et al. [22] put this figure between 1 and 2.

We agree with Unger that the surface coverage should be expressed as the number of silane molecules attached to the surface [22] and not as the number of alkylgroups as done by Karch [10, 23]. Not only have Karch's data been misinterpreted in the literature [1], but also the conversion from alkylgroups to silane molecules is not straightforward when the chlorosilanes possess two different alkylgroups. For example, in the case of dimethylchlorosilane the carbon content of 3.13% leads to a surface coverage with 4.35 μmol/m^2 of silane molecules, corresponding to Karch's figure of 8.7 μmol/m^2 methylgroups. However, in the case of dichloromethyldecylsilane the carbon content of 14.8% leads to 3.2 μmol/m^2 of silane molecules and hence also to 3.2 μmol/m^2 of decylgroups instead of 3.5 μmol/m^2 as stated by Karch.

Surface coverage data are obtained from carbon percentages derived from elemental analysis of the chemically bonded phase. However, when di- or trialkoxysilanes are used this conversion is by no means straightforward. Obviously, we must account for the increase in weight of the silica support. In addition, we must be sure that the reactions have been carried out under completely dry conditions to avoid crosslinking polymerization. The major problem, however, is the unknown contribution of unreacted alkoxy groups.

A case in point is provided by Engelhardt and Mathes [24], who use γ-aminopropyltriethoxysilane. From the carbon percentage of 6.28 % the authors' claim of 3 silane molecules per nm^2 can only be derived, if we assume that all three ethoxy groups have reacted with surface hydroxyl groups. This is not only improbable for steric reasons, but it would also require that the original silica has 9 hydroxyl groups per nm^2, which is much larger than the usually accepted number of 4.8 per nm^2 [25 - 29]. For the same reason the simultaneous reaction of two ethoxygroups is improbable since this requires 6 surface hydroxylgroups per nm^2. Now it may be true that non reacted ethoxygroups have been converted to hydroxylgroups (through washing with methanol/water), but even then the stated number of 3 γ-aminopropylsilane brushes per nm^2 requires that 6 bulky ethoxygroups can be positioned at each nm^2 of the silica surface during the reaction when one ethoxygroup reacts with the silica surface. In view of the size of the ethoxygroup we consider this impossible.

Much more acceptable results are obtained from Engelhardt and Mathes' data if we account for the contribution of remaining ethoxy groups. If two out of the three ethoxygroups of the γ-aminopropylsilane have reacted the remaining single ethoxygroup lowers the number of silane molecules to 1.8 per nm^2 requiring 3.6 hydroxylgroups per nm^2 of the silica surface. This is lower than the total number of 4.8 OH groups per nm^2. If only one ethoxygroup of the silane molecule reacts with the silica surface the carbon percentage corrected for the contribution of two remaining ethoxygroups yield only 1.3 silane brushes per nm^2 requiring only 1.3 hydroxyl groups per nm^2 of the silica surface. This is lower than the number of chemically reactive hydroxyl groups, which is estimated to range from 2.3 [30] to 2.7 [22] per nm^2 for monoreactive silanes. Obviously, the 1.3 silane brushes per nm^2 is substantially lower than the 3 brushes per nm^2 claimed by Engelhardt and Mathes [24].

This example demonstrates the difficulty in calculating surface coverage from carbon percentage when di- or trialkoxysilanes are used, Such problems are avoided when monochloro or monoalkoxysilanes are used as is done in the present study. In that case the carbon percentage, measured after preparation of the bonded phase, is readily expressed in the number of silane molecules attached to the silica surface as

$$P_C = \frac{100N.12n_C}{1+N(M-1)} \qquad (1)$$

where P_C is the measured carbon percentage

N is the number of moles organic phase per gram <u>unmodified</u> silica

n_C is the number of carbon atoms in the bonded silane molecule

M is the molecular weight of the silane molecule

Obviously, the term N(M-1) in the denominator of eq. (1) corrects for the weight increase of the silica (accounting for one hydrogen atom lost in the surface reaction) in order to obtain the number of silane brushes per unit of unmodified silica. Together with the specific surface area of the silica (S in m^2/g) we find from eq. (1) the surface coverage as

$$N\ (\text{mol/g}) = \frac{P_C}{1200n_C - P_C(M-1)} \tag{2a}$$

$$N\ (\mu\text{mol/m}^2) = \frac{10^6 . P_C}{1200n_C - P_C(M-1)} \cdot \frac{1}{S} \tag{2b}$$

$$N\ (\text{nm}^{-2}) = \frac{6.023 * 10^5 * P_C}{1200n_C - P_C(M-1)} \cdot \frac{1}{S} \tag{2c}$$

In a similar way the surface coverage can be derived from the hydrogen percentage, but in this case we must account for the contribution of remaining hydroxylgroups not only at the silica surface but also in the bulk of the material. From thermogravimetric and infrared measurements Erkelens and Linsen [31] determined the total number of hydroxyl groups in unmodified silica after pretreatment at 200°C; division by the specific area yielded values of 8.6 and 7.6 per nm^2, respectively. Note that this is significantly larger than the surface density of hydroxyl groups stated above as 4.8 nm^{-2}. The hydrogen percentage is then given as

$$P_H = \frac{100[N.n_H + q.S/6.023 * 10^5 - N]}{1+N(M-1)} \tag{3}$$

where P_H is the measured hydrogen percentage

n_H is the number of hydrogen atoms in the silane molecule

q is the 'surface' density of total hydroxyls in the original silica ($\sim 8\ nm^{-2}$)

and the other symbols have the same significance as in eq. (1). Note that ($q.S/6.023*10^5-N$) in the numerator of eq. (3) represents the contribution of hydroxyl groups to the hydrogen percentage after preparation of the bonded phase. Consequently, we find the surface coverage as

$$N\ (mol/g) = \frac{P_H-q.S/6.023*10^3}{100(n_H-1)-P_H(M-1)} \qquad (4a)$$

$$N\ (\mu mol/m^2) = \frac{(P_H/S-q/6.023*10^3)10^6}{100(n_H-1)-P_H(M-1)} \qquad (4b)$$

$$N\ (nm^{-2}) = \frac{6.023*10^5 P_H/S-100q}{100(n_H-1)-P_H(M-1)} \qquad (4c)$$

EXPERIMENTAL

The chemicals used in preparing the bonded phases and the solutes used in the chromatographic experiments were of the highest purity commercially available. Water used as mobile phase was doubly distilled and deionized. All bonded phases were prepared from a single batch of Lichrosorb SI100 silica of Merck (charge nr. 527.1302, batch nr. EF 72). The specific surface area of the 10 μm particles was determined in duplicate with the BET nitrogen adsorption method [32] as 299 m^2/g.

Elemental analysis for carbon and hydrogen was performed under dry nitrogen in duplicate, using 15-20 mg samples, which before the analysis were heated at 200°C and ∿1.5 torr during 12 hours.

Infrared spectra of the modified silicas were recorded with a Beckman model 4210 IR-spectrometer equipped with a beamcondensor. Since the spectra from KBr pellets were too weak, it was preferred to obtain spectra from the pure material. Unmodified silica is rather easily pressed into tablets. However, after surface modification with non-polar molecules this is much more difficult and the preparation of a suitable pellet is a matter of chance. After several attempts a moderately successful procedure was found. Some 20 mg of modified silica is pressed to between 100 and 150 bar; under vacuum the pressure is raised to 600 bar. After 10 minutes the pressure is released and generally the pellet falls into several pieces, of which the largest is selected to provide a suitable spectrum by means of a condensed light beam.

Chromatographic experiments were carried out with a liquid chromatograph assembled from Waters equipment (M6000 pump, U6K injector, models 440 UV and 401 RI detectors). Columns (300 mm, 4.6 mm ID) were slurry packed using the balanced density technique. All measurements were made under isothermal conditions either at 24 or 30°C (± 0.01°C) and peak positions were derived from first statistical moments using an on-line computer coupling and 5 Hz sampling rate.

Mobile phases were either isooctane/methylene chloride 90:10 v/v or methanol/water in various ratios. Solutes were introduced as 5 μl samples in either isooctane or methanol. At the end of the experiments the hold up of each column was determined with a dilute solution of sodiumdichromate in pure water as mobile phase, since this ion showed less retention than any of the four individual solvents used to compose the different mobile phases.

PREPARATION OF CHEMICALLY BONDED PHASES

Four different phases were prepared using trimethyl (TMS), phenyldimethyl (PDS), t-butyldimethyl (t-BuDS) and allyldimethyl monochlorosilane (ADS). The preparation followed the procedure described by Sebestian and Halász [33]. Before reaction the physically adsorbed water was removed from the silica by heating at 200°C and ∿ 1.5 torr during 12 hours. Pretreatment with acids as suggested in the literature to remove inorganic ions [10, 24, 23, 34, 35] was not done for fear of possible structural changes of the silica [31, 36] and the introduction of additional free hydroxyl groups.

The bonding reaction was carried out in an absolutely dry nitrogen atmosphere and in toluene dried over sodium. A four-fold excess of silane reagent (calculated for 4 chemically reactive silanol groups per nm^2) was added to 4 gram silica, 10 ml dry pyridine and 200 ml toluene. The mixture was heated for 12 hours under gentle stirring at a temperature 5-10°C below the boiling point of the most volatile component present.

The bonded phase was washed consecutively with dry toluene (to remove excess reagent), dry methanol, methanol/water 1:1, water (to remove the pyridine-HCl salt), methanol and ether. Finally, the modified silica was dried for 12 hours at 200°C in vacuo and samples for the elemental analysis were taken under dry nitrogen. Roughly half of the prepared material was post treated with a nine-fold excess of trimethylchlorosilane in the same way as described above. The material thus obtained was subjected to elemental analysis for carbon and hydrogen —special precautions were taken to avoid any contact with the atmosphere— and to chromatographic experiments identical to those performed with the original material. Consequently for each chemically bonded phase two different columns were packed, one containing the original reaction product, the other containing the same phase post-treated with excess TMS.

The trimethyl phase was also made from trimethylmonoethoxysilane, prepared from reacting trimethylchlorosilane with ethanol using diethylaniline as reaction-stimulant [37, 38]. This synthesis was carried out under the same conditions as described for the monochlorosilanes, except that no dry pyridine was added. Reactions were carried out with silica heated for 12 hours in vacuo at 200°C (as before) and with silica not subjected to this heating so that it still contained physically adsorbed water. The TMS-phases prepared in this way were washed with dry toluene, dry methanol and dry ether, respectively. After heating the product for 12 hours at 200°C under ∿ 1.5 torr samples were taken for the elemental analysis. Post-treatment with excess TMS was not carried out.

RESULTS OF ELEMENTAL ANALYSIS

Definite proof that the RP-phases are chemically bonded to the silica surface is obtained from the infrared spectra in figure 1, which we believe to be of better quality than previously published spectra [11, 39]. The characteristic absorption bands of the phenyl, the allyl and the t-butylgroup are readily discernable and are indicated in the IR-spectra. The spectra of the RP-phases with and without TMS post-treatment showed no differences.

The results of the elemental analysis are presented in table 1. If we start by discussing the data obtained with the monochlorosilanes, the first conclusion is that maximum coverage appears to have been realized for all four phases. Post-treatment with a large excess of TMS does not raise the carbon or hydrogen percentages (reliable to 0.1% absolute). In agreement with this is the observation that chromatographic retention is the same before and after post-treatment with TMS. Figure 2 shows a comparison of retention data for the two phenyl- and two t-butyl-

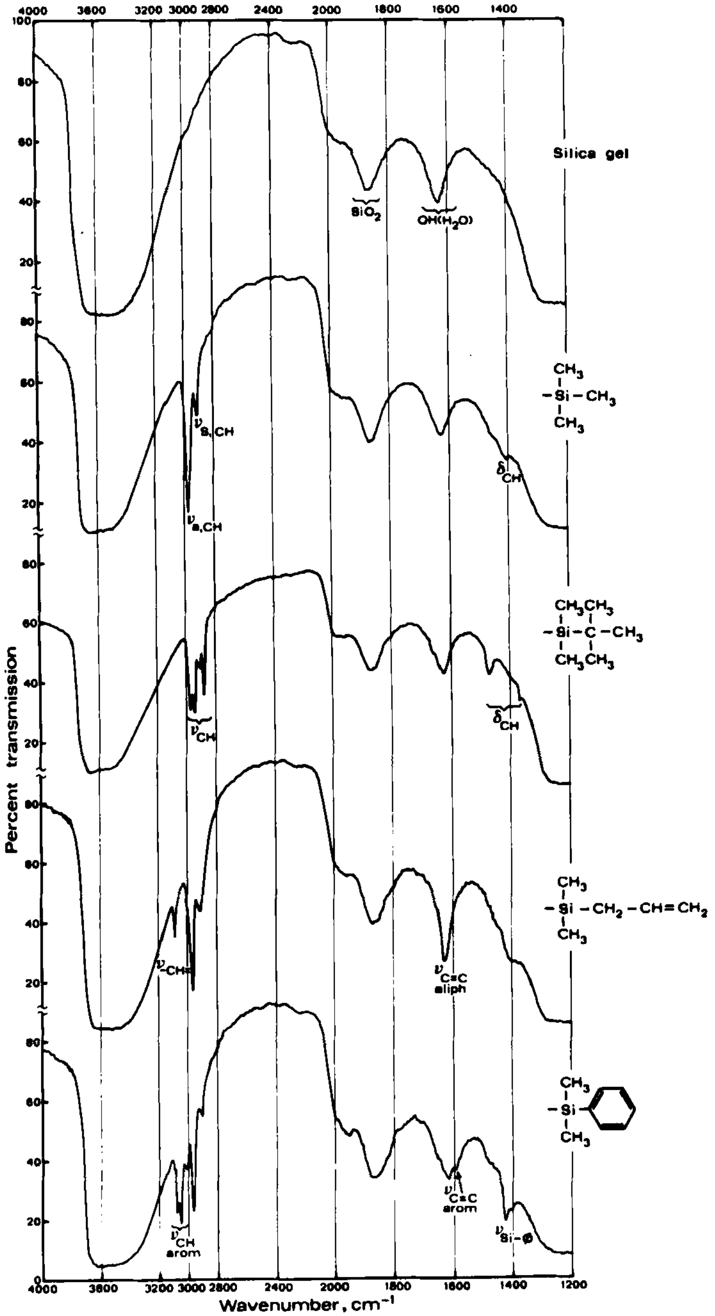

Figure 1. Infrared spectra of phases chemically bonded to silica gel.

TABLE 1

Results of the elemental analysis

monochlorosilanes	weight		percentages (*		surface		coverage	
	direct		after treatment with TMS		mmol/g		µmol/m²	nm⁻²
	C	H	C	H	C	H		
PDS	8.80	1.33	8.77	1.39	1.04	1.17	3.49	2.10
TMS	3.76	1.24	3.93	1.21	1.16	1.16	3.87	2.32
t-BuDS	3.95	1.14	3.73	1.02	0.57	0.54	1.90	1.14
ADS	5.52	1.24	5.30	1.24	1.00	0.96	3.34	2.00
monoethoxysilanes								
TMS**)	3.10	1.01	–	–	0.92	0.84	3.07	1.84
TMS***)	3.59	1.10	–	–	1.08	0.98	3.60	2.16
commercial RP-8 silane (Merck)	12.37	2.64	–	–	1.25	1.28	4.19	2.51

*) each weight percentage is the average of two measurements
**) reaction with activated silica
***) reaction with hydrated silica

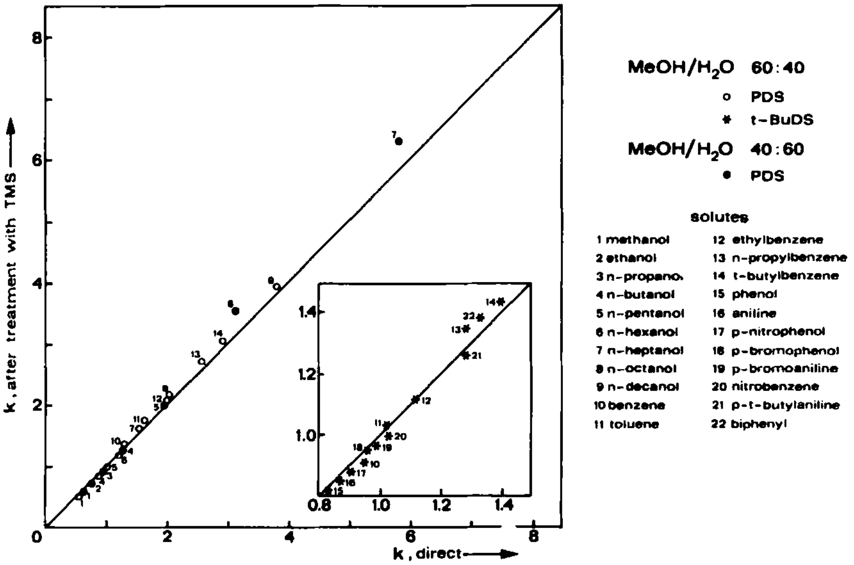

Figure 2. Constancy of chromatographic retention on PDS and t-BuDS phases after post-treatment with TMS.

phases, which according to table 1 have a high and a low surface coverage of 2.1 and only 1.1 silane molecules per nm^2 silica surface, respectively. Even for the t-butylphase post-treatment with TMS changes neither the carbon and hydrogen percentages nor the chromatographic retention behaviour. This allows us to average the two percentages for each element and to compute the surface coverages of each phase. So it can be concluded that post-treatment with TMS is not strictly necessary for monochlorosilanes, although it is essential for trichlorosilanes as found by Knox et al. [13].

When this is done, we obtain the remarkable result that the surface coverages calculated from the carbon and hydrogen percentage, respectively, agree within 10% for each phase. Such close agreement can only be obtained if the hydrogen percentage is corrected for the contribution of remaining hydroxyl groups, which amounts to about 0.4% by weight. The exact figure depends, of course, upon the hydroxyl density of pure silica (surface plus bulk hydroxyls), but moderate variations around the average value of 8 nm^{-2} taken from Erkelens and Linsen [31] does not impair the agreement between surface coverages calculated from carbon and hydrogen percentage, respectively. Nevertheless, the data derived from the carbon percentage must be considered as more reliable and these are used to derive the surface coverages in the final two columns of table 1.

Obviously, the surface coverage obtained for different silane phases varies from a low 1.14 nm^{-2} for t-butyl to a high 2.32 nm^{-2} for TMS. This latter value agrees well with the coverages of 2.4 and 2.7 nm^{-2} reported by Unger et al. [22]. By contrast our result for phenyldimethylsilane (2.1 nm^{-2}) is significantly larger than previous values reported for this phase by Kirkland [17], 1.5 nm^{-2}, and Unger et al. [22], 1.6 nm^{-2}. On the other hand it is quite close to the values of 2.2 and 2.3 nm^{-2} reported for a similar phase derived from phenyltrichlorosilane [11, 28]. This might indicate that only one of the three chlorine atoms reacts with surface silanol groups (take notice that the Van der

Waals radii of a CH_3-group and a chlorine atom are about the same, 2.0 and 1.8 Å, respectively).

All surface coverages found are much lower than the number density of surface hydroxyl groups in pure silica, which is generally taken as 4.8 nm^{-2}. Such high surface coverages are quite impossible, however, in view of the size of the silane molecules. This has been discussed more fully in a previous paper [30] where it has been shown that the data presented in table 1 for the TMS and the PDS phase represent nearly the geometrically maximum possible coverage. When the area taken up by remaining silanol groups (4.8 - 2.3 = 2.5 nm^{-2} in de case of TMS) is taken into account, it appears that for either phase only 15% of the silica surface is left completely bare. This corresponds to a quite efficient surface coverage. A similar conclusion would apply to the allyl phase, for which the coverage of 2.0 molecules per nm^2 is quite close to that of the PDS-phase.

By contrast, the coverage with t-butylphase is much lower. As stated above, the figure of 1.14 nm^{-2} still represents maximum coverage for this phase. Although the t-butylgroup is still more spacious than the PDS-molecule (its cross-section is 0.42 nm^2 against about 0.35 nm^2 for a PDS-group), the difference is insufficient to explain the low surface coverage. Indeed, multiplication of the molecular cross-section with the surface density data in table 1 shows that 74% of the silica surface is covered by PDS-molecules, whereas only 48% is covered by t-butylmolecules. This is attributed to a difference in molecular shape. The PDS-molecule is quite rectangular so that a close fit can be obtained. By contrast, the t-butylmolecule is nearly circular, so that after the bonding reaction relatively large free surface areas remain between the bulky t-butylgroups. The silanol groups present at the free surface areas are inaccessible for TMS molecules, as evidenced by the lack of change observed upon post-treatment with TMS. On the other hand, the relatively large amount of free silanols may well be chromatographically active, so that the t-butylphase is expected to behave more polar than any of the other chemically bonded phases. Consequently, in reversed-phase systems the solutes will be eluted more rapidly. As will be discussed below this is indeed observed.

Table 1 also includes data for a commercial octylsilane. If it assumed after Scott and Kucera [40] that this stationary phase is prepared from monochlorodimethyloctylsilane and if the specific surface area of these 10 µm particles is also 300 m^2/g, then the high carbon percentage corresponds to a coverage of 2.5 silane molecules per nm^2. In view of the above discussion this would mean a virtually complete coverage, so that this chemically bonded phase should constitute a nearly ideal non-polar stationary phase.

Finally table 1 contains data for a TMS-phase derived from monoethoxytrimethylsilane. It is seen that when physically adsorbed water is removed from the silica surface prior to the bonding reaction the surface coverage obtained for this TMS-phase is somewhat lower than realized with the monochlorotrimethylsilane (1.8 nm^{-2} versus 2.3 nm^{-2}). This agrees with the statement of Novotny et al. [19] that monochlorosilanes are more reactive than monoalkoxysilanes. However, this difference disappears, when fully hydrated silica is used for the bonding reaction. We agree, therefore, with the suggestion of Majors and Hopper [39] that physically adsorbed water stimulates the surface reaction, although this has been denied by Engelhardt and Mathes [24].

CHROMATOGRAPHIC PROPERTIES

a) Retention on chemically bonded phases with maximum coverage

The retention behaviour of the different chemically bonded phases and the commercial RP-8 phase has been tested in a reversed-phase system at 30°C with MeOH/H_2O 50:50 as mobile phase. Compounds used are several substituted benzenes as is indicated in figure 3. It is noticeable that the retention behaviour of

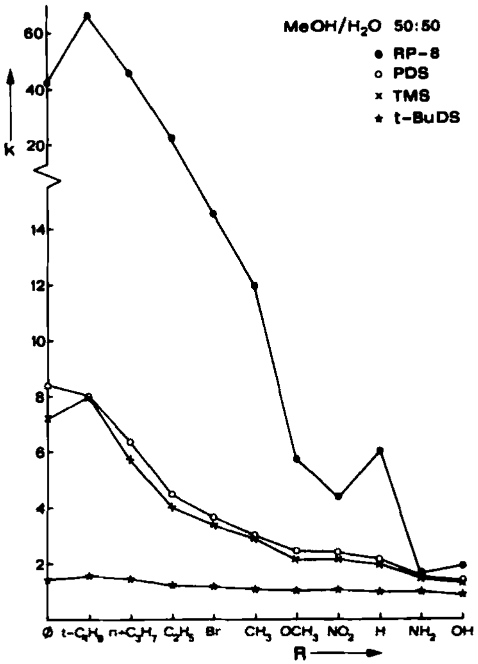

Figure 3. The capacity factor of monosubstituted benzenes, Ø-R, on various chemically bonded phases.

the PDS and TMS phases are more or less identical in spite of the much larger carbon percentage of the PDS phase. This points to an influence of the polar phenylring of the PDS-phase. The influence of the phenylring is confirmed by the specific π-π interaction of this ring with the substituted benzenes in comparison with n-alcohols as presented in figure 4. The retention-curve measured for n-alcohols on the PDS-phase is situated just below the curve for the TMS-phase whereas the reverse effect is shown in figure 3. Likewise, in going from t-butylbenzene to biphenyl the RP-8, TMS and t-butylphases all display a decrease in retention whereas PDS shows an increase in retention.

The retention data obtained with the t-butylphase are all remarkably low. Apparently, the selectivity is lost completely

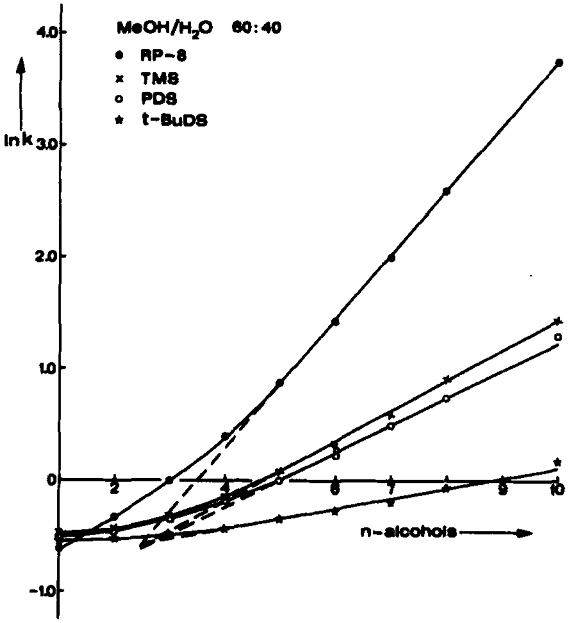

Figure 4. The logarithmic capacity factor versus carbon number of n-alcohols. Note the slightly more polar character of PDS in comparison to TMS. Also note the common intersection point of the extrapolated straight lines.

which must be attributed to the small coverage of only 1.1 t-BuDS groups per nm^{-2}. On the other hand the RP-8 phase constitutes a very nonpolar stationary phase. As a result, polar solutes such as aniline and phenol display very little retention in comparison to non-polar solutes. As expected the retention of the polar solutes methoxy- and nitrobenzene is smaller than that of benzene whereas such differences are not observed on the other phases which must be attributed to the influence of remaining silanols.

b) Trimethylsilane phases with various surface coverage

Chemically bonded stationary phases with less than maximum coverage can be obtained by reducing the time of the bonding reaction or by reducing the excess of silane reagent.
The resulting coverage cannot be predicted, but is readily derived from the carbon percentage of the prepared stationary phase. With monoethoxytrimethylsilane surface coverages of 1.6, 1.8 and 2.2 molecules per nm^2 were obtained. The chromatographic retention behaviour of these phases was determined in a straight-phase system using isooctane/methylenechloride 9:1 as the mobile phase. Retention data were measured in triplicate at 24°C with a flow rate of 2.0 ml/min. Capacity factors of seven components are presented in figure 5 as a function of surface coverage and compared with results obtained for pure silica. Note that isooctane itself shows some retention in comparison with sodiumdichromate (in water), which was taken as the unretained component.

It is seen that the retention of polar solutes decreases progressively with increasing coverage of the silica surface. At a surface coverage of 1.8 TMS-molecules per nm^2 the capacity factors drop sharply and when a maximum coverage of 2.3 TMS groups per nm^2 has been reached the TMS-phase virtually looses its selectivity in a straight-phase system. This behaviour can be understood by considering the size of the TMS molecule.

In a previous paper we have calculated the effective cross-section of the TMS-group parallel to the silica surface. This value depends upon the Si-O-Si bond angle through which the TMS-molecule is connected to the silica surface [30]. For a most probable angle between 120 and 140° it amounts to about 0.30 nm^2, so that theoretically 3.3 molecules per nm^2 are needed to completely cover the silica surface with close fitting TMS-groups. Now, if the lines connecting the first two data points for polar solutes are extrapolated towards zero retention, the abscissa values

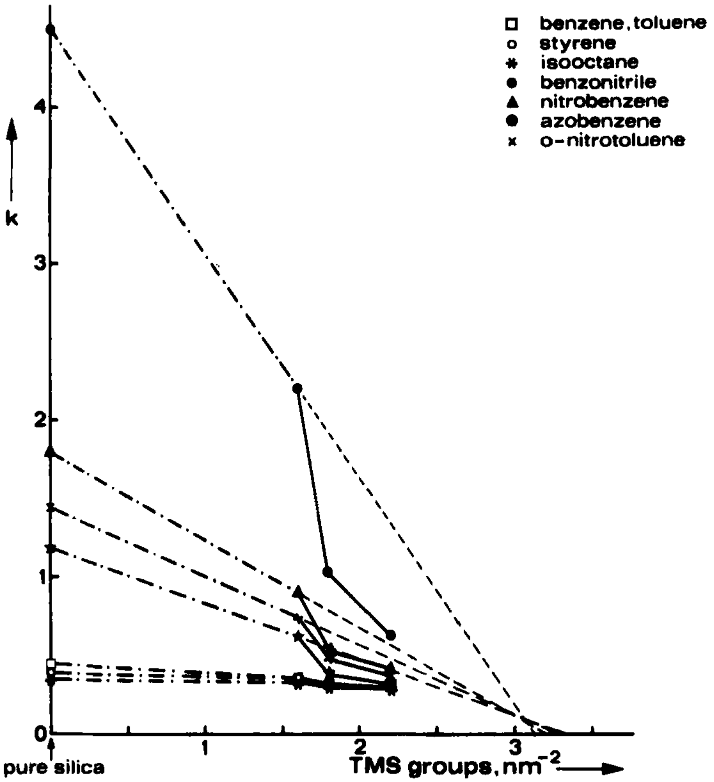

Figure 5. The capacity factor as a function of the TMS surface coverage (discussion see text).

correspond very closely to this value. Consequently, the initial decrease of the retention with increasing TMS-coverage simply reflects the decrease in the number of available silanol groups. Up to a surface coverage of 1.6 TMS-molecules per nm^2 the retention is fully determined by the surface density of silanol groups, just as for pure silica.

However, a complete surface coverage with 3.3 TMS molecules per nm^2 cannot be realized in practice. The curved shape of the methyl group make the necessary close fit impossible: there must remain free space between adjacent TMS-groups. Moreover, since the original silica surface contains 4.8 hydroxyl groups per

nm^2 and since these hydroxyls cannot hide under the methyl groups [30], there must remain room for at least 4.8-3.3=1.5 silanol groups. In practice, therefore, the maximum surface density of TMS is about 2.3 nm^{-2} covering some 70% of the surface. The remaining 30% is taken up by 2.5 silanol groups per nm^2 (partly hidden under TMS) and by bare silica surface. Obviously, the residual silanol groups are no longer chromatographically active, a conclusion reached previously by Scott and Kucera [40].

This analysis leads to the following description of the retention mechanism of modified silica in straight-phase systems. For pure silica the retention of polar components is determined by the surface silanol groups. When hydroxyl groups are modified through bonding reactions with nonpolar groups, such as TMS, the decrease in silanol density effectuates a proportional decrease of the capacity factor. At a certain surface coverage, however, the bonded molecules approach each other so closely that the hydroxyl groups in between are no longer accessible for solute molecules. For TMS this sets in at about 1.8 TMS molecules per nm^2, and correspondingly the capacity factors drop sharply. Soon thereafter maximum modification of the silica surface is reached and the chromatographic activity of residual silanol groups is reduced to long-range interactions extending over the length of the bonded molecules.

c. Retention behaviour of homologous series

Several authors have already reported a linear relation between logarithmic capacity factors and the number of carbon atoms of homologous compounds [10, 23, 40, 41]. There would thus appear little reason to repeat this observation. However, the data in figure 4 for alcohols eluted in a reversed phase system demonstrate another interesting point, that has not been previously noted.

When the linear relations between ln k and n_C (from $n_C = 5$ upward) are extrapolated for different chemically bonded stationary phases they are seem to intersect at a common point. This observation prompted us to analyse literature data for a similar property. The most extensive data have been published by Karch [23] and figure 6 presents his results (solid lines) for normal alkanes on chemically bonded alkylphases of different length.

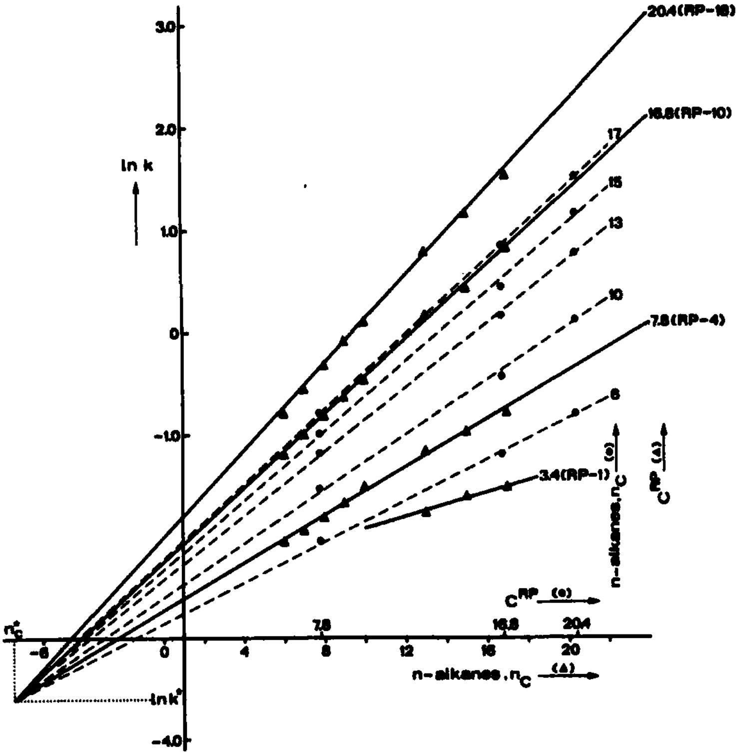

Figure 6. ln k versus carbon number of n-alkanes for different RP-phases (solid lines) and versus the corrected carbon content (see text) of the alkyl bonded phases for the n-alkanes (dotted lines), showing a common intersection point (data of Karch's thesis [23]).

Except for the RP-1 line, a common intersection point is again noted for the retention data of homologous alkanes on different alkyl phases. Other series of homologous compounds also obey this observation [10, 40] for different alkylphases, including RP-1. Mathematically this set of straight lines can be expressed as

$$\ln k = \ln k^{\bullet} + \alpha(n_C - n_C^{*}) \qquad (5)$$

where $\ln k^{\bullet}$ and n_C^{*} are the coordinates of the common intersection point. The slope factor α depends upon the mobile phase, the stationary phase and, probably, upon the nature of the eluted solutes. The coordinates $\ln k^{*}$ and n_C^{*} also depend upon the mobile phase and the nature of the eluted components, but they do not depend upon the stationary phase. The computed coordinates $\ln k^{*}$ and n_C^{*} of the intersection point for the solid lines of figure 6 are -3.7 and -8.0, respectively.

Because Karch's data refer to pure alkyl stationary phases a similar relation might be expected when the capacity factors are replotted, this time versus the chain-length of the bonded alkyl phase. However, if we substitute in eq. 5 for n_C the <u>nominal</u> number of carbon atoms in the bonded alkyl chain no straight line results. The reason is that we must account for differences in surface density of the bonded phase molecules. In eq. 2 this is expressed as the number of bonded phase molecules per unit of original silica, N. According to eq. 1 the product $N.n_C$ is proportional to the carbon percentage of the modified silica corrected for the weight increase upon modification. In fact

$$1200\ N.n_C^{RP} = P_C[1 + N(M - 1)] \qquad (6a)$$

where n_C^{RP} is the nominal carbon number and N is expressed in moles per gram original silica. Consequently, with eq. 2a we obtain

$$1200\ N.n_C^{RP} = P_C\left[1 + \frac{P_C[M - 1]}{1200n_C^{RP} - P_C[M - 1]}\right] \qquad (6b)$$

Now, when Karch's retention data for alkanes are plotted versus the righthand-side of eq. 6b we not only obtain straight lines for each alkane solute but again the straight lines possess a common intersection point. Moreover, the ordinate value of this intersection point is found to be -3.5 , which is virtually equal to the value of ln k^* obtained for the solid lines. The abscissa value of the intersection point is less defined for the following reason.

From the linear relations thus found we are tempted to call $N.n_C^{RP}$ in eq. 6 an effective carbon number for the bonded alkyl phases, but because N is not dimensionless, we retain an arbitrary scaling factor. This scaling factor does not affect the linear relationship between ln k and $N.n_C^{RP}$, but it changes the abscissa value of the common intersection point (unless this intersection point happens to fall at $N.n_C^{RP} = 0$). Since the ordinate value, ln k^*, remains unaltered, it is possible to find a scaling factor such that the intersection point falls exactly at the previously found value of $n_C^* = -8.0$. Regression analysis shows that the appropriate scaling factor is 1260 . Referring to eq. 6, we note that this scaling factor is very close to the value of 1200 used in eq. 6. This means that for the present system of n-alkanes retained on alkyl bonded phases the effective carbon number of the bonded alkyl chains is virtually equal to the weight percentage of carbon, corrected for the weight increase of the modified silica.

This situation is presented in fig. 6. As remarked before the solid lines indicate retention data for homologous alkanes on three different alkyl phases and plotted versus the nominal carbon number of the alkanes, n_C. The dotted lines represent the same data, but now plotted versus the corrected carbon percentage of the alkyl bonded phases, $C^{RP} = 1200\ Nn_C^{RP}$. The complete set of straight lines with a single common intersection point can now be expressed as

$$\ln k = \ln k^* + \beta(C^{RP} - n_C^*)(n_C - n_C^*) \qquad (7)$$

Computer analysis yields for the coordinates of the intersection point $\ln k^* = -3.6$ and $n_C^* = -7.5$.

The equivalence between the alkane carbon number, n_C, and the corrected carbon percentage of the bonded alkyl phase, C^{RP} is also clear from the location of the solid lines in fig. 6. Indeed, the solid line for the RP-10 phase, for which C^{RP} is equal to 16.8% runs very close to the dotted line for heptadecane ($n_C = 17$). A similar observation can be made for the RP-4 phase ($c^{RP} = 7.8\%$). The solid line nearly coincides with the dotted line for n-octane (deleted from fig. 6 for clarity).

Obviously, eq. 7 provides a possibility to predict the retention of an arbitrary n-alkane on any alkyl phase for which the carbon percentage is known, as long as the same mobile phase is used. In other words the system of alkanes on alkyl phases using a mobile phase with a certain composition is completely characterized by the coordinates $\ln k^*$ and n_C^* of the intersection point and the slope factor β.

The present data are too scarce to permit further generalization. For example, it remains to be seen whether the corrected carbon percentage also applies to chemically bonded phases other than alkyl. Furthermore, we expect that for low surface coverage of the modified silica the influence of remaining silanol groups manifests itself through a decrease of the slope factor β in eq. 7. Finally, the influence of the mobile phase upon the values of $\ln k^*$, n_C^* and β must be fully analysed. The results of such studies will be reported in forthcoming papers.

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