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Chromatographic activity of residual silanols of alkylsilane derivatized silica surfaces

Alex B. Scholten[a,*], Henk A. Claessens[b], Jan W. de Haan[b], Carel A. Cramers[b]

[a]*General Electric Plastics Europe, Plasticslaan 1, 4600 AC Bergen op Zoom, Netherlands*

[b]*Laboratory of Instrumental Analysis, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, Netherlands*

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Abstract

Residual silanols on *n*-octadecylsilane derivatized silica surfaces have a pronounced effect on the chromatographic performance of reversed-phase high-performance liquid chromatography (RP-HPLC) materials. The present study describes how the results of solid-state NMR investigations on two commercially available reversed-phases for HPLC are verified chromatographically. One phase is a dimethyl-*n*-octadecylsilane derivatized silica substrate (Rx-C_{18}), while the other is the same substrate derivatized with di-isobutyl-*n*-octadecylsilane ("stable bond", SB-C_{18}). Four column tests, taken from the literature, are performed in order to assess silanol activity and column hydrophobicity. It is concluded that on the SB-C_{18} phase, more residual silanols are analyte accessible and that the isobutyl groups contribute significantly to hydrophobicity. Generally, the results of the solid-state NMR method for determining shielded and accessible residual surface silanols on RP-HPLC stationary phases could be confirmed.

Keywords: Stationary phases, LC; Silanol groups; Residual silanols; Nuclear magnetic reasonance spectrometry

1. Introduction

Porous silica has certain well known properties that make it a useful substrate for reversed-phase high-performance liquid chromatography (RP-HPLC) stationary phases [1,2]. It has a high mechanical strength and nowadays it can be produced with controlled porosity and particle size. Its rigid SiO_2 matrix does not swell in organic modifiers and it has a large specific surface area that can be modified quite easily. In the majority of modern LC applications octadecylsilane (C_{18}) derivatized silica surfaces are employed. However, the long term stability of these materials is still an important issue in further improvement of RP-HPLC column technology. More precisely, the limited hydrolytic stability of the siloxane bond has encouraged the search for new, more stable silica based stationary phases [3,4].

It was proposed that bulky substituents on the silicon atom of octadecylsilanes would increase the chromatographic lifetime of column packings [5,6]. Indeed, Kirkland et al. have shown that phases with isobutyl substituted C_{18} silane groups exhibit a longer lifetime in low-pH aqueous organic mobile phases than their conventional methyl substituted analogues [7]. In an earlier paper we provided ^{29}Si solid-state NMR evidence for a decreased hydrogen bonding contribution of residual silanols to the ligand siloxane bond of di-isobutyl-*n*-octadecylsilane

*Corresponding author.

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ligands [8]. This was considered a result of the sterically protecting properties of the bulky side groups, hampering the siloxane bond hydrolysis by acidic mobile phases.

Another issue of prime importance in stationary phase research is the influence of residual surface silanols (remaining after silylation of the silica substrate) on chromatographic column performance [9–11]. On one hand, residual silanols may be held responsible for unwanted phenomena like irreversible adsorption and peak tailing of basic solutes. On the other hand, the porous silica substrate should have a maximum silanol surface concentration in order to yield a dense and homogenous surface layer of alkylsilanes after silylation [12]. However, the spatial requirements of *n*-octadecylsilanes prohibit the exhaustive silylation of all surface silanols. Inevitably, residual silanols remain at the silica surface after "full" coverage with C_{18} silane ligands. Thus the question is to what extent the chromatographic influence of these silanols can be mediated by altering the type of silylation reagent. Di-isobutyl C_{18} silanes, evidently, are sterically quite demanding and the maximum surface coverage will be less than for dimethyl C_{18} silanes. The better silanol shielding capacity of the isobutyl groups, however, is expected to compensate for the lack of silanol conversion in the silylation procedure. In this respect, the results of our work using solid-state NMR (published elsewhere [13]) have given a preliminary answer. This will be briefly outlined below.

Two commercially available C_{18} phases were studied by ^{29}Si cross-polarization magic-angle-spinning (CP MAS) NMR spectroscopy. Rx-C_{18} is a conventional silica based RP-HPLC column material with a dimethyl-*n*-octadecylsilane surface coverage of 3.37 μmol/m^2. SB-C_{18} is a so-called "stable bond" RP-HPLC stationary phase material that is based on the same silica substrate (Rx-Sil), but that has been reacted with di-isobutyl-*n*-octadecylsilanes to a surface concentration of 2.00 μmol/m^2. Upon exposure to an acetonitrile–D_2O (90:10, v/v) mixture, all surface silanols are deuterium exchanged [10,14,15]. The detection of deuterated silanol (SiOD) signals by means of ^1H and ^{29}Si CP MAS NMR then depends on a transfer of magnetization (cross-polarization) from protons in the alkylsilane ligands to silanol silicon atoms. Because this magnetization transfer is only feasible if the silanol silicon atoms are in the immediate vicinity of alkylsilane protons, the residual silanol signal of the RP-HPLC phases after deuterium exchange is assumed to represent surface silanols that are not directly accessible for analytes during chromatography for reasons of sterical constraints. Also, silanols that are buried inside the silica substrate matrix remain visible by the CP MAS NMR technique because they cannot be deuterated. These internal silanols are, however, irrelevant to chromatography.

The main results of the ^{29}Si NMR study are gathered in Table 1. The value of 6.2 μmol/m^2 as silanol surface concentration is lower than expected for a maximally hydroxylated porous silica surface, which is 8.2 μmol/m^2 (Kiselev–Zhuravlev constant [16,17]). It could be concluded that one di-isobutyl-*n*-octadecylsilane ligand sterically shields more residual surface silanols than one dimethyl-*n*-octadecylsilane (0.8 vs. 0.3), but that the lower surface concentration of the bulky silane leaves more residual silanols "free". In this respect "free" means not cross-polarizable by alkylsilane protons. It was surmised that these silanols will be analyte accessible during chromatography. From Table 1 it can be inferred that 60% of all silanols of the phase Rx-C_{18} and 45% of all silanols of the phase SB-C_{18} are not directly accessible for analytes during the chromatographic separation process. These percentages are probably minimum values, since larger analytes will have more difficulty penetrating the alkylsilane layer before possibly encountering a residual silanol. It is the purpose of the present study to investigate whether the NMR results and assumptions as described above can be verified chromatographically.

As there is a large variability in the separation

Table 1
Silanol surface concentrations (μmol/m^2) as determined by ^{29}Si CP MAS NMR analysis (±estimated maximum errors)

Silanol structure	Phase		
	Rx-Sil	Rx-C_{18}	SB-C_{18}
Internal	0.82±0.06	0.82±0.06	0.82±0.06
Surface	6.2±0.4	2.3±0.2	4.6±0.2
Shielded	–	1.1±0.2	1.6±0.2
Total	7.0	3.2	5.4

characteristics of marketed reversed-phase columns [18–20], there is a strong demand for test systems that allow precise and reliable classification of RP-column performance. Through the years, a large number of chromatographic test procedures have been developed [21], but it appears that there is yet no standard method that has earned general acceptance. The practitioner in chromatography has the task of selecting the proper column for his individual separation problems and therefore tends to design his own column testing procedure. In view of our goal, which is the chromatographic assessment of residual surface silanol activity of the phases Rx-C_{18} and SB-C_{18}, we in principle could have designed our own particular test system. Instead, to assure general comparability with other literature data, we have chosen to perform the column testing according to procedures proposed by authors who's intentions were to provide the chromatographer with standardized procedures for characterizing stationary phase hydrophobicity and polarity [18,22,23]. A total of four different characterization methods have been performed, which will be described in Section 3.

2. Experimental

The Zorbax Rx-C_{18} and SB-C_{18} columns (15×4.6 mm I.D.) were a gift from Rockland Technologies Inc. (Wilmington, DE, USA). The chromatographic system consisted of a Merck Hitachi L-6200A Intelligent Pump, an AS-200A Autosampler, a T-6300 Column Thermostat that was set at 30°C (unless stated otherwise) and a L-4250 UV-VIS detector operating at a wavelength of 254 nm. Data acquisition was controlled by a Nelson Model 2600 Chromatography Data System, connected by a PE Nelson 900 Series Interface to the UV-VIS detector.

In all experiments uracil was used as the dead-time marker. The eluent components methanol, acetonitrile (both Lichrosolv Gradient Grade, Merck, Darmstadt, Germany) and water (Millipore Milli-Q Water Purification System) were filtered and degassed before use. A constant flow of 1.0 ml/min was applied in all experiments.

To start the experiments on a fresh column, it was first rinsed with 100% methanol until the detector signal had stabilized. The experiments requiring the lowest percentage organic modifier (methanol) were conducted first. Before each experiment, columns were equilibrated with the new mobile phase composition by purging with at least 10 column volumes or by observation of a stable UV detector signal. Successive experiments requiring increasingly higher mobile phase methanol contents were then carried out and finally the column was purged again with 100% methanol. Then the mobile phase was switched to acetonitrile and the procedure as described for methanol was repeated. All retention times were measured in triplicate. Typically, a sample volume of 5 μl was introduced, consisting of test components dissolved in the mobile phase medium.

The check on the influence of TEA as silanol scavenger on the retention of the polar solute N,N-dimethylaniline (DMA) was performed as the final test on each column in order to exclude the possibility of irreversibly adsorbed triethylamine (TEA) influencing the other column tests.

3. Results and discussion

3.1. Column evaluation according to Engelhardt

Because many tests for evaluating the suitability of reversed-phases to separate basic solutes employ test components that are not readily available, Engelhardt et al. proposed the use of the following simple test solutes: three isomeric toluidines (*ortho*-, *meta*- and *para*-toluidine), aniline as a weak base and N,N-dimethylaniline as a strong base [22,23]. Phenol is proposed as a neutral polar compound, suitable for identifying polar interactions. The toluidines are especially interesting test solutes as they only differ in pK_a values and not in hydrophobic properties. Therefore, any separation of the toluidine isomers must be based on differences in silanophilic interaction potentials.

For checking column hydrophobicity, toluene and ethylbenzene are used. It is claimed that from the retention factors of toluene and ethylbenzene, the carbon content of a phase can be calculated to within 10% of that measured by elemental analysis, regardless of the type of silica substrate or the type of silane that was used for silylation of the silica

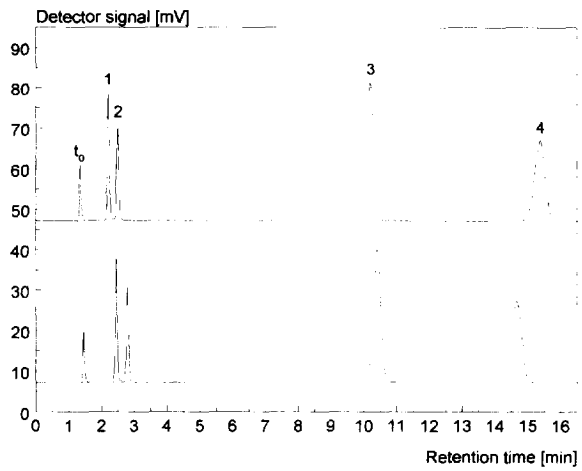

Fig. 1. Chromatograms of the silanophilic test mix according to Engelhardt on a Rx-C_{18} column (upper trace) and on a SB-C_{18} column (lower trace). t_0 = uracil, 1 = aniline (0.5 μg), 2 = phenol (0.8 μg), 3 = N,N-dimethylaniline (10 μg), 4 = toluene (12 μg). Eluent: MeOH–H_2O (55:45, v/v) at 30°C.

substrate [22]. Moreover, only below carbon contents of 12%, hydrophobic selectivity (defined as the ratio of retention factors of ethylbenzene and toluene) is shown to increase with increasing carbon content of the stationary phase.

Engelhardt classifies a stationary phase for RP-HPLC as good in the separation of basic compounds if the four following conditions are met when using MeOH–H_2O (55:45, v/v) as eluent:

1. Aniline elutes before phenol.
2. The ratio of peak asymmetries for aniline and phenol is smaller than 1.3.
3. The three isomeric toluidines are hardly separated, the ratio of their k values being smaller than 1.3.
4. N,N-dimethylaniline (DMA) elutes before toluene.

From Fig. 1 it is evident that both Rx-C_{18} and SB-C_{18} must be good phases according to this definition. The solute pairs aniline–phenol and DMA–toluene are clearly baseline separated. Surprisingly, at first sight there appears to be no dramatic difference between the chromatographic separations on the two phases under the chosen experimental conditions, despite the significant differences in alkylsilane type and coverage and residual silanol surface concentration. Comparing the numerical data in Table 2, however, the polar components aniline, phenol and DMA all have larger retention factors on the SB-C_{18} phase. Kirkland et al. already noted the increased retention of basic compounds on bulky alkylsilane phases when compared to the retention on dimethylalkylsilane phases [24]. Toluene retention factors, on the other hand, are almost identical on both phases.

Although from Table 2 the k values of the isomeric toluidines fulfil the requirement as stated under condition number 3, visual inspection of the chromatograms displayed in Fig. 2 clearly highlights the larger residual silanol activity of the phase SB-C_{18}. Recently it has been shown that pK_a values are not the only factors determining silanophilic interactions. Substituted pyridines were shown to elute in decreasing order of sterical shielding around the basic nitrogen atom in the solute molecule [25,26]. In other words, the elution order of the toluidines would remain the same due to steric effects, even if their pK_a values were identical. Thus, the improved separation of toluidines observed here for the SB-C_{18} phase may be interpreted as a lack of shielding of the residual silanols on this phase, permitting the easier access of the *para*-toluidine isomer to surface silanols.

The hydrophobicity of a stationary phase, according to Engelhardt, can be assessed by measuring the

Table 2
Retention factors of the test solutes used in the Engelhardt silanophilic column evaluation of the phases Rx-C_{18} and SB-C_{18}

Phase	Aniline	Phenol	Toluidine				DMA	Toluene
			Ortho-	*Meta-*	*Para-*	$\alpha^{a}_{P/O}$		
Rx-C_{18}	0.68	0.89	1.31	1.37	1.43	1.09	6.93	11.04
SB-C_{18}	0.77	1.06	1.30	1.41	1.51	1.16	7.33	10.95

[a] Ratio of retention factors for *ortho-* and *para*-toluidine.
Eluent: MeOH–H_2O (55:45, v/v).

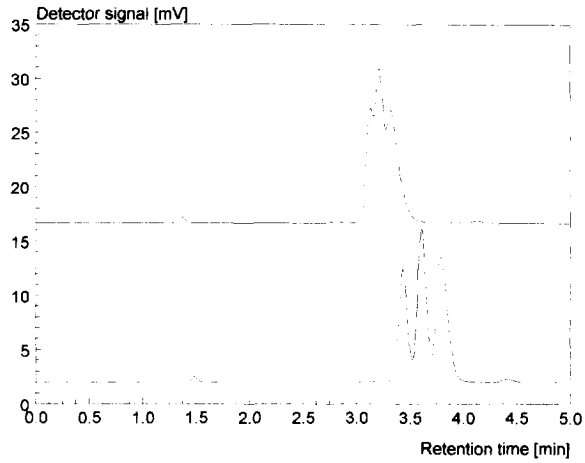

Fig. 2. Chromatograms of the toluidine isomers (elution order: *ortho*-, *meta*-, *para*-toluidine) on a Rx-C_{18} column (upper trace) and on a SB-C_{18} column (lower trace). Eluent: MeOH–H_2O (55:45, v/v). Flow-rate: 1.0 ml/min. Injected amount: 0.3 μg per component.

retention factors of toluene and ethylbenzene using methanol–water (65:35, w/w) as eluent. As already noted, the toluene retention factors are almost identical for the Rx-C_{18} and SB-C_{18} phases when eluting with a methanol–water (55:45, v/v) mixture. The actual hydrophobicity test is performed at 25°C using MeOH–H_2O (65:35, w/w) as eluent. Then, the k values of toluene and ethylbenzene should give an estimate of the %C of a stationary phase, through Eqs. (1,2) [22]:

$$\%C = 4.55 \cdot (k_{\text{toluene}} + 0.71) \quad (1)$$

$$\%C = 2.86 \cdot (k_{\text{ethylbenzene}} + 1.19) \quad (2)$$

The results in Table 3 illustrate that for both Rx-C_{18} and SB-C_{18} the weight percentage carbon (12.25 and 9.85% according to the data from elemental analysis) is significantly overestimated when applying Eqs. (1,2). Because there is almost no difference in hydrophobic selectivity of Rx-C_{18} and SB-C_{18} columns, it may be anticipated that the bulky di-isobutyl substituents of the stable bond material contribute significantly to hydrophobic retention, thereby compensating for the lack of C_{18} density when compared to the Rx-C_{18} phase.

3.2. The Galushko computational stationary phase characterization

An important goal of much research in chromatography is accurate prediction of solute retention based on known physico-chemical parameters of both solute and stationary phase. In this respect, the solvophobic retention theory [27–29] has earned significant acceptance. Based on this solvophobic theory, Galushko has proposed a calculatory model [30] for the retention of solutes in reversed-phase HPLC that appears able to predict retention factors for various solutes on different stationary phases for RP-HPLC with fairly good accuracy [31]. Basically, the method uses the retention data of just a few solutes with known molecular properties to calculate parameters describing the separation characteristics of the reversed phase. Inherent in the solvophobic theory is the assumption that solutes penetrate into the surface layer of alkyl chains that is partly solvated by the mobile phase (thus forming a liquid-like stationary phase). Retention is then expressed as a function of differences in free energies of solvation of the solute molecule in the surface layer and in the mobile phase. These energies are calculated by taking into account the known physico-chemical parameters of the solute, like molar volume and overall dipole moment. The assessment of stationary phase separation characteristics is made by using in the first instance the retention factors of aniline, phenol, benzene and toluene under defined conditions as input parameters. The calculatory model then assigns numerical values to the following

Table 3
Data of the Engelhardt evaluation of hydrophobicity of the phases Rx-C_{18} and SB-C_{18}

Phase	k_{toluene}	$k_{\text{ethylbenzene}}$	$k_{\text{ethylbenzene}}/k_{\text{toluene}}$	%C from Eq. (1)	%C from Eq. (2)	%C from elem. anal.
Rx-C_{18}	3.138	5.063	1.61	17.6	18.0	12
SB-C_{18}	3.052	5.059	1.66	17.1	17.9	10

stationary phase parameters: size selectivity, polarity and polar selectivity, hydrophobicity and hydrophobic selectivity and NH_2-interaction capacity. These data can then be used to describe the main differences between RP-HPLC stationary phase systems.

The results of the calculatory model of Galushko, using as input parameters the retention factors of aniline, phenol, benzene and toluene with MeOH–H_2O (60:40, v/v) as eluent, are given in Table 4. The overall conclusion is that there is a moderate, yet significant difference between the phases Rx-C_{18} and SB-C_{18}. The results are in general agreement with the Engelhardt test results. The phase Rx-C_{18} is slightly more hydrophobic than SB-C_{18} while it is clearly less polar. Both observations reflect the known composition of the surfaces of the two phases: Rx-C_{18} has a higher octadecyl ligand density and SB-C_{18} has a higher residual silanol concentration. However, one parameter is in conflict with the results of the Engelhardt test for silanophilic interactions. In Fig. 2 it is clearly illustrated that the toluidine isomers are better separated on the SB-C_{18} column. As the toluidines have about the same hydrophobic properties, this improved separation must be based on an increased NH_2-interaction capacity, regardless of the hydrophobicity of the stationary phase. The calculated values for NH_2-interaction of the Galushko model, however, are not significantly different for Rx-C_{18} and SB-C_{18}. We therefore assume that the retention data of aniline alone are not reliable enough for accurately describing the NH_2-interaction capacity of a stationary phase.

Table 4
Results of the Galushko calculatory model for the phases Rx-C_{18} and SB-C_{18}

Parameter[a]	Phase	
	Rx-C_{18}	SB-C_{18}
Size selectivity	0.2957	0.2852
Polarity	9.64	10.82
Polar selectivity	0.0622	0.0558
Hydrophobicity	10.05	8.45
Hydrophobic selectivity	1.88	1.84
NH_2-interactions	0.42	0.41

[a] Significant difference criterion = 0.075 [32].
Based on retention data for aniline, phenol, benzene and toluene using MeOH–H_2O (60:40, v/v) as eluent at 30°C.

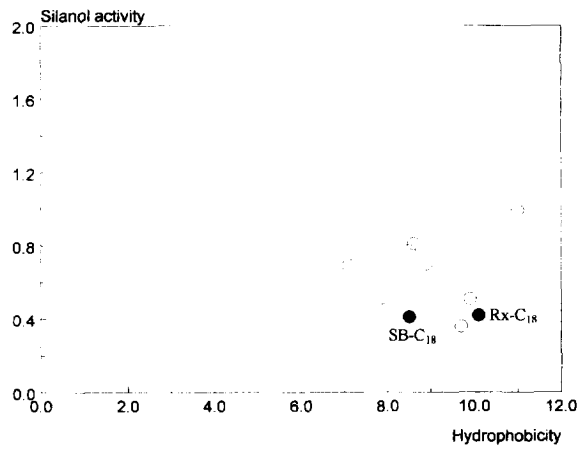

Fig. 3. Scatter plot of silanol activity versus hydrophobicity of several commercially available C_{18} RP-HPLC column materials according to the Galushko computational characterization.

To place these results in a broader context, Fig. 3 shows a scatter plot in which the hydrophobicity and silanol activity of ten popular commercially available RP-HPLC column materials are compared [32]. This figure implies that the phases under study here are not exceptional with respect to other RP-HPLC stationary phases. However, they appear to have a relatively low silanol activity. Because they are more or less average in hydrophobicity, this is probably related to the favorable properties of the Rx-Sil silica substrate: low degree of metal contaminations and maximum silanol surface concentration before silylation.

3.3. Column evaluation according to Walters

With the goal of accomplishing a standardized test system allowing marketed octadecyl columns to be classified in terms of silanophilic and hydrophobic interactions, Walters proposed a very simple, yet adequate procedure [18]. A silanol activity index is obtained by determining the retention of N,N-diethyltoluamide (DETA) relative to anthracene, with 100% acetonitrile as eluent. The retention of DETA is sensitive towards silanol activity, while anthracene retention behavior is assumed to be solely determined by hydrophobic interactions. The use of 100%

acetonitrile as mobile phase may be advantageous to our purpose, as acetonitrile solvates C_{18} alkyl chains rather well [33,34]. Solvation of the alkylsilane layer is assumed to facilitate access of solutes to residual silanol sites [11] and therefore eluents with a high acetonitrile content will show a RP-column's worst behavior towards basic analytes.

The ratio of retention factors of anthracene and benzene in an eluent mixture consisting of acetonitrile–water (65:35, v/v) is proposed as the hydrophobicity index.

In Table 5 the results of the column evaluation according to Walters are summarized. Again, both phases are classified as "good" RP-HPLC phases when using the criteria as defined for this particular test system:

–silanol index <0.9 (100% acetonitrile as mobile phase),

–hydrophobicity index >4.0 [acetonitrile–water (65:35, v/v) as mobile phase].

Nevertheless, some interesting differences are observable. The silanol index is significantly larger for the SB-C_{18} phase and this difference in residual silanol activity between the two phases seems to be expressed somewhat better in the acetonitrile–water test system than in the methanol–water test system used by Engelhardt. This is assumed to be an illustration of the better alkyl chain-solvating power of acetonitrile compared to methanol. Apparently, this also has consequences for the measured hydrophobicity index, which is larger for the Rx-C_{18} column. As the octadecyl chains are solvated by and extended into the mobile phase medium, the higher C_{18} ligand density of the Rx-C_{18} phase is more effective in the hydrophobic retention mechanism. The hydrophobic difference between the two phases with different ligand densities is less pronounced when the octadecyl chains are less solvated in the methanol–water mobile phase systems.

For the column characterization according to Walters a comparison with commercially available phases is also made. Fig. 4 displays a scatter plot similar to that used for presentation of the Galushko results: the silanol index is plotted vs. the hydrophobicity index (data as published in ref. [18]). It is evident that the distribution pattern again indicates that Rx-C_{18} and SB-C_{18} exhibit average hydrophobicity, but low silanol activity. Thus the general applicability of these column characterization methods is demonstrated.

Table 5
Results of the column evaluation according to Walters

Phase	Silanol index	Hydrophobicity index
Rx-C_{18}	0.47	4.84
SB-C_{18}	0.59	4.26

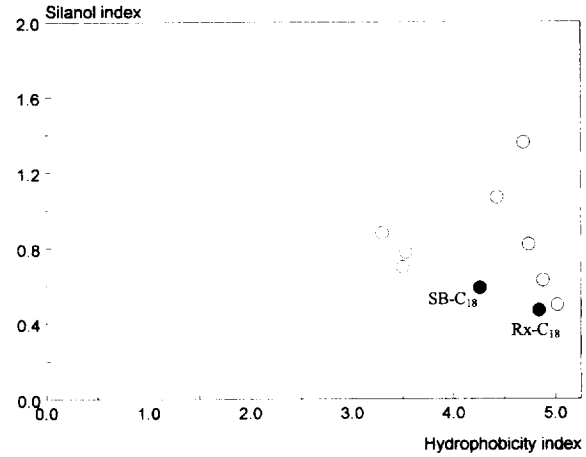

Fig. 4. Scatter plot of silanol index versus hydrophobicity index of several commercially available C_{18} RP-HPLC column materials according to Walters.

3.4. Silanol scavenging

Horváth et al. have described how surface silanols on RP-HPLC phases have a pronounced effect on the retention mechanism of polar solutes [35,36]. The basic assumptions underlying their dual retention mechanism seem rather sound and simple. Moreover, they illustrate how the contribution of silanophilic interactions to the overall retention can be assessed in a more quantitative manner. This will be outlined in some more detail.

The dual retention mechanism takes into account a hydrophobic (k_1) and a silanophilic contribution (k_2) to the overall retention factor (k_0), which is reflected in the expression for the observed retention factor of a solute:

$$k_0 = k_1 + k_2 \quad (3)$$

With the addition to the mobile phase of an amine, acting as silanol scavenger by strongly binding to accessible residual silanol sites, the phase ratio for silanophilic interactions with solutes is reduced. The extent of this reduction is dependent on the stability of the silanol–amine complex (represented by the equilibrium constant K_A) and the amine concentration in the mobile phase [A]:

$$k = k_1 + \frac{k_2}{1 + K_A[A]} \quad (4)$$

Subtraction of Eqs. (3,4) gives:

$$k_0 - k = \frac{k_2 K_A[A]}{1 + k_A[A]}. \quad (5)$$

Rearrangement of Eq. (5) results in:

$$\frac{[A]}{k_0 - k} = \frac{1}{k_2 K_A} + \frac{[A]}{k_2}. \quad (6)$$

First, k_0 is measured in the absence of the silanol scavenger. Then, gradually the amine concentration in the mobile phase is increased and the corresponding k is measured as a function of [A]. According to Eq. (6), a linear plot of $[A]/(k_0 - k)$ vs. [A] is obtained, from which the silanophilic retention factor k_2 and the stability of the silanol–amine complex (K_A) can be estimated by determining the slope and intercept of the regression line.

Because the phases Rx-C_{18} and SB-C_{18} probably differ significantly in residual silanol surface concentration and accessibility (as surmised on the basis of the ^{29}Si CP MAS NMR results), this method is expected to give a somewhat more quantitative result than the three stationary phase tests described in the previous subsections.

For evaluation of the contribution of silanophilic interactions to the total retention, N,N-dimethylaniline (DMA) was chosen as the test solute. It is a stronger base than aniline and therefore its retention behavior will depend more strongly on residual silanol availability. Moreover, DMA has a larger retention factor on both columns than aniline and this is favorable in terms of errors in the calculated values of k (small retention factors being disturbed much easier by small variations in measured absolute retention times). The mobile phase composition was acetonitrile–water (AcN–H_2O) (65:35, v/v), ensuring appropriate wetting of the octadecyl surface layer of the stationary phases and a convenient working range for DMA retention factors. In order to determine the silanophilic retention factor k_2 and the silanol binding constant (K_A) of triethylamine (TEA), the retention factors of DMA at different TEA concentrations in the mobile phase were measured. The TEA concentration was varied from 1.5 to 27 mM by mixing AcN–H_2O (65:35, v/v) and AcN–H_2O (65:35, v/v) 30 mM in TEA in the appropriate proportions.

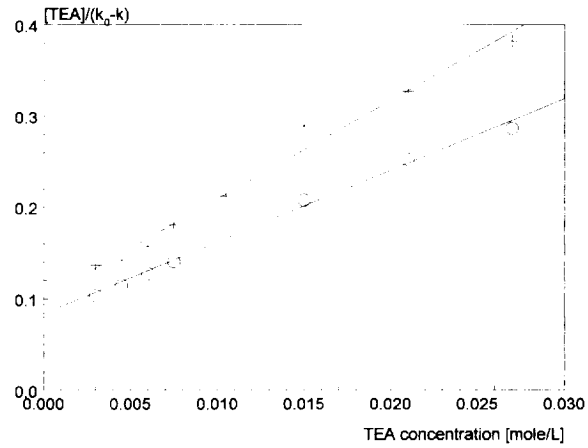

Fig. 5. Graph showing the linear plots of $[TEA]/(k_0 - k)$ vs. [TEA] for the retention of N,N-dimethylaniline on the phases Rx-C_{18} (+) and SB-C_{18} (○) from which the data in Table 6 are derived.

Fig. 5 shows the graphs from which k_2 and K_A can be derived as discussed. The results are summarized in Table 6 and it appears that they confirm the larger silanol activity of the SB-C_{18} phase quite well. The contribution to the overall retention of hydrophobic interactions is quite large. It was noted by Engelhardt [23] that DMA, despite being a strong

Table 6
Results of the determination of the amine–silanol stability constant (K_A) and the silanophilic and hydrophobic retention factor (k_2 and k_1) of N,N-dimethylaniline on the phases Rx-C_{18} and SB-C_{18} using an acetonitrile–water (65:35, v/v) eluent and triethylamine as silanol scavenger (±95% confidence interval as determined by regression analysis)

	K_A	k_2	k_1
Rx-C_{18}	107±21	0.092±0.012	1.78±0.02
SB-C_{18}	94±13	0.128±0.013	1.60±0.02

base, is mostly retained by hydrophobic interactions on reversed-phase materials. The contribution of the silanophilic retention factor is shown here to comprise only about 5–8% of the overall retention factor.

It is important to observe that the amine–silanol complex stability constant (K_A) is the same within experimental error for both columns. This indicates that the residual silanols on the Rx-C_{18} and SB-C_{18} surface are of the same average acidity. Therefore, it is demonstrated here that differences in silanophilic retention behavior of basic solutes on the two phases is related only to the difference in accessibility and number of residual surface silanols and not to an intrinsic difference in interaction strength.

Contrary to K_A, the retention factor for silanophilic interactions (k_2) is significantly larger for the SB-C_{18} phase. The capacity factor due to hydrophobic interactions (k_1) is of comparable value for both phases, indicating that the penetration of solutes in the C_{18} layer is comparable for the Rx-C_{18} and SB-C_{18} phases. Relating the larger k_2 to the larger number of accessible silanols, it can be concluded that the number of residual silanols accessible for N,N-dimethylaniline is a factor of 1.4 larger on the SB-C_{18} surface. On the basis of CP MAS NMR results, it was concluded that the number of silanols not shielded by the alkyl silane ligands, i.e. not cross-polarizable by alkylsilane protons, is roughly a factor of 2.5 larger [13]. The statements already made in the introduction are confirmed: the amount of sterically shielded silanols as determined by ^{29}Si CP MAS NMR were considered minimum values since they were based on the exchange with deuteriumoxide molecules, bigger molecules having more difficulty penetrating the octadecyl silane layer. The data presented here thus illustrate that some of the surface silanols that are not cross-polarizable after deuterium exchange are inaccessible for DMA.

4. Conclusions

All four column tests discussed here indicate that residual silanols play a significant role in the retention of polar and basic compounds on the phases Rx-C_{18} and SB-C_{18}. Nevertheless, both phases are "good" in terms of separation characteristics for these solutes. Moreover, residual silanol activity can play a beneficial role in the separation process as well. It can add just that extra selectivity that is needed to separate solutes with similar hydrophobic properties but with slightly different polarity or basicity.

The results of our earlier ^{29}Si CP MAS NMR study could be at least partly verified. The phase SB-C_{18} has a larger silanophilic index (Engelhardt, Walters), a higher polarity (Galushko) and a larger silanophilic contribution to the overall retention. The latter could be shown to stem from a larger number of accessible residual surface silanols. No difference in interaction strength between residual silanol sites and triethylamine on both phases was found. Because the observed retention differences of basic solutes on Rx-C_{18} and SB-C_{18} columns thus appear to be related to the sterical availability of the silanol sites, the use of bulky groups in the alkylsilane ligand is shown to leave more silanol functionalities accessible for interaction due to a less dense layer of octadecyl chains. This to some extent contradicts the conclusions of Kirkland et al. who claimed the silanols to be shielded more effectively by the bulky di-isobutyl side chains [7]. It is indeed evident that per single side chain more silanols are shielded from acid–base interactions with solutes, but overall (due to the lower surface density of bulky octadecyl groups) more silanols are accessible. Nevertheless, the same bulky side groups significantly contribute to the hydrophobic character of the SB-C_{18} phase, because hydrophobic differences between the two phases were shown to be much smaller than expected on the basis of octadecyl ligand density alone.

Altogether, it is surmised that the solid-state NMR method for determining the content of shielded and free silanols, as described in our earlier paper, has general applicability. This is especially true when the relatively low chromatographic silanol activity of the phases Rx-C_{18} and SB-C_{18} compared to other commercially available phases is considered.

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