Journal of Chromatography, 449 (1988) 1–24
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 611

REVIEW

INFLUENCE OF SILICA SURFACE CHEMISTRY AND STRUCTURE ON THE PROPERTIES, STRUCTURE AND COVERAGE OF ALKYL-BONDED PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received April 18th, 1988)

CONTENTS

1. INTRODUCTION

The vast variety of commercially available bonded alkyl phases from various manufacturers is often blamed for the irreproducibility of chromatographic results. This happens when one compares, *e.g.*, C_{18} bonded phases from various manufacturers and also various batches from the same manufacturer. Various methods for the preparation of the phases are used, various end-capping reactions are applied and various functionalities of the modifiers are introduced, which gives as a result a whole variety of "the same" phases. In this paper, we emphasize that the silica gel which is used in many methods can be a source of many differences among bonded phase materials. It has been generally assumed that silicas with the same physical characteristics such as surface area, pore radii and volume should be chemically the same, but this is not necessarily true.

2. CHEMICAL AND PHYSICAL PROPERTIES OF THE SILICA SURFACE

Silica is undoubtly the most frequently used material in chromatography. It is utilized as an adsorbent in both gas and high-performance liquid chromatography (HPLC). The surfaces of modern fused-silica capillary columns have the same chemical nature and in reversed-phase (RP) HPLC silica is the basic material for synthesis of the packings. Silica seems to be one of the best known inorganic, polymeric materials[1–3]. Despite this new papers devoted to the chemistry of silica surfaces are still being published and new analytical techniques permit unknown structures, behaviours and reactions to be revealed.

2.1. Physical criteria of silica gel for chromatography

The parameters used most often to characterize silica are specific surface area, mean pore diameter, specific pore volume and mean particle size. Although those properties seem to be the most important with the current state of the art, they appear to be inadequate and it is postulated that other important features such as trace metal content[4] and surface pH[5] should be included to characterize the material satisfactorily. It is beyond the scope of this paper to discuss in detail the importance of specific surface area, pore diameter and volume; the comprehensive book by Unger[2] is recommended. A review by Majors[6] is a source of data for commercial silicas.

Another feature of silica is the average wall thickness. Verzele *et al.*[4] calculated the wall thickness for 250–400 m^2/g silica as about 1–2 nm. They took a value of 1.9 g/cm^3 for the specific gravity of silica from papers by Berendsen and De Galan[7] and Strubert[8]; however, Unger[2] reported the specific gravity to be 2.2 g/cm^3. The latter value would obviously increase the average wall thickness to about 2.2 nm for 450 m^2/g silica. Hence we can say in general that the average silica wall consists of about 2–4 Si atoms. This illustrates the importance of trace metals in the silica network, as each metal atom can be located on the surface or just under the surface. In the latter instance such an atom has to interact with a surface silicon atom, which obviously would affect the surface properties of the silica. The influence of the trace metal activity on the properties of silica will be discussed below.

2.2. Silanols as adsorption centres

The surface of silica consists of various kinds of silanols and siloxane bonds. The silanols are considered as strong adsorption sites[9] and can easily be hydrated by adsorbing water molecules. Siloxane sites are usually considered as hydrophobic[2]. Silanols can exist on the surface in single, geminal or vicinal forms. A pair of vicinal silanols can form a so-called bonded pair, which was considered by Snyder[10] and Snyder and Ward[11] to be a "reactive" silanol. Miller *et al.*[12] postulated that geminal silanols can also form "reactive" centres. A variety of physical and chemical methods can be used to determine the surface concentration of silanols[2]. It is generally agreed that a fully hydroxylated surface contains about 8 $\mu mol/m^2$ of OH groups[2,13–15].

The existence of various forms of silanols on a silica surface is generally accepted, but there is a tacit assumption that all silanols interact or react with the same strength. Only rarely one can find in the literature a differentiation between silanols. It is an essential question for chromatographers whether various silanols interact (react) similarly or not. Snyder[10] assumed that silanols should have various "retention activities". However, Snyder and Poppe[14] neglected the interactions of other than single silanols, pointing out that single silanols comprise about 90% of the whole silanol population. Snyder and Ward[11] estimated "chromatographic-grade silica" to contain about 70% of all silanols in "reactive" form, whereas only 1% of hydroxyl groups would form "reactive" sites on a pyrogenic silica surface. In contrast to the "reactive" nature of the site as postulated by Snyder and Ward[11], Hair and Hertl[9] considered bonded vicinal silanols to be capable of adsorbing water only, whereas free silanols were suggested to be the main adsorption or reaction sites, in agreement with earlier studies by Kiselev and co-workers[16,17]. Clark-Monks and Ellis[18] determined that about 10% of pyrogenic silica silanols can form "reactive" sites.

The silica surface can also bear very unusual adsorption sites, sometimes with a very high adsorption strength. Such sites were described in a series of papers by Low *et al.*[19]. Specially prepared silica contained about 0.28 μmol/m^2 (ref. 20) of strong adsorption sites capable of dissociative adsorption of hydrocarbons.

Another strange property of the silica surface was found by Krasilnikov *et al.*[21]. They described the ability of the dehydroxylated silica surface to adsorb oxygen molecules. The surface concentration of the sites was determined to be 0.0001 μmol/m^2 (ref. 21).

2.3. *Apparent pH of silica surface, reactivity of silica silanols*

Commercially available silicas for chromatographic purposes have properties that differ greatly from idealized or theoretically predicted properties. Theoretically, silica should have pK_a = 7.1 ± 0.5 (refs. 22 and 23). However, the literature values of pK_a for silica are 1.5 (ref. 24), 6–8 (ref. 25), 5–7 (ref. 26), 9.5 (ref. 27) and 10 (ref. 28). Surprisingly large differences in the apparent surface pH among silica packings for HPLC were first noted by Engelhardt and Müller[5]. A comparison of the pH values of HPLC silicas is given in Table 1.

The highest pH value was 9.9 for LiChrospher 500 and the lowest 3.9 for Zorbax-SIL[5,29]. It was also shown that the most acidic and the most alkaline pH values were found for spherical silica particles whereas relatively neutral pH values were characteristic of irregular packings[29]. This is probably connected with the methods of manufacture of the silicas[29]. The differences in pH values may be reflected in different chromatographic properties of the silicas[29,30].

The variety of silica surface chemistry can probably also be reflected in the quality of alkyl-bonded phases for HPLC. Broadly observed ranges of pK_a and pH values may imply the existence of silanol groups differing in their acidity. It has been shown that the strength of hydrogen bonds of silanols depend on the acidity of the silanols[22]. One can easily imagine the existence of a broad range of hydrogen-bonded silanols on an amorphous silica surface having a whole variety of bond lengths, which would result in various acidities and reactivities of vicinal hydroxyl groups. The existence of such a broad range of hydrogen-bonded vicinal hydroxyls is shown by a broad and asymmetric IR band with a maximum at 3640 cm^{-1} (ref. 9).

Miller *et al.*[12] have shown that the silica surface contains a group of acidic, reactive silanols which are formed mainly by geminal and hydrogen-bonded species, *i.e.*, vicinal hydroxyls. However Bayer *et al.*[34] were not able to confirm the existence of geminal silanols by means of ^{29}Si cross-polarization-magic angle spinning (CP-MAS) NMR spectroscopy. A higher reactivity of geminal silanols was also reported by

TABLE 1

APPARENT SURFACE pH OF SELECTED SILICAS FOR HPLC

Silica		*Reference*				
		5, 29	*30*	*31*	*32*	*33*
LiChrosorb	Si 60	7.8/8.1*	7.4	7.2		7.12
	Si 100	7.0	7.5	6.7		6.9
LiChrospher	Si 100	5.3	5.8	4.6		
	Si 300	5.5	5.9	4.7		
	Si 500	9.9/8.8*	8.3	8.6		
	Si 1000	9.2	8.6	8.1		
Hypersil		9.0/8.1*	8.5			
Nucleosil 100			6.0		5.15	
100V		5.7	8.7			
Polygosil 60		8.0	7.1	6.5		
Partisil 10		7.5	5.6	4.9		
Spherisorb S5W		9.5	9.0			
Zorbax-SIL		3.9	4.1			
60				5.6	4.08	
150				4.8	4.27	
300				5.4	4.53	
Vydac 90			6.1	4.2		
TP				4.1	5.3	
Separon		4.5				4.5

* Different batches.

Sindorf and Maciel[35]. Blockage of the acidic, reactive silanols appeared to have unexpectedly great effect on the efficiency of the packing. Marshall *et al.*[36] have shown that the initial partial deactivation of about 5% of the total silanol population before the synthesis of an octadecyl-bonded phase leads to a packing with higher efficiency. In a continuation of that work Marshall and co-workers[37,38] drew a similar conclusion to Miller *et al.*[12], *i.e.*, that "on a silica surface a chemically distinct population of associated silanols of high reactivity remains". Marshall and co-workers[36–38] suggested that the reactive group of silanols is about 5% of the whole population of silica hydroxyls. All of the above studies[12,35–38] support in some sense the older concept of the "reactive silanols" of Snyder and Ward[11], which emphasizes a higher reactivity of hydrogen-bonded silanols.

However, Mauss and Engelhardt[39] have shown by Fourier transform IR spectrometry that molecules with basic properties are adsorbed preferentially on acidic, isolated silanols, whereas solutes with hydroxyl groups are able to interact with vicinal hydrogen-bonded silanols and are adsorbed on these sites. Moreover, they found evidence that only isolated silanols react with chlorosilanes. The selectivity of the silica was said to depend on the concentration of surface silanol groups and on the ratio between isolated and vicinal silanols. At about the same time, Köhler and co-workers published two papers[32,40] in which they traced the source of an undesirable adsorption of basis compounds on the silica surface. They concluded that the existence of highly acidic isolated SiOH groups is responsible for the unwanted adsorption of organic bases and also for the low hydrolytic stability of alkyl-bonded phases made of the silica containing such groups. This concept of a higher reactivity of isolated silanols[32,39,40] supports the earlier conclusions of Kiselev and co-workers[16,17] and those of Hair and Hertl[9].

Now, it would be interesting to answer two questions: how many deleterious, isolated silanols exist on silica surface?; and what is the chemical reason for the higher acidity of some silanols or why are some silanols more acidic than others?

Marshall *et al.*[36] estimated that about 5% of the total silanol population should be deactivated before the synthesis of alkyl bonded phases in order to obtain a phase with higher efficiency. In light of Köhler *et al.*'s paper[40], the population can be estimated as a much lower value, as 10 nmol of N,N-diethylaniline (N,N-DEA) were sufficient to deactivate the strongly adsorbing sites in an LC column. It was stated[40] that "NN-DEA is adsorbed on a relatively small concentration of high-energy sites...". This is in agreement with the findings of Nawrocki[41–44], who used an amine to block the strongest adsorption sites in a gas chromatographic column. It seems that a very low concentration of silanols (about 0.3% of the total population) may be responsible for 20% of the retention of benzene. This value was found for a silylated silica surface[41], but similar values have been found for bare silica[42] and trimethylsilylated silica surfaces[43]. It has also been shown that these few, deleterious silanols can considerably influence the column efficiency, which supports Marshall and co-workers'[36–38] efforts to obtain more efficient LC phases by prior deactivation of the silica surface. In a subsequent study[44], the concentration of strongly interacting silanols was found to be lower than 0.1 μmol/m^2 for three gas chromatography grade siliacas.

There is still no answer to the second question, but the content of trace metals seems a likely reason.

2.4. *Trace amounts of metals and their influence on the properties of silica*

The various acidities or reactivities of silanols may be caused by the structure of amorphous silica, *e.g.*, by variations in the distances of hydrogen-bonded silanols[9,12]. This, however, could apply if the strongly reactive silanols were hydrogen bonded as in the concept of Snyder and Ward[11]. It is much more difficult to explain the high acidity of single silanols.

Silica gels utilized in chromatography are not pure silicon dioxide. Chromatographic-grade silica usually contains 0.1–0.3% of metal oxides impurities. Verzele *et al.*[45,46] have shown that chromatographic materials may contain a whole variety of metals with Na, Ca, Al, Mg, Ti and Fe as the prevailing impurities. These traces of metals can obviously influence the chromatographic process[47]. One might expect that such low levels of impurities would not contribute substantially to the retention of solutes. However, taking into account the conclusions in earlier studies[12,36–38], particularly those of Nawrocki[41–44], even these low concentrations have to be considered. We believe that the properties of relatively pure pyrogenic silicas of the Cab-O-Sil or Aerosil type are attributed too often to chromatographic-grade silicas also (although, *e.g.*, Aerosil from Degussa, Frankfurt, F.R.G., has a guaranteed purity of *ca.* 99.8%[48], it is not pure silicon dioxide). Such differences were observed by Hendra *et al.*[49], who reported different behaviours of chromatographic-grade silica and Cab-O-Sil in the adsorption of pyrimidine bases. Similarly, Adams and Giam[50] did not expect localized adsorption of amines on Cab-O-Sil whereas adsorption took place on chromatographic-grade silica. The difference was explained[50] by the presence of metal oxides in the silica matrix which could generate additional adsorption centres with Brönsted acidity capable of chemisorbing amines[49,50].

An *ab initio* calculated of silanol model groups has shown that silanols with the highest absolute acidity are those which are associated with a Lewis acid like boron or aluminium atom[51]. A porous glass surface containing considerable amounts of boron was reported[52] to contain two types of acid centre. These centres differed in their pK_a values, which were 5.1 and 7. The $pK_a = 7$ centres were identified as silanols whereas the $pK_a = 5.1$ centres could not be identified as B–OH sites as these should have lower acidity. Magnesium atoms introduced into the silica structure form so-called "structural Lewis sites", which enhance the acidity of adjacent silanols[53]. Recently Sadek *et al.*[54] described the influence of traces of metals in a silica matrix on the acidity and consequently the hydrogen-bond donating capability of neighbouring silanol groups. They also determined the amount of such strong silanol sites in an HPLC column and, although no surface concentration was given, the values seem to be similar to those published by us[42,43]. The results of Sadek *et al.*[54] confirm our supposition[43] that metal impurities influence the acidity of silanols. According to Sadek *et al.*[54], silica can contain metals in three forms: surface species, internal and secluded. This can be confirmed by acid washing results[45–47]. According to Verzele and co-workers[45–47], acid washing can remove only one third to two thirds of metals from the silica.

A number of interesting facts can be found in the literature concerning the adsorption of amines or furan on a silica surface. It has long been known that the heats of adsorption of such compounds are much higher at very low coverages (of the order of 0.1 μmol/m^2) than at higher coverages[55–57]. These high values were connected with traces of metals in the silica matrix[56–58]. Traces of aluminium were shown to increase the catalytic activity of silica, although some intrinsic activity of the pure gel was suspected[58]. Hydrocarbons, both saturated (hexane) and unsaturated (benzene), appeared to have much higher heats of adsorption at very low coverages (below 0.1 μmol/m^2) than at higher surface concentrations[59]. Similarly, higher heats of adsorption of *n*-hexane and benzene on fume (pyrogenic) and precipitated silicas were found in the low coverage region[60]. Papirer *et al.*[60] also emphasized the difference in the distribution of surface silanol groups on precipitated and fume silicas of identical surface area.

Cusumano and Low[61,62] have shown that the residence time of an adsorbed molecule of benzene calculated from the Frenkel equation at low coverages can approach *ca.* 100 s, which is expected for chemisorption, whereas for higher coverages the residence time decreases to 10^{-8} s, *i.e.*, by ten orders of magnitude. Although this was calculated for the adsorption of benzene on porous glasses, the same must be true for porous silicas as the heats of adsorption of benzene are similar on both adsorbents.

2.5. *Heterogeneity of the silica surface*

Most known surfaces are heterogeneous. Heterogeneity of the surface is, in simple words the ability of some parts of the surface to interact more strongly with adsorbates than do other parts of the surface. The phenomenon of heterogeneity plays an important role in catalysis and chromatography. Acidity or basicity of the surface can be considered as a manifestation of the heterogeneity, as these phenomena are directly connected with particular centres on the surface. Acidity or basicity can be investigated by both static[63] and dynamic[64,65] methods.

Rudziński and co-workers[66,67] have found five different adsorption centres on the silica surface. These sites were said to represent various silanol and siloxane centres and were ordered in terms of their strength of interaction with hydrocarbons as: reactive > geminal > free > bonded > siloxane. This reminds one of Snyder's[10] classification of the adsorption activity of silanols: reactive > free > bond. Rudziński and co-workers[66,67] found that the strongest adsorption centres on the silica surface comprise 1.5–13% of the total number of centres, depending on the silica gel and the solute chromatographed. It has been suggested[66,67] that a considerable part of hydrocarbon adsorption proceeds on siloxane centres, which cannot be supported by other (spectroscopic) data. Hair and Hertl[9] have shown that the silanols are the adsorption centres, particularly single or free types, while bonded species interact weakly with hydrocarbons. In another study applying the same model of Rudziński and co-workers[66,67], only three adsorption sites were found on the silica surface, with strengths: reactive > free > Si^{4+} (ref. 68). The silica was thermally treated[68]; however, there is no spectroscopic evidence in the literature for adsorption on Si^{4+} sites on the silica surface. In another paper[69] from the same university it was stated that Rudziński and co-workers' method can be considered as a kind of approximation and the maxima on the energy curve cannot be ascribed to specific functional groups on the silica surface. The same theoretical model was applied to show how very small amounts of water block the strongest adsorption sites on a kaolinite surface[70].

An older concept of heterogeneity, which allowed the site energy distribution to be estimated from the experimental adsorption isotherm and an arbitrarily chosen local isotherm function, was used by Sorrell and Rowan[71] to show that modification of silica can hardly remove the highest energy sites from the surface. They also showed[72] that modification of the silica surface increases the efficiency of a chromatographic column.

^{29}Si CP-MAS-NMR has been applied to show that the silica surface can be considered to be heterogeneous, *i.e.*, consisting of separate regions resembling the 100 and 111 faces of β-crystobalite[73].

In the view of Farin and Avnir[74], the heterogeneity of adsorption sites originates from a homogeneous distribution on a geometrically irregular surface. They suggested that a concave zone increases the number of hydroxyl groups per unit whereas a convex zone decreases this number.

Deactivation was shown to eliminate sites with extremely high activity for an alumina surface[75]. The interactions with the sites were said to be the only mechanism that contributes to the c_k term in the plate-height equation for GSC capillary columns.

In high-performance adsorption chromatography, the presence of strong adsorption sites on an adsorbent (silica) surface requires the addition of water to the non-polar solvent to deactivate the sites. However, water was found to be inconvenient for such a purpose and organic modifiers were proposed instead[76]. Saunders[76] emphasized that the existence of these strongly adsorbing sites greatly affects the sample capacity. Generally, the chromatographic literature stresses the negative role of heterogeneity. However, it was found theoretically that energetically homogeneous surfaces may exhibit a lower selectivity than heterogeneous adsorbents having the same average adsorption energy in relation to the substances being chromatographed[77].

2.6. Small populations of highly retentive silanols and their influence on retention data and properties of chromatographic columns

There is agreement that the strongest adsorption sites constitute only a small part of the total number of sites on the silica surface[36,40,66,67]. This was fully confirmed by a recently developed method for the determination of the concentration of the strongest adsorption sites[41–44]. First, it was noted that on relatively poorly deactivated silica surfaces a very small amount of amine blocking the adsorption sites drastically reduces the retention of benzene[41]. The method was developed further and its usefulness for the evaluation of the distribution of the strength of retention sites on bare silica surfaces was demonstrated[42].

The principle of the method is very simple and is illustrated in Fig. 1. Although the experiments were carried out by means of gas chromatography, the method is

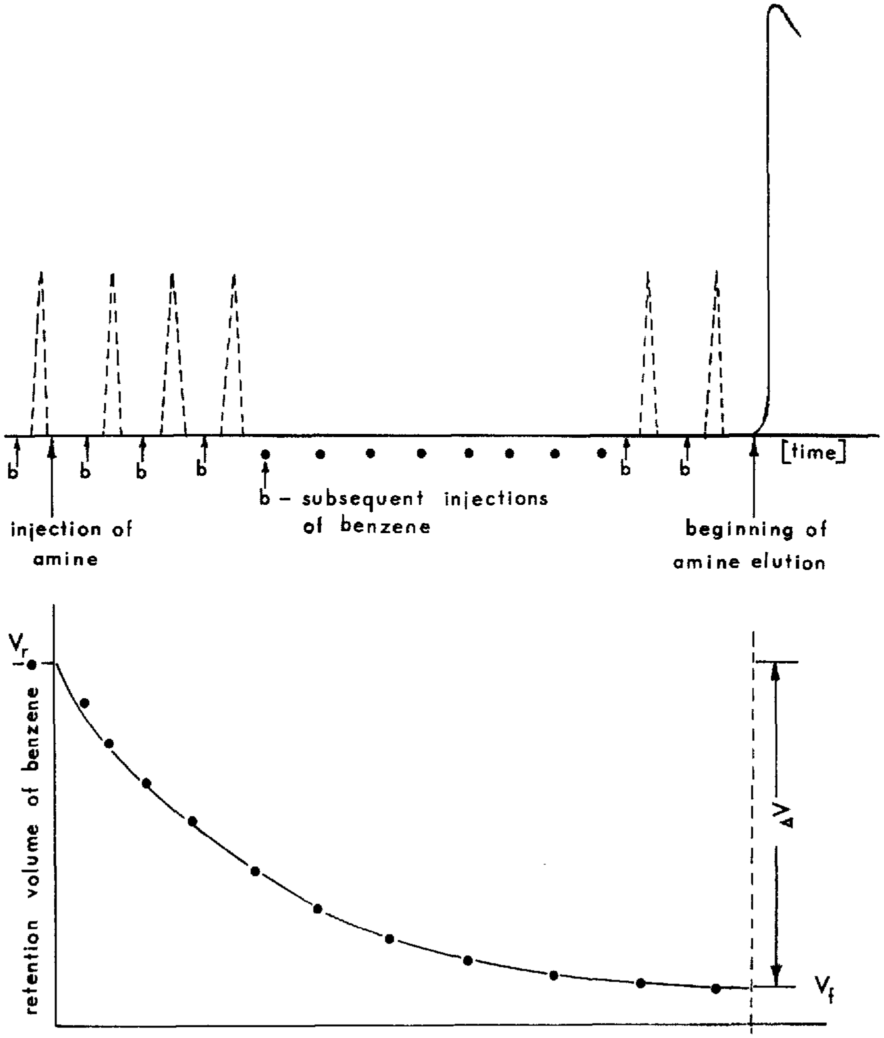


Fig. 1. Principle of the method.

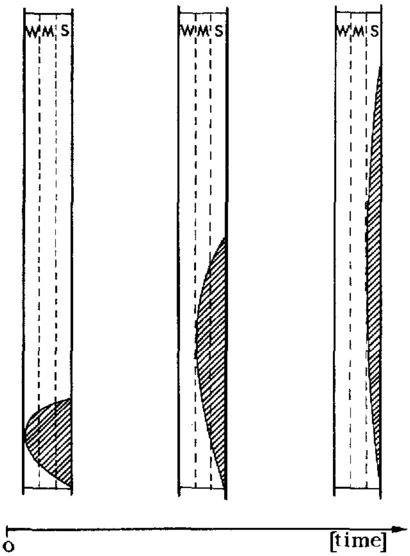


Fig. 2. Development of the amine band along the column. W, M, S = Weak, medium and strong adsorption sites, respectively, on the surface of the adsorbent.

generally chromatographic in nature and it can be performed using other chromatographic techniques. After measuring the retention volume of, *e.g.*, benzene or another hydrocarbon solute, a known amount of amine is injected into the column containing modified or bare silica. The amine is very strongly retained. Slowly a concentration profile is developed along the column. At the beginning the amine molecules will occupy the various, *i.e.*, weak (W), medium (M) and strong (S), adsorption sites. This is shown in Fig. 2. The desorption of the molecules will occur mainly from the weak (W) and medium (M) sites. Slowly the amine molecules will be adsorbed mainly on the strongest (S) adsorption sites available.

The very long retention time of the amine permits the retention of, *e.g.*, an unsaturated hydrocarbon to be measured in the presence of the amine in the column. Usually there is sufficient time to make several injections (up to tens) of the hydrocarbon before the amine begins to elute. Subsequent injections of the hydrocarbon reveal a decrease in retention time, as shown in Fig. 1. This gradual decrease in retention indicates that the amine blocks the strongest adsorption sites on the adsorbent surface.

According to Hair and Hertl[9] and Curthoys *et al.*[78], silanols on a silica surface can be considered to be the main adsorption sites and the adsorbate–adsorption site ratio can be assumed as 1:1. As a known amount of amine is injected into the column, the observed decrease in the retention volume of the hydrocarbon, ΔV, (Fig. 1) can be simply related to the blockage of a known number of adsorption centres. In other words, we can say that the amount of surface species blocked is responsible for the part which equals ΔV in the hydrocarbon retention.

Now, on injecting various amounts of the amine we shall obtain different ΔV values. These can be used for the evaluation of the distribution of the strength of the surface sites. The distribution curve can be obtained by plotting the relative ΔV values against the surface concentration of the blocking reagent, *i.e.*, amine. The relative ΔV values are calculated by dividing ΔV by the amount of the amine blocking agent, A (μmol):

$$\Delta V_R = \frac{\Delta V}{A}$$

The physical meaning of ΔV_R value is the decrease in the retention volume of the hydrocarbon caused by 1 μmol of the blocking reagent at a given surface concentration of the reagent. Since the surface concentration of the reagent is simply equal to the surface concentration of the blocked sites, we obtain direct information on the surface concentration of strongly adsorbing sites.

For this method the term "gas-phase titration" was proposed[43] as the distribution curves form straight lines with characteristic deflection points. Such a point can be considered as a "titration end-point" and it probably indicates the surface concentration of a specific subset of strongly interacting sites. It was shown that one or two such points can be found for bare or modified silica surfaces[42–44]. The method can also determine the relative strengths of interactions of strongly retaining silanols among various adsorbents[44]. However, the most interesting aspect for chromatographers was evidence that these few silanols are responsible for substantial broadening of the chromatographic band[43]. It was shown[43] that the blockage of less than 0.1% of the available silanols on a trimethylsilylated silica surface can considerably increase the efficiency of the column. This directly supports Marshall and co-workers'[36–38] attempts to make more efficient columns for HPLC by prior deactivation of the small, reactive population of silanols. This also supports much earlier theoretical predictions by Giddings[79] that "a few active sites on the solid support may lead to tailing".

2.7. *Conclusions*

The above-described facts can be summarized in the form of the following conclusions:

(i) the surface of chromatographic-grade silica differs from that of pyrogenic silica (Cab-O-Sil, Aero-Sil)[49,50,60];

(ii) there exists a small group of highly acidic, reactive silanols on the silica surface[12,32,37,39–44];

(iii) the reaction of silica surface silylation should proceed mainly on acidic sites[35], but probably for steric reasons some of these highly acidic sites remain unreacted[12,32,38];

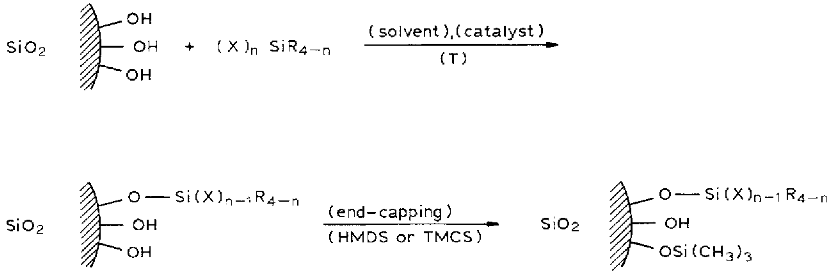
(iv) these few, highly reactive sites can have an important influence on column efficiency[36–38,43] and sample capacity[76,79], which is particularly important in preparative chromatography;

(v) metal impurities are the most likely reason for the enhanced acidity of some isolated silanols[54].

3. CHEMICALLY BONDED PHASES FOR HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHY

Bonded phases are widely used in HPLC and it is estimated that about 80% of chromatographic analyses are carried out with these phases[80]. However, widely differing capacity factors and selectivities are observed for the same solutes using various commercially available reversed-phase (RP) silica packings of the same type[81]. Thus, it seemed to be of interest to trace the possible sources of such differences. The aim of this section is to indicate the influence of various factors on the properties of reversed-phase silica packings.

Generally, the formation of an HPLC packing involves the reaction of an organosilane with a silica surface:



This scheme shows that the quality of the final result, *i.e.*, the RP packing, can depend on the following factors: silica surface, functionality of the silane, solvent, catalyst, temperature and end-capping reagent.

Statistical weighting[82,83] of the variables of the reaction has shown the following relative importance: functionality of the reagent, presence of acid scavenger (or catalyst as in the above scheme), number of silanizations with principal reagent, stoichiometry of silanizing reagent over the theoretical amount, hydroxylation of the silica surface, solvent pretreatment and type and functionality of the capping reagent. A further fourteen variables were said to be insignificant[82]. However, it must be stressed that carbon coverage was used for the judgement between good and poor results, which does not necessarily reflect the good or poor chromatographic quality of the phase. Moreover, only one kind of silica material was used[82,83], which seems to be insufficient to draw conclusions about the importance of the variables.

Many papers have been devoted to the influence of the functionality of the reagents on the reaction. Functionality can mean here both the number of functional groups able to react with surface silanols or the chemical nature of the groups. Monofunctional silanes are said to result in well defined products whereas di- and trifunctional silanes can produce oligomer or polymer layers on the silica surface. Although monomeric phases are usually considered to have the most reproducible properties, manufacturers use mono-, di- and trifunctional silanes to produce bonded phases[34,84]. Oligomer or polymer phases can sometimes have better chromatographic selectivity than monomer phases[31,85,86]. The reactivities of various functional groups were compared for *n*-octyldimethylsilanes by Lork *et al.*[87], who found the following sequence: $N(CH_3)_2 > OCOCF_3 > Cl \gg OH \approx OCH_3 \approx OC_2H_5 \gg$ –O– (siloxane).

Only limited studies have been carried out to establish the importance of the solvent[82,83,88]. The role of the solvent has probably been underestimated, and the solvent is probably much more important when a catalyst (amine) is used as an acid scavenger. Jones[82,83] proposed the application of a chlorinated solvent (1,1,2-trichloroethane) whereas Kinkel and Unger[88] found dichloromethane, dimethylformamide or acetonitrile to be the best choice. A similar recommendation of chlorinated hydrocarbons as solvents was made by Little *et al.*[89]. However, the densest coverages were obtained when *n*-hexane[90–97] or toluene[98,99] was applied as a diluent.

As the temperature of the reaction mainly depends on the solvent chosen, it will not be discussed in detail. The influence of temperature on the reactivity of various functional groups was reported by Lork *et al.*[87]. The role of amines and end-capping reactions will be discussed in section 3.2.

3.1. Physical and chemical requirements for silica

Silica is the base on which the silanization reaction proceeds, so it can obviously determine the resulting properties of bonded phase packings. It is obvious that silica should satisfy various requirements before it can be used efficiently for the preparation of phases, and these requirements are both physical and chemical in nature. Physical requirements can concern the silica macro- and micro-properties, and particle size, shape and distribution can be considered as macro-physical requirements. These parameters are usually well recognized by chromatographers and their influence on chromatographic efficiency is well known[2]. Apparent density, another macro-physical parameter, has been postulated to be important[4]. The apparent density of chromatographic-grade silica is usually between 0.4 and 0.6 g/cm^3 whereas the ideal value is said to be 0.45 g/cm^3 [4].

The influence of the micro-physical properties of silica on the quality of bonded-phases is not always realized and for this reason it will be reviewed below. Specific surface area, mean pore diameter, specific pore volume and pore size distribution will be taken into account. The first three of these parameters depend on each other. Hence the influence of one of these parameters on the quality of a bonded phase cannot be checked by keeping the other two parameters constant and changing only the one being investigated.

The specific surface area of the silica used for the synthesis of bonded phases usually lies in the range 150–400 m^2/g, but it must be pointed out that for size-exclusion chromatography much lower values are required[2,4]. The mean pore diameter should be in the range 6–10 nm, with an ideal value of 8 nm according to Verzele *et al.*[4]. The specific pore volume of silica is usually higher than 0.2–0.3 ml/g and lower than 1.5 ml/g; Verzele *et al.*[4] consider 0.7 ml/g to be an ideal value. Pore size distribution seems to be one of the most important physical parameters. A homogeneous distribution of pore sizes, *i.e.*, resembling a Gaussian distribution, should be the main requirement. The narrower the distribution curve the better is the silica material, but it should be emphasized that the actual range of pore diameters usually spans an order of magnitude and the mean pore diameter which is given by the manufacturer is only an average. This means that on any silica surface a variety of pores exist.

Pores of diameter less than 2 nm (micropores) are particularly undesirable.

Berendsen *et al.*[100] have shown how the curvature of the pores can influence the final coverage of the bonded phase. This effect is of greater importance the longer is the alkyl chain anchored on the pore surface, *i.e.*, the effect has to be taken into account particularly for the most common octadecyl phases. Bearing in mind that an extended conformation of a C_{18} phase is 2.45 nm long, the maximum coverage cannot be obtained on silicas with narrow pores. Berendsen *et al.*[100] stated that for pore diameters smaller than 12 nm the pore curvature will decrease the coverage. Silicas with narrower pores would probably show a tendency to produce ink-bottle-shaped pores after silanization[100], as the initial silanization of the cylindrical pores at the edges would prevent other molecules of ODS reagent diffusing inside the pores. Smaller pores existing in the structure will be only partly silanized, which is why an "end-capping" procedure is necessary to cover as many silanols as possible. Sander and Wise[31,85,86] tried to evaluate the bonded-phase selectivity differences for various pore diameters. For monomeric phases they observed a decrease in retention with increasing pore diameter. The observed decrease in retention was caused by the decrease in the specific surface area for packings with large pore diameters. However, it must be emphasized that the phases in question had much lower coverages than predicted theoretically. For polymeric phases synthesized on the same silicas, the selectivity was observed to change as a function of the pore diameter. The best separations were achieved on packings of large pore diameter.

Similar investigations were carried out by Engelhardt *et al.*[101], who showed that wider pore silica permitted phases with higher coverage to be obtained. A packing obtained from narrow-pore silica (mean pore diameter 3.5 nm) was shown to be much less efficient than the wider pore materials.

Chemical requirements are considered much less often than physical requirements by most chromatographers. The most important chemical criteria are a fully hydroxylated silica surface, suitable pH of the silica surface and a low content of trace metal impurities.

It has been known for many years that silica used for the synthesis of bonded phases must be dry, *i.e.*, it must not contain physically adsorbed water. Water was shown to have a deleterious effect on the coverage of alkylsilyl groups on the silica surface[87]. For this reason, silica is always thermally and vacuum treated before the reaction in order to remove physically adsorbed water[86,87,102–107]. Thus it seems surprising that the preliminary dehydration of silica was not considered to be a significant variable in the statistical optimization of the silanization process, whereas hydroxylation of the silica surface appeared to be an important variable[82]. Extended investigations of various methods for the rehydroxylation of silica surfaces were recently carried out by Köhler and Kirkland[32]. In view of their findings, the hydroxylation of the surface seems previously to have been an underestimated parameter. They stated[32] that fully hydroxylated silica exhibits (1) a larger number of associated silanols, (2) a higher pH, (3) a markedly lower adsorptivity for basic compounds, (4) a significantly improved hydrolytic stability of bonded phase ligands and (5) increased mechanical stability. Köhler and co-workers[32,40] found that chromatographic-grade silicas often contain only 5–6 μmol/m^2 of silanols. Rehydroxylation has so far usually been accomplished by heating in aqueous acids or by extensive boiling in water. For example, Hennion *et al.*[103] and Kingston and Gerhart[108] boiled silica in concentrated nitric acid–sulphuric acid (1:1) to obtain a fully hydroxylated surface.

Gobet and Kováts[109] have proposed a reproducible method for obtaining a fully hydroxylated surface of fume silica (Cab-O-Sil). Their procedure consisted of thermal treatment at 1173 K for 120 h, then rehydratation for 70 h in boiling water and subsequent freeze-drying of the wet silica. Such a procedure was sufficient to ensure maximum coverage with trimethyldimethylaminosilane of the order of 4.75 μmol/m^2. Köhler and Kirkland[32] found this procedure to be inadequate to give total rehydroxylation of the silica surface, which again confirms our belief (section 2) that the surfaces of fume and chromatographic-grade silicas are not completely identical. Köhler and Kirkland[32] recommend rehydroxylation of the silica surface with certain basic activators, *e.g.*, treatment with dilute ammonia solution at room temperature, tetrabutylammonium hydroxide or ethylenediamine. They also found that treatment with 100 ppm of hydrofluoric acid for 24 h led to a support with a low adsorptivity for organic bases[32].

A different concept concerning the silanol concentration was proposed by Scott and Kucera[110], who employed a thermal treatment for reducing the silanol content on silica. Later it was found by Van de Venne[111] that thermal pretreatment up to 400°C reduces the number silanols but does not affect the carbon content.

Welsch and Frank[112] developed the so-called "high temperature silylation" at 250°C, which led to sufficient alkyl coverages (*ca.* 3.6–3.8 μmol/m^2) and simultaneously reduced the number of remaining silanols. The resulting materials were shown to be comparable to commercial bonded phase packings. Frank[113] also showed that asymmetry of phenol and benzyl alcohol peaks was highest for certain silanol surface concentrations (*ca.* 3 μmol/m^2) whereas higher or lower silanol concentrations greatly improved the symmetry of the peaks.

The next question to consider is the problem of surface pH. As discussed in section 2.3, different surface pH values can manifest themselves in various chromatographic properties when normal-phase chromatography is considered[29,30]. However, the surface pH was shown to affect the packing properties of alkyl bonded phases made of silica. Engelhardt *et al.*[101] have shown that bonded phases made of basic silica were more suitable for the separation of basic solutes, whereas the opposite was true and bonded phases based on acidic silica gave better efficiencies for the separation of acidic compounds.

Köhler and Kirkland[32] pointed out that the rehydratation procedure usually increase the pH and a low adsorptivity of bases was observed for silicas with surface pH ranging from 4.80 to 6.89. However, these two extreme values seem to be exceptions as the remaining silicas have pH values in a much narrower range of 5.15–5.59. Trimethylsilylated silicas obtained from these rehydrated silicas were covered with 3.47–5.43 μmol/m^2 of TMS[32,40]. The last value seems surprising in view of the Van der Waal's radius of the trimethylsilyl group. The concentration of the densest trimethylsiloxy layer was calculated by Boksanyi *et al.*[114] to be of the order of 4.7 μmol/m^2 [trimethylsilyl enolate (enolate of pentane-2,4-dione) was used for the synthesis of the densest layer[32,40] and the same reagent was earlier shown to give an even higher density of 6.6 μmol/m^2 (ref. 115)]. Their "good" chromatographic properties were judged by chromatographic experiments rather than from the coverage values.

The final chemical requirement for silica is a low trace metal content. As discussed in section 2.4, traces of metals can seriously affect the chromatographic process, as was shown by Verzele and co-workers[45–47] and Sadek *et al.*[54]. Boiling on an RP C_{18} packing in hydrochloric acid was recommended for removal of the metals, whereas the application of EDTA was said to be ineffective[45–47]. In contrast, Köhler *et al.*[40] have shown that washing silica with 1% Na_2EDTA solution can remove all the trace metals from the matrix. This seems surprising in view of Verzele and co-workers' attemps to remove traces of metals by extensive washing with acids[45–47]. Our experience[116] confirms Verzele and co-workers' results. As the procedures described above for the rehydration of a silica surface would be also effective in removing some metal traces, it is possible that the removable part of the trace metals is washed out during the rehydration process, which should obviously improve the chromatographic properties of the silica.

3.2. Dense coverage packings

The term "dense coverage" can be reasonably used only for chemically bonded phases prepared from monofunctional silanes. Attempts to make the densest possible organic layers bonded to a silica surface were begun in order to suppress the interactions of unreacted silanols. Colin and Guiochon[117] stated that "For extensive coverages ... the unreacted silanols do not have a critical role. Theoretically it is possible to change them into inactive species by reaction with DMCS or TMCS". This statement was made 11 years ago during the early development of chemically bonded phases. Today it is generally accepted that the unreacted silanols play an important role in the mechanism of retention[118,119] and for steric reasons the remaining silanols cannot be converted into inactive species with TMCS reagent and DMCS is no longer used for "end-capping". The maximum coverage on a silica surface can be calculated from the space requirements for a given molecule. This, however, needs a further assumption of a flat silica surface. This assumption can be true only for small molecules. The space requirement for a trimethylsilyl (TMS) group has been calculated to be 0.37, 0.35 or 0.43 nm^2, depending on the equation or method applied[2]. From these data, an average value 0.38 nm^2 per TMS group gave a maximum surface concentration of 4.3 μmol/m^2 of TMS. A very close value, namely 4.2 μmol/m^2, was obtained by Unger *et al.*[104]. The surface concentration of TMS groups calculated from the density of trimethylchlorosilane is about 4.7 μmol/m^2 and Boksanyi *et al.*[114] obtained 4.7 μmol/m^2 by the ammonia-catalysed reaction of trimethylsilanol with Cab-O-Sil M5. The space requirement calculated from such a coverage equals 0.32 or 0.28 nm^2 per group, depending on the arrangement of the groups on the surface[114]. These values, according to Berendsen's[120] calculations, would indicate a rigid position of the TMS group and an Si–O–Si angle of 110–150°. Berendsen[120] has shown that the cross-sectional area of the TMS group depends on the Si–O–Si angle and the rotation of the group. Taking into account the space requirements of the TMS group calculated for angles in the range 110–180° with and without rotation, he assumed the highest surface coverage with the group to be 3.82 μmol/m^2 (2.3 nm^2 per TMS group), despite the fact that the author[20] easily exceeded this value, obtaining 4.16 μmol/m^2, in the same work. However, for longer alkyl radicals lower coverages were obtained, which was explained by hindered diffusion of the reactant into smaller pores. Unger *et al.*[104] considered prolonged heating at high temperature would ensure a dense surface coverage. However, attempts to obtain the dense coverages soon went in other directions, *i.e.*, a search for a silane with a more reactive functional group. A variety of such groups were examined[87,115] and the most reactive seems to be the N,N-dimethylamino group, which was introduced into chromatography by Kováts and co-workers[90,121]. The application of N,N-dimethylaminotriorganylsilanes permitted a number of densely silylated silica surfaces to be obtained with coverages above 4 μmol/m^2 (refs. 91, 109, 122, 123). Trimethyl(dimethylamino)silane applied at 125–275°C for 120–170 h gave a theoretically calculated[114] value of 4.77 μmol/m^2 of TMS[91], whereas for longer radicals the values decreased as the alkyl chain length increased. The coverages obtained were 4.52 μmol/m^2 for ethyl, 4.31 μmol/m^2 for propyl, 4.24 μmol/m^2 for tetradecyl and 4.20 μmol/m^2 for octadecyl[91]. All of these results were achieved on Cab-O-Sil M5 fume silica hydrated according to the described procedure[109] and are much higher than the values predicted by Berendsen *et al.*[100]. No value for the pore volume of hydrated Cab-O-Sil silica was given by Kováts and co-workers[48,91,109,122,123], but it can be calculated roughly from the nitrogen adsorption isotherm[109] to be *ca.* 0.41 cm^3/g. This value and specific surface area of 179 m^2/g allow the mean pore diameter (D) to be calculated according to the Wheeler equation[2] as D = 9.2 nm. This low value, according to Berendsen *et al.*[100], would permit a coverage of C_{18} ligands lower than 2.5 μmol/m^2 to be obtained. Although hydrated and silanized samples of Cab-O-Sil were not used in HPLC, probably because of their low apparent density of 0.15 g/cm^3, very similar coverages with TMS groups were obtained on precipitated silica surfaces and on LiChrosorb Si 100 chromatographic-grade silica[48].

Approximately at the same time, Serpinet's group started to investigate phase transitions in very compact octadecyl bonded phases. The phases were synthesized according to Kováts' procedure[90] on wide-pore silica gels[92,93]. Morel and Sepinet[92,93] showed that under gas chromatographic conditions the grafted alkyl chains undergo a melting-like phase transition and the transition for the octadecyl radical occurred at ambient temperature, which was said to change the retention characteristics of the phase. The coverages obtained were 4.13–4.64 μmol/m^2, depending on the support. Subsequently the same authors[94] confirmed the occurrence of the phase transition under liquid chromatographic conditions for a C_{22} bonded silica. The work on densely covered phases was continued[95–97], always using phases with surface concentrations higher than 4 μmol/m^2. The authors[97] concluded that the widespread choice of the C_{18} ligand was probably unfortunate as the phase undergoes a transition over the entire laboratory temperature range. For that reason, Morel *et al.*[97] advocated a C_{16} phase instead of the C_{18} phase.

The same reagent, N,N′-dimethylaminooctadecyldimethylsilane, and the same procedure according to Kováts[90] were shown to produce a densely covered ODS phase for HPLC[124] on wide-pore silica. The packing obtained had much better chromatographic properties than sparsely covered phases obtained from chloro and methoxy derivatives[124]. A high-density (>4 μmol/m^2) of grafted alkyl ligands was shown to be relatively easily obtainable even with chloro derivatives on controlled porosity glasses[125–128].

The application of basic catalysts seems to be another means of producing high-density reversed phases. Pyridine was used as a hydrochloric acid scavenger[102,129] in the reaction of trichlorosilanes with silica. Berendsen *et al.*[100] also used pyridine when bonding with monochlorosilanes. All of these reactions[100,102,129] were carried out in toluene. The coverages for the ODS phase obtained by Berendsen *et* *al.*[100] were 3.04 and 3.28 μmol/m^2. Kinkel and Unger[88] have shown that the role of both the amine and the solvent are much more complicated than had previously been believed. According to Kinkel and Unger[88], the solvent can have a considerable effect on the Si–O bond strength, can also activate the silica atom of an organohalosilane and finally can influence the reactivity of the added base. However, in order to be able to predict an optimum solvent–base combination, much more work has to be done. It was shown also that the type of base does not cause substantial differences in the kinetics of the silanization reaction. The *n*-octyl ligand densities obtained were scattered around 3.01 μmol/m^2 for most of the bases. The best result of 3.53 μmol/m^2 was obtained when the reaction was catalysed by imidazole. Berendsen *et al.*'s values[100] for the *n*-octyl phase were 3.49 and 3.45 μmol/m^2. A comparison of the results[88,100] favours Berendsen *et al.*'s methodology, especially as Kinkel and Unger[88] used wider pore silica. All of the results are much lower, however, than the "true" dense phase obtained via N,N′-dimethylaminoalkylsilanes[92,94].

In contrast to Kinkel and Unger's[88] investigations of the role of base catalysts, Buszewski and co-workers[98,99] found a clear relationship between pK_a value of the base and the obtained bonded phase coverage obtained. Morpholine (pK_a = 8.33) was shown to be the most efficient catalyst for the bonding reaction. The same activator was also used in the synthesis of densely covered controlled porosity glasses[130]. As the dense coverages of 4.24 μmol/m^2 obtained are higher than predicted from Berendsen *et al.*'s model[100], Buszewski and Suprynowicz[99] criticized the model as unrealistic.

The importance of amines as catalysts and not as simple acid scavengers was shown by Boksanyi *et al.*[114], who tried numerous compounds as catalysts of the reaction of triorganylsilanols with silica and found amines to be the best.

3.3. *Models of bonded phase structure*

Since the initial experiments on surface-anchored groups, attention has been devoted to the explanation of the chromatographic behaviour of the bonded phases and for this purpose various models of the phases have been proposed. The first models were static whereas the later models assumed flexibility and mobility of the ligands.

Historically, Halász and Sebestian[131] were the first to introduce the static model of an esterified surface of Porasil. The model, well known now as a "bristle" or "brush", dominated the thinking of chromatographers for about 10 years[132]. Hemetsberger *et al.*[133] noticed that the bonded alkyl radicals are not rigid rod-like structures but are rather folded or pressed into the surface. This description has been known since then as a "blanket" model. Another static model of "alkyl grass" was proposed by Scott and Simpson[134]. They noticed that hydrocarbon chains anchored on the silica surface can associate with themselves at low methanol or high water concentrations. They also showed that the phase needs a significant time to reach equilibrium with the mobile phase. In contrast to monomeric phases, polymeric bonded phases were said to be held fairly rigidly in the structure of the phase.

Scott and Simpson's findings were generally in agreement with Lochmüller and Wilder's[135] concept of microdroplets and Gilpin and Squires'[136] suggestion of considerable interactions of hydrocarbon chains among themselves under certain conditions. Lochmüller and Wilder's first concept of aggregation of alkyl chains to form "bundles"[135] was later confirmed in a study of the luminescence of pyrene molecules chemically bonded to microparticulate silica gel. That study resulted in the "microdroplet" model[137,138], which assumes a non-homogeneous distribution of silanols on a fully hydroxylated silica surface, which subsequently leads to clusters of covalently bonded molecules. Their conclusions were recently criticized by Farin and Avnir[74,139], who pointed out that the silanols are homogeneously distributed on the silica surface but various curvatures of the surface may result in different accessibilities of the silanols. First, Lochmüller and Wilder's assumption of a flat silica surface was said to be unrealistic, in addition to their view of the pore network, *i.e.*, with the silica being treated as a zeolite-like material with one typical pore size[74,139], whereas in fact the pore diameters of silica are usually distributed over a fairly wide range. However, the idea of a non-uniform distribution of silanols and consequently of bonded alkyl chains was supported by a chromatographic study of the entrapment and release of solvent[140] and also by NMR[141] and infrared investigations[142]. On the other hand, taking into account Farin and Avnir's[74,139] arguments and the pore size distribution in chromatographic-grade silica, it is still disputable whether the observed clustering of bonded alkyl groups occurs because of a non-homogeneous distribution of silanol or whether it occurs in pores of smaller diameters. To judge between these two possibilities one would have to have access to more detailed data on pore size distribution, *e.g.*, for Partisil 10, which was used by Lochmüller *et al.*[137,138]. Consequently, similar[137,138] experiments could be carried out on very wide-pore silica to avoid the influence of small diameter pores. The above discussion emphasizes again our belief of the importance of pore size distribution in the production and understanding of bonded phases for HPLC.

Another model was proposed by Gilpin and co-workers[136,140] in which both folded and bristle states are possible and transitions between the two take place with changes in temperature or solvent. Such a model was confirmed by Fourier transform IR spectrometry by Sander *et al.*[143]. According to their study, a disordered "folded" state is favoured when the packing is either dry or in the presence of a non-wetting mobile phase, and a more ordered "bristle" state is favoured when the chains are wetted with a mobile phase of high organic content. At that time it became increasingly clear that the alkyl chains possess a certain molecular mobility. Such a motion was confirmed mainly by numerous NMR studies. CP-MAS NMR investigations have shown an increase in motion towards the end of the chain[144]. Measurements of spin–lattice relaxation data for ^{13}C-labelled bonded alkyl chains indicated that rotation of the terminal methyl carbon atom is the dominant factor in the overall mobility of the chains in solvated conditions[141,145]. A decrease in end-group mobility was also observed to occur when a certain critical surface concentration of the alkyl groups was attained. These conclusions were in agreement with earlier suggestions by Sindorf and Maciel[144]. The results of ^{29}Si and ^{13}C CP-MAS NMR studies of RP-HPLC packings[146] have shown that under conditions typical of chromatographic separations the alkyl chains assume different conformations. There are several factors governing the behaviour of the chains: interactions of the chains with themselves, with neighbouring chains, with trimethylsilyl groups, with the mobile phase and with the solutes. The extended conformation of the chain appears to be more flexible while the increasing polarity of the mobile phase increases the rigidity of the bonded alkyl groups, *i.e.*, the collapsed structure appears to be more rigid. Bayer *et al.*[146] noticed that the process of end-capping reduces the mobility of long chains. This means that in uncapped packings the alkyl chains will be more extended whereas in capped packings they will have a tendency to collapse. This indicates the importance of the unreacted silanols for interactions with solute and solvent molecules.

The investigations of densely grafted bonded phases by Serpinet and co-workers[92–97,147] led to the proposal of another model, a "solid condensed–liquid expanded" phase. Based mainly on gas chromatographic experiments, the model assumes the existence of two temperature-controlled states of the phase. At lower temperatures the "solid condensed" state is the most likely whereas an increase of the temperature causes a transition of the phase into a "liquid expanded" state. The transition results in an irregular retention behaviour of the phase, *i.e.*, an increase in the absolute retention volume with increase in temperature. The transition appears to be a sort of two-dimensional "melting" which gives the solute access to new retention sites. As for the C_{18} bonded phase the phenomenon takes place at ambient temperatures, Serpinet's group is advocating the use of shorter or longer alkyl grafts instead of the octadecyl type.

Martire and Boehm[148] have proposed a "breathing" surface model, which describes mathematically the dynamic nature of the bonded layer. The surface layer can adjust itself depending on the mobile phase composition. The model assumes a swelling of the bonded phase in solvents compatible with *n*-alkanes, *i.e.*, promoting extension of the bonded alkyl chains ("brush" structure) and the collapse of the chains in fairly polar solvents. However, the "breathing" model involves some simplifications which do not allow the retention behaviour of some polar molecules to be predicted. The model assumes no interactions with residual silanols, but such interactions can play a major role particularly when an organic-rich mobile phase is used and molecules containing heteroatoms are chromatographed[118,119].

3.4. Residual silanols and their influence on retention

Early attempts to explain the retention mechanism in HPLC on bonded alkyl phases mostly neglected any influence from unreacted silanols. Essentially three possibilities of the mechanism were considered: partition, adsorption and partition between the mobile phase and a new adsorbed, mixed stationary phase having a composition different to that of the mobile phase. The controversy regarding the retention mechanism deepened because of the difficulties encountered in preparing batches of modified silica gels with sufficient reproducibility. An interesting discussion of those early attempts can be found in the review by Colin and Guiochon[117]. The neglect of the participation of residual silanols in the chromatographic process led to the development of a solvophobic theory by Horváth *et al.*[149]. According to this theory, retention is governed by the formation of reversible complexes due to solvophobic interactions between solute molecules and chemically bonded organic chains on the surface of silica. The very important role of the mobile phase was emphasized. Although the theory appeared valid in numerous chromatographic systems, particularly when water-rich solvents were used, irregularities were noted by many workers[150–152]. Some of these irregularities could be explained by changes in the conformations of the chromatographed compounds[153]. However, more and more facts supported the idea of the availability of the residual silanols on the stationary phase surface for interaction with solute molecules[154–156]. Simple tests were proposed for the detection of silanophilic interactions[157,158].

Generally, unreacted silanols are considered undesirable[159–162]. However, as

Nahum and Horváth[118] have emphasized, no detailed study has so far focused on their role in determining retention behaviour. On the basis of some recently published papers[32,36–44,163], we believe that not all unreacted silanols are undesirable but only a very small population of highly acidic, isolated silanols. The role of this strongly interacting group of silanols was described in detail in section 2.3. The other silanols probably play a "positive" role in the separation mechanism. Bij *et al.*[119] have shown that blocking of silanols may sometimes lead to deterioration of a separation. A similar example was given by Gonnet *et al.*[96]. The silanols on the surface of the bonded phase generate some kind of heterogeneity and it was shown theoretically that heterogeneous surfaces may exhibit a higher selectivity than homogeneous surfaces with the same average adsorption energy[77]. Heterogeneity of alkyl bonded phases was recently confirmed by an investigation of sorption–desorption dynamics by means of a relaxation kinetic method[164,165]. On the other hand, the residual silanols produce a polar surface environment, which was detected by means of fluorescence spectroscopy of chemically modified surfaces[166]. If some of the silanols are considered "undesirable" than it becomes obvious that their influence on chromatographed solutes should be suppressed. Such a suppression can be achieved when the mobile phase contains a strongly adsorbing compound, usually an amine. This is advisable when chromatographed compounds have an unprotected nucleophilic function. The advantages of the silanol blockage were demonstrated by Wehrli *et al.*[167], Wahlund and Sokołowski[168] and Moats[169], who used an amine to block the active sites on the bonded phase surface. In supercritical fluid chromatography, dioxane was used for "on-column" silica surface modification, which improved the speed and efficiency of the separation[170]. Other advantages of silanol masking are a reduction of the dependence of retention on sample size and improvements in column efficiency and sample recovery[109]. Silanophilic retention can also be eliminated by protecting the function of the solute molecule[109].

We believe that the above advantages of the partial masking of unreacted silanols confirm our idea of a small, highly retentive population of silanols[43]. We have already shown[43] that the blocking of a very few strongly adsorbing sites can dramatically improve the column efficiency. A similar phenomenon was observed by Marshall *et al.*[38] after treatment of a fully end-capped packing with a highly reactive reagent, trimethylsilylphosphine. The reagent did not increase the carbon coverage but it did greatly improve the column efficiency. As the blocking improves the solute peak shape[43] it will obviously reduce the dependence of retention on sample size[119]. Sample recovery also depends on the strong adsorption sites, so the blocking should improve the recovery[119].

4. CONCLUSIONS

We have shown that the properties of silicas may differ, despite them having the same surface area, mean pore diameter or pore volume, because of slight differences in the chemistry of the surface. Several reports[32,36–44,54,163] support the idea of a small population of silanols which can be blamed for the undesirable, strong adsorption of compounds with a nucleophilic character. Part of these silanols remain active even on octadecyl bonded phase surfaces and may reduce the efficiency of chromatographic separations of compounds with unprotected nucleophilic groups. The chemistry of this population is still unknown, although traces of metals in the silica matrix are suspected to be a possible cause[54]. Blocking of these highly retentive sites can result in an improvement in column efficiency, a reduced dependence of retention on sample size, an improved sample recovery, an improved hydrolysis of the phase, a lower adsorptivity for basic compounds and an increased mechanical stability[32,36–38,40,43,119]. Three strategies have been adopted in the literature for avoiding adsorption on the strongly retentive sites:

(1) rehydration of the silica surface to obtain a fully hydroxylated surface[32,40];

(2) synthesis of the bonded phase by a method that permits these strongly interacting sites to react[36,37];

(3) blocking of the sites in the chromatographic column with an amine in the mobile phase[119,163,167–169].

We have also shown that the physical properties of silica may influence the coverage, and the importance of the pore size distribution is emphasized.

An excellent, recently published review on HPLC packing development can be recommended to the reader for other aspects of the synthesis and behaviour of packings[171].

5. SUMMARY

This review does not pretend to cover all the achievements of the technology for the synthesis of bonded phases for HPLC. We have rather reviewed some selected topics connected with the preparation of alkyl bonded phases. Particular emphasis is put on the undesirable adsorption of nucleophilic compounds on both modified and unmodified silica surfaces. The advantages of reducing the number of strong adsorption sites are pointed out. A more detailed discussion is given of silanols as adsorption centres, metal impurities in the silica matrix and the highly retentive population of silanols.

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