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Review

The influence of silica structure on reversed-phase retention

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ABSTRACT

The literature concerning the deleterious effects of silanol groups on reversed-phase separations is reviewed. Methods for their estimation on the surface of silica range from chemical, by reaction of surface silanols with a suitable reagent to physical procedures such as infrared and NMR spectroscopy. Most procedures are seen to be dependent upon the equilibrium between silanol groups and surface adsorbed water. The possible interactions between solutes and silanol groups, ion exchange, hydrophobic and silanophilic, all contribute to the overall retention mechanism; the performance problems with basic molecules in reversed-phase chromatography are mainly a consequence of ion exchange on active silanol groups. The acidity of silanol groups has been ascribed to incomplete rehydroxylation of silicas and to the presence of metal atoms within the silica matrix. The available evidence currently points to the latter as being the main source of the deleterious effects of silica structure on reversed-phase separations.

CONTENTS

1. INTRODUCTION

Since its inception in the 1960s, HPLC has relied upon silica gel in some form as the primary column packing medium. This was a result of its ready availability in appropriate

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particle size ranges and the ease of use of the medium for adsorption chromatography. It is not surprising that silica became the base support first for partition phases in which a liquid phase was coated on the surface of the packing (either statically or dynamically) and later, following the work of Stewart and Perry [1], as the support of choice for the development of bonded phase packings. It is hardly necessary to expand upon the importance of bonded phases in this edition of the Journal, other than to note that the use of reversed-phase packings very rapidly eclipsed that of silica until by the early 1970s, most separations under development were on reversed-phase packings. It is currently estimated that approximately 50% of all separations are carried out in the reversed-phase mode [2].

Despite the success story of reversed-phase packings for chromatography, there has been a number of occasions on which they were noted to fail dismally in achieving a separation. The vast majority of these separations were those of compounds of pharmaceutical origin, almost all of them containing basic amino groups. A review of the problems in the chromatography of such basic compounds which also proposes a number of possible solutions has appeared [3]. Many of the problems can be overcome by the use of buffered mobile phases at a carefully chosen pH [4]. Although this technique was suitable for a number of solutes, some others appeared to be separated with much less success. The situation became more confusing when it became apparent that not all bonded phase silicas were equal in their ability to separate such compounds. In some cases, differences were noted between different lots of what was ostensibly the same reversed-phase packing from the same manufacturer [5].

Similar problems were noted in the field of peptide and protein separations [6]. The recovery of protein from the column was found to vary quite dramatically from one column type to another, even in cases where the chromatography was acceptable in terms of peak shape. Further, the chromatography was found to vary with the packing material, to the extent that solutes would elute from one packing but would be irreversibly retained on others.

One aspect that all of these problems had in common was a growing realisation that the problem did not lie in the bonded phase but resided in the underlying silica. The presence of unbonded silanol groups was known, and all the problems of chromatography were assigned to their presence.

This paper addresses the question of the influence of the structure of silica on reversed-phase chromatography. It touches upon the validity of the view that silanol groups are in general undesirable, addresses the question of what makes a silanol group a "bad" one and also considers what steps to take to avoid meeting silanol-related problems in reversed-phase chromatography.

2. SILICA STRUCTURE

Before considering the effect of silica structure on the chromatography, it is profitable to spend a little time in describing its salient features. For greater detail, the reader is referred to two books, those of Iler [7] and Unger [8].

Silica has a number of crystalline forms and is also found naturally in an amorphous state. It is made up of three-dimensional branched chains of alternating silicon and oxygen atoms. The chains terminate in hydroxyl groups linked to the silicon atom; these are conventionally called silanol groups. These are the groups which predominate at the surface. They may be single or geminal (one or two hydroxyl groups on the silicon atom); it is considered unlikely that a species with three hydroxyl groups will occur in silica [8].

Silica as used for chromatographic purposes is amorphous in nature, generally prepared by polymerisation of silicic acid [7]. Although the structure of the many crystalline forms of silica is normally only of academic interest to the chromatographer, the chemical nature of all silicas is the same and a number of the properties of amorphous silica have been deduced from the study of related crystalline species. Amongst these is the density of silanol groups on the surface of amorphous silica which has been calculated from a knowledge of the structure of crystalline silica of similar density. The values

have ranged from 4.6 [9] to 8 [10] hydroxyls per nm^2, depending upon the model chosen. Thus, it has been left to experiment to determine the actual number of silanol groups on the surface.

Silica adsorbs water very readily and is extensively hydrated under normal conditions; thus, a major problem in the determination of surface silanol groups lies in the presence of the adsorbed water layer. Most methods of measurement cannot distinguish between the surface hydroxyls and adsorbed water and thus must rely upon initial drying of the silica, which can influence the ultimate result. The surface of silica is reversibly and easily dehydrated, since two adjacent silanols may readily be converted to a siloxane bond with the loss of water, and equally, the siloxane groups may be hydrolysed to two silanols. It is in part because of this propensity to change surface configuration that silica has been termed a "living polymer" [7]. This surface lability implies that the task of removing water without affecting the silanol concentration is close to impossible and therefore great care has to be taken with the preparation of the sample. Although the difference between hydroxyl groups in physically adsorbed water and in the silanol groups can be differentiated by physical techniques, there appears to be no single set of experimental conditions which results in a completely dry silica surface without at least partial dehydration of silanol groups occurring [8]. Because the dehydration reaction is reversible (if the silica has not been heated to the point at which its structure is changed) this dry but fully hydroxylated state is difficult to realise, and most workers rely upon heating to moderate temperature (*ca.* 120°C) *in vacuo* to reach at least a reasonably reproducible condition.

Methods for the measurement of silanols have relied upon weight loss by thermogravimetric experiments [11], deuterium [12] or tritium [13] exchange reactions or chemical methods in which a reagent is used which liberates a readily detectable species on reaction with a silanol group [14,15]. These last methods assume that the reagent is small enough to penetrate the pores of the silica in order to reach all of the hydroxyls. Even for a reagent as small as methyl lithium, it has been estimated that a pore diameter of at least 5 nm is required for complete access to the surface hydroxyls and the results from such procedures are heavily reliant upon adequate pore distributions. The results of the determination of the surface concentrations of silanols depend upon the method and the silica chosen for study, but it is generally agreed that the surface concentration is of the order of 8 μmoles/m^2, which corresponds to between 5 and 6 hydroxyl groups per nm^2 for a fully hydroxylated silica.

A value of 6 silanol groups per nm^2 implies that the average distance between two silanols is of the order of 0.4 nm. Given the amorphous nature and therefore unstructured surface of the silica, this means that some silanol groups will approach each other within the distance required for hydrogen bonding (less than 0.3 nm [16]). These are usually termed "vicinal" silanols. It is clear, given the intergroup distance, that the numbers of hydrogen bonded silanols will be sensitive to the surface coverage and a small reduction in the surface concentration could easily result in large changes in the numbers of silanols which approach each other closely enough for hydrogen bonding. Other silanols will remain outside this distance from their nearest neighbour and will therefore remain isolated. In addition, two silanol groups (geminal silanols) on a single silicon atom can occur [17]. At least one of these can presumably be hydrogen bonded, or both can remain isolated. The presence of geminal groups in large numbers on the surface presumably will mean that the single silanols are spread a little more thinly than suggested above, which will further influence the numbers of hydrogen bonded silanols.

3. STUDIES OF SILANOL GROUPS

3.1 Infrared studies

The infrared spectrum of silica is characterised (in the hydroxyl stretch region) by a sharp band at around 3750 cm^{-1} and a broad band which starts at 3750 cm^{-1}, is centred around 3550 cm^{-1} and tails out to around 3100 cm^{-1} [11]. The sharp band has been identified as being due to the isolated silanols whilst the broad band is due to the groups which are in close enough proximi-

ty for hydrogen bond interaction. From the frequency range of these hydrogen bonded groups, there is clearly a wide range of hydrogen bonding strengths which presumably depend, as suggested above, upon the inter-silanol distance. A study using DRIFT [11] (diffuse reflectance FT infrared spectroscopy) reported that the spectra of fully hydroxylated silicas indicated that they contained higher concentrations of hydrogen bonded silanols with lower average frequencies than did less hydroxylated silicas. The frequency of the maximum of the sharp adsorption band was reported to vary in position over a range of around 2 to 4 wavenumbers and to be correlated with the acidity of the silanol groups; the more acidic silicas show an isolated silanol frequency higher than the less acidic media. Given that the experimental error was reported to be ± 1 cm^{-1}, these measurements were difficult. The same study indicated that it was the isolated silanols which preferentially reacted with the silane reagents. It was also found that after full reaction with trimethylsilylchloride approximately 30% of the silanols remained unbonded, presumably due to their inaccessibility to the reagent once the neighbouring groups had reacted.

3.2 NMR studies

Recent studies of the silanol groups on the surface have been undertaken with the aid of ^{29}Si Fourier transform NMR, using the magic angle spinning technique for solid samples [18]. One drawback of this is its low resolution; one group early in this field cast strong doubts upon the existence of the geminal silanols because of their inability to detect them [19]. The difficulty of this low resolution between the signals may be solved either by deconvolution [11] or by saturation of the silica with water [20]. After the latter treatment, the NMR spectra of silica were found to be characterised by signals due to the single silanols, the geminal silanols and the siloxane backbone, as shown in Fig. 1 [20]. The spectra are modified after bonding reactions when the signal due to the geminal groups disappears [11] or is reduced [18] and that due to the single silanols decreases. A signal due to the silicon atom in the bonded phase group appears. It is

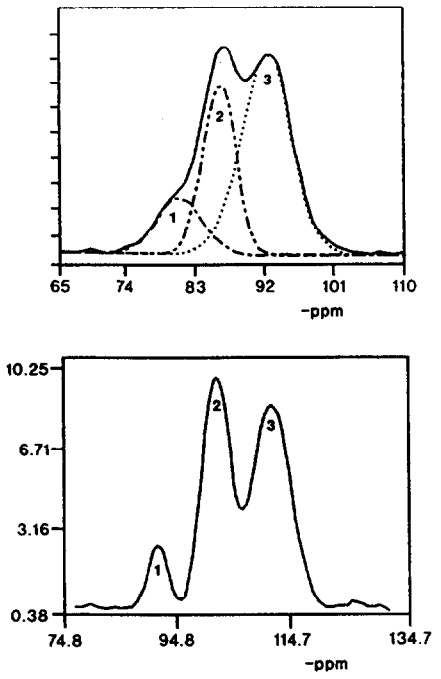

Fig. 1. Comparison of ^{29}Si cross-polarised magic angle spinning NMR spectra. Top: dry silica. Bottom: silica saturated with water vapour. Peaks: 1 = geminal silanols; 2 = isolated silanols; 3 = structural siloxane bridges. Figure reproduced from refs. 11 and 20.

apparent that the ^{29}Si NMR spectra do not differentiate between hydrogen bonded and isolated silanols.

Proton NMR spectra allow the calculation of the relative amounts of geminal and single silanols [11]. Typically, 32% of the silanols are geminal. This study also indicated the preferential reaction of the geminal silanols with silylating reagents. Correlation of this with the infrared data suggests that many of the geminal silanols are isolated and are not hydrogen bonded, although given the lack of quantitative discrimination between the different types of silanol, this cannot be stated with certainty.

4. SILANOL ACIDITY

Silanol groups are acidic, although there has been some controversy over the origin of the acidity and the variability of this acidity between different silanol groups on the same silica surface and similar silanol groups on silica surfaces

which have had different histories. There have been a number of attempts to define silica acidity as a measurable parameter for the selection and characterisation of silicas. One method reported is the measurement of the pH of an aqueous suspension of the silica [21]. Such a measurement can lead to strange results, as pH values ranging from 3 to 9.5 were found. Reference to the history of the silica usually accounts for these. One problem in the synthesis of silica is the removal of traces of acid or alkalies used in the synthesis and washing of the packing. Silicas which have very high pH values in the tests generally have high residual content of sodium or other metal ions present within the matrix. The material (Zorbax SIL) reported to have the lowest pH has an extensive acid treatment for rehydroxylation and even though this is one of the more acidic silicas, it is highly probable that the pH measurement indicated more the degree to which the subsequent washing step had removed the acid rather than any more fundamental property of the silica. Because of the need for extremely careful pretreatment prior to pH measurement or titration of the silica, much of the measurement of acidity has relied upon physicochemical procedures such as IR, where the stretching frequency of the hydroxyl band is related to its acidity. Such infrared studies have suggested that the pK_a of the silanol groups is around 7.1 [22], although such a result must be treated with caution since these workers noted that although the average pK_a of the surface hydroxyls for a silica/alumina catalyst was 7.1, there were a few groups with a pK_a of less than -0.4 present. Thus, such measurements must be regarded as giving average values and will not detect a small number of extremely active silanols.

5. INFLUENCE OF THE DEGREE OF HYDRATION OF THE SILICA SURFACE

A number of studies have been made of the effects of heat treatment of silica prior to use in chromatography either as an adsorbent or after chemically bonding a phase to the dehydroxylated surface. Scott heated silica samples to a range of temperatures [23]. These were investigated by adsorption chromatography and the retention could be related to the loss of surface silanols. Further, it was stated that, for such normal phase separations the surface of silicas heated above 600°C was uniform and reproducible. A more recent study, which used both infrared data and chromatographic retention to characterise the resulting surface [24] indicated that the vicinal silanol concentration decreased upon heating with an increase in that of the isolated groups, indicating (not surprisingly) that it is the hydrogen bonded silanol groups which most easily dehydrate. It was found that the silicas used for this study could not be returned to their original condition by rehydration techniques if they had been heated above 400°C.

Other workers have tried to use preliminary heat treatment to eliminate unwanted silanol groups from the surface of bonded phase silicas [25]. Obviously, such an approach will not be useful in reversed-phase chromatography since there will always be at least a slow rehydration of the silica, even if the temperature of the initial treatment is well over the 400°C limit for full rehydroxylation found by Engelhardt [24]. This was illustrated by Köhler *et al.* [11], who heat treated silica to reduce the available silanol groups, reacted the product exhaustively with a silane and subsequently found further silanol groups which had appeared through rehydration reactions of the siloxane bridges in the product. Further, in contradiction to the philosophy that reduction of the silanol concentration would improve the packing materials, this group concluded that the best performance in terms of peak shapes and bonded phase stability is obtained with silicas bearing the maximum number of surface silanols.

The group of Kirkland [11] also deduced that the acidity of the silanol groups was affected by their surface concentration. In contrast to the model of Snyder and Ward [26], which postulates that hydrogen bonded silanol groups are the most acidic, the results from the later experiments indicate that the surface acidity is increased upon decreasing the silanol concentration. Since a decrease in the surface silanol concentration should result in a decrease in the numbers of hydrogen bonded silanols and an

increase in the relative numbers of isolated silanols, these latter groups must be the more acidic. This conclusion agrees with that of Kiselev's group [27].

6. THE INFLUENCE OF IMPURITIES ON THE STRUCTURE AND PROPERTIES OF SILICA

The majority of silicas which are used in chromatography are not pure. This is because of the source of the silica sol from which they are prepared. Most silicas are synthesised from silica sols which are themselves obtained by acidification of sodium silicate under controlled conditions. The sodium silicates are usually prepared from dissolution of silica sands in sodium hydroxide. The initial silica usually contains significant levels of impurities, mostly metals and alumina. In addition, the sodium hydroxide also often contains trace levels of heavy metal impurities which may be concentrated within the silica sol by virtue of the ion exchange character of the silanol groups.

It is difficult to remove the impurities from silica once it is prepared. Only a proportion of the metals can be removed from silica by prolonged treatment with acids [28] and it has also been noted that the removal of metals from spherical silicas (which normally include a heat treatment in their synthesis) was difficult [25]. This is presumably because of occlusion of the metals within the structure of the silica. It has been estimated that the walls within porous silica particles are on average between 2 and 4 silicon atoms thick [29]. Thus, impurities can be trapped within the thicker sections of the walls.

Therefore, unless the silica is prepared from sols derived from carefully purified tetraethylorthosilicate (which can be distilled free of metal impurities) or from fume silica together with carefully purified reagents, it is certain to contain at least trace quantities of impurities. Typical levels of impurities found in silica gel are shown in the Table. The first four rows are from silica samples prepared by Ohtsu *et al.* [30]. Silica I was prepared from an impure sodium silicate. Silica II is silica I after extensive acid washing. Silica III was prepared from carefully purified sodium silicate, and silica IV is the result of acid washing this material. The other data were from measurement of impurities in commercial silicas [31], Develosil (a silica prepared from a sodium silicate derived sol) Kromasil (prepared from tetraethylorthosilicate (TEOS)), Nucleosil, LiChrospher Zorbax SIL [11] and Zorbax Rx SIL [32]. It is noteworthy that even the material prepared from very carefully purified sodium silicate had appreciable remaining levels of aluminium, titanium and zirconium; only the TEOS derived silicas are pure.

The effect of the metals may be seen in Figs. 2 and 3. The separation of chelating compounds

TABLE 1

TYPICAL IMPURITY LEVELS IN SILICAS FROM VARIOUS SOURCES

Silica	Metal concentration (μg/g)								Ref.
	Na	Mg	Al	K	Ca	Ti	Fe	Zr	
I	308	11	264	42	16	230	154	11	28
II	0	8	188	0	13	162	33	13	28
III	66	0.1	8	0	1	10	15	3	28
IV	0	0	6	0	0	7	1	3	28
Develosil	25	51	54	0	215	38	0	5	29
Kromasil	25	0	0	0	0	2	0	0	29
Nucleosil	56	NA	0	NA	130	57	76	0	9
LiChrospher	2900	NA	1100	NA	0	235	445	0	9
Zorbax PSM 60[a]	105	NA	0	NA	0	41	68	<25	9
Zorbax Rx SIL	10	<4	<10	<3	4	–	–	–	32

[a] This sample also contained 245 ppm copper and 115 ppm chromium.

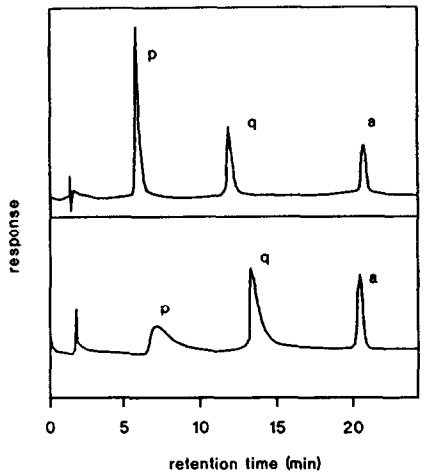

Fig. 2. Comparison of the chromatography of chelating species on silicas with different metal contents. Top: Kromasil C18; bottom: Develosil C18. Peaks: p = purpurin; q = quinazarin; a = amylbenzene. Mobile phase: 75% methanol–0.05 *M* phosphate buffer, pH 3. Figure reproduced from ref. 31.

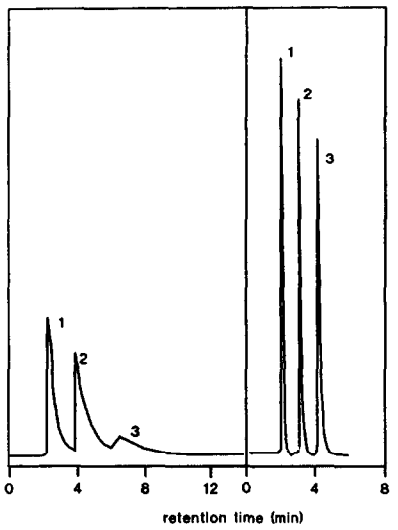

Fig. 3. Comparison of the effects of metal content upon the reversed-phase chromatography of amines on capped ODS packings. Left, silica containing a high concentration of metals; right, silica prepared from purified sodium silicate. Peaks: 1 = aniline; 2 = N-methylaniline; 3 = N,N-diethylaniline. Mobile phase: 5% methanol–0.02 *M* phosphate buffer, pH 2.7. Figure reproduced from ref. 30.

[31], purpurin and quinizarin together with amylbenzene is shown in Fig. 2. The Kromasil packing clearly gave a result superior to that of the Develosil when bonded in an analogous manner. This may readily be related to the difference in the metal content of the media. These workers did not compare these packings for basic components. Fig. 3 shows the difference between the silica with high levels of metal contaminants relative to that with only a few μg/g of metals [30]. This depicts the separation on an end-capped ODS packing of aniline, N-methylaniline and N,N-diethylaniline, the latter being the test probe used by other workers [11] to differentiate between acidic and non-acidic silicas. The material with high metal concentrations gave very poor peaks in this experiment, whilst the purified silica gave acceptable performance. Even after end capping, the heavily contaminated material did not give acceptable performance.

The problem of metal impurities can be further exacerbated if the silica is heat treated for strengthening purposes during its processing. As noted above, this is frequently part of the processes to synthesise spherical silicas. If the silica is heated above a certain temperature (approximately 550°C), the metals are incorporated into the silica structure. This phenomenon has been used to prepare a range of silicas which had an enhanced stability to hydrolysis [33]. This involved the coating of a transition metal on the surface of the silica, although not in sufficient quantities fully to coat the surface, followed by firing the material at elevated temperatures to incorporate the metal ion into the matrix. It was shown that although the metal ion coating could easily be removed by washing prior to the heat treatment, neither acid nor alkali washing could remove it afterward, indicating that the ions had been incorporated into the silica structure. The process greatly enhanced the stability of the silica to alkaline hydrolysis, indicating that a significant change in its structure had taken place. Since the quantities of transition metals were insufficient fully to coat the surface, clearly there must be remaining patches of silica which would be vulnerable to hydrolytic attack; this suggests that the introduction of the metals into the surface structure has an effect which is passed through the remaining silica network. Although most of the work in this area has

concentrated on the use of zirconium in this regard, aluminium [7] and other metals were also shown to have similar effects and to be incorporated into the silica structure [34]. A feature of these stabilised silicas was the stronger adsorption of basic molecules [35]. This enhanced adsorption suggests strongly that the incorporation of foreign metals into the surface of the silica results in a higher acidity of the silanols residing on adjacent silicon atoms.

Other workers have also noted the effects of metals which are incorporated in the silica matrix. It has been calculated that aluminium present in the structure should confer a high acidity upon adjacent silanol groups [36] whilst Sadek and co-workers [37] have shown that removal of metal impurities from silicas leads to fundamentally different retention behavior of hydrogen bond acceptor solutes which was attributed to the decreased numbers of metal hydroxides and the reduced influence of metals upon the surface silanol groups. These data, together with observations on the gas chromatographic adsorption of amines, have led Nawrocki [38] to the conclusion that the metals play an all important part in enhancing the acidity of the surface silanol groups in chromatographic silicas.

7. CHROMATOGRAPHY ON SILICA WITH AQUEOUS SOLVENT SYSTEMS

7.1 Reversed-phase character of silica

Besides the polar silanol groups, silica possesses non-polar siloxane bonds. Where these are at the surface, they are expected to reduce the polarity of the surface. This reduction has been observed for heat treated silicas described earlier. It should also be possible, under the right circumstances, to observe retention of non-polar molecules on these siloxane bridges. This has been noted by Horváth and co-workers [39] and by Cox and Stout [40]. Plots of log capacity factor for both propylbenzene [39] and toluene [40] against methanol concentration were linear, as expected for a reversed-phase mechanism. The retention was, however, small compared with that found for an alkyl bonded phase packing, although the contribution of reversed-phase mechanisms to the separations of basic compounds on bare silica using predominantly aqueous mobile phases were shown to be significant [40]. The reversed-phase component of the retention mechanism was demonstrated to be a function of the degree of hydroxylation of the silica, with the more highly rehydroxylated silicas having a significantly lower reversed-phase character than the others. This confirms that the siloxane groups are the site of the reversed-phase interactions on bare silica.

7.2 Silanophilic interactions

Some time ago, it was noted that not all reversed-phase separations proceeded according to the hydrophobic theory. Crown ethers, for example, showed anomalous behaviour. A silanophilic theory was developed by Horváth and co-workers [41] in order to account for the observed deviations from the hydrophobic theory developed by the same group at an earlier stage. The crown ethers were found to behave as a normal phase system in predominantly alcoholic mobile phases, with linear plots of the inverse of capacity factor against water concentration in the mobile phase.

Such behaviour has also been documented for peptides [42]. Both normal and reversed-phase behaviour have been observed for a wide range of species when separated using reversed-phase conditions. At low concentrations of organic solvent, the behaviour is predominantly reversed-phase, but at higher concentrations the solutes show increasing normal phase behaviour. This results in typical U-shaped plots of log k' *vs.* organic solvent concentration. In the absence of silanophilic interactions, these plots are linear, with negative slope.

7.3 Ionic interactions

During the course of the reversed-phase chromatography of basic compounds, it was noted that peak shapes were poor and efficiencies were low. Some of the difficulties in the chromatography were resolved by Jane [43], who employed the expedient of using bare silica rather than reversed-phase packings with a methanol mobile phase containing a small percentage of water buffered to high pH. During the course of the development of reversed-phase analytical meth-

odologies for quaternary ammonium compounds used as animal feed additives, it was noticed that the retention of the components was strongly influenced by pH and buffer concentration [44]. The substitution of a bare silica column surprisingly gave almost exactly the identical separation. The result prompted a study of the chromatography of basic compounds on silica [45] using methanol–water mobile phases which were buffered to less extreme pH values than those of Jane. The next few years saw a number of studies in this area [46,47] which concluded that, in contrast to the silanophilic effects noted for the crown ethers, the principle mechanism of retention was ion exchange chromatography on the acidic silanol groups.

In a detailed study, Cox and Stout [40] found that different silicas exhibited different ion exchange properties. By studying the separations of several probes of different basicity using packings of different surface coverages of bonded phase groups, the retention mechanisms were shown to comprise a number of competing interactions which included hydrophobic interactions with the siloxane bridges and silanophilic interactions beside the ion exchange. A method for the assessment of the ion exchange character of retention was described by Stout *et al.* [48]. This work arose from a study of the chromatography of basic, ionogenic solutes on unbonded silica under conditions closely analogous to those used for reversed-phase chromatography. It was shown that the predominant retention mechanism of such solutes in buffered aqueous organic solvents was one of ion exchange. Under such a retention mechanism, plots of capacity factor against the inverse of competing ion concentration are linear. If ion exchange is the sole mechanism, such a plot should pass through the origin. When separation mechanisms which are not influenced by ionic species are present, there will be an intercept, corresponding to the retention which should be observed at an infinite competing ion concentration. Such an intercept is typical where silanophilic or reversed-phase retention mechanisms occur.

The slope of the plot of capacity factor of an ionic solute vs the inverse of ionic strength was shown to be directly related to (amongst other parameters —the phase ratio and degree of ionisation of the silica— for example) the ion exchange distribution coefficient [48]. For silicas of similar surface area, the slope of this plot acted as an indicator of the ion exchange character of the material. It was shown that silicas with "good" properties with regard to the reversed-phase chromatography of basic compounds had low values of the slope, whilst those silicas which were poor for the chromatography of bases had high slope values. This technique has the merit of measuring the property closest to the problem in that the ion exchange character of the silica is directly related to the acidity of the silanol groups, and further, the most acidic silanols will have a strong effect, in contrast to the case of infrared measurements where a bulk property is measured which is hardly influenced by the most acidic silanols. Although this measurement was not converted into a quantitative technique by the authors, it nevertheless provides useful data for comparison of similar packing materials and has been used to provide a method for the comparison of silanol acidity in silicas of different provenances.

8. REDUCTION OF THE EFFECTS OF SILANOL GROUPS

8.1 End capping: the minimisation of silanol groups

The first attempts to remove the effects of silanol groups were through the use of end capping. The reaction of silica with long chain alkylsilyl halides does not cover all the available silanol groups. A fully bonded C_{18} phase, for example, covers perhaps two of the four or five silanol groups on each square nanometer. Those remaining are available to interact with any molecule which can reach them. The logical step to eliminate the effects of the silanols then is to react them with some other, smaller, reagent which will cover as many of the remaining groups as possible. The usual procedure is to replace the hydrogen atom of the silanol with a trimethylsilyl group. The reagents used for this are usually hexamethyldisilazane or trimethylsilyl chloride. Although the incremental carbon content of the bonded phase is hardly noticeable

(especially within the confines of the accuracy of carbon–hydrogen analysis of bonded phase silicas) the effects can be appreciable [49]. Typical results are shown in Fig. 4. Here a separation analogous to that of Fig. 3 is shown which is performed on an ODS phase prepared on an acid-washed impure silica (Silica II of the Table) before and after end capping. The improvement in the chromatography demonstrates that the end capping process either blocks the active silanols by bonding to them or by rendering them inaccessible sterically.

End-capping the silica with trimethylsilyl groups does not react all of the silanols. After extensive bonding with trimethylchlorosilane only around 60% of the silanols were found by NMR to have reacted [11]. Given the relative size of the organosilane and the silanol groups this is not unexpected. It has been shown by the use of small probes that the silanols are still accessible. By a combination of deuterium exchange and measurement of the adsorption isotherms of methanol and ethanol, it was suggested that solutes could still access surface silanols through transient holes in the bonded phase caused by its lateral vibrations [50]. Other workers showed that the peak symmetry of even simple solutes such as benzyl alcohol, phenol and aniline was a function of the surface coverage [51]. They measured the peak asymmetry value for a number of bonded phases which had been prepared with different surface coverages. A plot of the asymmetry versus the remaining silanol group concentration after bonding showed a maximum at a value of around 700 to 1100 μmole/g. This, it was pointed out, was a value commonly found after conventional bonding reactions were complete and it was postulated that it was the restricted access to the surface which caused the asymmetry. End capping processes reduce the silanol concentration to levels sufficiently low that the peak symmetry is improved.

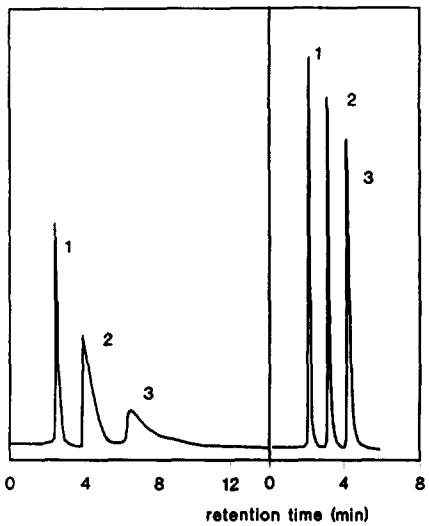

Fig. 4. Illustration of the beneficial effects of end capping. Left, uncapped C18 silica, prepared from a silica containing moderate metal concentrations; right, separation performed on the same material after end capping. Samples and conditions as in Fig. 3. Figure reproduced from ref. 30.

One feature of bonded phases which is not necessarily appreciated is the speed with which they are hydrolysed in aqueous solution. In the process of studies on the preparation of "ideal" silicas for use in HPLC, Köhler and co-workers observed the rapid hydrolysis of trimethylsilyl bonded phases [11]. They noted that the chromatographic parameters of the test solute (N,N-diethylaniline) such as asymmetry and plate count changed with hydrolysis in ways particular to the silica used rather than with any function of the surface silanols exposed by the hydrolysis. This was taken further by Glajch, Kirkland and Köhler [52] who measured the rates of hydrolysis of phases under the usual conditions of peptide analysis (0.1% aqueous trifluoroacetic acid–acetonitrile gradients). They found that after a very short time much of the end capping was removed from the packing material. Paradoxically, the chromatography even of very basic peptides such as melittin did not deteriorate when the chromatography was good on the freshly end-capped packing. Fig. 5 shows a comparison of a freshly prepared TMS silica and the separation on the material after 50% of the phase had hydrolysed. For comparison, Fig. 6 shows the chromatography of lysozyme, melittin and ovalbumin on the freshly prepared TMS packing and a TMS phase prepared upon an inadequately rehydroxylated silica. These results suggested that it is not the quantity of silanols on the surface which can

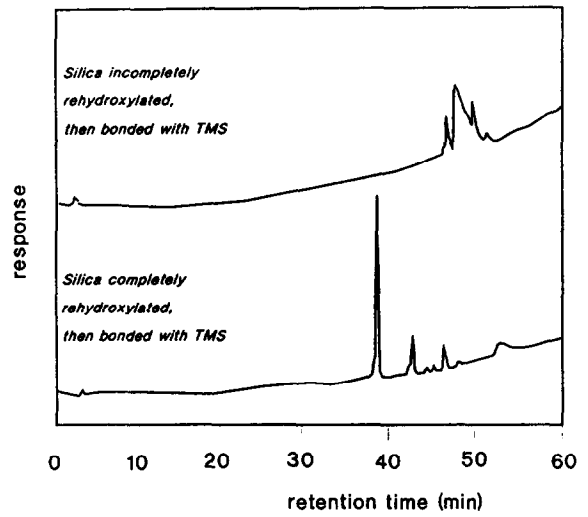

Fig. 5. Effect of bonded phase hydrolysis on the chromatography of a basic peptide (melittin). Top, freshly prepared TMS phase on fully rehydroxylated Zorbax PSM 60; bottom, the same separation after 50% hydrolysis of the TMS phase (1 month of operation). Mobile phase: 0 to 100% acetonitrile in 0.1% trifluoroacetic acid over 80 min, 1 ml/min at 50°C. Figure reproduced from ref. 52.

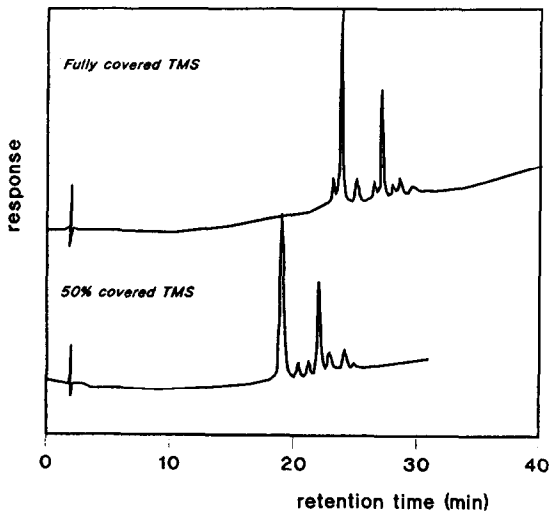

Fig. 6. Effect of the degree of rehydroxylation of the silica upon the separation of lysozyme, melittin and ovalbumin. Top, TMS phase prepared from inadequately rehydroxylated silica; bottom, TMS phase prepared from fully rehydroxylated silica. Other conditions as in Fig. 5. Figure reproduced from ref. 52.

cause problems with chromatographic separations but some function of their quality.

8.2 Rehydroxylation: the maximisation of silanol groups.

Kirkland's group found that the chromatography of diethylaniline in unbuffered aqueous–organic eluents could be used as a diagnostic of the likely behaviour of packings for more difficult separations of basic molecules [11]. Good performance with diethyl aniline under the test conditions was taken as an indication of a lack of deleterious silanol interactions. Silicas were divided into Type B and Type A groups according to this test. (Type B silicas are preferred for separations of basic solutes. It should be noted, incidentally, that many Type A silicas perform extremely well in other tests or other applications.) Interestingly, the test results also correlated with the rate of hydrolysis of the trimethylsilyl bonded phases investigated on the silicas. Further, as the phases hydrolysed, the Type B packings remained good, whilst the Type A packings generally became worse, with the diethylaniline peak moving to longer retention time with increased skew.

The conclusion was reached that the number of active sites which were the cause of the poor performance was not necessarily large. The chromatography of N,N-diethylaniline was found in some cases to improve after successive injections of the sample. This was ascribed to the coating of the active sites with the amine, thus removing them from interaction upon future injections. For a column with a relatively high surface coverage of trimethylsilyl groups, only around 10 nmole of diethylaniline were needed to "block" the active sites to a significant extent.

If we assume that there are no steric effects which block access to these active sites, 10 nmoles corresponds to a concentration of the active sites of 2.5 ppm. Since around 50% of the silanols are bonded, this raises the concentration of active sites to 5 ppm. It was shown that as the bonded phase hydrolysed, exposing more silica surface, the diethylaniline adsorption increased. The increase in diethylaniline retention was of the same order as the decrease in the retention of the non-polar solutes, suggesting that the

hydrolysis exposed Type B and Type A active sites in a relatively constant proportion. A TMS silica with a low surface coverage was synthesised to investigate this phenomenon. Paradoxically, this packing adsorbed diethylaniline totally, even though its bonded phase concentration as estimated by the retention of non-polar constituents was similar to the partially hydrolysed material. No diethylaniline was eluted even after injection of 100 nmole. This suggests that the hydrolysis preferentially liberated the less active sites rather than a representative sample of all sites, which places the number of active sites on the silica considerably higher than the 5 ppm estimated above. Using this figure raises the active site concentration to at least 80 ppm and (since no diethylaniline was eluted even after this quantity had been injected) probably the figure is of the order of several hundred ppm. It should be noted, however, that the bonding reaction used was different, in that no acid scavenging catalyst was used in the synthesis of the silica with low surface coverage and a gas purge was used to remove HCl liberated in the reaction. The hydrogen chloride could have attacked the silica surface during the bonding reaction, producing more active sites than were present in the silica initially.

Köhler *et al.* [11] resolved the differences in the packings by study of the surface silanols by a combination of DRIFT and ^{29}Si NMR. The Type B packings were found to have higher surface silanol concentrations and lower acidity silanols than the Type A silicas. The two observations were correlated and methods for the improvement of the rehydroxylation of the silicas were investigated. It is relevant to note that the two sets of silicas also correlated well with the observation that all of the Type A silicas had been heat treated during their synthesis, whilst the Type B silicas had not. It was assumed at the time that the problem was one of rehydroxylation. This meant that in contrast to the previous ideas of silanol groups, the best materials were those with the maximum number of such groups rather than the minimum.

Several possible methods for the full rehydroxylation of silicas were proposed [18], mostly consisting of prolonged treatment with dilute aqueous reagents, either basic or acidic. The mechanism for the rehydroxylation was thought to be one of dissolution and reprecipitation of the silica, with the redeposition forming a chromatographically favourable surface involving a maximum number of hydrogen bonded silanols. Given the average thickness of the walls in a silica particle, this dissolution clearly cannot happen quickly, since it would be expected to destroy the silica structure. Indeed, Köhler and Kirkland observed this with concentrated reagents and vigourous conditions; hot aqueous ammonia or calcium carbonate both resulted in silicas with greatly reduced surface areas. Although these latter materials had a high level of surface silanols, they behaved poorly in the chromatographic tests. This prompted the postulation that the surface had to be deposited in a "homogenous" state; a rapid, destructive process was suggested to deposit a "heterogenous" surface with a large number of acidic silanols. Slower reactions were found to yield products with better chromatographic properties. It is possible that these result in the dissolution of only a little of the silica or even the breaking of some but not all of the siloxanes anchoring a silicon atom to the surface. Although the starting materials were analysed for impurities, no analyses were reported on the metal content of the products; it would be of interest to find if the dissolution and redeposition of silica suggested also dissolved the metals from the matrix.

The method of choice turned out to be a prolonged boiling with very dilute hydrofluoric acid [53]. This was thought to dissolve and reprecipitate a "homogenous" (*i.e.*, strongly hydrogen bonded) layer of silica at the surface of the packing material. Given the average thickness of the silica walls, this could not have been an especially thick layer, but it seemed to be adequate to give the heat treated spherical silica used in the experiments the desired surface, with a high concentration of surface silanols with a low acidity. The description of the layer of reprecipitated silica as being homogenous may be misleading when it is considered that a strictly uniform surface would have each silanol group 4 Å from the next with no prospect of hydrogen bonding to it. Presumably the process has to

operate slowly so that an energetically favourable surface with as many silanols hydrogen bonded as possible can be developed. Rapid reactions, such as those with calcium carbonate or concentrated ammonia solutions are less likely to result in an energetically favourable surface.

Meanwhile, Stout *et al.* [48] were developing other procedures for the hydroxylation of silica. They found that a very rapid treatment of silica with sodium bifluoride produced materials with very low the ion exchange distribution coefficients, performed a good rehydroxylation of the surface and also resulted in a marked improvement in the properties of the silica for basic compounds. The process involved addition of sufficient ammonium bifluoride to dissolve a layer of silica from the packing. Once sufficient silica had been dissolved, the properties of the silica remained constant, suggesting that the surface of the material was different from the remainder of the particle. Measurement of the quantity of silica removed made it apparent that the reaction was complete within a short space of time— reactions of 15 minutes duration were common. This process could not, therefore, proceed with the slow rearrangement of the surface as was postulated for the HF mediated process. The products were monitored by measurement of the slope and intercept of plots of capacity factor of thiamine (a quaternary ammonium compound, shown earlier to be retained by a predominantly ion exchange mechanism) against sodium ion concentration in a 15% methanol–aqueous phosphate buffer solvent system at pH 4.6. The slope of the plot (which, as mentioned earlier, is directly related to the ion exchange distribution coefficient) was normalised for the surface area of the silica. Results were obtained for very pure silicas, derived from tetraethylorthosilicate, silicas containing significant amounts of metal ion contaminants and a silica derived from the work of Köhler and Kirkland. The smallest values of the normalised slopes were obtained for the pure silicas. The material prepared from HF treatment of a silica had a slope a little larger than these, whilst the materials which were known to contain metal impurities had significantly higher slopes and therefore ion exchange properties. The intercepts of all products, which relate to the hydrophobic interactions of the solute, were uniformly low, indicating that the reversed-phase interaction with siloxane bridges was minimal. It can be concluded from this that all the surfaces were hydroxylated to similar extent: thus differences between the silicas were probably not due to marked differences in silanol concentration. In all cases the slopes and intercepts were much lower than had been observed for the traditional acidic rehydroxylation treatment.

The above results, together with the correlation of Type A and Type B performance with heat treatment and the known effect of the incorporation of metals into the surface of silica suggest the hypothesis that the poor performance is due to the combination of heavy metals and heat treatment. The postulated relayering of silica by the HF treatment of Köhler *et al.* could conceivably cover small amounts of surface metals whilst the ammonium bifluoride treatment would not. Neither process should recover silicas with high metal content, since appreciable quantities of the metals would always be close to the surface and would influence the silanol groups. Subsequent experiments have supported this theory. One such piece of work is the comparison of Develosil with Kromasil, mentioned above [30]. Kromasil is a very pure spherical silica which has been heat treated and subsequently rehydroxylated. This material was reported to give good results relative to the other packing which contained a variety of metal ions within the structure.

It therefore appears that there are two theories to account for the poor performance of the silicas. One is that the heat treated silicas which contain metal impurities may not be fully hydroxylated without special treatment. This follows the earlier observation of Engelhardt that heat treatment above 400°C results in materials which cannot be fully returned to their original level of hydration. The success of very pure silicas, such as Kromasil and Zorbax R_x, in the separation of basic materials implies that these silicas, at least, may be rehydroxylated successfully. Thus, one can conclude that perhaps the presence of the metals embedded in the surface of the silicas prevents its full rehydrox-

ylation due to the changes that such embedding causes in the solubility of the material. This would ensure that the remaining silanol groups are acidic. The other possible theory is that the metals in the matrix cause the neighbouring silanols to be more acidic than those in a pure silica because of their electron withdrawing effects, irrespective of the state of rehydroxylation of the silica.

Some of the evidence points to there being a limited but active subset of silanol groups on the silica surface which can be saturated with relatively small quantities of basic solute and this can be used to support the latter view. It should be noted, however, that the performance of the packings deteriorates with hydrolysis as more active sites are uncovered and may be much worse on partially bonded silicas, which suggests that the subset is probably larger than first appears; it is merely covered by the bonded groups. The uniformly low level of reversed-phase character of packings prepared from ammonium bifluoride treatment together with the variation in ionic character with the metal content of the silica argues in favour of the effects being due to the interaction of the metals with the adjacent silanol groups.

The numbers of silanol groups which may be so affected can be estimated by consideration of the numbers of metal atoms which are close to the surface. If the metal influences the silanols adjacent to it, then any metal atom should influence between one and four silanols. This is due to the average thickness of the walls being between 2 and 4 atoms: even a metal ion which is buried in the structure can only be one layer away from a surface. If we suppose that a packing has 100 ppm of metal atoms in the matrix and the metal influences the acidity of only one silanol group, this will lead to the formation of around 150 nmoles of active groups free per gram of packing after TMS bonding. This value appears to be of the right order of magnitude to explain the effects observed by Köhler [11] and Nawrocki [29].

Weighing the data available, it is apparent that insufficient rehydroxylation can cause problems with reversed-phase separations by the mechanism proposed by Köhler *et al.* [11]. Silicas which have been extensively dehydroxylated are known to give poor reversed-phase separations. This is not, however the sole cause of the problems which are seen. Even with silicas which are thought to be fully hydroxylated, there are differences in the retention and chromatographic performance of basic molecules which point to a remaining set of acidic silanols. These are almost certainly those silanols adjacent to metals fixed into the silica matrix.

9. CONCLUSIONS

The poor performance of reversed-phase packings for basic compounds has been shown to be due to the presence of strongly acidic silanol groups which occur on the surface of some silicas. The majority of the silicas which show poor performance for basic compounds are those which contain significant levels of heavy metal impurities and which have been heat treated during their synthesis. The heat treatment is believed to incorporate the metals into the surface of the silica where they act to enhance the acidity of silanol groups in their immediate vicinity. Rehydroxylation processes alone do not allow such materials to function well in the chromatography of basic compounds.

Pure silicas which have been heat treated are able, after appropriate rehydroxylation procedures, to perform well in the chromatography of bases. Silicas which have never been heat treated but which contain metal ions are also able to perform well. It appears that the metals present in the silica render local silanol groups more acidic irrespective of the hydroxylation level.

The chromatography of bases should be carried out on silicas which are pure and to which care has been given in their rehydroxylation, if they have been heat treated during their preparation. The bonding chemistry is probably not as important, given the results from the hydrolysis of packings, although end capping appears to block some of the more active sites, resulting in better performance. It should be remembered, however, that the packings which are most suitable for bases may not necessarily be the best for the separation of other types of compound

where a more acidic silica may perform to advantage.

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