

CHROMSYMP. 207

MIXED RETENTION MECHANISMS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ALKYL-BONDED SILICA

R. N. NIKOLOV

Institute of Chemical Technology, Central Research Laboratory, BG-1156 Sofia (Bulgaria)

SUMMARY

A model of "monomeric" alkyl-bonded silica for high-performance liquid chromatography is proposed, accounting for the presence of unreacted silanol groups on the surface of the derivatized silica matrix. Under chromatographic conditions the silanols are considered to be hydrated, while the anchored alkyl ligates can be partially or fully solvated by organic molecules of the eluent. With aqueous-organic eluents the solute retention is explained as being due to both silanophilic and hydrophobic-dispersive interactions of the solute with the silanols and the alkyl ligates, respectively. A retention equation is presented, the peculiarities of which are discussed in detail. The procedure for the identification of the retention mechanisms and for the evaluation of their contributions to the solute retention is verified with published experimental data.

INTRODUCTION

The problems of retention in high-performance liquid chromatography (HPLC) with chemically bonded phases, especially with the "monomeric" alkyl-bonded types, have been the subject of numerous investigations and publications. Many models of the so-called reversed phases and different mechanisms attempting to explain retention have been proposed: adsorption¹⁻⁷, absorption (partition)⁸⁻¹¹, mixed adsorption and absorption¹², dispersive interaction¹³, solubility in the eluent¹⁴, solvophobic adsorption^{15,16} and compulsory absorption¹⁷. In all cases it was suggested that retention is governed by only one mechanism.

Horváth and co-workers^{18,19} were the first to report the observation of a dual retention mechanism in the reversed-phase HPLC of crown ethers with alkyl-bonded silica. They assumed that the retention is caused not only by solvophobic, but also by the so-called "silanophilic" interactions between the solute and the accessible silanol groups at the surface of the derivatized silica matrix.

This fact, the recent investigations by Scott²⁰ and Simpson²¹ on the role of adsorbed water on the superficial silanols in the activated silica, and many published plots of k versus percentage of carbon that do not pass through zero k on extrapolation to zero carbon content, allowed an improved model of monomeric

alkyl-bonded silica to be proposed²². It accounts for the presence of hydrated silanols under chromatographic conditions, while the anchored alkyl ligates are considered to be partially or fully solvated by organic molecules of the eluent. The possibility of internal rotation for most sigma C-C bonds in the solvated alkyl chains determines their relative freedom of motion.

It was suggested that the solute retention in the reversed-phase systems (if solute dissociation is avoided) is a complex phenomenon governed by mixed mechanisms, including solvophobic and silanophilic effects. With aqueous-organic eluents the water causes the appearance of hydrophobic interactions between the ligates and the solute molecules. Thus, the attraction between them, initially affected by the weak dispersive forces, strongly increases and can be considered as due to hydrophobic-dispersive (HD) interactions. With organic-enriched eluents the relatively high mobility of the solvated ligates allows the penetration of solute molecules among them, ensuring strong bulk hydrophobic-dispersive (BHD) interactions. For the same reason the hydrated silanols are also more accessible for silanophilic (SPH) interactions. The latter are considered here as all possible interactions (specific and/or non-specific) between the silanols and the solute molecules. Hence, the solute retention in this instance is controlled by both SPH and HD mechanisms.

An increase in the water content of the eluent to about 50% leads to weaker solvation of the alkyl ligates and to more intensive BHD interactions. Simultaneously, the HD interactions between the ligates become stronger, thus giving rise to the influence of steric effects. The hydrated silanols remain inaccessible to certain solutes, the latter being retained by means of the HD mechanism only. With more than 50% water in the eluent, strong HD interactions between the ligates lead to their shrinkage. They form a relatively compact layer-like structure that limits the solute penetration. Thus the probability of both BHD and SPH interactions occurring is reduced, leaving only the possibility of superficial hydrophobic-dispersive (SHD) interactions of the solute with the shrunken organic layer. The retention of most solutes in such a system is obviously effected only by the HD mechanism, determined here by both BHD and SHD interactions. With pure water as the eluent an impenetrable layer of entangled ligates is formed on the silica surface, the solute retention being controlled by SHD interactions only.

In this paper an attempt is made to explain and quantitate the retention of non-dissociated solutes in reversed-phase chromatographic systems, in part those with monomeric alkyl-bonded silica. The peculiarities of the retention equation ensuing from the stationary phase model introduced, and from the probable retention mechanisms, are discussed in detail. The possibilities for the identification of the retention mechanisms and for the evaluation of their contributions to the solute retention are considered and verified with published experimental data.

THEORETICAL

As shown elsewhere²³, the generalized retention equation for all column chromatographic systems can be written in the form

$$E = \sum_i S_i K_i \quad (1)$$

where E is the integral retention effect, representing the net retention volume per gram of packing, K_i are the appropriate partial partition coefficients for the acting mixed retention mechanisms and S_i are the corresponding specific phase characteristics of the packing. A particular representation of eqn. 1 applicable to HPLC with chemically bonded phases appears to be

$$E = N_{\text{AH}}K_{\text{SP}} + W_{\text{OL}}K_{\text{SD}} + N_{\text{PG}}K_{\text{FS}} \quad (2)$$

which accounts for the action of the silanophilic (K_{SP}), solvophobic-dispersive (K_{SD}) and functional-specific (K_{FS}) mechanisms. The last is determined by the specific solute interactions with polar functional groups ($-\text{OH}$, $-\text{NH}_2$ or $-\text{CN}$) usually located in the ω -position to the main alkyl chains of some phases. N_{AH} and N_{PG} are the number of moles of the accessible silanols and of the polar functional groups, respectively, and W_{OL} is the weight of the organic ligates (hydrocarbonaceous part only). All phase characteristics in eqn. 2 are expressed per gram of column packing.

Most systems employed for HPLC contain monomeric alkyl-bonded silica as stationary phases and aqueous-organic eluents. For such systems, eqn. 2 can be reduced to

$$E = N_{\text{AH}}K_{\text{SP}} + W_{\text{AL}}K_{\text{HD}} \quad (3)$$

where K_{HD} is related to the HD mechanism and W_{AL} represents the weight of alkyl ligates per gram of packing. As mentioned above, depending on the eluent composition SPH and/or HD mechanisms can govern the solute retention in these systems. Analogously, the same mechanisms act in the systems when, holding the eluent composition constant, one changes the surface concentration of the alkyl ligates or simply W_{AL} . Then, at small W_{AL} the SPH will prevail over the HD interactions, whereas at moderate W_{AL} the situation will be the opposite. With higher values of W_{AL} , BHD and SHD interactions will mainly determine the solute retention. Hence, it is clear that with each W_{AL} the HD contribution to the solute retention is guaranteed. The SPH contribution could be considerable with small W_{AL} only, depending on the nature of the solute.

Obviously, the problem of identification of the retention mechanisms acting in the systems discussed reduces to solving eqn. 3, *i.e.*, to determining K_{SP} and K_{HD} values. Prior to considering the means of finding the solution of this equation, two main problems should be solved: the evaluation of W_{AL} and N_{AH} and overcoming the difficulties due to the dependence of K_{HD} on W_{AL} (with constant eluent composition), which transforms eqn. 3 into a non-linear equation and does not allow it to be solved by matrix algebra²⁴.

Evaluation of specific phase characteristics

The values of W_{AL} and N_{AH} can be calculated approximately from the carbon percentage, P_{C} , of the packing and from the number of moles of superficial silanols, N_{OH}^{s} , per gram of silica used for preparation of the bonded phase. Usually, P_{C} is determined by microanalysis²⁵, while for evaluation of N_{OH}^{s} either methods using methyllithium or methylmagnesium iodide²⁶ or the method of deuterium exchange^{27,28} could be applied. A good approximate value of N_{OH}^{s} can be obtained from expression

$$N_{\text{OH}}^{\text{b}} = 4.6 \cdot 10^{14} A_{\text{S}} / Z_{\text{A}} = 7.64 \cdot 10^{-10} A_{\text{S}} \quad (4)$$

where Z_{A} is Avogadro's number and A_{S} (cm^2g^{-1}) is the specific surface area of the silica. Here 4.6 represents the statistically mean number of silanols per nm^2 of the silica surface²⁹.

To evaluate W_{AL} and N_{AH} correctly, the following facts and peculiarities should be accounted for: the type of silanizing reagent (form and length of its alkyl chain, its functionality); the formation of a new siloxane bond with the derivatization of each silanol group, thus anchoring a molecular fragment of the reagent to the silica surface (e.g., $-\text{SiCl}_2\text{R}$, $\frac{1}{2}\text{SiClR}$, $-\text{Si}(\text{CH}_3)_2\text{R}$); the formation of new silanols by hydrolysis of unreacted chlorine atoms in the fragments; the eventual effect of the so-called "end-capping" procedure; the presence of unreacted silanols at the surface of the silica matrix; and the "screening" effect of the chemically bonded fragments and especially of the alkyl ligates towards the unreacted silanols.

Taking all this into account, the following expressions can be derived.

Expression for W_{AL} . This is

$$W_{\text{AL}} = M_{\text{A}} N_{\text{F}} \quad (5)$$

where M_{A} is the molecular weight of all alkyl groups in the molecule of the silanizing reagent and N_{F} is the number of moles of the fragments bonded to the silica surface per gram of packing. Then,

$$N_{\text{F}} = P_{\text{C}} / 1200 Z_{\text{C}} \quad (6)$$

where Z_{C} is the total number of carbon atoms in the silanizing molecule.

Expression for N_{AH} . The molar content of the accessible silanols can be expressed by the equation

$$N_{\text{AH}} = \alpha [N_{\text{OH}}^{\text{b}}(1 - M_{\text{F}} N_{\text{F}}) - N_{\text{F}} / N_{\text{SE}} + N_{\text{OH}}^{\text{b}}] = \alpha N_{\text{H}} \quad (7)$$

where the term $N_{\text{OH}}^{\text{b}}(1 - M_{\text{F}} N_{\text{F}})$ represents the total number of moles of silanols on the surface of that amount of the silica transformed into 1 g of bonded phase. $N_{\text{F}} / N_{\text{SE}}$ and N_{OH}^{b} represent the corresponding molar amounts of derivatized and of additionally generated silanols, respectively, per gram of bonded phase. M_{F} is the mean molecular weight of the bonded fragments (including silicon atoms) and N_{SE} represents the molar ratio of fragments to derivatized silanols and will be called here the "silanol equivalent". Hence, the expression in square brackets in eqn. 7 represents the molar content of all available silanols in 1 g of bonded phase, N_{H} . Then, the ratio $N_{\text{AH}} / N_{\text{H}}$, denoted here by α , will be called the "accessibility coefficient".

It follows from eqn. 7 that the values of N_{SE} , M_{F} , N_{OH}^{b} and α have to be known in order to be able to obtain a reliable evaluation of N_{AH} . There exist no methods for their experimental determination, but they can be calculated. Some details of the calculations are considered below.

Problems with evaluation of N_{SE} . Formally, the silanol equivalent of a silanizing molecule is determined by the reciprocal of the number of chlorine atoms participating in the derivatization procedure. With a monofunctional alkylchlorosilane

there is only one chlorine atom that can react and hence $N_{SE} = N_{SE}^{(1)} = 1$ (the superscript corresponds to the number of reacted chlorine atoms). With bi- and trifunctional reagents the reaction can proceed, at least theoretically, in two or three directions, respectively. Then for some silanizing molecules there will $N_{SE}^{(1)} = 1$, for others $N_{SE}^{(2)} = 1/2$, and for others $N_{SE}^{(3)} = 1/3$.

Let us assume that the probability of reacting three chlorine atoms in a trifunctional molecule is negligibly small for steric reasons (this will be confirmed rigorously below). Hence, there remain practically the possibilities of $N_{SE}^{(1)}$ and $N_{SE}^{(2)}$ for both bi- and trifunctional silanizing reagents. Consequently, N_{SE} must be considered as a mean value for the reagent as a whole. Then, if ν is the molar fraction of the derivatized silanols, reacted in a ratio of one silanol group per one silanizing molecule, the latter will have $N_{SE}^{(1)} = 1$. Alternatively, $1 - \nu$ will be the molar fraction of the derivatized silanols reacted in a ratio of two silanol groups per one silanizing molecule with $N_{SE}^{(2)} = 1/2$. Hence, we obtain

$$N_{SE} = (1 + \nu)/2 \quad (8)$$

which means that a knowledge of ν is required for evaluation of N_{SE} .

Problems with evaluation of M_F and N_{OH}^a . With monofunctional reagents the molecular weight of the bonded fragments, M_F , is clearly determined. However, with bi- and trifunctional reagents, M_F will be a mean value that depends on the ratio of the molecules participating in the derivatization with $N_{SE}^{(1)}$ and $N_{SE}^{(2)}$. It can be shown that

$$M_F = [2\nu/(1 + \nu)]M_{F1} + [(1 - \nu)/(1 + \nu)]M_{F2} \quad (9)$$

where M_{F1} and M_{F2} are the molecular weights of the bonded fragments from molecules with $N_{SE}^{(1)}$ and $N_{SE}^{(2)}$, respectively. As M_{F1} and M_{F2} are easy to determine, the main requirement for evaluating M_F remains a knowledge of ν .

Taking into account the above considerations, it is no problem to find expressions for determining the additionally generated silanol groups, N_{OH}^a , after hydrolysis of the unreacted chlorine atoms. It is clear that with monofunctional reagents $N_{OH}^a = 0$, while with bi- and trifunctional reagents the expressions will be

$$N_{OH}^a = [2\nu/(1 + \nu)]N_F \quad (10)$$

and

$$N_{OH}^a = [(1 + 3\nu)/(1 + \nu)]N_F \quad (11)$$

respectively. Again, a knowledge of ν appears to be the main requirement for the calculation of N_{OH}^a .

The importance of ν for the calculation of N_{SE} , M_F and N_{OH}^a is obvious. There is no method for its determination, but an approximate evaluation is possible.

Probability approach for the evaluation of ν . It follows from the definition of ν that it corresponds to the probability, p_1 , of reacting one chlorine atom from the silanizing molecule with one silanol group. On the other hand, $1 - \nu$ corresponds to

the probability, p_2 , of reacting two chlorine atoms of a molecule with two neighbouring silanols. Both probabilities are determined by corresponding sets of concentration, diffusion and steric factors. The first two types of factors are in general identical for both sets. The difference between the sets and, hence, between p_1 and p_2 , is due mainly to the non-identical steric factors.

More precisely, this means the distance between the hydrogen atoms, d_H , of two neighbouring silanols is a probability function, because of rotation of the hydrogen atom around the Si-O bond. It seems logical to suppose that two chlorine atoms of a silanizing molecule could react with two silanol groups only in the case of coincidental distances between the hydrogen and chlorine atoms. Because of oscillation of the chlorine atoms, the distance between them will vary in the range $d_{Cl} \pm \Delta$, where d_{Cl} is a mean value. Consequently, the probability $p(d_H < d_{Cl} \pm \Delta)$ of coincidence of d_H with $d_{Cl} \pm \Delta$ should be calculated.

The mean value of d_H and its variance, as well as $d_{Cl} \pm \Delta$, can be calculated from the available data of interatomic distances and angles³⁰. As a first approximation the silanols can be considered as distributed uniformly on the silica surface with a concentration of 4.6 groups per nm² (ref. 29). Then d_H is 0.5 nm and, assuming a normal law of its distribution, we obtain $p(d_H < d_{Cl} \pm \Delta) = 0.020$ with the bifunctional reagents, and $p(d_H < d_{Cl} \pm \Delta) = 0.025$ with trifunctional reagents.

With six possible pairs of neighbouring silanols, the probability of reacting two chlorine atoms of a silanizing molecule will be

$$p_2 = 6p(d_H < d_{Cl} \pm \Delta) = 0.12 \quad (12)$$

for bifunctional reagents and

$$p_2 = 18p(d_H < d_{Cl} \pm \Delta) = 0.45 \quad (13)$$

for trifunctional reagents because of three equal possibilities of combining the chlorine pairs. Following an analogous approach, it can be shown that the probability of reacting three chlorine atoms of a trifunctional molecule is $9.4 \cdot 10^{-5}$. Obviously, this value is negligibly small.

Further, considering $p_1 + p_2 = 1$, it follows that

$$(1 - v)/v = p_2/(1 - p_2)$$

or

$$v = 1 - p_2 \quad (14)$$

Combinations of eqns. 12, 13 and 14 lead to the evaluation of v for different silanizing reagents. These values are presented in Table I together with the corresponding values or equations for N_{SE} , N_{OH}^3 and M_F . The information from Table I can facilitate the evaluation of N_{AH} if the accessibility coefficient, α , is also available.

Problems with evaluation of α . As a ratio of accessible to available silanols in the derivatized silica, α accounts for the influence of the steric factors in a chromatographic system and should depend on the following: the structure and the chain

TABLE I
VALUES AND EQUATIONS FOR VARIOUS PARAMETERS DEPENDING ON THE FUNCTIONALITY OF THE SILANIZING REAGENT

Parameter	Silanizing reagent		
	Monofunctional	Bifunctional	Trifunctional
ν	1	0.88	0.55
N_{SE}	1	0.94	0.78
N_{OH} =	0	$0.94N_F$	$1.71N_F$
M_F =	M_{F1}	$0.94M_{F1} + 0.06M_{F2}$	$0.71M_{F1} + 0.29M_{F2}$

length of the alkyl ligates; their surface concentration; the nature of the eluent or its composition in the case of aqueous-organic media; and the structure and the molecular size of the chromatographed solute.

The most important factor is the surface concentration of the ligates. Its reciprocal value, *i.e.*, the silica surface area, S_L , belonging to an alkyl ligate can be defined as

$$S_L = A_S(1 - M_F N_F) / Z_A N_F \quad (15)$$

It is assumed in eqn. 15 that the number of moles of the ligate is identical with that of the bonded fragments, N_F . Of course, this cannot be generally valid, but it is true for many practically applicable phases.

It seems logical to suppose that the surface area, S'_L , occupied by the ligate will depend on its configuration, the latter being determined by both the conformation and the orientation of the alkyl chain towards the silica surface. Depending on the eluent composition, the alkyl chain acquires various conformations, which are generally different from its linear form. Further, we shall introduce the term "standard" configuration in order to denote a state of the ligate in which the alkyl chain is linear and orthogonally oriented to the area sector of bonding. All other states, in which the ligate chain is not linear and hence not orthogonally oriented, will be denoted as ligate "non-standard" configurations. Then, S'_L can be divided in two constituents: S_R , representing the area sector of bonding with a standard ligate configuration, and S_E , representing the area additionally screened by the ligate in the case of a non-standard configuration.

A calculation based on the available data^{30,31} gives 0.193 nm^2 for S_R , which can be considered also as the alkyl chain cross-sectional area. However, the calculation of S_E appears to be a more serious problem and it will be considered later.

With a standard configuration of the ligate, the mean free silica surface area ($S_L - S_R$) belonging to it will contain unreacted silanols. Their number, Z_S , can be defined as the ratio

$$Z_S = (S_L - S_R) / S_S \quad (16)$$

where S_S is the silica surface area belonging to a hydrated silanol group in the bonded phase. S_S can be easily calculated, but it is not necessary, as will be shown below.

Hence, Z_S is the number of available silanols around a ligate.

With a non-standard configuration, the silanols on the screened area, S_E , remain inaccessible to the solute molecules. Thus, the number of the theoretically accessible silanol groups will be

$$Z_{ST} = (S_L - S_R - S_E)/S_S \quad (17)$$

Then, the ratio

$$\alpha' = Z_{ST}/Z_S = (S_L - S_R - S_E)/(S_L - S_R) \quad (18)$$

can be called the "apparent" accessibility coefficient, which accounts for the influence of the ligate surface concentration (by means of S_L) and for the role of the structure and the chain length (by means of S_R), as well as the nature of the eluent (by means of S_E).

The real accessibility coefficient, α , must account also for the molecular size of the solute. The smaller is the surface area, S_C , occupied by the solute molecule with adsorption, the greater will be the probability that the molecule will reach the free surface area around the ligate. Denoting this probability by p_C , we can write

$$\alpha = \alpha' p_C \quad (19)$$

where p_C is determined by the ratio

$$p_C = [(S_L - S_R - S_E) - S_C]/(S_L - S_R - S_E) \quad (20)$$

Combining eqns. 18, 19 and 20, we obtain

$$\alpha = 1 - (S_E + S_C)/(S_L - S_R) \quad (21)$$

In eqn. 21, S_L and S_R are easily determined. Values of S_C for some substances are also obtainable, and for others they can be calculated by taking the geometrical structure into consideration. Hence, there remains the question of the calculation of S_E .

Problems with evaluation of S_E . The surface area, additionally screened by the anchored ligate, can be presented in the following most common form:

$$S_E = \eta S_M \quad (22)$$

where S_M is the maximal area that can be screened by the ligate at a definite surface concentration and η is a parameter that will be explained later.

With a small concentration of the ligates, $S_L - S_R$ is large enough to permit a parallel orientation of the ligate towards the silica surface. In this state the screened area will be maximal and will depend on the chain structure and length. Then, denoting this area by S_P , it follows that S_M will be identical with it. With a higher ligate concentration $S_L - S_R$ becomes smaller and does not allow a parallel orientation of the ligate. Hence, S_M cannot exceed the available free area ($S_L - S_R$). Summarizing the above conclusions, we can write

$$\text{if } S_L - S_R \geq S_P, \text{ then } S_M \equiv S_P \quad (23)$$

$$\text{if } S_L - S_R < S_P, \text{ then } S_M \equiv S_L - S_R$$

For evaluation of S_P the length and the mean diameter of the hydrocarbonaceous chain have to be taken into account. It follows from the available data³⁰⁻³² that for a C_{18} ligate $S_P = 0.941 \text{ nm}^2$. Analogously, S_P values for different types of alkyl ligates can also be calculated.

Parameter η . It follows from eqn. 22 that η represents the fraction of S_M that is really screened by the ligate. The screening effect is dependent on the mobility of the anchored alkyl chain. A movable ligate makes the surface silanols around it more accessible to the solute molecules. Then, the greater the ligate mobility, the smaller will be the value of η . Consequently, η can be considered as the reciprocal of the mobility, the latter being evaluated by means of the total number of configurations, Z_T , really gained by the ligate, *i.e.*,

$$\eta = 1/Z_T \quad (24)$$

Remembering that there exists only one standard configuration for a ligate, we can write

$$Z_T = 1 + Z_N \quad (25)$$

where Z_N is the number of the really gained non-standard configurations. Further, Z_N can be presented as

$$Z_N = p_L Z_M \quad (26)$$

where Z_M is the total number of possible non-standard configurations and p_L is the probability of such a configuration being gained by the ligate.

It could be supposed that Z_M is directly proportional to the size of the free space around the ligate and inversely proportional to the space occupied by it. Considering these terms as linearly dependent on the corresponding $S_L - S_R$ and S_R areas, it can be postulated that

$$Z_M = \lambda(S_L - S_R)/S_R \quad (27)$$

where λ is the maximal number of non-standard configurations at $S_L - S_R = S_R$.

The probability of gaining a non-standard configuration is complex, determined by both the probabilities of finding the ligate in a definite conformation, p_D , and of changing the latter with another one, p_T , *i.e.*,

$$p_L = p_D p_T \quad (28)$$

It is evident from the definition of λ that

$$p_D = 1/\lambda \quad (29)$$

On the other hand, the change of ligate conformation depends on the composition of the aqueous-organic eluent and on the nature of the organic constituent. Denoting the organic weight fraction in the eluent by θ , maximal solvation can be expected and, hence, a maximal mobility of the ligate at $\theta = 1$. With different organic eluents the maximal ligate mobility will depend on the solvating ability of their molecules. As this ability is determined by the molecule affinity towards dispersive interactions with the alkyl ligate, an approximate evaluation of the eluent solvating power can be obtained from the ratio

$$\psi = \delta_a^2 / \delta_o^2 \quad (30)$$

where δ_o is the total solubility parameter and δ_a its dispersive component³³ for the organic constituent. Then, p_T can be defined as

$$p_T = \theta\psi = \theta\delta_a^2 / \delta_o^2 \quad (31)$$

where with pure water as the eluent ($\theta = 0$), $p_T = 0$, as the ligate is fixed owing to the strong HD interactions and hence cannot change its configuration. With $\theta = 1$ the probability of changing the configuration is maximal, *i.e.*, $p_T = \psi$. If θ varies between 0 and 1, the intermediate mobility of the ligate allows its configuration to be changed with a probability determined by eqn. 31.

Combining eqns. 24-31, we obtain

$$\eta = \delta_o^2 S_R / [\delta_o^2 S_R + \theta\delta_a^2 (S_L - S_R)] \quad (32)$$

which permits S_E to be evaluated from eqn. 22 in accordance with conditions 23 for determining S_M .

Evaluation of partial partition coefficients K_{SP} and K_{HD}

Usually, a retention equation is linear and the corresponding K values can be obtained as the solution of a system of linear equations, based on the retention equation. This requires a series of chromatographic experiments on similar packings differing in their phase characteristics. As mentioned above, the dependence of K_{HD} on W_{AL} frustrates the possibility of solving eqn. 3 in the usual manner because of its non-linearity. Hence, it is necessary to obtain K_{SP} directly by eluting the solute from a column packed with the pure silica used for the preparation of the alkyl-bonded packings. Further, rearranging eqn. 3 in the form

$$E^i = \alpha^i N_H^i K_{SP} + W_{AL}^i K_{HD}^i \quad (33)$$

where the superscript i relates the corresponding terms to the packings with different carbon contents (*i.e.*, different W_{AL}), we can obtain K_{HD}^i . A necessary condition for the correct evaluation of K_{SP} and K_{HD}^i is to carry out the chromatographic experiments with all the silica and the alkyl-bonded packings at constant temperature with a constant eluent composition.

TABLE II
CHARACTERISTICS OF ORIGINAL SILICA AND SILANIZING REAGENT USED FOR PREPARATION OF ALKYL-BONDED PACKINGS

Material	Parameter	Value and dimensions	Notes	
Silica: Partisil	Average particle size	6 μm	Ref. 34	
	Specific surface area, A_s	402 $\text{m}^2 \cdot \text{g}^{-1}$	BET method, ref. 34	
	Superficial silanol groups, N_{OH}	$3.07 \cdot 10^{-3} \text{ mol} \cdot \text{g}^{-1}$	Calculated from eqn. 4, this paper	
Silanizing reagent: octadecyltrichlorosilane	Carbon atoms, Z_C	18		
	Molar fraction, ν	0.55	See Table I, this paper	
	Silanizing equivalent, N_{SE}	0.78	See Table I, this paper	
	Molecular weights:			
	M_A	253		
	M_{F1}	315		
	M_{F2}	298		
	M_F	310	Calculated from eqn. 9, this paper	

CALCULATIONS, RESULTS AND DISCUSSION

To illustrate the validity of the proposed model of alkyl-bonded silica, we employed the experimental data published by Hennion *et al.*³⁴. All necessary characteristics of both the silica and the silanizing reagent used by the authors for preparing the alkyl-bonded packings are presented in Table II. Some of the data have been obtained experimentally by the authors and others were calculated by us.

The specific phase characteristics of the various packings are presented in Table III. N_F and W_{AL} values were calculated from the carbon percentage, P_C , while the

TABLE III
CALCULATED SPECIFIC PHASE CHARACTERISTICS OF THE ALKYL-BONDED PACKINGS

Packing No. (i)	P_C^* (%)	N_F^{**} ($\times 10^{-4} \text{ mol} \cdot \text{g}^{-1}$)	W_{AL}^{***} ($\times 10^{-2} \text{ g} \cdot \text{g}^{-1}$)	N_H^{\S} ($\times 10^{-3} \text{ mol} \cdot \text{g}^{-1}$)
0	0	0	0	3.07 ^{\S\S}
1	2.9	1.34	3.39	3.00
2	4.8	2.22	5.62	2.95
3	6.5	3.01	7.62	2.91
4	9.4	4.35	11.0	2.84
5	11.5	5.32	13.5	2.79
6	14.4	6.67	16.9	2.71
7	23.0	10.6	26.8	2.51

* All P_C values were readed from the log k vs. P_C plots in ref. 34.

** Calculated from eqn. 6.

*** Calculated from eqn. 5.

^{\S} Calculated from eqn. 7.

^{\S\S} Calculated as N_{OH} from eqn. 4.

TABLE IV

PARAMETERS NECESSARY FOR EVALUATION OF THE ACCESSIBILITY COEFFICIENT, α , WITH TWO ELUENT COMPOSITIONS: 70:30 AND 40:60 METHANOL-WATER

Conditions	<i>i</i>	S_L^* (nm ²)	$S_L - S_R^{**}$ (nm ²)	S_M^{***} (nm ²)	70:30		40:60	
					η^\S	$S_E^{\S\S}$ (nm ²)	η^\S	$S_E^{\S\S}$ (nm ²)
$S_L - S_R \geq S_P^{\S\S\S}$	1	4.77	4.58	0.941	0.188	0.177	0.288	0.271
	2	2.80	2.61	0.941	0.289	0.272	0.415	0.391
	3	2.01	1.82	0.941	0.368	0.346	0.504	0.474
$S_L - S_R < S_P^{\S\S\S}$	4	1.33	1.14	0.941	0.481	0.453	0.619	0.583
	5	1.05	0.857	0.857	0.553	0.474	0.684	0.586
	6	0.794	0.601	0.601	0.638	0.383	0.755	0.454
	7	0.423	0.230	0.230	0.822	0.189	0.890	0.205

* Calculated from eqn. 15.

** For all alkyl ligates with normal carbon chain, $S_R = 0.193$ nm², calculated from the molecular structure.

*** Calculated according to relations 23.

§ Calculated from eqn. 32.

§§ Calculated from eqn. 27.

§§§ For C₁₈ alkyl ligates $S_P = 0.941$ nm².

values of N_H were obtained from eqn. 7 on the basis of the N_{OH}^B value. The latter, calculated from eqn. 4, is given in the N_H column of Table III for the "zero" number of the packing, *i*, corresponding to the pure silica. Although the authors have not experimented with such a packing, this value is important and it should be presented. As all experiments were carried out with water-methanol eluents, the values of both the total solubility parameter for methanol, δ_o , and its dispersive component, δ_d , were taken from ref. 33 [14.5 and 7.4 (cal · cm⁻³)^{1/2}, respectively].

TABLE V

VALUES OF ACCESSIBILITY COEFFICIENT, α , FOR SOLUTES IN THE ALKYL-BONDED PACKINGS

Solvent	Solute	S_C^* (nm ²)	α^{**} in the corresponding packings, <i>i</i>						
			1	2	3	4	5	6	7
Methanol-water (70:30)	Benzene	0.51	0.85	0.70	0.53	0.16	—	—	—
	Naphthalene	0.85	0.78	0.57	0.34	—	—	—	—
	Phenanthrene	1.19	0.70	0.44	0.16	—	—	—	—
	Anthracene	1.19	0.70	0.44	0.16	—	—	—	—
	Pyrene	1.36	0.66	0.37	0.06	—	—	—	—
Methanol-water (40:60)	Hydroquinone	0.32	0.87	0.73	0.56	0.21	—	—	—
	Resorcinol	0.82	0.76	0.54	0.29	—	—	—	—
	Methylresorcinol	1.33	0.65	0.34	0.01	—	—	—	—
	Phenol	0.32	0.87	0.73	0.56	0.21	—	—	—

* Calculated from the molecular structure of the solute in a state of interaction with the silanol groups.

** Calculated from eqn. 21.

All parameters necessary for the evaluation of the accessibility coefficient, α , are given in Table IV. It is evident that the increase in the amount of ligate, W_{AL} , corresponding to the increase in the packing number, i , causes a gradual decrease in the silica surface area, S_L , belonging to an alkyl ligate. Correspondingly, $S_L - S_R$ also decreases, while the surface area, S_E , additionally screened by the ligate, passes through a maximum located approximately at 11% carbon in the packing. Moreover, the S_E values with the water-enriched eluent (40:60 methanol-water) are always greater than those with the methanol-enriched eluent (70:30 methanol-water). These facts agree well with the model of alkyl-bonded silica discussed and also confirm its adequacy.

Values of the accessibility coefficient, α , for the solutes chromatographed are presented in Table V, which also gives values of the surface area, S_C , required by the different solute molecules when they are in a state of interaction with the silanol groups. For each solute a gradual decrease in α is observed with increase in W_{AL} in the packings. With more than a 9% carbon content, α becomes zero for nearly all solutes, indicating the absence of SPH interactions because of steric hindrances. The greater is S_C for a solute molecule, the smaller are the corresponding α values, which is in agreement with the model considered.

The partial partition coefficients K_{SP} and K_{HD} evaluated for the solutes in the various alkyl-bonded packings are presented in Table VI. Relatively small to moderate values of K_{SP} are observed for all solutes, bearing in mind that this coefficient is per mole of silanols. For the aromatic and polyaromatic hydrocarbons this evidence agrees well with the weak SPH interactions, which are to be expected because of their non-specific character. In contrast, the hydroxy compounds can interact specifically with the silanols, but in the chromatographic system considered these interactions are greatly influenced by the competition of the water molecules.

The HD partition coefficient, K_{HD} , with increase in W_{AL} , increases for each solute up to a certain value. When W_{AL} becomes large (e.g., for hydrocarbons with more than 14% of carbon in the packing and for hydroxy compounds with more than 11%) K_{HD} starts to decrease. With small to moderate W_{AL} values the solute molecules enter into BHD interactions with the alkyl ligates. Obviously, these inter-

TABLE VI

PARTIAL PARTITION COEFFICIENTS, K_{SP} AND K_{HD} , FOR SOLUTES IN THE ALKYL-BONDED PACKINGS

Solvent	Solute	K_{SP} ($cm^3 \cdot mol^{-1}$)	K_{HD} ($cm^3 \cdot g^{-1}$) in the corresponding packings, i						
			1	2	3	4	5	6	7
Methanol- water (70:30)	Benzene	136	1.83	2.66	3.94	7.94	10.6	11.8	6.65
	Naphthalene	245	6.27	9.61	13.3	21.0	27.2	33.5	19.7
	Phenanthrene	406	23.9	28.7	35.4	50.3	76.3	101	66.5
	Anthracene	444	26.7	32.7	44.2	63.8	99.4	113	84.7
	Pyrene	735	34.9	46.9	64.2	94.0	141	193	136
Methanol- water (40:60)	Hydroquinone	138	8.28	11.3	13.5	12.3	10.5	8.51	4.91
	Resorcinol	174	14.8	18.2	21.2	22.7	19.8	16.2	9.41
	Methylresorcinol	198	23.1	31.0	37.4	42.4	40.4	34.9	18.0
	Phenol	707	65.9	83.6	110	149	175	167	87.2

TABLE VII

RELATIVE CONTRIBUTIONS OF PARTIAL RETENTION EFFECTS (PRE) TO INTEGRAL RETENTION IN THE ALKYL-BONDED PACKINGS

Solvent	Solute	PRE	Alkyl-bonded packings, <i>i</i>						
			1	2	3	4	5	6	7
Methanol-water (70:30)	Benzene	$N_{AH}K_{SP}$	84.8	65.3	41.1	6.6	—	—	—
		$W_{AL}K_{HD}$	15.2	34.7	58.9	93.4	100	100	100
	Naphthalene	$N_{AH}K_{SP}$	73.0	43.3	19.3	—	—	—	—
		$W_{AL}K_{HD}$	27.0	56.7	80.7	100	100	100	100
	Phenanthrene	$N_{AH}K_{SP}$	51.3	24.6	6.6	—	—	—	—
		$W_{AL}K_{HD}$	48.7	75.4	93.4	100	100	100	100
	Anthracene	$N_{AH}K_{SP}$	50.7	23.9	5.8	—	—	—	—
		$W_{AL}K_{HD}$	49.3	76.1	94.2	100	100	100	100
	Pyrene	$N_{AH}K_{SP}$	54.8	23.3	2.6	—	—	—	—
		$W_{AL}K_{HD}$	45.2	76.7	97.4	100	100	100	100
Methanol-water (40:60)	Hydroquinone	$N_{AH}K_{SP}$	56.2	31.9	17.9	5.7	—	—	—
		$W_{AL}K_{HD}$	43.8	68.1	82.1	94.3	100	100	100
	Resorcinol	$N_{AH}K_{SP}$	44.2	21.3	8.3	—	—	—	—
		$W_{AL}K_{HD}$	55.8	78.7	91.7	100	100	100	100
	Methylresorcinol	$N_{AH}K_{SP}$	33.0	10.2	0.2	—	—	—	—
		$W_{AL}K_{HD}$	67.0	89.8	99.8	100	100	100	100
	Phenol	$N_{AH}K_{SP}$	45.2	24.5	12.1	2.5	—	—	—
		$W_{AL}K_{HD}$	54.8	75.5	87.9	97.5	100	100	100

actions become more effective with increase in W_{AL} , thus corresponding to the increase in K_{HD} . However, when a sufficiently high surface concentration of the ligates is achieved, the penetration of the solute molecules among them becomes more complicated. Then, the possibility of BHD interactions is reduced, whereas that of SHD interactions increases. Nevertheless, as the latter are less effective than BHD interactions, K_{HD} tends to decrease.

It can be seen from Table VI that the K_{HD} values of polyaromatic hydrocarbons are strongly dependent on the number of aromatic rings in the molecule and on the molecular configuration. The more rings, the larger are the K_{HD} values. This can be well explained with the increase in the molecular contact surface, contributing mainly to both BHD and SHD interactions. With anthracene and phenanthrene, however, the molecular configuration plays an important role. The order of aromatic rings in the anthracene molecule favours its penetration among the ligates, resulting in more effective interactions. In this respect the phenanthrene configuration is unfavourable, which is reflected in weaker interactions and hence in smaller K_{HD} values.

For hydroxy compounds, the K_{HD} values are essentially dependent on the molecular structure. In comparison with resorcinol, the *para*-position of the two hydroxyl groups in hydroquinone gives rise to more obstructions with regard to BHD and SHD interactions. As a larger part of the aromatic ring in the resorcinol molecule is accessible to interactions, its K_{HD} values are greater than those of hydroquinone. As the presence of a methyl group in the molecule will extend the hydrophobic moiety, it is reasonable that the K_{HD} values for methylresorcinol will always be larger than the corresponding values for resorcinol. Finally, it is also clear that, because of

the absence of special steric hindrances for the phenol molecule, its K_{HD} values are relatively large.

It is clear that the evaluation of K_{SP} and K_{HD} obtained above leads to the identification of the retention mechanisms acting in a reversed-phase system with alkyl-bonded silica. Having these values for different solutes, we can evaluate the contributions of both SPH and HD retention mechanisms to the integral solute retention. Results for the alkyl-bonded packings considered are presented in Table VII. For each solute the partial retention effects (PRE) are indicated in accordance with the corresponding retention mechanisms identified on the packings. All calculated values are percentages, thus allowing comparisons of the relative contributions.

It follows from Table VII that SPH interactions contribute mainly with low W_{AL} values. With increase in the molecular size of the solute the SPH contribution gradually decreases. It also decreases with increasing W_{AL} , becoming zero at a certain W_{AL} value. Simultaneously, an increase in the HD contribution to the retention is observed, the latter remaining unique with large values of W_{AL} .

In conclusion, the proposed model of alkyl-bonded silica accounts for the peculiarities of the most commonly employed reversed-phase chromatographic systems and allows a quantitative evaluation of both SPH and HD contributions to the integral solute retention. In order to illustrate the validity of this model, we employed published experimental data³⁴ for the calculations. The utilization of such data, which in this instance was connected with the application of some interpolation and extrapolation procedures, often leads to unavoidable inaccuracy of the results obtained. In our opinion the latter are approximate and serve only to demonstrate the possibilities of the model discussed for a quantitative interpretation of the mixed retention mechanisms in the HPLC systems considered.

ACKNOWLEDGEMENTS

I gratefully acknowledge the usefulness of the experimental data published by M. C. Hennion, C. Picard and M. Caude³⁴, Laboratoire de Chimie Analytique, École Supérieure de Physique et de Chimie de Paris, Paris, France.

REFERENCES

- 1 M. J. Telepchak, *Chromatographia*, 6 (1973) 234.
- 2 J. J. Kirkland, *J. Chromatogr. Sci.*, 9 (1975) 171.
- 3 T. Hanai and K. Fujimura, *J. Chromatogr. Sci.*, 14 (1976) 140.
- 4 K. K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
- 5 H. Hemetsberger, W. Maasfeld and H. Ricken, *Chromatographia*, 9 (1976) 303.
- 6 H. Hemetsberger, M. Kellermann and H. Ricken, *Chromatographia*, 10 (1977) 726.
- 7 H. Colin and G. Guiochon, *J. Chromatogr.*, 158 (1978) 183.
- 8 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1975) 171.
- 9 E. J. Kikta and E. Grushka, *Anal. Chem.*, 48 (1976) 1098.
- 10 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213.
- 11 C. H. Löchmüller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
- 12 V. Reháková and E. Smolková, *Chromatographia*, 9 (1976) 219.
- 13 K. Karch, I. Sebestian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.
- 14 D. C. Locke, *J. Chromatogr. Sci.*, 12 (1974) 433.
- 15 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
- 16 B. L. Karger, J. R. Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.

- 17 G. E. Berendsen and L. de Galan, *J. Chromatogr.*, 196 (1980) 21.
- 18 A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
- 19 K. E. Bij, Cs. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
- 20 R. P. W. Scott, *J. Chromatogr. Sci.*, 18 (1980) 297.
- 21 R. P. W. Scott and C. F. Simpson, *J. Chromatogr.*, 197 (1980) 11.
- 22 R. N. Nikolov, *Izv. Khim.*, 15 (1982) 36.
- 23 R. N. Nikolov, in E. Kováts (Editor), *Column Chromatography (Proceedings of Symposium, Lausanne, October 7-10, 1969)*, Swiss Chemists' Association, 1970, p. 156.
- 24 R. N. Nikolov, *Chromatographia*, 4 (1971) 565.
- 25 H. Engelhardt, *Hochdruck-Flüssigkeits-Chromatographie*, Springer-Verlag, Berlin, 1977, p. 133.
- 26 V. Ya. Davydov, A. V. Kiselev and L. T. Zhuravlev, *Trans. Faraday Soc.*, 60 (1964) 2254.
- 27 L. T. Zhuravlev and A. V. Kiselev, *Kolloidn. Zh.*, 24 (1962) 22.
- 28 A. A. Agzamkhodzhaev, L. T. Zhuravlev, A. V. Kiselev and K. Ya. Shengeliya, *Izv. Akad. Nauk SSSR, Ser Khim.*, 10 (1969) 2111.
- 29 R. K. Iler, *The Chemistry of Silica*, Wiley-Interscience, New York, 1979, p. 633.
- 30 *Tables of Interatomic Distances and Configuration in Molecules and Ions, Special Publication No. 11*, Chemical Society, London, 1958.
- 31 S. Fitzwater and L. S. Bartell, *J. Amer. Chem. Soc.*, 98 (1976) 8338.
- 32 L. Pauling, *The Nature of the Chemical Bond*, Cornell University Press, Ithaca, New York, 1960, pp. 257-264.
- 33 A. F. M. Barton, *Chem. Rev.*, 75 (1975) 731.
- 34 M. C. Hennion, C. Picard and M. Caude, *J. Chromatogr.*, 166 (1978) 21.