Journal of Chromatography, 446 (1988) 191–201
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1359

INFLUENCE OF THE POROUS SILICA GEL STRUCTURE ON THE COVERAGE DENSITY OF A CHEMICALLY BONDED C_{18} PHASE FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

BOGUSŁAW BUSZEWSKI*
Department of Chemical Physics, Institute of Chemistry, Maria Curie Sklodowska University, PL-20 031 Lublin (Poland)
DUŠAN BEREK
Polymer Institute, CChR, Slovak Academy of Sciences, CS-812 36 Bratislava (Czechoslovakia)
JÁN GARAJ
Department of Analytical Chemistry, Faculty of Chemical Technology, Slovak Technical University, CS-812 37 Bratislava (Czechoslovakia)
IVAN NOVÁK
Polymer Institute, CChR, Slovak Academy of Sciences, CS-812 36 Bratislava (Czechoslovakia)
and
ZDZISŁAW SUPRYNOWICZ
Department of Chemical Physics, Institute of Chemistry, Maria Curie Sklodowska University, PL-20 031 Lublin (Poland)

SUMMARY

In high-performance liquid chromatography (HPLC), silica gels are the most often used supports for chemically bonded stationary phases. The influence of the physical (volume and diameter of pores) and chemical (hydroxyl group concentration) structures of various silica gel supports on blocking effectively the binding of silanol groups by monochlorooctadecylsilane was investigated. Using silica gel supports with a pore volume $\geqslant 1.5$ cm^3/g and a mean pore diameter $\geqslant 14$ nm as starting materials, reproducible packings for HPLC having a high coverage density ($\alpha_{RP} \geqslant 4$ μmol/m^2) were obtained. Retention behaviour is discussed in terms of a schematic representation of bonded-phase structure.

INTRODUCTION

In previous papers[1–4] we discussed the effect of modifying the surface of wide-pore silica gel with different monooctadecylsilanes. On the basis of a geometric model of the pores and geometric dimensions of alkylsilyl ligands, we concluded that the maximum attainable coverage of the surface of silica gel is approximately 4.3 μmol/m^2, which corresponds to 2.8 C_{18} groups/nm^2.

However, the silica gels mainly used as supports of chemically bonded phases in high-performance liquid chromatography (HPLC) have chemical and physical

0021-9673/88/$03.50

parameters that differ significantly from those of the silica gels described previously[1–4]. It may be stated in general that supports of chemically bonded phases should be characterized by the following features:

(i) High mechanical stability of the solid silica skeleton by maintenance of a sufficiently great thickness of the pore walls[5–9].

(ii) Sufficiently large diameter and volume of pores and also a cylindrical geometry and a properly defined specific surface area. Such parameters permit good penetration of long alkylsilyl C_{18} chains into the pore interior and guarantee optimal retention and rapid mass exchange[2,3,5,9–16].

(iii) Optimal number of free accessible silanol groups per specific surface area and uniform distribution[5,6,8,9,14,15,17–22].

When strictly controlled conditions are maintained in the process of chemical modification (time, temperature, reagent concentration) and monochlorooctadecylsilane is used in the presence of an activator, reproducible monomeric structures of chemically bonded phases are certain to be obtained[1–4,9,21,22]. This is especially important in the case of routine chromatographic analyses of substances prepared from natural materials and differing significantly in chemical character. For this reason, increasing attention is being paid to the optimization of conditions for the preparation of packings with regard both to choosing the supports and to determining the density and homogeneity of the coverage of its surface by the chemically bonded organic phase[3,4,9,11–16,23,24].

This paper describes the results of investigations on the effects of physical (volume and dimensions of the pores) and chemical (silanol group concentration) features of different supports, prepared from silica gel, on effectively blocking the coverage of silanol groups by monochlorooctadecylsilane molecules. The chromatographic properties of the packings prepared were compared in applications to reversed-phase (RP) HPLC.

EXPERIMENTAL

Materials

The silica gels used to prepare packings with C_{18} chemically bonded phases where Silpearl (Sklo Union-Kawalier, Votice, Czechoslovakia), LiChrosorb Si-60 and Si-100 (Merck, Darmstadt, F.R.G.), Separon SIX (Laboratorní Přístroje, Prague, Czechoslovakia) and silica gel SG-7/G (Polymer Institute of the Slovak Academy of Sciences, Bratislava, Czechoslovakia)[25,26]. The physico-chemical properties of the bare silica gels are listed in Table I.

Monochlorooctadecylsilane (ODMCS) (Petrarch Systems, Bristol, PA, U.S.A.), mixed with specially dried morpholine[27] (Reakhim, Moscow, U.S.S.R.) and toluene (POCh, Gliwice, Poland), was used as the modifying reagent.

Binary eluents (methanol–water) for RP-HPLC systems were prepared as described previously[28] with water having a conductivity of 10^{-5} S/m. In measurements carried out by gel permeation chromatography, tetrahydrofuran (THF) (VEB Laborchemie, Apolda, G.D.R.) was used as the mobile phase. The solvents used were benzene, methanol and diethyl ether (Lachema, Brno, Czechoslovakia), all of analytical-reagent grade.

Stainless-steel columns (100 × 4 mm I.D.) were purchased from Laboratorní Přístroje.

TABLE I

PHYSICAL CHARACTERISTICS OF UNMODIFIED SORBENTS

Symbols: d_p = particle diameter; S_{BET} = specific surface area, determined by the BET method; D = pore diameter; V_p = pore volume; ρ_s = bulk density.

No.	*Sorbent*	*Particle shape**	d_p *(μm)*	S_{BET} *(m²/g)*	D *(nm)*	V_p *(cm³/g)*	ρ_s *(g/cm³)*	α_{SiOH} *(μmol/m²)*	*pH of 5% aqueous suspension*
1	Silpearl	R	6.8	612	4.6	0.67	0.59	3.69	7.05
2	LiChrosorb Si-60	I	7.2	335	8.5	0.86	0.42	4.24	7.17
3	LiChrosorb Si-100	I	10.3	268	13.3	1.20	0.37	4.52	6.90
4	Separon SIX	R	8.6	344	16.4	1.60	0.32	4.46	4.50
5	SG-7/G	R	7.5	361	20.0	2.10	0.23	5.21	5.45

* R = Regular; I = irregular.

Synthesis of chemically bonded phases

After placing the individual bare silica gels in glass reactors having the shape of ampoules, which permits contact of the reagents with the environment[9], they were heated at 200°C under vacuum (10^{-3} Pa) for 6 h. All packings were synthesized under the standard conditions described previously[2,9]. The physico-chemical characteristics of the prepared packings are listed in Table II.

Columns packings

The columns were packed under a pressure of 45 MPa, using the laboratory-built apparatus described previously[29]. A mixture of 8% (v/v) of carbon tetrachloride in methanol[9] was used as the dispersing medium.

Physico-chemical and chromatographic measurements

The parameters characterizing porosity, *i.e.*, specific surface area (S_{BET}), pore volume (V_p) and mean pore diameter (D) of the packings before and after chemical

TABLE II

PHYSICAL AND CHEMICAL CHARACTERISTIC OF SORBENTS MODIFIED WITH C_{18} STATIONARY PHASE

No.	*Surface coverage*				S_{BET} *(m²/g)*	V_p *(cm³/g)*	D *(nm)*	ρ_s *(g/cm³)*
	C (%)	*C (%)**	α_{RP} *(μmol/m²)*	α_{nRP} *(nm⁻²)*				
6	22.9	23.2	2.2	1.32	378	0.57	4.6	0.79
7	20.3	21.5	3.45	2.08	166	0.623	6.78	0.64
8	18.5	19.2	3.85	2.33	115	0.798	10.39	0.63
9	23.4	23.9	4.05	2.45	179	1.172	13.06	0.61
10	24.55	25.3	4.25	2.53	175	1.480	14.98	0.58

* Data after modification with HMDS.

modification, were determined by the low-temperature nitrogen adsorption–desorption method, using a Model 1800 Sorptomatic instrument (Carlo Erba, Milan, Italy).

The bulk density (ρ_s) was determined by a modified pycnometric method[30].

The concentration of surface silanol groups (α_{SiOH}) was determined by the method proposed by Nondek and Vyskočil[17], based on the determination of methane formed during the reaction of the $(CH_3)_2Zn$–THF complex with silanol groups.

The pH values of aqueous suspensions of bare silica gels (5%, v/v) were determined with a Model PHM-84 pH meter (Radiometer, Copenhagen, Denmark) according to Engelhardt and Müller[31,32].

The degree of coverage of the surface by alkylsilyl ligands (α_{RP} and α_{nRP}) was calculated on the basis of the carbon percentage, determined with a Model 240 CHN analyser (Perkin-Elmer, Norwalk, CT, U.S.A.) using the equations proposed by Berendsen and co-workers[10,33]:

$$\alpha_{RP}\ (\mu\text{mol/m}^2) = \frac{10^6\ P_c}{1200\ n_c - P_c\ (M - 1)} \cdot \frac{1}{S_{BET}} \qquad (1)$$

$$\alpha_{nRP}\ (\text{nm}^{-2}) = \frac{6.023 \cdot 10^5\ P_c}{1200\ n_c - P_c\ (M - 1)} \cdot \frac{1}{S_{BET}} \qquad (2)$$

where P_c = measured carbon percentage (%C); n_c = number of carbon atoms in the molecule of bonded silane (for dimethyloctadecylsilane, n_c = 20); M = molecular weight of the silane (311); S_{BET} = specific surface area of unmodified silica (m^2/g).

Chromatographic measurements were carried out with a liquid chromatograph, consisting of a reciprocating membrane pump (Model VCM-300; Workshop of Czechoslovak Academy of Sciences, Prague, Czechoslovakia), a pulse-damping device, a sample valve (Model 7120; Rheodyne, Berkeley, CA, U.S.A.) equipped with a 14-μl injection loop. Also, a UV–visible detector (254 nm) (Workshop of Czechoslovak Academy of Sciences) and a linear recorder (TZ-4200; Laboratorní Přístroje) were used. The dead volume (V_0) of the system was determined on the basis of solvent peaks, according to the method described previously[9].

RESULTS AND DISCUSSION

On the basis of the structural characteristics of the silica gels used, it can be seen that individual adsorbents differ in the volume and dimensions of the pores. As a consequence, they also differ in their specific surface areas and bulk densities. Unfortunately, choosing the optimal structure of the sorbent is very complicated because the experimental determination of the shape of the pores is almost impossible. According to De Boer[34] and others[5,6,10,22,33], some information can be obtained on the basis of the course of nitrogen adsorption–desorption curves (see Fig. 1).

From the course of the isotherms determined for packings 2–5 (Table I), it can be concluded that the shape of the pores is approximately cylindrical, because the adsorption and desorption branches are parallel, except for isotherm 1, corresponding to Silpearl, which, owing to its microporous structure ($D \leqslant 2$ nm) (Fig. 1, Table I), may have a non-cylindrical pore shape. This is especially important in drawing conclusions relating to the effectiveness in blocking silanol groups by modifier molecules. The same

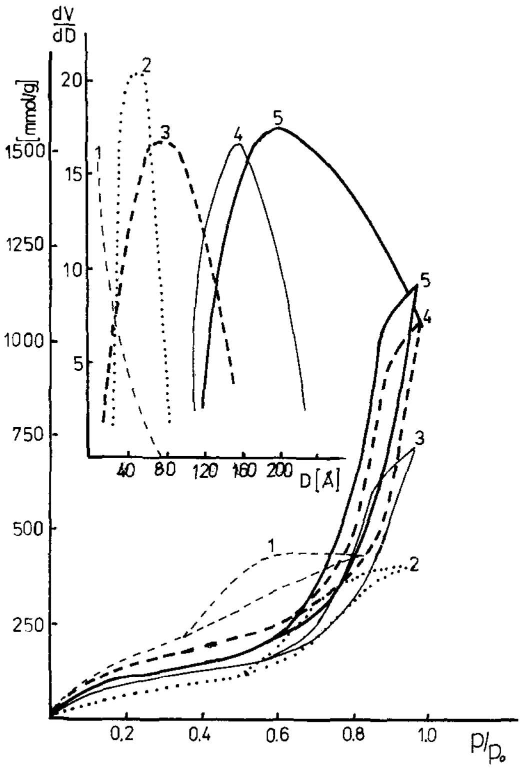

Fig. 1. Adsorption–desorption isotherms of nitrogen and pore distribution curves. 1 = Silpearl; 2 = LiChrosorb Si-60; 3 = LiChrosorb Si-100; 4 = Separon SIX; 5 = SG-7/G.

regularities relate to the concentration of surface silanol groups that can be blocked.

On the basis of the data presented in Fig. 1 and Table I, it can be assumed that an increase in the number of micropores correlates with a decrease in the α_{SiOH} value. This is probably due to limited accessibility of active OH groups located in the interior of the micropores for the large molecules of the $(CH_3)_2Zn$–THF complex. It should be noted that the dimensions of the bottom part of $(CH_3)_2Zn$–THF complex molecule are comparable to those of the same part of the modifier (ODMCS) molecule. Moreover, some silica gels, especially those possessing a high specific surface area and small pore diameter ($D < 8$ nm), may contain some micropores. Probably for the above reason, the α_{SiOH} values for silica gels with large pore dimensions differ significantly from those generally accepted in the literature, *i.e.*, 7–8 μmol/m^2 (refs. 5 and 6) (see Table I).

Table II lists the results of the physico-chemical measurements (see Experimental) used to evaluate the effectiveness of blocking free silanol groups. It appears that the surface coverage of the packings investigated is relatively high and changes from 18.5 to 22.55 ± 0.5% C. Using eqns. 1 and 2, we can state that these values correspond to $\alpha_{RP} = 2.2$–$4.23 \pm 0.1\%$ μmol/m^2 or 1.32–2.73 RP ligands/nm^2. Additional, secondary silanization (end-capping) of the surface with hexamethyldisilazane (HMDS) does not influence the total concentration, expressed in terms of carbon percentage. This may indicate effective blocking of free silanol groups during

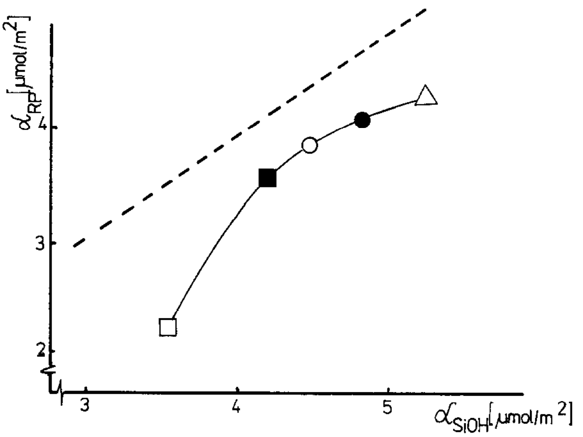

Fig. 2. Dependence of alkylsilyl ligand concentration (α_{RP}) on the concentration of silanol surface groups (α_{SiOH}) for (□) Silpearl, (■) LiChrosorb Si-60, (○) LiChrosorb Si-100, (●) Separon SIX and (△) SG-7/G.

the first stage of modification. Because end-capping has virtually no influence on the α_{RP} and α_{nRP} values, we evaluated the packings prepared during the first silanization only.

Fig. 2 presents the relationship between the degree of coverage of the surface with C_{18} groups (α_{RP}) and the concentration of silanol groups on the unmodified support (α_{SiOH}). When the surface is ideally covered with monofunctional modifier, each OH group should react (broken line). When the D values of individual silica gels increase, the pores of such gels become more accessible to large silane molecules (owing to a decrease in the number of micropores that are less accessible or inaccessible to silane molecules) and the experimental dependence of α_{RP} on α_{SiOH} becomes similar to the theoretical dependence (continuous line). On the other hand, the increase in α_{RP} causes some silanol groups to become inaccessible to ODMCS molecules, because of the possible interaction with already bonded C_{18} ligands. For this reason, the curve presented in Fig. 2 is lower than the ideal linear relationship. According to the observations of Lochmüller and co-workers[18,20], this effect is characterized by significant interactions between neighbouring alkylsilyl ligands. This confirms our earlier suggestions that, because of steric effects, it is impossible to obtain a coverage greater than 4.3 μmol/m^2 when a monosilane containing 18 carbon atoms in the molecule is used as modifier[1–4,9,21–23]. Moreover, apart from the steric effect (*i.e.*, limited acessibility of the pores and inhibitory interactions of already bonded silane molecules), the relative concentration of the silanols also influences the character of the dependence presented in Fig. 2. The optimal value of this relative concentration is 5.21 μmol/m^2, and it appears that additional hydroxylation of the support surface will not increase the coverage density of the surface by C_{18} ligands. This is in good agreement with an earlier suggestion of Engelhardt and co-workers[12,35]. In contrast, generation of additional silanols may lead to undesirable changes in the structure of the silica support. This may lead to changes in D and V_p values and hence to changes in S_{BET}.

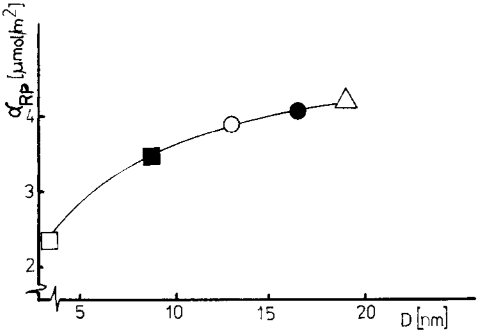

Fig. 3. Effect of primary silanization on bonding of C_{18} phase. Symbols as in Fig. 2.

From the data in Table II it appears that as the concentration of C_{18} increases with increasing pore dimensions, the effect of pore volume is only indirect, because the possibility of penetration of alkylsilyl C_{18} molecules depends not on the total pore volume but only on the pore inlet diameter. The numerical values relating to porosity of the material prepared and listed in Table II represent the differences between the values characteristic of bare silica (Table I) and of the same silica after chemical modification with C_{18} ligands. Hence, if we wish to obtain so-called "wide-pore" gels (large D values) and ensure a proper course of silanization when the specific surface area is relatively large, the V_p value must be also be relatively large. This indicates that silicas with a greater pore size and more reactive silanols are more accessible to modifier molecules. It should be noted that pore size is the parameter that gives actual information about the accessibility of pores to C_{18} molecules, whereas the pore volume may lead to incorrect information. It appears that this is due to difficulties relating to obtaining a strictly defined pore shape. For this reason, if we wish to increase the accessibility of pores to modifier molecules, we should utilize the dependence of α_{RP} on pore diameter (D) (Fig. 3).

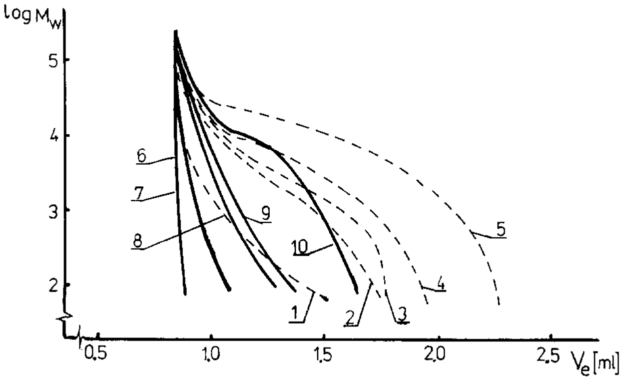

Fig. 4. GPC curves of the packings investigated: unmodified sorbents (broken lines); sorbents modified with ODMCS (continuous lines). 1 and 6 = Silpearl; 2 and 7 = LiChrosorb Si-60; 3 and 8 = LiChrosorb Si-100; 4 and 9 = Separon SIX; 5 and 10 = SG-7/G.

TABLE III

CHARACTERISTICS OF RP COLUMNS

Mobile phase: methanol–water (70:30, v/v). Symbols: k' = capacity factor; n_T = theoretical plates; φ = column resistance parameter; E = separation impedance; f_{As} = peak asymmetry coefficient.

Column	*Solute (naphthalene)*				
	k'	n_T	φ	E	f_{As}
A	6.4	3080	1616	50 640	1.12
B	4.3	3460	1265	55 750	1.05
C	3.8	2690	480	6630	0.90
D	4.2	3400	1293	15 125	1.08
E	4.9	3260	972	17 160	0.92

From the course of this dependence, it appears that a dense coverage ($\alpha_{RP} \geqslant 4.0$ μmol/m^2) is obtained at $D \geqslant 13$–14 nm and that at $D = 16$ nm a "plateau" region is reached. This indicates a lack of influence of pore size. For separation by HPLC, a "good" packing is usually characterized by $\alpha_{RP} \geqslant 3.0$ μmol/m^2, which corresponds to $D \geqslant 7.0$ nm[11–13]. According to earlier data[1,4–9] and under hypothetically optimal conditions, if the length of the C_{18} chain is approximately 2.3 nm, it may be stated that the entry of a given molecule into the pores of a modified support will not be completely blocked. This effect was investigated by gel-permeation chromatography (GPC), where the THF mobile phase was pumped through the column at a flow-rate of 0.5 ml/min. Polystyrene standards of relative molecular weight ranging from 600 to 650 000 were used as test substances. Typical GPC curves are presented in Fig. 4.

From the course of the curves in Fig. 4 it appears that silica gel SG-7/G as the greatest exclusion range, which is in good agreement with sorptomatic data (packing 10). The courses of all other curves correlate with the sequence presented in Table I. Chemical modification causes a decrease in elution volume (V_e). Moreover, Fig. 4 shows that the chains of the chemically bonded C_{18} phase have almost blocked the narrow pores of Silpearl and LiChrosorb Si-60 (packings 1 and 2). The greatest selectivity in GPC (which is characterized by large pore volumes) is shown by a modified SG-7/G gel (packing 10). Therefore, the determination of V_p and D values for Silpearl (Table II) was almost impossible.

Table III lists chromatographic parameters characterizing the quality of the columns prepared[36,37], packed with individual sorbents. In this instance, naphthalene was used as a test substance in the RP-HPLC systems and the flow-rate was 1 ml/min.

Table III indicates that the columns prepared are characterized by a relatively large number of theoretically plates (n_T) for packings having a particle diameter of 6.8–10.3 μm. The parameters characterizing the permeability of a packed bed (column resistance parameter, φ, and separation impedance, E) were in the range of permissible values. This relates also to peak asymmetry coefficients (f_{As})[36,37]. According to the nomenclature of Colin *et al.*[38], these columns may be considered as "good" or even "very good", considering that the quantities listed in the tables correspond to beds measuring 100 × 4 mm I.D. However, in this instance the effect of blocking the pores by C_{18} ligands become more significant (φ values for column A, Table III). This effect

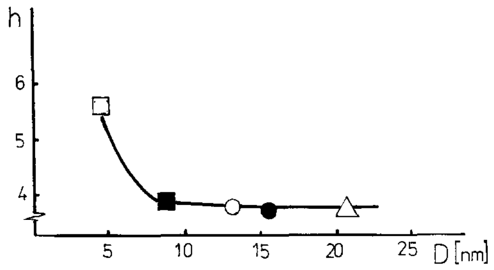

Fig. 5. Dependence of *h* on pore diameter, *D*. Symbols as in Fig. 2.

will become more significant if we consider the relationship between reduced HETP (*h*) and pore diameter (Fig. 5).

From Fig. 5 it appears that, in spite of a smaller particle size (d_p = 6.8 μm), column A (packed with Silpearl C_{18}) is characterized by the largest *h* value. It decreases for other columns and the *h* values for individual columns differ slightly. This may mean that with column A mass exchange is the least intense, which may be caused by a very dense packing (blocking of pores) of C_{18} ligands, resembling the membrane. If we assume now that the L_{RP} (= length of the alkyl chain) value for C_{18} molecules is 2.3 nm and the mean pore radius of Silpearl is D = 4.6 nm, then our calculation has a logical basis. This conclusion is confirmed also by the course of the GPC curve for Silpearl C_{18} (Fig. 4).

The effect of the coverage density of the support surface by a chemically bonded C_{18} phase on retention may be illustrated by the relationship between the selectivity (α_{ij}) of two substances, one of which is polar (phenol) and the other non-polar (benzene), and the concentration of methanol in the aqueous mobile phase (χ) (Fig. 6). Such a relationship was recommended by Guiochon and co-workers[38,39] especially for the determination of the degree of hydrophobility of the packing and the selectivity of

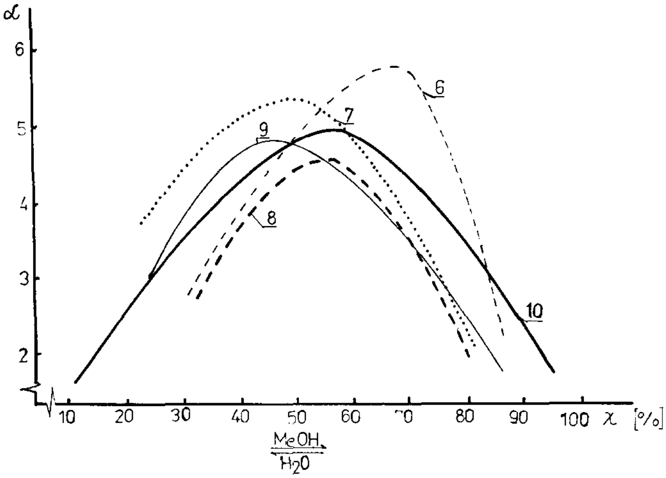

Fig. 6. Selectivity of separation of phenol–benzene mixture as a function of mobile phase composition. 6 = Silpearl; 7 = LiChrosorb Si-60; 8 = LiChrosorb Si-100; 9 = Separon SIX; 10 = SG-7/G. MeOH = Methanol.

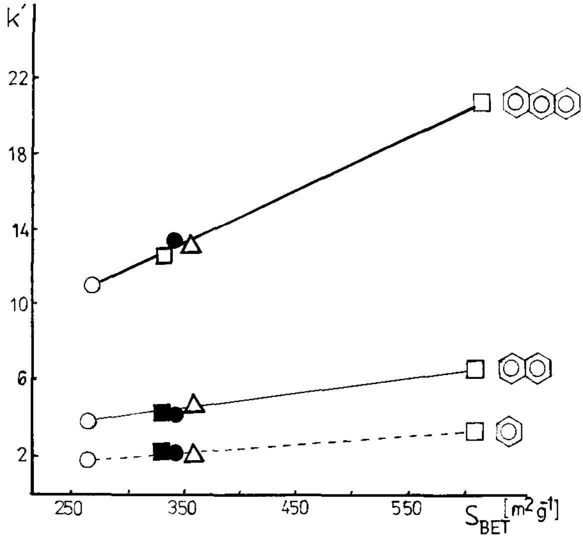

Fig. 7. Effect of specific surface area of the packings on retention of aromatic hydrocarbons. Symbols as in Fig. 2.

separation utilizing the interaction of test substances between the eluent and the packing. From the course of the relationship between α_{ij} and χ, it can be seen that the packings prepared are characterized by a relatively wide range of separation selectivity. This means that the surface is hydrophobic and densely covered by alkylsilyl ligands. However, the localization of the maximum on the $\alpha_{ij} = f(\chi)$ curve plotted for Silpearl C_{18} (column A, Fig. 6) differs significantly from that of the maxima on other curves. It appears that this is due to greater interactions between the mobile phase and alkylsilyl C_{18} ligands[1–4,9,18,22], which block the access to smaller pores. The course of the relationship presented in Fig. 6 also indicates that the greatest selectivity, extension of the curve and application range occur with the SG-7/G C_{18} packing (packing 10, Table II; column E, Table III). This means that for separations by RP-HPLC, this packing will be the most useful in the determination of substances having small differences in k' values.

The relationship between k' and S_{BET} (for bare silica gels) (Fig. 7), determined for aromatic hydrocarbons having different numbers of aromatic rings, indicates that the packings with smaller particle size and pore volume and the largest specific surface area have the highest capacity factors (k'). The course of this relationship is in good agreement with the results published by Guillemin *et al.*[40] and by Snyder and Kirkland[41]. It appears that in this instance the effect of interactions between the molecules separated and the alkylsilyl ligands of the C_{18} phase is observed, which confirms our earlier observations[3,4].

CONCLUSIONS

On the basis of the results presented, it can be stated that in general the parameters characterizing the porosity of the starting support material influence significantly the coverage density of a chemically bonded octadecyl phase. High coverages ($\alpha_{RP} \geqslant 4$ μmol/m^2) by chemically bonded phases can be obtained in this instance when the support is characterized by pore diameters greater than 14 nm and the concentration of silanol groups is greater than 4.5 μmol/m^2.

The columns prepared are characterized by good performance ($n_T \geqslant 3000$ theoretical plates for a bed of 100 × 4 mm I.D.) and by good separation selectivity. They are characterized, moreover, by a high separation capacity (for the packings of $V_p \geqslant 1.6$ cm^3/g) and by a high permeability, which can be especially significant in separations of substances isolated from biological materials.

REFERENCES

1 B. Buszewski, L. Nondek, A. Jurášek and D. Berek, *Chromatographia*, 23 (1987) 442.
2 B. Buszewski, A. Jurášek, J. Garaj, L. Nondek, I. Novák and D. Berek, *J. Liq. Chromatogr.*, 10 (1987) 2325.
3 B. Buszewski and Z. Suprynowicz, *Chromatographia*, 24 (1987) 573.
4 B. Buszewski and Z. Suprynowicz, *Anal. Chim. Acta*, (1988) in press.
5 K. K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979.
6 R. K. Iler, *The Chemistry of Silica*, Wiley, New York, 1979.
7 M. Verzele, C. Dewaele and D. Duquet, *J. Chromatogr.*, 329 (1985) 351.
8 K. K. Unger, J. N. Kinkel, B. Anspach and H. Giesche, *J. Chromatogr.*, 296 (1984) 3.
9 B. Buszewski, *Ph.D. Thesis*, Slovak Technical University, Bratislava, 1986.
10 G. E. Berendsen, K. A. Pikaart and L. de Galan, *J. Liq. Chromatogr.*, 3 (1980) 1437.
11 L. C. Sander and S. A. Wise, *J. Chromatogr.*, 316 (1984) 163.
12 H. Engelhardt, B. Dreyer and H. Schmidt, *Chromatographia*, 16 (1982) 11.
13 B. W. Sands, Y. S. Kim and J. L. Bass, *J. Chromatogr.*, 360 (1986) 353.
14 R. K. Gilpin, *J. Chromatogr. Sci.*, 22 (1984) 371.
15 E. Bayer, K. Albert, J. Reiners, H. Nieder and D. Müller, *J. Chromatogr.*, 264 (1983) 197.
16 E. Bayer, A. Paulus, B. Peters, G. Laupp, J. Reiners and K. Albert, *J. Chromatogr.*, 364 (1986) 25.
17 L. Nondek and V. Vyskočil, *J. Chromatogr.*, 206 (1981) 581.
18 C. H. Lochmüller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
19 M. L. Miller, R. W. Linton, G. Maciel and B. L. Hawkins, *J. Chromatogr.*, 319 (1985) 9.
20 D. B. Marshall, K. A. Stutler and C. H. Lochmüller, *J. Chromatogr. Sci.*, 22 (1984) 217.
21 L. Nondek, B. Buszewski and D. Berek, *J. Chromatogr.*, 360 (1986) 241.
22 B. Buszewski, *Chem. Stosow.*, 32 (1988) 201.
23 B. Buszewski, K. Šebeková, P. Božek and D. Berek, *J. Chromatogr.*, 367 (1986) 171.
24 M. Okamoto and K. Jinno, *J. Chromatogr.*, 395 (1987) 171.
25 D. Berek and I. Novák, *U.S. Pat.*, 4 255 286 (1981).
26 I. Novák and D. Berek, *U.S. Pat.*, 4 382 070 (1983).
27 B. Buszewski, R. Lodkowski and J. Trocewicz, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 527.
28 B. Buszewski, T. Bleha and D. Berek, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 860.
29 B. Buszewski, Z. Suprynowicz and T. Pastuszak, *Chem. Anal. (Warsaw)*, 28 (1983) 527.
30 I. Novák, *Ph.D. Thesis*, Slovak Academy of Sciences, Bratislava, 1969.
31 H. Engelhardt and H. Müller, *J. Chromatogr.*, 218 (1981) 395.
32 H. Müller and H. Engelhardt, in I. Molnar (Editor), *Practical Aspects of Modern HPLC*, W. de Gruyter, Berlin, New York, 1982, p. 25.
33 G. E. Berendsen and L. de Galan, J. Liq. Chromatogr., 1 (1978) 561.
34 J. H. De Boer, *The Structure and Properties of Porous Materials*, Butterworths, London, 1958.
35 H. Engelhardt and G. Ahr, Chromatographia, 14 (1981) 227.
36 P. A. Bristow and J. H. Knox, *Chromatographia*, 10 (1977) 279.
37 B. Buszewski, D. Berek and J. Garaj, *Wiad. Chem.*, 40 (1986) 369.
38 H. Colin, N. Ward and G. Guiochon, *J. Chromatogr.*, 149 (1978) 169.
39 H. Colin, G. Eon and G. J. Guiochon, *J. Chromatogr.*, 119 (1976) 41.
40 G. L. Guillemin, J. P. Thomas, S. Thiault and J. P. Bounine, *J. Chromatogr.*, 142 (1977) 321.
41 R. L. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979.