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EFFECT OF COVERAGE DENSITY ON THE RETENTION MECHANISM IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The chromatographic properties of reversed-phase high-performance liquid chromatographic (RP-HPLC) packings with chemically bonded C_{18} groups of controlled coverage were characterized by correlation of the capacity factor and the composition of the mobile phase, taking physico-chemical properties of the packings into account. On the basis of a cylindrical model of pores and the dependence of retention on density and homogeneity of the surface coverage with alkylsilyl ligands, an attempt was made to describe the retention mechanism in the RP-HPLC system. It was found that packings with a coverage density higher than 3.8 μmol/m^2 were dense, homogeneous and shielded the unblocked surface silanol groups sufficiently.

INTRODUCTION

The separation mechanism in reversed-phase high-performance liquid chromatography (RP-HPLC) is based on the hydrophobic interactions between the solute and alkylsilyl groups chemically bonded to the support surface[1–6]. In previous papers[7–11] the controlled preparation of packings with chemically bonded C_{18} groups resulting in materials with various surface coverages of alkylsilyl ligands was discussed. A maximum density of the stationary phase of $\alpha_{RP} > 4$ μmol/m^2 can be obtained. In most of the previous papers[7–14], however, the correlation between the physico-chemical properties of the phases and the separation of various substances was only partially discussed.

The determination of the free surface energy of packings has been demonstrated to be very useful[13]. Depending on the coverage with octadecyl ligands[15–18], the surface of the packing showed significantly different properties and interactions, especially[13] for C_{18} ligands with a coverage density $\alpha_{RP} \geqslant 3.8$ μmol/m^2. It follows from the literature[1–3,7,10–12,17–20] that during elution in the RP-HPLC system, C_{18} ligands

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change their conformation[13,15,21] which significantly influences the retention, reso-
lution and peak shape. This can result from (i) the presence of free, unblocked silanol
groups, which causes an increase in the interactions at the mobile phase–solute–
stationary phase interface, (ii) specific interactions between individual alkylsilyl
ligands (especially when the surfaces are blocked homogeneously) and (iii) the
composition of the mobile phase and the nature of the sample.

Investigation of the separation mechanism in columns of packings with different
surface coverages of C_{18} alkylsilyl ligands therefore appears to be very interesting.
Correlations between the retention data of the solutes and the coverage density (α_{RP})
and the mobile phase composition were therefore studied. In addition, an evaluation of
the density and the homogeneity of the coverage density of the packings was attempted
on the basis of the experimental data.

EXPERIMENTAL

Materials

Monochlorooctadecylsilane (Petrarch Systems, Bristol, PA, U.S.A.) mixed with
dried morpholine (Reakhim, Moscow, U.S.S.R.) and specially dried toluene[22] (POCh,
Gliwice, Poland) was used as the modifying reagent.

TABLE I

PHYSICO-CHEMICAL CHARACTERISTICS OF THE PREPARED PACKING MATERIALS

A = activator; ODMCS = monochlorooctadecylsilane; α_{RP} = concentration of chemically bonded C_{18}
groups ($\mu mol/m^2$); α_{SiOH} = concentration of accessible silanol groups ($'mol/m^2$); D = mean pore diameter
(nm); V_p = pore volume (cm^3/g); S_{BET} = specific surface area (m^2/g).

No. of packing	Type of packing	A:ODMCS ratio (mol/mol)	Surface coverage			Porosity		
			C (%)	α_{RP}	α_{SiOH}	D	V_p	S_{BET}
	Bare silica gel (SG)	–	–	–	5.21	20.00	2.10	361
1	Silica gel C_{18}	2.60:1	5.08	0.72	4.53	18.28	1.92	338
2	monomeric structure	2.35:1	11.69	1.61	3.45	17.37	1.81	323
3		2.10:1	17.20	2.60	2.52	15.71	1.65	314
4		1.85:1	21.20	3.46	1.58	15.24	1.60	203
5		1.60:1	24.50	4.24	0.42	14.28	1.55	175

Spherical silica gel (SG-7/G) (Polymer Institute, Bratislava, Czechoslovakia)[23]
was used as the support for the chemically bonded phase. Its characteristic data are
given in Table I. The solvents used (analytical-reagent grade) were toluene, benzene,
methanol and dimethyl ether (POCh). Binary eluents for RP-HPLC systems
(methanol–water) were prepared as described previously[22] using water with a con-
ductivity of 10^{-5} S/m. In measurements carried out by gel permeation chromatography
(GPC), tetrahydrofuran (THF) (VEB Laborchemie, Apolda, G.D.R.) was used as the
mobile phase. Stainless-steel tubes (100 × 4 mm I.D.) were purchased from the
Chemical Reagent Factory–ZOCh (Lublin, Poland).

Apparatus

Chromatographic measurements were made using a liquid chromatograph consisting of an LC-20 piston pump (Pye Unicam, Cambridge, U.K.), a Model 7120 injection valve (Rheodyne, Berkeley, CA, U.S.A.) equipped with a 10-μl injection loop, a Model 254 M ultraviolet detector (Techma Robot, Warsaw, Poland) equipped with an 8-μl flowcell and a TZ-4100 linear recorder (Laboratorní Přístroje, Prague, Czechoslovakia). The dead volume was determined on the basis of the solvent peaks.

The porosity parameters characterizing the starting material and chemically modified packings (the specific surface area, S_{BET}, the pore volume, V_p, and the mean pore diameter, D) were determined by low-temperature adsorption and desorption of nitrogen using a Model 1800 automatic apparatus (Carlo Erba, Milan, Italy).

The concentration of surface silanol groups (α_{SiOH}) before and after modification was determined using the method proposed by Nondek and Vyskočil[24]. The degree of coverage of the surface with alkyl ligands (α_{RP}) was calculated as described elsewhere[25]. The percentage of carbon was determined using a Model 185 CHN analyser (Hewlett-Packard, Palo Alto, CA, U.S.A.).

Solid-state NMR measurements were performed on a Bruker MSL 200 spectrometer with samples of 200–300 mg in double-bearing rotors of ZrO_2. Magic-angle spinning (MAS) was carried out at a spinning rate of 4 kHz. ^{29}Si cross-polarization (CP)-MAS-NMR spectra were recorded with a pulse length of 5 μs together with a contact time of 5 ms and a pulse repetition time of 2 s. For ^{13}C CP-MAS-NMR spectra a contact time of 12 ms was used. All NMR spectra were externally referenced to liquid tetramethylsilane (TMS) and the chemical shifts are given in parts per million (ppm).

Chemical bonding procedure

Chemical modification of the surface of silica supports was carried out as described elsewhere using only monofunctional silanes[8,9]. Changes in the molar ratio of the modifier monochlorooctadecylsilane (ODMCS) and activator (morpholine) permit the preparation of supports with strictly defined amounts of carbon on the support surface[10,11]. Numerical volumes of the molar ratio are given in Table I, which also contains the physico-chemical characteristics of the surface of the prepared packings after their chemical modification.

Column packing procedure

The columns were packed under a pressure of 450 Pa using a laboratory-built apparatus described previously[26]. An 8% (v/v) solution of carbon tetrachloride in methanol was used as the dispersing medium. The prepared columns were evaluated by determining characteristic basic parameters such as the number of theoretical plates (N_T), reduced parameters: h (plate height) and v (velocity), column resistance (φ) and asymmetry coefficient (f_{As})[27,28]. The columns prepared can be described according to Bristow and Knox[28] as "good" or even "very good", as confirmed by the data presented in Table II.

RESULTS AND DISCUSSON

Table I lists the physico-chemical data of the monofunctionally modified silica gels (C_{18}) with different carbon contents. The data indicate that a change in the activator—modifier (ODMCS) molar ratio permits the preparation of packings with different carbon contents. The data show that with increasing α_{RP} a decrease in α_{SiOH} is obtained. The sum of α_{RP} and α_{SiOH} is approximately constant and equal to the initial α_{SiOH} value. The densest packing (No. 5) is an exception, because the sum of α_{RP} and α_{SiOH} is significantly smaller. This can be explained by steric masking of unreacted silanol groups[10-12,15-18,29,30] on the whole surface by the dense population of alkylsilyl ligands. An analysis of the porosimetric data indicates similar conclusions (Table I).

In all instances the decrease in D, V_p and S_{BET} values can be correlated with an increase in the percentage of carbon. From the typical GPC curves presented in Fig. 1 it can be concluded that in spite of the increase in carbon content, even packing 5, with the highest degree of modification, is capable of typical GPC separations, which means that the pores are not blocked by long C_{18} chains. A correlation between porosimetric (Table I) and chromatographic data is possible. The increase in α_{RP} values leads to an decrease in the elution volume (V_e)[10,14].
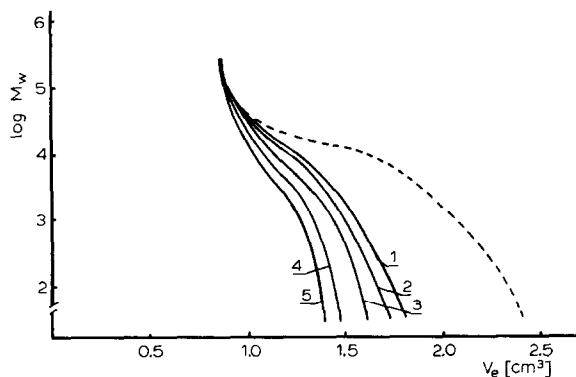

Fig. 1. Plot of log mol.wt. (M_w) of polystyrene standards *vs.* elution volume (V_e) for unmodified support (dashed line) and for packings with chemically bonded C_{18} groups (continuous line). α_{RP}: (1) 0.72; (2) 1.61; (3) 2.60; (4) 3.46; (5) 4.25 $\mu mol/m^2$. Chromatographic conditions: mobile phase, THF; flow-rate, 0.5 ml/min; detection, UV (254 nm).

We showed recently that in material of dense coverage ($\alpha_{RP} \geqslant 4.0$ $\mu mol/m^2$), about 85% of the silanol groups are blocked and the surface area decreases by about 60%[10,11]. These findings are supported by ^{29}Si CP-MAS-NMR data. In Fig. 2A the ^{29}Si CP-MAS-NMR spectrum of native silica gel is depicted. The existence of geminal silanol groups Q_2, silanol groups Q_3 and siloxane units Q_4 can easily be recognized by the corresponding signals at -90, -100 and -110 ppm, respectively[15,16]. With modified silica gel with an 11.69% carbon loading (packing 2), a new signal arises at 14.0 ppm due to the introduced Si–O–Si moiety (Fig. 2B). Correspondingly, the signal intensity of the Q_2 and Q_3 units decreases.
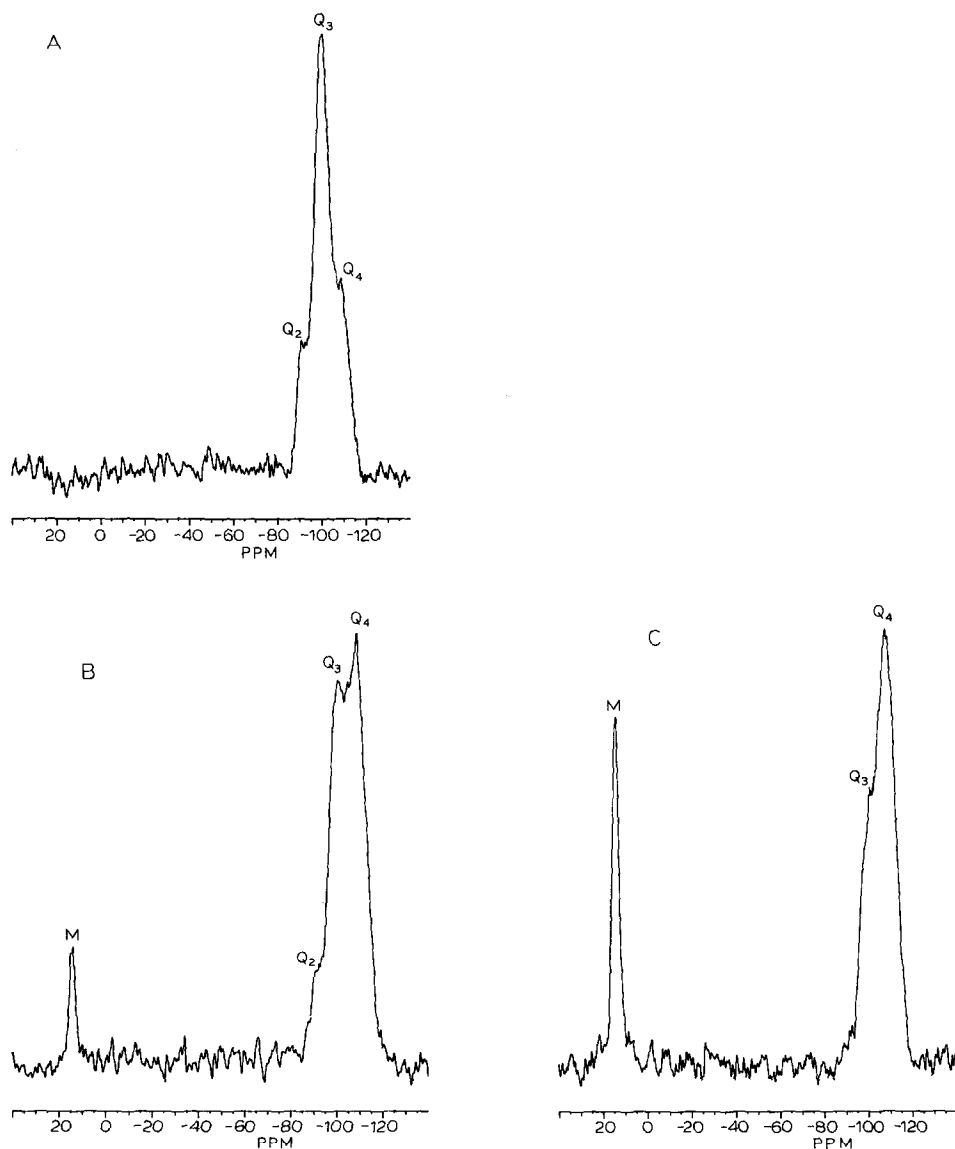
Fig. 2. ^{29}Si CP-MAS-NMR spectra (39.75 MHz) of silica gel samples: (A) unmodified silica gel; (B) monofunctionally modified silica gel with a carbon loading of 11.67% (packing 2); (C) monofunctionally modified silica gel with a carbon loading of 24.5% (packing 5).

In the spectrum of the modified silica gel with the highest carbon loading (packing 5, Fig. 2C), the Q_2 units can no longer be detected and the signal intensity of Q_3 units is also dramatically reduced. In contrast to Fig. 2A, the signal of Q_4 units dominates the spectral pattern between -90 and -110 ppm.

The CP excitation technique used for recording of the ^{29}Si NMR spectra is dependent on the number of protons associated with the investigated species.

Therefore, at a distinct contact time, only the $M/(Q_2 + Q_3)$ ratio can be quantitatively determined[16]. The decreasing signal intensity of Q_2 and Q_3 units in Fig. 2B and C is consistent with an increasing signal intensity of the M unit. This clearly confirms the formation of a covalent Si–O–Si linkage even at a high surface loading.

In the ^{13}C CP-MAS-NMR spectrum of the modified material with the highest carbon loading (packing 5), no signal of adsorbed material can be found, in contrast to earlier observations[15] (Fig. 3).

Experiments permitting the determination of the free surface energy of packings prepared were also carried out[13]. From these investigations it follows that the materials with a coverage above 3.8 μmol/m^2 are so dense and homogeneous that the free surface energy decreases by a factor of 5 in comparison with unmodified silica gel and is very similar to that of a hydrocarbon surface. These data are consistent with the results obtained from elemental and porosimetric analysis[14].
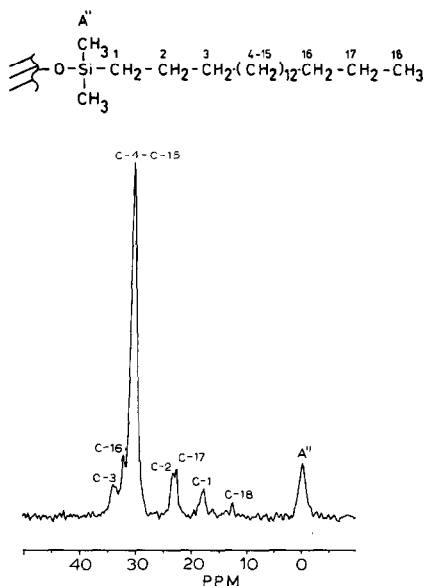

Fig. 3. ^{13}C CP-MAS-NMR spectrum (50.33 MHz) of monofunctionally modified silica gel with a carbon loading of 24.5% (packing 5).

The prepared phases were investigated in the RP-HPLC system and compounds of different chemical natures were used as test substances. Fig. 4 presents the results for the five columns packed with the materials described in Table I. The column data are given in Table II. Correlation of the capacity factors (k') of benzene derivatives and the composition of the binary mobile phase (Φ) showed a dependence of the selectivity on the surface loading. An increase in the coverage density results in an increase in the separation selectivity for different substances. In all instances the k' values of individual substances increase with increase in the water content in the mobile phase (when the water content exceeds 50%, v/v). This effect, however, is most significant for materials with α_{RP} values of 3.46 and 4.24 μmol/m^2 (Fig. 4D and E, respectively). The selectivity improves when the mobile phase contains 60–80% of methanol.
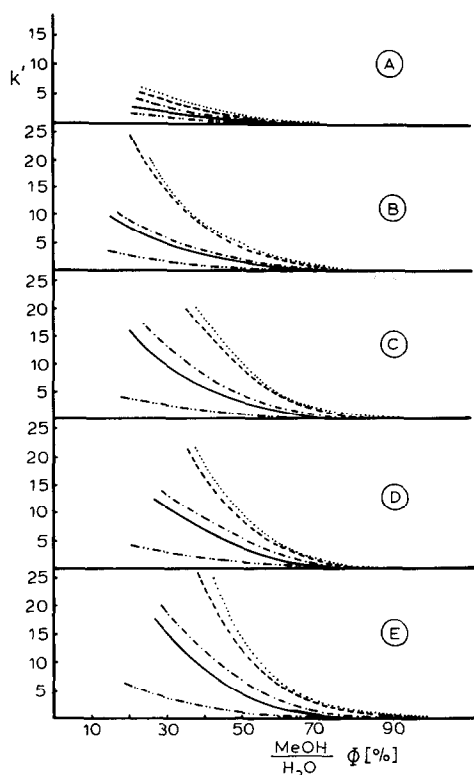
Fig. 4. Relationship between the capacity factor (k') and the composition of the mobile phase (Φ)for benzene derivatives obtained on columns packed with materials with a controlled content of C_{18} groups on the surface. α_{RP}: (A) 0.72; (B) 1.60; (C) 2.60; (D) 3.46; (E) 4.25 $\mu mol/m^2$. ($\cdots$) chlorobenzene; (------) toluene; (-----) benzene; (———) nitrobenzene; (-·-··-) phenol. MeOH = Methanol.

It can be concluded that hydrophobic interactions between the mobile phase and chemically bonded hydrophobic C_{18} ligands should be especially observed for materials with high surface loading (α_{RP} = 4.24 $\mu mol/m^2$). In such materials, polar molecules of benzene derivatives cannot interact because of great cohesive (chain–

TABLE II

COLUMN PROPERTIES

Mobile phase: methanol–water (70:30, v/v). k' = capacity factor; N_T = number of theoretical plates per 100 mm column length; h = reduced plate height; v = reduced velocity; φ = column resistance parameter; f_{As} = asymmetry factor.

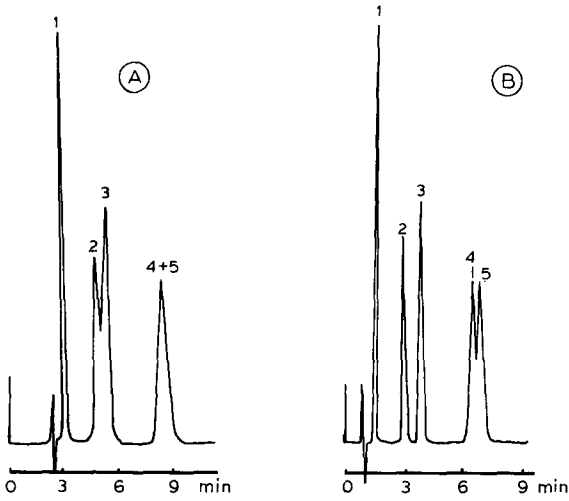| No. of packing	Solute naphthalene					
	k'	N_T	h	v	φ	f_{As}
1	0.29	2620	5.45	6.2	782	1.42
2	1.09	2835	5.05	5.95	778	1.39
3	2.79	2978	4.80	5.80	742	1.20
4	3.26	3215	4.45	5.09	717	1.19
5	4.40	3605	3.96	4.90	695	1.11

Fig. 5. Examples of the separation of benzene derivatives on columns packed with materials with different coverage densities of C_{18} groups. α_{RP}: (A) 3.46; (B) 4.25 μmol/m^2. Chromatographic conditions: mobile phase, methanol–water (60:40, v/v); flow-rate, 1 ml/min; detection, UV (254 nm). Peaks: 1 = phenol; 2 = nitrobenzene; 3 = benzene; 4 = toluene; 5 = chlorobenzene.
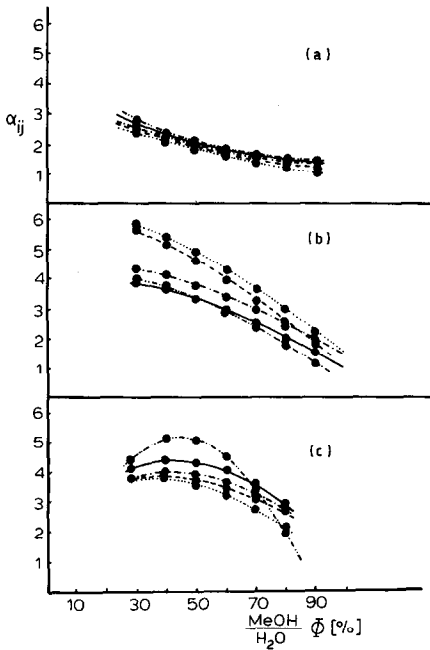

Fig. 6. Selectivity coefficients (α_{ij}) for three pairs of substances as a function of the mobile phase composition (Φ). (a) $\alpha_{ij} = k'_{toluene}/k'_{benzene}$; (b) $\alpha_{ij} = k'_{nitrobenzene}/k'_{phenol}$; (c) $\alpha_{ij} = k'_{benzene}/k'_{phenol}$; ($\cdots$) packing 1; (– – –) packing 2; (– $\cdot$ – $\cdot$ –) packing 3; (———) packing 4; (– $\cdot$ $\cdot$ – $\cdot$ $\cdot$ –) packing 5. For column packing characteristics, see Tables I and II.

chain) interactions. It seems that for the materials with a high coverage density, the mechanism of separation can be described as a partition process[1–6], because the resolution of peaks 2 and 3 and 4 and 5 is improved with increasing coverage density. This suggestion is confirmed by the chromatograms in Fig. 5, which shows the separation of the test mixture obtained on columns containing packings 4 and 5 (Table I).

In order to explain more precisely the processes that occur in the packings during the HPLC test, three other test mixtures were used: benzene–toluene, phenol–nitrobenzene and phenol–benzene. The relationship between the selectivity of separation ($\alpha_{ij} = k'_j/k'_i$) and composition of the mobile phase is plotted in Fig. 6, from which it can be concluded that their slopes change depending on the chemical nature of the test substances and on the properties of the surface[14]. In two cases (Fig. 6A and C) the elution order correlates with the changes in percentage of carbon (Table I) and with increasing α_{ij}. In Fig. 6B, the sequence of the curves is the opposite of that in Fig. 6A and C. In this connection, it can be assumed that the mechanism of the elution is slightly different.

Fig. 6A presents the plots determined for the non-polar mixture, benzene–toluene. The form of these plots is consistent with hydrophobic theory[1–7,12,29,31–33], where interactions between the mobile phase and the support surface dominate. Thus α_{ij} values will change with increase in the hydrophobic nature of the prepared packing and a small concave in the course of the curves can be observed. Fig. 6B presents the curves plotted for the phase containing polar components, which can interact with the unblocked surface silanol groups. The lack of any interactions results in a smaller selectivity for the separation of polar substances chromatographed on the material of high coverage (packing 5). The resolution improves, however, owing to prolongation of the retention time on the materials characterized by less dense coverage (packings 1 and 2). This behaviour is indicated by different slopes of the curves corresponding to the materials characterized by a low percentage of carbon (the α_{RP} values for these materials are 0.72 and 1.61 μmol/m^2, respectively, see Table I). Hence the previous conclusion about a different mechanism (adsorption type) for these materials is supported. Free silanol groups also seem to play a role. With a low surface loading the surface is not masked sufficiently by the "mobile" bonded C_{18} chains, in contrast to materials with a high surface loading[9,12,29,30].

The curves corresponding to packings 3, 4 and 5 are similar in general, but the significantly hydrophobic nature of the packing with a coverage density $\alpha_{RP} = 4.24$ μmol/m^2 increases with increase in methanol content in the mobile phase (stronger interactions of the chain–chain type). This phenomenon is probably responsible for the penetration of polar solute molecules to silanol groups. For this reason, the α_{ij} values are significantly lower. Considering Fig. 6C, where the plots of the mixture of non-polar (benzene) and polar (phenol) solutes are shown, typical slopes for the materials of a hydrophobic nature are observed[10,14]. The high-coverage material (packing 5) shows the smallest retention of phenol, because the $\alpha_{ij} = k'_{benzene}/k'_{phenol}$ values are the highest. This is due to the shielding of residual silanol groups by C_{18} ligands. From the point of view of separation selectivity, the slope of the curve corresponding to packing 5 ($\alpha_{RP} = 4.24$ μmol/m^2) seems to be optimal.

The significant maximum of the selectivity (α_{ij}) in relation to the changes in composition of mobile phase (Φ) indicates different interactions of the substances

separated between the mobile phase and the packing surface[31–33]. The 6-fold increase in coverage density for packing 5 compared with packing 1 (Table I) leads to an increase in separation selectivity of about 30%.

The slopes of the curves for packings 1, 2 and 3 are similar, but differ significantly from those of packings 4 and 5. The slight differences in selectivity probably result from the increase in interactions between the test substances and the stationary phases and the increase in the hydrophobic nature of the packings investigated. This may also indicate a different accessibility of these surfaces for polar phenol molecules.

Convergence of the results is observed as in the calculations of surface free energy[13] on the basis of adsorption of two different substances, n-octane and water. Similar conclusions can also be drawn from the linear relationship between log (k'/S_{BET}) and the number of carbon atoms (n_c) for the benzene homologues, which are presented in Fig. 7.
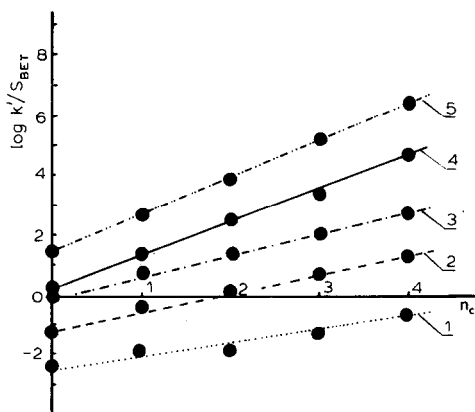

Fig. 7. Dependence of log (k'/S_{BET}) on the number of methylene groups (n_c) in the homologous series of alkylbenzenes (benzene, toluene, ethylbenzene, propylbenzene and butylbenzene). For packings 1–5, see Tables I and II.

Correlations with the slopes in Fig. 4A–E can be found. A different slope corresponding to the materials with higher α_{RP} values shows a homogeneous coverage of the support surface, and the unblocked silanols are shielded by the C_{18} ligands in materials with a high surface loading.

The slopes of the straight lines corresponding to packings 1, 2 and 3 (Fig. 7) characterized by smaller degrees of coverage (specific solvent–surface interactions are preferred to cohesive and hydrophobic interactions), in contrast, show a different elution mechanism and it seems that these materials show an adsorption mechanism. This can only be explained by a larger number of free silanol groups. These unblocked silanol groups are especially susceptible to interactions with polar substances having free electron pairs or π electrons. For this reason, the most sensitive test to define the nature of the surface and its density and homogeneity is undoubtedly the elution of organic bases, e.g., amines[12,29]. In these instances not only the retention data but also

the tailing effect due to the specific interactions with unblocked silanols can reveal the separation mechanism. For these investigations, pyridine and 2,6-dimethylpyridine (lutidine) were used as the test substances in the RP-HPLC system [methanol–water (70:30, v/v)] (Fig. 8).
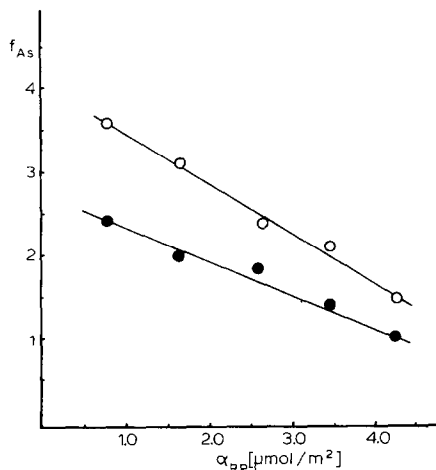

Fig. 8. Effect of coverage density of C_{18} groups (α_{RP}) on the peak asymmetry (f_{As}) for ($\bigcirc$) lutidine and ($\bullet$) pyridine. Chromatographic conditions: mobile phase, methanol–water (70:30, v/v); flow-rate, 1 ml/min; detection, UV (254 nm).

The relationship between the asymmetry coefficient (f_{As}) and concentration of C_{18} ligands (α_{RP}) indicates that the more hydrophobic packing No. 5 ($\alpha_{RP} = 4.24$ μmol/m^2) shows for both amines the smallest f_{As} value calculated according to Buszewski et al.[27] and Bristow and Knox[28]. The increase in the amount of free silanol groups leads to an increase in tailing (i.e., f_{As} value)[10]. It can be concluded that the presence of polar basic substances changes not only the mechanism of the process, but also the "mobility" and the conformation of C_{18} ligands[12,16,17,19]. In this connection, the assumption that for the materials with a coverage density $\alpha_{RP} \geqslant 3.46$ μmol/m^2 (packing 4) the f_{As} values are less than 2 seems to be reasonable. The behaviour of the packings with a coverage density of $\alpha_{RP} \geqslant 3.46$ μmol/m^2 is similar to that with $\alpha_{RP} \geqslant 4$ μmol/m^2 (No. 5), although the coverage is not dense.

CONCLUSIONS

The CP-MAS-NMR data confirm the high surface coverage of the investigated materials. In materials with a high surface loading (4.24 μmol/m^2), the n-alkyl ligands are exclusively covalently bonded to the silica surface. For dense coverages ($\alpha_{RP} = 4.24$ μmol/m^2), 85% of the accessible silanol groups are blocked by alkylsilyl ligands.

The retention mechanism based on hydrophobic interactions is dependent on the surface coverage. In materials having a dense coverage ($\alpha_{RP} \geqslant 3.8$ μmol/m^2), the partition process predominates, whereas in materials with less dense coverages ($\alpha_{RP} < 3.8$ μmol/m^2), the adsorption process is of increasing importance.

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