Influence of the modification conditions of alkyl bonded ligands on the characteristics of reversed-phase liquid chromatography

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Abstract

The parameters characterizing retention and the mass transfer mechanisms in reversed-phase liquid chromatography were derived from data acquired for a series of compounds on a silica gel bonded to alkyl ligands of various lengths and ligand densities. The adsorption equilibrium constant, the absolute value of the isosteric heat of adsorption, and the activation energy of surface diffusion increase while the surface diffusion coefficient decreases with increasing length of the alkyl chain and density of the C_{18} ligand. These results are consistent with increasing restriction of the mobility of an increasingly adsorbable molecule. There is a critical level of carbon content above which the four parameters just listed no longer change with increasing alkyl ligand density. This level is likely to depend on the sizes of the alkyl ligand and the sample molecule. Even at high densities of C_{18} chains, there may be a limitation in the contact area of the sample molecule and the alkyl ligand. The retention behavior on the stationary phases studied is explained by assuming that the sample molecules penetrate into the layer of alkyl ligands. Enthalpy–entropy compensation was demonstrated for surface diffusion. Its mechanism is probably similar on the different stationary phases studied, irrespective of the length and density of the bonded ligand. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

A systematic web search reveals that slightly more than 400 commercial brands of C_{18} bonded silica are currently available as stationary phases for liquid chromatography [1]. In addition numerous phases based on silica gels bonded to other alkyl ligands (mainly but not uniquely C_1, C_4 and C_8) and to various functional ligands are also offered. Retention on these phases arises from the different adsorption properties imparted to the surface of a common adsorbent by different functional groups or ligands bonded at a sufficiently high density. The abundance of reversed-phase liquid chromatography (RPLC) stationary phases having similar but quantitatively different physicochemical and physical properties is the essential reason why chromatography is an effective separation technique for both analytical and preparative applications [2]. Still alkyl-bonded silica gels are the most popular packing material for RPLC

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because of their flexibility. Numerous studies have been published discussing the effects of changing the length and/or the density of the alkyl-chain ligand on the retention behavior and the column efficiency in connection with the fundamentals of the separation mechanism in RPLC [3].

The scientific literature contains contradictory results regarding the influence of the alkyl chain length and/or density on the retention and the separation factors in RPLC [4–9]. Berendsen and De Galan [4] studied the influence of the alkyl chain length on the retention behavior in RPLC and showed that retention (k') and the separation (α) factors tend toward constant values with increasing chain length. These constant values were reached when the chain was longer than a critical length. Values of the corresponding carbon number between 6 and 14 were found, regardless of the mobile phase composition. From these results, the authors concluded that only a part of long alkyl chains contribute to retention and to the separation behavior in RPLC. Löchmuller and Wilder [5] studied the correlation between the capacity factor (k') and the bonded phase coverage (% carbon). They found that a limit value of k' was observed for smaller solutes at high phase coverages. However, the correlations between k' and the carbon content became more nearly linear with increasing solute size and k' did not reach a limit for larger molecules (anthracene and chrysene). Similar results were reported for the correlation between the logarithm of the separation factor and the length of the bonded alkyl chain.

However, different results were reported by other authors. Roumeliotis and Unger [6] investigated the dependence of the retention factor normalized by the surface area of the bare silica gel on the hydrophobic surface area of the alkyl chains under various RPLC conditions and observed linear correlations between them for all RP stationary phases, except a C_1-silica gel. They concluded that the entire alkyl chain contributes to the hydrophobic interactions between sample molecules and alkyl ligands. Several other papers reported a monotonous increase of the retention and/or the separation factors with increasing alkyl chain length [7–9].

Contradictory conclusions were also reported for the influence of the alkyl chain length on the column efficiency and the mass transfer rate in RPLC. Knox

and Pryde [10] indicated that the mass transfer rate was faster for RPLC packing materials chemically modified with an n-alkyl ligand than for the bare silica gel and that it increased with decreasing length of the alkyl chain. On the other hand, Karch et al. [7] suggested that the column efficiency was independent of the alkyl chain length.

These contradictory results demand deeper fundamental investigations on the influence of the length and density of the bonded alkyl chains on the chromatographic behavior of RPLC phases to clarify this issue. It would be useful to measure intrinsic mass transfer kinetics and thermodynamics properties beyond the retention equilibrium at infinite dilution. In a previous paper [11], we studied the chromatographic behavior of four packing materials chemically modified with C_1, C_4, C_8 and C_{18} alkyl ligands. We found that the adsorption equilibrium constant (K), the isosteric heat of adsorption (Q_{st}), and the activation energy of surface diffusion (E_s) increased with increasing chain length while the surface diffusion coefficient (D_s) decreased. However, the rate of change of these four parameters decreased for carbon numbers larger than 8. Almost the same values of these four parameters were observed for C_8 and C_{18} silica gels. From these results, it was concluded that only parts of the long alkyl ligands contribute to the retention and the mass transfer mechanisms in RPLC.

This paper deals with the effects of changes in the composition of the surface of the stationary phase on the retention mechanism in RPLC. Using columns packed with phases prepared from the same silica gel, bonded to alkyl chains of three different lengths (C_1, C_4, and C_{18}), and, for C_{18}, with four different bonding densities, chromatographic data were acquired and analyzed using approaches recently discussed [11]. Information regarding novel correlations between the parameters describing the retention equilibrium, the mass transfer kinetics, and the thermodynamic properties is reported.

2. Experimental

2.1. Columns and reagents

Table 1 lists the relevant information on the

Table 1
Physical properties of RP columns

	Column/stationary phase no.					
	1	2	3	4	5	6
Main alkyl ligand	C_1	C_4	C_{18}	C_{18}	C_{18}	C_{18}
Particle density, r_p (g cm^{-3})	0.74	0.73	0.64	0.71	0.79	0.86
Porosity, ε_p (−)	0.62	0.61	0.64	0.57	0.50	0.46
Tortuosity factor, k^2 (−)	3.9	4.1	3.8	4.1	4.4	4.5
Carbon content (%)						
Before end-capping	4.1	6.7	3.6	6.4	12.8	17.1
After end-capping	−[a]	−[a]	6.6	8.6	13.7	17.1
Main ligand density, (mmol m^{-2})[b]	13.4	3.7	0.59	1.1	2.3	3.2
Distance between ligands (nm)[b]	0.40	0.76	1.9	1.4	1.0	0.81
Reaction ratio of silanol group (%)[b]	56	46	7.3	13	29	40

[a] No end-capping treatment was carried out.

[b] Calculated from the carbon content before end-capping, the surface area of the base silica gel (290 m^2 g^{-1}), and the density of silanol group on the surface of the base silica gel (assumed to be 8 μmol m^{-2}).

physical properties of the different columns and stationary phases used. These columns and most of the information were obtained from YMC (Kyoto, Japan). The columns were slurry packed with materials synthesized by chemically bonding *n*-alkyldimethylsilyl ligands onto the surface of the same base silica gel. The density of alkyl ligands on the surface of the base silica gel was estimated from the carbon content of the packing materials. Regarding C_{18}-silica gels, the density of the alkyl ligand ranges between 0.59 and 3.2 μmol m^{-2} in this study. This range is relatively low. However, the highest density of alkyl ligands is comparable to the highest values (3.0–3.5 μmol m^{-2}) encountered for most commercially available monofunctional C_{18} packing materials. The amount of carbon of the first three octadecyl phases (8.6 to 13.7% C) increased significantly upon end-capping treatment with trimethylsilyl ligand. The end-capping caused no substantial increase in the carbon content of the third C_{18} phase (17.1% C). No end-capping was carried out for the C_1 and C_4 phases. Assuming a typical density of the silanol groups of about 8 μmol m^{-2} on the surface of the silica gel [12], it seems that 7.3–40% of the silanol groups reacted with the C_{18} ligand for the four C_{18} phases (6.6–17.1% C).

The average particle diameter of the base silica gel is 45 μm. Its specific surface area measured by nitrogen BET is 290 m^2 g^{-1}. The average distance between two alkyl ligands on the surface of each packing material was derived from the density of the alkyl ligands. The distance between two adjacent ligands is about 0.5–2.5 times the molecular size of the samples studied. For instance, the molecular radii of benzene and hexylbenzene estimated approximately from their molar volumes at their respective normal boiling points are 0.34 and 0.45 nm, respectively. In all cases, the mobile phase was a methanol–water (70:30, v/v) mixture. Benzene, ethylbenzene, butylbenzene and hexylbenzene were used as the samples and uracil as the inert tracer.

2.2. Apparatus

Pulse response experiments (i.e. elution chromatography) were made using a high-performance liquid chromatographic system (LC-6A, Shimadzu, Kyoto, Japan) to acquire the data. A small amount of sample solution was injected with a Rheodyne (Cotati, CA, USA) valve injector (Model 7125). The column temperature was kept constant by circulating temperature-controlled water around the column. The concentration of the sample at the column exit was monitored with the ultraviolet detector of the HPLC system.

2.3. Procedure

The elution peaks were recorded under linear-isotherm conditions, at different column temperatures (288–308 K) and mobile phase flow-rates (1.0–2.0 ml min^{-1}). The first and second moments

of the elution peaks were calculated. Information on the phase equilibrium and the mass transfer rates in the column was derived from these moments [11]. The procedures followed for the data analysis are briefly described here. Further details on the moment analysis method can be found in the literature [2,11,13,14].

Information on the K value was derived from the first moment (μ_1). The value of D_s was estimated from the second central moment (μ_2'), by subtracting the contributions of axial dispersion, fluid-to-particle mass transfer, and pore diffusion to band broadening. The equations proposed by Wilson–Geankoplis [15] and Wilke–Chang [16,17] were used to estimate the fluid-to-particle mass transfer coefficient and the molecular diffusivity (D_m) of the elutes, respectively. Taking advantage of their different flow-rate dependence, the contributions of axial dispersion and intraparticle diffusion were separated. It was assumed that intraparticle diffusion arose from the parallel contributions of pore and surface diffusion [13,14]. The value of D_s was derived from the intraparticle diffusivity (D_e) by subtracting the contribution of pore diffusivity (D_p), estimated from D_m, the intraparticle porosity of the stationary particle, and the tortuosity factor of the pores, according to the parallel pore model [13,14]. The tortuosity factor was determined from pulse response experiments made with an inert tracer. The influence of the extra-column volumes on μ_1 and μ_2' was measured by performing the same measurements without column and using the results as corrections.

Corrections were also made for the influence of the peak distortion on the determination of the moments μ_1 and μ_2' of the elution peaks. Several interpretations were proposed for the origin of peak asymmetry (tailing and fronting) [2]. In this study, the radial heterogeneity of the column was regarded as the only origin of the peak skewness [18–20]. Possible heterogeneous mass transfer kinetics was neglected because the surface of the alkyl bonded silica gels seemed to be energetically homogeneous. For instance, apparent uniformity of the surface of C_{18}-silica gel is suggested by experimental results showing that the phase equilibrium isotherm can usually be accounted for by the simple Langmuir model and that the isosteric heat of adsorption, Q_{st}, and the activation energy of surface diffusion, E_s,

were nearly constant, irrespective of the amount adsorbed [11]. Although the influence on the values of μ_1 and μ_2' of the width of the sample pulses injected was also corrected for by assuming that these pulses had a rectangular profile, this influence could have been neglected as well because of the extremely small size of the samples injected. These corrections are responsible for the error made in the determination of D_s, an error which is estimated at about several percent, as previously discussed [11].

3. Results and discussion

3.1. Retention equilibrium

Fig. 1 illustrates the dependence of K at 298 K on the carbon content of the RPLC phase. The increase in carbon content is accompanied by an increase of K for all the compounds. However, the slope of the curves gradually decreases above 8% carbon. Almost the same values of K are observed at 13.7 and 17.1%
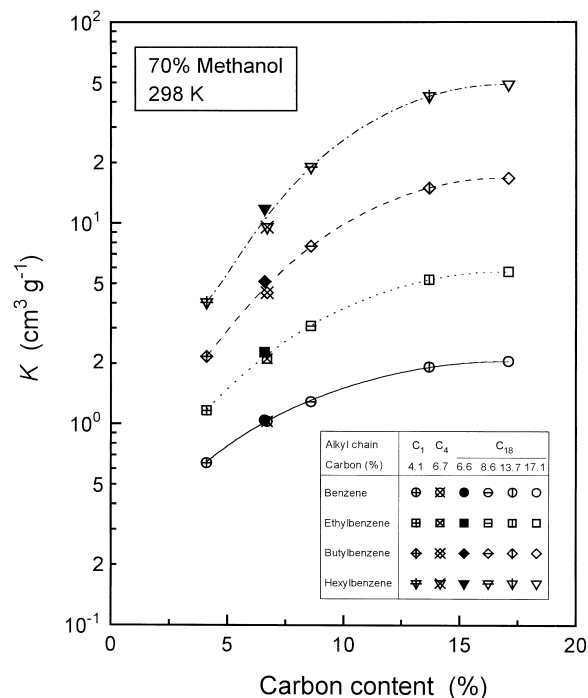

Fig. 1. Correlation between the adsorption equilibrium constant and the carbon content of the stationary phase.

carbon. The trend of the curves in Fig. 1 is similar to that of the correlation between K and the alkyl chain length on the same stationary phases reported previously [11]. The value of K tends toward a limit corresponding to an alkyl chain length slightly larger than C_8. We conclude that only part of longer alkyl ligands may contribute to the retention behavior in RPLC. The experimental data obtained for the C_1- and C_4-silica gels are also shown on each line in Fig. 1.

Almost the same values of K are observed for the stationary phases C_4 (6.7% C) and C_{18} (6.6% C). However, it is unclear whether this result is general or a coincidence. Table 1 shows that the density of the main alkyl ligands (excluding the endcapping methyl groups) is six times larger for C_4-(6.7% C) than for C_{18}-silica gel (6.6% C). The average distance between two C_4 ligands was estimated as 0.76 nm, equal to the molecular size of the compounds studied. By contrast, the distance between two alkyl ligands is 2.5 times larger on phase C_{18} (6.6% C) than on phase C_4 (6.7% C). The retention mechanism is probably different on the two phases because of the different flexibility of the two alkyl chains (C_4 and C_{18}) and because the ratios of the size of the sample molecule and the average distance between two alkyl ligands are so different on the C_4- and C_{18}-silica gel.

Fig. 2 shows the correlation of K at 298 K with the density of C_{18} ligand on the four C_{18} phases. Almost linear correlations are observed for all four sample components on the three C_{18} phases of carbon content between 6.6 and 13.7%. However, the values of K for the stationary phase C_{18} (17.1% C) are smaller than predicted by an extrapolation of these linear correlations. Although the average distance between two C_{18} ligands bonded to the phases C_{18} (13.7% C) and C_{18} (17.1% C) (Table 1) is estimated to be nearly the same as the molecular size of the sample compounds, a certain flexibility of the C_{18} chain may allow interactions of the sample molecules with several alkyl ligands. However, if this were actually to take place, K would be larger than predicted by the extrapolation of the straight line in Fig. 2, which is in contradiction with what Fig. 2 shows. On the other hand, K increases almost linearly with increasing ligand density at low densities [phases C_{18} (6.6% C) to C_{18} (13.7% C)]. Accordingly, it is likely that (1) a solute molecule, at
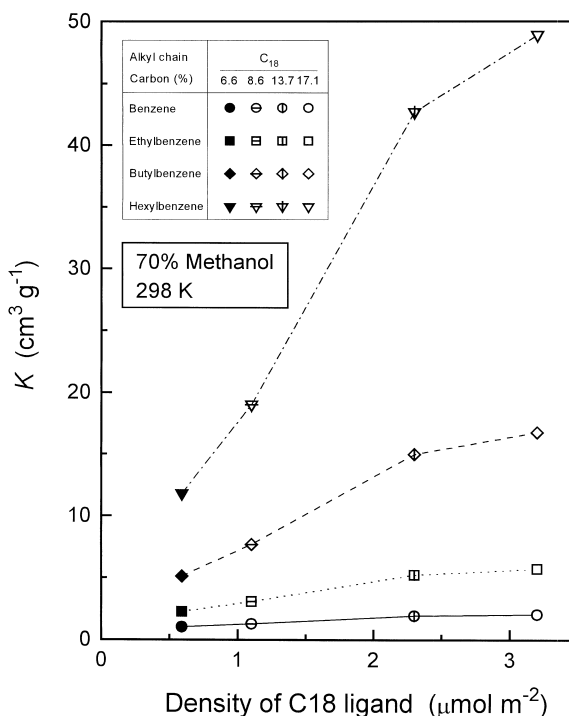

Fig. 2. Correlation between the adsorption equilibrium constant and the modification density of C_{18} ligand of the stationary phase.

least for those as small as the benzene derivatives studied here, interacts with only one alkyl ligand, even when the density of these ligand is high; and (2) all the C_{18} ligands do not necessarily contribute to the retention of the solutes at ligand densities higher than ~2.3 μmol m^{-2}.

Fig. 3 shows the linear correlations between the logarithm of K and the hydrophobic surface area of the sample compounds (A). This area was calculated by summing the surface area increments of each group of their molecules [21]. The slope of the straight lines is slightly different for the six phases, suggesting that the retention mechanism of the sample compound depends somewhat on the nature of the modified surface of the adsorbent, the alkyl chain length, and the C_{18} ligand density. In the solvophobic theory [9,22–24], it is assumed: (1) that the contact area between the polar solvent molecules and the hydrophobic surfaces of solute molecules and bonded alkyl ligands decreases when the solute molecule is adsorbed on the alkyl ligand and (2) that the magnitude of this reduction of the total hydro-
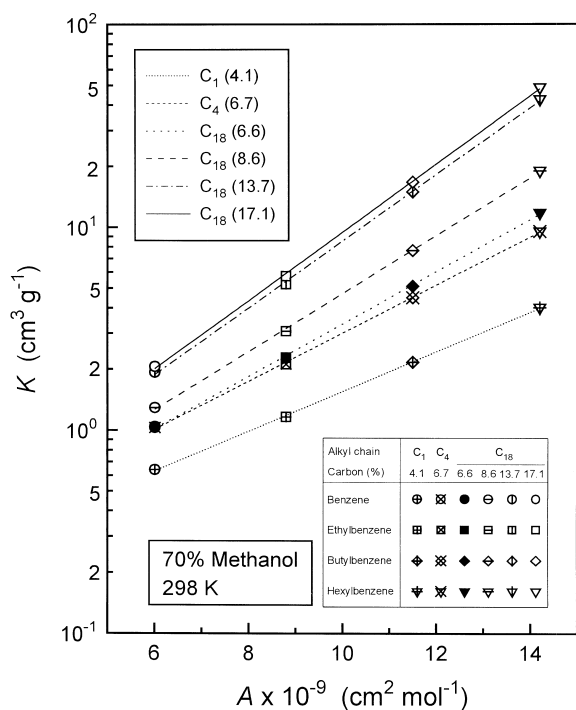
Fig. 3. Correlation between the adsorption equilibrium constant and the hydrophobic surface area of the sample compound.

phobic surface area (ΔA) is proportional to A. The ratio $\Delta A/2A$ and the value of $(1 - \Delta A/2A)$ probably represent the relative fractions of the hydrophobic surface of the sample molecule in contact with the alkyl ligand and the polar mobile phase solvent, respectively. According to the solvophobic theory, the ratio $\Delta A/A$ can be estimated from the slope of the linear correlations in Fig. 3.

Values of the ratio $\Delta A/A$ ranging from 0.20 to 0.35 were calculated from the slope of the linear correlations in Fig. 3. In RPLC, the solvation interaction between the molecules of sample and their solvent neighbors in the mobile phase seems to be reduced to 82–90% of its initial value because the adsorption of the sample molecule on the stationary phase is accompanied with a reduction of the hydrophobic surface area of the sample molecule exposed to the polar solvent. Some values of $\Delta A/A$ reported in RPLC on C_{18}-silica gels, are 0.30–0.35 in 70% methanol [11], 0.18 in 70% acetonitrile [25], 0.26 in 70% ethanol [26], and 0.35 in an aqueous buffer [22]. A similar value for $\Delta A/A$ (0.20–0.30) was also

reported for a liquid phase system consisting of activated carbon and water [24]. The results in Fig. 3 do not provide a definitive conclusion regarding the number of alkyl groups with which a solute molecule may interact. It is likely, however, that no more than about 10–18% of the hydrophobic surface area of the solute molecule can interact with the long alkyl ligands because of steric hindrance when the density of the bonded ligand is high.

The structural characteristics of the alkyl ligand bonded to the packing material influence the retention behavior of a solute and the ratio $\Delta A/A$. As indicated in Fig. 3, the logarithm of K increases linearly with increasing number of methylene groups in the solute molecule. The increment in ln K caused by a given increase in the molecular size of the sample compound is larger on the packing material having the longer alkyl chains or the higher density of C_{18} ligands than on that having a shorter chain or a lower density. This situation may arise from the difference in the degree of steric interactions between the bonded alkyl ligand and the sample molecule. Although only planar interaction seems to be possible in the case of the C_1-silica gel, a three-dimensional interaction of the sample molecule with C_{18} ligands could be considered. The penetration of the sample molecule into the layer of long alkyl ligands on the packing material was suggested by Tchapla and co-workers [27,28], on the basis of the results of their detailed studies on the retention of many homologous families. The difference in the slope of the linear correlations for the stationary phases C_4 (6.7% C) and C_{18} (6.6% C) is probably accounted for by this penetration model. The slope of the straight lines is slightly larger for the C_{18}-silica gel (6.6% C) than for the C_4-silica gel (6.7% C), although the carbon contents of the both RP packing materials are almost same.

Löchmuller and Wilder [5] reported a correlation between k' and the coverage of the stationary phase by bonded alkyl groups. They indicated that, although k' for small solutes tends toward a limit with increasing length of the alkyl chain, the correlations between k' and the carbon content are straighter for larger solutes (anthracene and chrysene). They explained the characteristics of the retention behavior by considering a liquid-like partitioning of the hydrocarbon part of the solutes into the bonded

alkanes. They suggested that the retention behavior of a bonded phase depends on the solute size and the bonded chain length.

The retention behavior of solutes seems to be affected by the degree of their penetration into the alkyl ligand layer. This penetration depends on the relative molecular size of the solute and the alkyl ligand, on their steric properties, on the bonding conditions and on other chromatographic conditions, for instance, the mobile phase composition. The contradictory results concerning the correlation between retention behavior and bonding conditions that were previously reported might be interpreted by considering the influence of these factors on the retention interactions between solute molecules and alkyl ligands.

3.2. Mass transfer kinetics

The influence of the length and/or density of bonded alkyl ligand on the mass transfer properties in chromatography was also the topic of many studies. However, in most cases, an overall kinetic parameter, the number of theoretical plates or the height equivalent to a theoretical plate (HETP), was used. It is well known that peak broadening depends on the contributions of several mass transfer processes [2]. The following four processes are usually considered as the most important: (1) axial dispersion, (2) fluid-to-particle mass transfer, (3) intraparticle diffusion and (4) adsorption/desorption. The contributions of the first two processes are essentially independent of the surface chemistry and that of adsorption/desorption is usually neglected in RPLC because it is small [29]. Therefore, the study of the kinetic influence of the alkyl ligand bonded to the stationary phase should be made by analyzing the properties of intraparticle mass transfer. Fig. 4 shows the dependence of intraparticle diffusivity (D_e) on the carbon content of the stationary phase. As in Fig. 1, D_e increases with increasing carbon content at 298 K, tending toward a limit.

It is usually assumed that intraparticle diffusion consists of two mechanisms, pore and surface diffusions [13,14]:
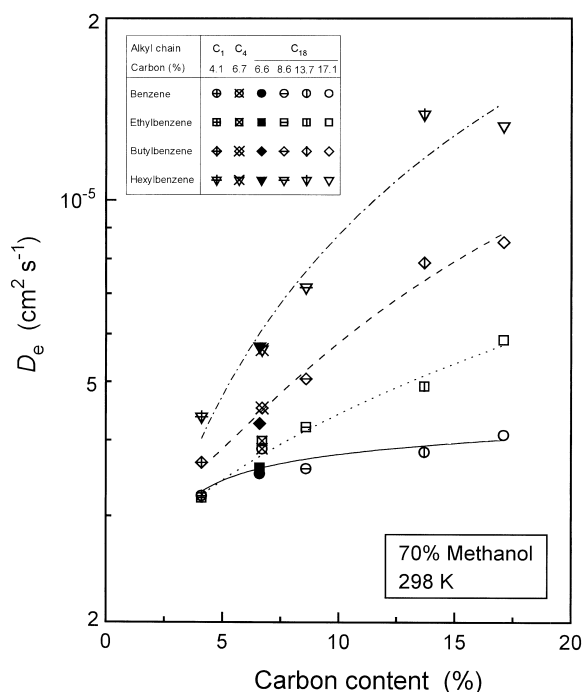
$$D_e = D_p + \rho_p K D_s \tag{1}$$

Fig. 4. Correlation between the intraparticle diffusivity and the carbon content of the stationary phase.

where ρ_p is the particle density. In this study, D_p was estimated by assuming the parallel pore model, with:

$$D_p = \frac{\varepsilon_p}{k^2} \cdot D_m \tag{2}$$

where ε_p is the porosity of the packing material and k the tortuosity factor. The value of D_m was calculated with the Wilke–Chang equation [16,17]:

$$D_{m,s} = 7.4 \cdot 10^{-8} \cdot \frac{(\alpha_{A,sv} M_{sv})^{1/2} T}{\eta_{sv} V_{b,s}^{0.6}} \tag{3}$$

where the subscripts s and sv denote the solute and the solvent, respectively; α_A is an association coefficient, M the molecular mass, η the viscosity, T the absolute temperature, and V_b the molar volume at the normal boiling point. Fig. 5 compares D_e and D_p. D_p is several times to one order of magnitude smaller than D_e, indicating that surface diffusion has an influential contribution to intraparticle diffusion. The difference between D_e and D_p corresponds to the
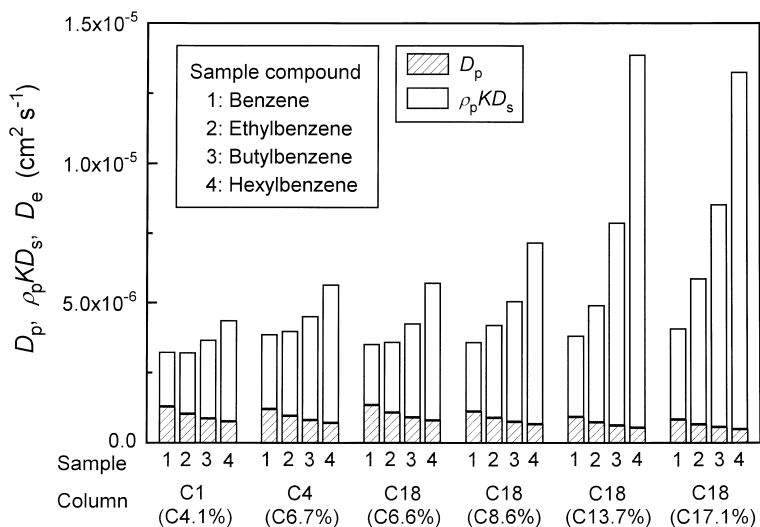
Fig. 5. Comparison of the contributions of the pore and surface diffusions to intraparticle diffusion.

contribution of surface diffusion, i.e. $\rho_p K D_s$, from which D_s was calculated. In this study, a further analysis was made of the surface diffusion phenomena because this type of mass transfer mechanism is the most significantly influenced by changes in the density and the length of the alkyl ligand. Pore diffusion also seems to be independent of the density and length of the bonded alkyl chain.

Fig. 6 illustrates the correlation between D_s and the carbon content of the stationary phase. By contrast to D_e (Fig. 4), D_s increases with decreasing carbon content and seems to tend toward D_m. The values of D_m at 298 K derived from the Wilke–Chang equation are as follows, $8.2 \cdot 10^{-6}$ cm^2 s^{-1} (benzene), $6.5 \cdot 10^{-6}$ cm^2 s^{-1} (ethylbenzene), $5.5 \cdot 10^{-6}$ cm^2 s^{-1} (butylbenzene), and $4.8 \cdot 10^{-6}$ cm^2 s^{-1} (hexylbenzene). No hydrophobic adsorption takes place when no alkyl ligands are bonded to the surface of the silica gel. The results in Fig. 6 suggest that D_s becomes close to D_m when the retention of the analyte becomes negligible and that the diffusivity of the sample molecule in the potential field of adsorption is reduced according to the extent of the adsorptive interactions of the sample component with the surface of the stationary phase. Fig. 6 also shows that D_s is approximately the same for a given analyte at high carbon content, suggesting that the restriction caused by the C$_{18}$ ligand reaches a limit when the

carbon content exceeds a critical value. This trend is consistent with the correlation between K and the carbon content in Fig. 1.
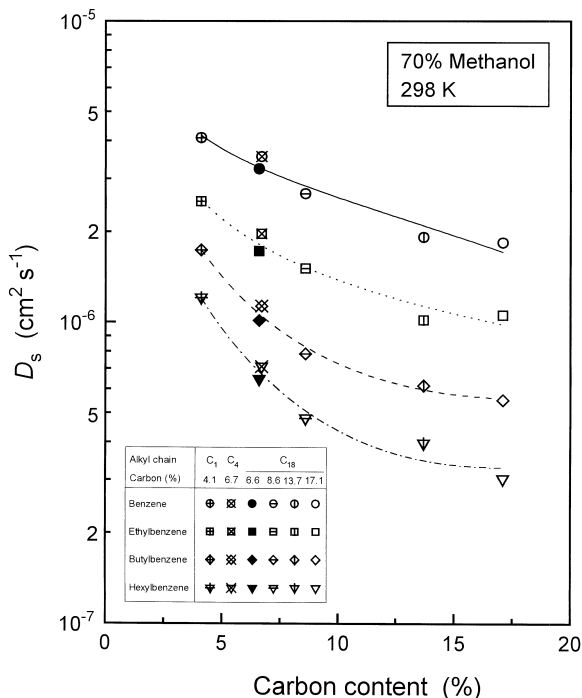

Fig. 6. Correlation between the surface diffusion coefficient and the carbon content of the stationary phase.

3.3. Thermodynamic properties

Earlier, we studied the influence of the length and density of the alkyl chains on the chromatographic properties of the stationary phase, taking K and D_s as the parameters. In the following, we study the thermodynamic properties of the retention equilibrium and of surface diffusion in RPLC systems using these different stationary phases.

Fig. 7 shows the correlation between the isosteric heat of adsorption, Q_{st}, and the carbon content of the phase. The values of K at different temperatures were analyzed using the Van't Hoff equation in order to determine Q_{st}, with:

$$K = K_0 \exp\left(\frac{-Q_{st}}{RT}\right) \qquad (4)$$

where K_0 is K at $1/T = 0$ or $Q_{st} = 0$ and R the universal gas constant. Almost the same trend was observed for all the sample compounds. Whereas $-Q_{st}$ gradually increases with increasing carbon content below ~10%, almost constant values of Q_{st}

are observed at higher carbon content. The temperature dependence of D_s was analyzed using the Arrhenius equation:

$$D_s = D_{s0} \exp\left(\frac{-E_s}{RT}\right) \qquad (5)$$

where D_{s0} and E_s are the frequency factor and the activation energy of surface diffusion, respectively. The value of E_s is plotted against the carbon content of the phase in Fig. 8. The trend observed in Fig. 8 is essentially the same as in Figs. 1, 6 and 7, leading to the following conclusion. The interactions of the sample compound and the stationary phase increase with increasing length of the alkyl chain and with increasing ligand density for a given ligand (C_{18}). However, there is a critical carbon content above which the chromatographic properties studied, i.e. K, D_s, Q_{st}, and E_s, no longer significantly change with increasing carbon content. This critical level depends probably on the size of both the alkyl ligand and the sample molecule. Even when the density of C_{18} chains is high and the corresponding carbon content
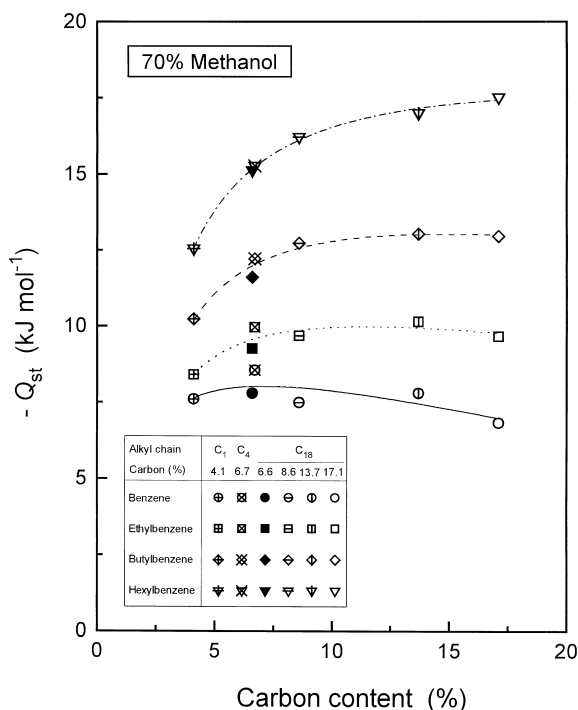

Fig. 7. Correlation between the isosteric heat of adsorption and the carbon content of the stationary phase.
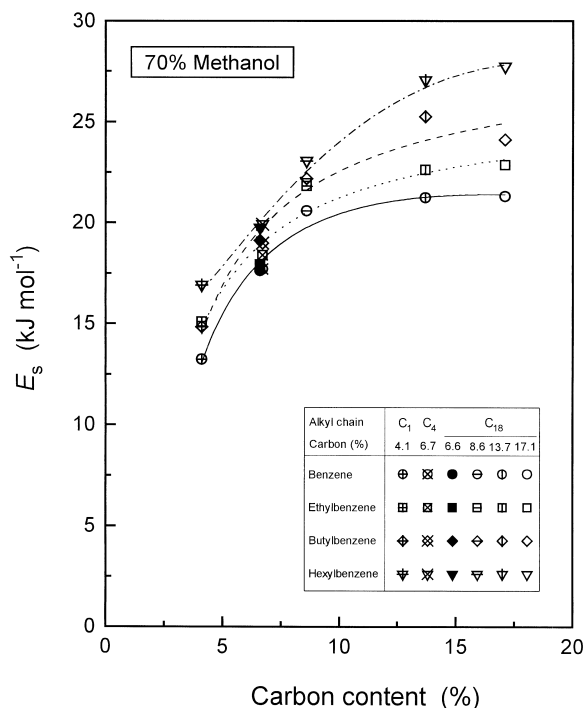

Fig. 8. Correlation between the activation energy of surface diffusion and the carbon content of the stationary phase.

exceeds the critical level, there may be a limitation in the contact area of the sample molecule and the alkyl ligand. As described earlier, the ratio $\Delta A/A$ was estimated from the slope of the linear correlations between ln K and A in Fig. 3, according to the solvophobic theory. It was found to be between 0.20 and 0.35. The ratio $\Delta A/2A$ and the value of $(1-\Delta A/2A)$ probably represent the relative fractions of the hydrophobic surface of the sample molecule in contact with the alkyl ligand and the polar mobile phase solvent, respectively. As a consequence, it is likely that the solvation interaction between the molecules of sample and their solvent neighbors in the mobile phase is reduced to 82–90% of its initial value. It also seems probable that no more than 10–18% of the hydrophobic surface area of the sample molecule may be in contact with the long alkyl ligand, although it is possible that one sample molecule interacts with several alkyl ligands. A definitive conclusion regarding the number of alkyl chains with which a sample molecule can interact could not obtained in this study.

Fig. 9 shows the correlation between D_{s0} and E_s for each compound studied. The values were determined from the analysis of the temperature dependence of D_s by the Arrhenius equation. In previous papers [11,25,26], we demonstrated an enthalpy–entropy compensation for surface diffusion under all RPLC experimental conditions, independently of the nature of the sample compound, the type and composition of the organic modifiers of the aqueous mobile phase, and the length of the alkyl groups bonded to the surface of the stationary phase. The mechanism of surface diffusion seems to be similar, irrespective of the particular experimental conditions in RPLC. The enthalpy–entropy compensation was already reported for the retention equilibrium in some chromatographic systems [30–36]. Boots and de Bokx [37] and Vailaya and Horváth [38] made detailed analyses of this enthalpy–entropy compensation.

Although the intercepts are different for the different compounds, linear, parallel correlations are observed between ln D_{s0} and E_s in Fig. 9. The thermodynamic properties of surface diffusion, i.e. D_{s0} and E_s, on the different RP stationary phases are linearly correlated. The results illustrated in Fig. 9 imply that the migration mechanism of the com-
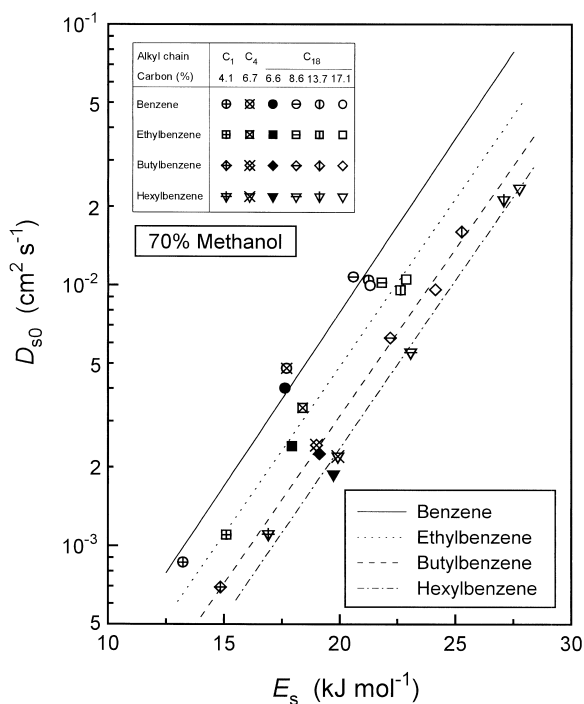

Fig. 9. Correlation between the frequency factor and the activation energy of surface diffusion.

pounds studied by surface diffusion is similar, irrespective of the modifications made to the stationary phase. As described in Fig. 6, D_s seems to tend toward a value close to D_m with decreasing retention, suggesting a sort of correlation between surface and molecular diffusions [11,39]. As some theories and models have been suggested to explain the retention behavior in RPLC [23], a consistent interpretation of the migration mechanism of surface diffusion may be possible. This subject is currently under investigation [40,41].

4. Conclusion

The influence on their chromatographic characteristics of the length and the density of alkyl chains bonded to an adsorbent surface was studied from the viewpoints of the retention equilibrium, the surface diffusion, and their thermodynamic properties. The values of K, $-Q_{st}$ and E_s increase toward constant limits with increasing alkyl chain length and C_{18}

density. Above a critical carbon content, these three parameters remain constant when the amount of bonded alkyl groups increases. This level probably depends on the size of both the alkyl chain and the sample molecule. Even when the density of C_{18} chain and the carbon content of the stationary phase exceed the critical level, the area of contact of the sample molecule and the alkyl ligand may be limited. Although no definitive conclusion could be obtained regarding the number of alkyl chains with which one sample molecule may interact, it was shown that no more than ~10–18% of the hydrophobic surface area of the sample molecule could contact long alkyl chains. The penetration of the sample molecule into the layer of alkyl ligands probably explains the changes observed in the stationary phase characteristics and its retention behavior.

Although D_s decreases with increasing carbon content, it also tends toward a constant value for a similar critical carbon content. The profiles of the correlations of K, Q_{st}, E_s and D_s with the carbon content consistently suggest that the mobility of the adsorbate is inversely related to its retention. With decreasing carbon content of the stationary phase, D_s tends toward D_m, suggesting a correlation between surface and molecular diffusions. An enthalpy–entropy compensation was demonstrated for surface diffusion. The mechanism of surface diffusion may be the same, irrespective of the chemical changes made to the stationary phase. It is expected that a theoretical model could afford a consistent interpretation of the characteristics of the mechanism of surface diffusion in RPLC based on these experimental results.

5. Nomenclature

A	Hydrophobic surface area of the sample compound (cm^2)
ΔA	Reduction of total hydrophobic surface area due to adsorption (cm^2)
D_e	Intraparticle diffusivity ($cm^2\,s^{-1}$)
D_m	Molecular diffusivity ($cm^2\,s^{-1}$)
D_p	Pore diffusivity ($cm^2\,s^{-1}$)
D_s	Surface diffusion coefficient ($cm^2\,s^{-1}$)

D_{s0}	Frequency factor of surface diffusion ($cm^2\,s^{-1}$)
E_s	Activation energy of surface diffusion ($kJ\,mol^{-1}$)
k	Tortuosity factor (−)
k'	Retention factor (−)
K	Adsorption equilibrium constant ($cm^3\,g^{-1}$)
K_0	K at $1/T=0$ or $Q_{st}=0$ ($cm^3\,g^{-1}$)
M	Molecular mass (−)
Q_{st}	Isosteric heat of adsorption ($kJ\,mol^{-1}$)
R	Gas constant ($J\,mol^{-1}\,K^{-1}$)
T	Absolute temperature (K)
V_b	Molar volume at the normal boiling point ($cm^3\,mol^{-1}$)

Greek symbols

α_A	Association coefficient (−)
ϵ_p	Intraparticle porosity (−)
η	Viscosity (Pa s)
μ_1'	First moment (s)
μ_2'	Second central moment (s^2)
ρ_p	Particle density ($g\,cm^{-3}$)

Subscripts

s	Solute
sv	Solvent

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