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SIMPLE RELATIONSHIPS CONCERNING MOBILE AND STATIONARY PHASES IN NORMAL- AND REVERSED-PHASE CHROMATOGRAPHY*

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SUMMARY

For normal-phase chromatography on aminopropyl-bonded silicas, the adsorption of the polar modifier of various mixtures of hexane with *n*-alkanols, benzyl alcohol, acetone, dimethylformamide, nitromethane, chloroform and diisopropyl ether were measured. One to three molecules of the polar solvent per bonded aminopropyl chain can be fixed. Simple calculations allow the determination of the relative proportions of amino groups free or bonded with one, two or three modifier molecules for each mobile phase composition. The amount of the polar modifier in the stationary phase increases with its ability to form hydrogen bonds with the amino groups for a given composition of the mobile phase. The amount is larger with any alcohol than with nitromethane, acetone or dimethylformamide.

The capacity factor (k') of various polar solutes (phenols, amines and pyridines) and non-polar solutes (polyaromatic hydrocarbons) decreases when the concentration of polar solvent in the mobile phase increases. When the average number of molecules of the polar modifier in the stationary phase remains less than one per amino group, a linear relationship exists between $1/k'$ and the volume fraction of the polar solvent in the mobile phase. When more than one molecule per amino chain is fixed, the variations of $1/k'$ are not easily linked to the eluent composition.

For reversed-phase chromatography on alkyl-bonded silicas, the ln k' and the selectivity for non-polar and polar solutes vary linearly with NS (N being the total number of chains bonded on the silica surface and S the hydrocarbonaceous surface area of a chain). For instance, the retention time of a solute will be the same using C_8, C_{12} or C_{22} bonded phases with the same value of NS and consequently the same carbon content.

Retentions of polar and non-polar solutes were measured with five different water-miscible organic modifiers (methanol, ethanol, 1-propanol, acetonitrile and tetrahydrofuran). The variations of k' can be related to the volume fraction (x) of

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water by $\ln k' = ax^n + b$ ($0 < x < 0.85$), n depending on the organic solvent nature and a on the solute properties. For each organic modifier, a linear relationship exists between a and the molar volume of the solute. The solubility of solutes was measured in the same mixtures used as mobile phases. The solubility of a given solute in any water–modifier mixture is related to x by $\ln s = -ax^n + b'$, a and n having the same values as those obtained for the retention variation. This equation is verified for x values corresponding to $1 < k' < 200$. Hence a very simple relationship between k' and s ($k's = C$) is valid for all mobile phase compositions.

INTRODUCTION

In partition by normal- and reversed-phase chromatography, the retention of a solute depends on the amount and polarity of the bonded stationary phase, the mobile phase composition, the temperature and the nature of the solute.

In reversed-phase chromatography, Locke[1] has shown that the interactions between *n*-alkyl-bonded chains and solutes are weak and non-selective, and suggested that the relative retention for closely related solutes could be determined solely by the difference in solubility of the two components in the mobile phase. This idea was considered by Karger *et al.*[2], who studied the water solubility theory and predicted retention by using a topological index called molecular connectivity, in which the solute surface area is estimated from the hydrocarbonaceous skeleton of the solute. They obtained good correlations for alkyl and cycloalkyl compounds. Horváth and co-workers[3,4] also showed that the solvophobic effect is mainly responsible for the solute retention. Therefore, even if the hydrocarbonaceous stationary phase plays some role in retention[5], the mobile phase and the solute solubility play a more important role[6–9].

In contrast, in normal-phase or adsorption chromatography, the solute retention is often described as being mainly due to the interactions in the stationary phase. Two approaches to the chromatographic behavior of solutes have been developed. The first describes the stationary phase composition in equilibrium with the eluent. Snyder's[10] and Oscik's[11] theories are two examples of the first approach. According to Snyder[10,12,13], the interaction energy of a solute with any component in the mobile phase is always negligible in comparison with the adsorption energy of the solute or the solvent molecules on the stationary phase surface. This assumption was also used when this theory was extended to non-ideal mobile phases[14] or heterogeneous surfaces[15]. However, the behaviour of aminoalkyl-bonded phases is different and cannot be described accurately by Snyder's theory[15]. Consequently, the activity of this phase should be very low compared with silica or other bonded phases. Moreover, Hammers *et al.*[16] noted that strong interactions occurred between dichloromethane molecules and the bonded amino groups. Strong interactions must occur in the mobile phase between the dichloromethane and amino groups of any solute and this is therefore inconsistent with assumptions about interactions in the mobile phase with polar solutes.

The second approach describes the solute partition between both phases[17,18]. Polar forces are the main forces in the bulk phase, whereas dispersive and polar forces can occur in the eluent. Scott and Kucera[17] observed a linear relationship between the

inverse of the retention volume of a solute and the concentration of the polar modifier in the mobile phase. Another linear relationship is obtained with the eluent density when the polar solvent concentration remains constant. However, this theory only describes dipole–dipole forces in both phases and not hydrogen bond or complex formation, which seem more important with aminoalkyl-bonded phases.

As no theory accurately describes the behaviour of amino-bonded phases, we have tried to define the nature and the composition of the stationary phase in equilibrium with mixtures of *n*-hexane and various polar modifiers and then to explain the variations of the capacity ratio of the solutes with the mobile phase composition.

EXPERIMENTAL

Apparatus

Experiments were performed with a liquid chromatograph assembled from commercially available modules consisting of an Altex 380 pump (Altex, Berkeley, CA, U.S.A.), a Valco six-port sampling valve with a 20-μl loop, a Waters R-401 refractometer (Waters Assoc., Milford, MA, U.S.A.), or a fixed-wavelength UV detector (Altex), and also with a Hewlett-Packard 1084 B liquid chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.). The temperature was maintained at 30 $\pm$ 0.1°C with a constant-temperature water-bath. Solute solubilities were measured by the UV technique on a Variscan variable-wavelength spectrophotometer (Varian, Palo Alto, CA, U.S.A.).

Stationary phases

Several chromatographic columns of different lengths were packed with home-made stationary phases described previously[5] and with the commercially available LiChrosorb-NH_2 and RP-8 (10 μm) (Merck, Darmstadt, G.F.R.). The dead times of reversed-phase columns were measured for each mobile phase composition by determining the retention time of sodium nitrate.

Chemicals

All of the alcohols used were of analytical-reagent grade and were obtained from Prolabo (Paris, France). Chloroform and methylene chloride were of LiChrosolv grade and were purchased from Merck. *n*-Hexane, tetrahydrofuran and acetonitrile were of Chromasol grade and were purchased from SDS (Valdone, France). The water was doubly distilled.

Calculations

All calculator-generated data were obtained with a Hewlett-Packard 9825A programmable calculator.

NORMAL-PHASE CHROMATOGRAPHY ON AMINOPROPYL-BONDED SILICAS

Adsorption isotherms

Experimental results. Adsorption of the polar modifier from various mixtures of *n*-hexane with *n*-alkanols, benzyl alcohol, N,N-dimethylformamide, acetone, nitromethane and chloroform was measured on an aminopropyl-bonded stationary phase (LiChrosorb-NH_2) using a frontal analysis technique.

The adsorption isotherms in Fig. 1 show the affinity order of the polar modifier for the amino groups. There is no satisfactory correlation between affinity order and classical measurement of polarity as Snyder's eluent strength ε_0 or Rohrschneider's polarity parameter P' for all of the solvents considered. For instance, acetone has more affinity for amino groups than nitromethane, although acetone is always considered to be less polar than nitromethane.

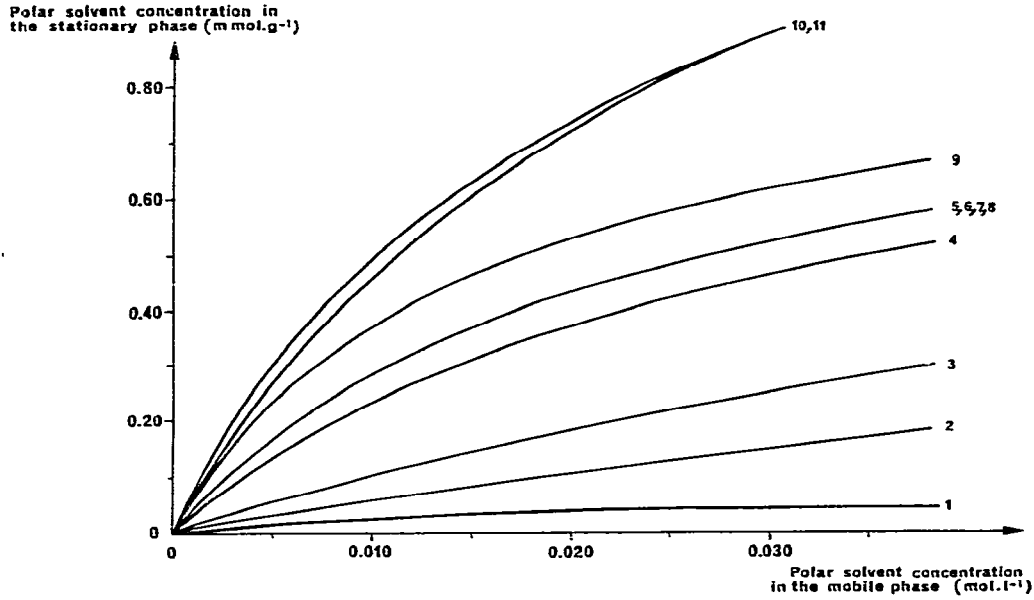

Fig. 1. Adsorption isotherms of polar solvents on LiChrosorb-NH_2 containing 0.9 mmol·g^{-1} of amino chains. Solvents: 1, chloroform; 2, nitromethane; 3, acetone; 4, N,N-dimethylformamide; 5, 1-decanol; 6, 1-octanol; 7, 1-pentanol; 8, 1-propanol; 9, ethanol; 10, methanol; 11, benzylmethanol.

However, as usual, a good correlation is observed for *n*-alkanols:methanol > ethanol > 1-propanol > 1-pentanol > 1-octanol > 1-decanol.

Description of an adsorption model for aminopropyl-bonded silica. We consider that molecules of a polar modifier (P) interact with the amino groups according to the following equilibria:

$$-\overline{NH_2} + P \rightleftharpoons -\overline{NH_2\text{–}P} \qquad K_1 = \frac{|-\overline{NH_2\text{–}P}|}{|-\overline{NH_2}|\,|P|} \tag{1}$$

$$-\overline{NH_2\text{–}P} + P \rightleftharpoons -\overline{NH_2\text{–}P_2} \qquad K_2 = \frac{|-\overline{NH_2\text{–}P_2}|}{|-\overline{NH_2\text{–}P}|\,|P|} \tag{2}$$

$$-\overline{NH_2\text{–}P_2} + P \rightleftharpoons -\overline{NH_2\text{–}P_3} \qquad K_3 = \frac{|-\overline{NH_2\text{–}P_3}|}{|-\overline{NH_2\text{–}P_2}|\,|P|} \tag{3}$$

The number of molecules of P fixed per amino group depends on the polarity of P. For instance, with a slightly polar molecule such as chloroform one obtains only adsorption of one molecule per amino group (Langmuir isotherm). For a strongly polar solvent such as ethanol one obtains adsorption of three alcohol molecules per amino group.

Table I gives equilibrium constants for various *n*-hexane–polar solvent mixtures on aminopropyl-bonded silica (LiChrosorb-NH_2 with 0.9 mmol $\cdot g^{-1}$ of bonded chain). These were determined by regression of the experimental adsorption isotherms.

TABLE I

EQUILIBRIUM CONSTANTS OBTAINED WITH *n*-HEXANE–POLAR MODIFIERS ON LICHROSORB-NH_2 CONTAINING 0.9 mmol $\cdot g^{-1}$ OF BONDED CHAINS

Polar modifier	*Equilibrium constants* ($mol^{-1} \cdot l$)		
	K_1	K_2	K_3
Benzylmethanol	87	12	4
Methanol	71	15	8
Ethanol	50	8,8	1,2
1-Propanol	45.5	–	–
1-Pentanol	45.5	–	–
1-Octanol	46.5	–	–
1-Decanol	46	–	–
N,N-Dimethylformamide	35		
Acetone	13		
Nitromethane	6.7		
Chloroform	0.8		
Dichloromethane	0.7		

We discuss two cases: adsorption of a slightly polar solvent (chloroform) and adsorption of a strongly polar solvent (ethanol).

Adsorption of a slightly polar solvent (chloroform). In this case only one molecule of chloroform is adsorbed per amino group (Fig. 2a) and an adsorption isotherm of the Langmuir type is obtained:

$$|P|_{total} = \frac{K_1 N |P|}{1 + K_1 |P|}$$

Fig. 2b represents fractions of free amino groups and solvated amino groups (with only one chloroform molecule) plotted against chloroform concentration in the mobile phase (calculated by computer from the K_1 value).

Adsorption of a strongly polar solvent (ethanol). Here, the results indicate that one amino group can adsorb up to three ethanol molecules (Fig. 2c). Fig. 2d shows variations of the number of free and solvated amino groups (with one, two or three ethanol molecules) *versus* ethanol concentration in the mobile phase. The free amino group concentration decreases considerably as the ethanol concentration increases in ethanol–*n*-hexane mixtures.

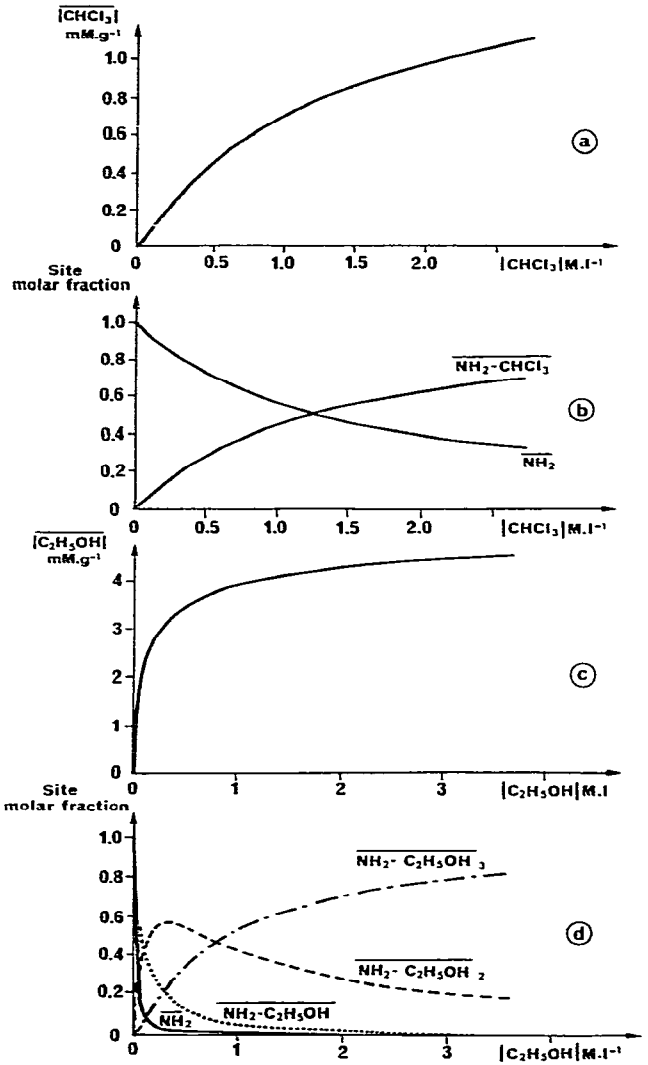

Fig. 2. Adsorption isotherms of chloroform (a) and ethanol (c) on a home-made stationary phase (1.5 mmol $\cdot g^{-1}$ of amino bonded chains), and computed molar fraction of free and "solvated" groups when varying the polar modifier concentration in the mobile phase: chloroform–*n*-hexane (b) and ethanol–*n*-hexane (d).

Retention of solutes on amino-bonded phases

Description of the retention model. Although the decrease in the capacity ratio of solutes with increasing concentration of the polar modifier in the eluent is well known in normal-phase chromatography, only a few data on these variations with amino-bonded phases are available. Linear relationships between the logarithm of the capacity ratio and the polar solvent concentration were mainly observed with various eluent mixtures[19,20].

(a) Mixture of an apolar solvent with a slightly polar solvent. The mobile phase can be considered as ideal and the activity of any species is equal to its concentration. However, the isotherm determination always shows only one molecule of the most polar solvent per amino-bonded group, even with high concentrations. If the solute S can form complexes with the polar solvent in the mobile phase according to

$$S + P \rightleftharpoons SP \qquad K = \frac{|SP|}{|S|\,|P|} \tag{4}$$

and assuming that the solute molecule mainly interacts with free amino groups in the stationary phase according to

$$\overline{-NH_2} + S \rightleftharpoons \overline{-NH_2-S} \qquad K_S = \frac{|\overline{-NH_2-S}|}{|\overline{-NH_2}|\,|S|} \tag{5}$$

the following relationship is obtained:

$$\frac{1}{k'} = \frac{V}{mr}\,\frac{1 + (K_1 + K)\,|P| + K_1K\,|P|^2}{K_S} \tag{6}$$

where m is the mass of the stationary phase, V the volume of the mobile phase in the chromatographic column and r the total concentration of free and solvated amino groups ($r = (\overline{-NH_2}) + (\overline{-NH_2-S}) + (\overline{-NH_2-P})$). Hence the inverse of the capacity ratio of a solute is related to the concentration of the most polar solvent by a parabolic equation.

(b) Mixtures of an apolar solvent with a strongly polar solvent. For ethanol–*n*-hexane mixtures, polar solvent molecules solvate each amino group when the polar modifier concentration in the mobile phase is increased. In fact, the interaction energy between amino groups and alcohols is so large that the fixation of one molecule of the polar solvent per amino group occurs only for very low concentrations, generally less than 0.2% (v/v). The activities of the various species are roughly proportional to their concentrations and eqn. 6 can be written as

$$\frac{1}{k'} = \frac{V}{mr}\cdot\frac{1 + (K_1 + K)\,\gamma(P) + K_1K\gamma^2(P)^2}{K_S} \tag{7}$$

where γ is the mean activity coefficient of the polar eluent in the mixture, roughly equal to its infinite dilution activity coefficient. For very low concentrations of the polar solvent, the term $K_1K\gamma^2(P)^2$ is negligible and eqn. 7 can be written as

$$\frac{1}{k'} = \frac{V}{mr}\cdot\frac{1 + (K_1 + K)\,\gamma(P)}{K_S} \tag{8}$$

Then, the inverse of the capacity ratio varies linearly with the concentration of the polar solvent in the eluent.

Experimental verification. Two sets of experiments were performed with amino-

bonded phases and various solvent mixtures: the first with *n*-hexane and dichloromethane mixtures for aniline or phenol derivatives and the second with *n*-hexane and *n*-alkanol mixtures for the pyridine derivative separations.

(a) Mixture of an apolar solvent with a slightly polar solvent. Fig. 3. shows the parabolic variations of the capacity ratio of some aniline derivatives eluted with cyclohexane–dichloromethane mixtures (Fig. 3a) and some phenol derivatives eluted with cyclohexane–trichloromethane mixtures (Fig. 3b). According to eqn. 6, we observe that the ratio of the second degree coefficient to the constant term is equal to the product K_1K. Similarly, the ratio of the first degree coefficient of the constant term is equal to the sum $K_1 + K$. Then the calculation of K and K_1 is easy.

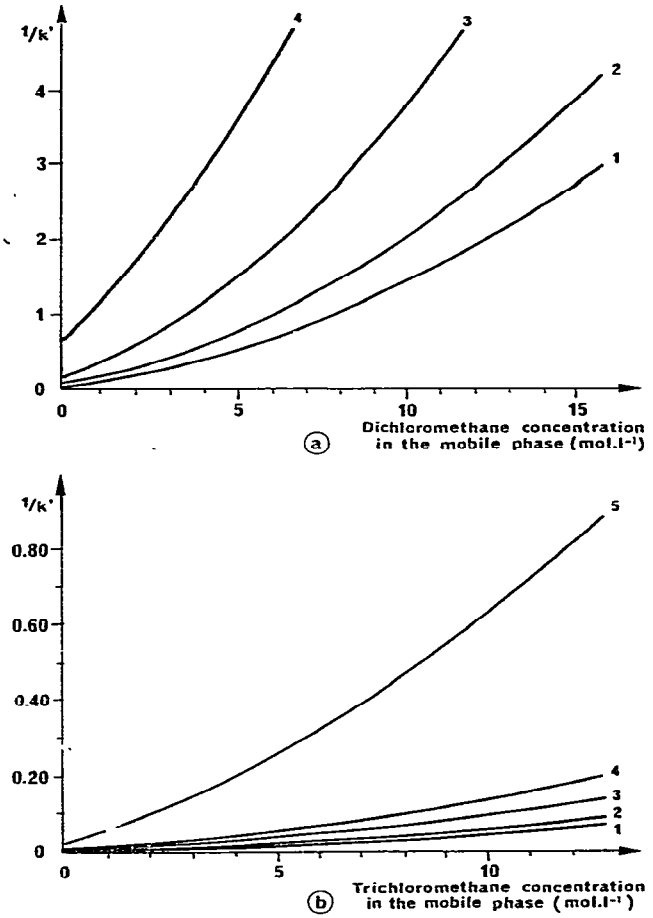

Fig. 3. (a) Variation of $1/k'$ with dichloromethane concentration in the mobile phase (dichloromethane–*n*-hexane mixtures) for aniline derivatives. Column: 15 cm × 4 mm I.D. packed with a stationary phase containing 1.5 mmol · g^{-1} of bonded aminopropyl chains. Flow-rate, 1 ml/min; UV detection at 254 nm. Solutes: 1, aniline; 2, *o*-toluidine; 3, N-methylaniline; 4, N,N-dimethylaniline. (b) Variation of $1/k'$ with trichloromethane concentration in the mobile phase for phenol derivatives. Solutes: 1, *m*-cresol; 2, 3,4-dimethylphenol; 3, *o*-cresol; 4, 2,5-dimethylphenol; 5, 2,6-dimethylphenol.

Table II gives the coefficients of the parabolic curves for the aniline derivatives, as determined by regression analysis, and the two equilibrium constants K and K_1. Table II shows that the equilibrium constant K_1 between the polar solvent and the amino-bonded chains does not depend on the nature of the solute and remains roughly equal to 0.6 $mol^{-1} \cdot l$, which is very close to the value calculated from isotherm determination, *viz.*, 0.7 $mol^{-1} \cdot l$.

TABLE II

COEFFICIENT OF THE FITTED PARABOLIC CURVE FOR VARIATIONS OF $1/k'$ *VERSUS* THE POLAR MODIFIER CONCENTRATION IN THE MOBILE PHASE FOR ANILINE DERIVATIVES

Conditions as in Fig. 3a.

Solute	$\frac{V}{mr} \cdot \frac{1}{K_S}$	$\frac{V}{mr} \cdot \frac{K_1 + K}{K_S}$	$\frac{V}{mr} \cdot \frac{K_1 K}{K_S}$	K_1	K
		$(mol^{-1} \cdot l)$	$(mol^{-2} \cdot l^2)$	$(mol^{-1} \cdot l)$	$(mol^{-1} \cdot l)$
N,N-Dimethylaniline	0.688	0.439	0.025	0.57	0.06
N-methylaniline	0.202	0.148	0.002	0.56	0.18
o-Toluidine	0.087	0.071	0.0116	0.58	0.23
Aniline	0.058	0.051	0.008	0.66	0.22

In contrast, the association constant K between the solute molecule and dichloromethane increases from N,N-dimethylaniline to aniline, that is, with increasing accessibility of the nitrogen atom of the solute molecule. As the constant increases simultaneously with the order of elution of the aniline derivatives, the distribution constant K_S of the solute between the two phases also increases from N,N-dimethylaniline to aniline.

(b) Mixture of an apolar and a strongly polar solvent. As predicted by eqn. 8, the inverse of the capacity ratio of various alkylpyridines eluted with *n*-hexane–methanol mixtures is linearly related to the concentration of the polar solvent in the mobile phase (Fig. 4a). The slopes of the straight lines obtained depend on the structure of the solute and, for instance, the influence of the alcohol concentration is less with 3,4-dimethylpyridine than with 2,6-dimethylpyridine or pyridine.

Fig. 4b shows the influence of the hydrocarbonaceous chain of the alcohol on the elution of 2,6-dimethylpyridine. For each alcohol a linear relationship was observed between the inverse of the capacity ratio of the solute and the mobile phase composition. Further, for a constant composition of the mobile phase (expressed as the volume fraction of the alcohol in the eluent) the solute retention is lower when the number of carbon atoms in the alcohol molecule decreases.

REVERSED-PHASE CHROMATOGRAPHY ON *n*-ALKYL BONDED PHASES

Role of the stationary phase

The influence of the surface concentration and of the length of bonded *n*-alkyl chains was studied previously[5]. C_{18} bonded phases with various surface coverages were synthesized and it was shown that the solute capacity factors and relative reten-

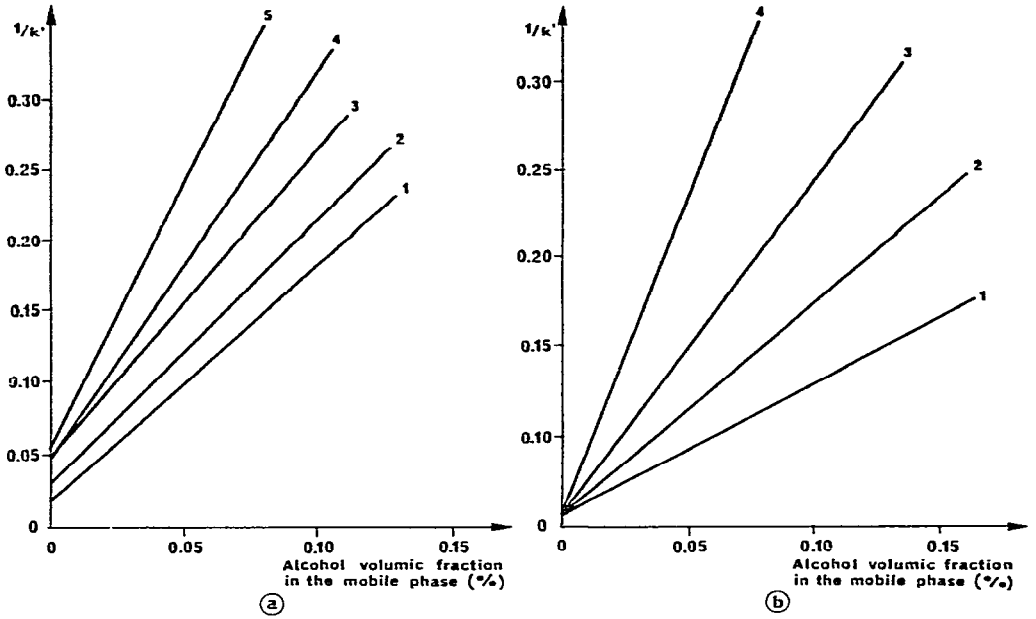

Fig. 4. (a) Variation of $1/k'$ with volume fraction of methanol in methanol–*n*-hexane mixtures for pyridine derivatives. Flow-rate, 1 ml/min; UV detection at 254 nm. Solutes: 1, 3,4-dimethylpyridine; 2, 4-dimethylpyridine; 3, 3-methylpyridine; 4, 2-methylpyridine; 5, 2,6-dimethylpyridine. (b) Variation of $1/k'$ with polar modifier concentration in the mobile phase for 2,6-dimethylpyridine. Solutes: 1, 1-octanol; 2, 1-pentanol; 3, 1-propanol; 4, methanol.

tions increase with increasing surface concentration. For a constant surface concentration of bonded chains, the capacity factor and relative retention increase with increasing chain length. Roumeliotis and Unger[21] correlated k'/S_{BET} (where S_{BET} is the specific surface area of silica) with the hydrocarbonaceous surface area of the bonded chain.

In this study, several stationary phases with various surface concentrations and various lengths of bonded chains (C_4–C_{22}) were synthesized from the same batch of silica and using the same bonding method (from *n*-alkyltrichlorosilane). The hydrocarbonaceous surface area (S) of a chain was calculated from Bondi's relationship[22] and the number of bonded chains per square nanometre of silica (N) from carbon microanalysis[23]. The capacity factors of various polar and non-polar solutes were measured with the above stationary phases, the eluting mixture being the same. The results are shown in Fig. 5. A linear relationship is observed between the capacity factor of pyrene (Fig. 5a) and the bonded hydrocarbonaceous surface per square nanometre of silica. A similar relationship is obtained for the relative retentions of pyrene and phenanthrene (Fig. 5b). So, using a C_{12}, C_{16}, C_{18} or C_{22} bonded phase having about the same hydrocarbonaceous surface area NS and the same eluent, the capacity factors and relative retentions obtained are very similar.

The same results were observed for a solute with a long alkyl chain such as 1-tetradecanol (Fig. 5c). Thus, the chain length has no specific role in the retention mechanism. Berendsen and De Galan[24] have shown from a geometrical model that

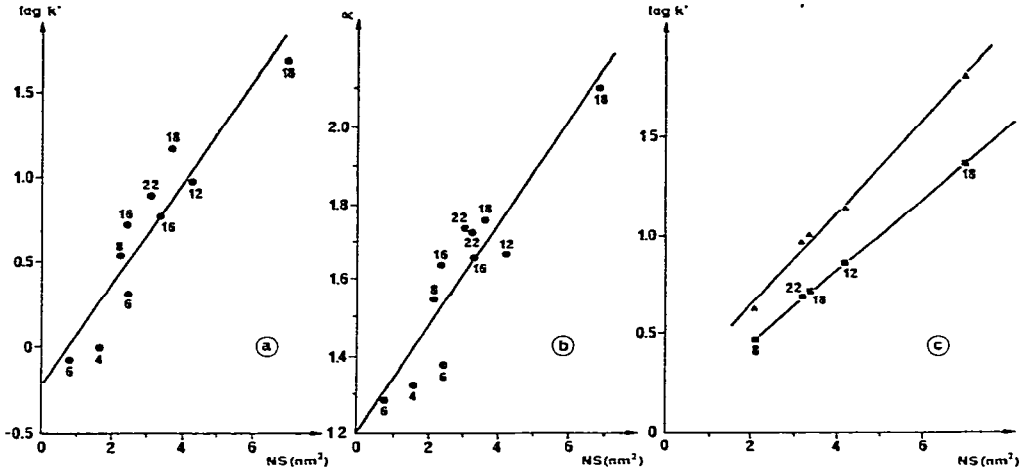

Fig. 5. Variation of logarithm of capacity factor and relative retention with hydrocarbonaceous surface area of the bonded phase (*NS*) per nm^2 of silica. Stationary phase: *n*-alkyl chains bonded on experimental Spherosil (specific surface area, 520 $m^2\ g^{-1}$; average particle diameter, 5 μm). Column: 15 cm × 4 mm I.D. (a) Capacity factor of pyrene eluted with methanol–water (70:30); (b) relative retention of pyrene and anthracene with the same eluent; (c) capacity factor of 1-tetradecanol (▲) and 1-decanol (■) eluted with acetonitrile–water (80:20). The numbers above the experimental points correspond to the bonded chain lengths.

the surface concentration is limited to 3.8 μmole $\cdot\ m^{-2}$ for a trimethyl chain and a lower value for a longer chain. The advantages of bonding a longer chain are to increase the hydrocarbonaceous surface area and to obtain a more selective bonded phase. For instance, Takayama *et al.*[25] synthesized a C_{30} bonded phase and so achieved a very difficult separation of fatty acids.

Role of the mobile phase

Relationship between retention and mobile phase composition. Retentions of non-polar solutes (various polycyclic aromatic compounds) and polar solutes (*n*-alkanols) were measured with various mixtures of water and a water-miscible organic solvent. Five solvents were studied: methanol, ethanol, 1-propanol, acetonitrile and tetrahydrofuran. The relationship between ln k' for the solutes and the volume fraction of water (x) in the eluting mixture is shown in Fig. 6. A linear relationship exists only with methanol as the organic modifier (Fig. 6a). For non-polar solutes the rate of change of ln k' with x increases from acetonitrile to ethanol, to tetrahydrofuran and then to 1-propanol (Fig. 6b–e). Polar solutes eluted with acetonitrile–water mixtures show the same behaviour as non-polar solutes (Fig. 6f). The order of elution of non-polar solutes is the same for each modifier and is related to the solute size; therefore, the heaviest compounds are retained most in the column. The curves obtained with tetrahydrofuran–water mixtures are not well separated; a consequence is that the selectivity between these solutes is not as high as with the other modifier–water mixtures.

Another observation is that for each organic modifier the recorded capacity factor is smaller than that expected from the beginning of the curve when the volume

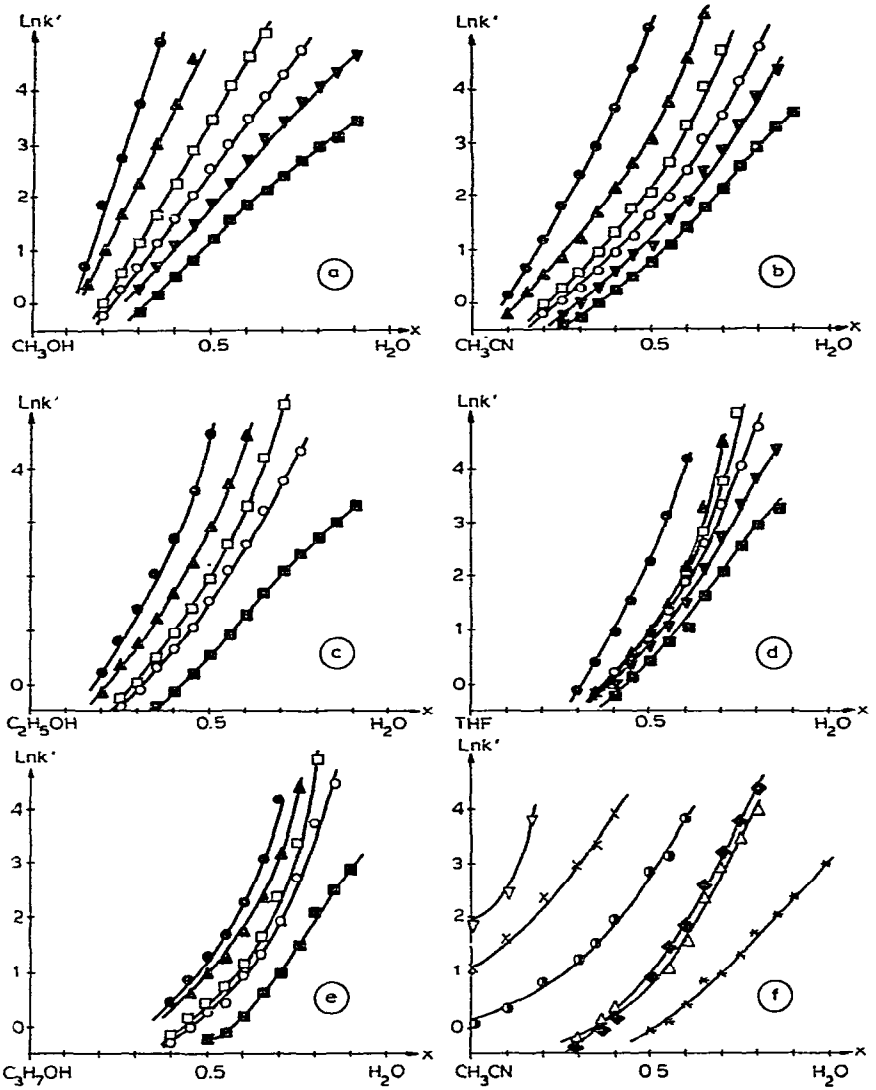

Fig. 6. Variation of logarithm of capacity factor with volume fraction of water in the eluent for various solutes. Column: 5 cm (a–e) and 15 cm for (f); I.D. 4 mm. Packing: *n*-octylsilica (RP-8). Eluent: mixtures of water and (a) methanol, (b) acetonitrile, (c) ethanol, (d) tetrahydrofuran, (e) 1-propanol and (f) acetonitrile. Solute: ■, benzene; ▼, toluene; ○, *p*-xylene; □, phenanthrene; ▲, perylene; ●, tetraphenylnaphthalene; ⋆, 1-pentanol; ◑, 1-decanol; ×, 1-tetradecanol; ▽, 1-hexadecanol; ◆, 1-naphthol; △, 2-naphthylamine.

fraction of water is higher than 0.80 or 0.85. This effect is particularly noticeable with the benzene and toluene curves.

In reversed-phase studies, a linear relationship between ln k' and x was frequently observed with methanol–water mixtures and with other modifiers at low water contents. Some workers[2,3,26,27] also noticed that the linear relationship was not always checked, especially when the water content was high. Similar observations

have been reported in thin-layer chromatography on silanized silica gel or on silica gel coated with paraffin oil[29–31]. Recently, Schoenmakers and co-workers[26–28] proposed the parabolic dependence $\ln k' = ax^2 + bx + c$ to represent the experimental curves mathematically.

As we observed that the rate of change in $\ln k'$ with x was dependent on the nature of the modifier, the mathematical model chosen for the study of the experimental curves was $\ln k' = ax^n + b$. For each organic modifier and up to $x = 0.8$, a and n were calculated for each solute (Table III).

(a) Examination of n. Values of n vary between 1.9 and 2.5 for aromatic hydrocarbons with acetonitrile as modifier; with ethanol n varies between 2.5 and 3, with tetrahydrofuran between 3.5 and 4.8 and with 1-propanol between 3.5 and 5. Hence, for this type of solute, n depends mainly on the organic modifier. However, if we look at each column in Table III, we can see that n also depends on the solutes and, except for tetrahydrofuran, for any modifier n decreases as the solute size increases.

If n is about 2 for aromatic hydrocarbons eluted with acetonitrile–water mixtures, n does not have the same value for n-alkanol solutes eluted with the same

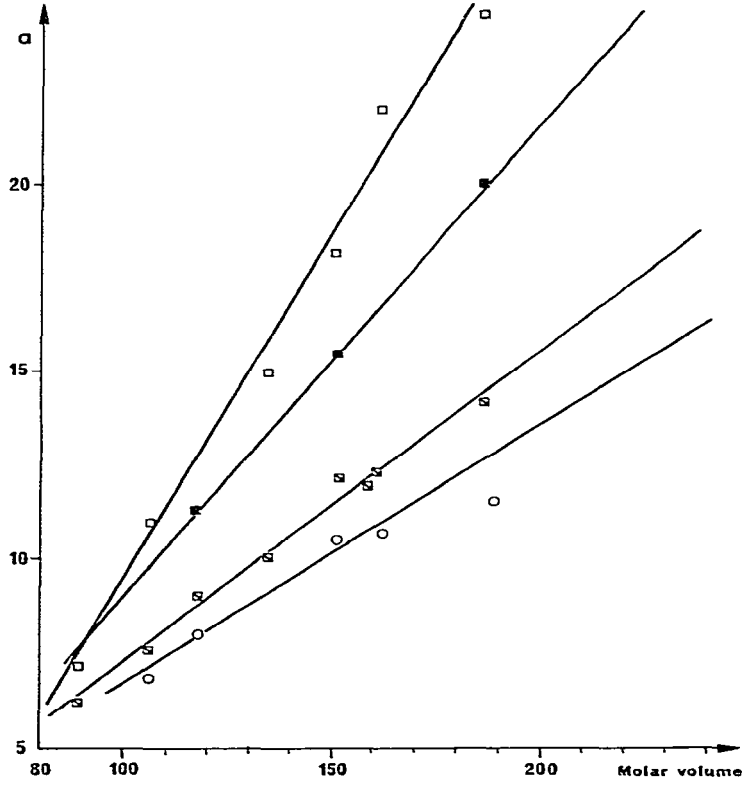

Fig. 7. Variation of the coefficient a obtained from $\ln k' = ax^n + b$ with the molar volume of aromatic compounds for different organic modifiers. Experimental conditions as in Fig. 6. ⊠, Methanol; ○, acetonitrile; ■, ethanol; ⊔, tetrahydrofuran.

TABLE III

a AND n VALUES OBTAINED BY THE RELATIONSHIP $\ln k' = a\lambda^n + b$ FOR AROMATIC HYDROCARBONS ELUTED WITH WATER–METHANOL (OR ACETONITRILE, ETHANOL, TETRAHYDROFURAN OR 1-PROPANOL) MIXTURES AND FOR POLAR SOLUTES ELUTED WITH WATER–ACETONITRILE MIXTURES

For experimental conditions, see Fig. 6.

Solute	CH_3OH		CH_3CN		C_2H_5OH		THF		C_3H_7OH		*Solute*	CH_3CN	
	a	*n*	*a*	*n*	*a*	*n*	*a*	*n*	*a*	*n*		*a*	*n*
Benzene	6.2	1		2.6							1-Pentanol	3.2	2.7
Toluene	7.5	1	6.8	2.5			7.2	3.0			1-Decanol	8.7	1.8
p-Xylene	9	1	8.0	2.3	11.2	3.0	11.0	3.5	9.8	4.5	1-Tetradecanol	8.2	1
2-Ethyltoluene	10	1	8.9	2.2			15.1	3.8			1-Naphthol	9.8	3.2
Phenanthrene	12.2	1	10.2	2.1	15.7	3.0	18.1	4.2	15.0	5.0	2-Naphthylamine	9	3.4
Fluoranthene	12.3	1	10.7	2.1			22	4.8					
Perylene	14.2	1	11.5	1.9	20	2.6	24.5	4.7	12.2	3.7			
Tetraphenylnaphthalene	19	1	15.3	1.7	27.5	2.5	25	3.5	14.4	3.5			

mixtures and it varies from 2.5 for 1-pentanol to 1 for 1-tetradecanol. 1-Naphthol and 2-naphthylamine give the same n value.

(b) Examination of a. In Table III for aromatic hydrocarbons, a increases with increasing solute size; Fig. 7 shows a linear relationship between a and the molar volume of solutes. It has been shown[32] that for homologous compounds the logarithm of the water solubility is linearly related to the molar volume.

Thus, for non-polar solutes, as n depends mainly on the organic modifier, it is possible to predict retention using the molar volume of the solute.

Relationship between solubility of the solute and mobile phase composition. The solubility (s) of phenanthrene was measured in the same mixtures as the eluting mixtures previously studied. Fig. 8 shows the logarithms of phenanthrene solubility *versus* the volume fraction of water (x). There is a linear relationship between the solubility and x for methanol–water mixtures, but not for the other organic modifiers

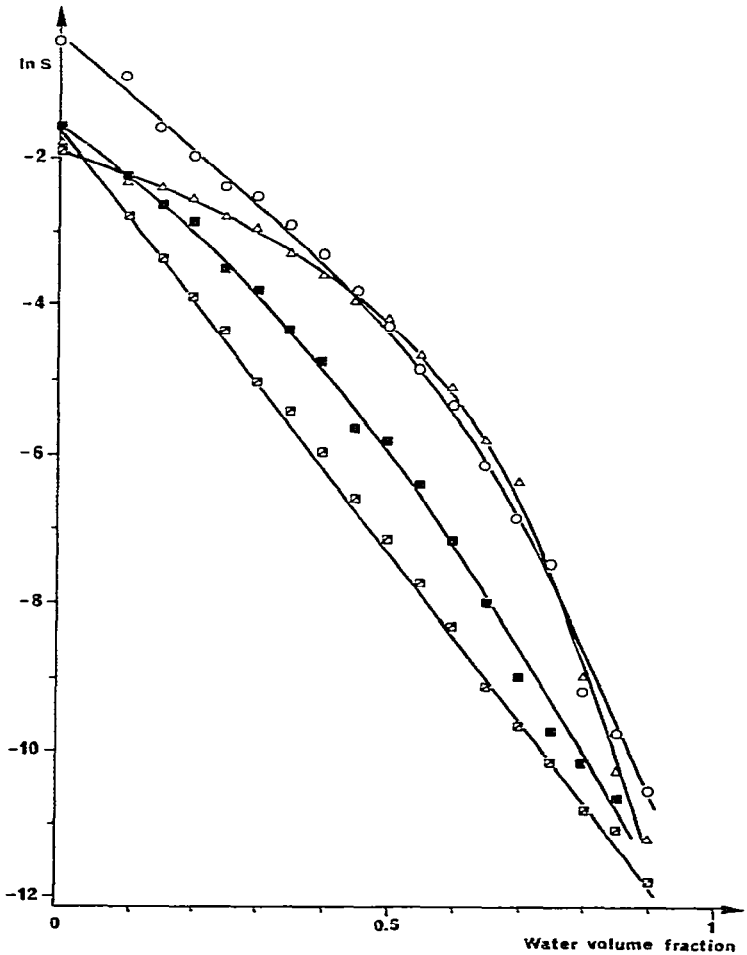

Fig. 8. Variation of logarithm of solubility (mole·l^{-1}) of phenanthrene in water–organic solvent mixtures with the volume fraction of water. ⊠, Methanol; ○, acetonitrile; ■, ethanol; △, 1-propanol.

(the solubility in water–tetrahydrofuran mixtures could not be measured because separation occurred).

Because of this linear relationship with methanol–water mixtures, the same mathematical analysis of experimental curves was carried out, using the equation $\ln s = a'x^{n'} + b'$. Experimental values of a' and n' are given in Table IV.

TABLE IV

a' AND n' VALUES OBTAINED ON REPRESENTING EXPERIMENTAL CURVES OF THE SOLUBILITY OF PHENANTHRENE IN WATER–METHANOL (OR ETHANOL, ACETONITRILE OR 1-PROPANOL) MIXTURES BY THE RELATIONSHIP $\ln s = a'x^{n'} + b'$

This relationship is checked in the x range for k' between 1 and 200.

Parameter	*Organic modifier*			
	CH_3OH	CH_3CN	C_2H_5OH	C_3H_7OH
a'	−11.4	−10.5	−15.5	−15.1
n'	1	2.1	3.0	5.0
x range for $1 < k' < 200$	$0.15 < x < 0.65$	$0.20 < x < 0.75$	$0.25 < x < 0.75$	$0.35 < x < 0.85$

Comparison of these values with those obtained by $\ln k' = ax^n + b$ for phenanthrene in Table III shows that $a' \approx -a$ and $n' = n$. Hence for phenanthrene and one given modifier the capacity factor is related to x by $\ln k' = ax^n + b$ and solubility is related to x by $\ln s = -ax^n + b'$. Note that the relationship $\ln s = -ax^n + b$ is checked for x values corresponding to the range of k' between 1 and 200.

It appears that the retention curves look like solubility curves. This analogy has been mentioned in reversed-phase thin-layer chromatography by Markowski *et al.*[33], and indicates the predominating role of the mobile phase.

Relationship between retention and solubility. If we compare the two experimental relationships between s and x and between k' and n, the capacity factor and the solubility are directly related by $k's = e^{b+b'} = C$, where C is a constant. For a given organic modifier this constant is independant of x. This means that when the only variable parameter is the water content in the eluting mixture, the change in retention is governed only by the change in solubility in the mobile phase.

The retention can be defined by[17]

$$k' = \frac{\Sigma \text{ (solute–stationary phase interactions)}}{\Sigma \text{ (solute–mobile phase interactions)}}$$

The solubility can be considered as a measure of the solute–mobile phase interactions. Hence the $k's$ reflects the solute–stationary phase interactions. As this product is independent of the water content of the mobile phase, x, the solute–stationary phase interactions are independent of the water content. Hence the stationary phase has the same composition (in the x range checked for the above relationship, *i.e.*, $x < 0.8$).

Considering the constant $k's$ value of phenanthrene obtained with four organic

modifiers in Table V, this constant is nearly the same for the three alcohols (about 0.02) and is much higher for acetonitrile (0.13). The solubility of phenanthrene in the three pure alcohols (s_0) is also the same and is much higher in pure acetonitrile. If, for each modifier, the water content of the mobile phase is adjusted so as to provide the same solubility (s) of phenanthrene, the phenanthrene capacity factor will be the same for the three alcohols (as $k' = C/s$). It will be about six times higher for acetonitrile as the modifier.

TABLE V

$k's$ VALUES OBTAINED FOR PHENANTHRENE ELUTED WITH WATER–ORGANIC MODIFIER MIXTURES AND PHENANTHRENE SOLUBILITY (s_0) IN THE PURE ORGANIC MODIFIERS (FOR $x = 0$)

Parameter	*Organic modifier*			
	CH_3OH	C_2H_5OH	C_3H_7OH	CH_3CN
$k's$ (mole·l^{-1})	0.019 ± 0.001	0.021 ± 0.001	0.020 ± 0.001	0.13 ± 0.01
s_0 (mole·l^{-1} at 25°C)	0.15	0.19	0.17	0.65

This difference in retention can be easily explained if we consider that the non-polar alkyl-bonded chains are covered with one or several layers of pure organic modifier (or of a mixture very enriched with pure modifier). Table VI gives the $k's$ values of some solutes eluted with ethanol–water mixtures and solute solubilities (s_0) in ethanol.

TABLE VI

VALUES OF $k's$ OBTAINED WITH SOME SOLUTES ELUTED WITH WATER–ETHANOL MIXTURES (15 cm COLUMN PACKED WITH RP-18) AND SOLUBILITIES OF THESE SOLUTES IN PURE ETHANOL AT 25°C

Parameter	*Solute*				
	Acetanilide	*p-Ethoxy-acetanilide*	*p-Methyl-acetanilide*	*Benzamide*	*o-Nitrophenol*
$k's$ (mole·l^{-1})	0.30 ± 0.04	0.08 ± 0.01	0.11 ± 0.01	0.24 ± 0.03	0.36 ± 0.04
s_0 (mole·l^{-1})	1.83	0.37	0.62	1.20	2.25
$k's/s_0$	0.16	0.22	0.18	0.20	0.16

It can be seen that the constant $k's$ is related to s_0, as the ratio $k's/s_0$ is constant. Therefore, $k' = C's_0/s$ and the solute–stationary phase interactions are in fact the interactions between the solute and the organic solvent coated on the stationary phase; the more soluble the solute is in the organic solvent, the stronger these interactions are.

Scott and Kucera[34] showed that for a low water content the bonded chains were wetted by the organic solvent; our results are in complete agreement.

We have seen above that the bonded chains play no specific role in the retention mechanism and that the only important parameter for solute capacity factors and relative retentions is the total hydrocarbonaceous surface area *NS*. In fact, the hydrophobic hydrocarbonaceous chains are only a support for a layer of organic solvent and, as the solute does not interact directly with the bonded chains, there is no specific interaction between the bonded bristle and the solute.

CONCLUSION

In this study, some simple relationships concerning solute retention have been given. The optimization of chromatographic separations can easily be attained with these relationships, particularly in reversed-phase chromatography. The very simple relationship between the k' and s for a solute in the mobile phase provides a useful means for the rapid choice of operating conditions.

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