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Molecular interactions in liquid chromatography[a]

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ABSTRACT

An approach to the description of molecular interactions in normal- and reversed-phase high-performance liquid chromatography as quasi-chemical equilibria on the sorbent and in solution (of solute–sorbent, modifier–sorbent, solute–modifier and modifier–modifier types) is presented. Such an approach is of help in understanding better the role of various parameters of chromatographic systems that determine the retention of substances in HPLC. The mathematical mechanism used is the same to describe the equilibrium distribution of the sorbate between the liquid and solid phases (adsorption mechanism) or between the liquid and bonded phases (absorption mechanism) and the final equations do not take into account the type of mechanism. A general expression correlating the retention of solute molecules with the concentration of the modifying additive of the mobile phase (over a wide range of concentrations) was obtained. Numerous examples have shown that, depending on the prevalence of certain types of interactions, this relationship makes it possible to describe quantitatively and to explain various deviations of the retention dependence from linearity.

The study of molecular interactions in gas and liquid chromatography is of great importance for understanding the mechanisms of chromatographic separations of substances and for developing selectivity theory in chromatography. Chromatographic parameters of retention in gas adsorption chromatography at small (zero) surface coverage are determined by adsorbate–adsorbent interactions which depend on the nature of the adsorbent and the adsorbed molecules. Pioneering studies by Kiselev and co-workers [1–3] resulted in the development of the fundamentals of the molecular statistical theory of adsorption on homogeneous surfaces. This theory enables one to calculate the thermodynamic characteristics of adsorption of various organic compounds for homogeneous adsorbents [graphitized thermal carbon black (GTCB), zeolites] (the direct approach), while the chromatographic data on the adsorption on GTCB made it possible to identify, in a number of cases, the structure of the adsorbed molecules (the reverse approach, *i.e.*, chromatographic structure analysis or "chromatoscopy" [4]).

The retention mechanisms in liquid–solid chromatography (LSC) are much more complicated. In addition to sorbate–sorbent molecular interactions, also of great

[a] Dedicated to Professor J. C. Giddings on the occasion of his 60th birthday.

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importance are the sorbate–mobile phase and sorbent–mobile phase interactions, in particular the interactions between the components of binary, ternary, etc., mobile phases. At present, it is impossible to calculate quantitatively the thermodynamic characteristics of retention in such a complicated system. Further development of the theory of liquid chromatography is largely due to the determination of correlation dependences between the retention parameters and various properties of sorbates [5–12], mobile phases (MP) [10–16] and stationary phases (SP) [17–21].

Kiselev and co-workers suggested that the theory of chromatographic retention and separation in liquid–solid chromatography should be based on the theory of sorption from multi-component solutions. Such an approach provides a common basis for studying the normal-phase (NP) and reversed-phase (RP) variants of high-performance liquid chromatography (HPLC). The main difference between NP- and RP-HPLC consists in the different characters of molecular interactions between the solute molecules on the one hand, and the mobile and stationary phases on the other. In NP-HPLC, the main contribution to retention is made by specific interactions of the solute molecules with the SP surface. In RP-HPLC the non-specific interactions of the solute molecules with the hydrophobic sorbent predominate over specific and non-specific interactions of the solute with components of the mobile liquid phase.

Two problems are usually considered in the theory of HPLC: (1) the effect of the mobile phase and its composition on the retention and (2) the dependence of the retention parameters on the nature and structure of the molecules of adsorbed substances. Much less study has been devoted to the effect of the adsorbent surface chemistry on retention in liquid–solid chromatography than in gas–solid chromatography, since in HPLC the regulation of retention and separation of analyte substances are often performed by changing the type and composition of the mobile phase rather than those of the adsorbent in the column.

Now let us consider the most frequently applied system in HPLC: an adsorbent (A), a substance being adsorbed, *i.e.*, the solute (S), and a two-component mobile phase consisting of the basic component, *i.e.*, the solvent (L), and a modifying additive (M). As a rule, RP-HPLC involves L = water and M = organic solvent (*e.g.*, methanol, acetonitrile, tetrahydrofuran) and NP-HPLC involves L = alkane and M = weakly polar or polar organic solvent (chloroform, alcohol). In this instance, we are dealing with sorption from a three-component solution (S, M, L), the concentration of the solute S being 3–4 orders of magnitude smaller than that of the modifier M and solvent L.

We take into account the following assumptions: the sorbent surface is chemically and geometrically homogeneous and therefore the sorption energy of the components in the mobile phase is constant on any part of the surface; sorption is of an exchange (competitive) character; the absence of molecular associations in the adsorption layer; and the surface solution and bulk solution are ideal. Considering the sorption as a quasi-chemical reversible reaction of exchange, in the simplest case, where the areas occupied by molecules S, M and L in the surface layer are equal, we have equilibria for the adsorbate:

$$S_m + L_s \overset{K_S}{\rightleftharpoons} S_s + L_m \tag{1}$$

$$K_S = S_s L_m / S_m L_s \tag{2}$$

and for the modifier:

$$M_m + L_s \overset{K_M}{\rightleftharpoons} M_s + L_m \tag{3}$$

$$K_M = M_s L_m / M_m L_s \tag{4}$$

where K_S and K_M are equilibrium constants and S_s and S_m, M_s and M_m and L_s and L_m are mole fractions of the solute, modifier and solvent in the stationary phase (s) and mobile phase (m). It should be noted that eqns. 1–4, which are of general character, can describe the equilibria distribution of the adsorbate or modifier between the mobile and stationary phases for both the adsorption and partition mechanisms of retention. For the partition mechanism the assumption is made of a constant binding capacity of the sorption layer; the composition of the surface solution and that of the bulk solution are, of course, different. A similar approach was used also by Murakami [16] to describe the retention of aromatic compounds by reversed phases and by Arvidsson *et al.* [22] to describe the ion-pair chromatography.

Assuming that the bulk and surface solutions are ideal and the processes of molecular association are absent, we can easily obtain the sorption equations for the solute and modifier.

From eqns. 2 and 4, we obtain

$$M_s/S_s = K_M M_m / K_S S_m \tag{5}$$

Also,

$$L_s + M_s + S_s = 1 \tag{6}$$

where L_s, M_s, S_s are mole fractions of non-associated molecules of the solvent, modifier and sorbate, respectively, in the stationary phase.

$$M_s + M_s K_S S_m / K_M M_m + M_s L_m / K_M M_m = 1 \tag{7}$$

As $S_m \ll M_m$ and $S_m \ll L_m$, then

$$L_m \leqslant 1 - M_m \tag{8}$$

and

$$M_s[1 + K_S S_m / K_M M_m + (1 - M_m)/K_M M_m] = 1 \tag{9}$$

$$M_s = K_M M_m / [1 + (K_M - 1)M_m + K_S S_m] \tag{10}$$

Dividing eqn. 2 by eqn. 4, we obtain

$$K_S/K_M = S_s M_m / S_m M_s \tag{11}$$

It is known [23] that

$$k' = \Phi K = \Phi S_s/S_m \tag{12}$$

where k' is the capacity factor, Φ is the phase ratio (the ratio of stationary to mobile phase volumes) and K is the constant of the adsorption equilibrium of the solute (the ratio of the total mole fraction of the solute in the stationary and mobile phases).

Then, from eqns. 11 and 12, we obtain

$$K_S/K_M = k'M_m/(\Phi M_s) \tag{13}$$

and

$$1/k' = K_M M_m/(\Phi K_S M_s) \tag{14}$$

Substituting eqn. 10 in eqn. 14, we obtain

$$1/k' = [1 + (K_M - 1)M_m + K_S S_m]/(\Phi K_S) \tag{15}$$

Usually K_S is not drastically different from K_M and $S_m \ll M_m$. Consequently, the term $K_S S_m$ in eqn. 15 may be neglected. Then we have

$$1/k' = [1 + (K_M - 1)M_m]/(\Phi K_S) \tag{16}$$

It follows from eqns. 12 and 15 that the equations of adsorption for the solute, modifier and solvent acquire the forms

$$S_s = K_S S_m/[1 + K_S S_m + (K_M - 1)M_m] \tag{17}$$

$$M_s = K_M M_m/[1 + K_S S_m + (K_M - 1)M_m] \tag{10}$$

$$L_s = (1 - M_m)/[1 + K_S S_m + (K_M - 1)M_m] \tag{18}$$

A rectilinear dependence of the reciprocal of the capacity factor on the content of the modifier in the eluent was initially applied by Scott [24]. In many instances this dependence can be successfully used to describe the decreasing retention of the solute with increasing content of the modifier in the solution and also reflects the gradual blocking of the active sites on the homogeneous surface of the sorbent by the modifier molecules (Figs. 1 and 2). It is applied more often and much better in NP-HPLC than in RP-HPLC [25].

Under favourable conditions (homogeneous surface, absence of adsorption on the surface covered with the modifier molecules, absence of associate formation in the solution and on the surface), the dependence in eqn. 16 should be observed until the surface is completely covered with a monolayer of the modifier and hence full deactivation is attained. However, in real circumstances the surface covered with the modifier retains its ability to have molecular interactions with the mobile phase components. Moreover, in this instance not only the interaction force may change (a

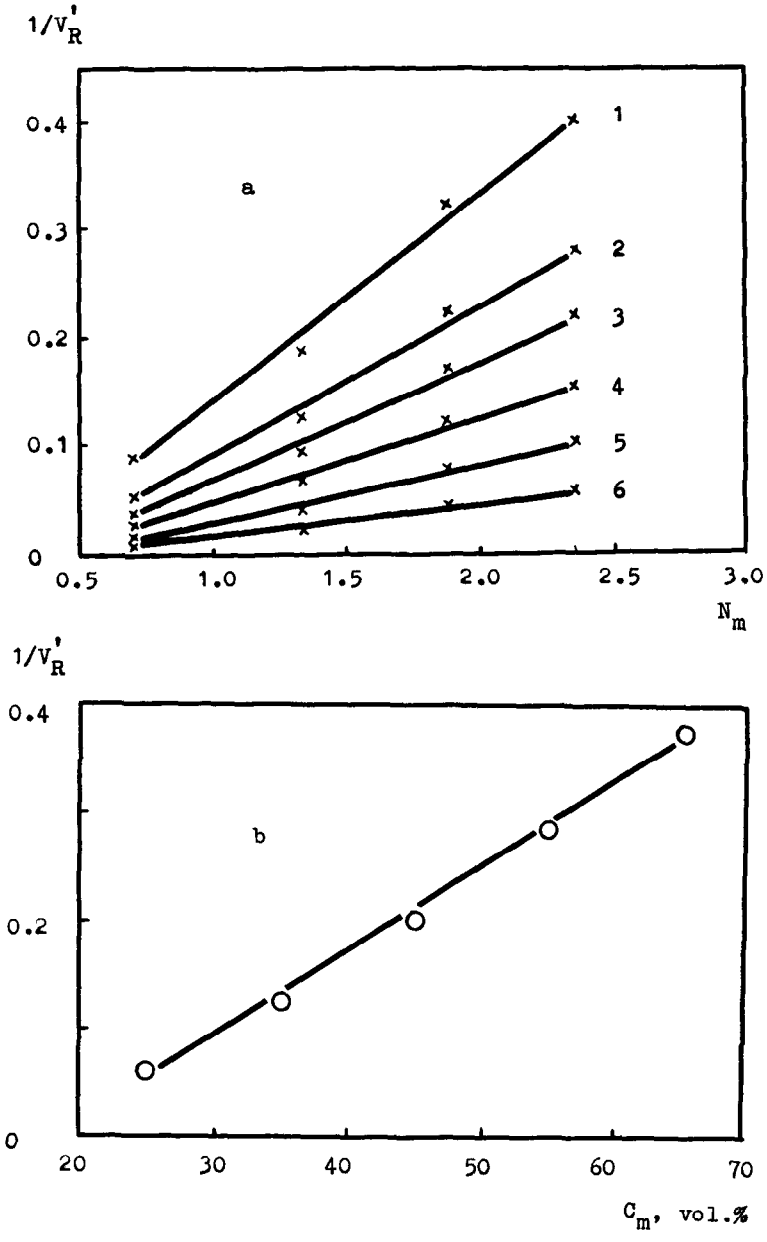

Fig. 1. Dependence of the reciprocal of the corrected retention volume ($1/V'_R$) on the mobile phase composition: the mole fraction ($N_m \times 10$) or volume percent (C_m, %, v/v) of the modifier. (a) Adsorbent, silica gel L; mobile phase, *n*-hexane–dioxane. 1 = *o*-Cresol; 2 = phenol; 3 = *p*-chlorophenol; 4 = *p*-methoxyphenol; 5 = *m*-nitrophenol; 6 = *p*-nitrophenol. (b) Adsorbent, Suplex pKb-100; mobile phase, water–acetonitrile; solute, *p*-toluic acid [25].

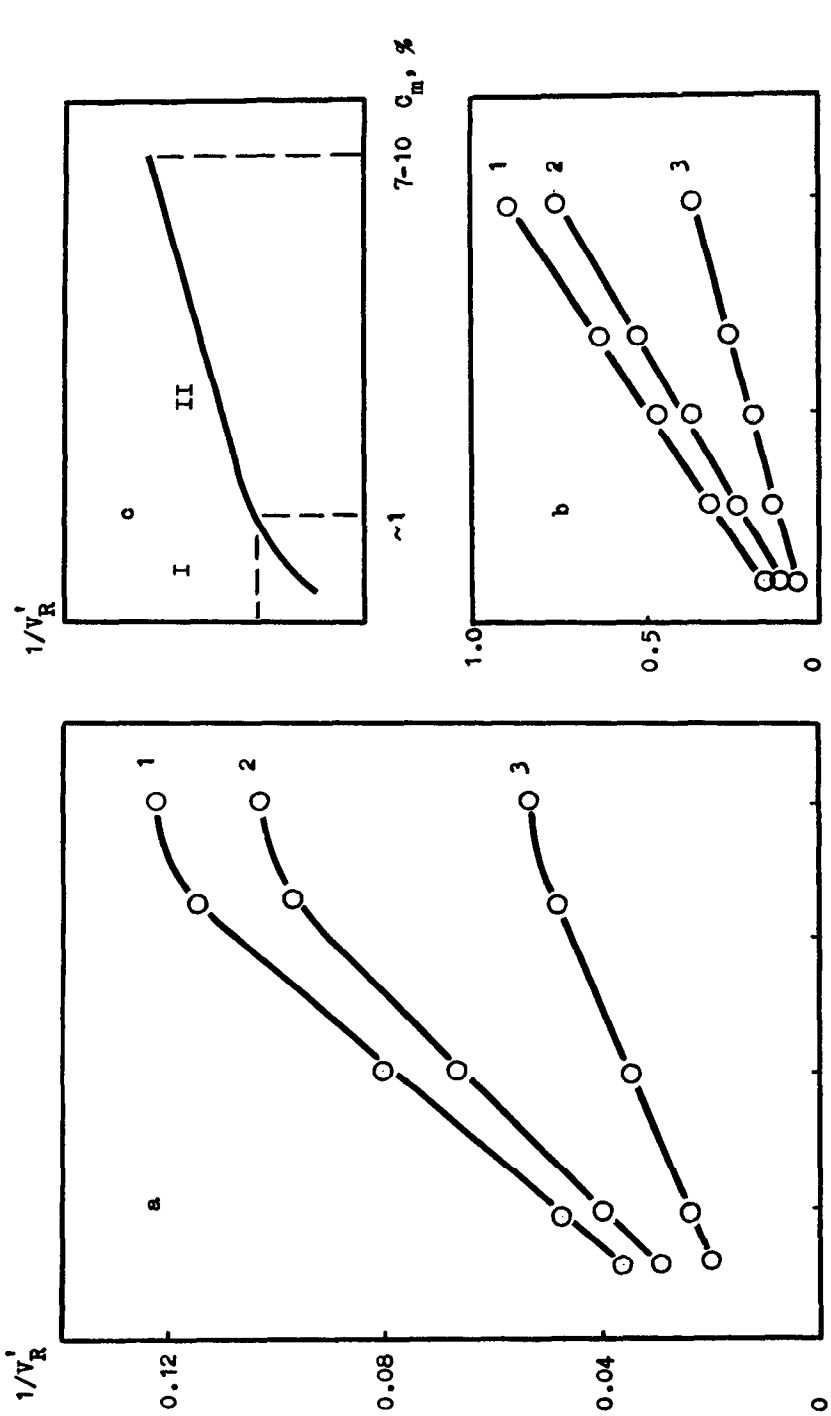

Fig. 2. Dependence of the reciprocal of the corrected retention volumes ($1/V'_R$) of some substituted benzoic acids on the content of polar modifier (C, %, v/v, of propionic acid) in the mobile phase. Adsorbent, silica gel L, 10 μm; mobile phase, *n*-hexane–propionic acid. C: (a) 0–1; (b) 1–10; (c) 0–10% (v/v). 1 = *p*-Toluic acid; 2 = *p*-brombenzoic acid; 3 = *p*-methoxybenzoic acid.

change in K_S and K_M on transition from the initial to the modified surface of the adsorbent) but also the very character of interactions may undergo certain changes. Hence, in studying the retention of substituted benzoic acids on silica gel with the use of an *n*-alkane and a polar additive (acetic or propionic acid) mixture as the mobile phase, it was found that the retention mechanism of benzoic acids on transition from low to higher concentrations of the polar additive in the mobile phase undergoes certain changes [26]. When the concentration of the polar additive, an aliphatic acid, is less than 1 vol.-%, the solute molecules can directly interact with silanol groups of the silica gel surface, that is, retention is mainly determined by interactions of the hydrogen bond type. In Fig. 2c this mechanism is represented by the initial, steep part (I) of the dependence of $1/V_R'$ on the concentration C of the polar additive in the mobile phase (V_R' is the corrected retention volume of the solute). When the content of the polar additive increases from 1 to 7–9 vol.-% (II), the silica gel surface is covered more with the modifier molecules and the retention of substituted benzoic acids proceeds via adsorption on the modified surface of silica gel. In this case ($C > 1\%$, v/v), the slope of dependence of $1/V_R'$ on C is smaller and the intercept on the ordinate at $C = 0$ is greater than that for the region of low concentrations ($C < 1\%$, v/v), which is indicative of a weaker interaction of benzoic acids with the modified silica gel surface compared with the initial hydroxylated one.

Fig. 3 shows the adsorption isotherm of acetic acid from solution in cetane on silica gel [27], which is characterized by the presence of two horizontal sections. We believe that the first (A) corresponds to the surface coating of the adsorbent with monomeric molecules of acetic acid oriented probably parallel to the surface. The second horizontal section (B) corresponds to the coating of the surface with dimeric associates of the molecules which are either perpendicular or inclined to the surface. As is seen from Fig. 3, the surface coating with monomeric molecules of acetic acid proceeds at a concentration in solution of 150–200 mmole/l, which approximately corresponds to 1–1.3 vol.-%. Also of importance is that at modifier concentrations above 1–2 vol.-%, aliphatic acids in solution are basically in the state of dimers [28].

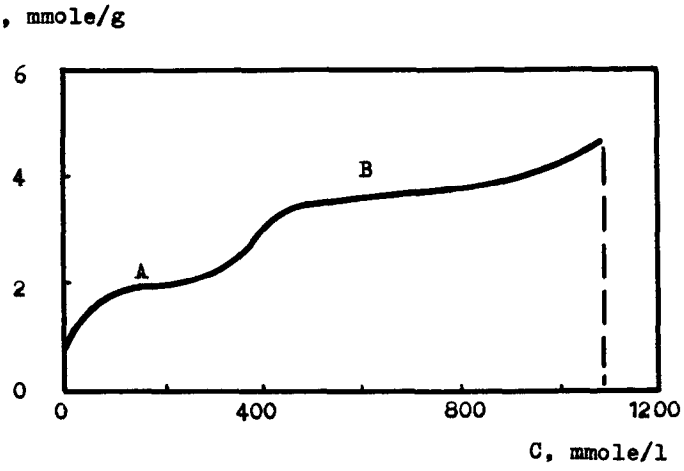

Fig. 3. Adsorption isotherm of acetic acid on silica gel KCK from solution in cetane at 20°C.

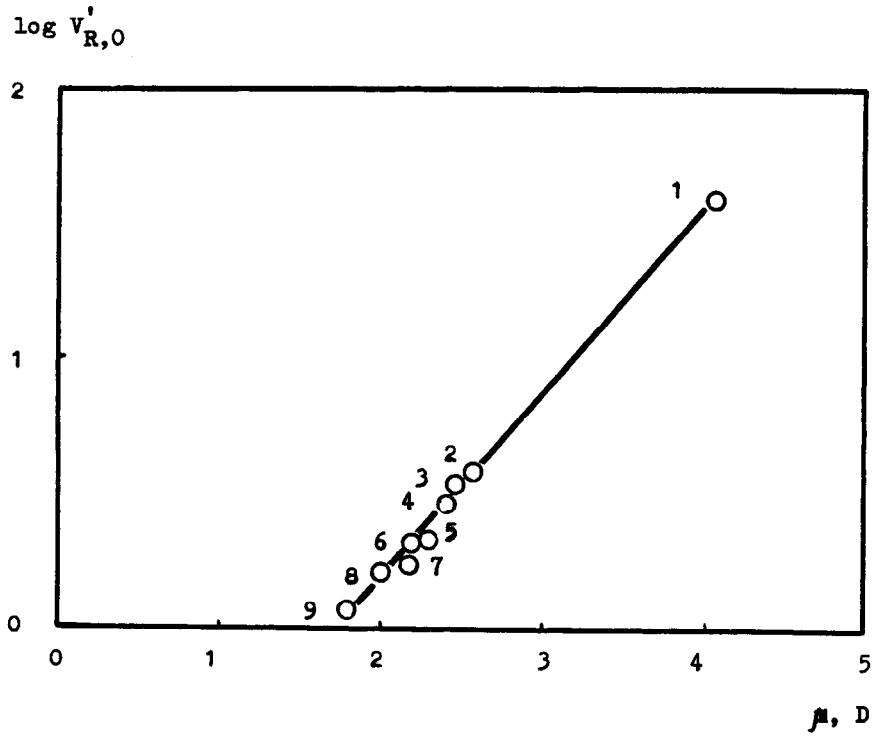

Fig. 4. Dependence of logarithm of corrected retention volume (log V'_R) of substituted benzoic acids on their dipole moment (μ, D). Adsorbent, silica gel L, 10 μm; mobile phase, *n*-hexane–propionic acid (20:1). 1 = *m*-nitrobenzoic acid; 2 = *o*-bromobenzoic acid; 3 = *o*-chlorobenzoic acid; 4 = *p*-methoxybenzoic acid; 5 = *m*-chlorobenzoic acid; 6 = *m*-bromobenzoic acid; 7 = *p*-bromobenzoic acid; 8 = *p*-chlorobenzoic acid; 9 = benzoic acid.

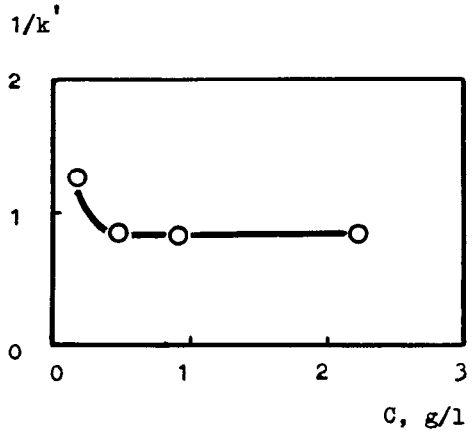

Fig. 5. Dependence of the reciprocal of the capacity factor ($1/k'$) of glucose on the piperazine concentration (C, g/l) in the mobile phase [acetone–water (7:3)]. Adsorbent, LiChrosorb Si 100 silica gel, 7 μm.

The mechanism of retention of benzoic acids, which under these conditions yield cyclic dimers ("aromatic acid–aliphatic acid"), seems to be of pure electrostatic character. This is confirmed by a good linear correlation of the logarithm of retention with the dipole moments of substituted benzoic acids (Fig. 4). It is known [29] that substituted benzoic acids are retained on the surface of anion exchangers in conformity with the values of the Taft constants rather than with their dipole moments.

Introduction of the modifier into the mobile phase may not only result in a decrease in the activity of the adsorbent and, hence, a weaker retention of the solute molecules, but also, in certain circumstances, modification creates a more active surface. Thus, adsorption of diamines on silica gel from an acetone–water mobile phase was used for the liquid chromatography of carbohydrates [30]. Fig. 5 shows that an increase in the concentration of a cyclic diamine, piperazine, in the mobile phase first results in a considerable increase in the retention of glucose (the $1/k'$ value decreases) and then, at concentrations above 0.44 mg/cm^3 (*i.e.*, after the formation of a piperazine monolayer on the surface of silica), the retention hardly changes (or decreases slightly). Mono-, di- and trisaccharides are well separated on a silica surface modified by piperazine directly from the mobile phase (Fig. 6).

Modification of the adsorbent surface due to adsorption of the modifier from the mobile phase, often referred to as dynamic modification, has been widely applied in liquid chromatography. It enables one to change both the retention value of the separated substances and the selectivity of separation. Non-polar reversed phases (such as ODS type phases) are often used as sorbents. Compounds of various types, such as molecular, ionic and complex-forming, are employed as modifiers of two-component water–organic mobile phases. However, a quantitative description of retention depending on the mobile phase composition in RP-HPLC becomes even more complicated as the mobile phase in this instance contains at least four components: solute, modifier, organic solvent and water.

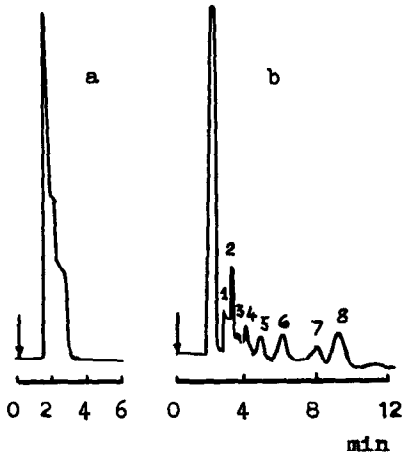

Fig. 6. Chromatogram of a carbohydrate mixture in a column (125 × 4.8 mm I.D.) packed with LiChrosorb Si 100 silica gel, 7 μm. Mobile phase, acetone–water (4:1); volume flow-rate F = 1 cm^3/min; temperature, 30°C. Adsorbent: (a) hydroxylated silica gel; (b) silica gel modified by adsorption of piperazine from the mobile phase. Piperazine concentration in the mobile phase, 0.44 mg cm^{-3}. 1 = Ribose; 2 = xylose; 3 = fructose; 4 = glucose; 5 = saccharose; 6 = cellulose; 7 = melezitose; 8 = raffinose.

In real systems the solute retention is influenced not only by molecular interactions with the stationary phase but also by molecular interactions in the mobile phase. Jaroniec and Jaroniec [31,32] proposed to take into account the association of sorbate molecules and modifier in solution (S_m–M_m) and self-association of the modifier molecules (M_m–M_m). In the simplest case of dimer formation the following equilibrium is attained in solution:

$$S_m + M_m \overset{K_{SM}}{\rightleftharpoons} (SM)_m \tag{19}$$

and

$$M_m + M_m \overset{K_{MM}}{\rightleftharpoons} (MM)_m \tag{20}$$

Then the dependence of the solute retention on the modifier concentration in the mobile phase, in the case of association $(SM)_m$, is expressed by the equation

$$1/k' = [1 + (K_M - 1)M_m](1 + K_{SM}M_m)/(\Phi K_S) \tag{21}$$

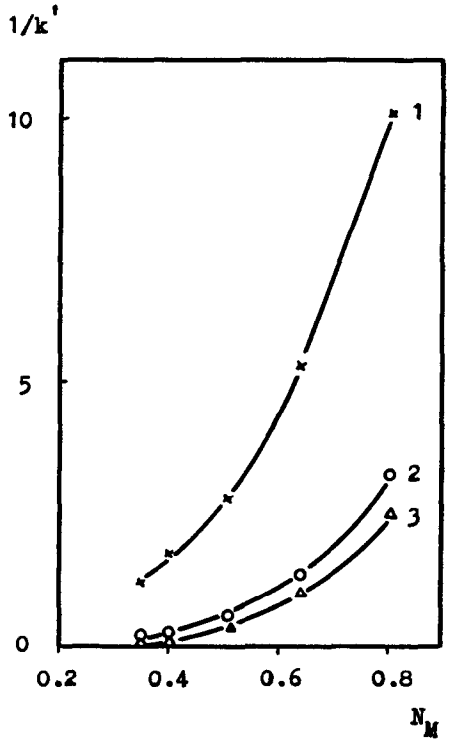

Fig. 7. Dependence of the reciprocal of the capacity factor ($1/k'$) of phenols on the mole fraction (N_M) of methanol in the binary water–organic mobile phase. Adsorbent, LiChrosorb RP-18, 5 μm. 1 = Phenol; 2 = 3-*tert.*-butylphenol; 3 = 2-*tert.*-butylphenol.

and for association $(MM)_m$ by the equation

$$1/k' = [1 + (K_M - 1)M_m - 2K_{MM}M_m^2]/(\Phi K_S) \quad (22)$$

The dependences of $1/k'$ on M_m described by eqns. 21 and 22 are non-linear. Only in the region of small M_m and at small values of K_{MM} and K_{SM} are they transformed into a linear equation (eqn. 16) as previously considered. The molecular solute–modifier (S–M) interaction promotes a faster decrease of the solute retention with increasing concentration M in the mobile phase than in the absence of these interactions (Fig. 7) [33]. The formation of associates between the modifier molecules (*e.g.*, due to non-specific interactions in RP-HPLC), on the other hand, slows down the decrease in retention (Fig. 8).

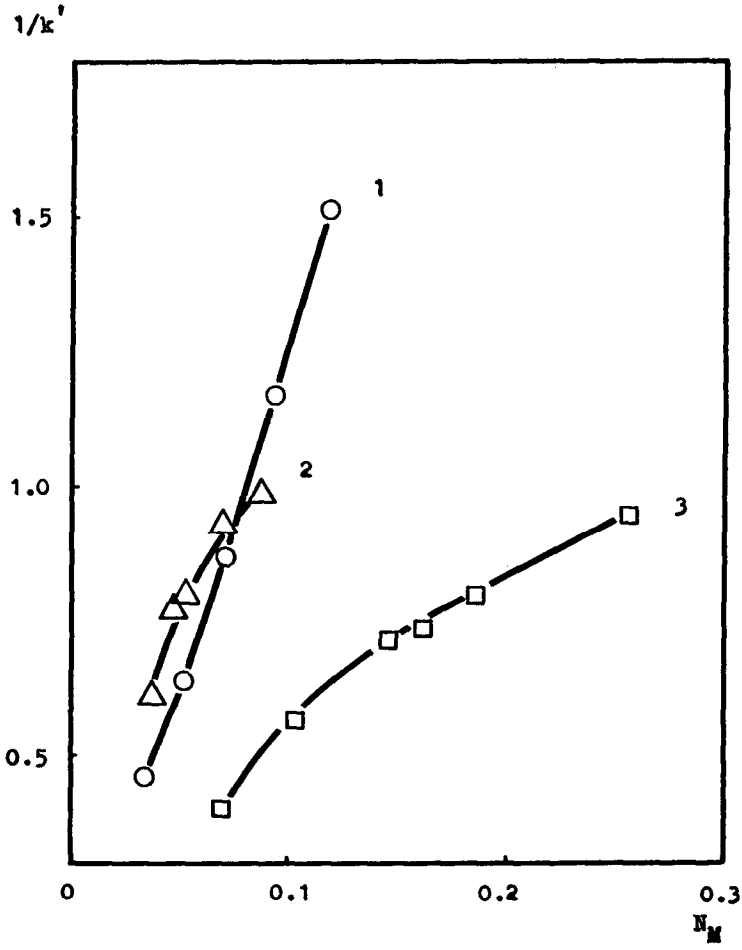

Fig. 8. Dependence of the reciprocal of the capacity factor ($1/k'$) of nivalenol on the nature and mole fraction (N_M) of the organic modifier in the binary water–organic mobile phase. Adsorbent, Nucleosil RP-18, 5 μm. Modifier: 1 = ethanol; 2 = tetrahydrofuran; 3 = acetonitrile.

Now consider the general case when (1) the molecules of solute S and modifier M are able to form in solution polymolecular ($C+1$-molecular) associates and (2) the molecules of modifier M form in the mobile phase polymolecular (d-molecular) associates.

Sorption of S and M molecules occurs only on that part of the sorbent which is occupied with the solvent molecules L and is described by eqns. 1–4. Solvation of the solute molecules by the modifier molecules proceeds in the mobile phase:

$$S_m + cM_m \overset{K_{SM}}{\rightleftharpoons} (SM_c)_m \tag{23}$$

for $c = 1,2,3, \ldots, C$ with the equilibrium constant

$$K_{SM} = (SM_c)_m / S_m M_m^c = Z_c / S_m M_m^c \tag{24}$$

where Z_c is the mole fraction of $c+1$-molecular associates in the mobile phase.

Simultaneously, association of the modifier molecules occurs in the mobile phase:

$$M_m + (d-1)M_m \overset{K_{MM}}{\rightleftharpoons} (M_d)_m \tag{25}$$

for $d = 2,3,4, \ldots, D$, with the equilibrium constant

$$K_{MM} = (M_d)_m / M_m^d = Z_d M_m^d \tag{26}$$

where Z_d is the mole fraction of d-molecular associates in the mobile phase.

As no associates appear on the sorbent,

$$S_s = [S_s^0] \tag{27}$$

$$L_s = [L_s^0] \tag{28}$$

$$M_s = [M_s^0] \tag{29}$$

and eqn. 6 remains correct for the case in question.

In the mobile phase we have

$$[S_m^0] + [M_m^0] + L_m = 1 \tag{30}$$

where $[S_m^0]$ and $[M_m^0]$ are the total concentrations of the different forms of solute and modifier in the mobile phase.

Assuming that $S_m \ll M_m$, L_m and $S_s \ll M_s$, L_s, we have

$$L_m + [M_m^0] \approx 1 \tag{31}$$

and

$$L_s + M_s \approx 1 \tag{32}$$

On the other hand,

$$[S_m^0] = S_m + \sum_{c=1}^{C} Z_c = S_m\left(1 + \sum_{c=1}^{C} K_{SM} M_m^c\right) \tag{33}$$

and

$$[M_m^0] = M_m + \sum_{c=1}^{C} cZ_c + \sum_{d=2}^{D} dZ_d \tag{34}$$

$$[M_m^0] = M_m + \sum_{c=1}^{C} cK_{SM} S_m M_m^c + \sum_{d=2}^{D} dK_{MM} M_m^d$$

$$= M_m\left(1 + K_{SM} \sum_{c=1}^{C} cS_m M_m^{c-1} + K_{MM} \sum_{d=2}^{D} dM_m^{d-1}\right) \tag{35}$$

As S_m is a factor of 10^2–10^3 smaller than M_m, and K_{SM} is commensurate with K_{MM}, then

$$[M_m^0] = M_m\left(1 + K_{MM} \sum_{d=2}^{D} dM_m^{d-1}\right) \tag{36}$$

It follows from eqns. 2 and 4 that

$$K_S = (S_s/S_m)\frac{K_M M_m}{M_s} \tag{37}$$

From eqns. 4, 31 and 32 we have

$$K_M = M_s(1 - [M_m^0])/M_m(1 - M_s) \tag{38}$$

Introducing eqn. 35 into eqn. 38, we obtain

$$M_s - M_s[M_m^0] = K_M M_m - K_M M_m M_s$$

$$= M_s - M_s\left[M_m(1 + K_{SM} S_m \sum_{c=1}^{C} cM_m^{c-1} + K_{MM} \sum_{d=2}^{D} dM_m^{d-1})\right] \tag{39}$$

and

$$M_s = K_M M_m / \left[1 - M_m\left(1 + K_{SM} S_m \sum_{c=1}^{C} cM_m^{c-1} + K_{MM} \sum_{d=2}^{D} dM_m^{d-1}\right) + K_M M_m\right] \tag{40}$$

$$M_s = K_M M_m / \left[1 + M_m(K_M - 1) - K_{SM} S_m \sum_{c=1}^{C} cM_m^c + K_{MM} \sum_{d=2}^{D} dM_m^d\right] \tag{41}$$

Introducing eqn. 41 into eqn. 37, we obtain

$$K_S = (S_s/S_m)\left[1 + (K_M - 1)M_m - K_{SM}S_m \sum_{c=1}^{C} cM_m^c + K_{MM} \sum_{d=2}^{D} dM_m^d\right] \quad (42)$$

Since according to analogy with eqn. 12

$$k' = \Phi(S_s/[S_m^0]) = \Phi K \quad (43)$$

then, introducing eqn. 33 into eqn. 43, we obtain

$$k' = \Phi(S_s/S_m)\left(1 + K_{SM} \sum_{c=1}^{C} M_m^c\right)^{-1} \quad (44)$$

Introducing the values of S_s/S_m from eqn. 42 into eqn. 44, we obtain

$$k' = \Phi K_S\left(1 + \sum_{c=1}^{C} K_{SM}M_m^c\right)^{-1}\left[1 + M_m(K_M - 1) - K_{SM}S_m \sum_{c=1}^{C} cM_m^c - K_{MM} \sum_{d=2}^{D} dM_m^d\right]^{-1} \quad (45)$$

and

$$1/k' = (1/\Phi K_S)\left(1 + \sum_{c=1}^{C} K_{SM}M_m^c\right)\left[1 + (K_M - 1)M_m - K_{SM}S_m \sum_{c=1}^{C} cM_m^c - K_{MM} \sum_{d=2}^{D} dM_m^d\right] \quad (46)$$

In the absence of associates in the solution, *i.e.*, $K_{SM} = 0$ and $K_{MM} = 0$, we obtain eqn. 16 of the linear dependence of $1/k'$ on M_m. When associates are formed between the molecules of the modifier and the solute, $K_{SM} > 0$, and in the absence of association of the modifier molecules, $K_{MM} = 0$, the value of $1/k'$ increases faster (with increasing content of the modifier M_m in the mobile phase) than to the first power of M_m. In contrast, the formation of associates of modifier molecules decreases $1/k'$ with respect to the linear dependence. Thus, in the general case, eqn. 46 represents a complex curvilinear dependence of the solute retention on the modifier concentration, its character being determined by the ratio of the constants K_{SM}, K_{MM}, K_M and K_S corresponding to various types of intermolecular processes occurring in a chromatographic system.

When the interaction between the molecules of the solute and modifier with the sorbent (K_S and K_M) is stronger than the molecular interactions in the solution (K_{SM}), which is typical for NP-HPLC, the dependence of $1/k'$ on M_m should approach linearity. For example, phenols, which are strongly adsorbed from solution onto a silica gel surface in the *n*-hexane–dioxane system, fit eqn. 16 (see Fig. 1).

Specific and non-specific molecular interactions in the mobile phase are more strongly exhibited in RP-HPLC, which is well seen in Fig. 7, where the dependence of $1/k'$ on N_m is non-linear. This character of the dependence shows that in RP-HPLC the contribution of specific and non-specific interactions of phenol molecules with the molecule of the mobile phase is comparable to the non-specific interactions of the solute molecules with the hydrophobic surface of the stationary phase.

In the limiting case, where adsorption of the modifier (K_M) is much less than the interaction of the solute with the modifier (K_{SM}), the retention of substances is approximately described by the equation

$$1/k' = (1/\Phi K_S)\left(1 + K_{SM}\sum_{c=1}^{C} M_m^c\right) = (1/\Phi K_S)(1 + K_{SM}M_m^n) \quad (47)$$

where n characterizes some mean solvation number of the solute molecules in the mobile phase. Then, in the coordinates of $1/k'$ *versus* M_m^n, a certain linearity is observed for phenol at $n = 3$ (Fig. 9a) and for 3-*tert.*-butylphenol at $n = 4$ (Fig. 9b); n is higher for the larger and more hydrophobic 3-*tert.*-butylphenol molecule than for phenol.

Considering the chromatographic retention of mycotoxins of the trichothecene series (nivalenol, deoxynivalenol and deoxynivalenol 15-acetate), which differ in the number of polar groups, we can see (Fig. 10) that the rectilinear dependence of $1/k'$ on M_m is well realized for deoxynivalenol 15-acetate for all investigated modifiers of the mobile phase (acetonitrile, tetrahydrofuran, ethanol). The molecule of deoxynivalenol 15-acetate is the most hydrophobic among the investigated mycotoxins and is most strongly retained by the reversed phase.

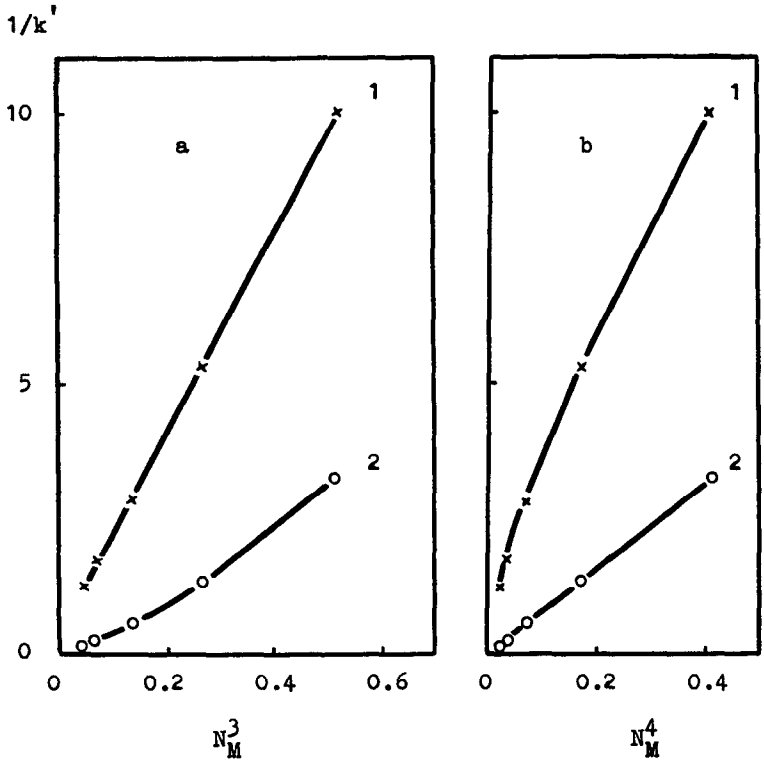

Fig. 9. Dependence of the reciprocal of the capacity factor ($1/k'$) on the mole fraction (N_M) of methanol in the mobile phase. Conditions as in Fig. 7. (a) N_M^3; (b) N_M^4. 1 = Phenol; 2 = 3-*tert.*-butylphenol.

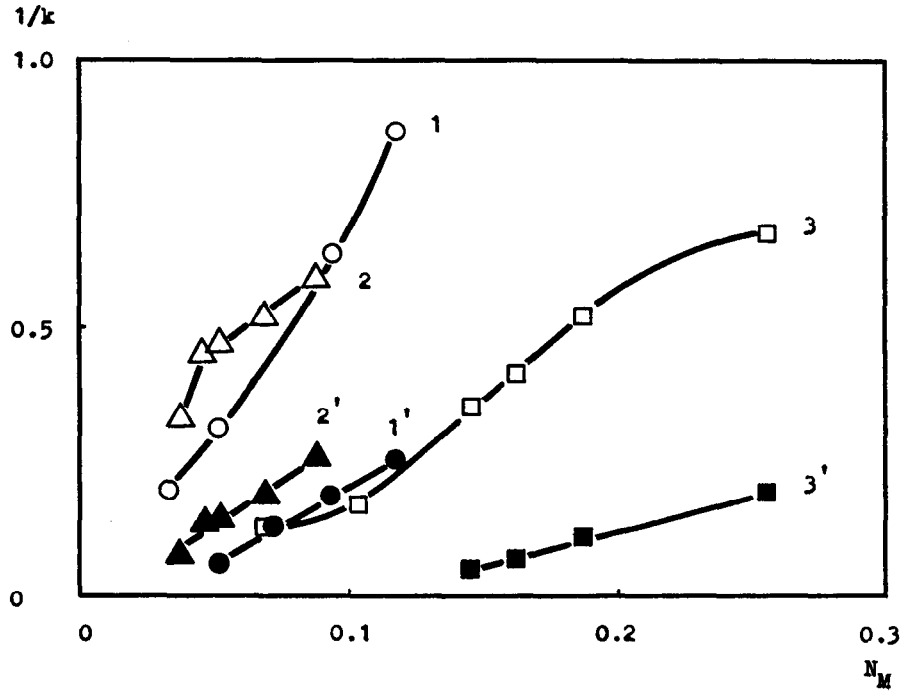

Fig. 10. Dependence of the reciprocal of the capacity factor ($1/k'$) on the mole fraction (N_M) of the organic component in binary water–organic mobile phases. Conditions as in Fig. 8. (1–3) Deoxynivalenol; (1′–3′) deoxynivalenol 15-acetate.

With the more polar nivalenol, which is less strongly adsorbed on the stationary phase, a dependence close to linearity is observed only for the ethanol mobile phase (Fig. 8). The curvilinear dependence which, according to eqn. 46, indicates a considerable contribution of interactions of the modifier molecules with each other in the mobile phase, is more typical for the more hydrophobic modifiers tetrahydrofuran and acetonitrile. For deoxynivalenol, the character of the retention dependence in different mobile phases is similar to that observed for nivalenol.

As is seen in eqn. 46, at M_m approaching zero the values of k' must be equal to the value of ΦK_S, *i.e.* the retention k' of the solute with water as the mobile phase. Fig. 8 shows that on extrapolation of the initial parts of the dependence of $1/k'$ on M_m towards $M_m = 0$ for three different modifiers, the points of intersection with the ordinate are actually close to each other and very close to zero, which is indicative of a very strong retention when water is used as the mobile phase.

The calculation of the first three points of the curves (Fig. 8) according to eqn. 48:

$$1/k' = a + bN_m \tag{48}$$

where a and b are constants resulted in values for the intersection points of $a = 0.1 \pm 0.46$, 0.13 ± 0.58 and 0.15 ± 2.2 for ethanol, acetonitrile and tetrahydrofuran, respectively, as modifiers.

Hence eqn. 46 allows one to describe a change in the retention of substances in NP- and RP-HPLC for various cases of molecular interactions in the chromatographic system (solute–mobile phase–stationary phase).

Such an approach to the description of molecular interactions in NP- and RP-HPLC as quasi-chemical equilibria on the sorbent and in solution (of solute–sorbent, modifier–sorbent, solute–modifier and modifier–modifier types) helps in obtaining a better understanding of the retention mechanism in HPLC. It is necessary for a quantitative description of the retention dependence on the mobile phase composition to know the constants of these equilibria (K_S, K_M, K_{SM}, K_{MM}). For this purpose it is necessary to intensify studies on measuring the isotherms of adsorption from solutions of modifying additives over a wide range of concentrations and also on the determination of the association constants of substances in solutions with the use of independent techniques.

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