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# Effect of the organic modifier concentration on the retention in reversed-phase liquid chromatography

## I. General semi-thermodynamic treatment for adsorption and partition mechanisms

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### Abstract

A semi-thermodynamic treatment is adopted to account for adsorption or partition of solute molecules from aqueous mobile phases on/in reversed-phase liquid chromatography stationary phases. The theoretical expressions of  $\ln k'$  versus organic modifier content are tested against 10 data sets covering a variety of solute molecules. It is shown that the mean field approximation, adopted widely in previous studies, is marginally valid in aqueous mobile phases, especially in the presence of solute molecules, and the lattice model approximation, which is also used in relevant studies, is a poor approximation. Clear conclusions about the validity of either the adsorption or the partition model for the retention mechanism could not be drawn. The equations of the adsorption model describe all data sets absolutely satisfactorily and yield a physically reasonable picture about the behavior of modifier and solvent at the adsorbed layer. However, the high applicability of the adsorption model may not safely entail the validity of the adsorption mechanism at a molecular level, especially in the case of solutes with small and non-polar molecules, where our analysis gives strong indications about the validity of the partition mechanism. The next steps needed for the final elucidation of the retention mechanism in reversed-phase chromatographic columns are indicated. © 2002 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

The mechanism of retention in chromatographic columns has attracted much attention from the very beginning of liquid chromatography. Two mechanisms have been examined in detail: adsorption and partition. The wide use of reversed-phase liquid

chromatography (RPLC), where the stationary phase is characterized by alkyl chains grafted onto silica or polymer surfaces, led to the development of more advanced models especially for the partition mechanism, where the partitioning process takes place into a phase of disordered alkyl chains [1–11]. Adsorption models have also been proposed to explain the retention in RPLC [2,12–16].

Despite the intensive studies on this issue, there is not a clear picture about the retention mechanism yet. The reason is the following: In order to study the

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mechanism of retention of a solute we usually add an organic modifier to the mobile phase and examine the effect of the modifier concentration on the retention of the solute. However, the curve describing this dependence is so simple that even very primitive models may describe it. Thus the experimental data can hardly help in the selection of the proper model, i.e. the model that is close to the physical reality of the retention mechanism. In addition, a strict treatment leads to equations with a great number of adjustable parameters. However, the greater the number of adjustable parameters, the better the model describes every feature of the retention curve even artificial. But this is another obstacle for gaining a correct picture of the retention mechanism.

The present paper is a contribution to the same issue of the retention mechanism. Our approach is to model both the adsorption and partition mechanisms using the least number of assumptions and approximations keeping at the same time the number of the adjustable parameters to a minimum. In addition, we are going to use a great number of experimental data to test the theory in order to avoid accidental agreements between theory and experiment. The tests will concern not only the agreement between theoretical and experimental retention curves but also whether the adjustable parameters produce physically coherent values.

## 2. Retention models

Up to now, as referred above, two principal mechanisms for retention in RPLC have been proposed: adsorption and partition. However, the models of the stationary phase adopted to treat the retention mechanism are more than two. In a very instructive review article, Vailaya and Horváth [11] have identified the following five models for the stationary phase: (1) liquid hydrocarbon partition model, (2) liquid-crystalline hydrocarbon partition model, (3) amorphous-crystalline hydrocarbon partition model, (4) adsorptive hydrocarbon monolayer model, and (5) isolated solvated hydrocarbon chains model.

The first three models have been proposed for the partition mechanism and they differ in the configurations of the hydrocarbon chains [11]. The bonded

hydrocarbon chains of the stationary phase may form a bulk liquid hydrocarbon layer, a liquid crystalline or an amorphous crystalline hydrocarbon layer over the siliceous surface. In all cases only solute A molecules can penetrate this layer. Thus the partition of molecules A is established between the mobile phase and vacant sites into the grafted chains (hydrocarbon layer) of the stationary phase, usually called the interface region. Such a model was described by Dill [2].

However, Dill's model may raise questions whether solute molecules with dimensions three or more times the dimensions of solvent molecules can penetrate the hydrocarbon layer, whereas the solvent, usually water, and the organic modifier molecules are excluded from this layer. A speculation for such a behavior is based on the hydrophobic interactions between water or small polar molecules and the hydrocarbon chains of the stationary phase. However, these interactions are of short range and if there are vacant sites in the hydrocarbon layer due to the thermal mobility of the hydrocarbon chains, water and modifier molecules may enter the interface region, especially in high organic content mobile phases, since in this case the chains extend towards the mobile phase [11].

The above case is interesting from a thermodynamic point of view. The equilibrium between two phases, which are the hydrocarbon layer and the mobile phase inside the chromatographic column, should be governed by Gibbs phase rule,  $F=C-P+2$ , where  $F$  is the number of degrees of freedom, i.e. the number of independent variables,  $C$  is the number of components, and  $P$  is the number of phases, in our case  $P=2$ . If solute, modifier and solvent molecules can co-exist within the hydrocarbon layer forming an independent phase, then the equilibrium between this phase and the mobile phase has three degrees of freedom, since  $F=3-2+2=3$ . Thus at constant temperature and pressure there is just one degree of freedom, which means that we cannot fix the composition of the mobile phase arbitrarily. Therefore, such equilibrium cannot be established in a chromatographic column with different compositions of the mobile phase. It is seen that, from a thermodynamic point of view, either solvent and modifier molecules cannot penetrate the hydrocarbon layer or, if these substances do co-exist in the

hydrocarbon layer, they do not constitute an independent phase. In the latter case  $P=1$ , which yields  $F=4$ . Therefore, at constant temperature and pressure we have  $F=2$ , which shows that we can change the composition of the mobile phase as we wish.

In contrast, Dill's model is thermodynamically consistent, because modifier and solvent molecules cannot penetrate the hydrocarbon layer. In order to prove it we will follow a slightly different approach, because in this case all the components do not exist in the two phases. If we denote by superscript (s) the hydrocarbon layer of the stationary phase and by superscript (m) the mobile phase, then the variables that describe the equilibrium in the column are the following:  $T^s, T^m, p^s, p^m, x_A^s, x_A^m, x_B^m$  and  $x_S^m$ , where  $T$  is the temperature,  $p$  is the pressure and  $x_A, x_B, x_S$  are the mol fractions of the solute, the modifier and the solvent, respectively. However, all these variables are not independent, since there are the following equilibrium restraints:  $T^s=T^m, p^s=p^m, x_A^m + x_B^m + x_S^m = 1$  and  $\mu_A^m = \mu_A^s$ , where  $\mu_A$  is the chemical potential of A. It is seen that the total number of variables that describe the equilibrium is eight but there are four relationships among them. Therefore, there are four independent variables, which means that at constant pressure and temperature we can fix the composition of the mobile phase arbitrarily. Finally, we should point out that we could not assume that solute and solvent molecules can insert among the chains of the hydrocarbon layer forming an independent phase, because again this is not allowed from a thermodynamic point of view. Indeed in this case the total number of variables is nine ( $T^s, T^m, p^s, p^m, x_A^s, x_S^s, x_A^m, x_B^m, x_S^m$ ) with six relationships among them ( $T^s=T^m, p^s=p^m, x_A^s + x_S^s = 1, x_A^m + x_B^m + x_S^m = 1, \mu_A^m = \mu_A^s, \mu_S^m = \mu_S^s$ ). Thus, there are only three independent variables, which do not allow the free change in composition of the mobile phase, at constant pressure and temperature.

The above observations clearly show that the classical picture of the partition, i.e. the partition of a solute between the mobile phase and a similar phase formed within the hydrocarbon chains of the stationary phase, cannot be realized in chromatographic columns. Therefore, if the retention is governed by a partition mechanism, this mechanism should be closely described by Dill's model [2]. However, this

does not mean that Dill's model gives a precise picture of the reality inside a RPLC column. The work on this issue carried out by Klatté and Beck [17–19] is quite interesting. These authors presented molecular dynamic simulations of the RPLC interface in order to gain a microscopic and realistic picture of the local structure and the driving forces that govern the retention. Their results showed the existence of a number of specific interfacial effects that cannot be fully described by bulk partition models, like Dill's model. Note that the bulk partition models have also been questioned by Tijssen et al. [16,20].

The fourth model for the stationary phase concerns the adsorption mechanism, which is in fact a displacement mechanism [12–15]. It is assumed that the solute molecules and the modifier molecules are co-adsorbed on the tips of the hydrocarbon chains displacing solvent molecules. Speculations about combined adsorption and partition processes have also been made [8,13,15]. Note the landmark paper by Jaroniec and Martire on the combined adsorption and partition model, which is formulated in terms of classical thermodynamics [13]. This approach leads easily to a general expression for the dependence of the retention factor upon the modifiers content in the mobile phase, from which the limiting equations based on either the adsorption or partition model may be deduced.

The last model for the stationary phase is for the solvophobic theory [11,21,22], which does not distinguish between adsorption and partition. This model assumes a displacement process of solvent molecules from the solvated hydrocarbon chains by the solute molecules, which is followed by the association of the solute molecules with the chains. However, such a process is in fact an adsorption process, despite the fact that the solvophobic theory is completely different from the corresponding adsorption theories.

The partition model suggested by Dill [2] may explain the retention mechanism of small non-polar solute molecules. However, reversed-phase columns are used in separation processes of mixtures containing small or large molecules of different polarity [23,24]. Thus it would be interesting to examine the retention mechanism of all kind of molecules. This is attempted in the present paper. Our treatment on the

adsorption and partition mechanisms is not based on lattice statistical thermodynamics but on classical thermodynamics. Note that a similar approach has been adopted by Jaroniec and Martire [13] to treat the combined adsorption and partition model. This approach has the advantage that it is not interested in the detail structure of either the stationary or the mobile phase. Thus for the partition mechanism we are based on the arguments presented above about the prerequisites for a partitioning process to be valid from a thermodynamic point of view and we consequently assume that only the solute molecules are distributed between the mobile phase and cavities within the hydrocarbon chains on the stationary phase. Concerning the adsorption mechanism, we adopt a co-adsorption process of solute and modifier molecules either on the tips or the stem of the hydrocarbon chains. This co-adsorption process is in fact a displacement process of solvent molecules by the molecules of solute and modifier.

### 3. Basic expressions for the retention factor

Consider that the mobile phase consists of a polar solvent S, like water, the organic modifier B and the eluite A. The retention factor,  $k'$ , for eluite A is given by [2,25,26]:

$$\ln k' = \lim_{n_A^m \rightarrow 0} \ln \frac{n_A^s}{n_A^m} \quad (1)$$

where  $n_A^s$ ,  $n_A^m$  are the numbers of moles of solute A on/in the stationary phase and in the mobile phase inside the chromatographic column, respectively. Thus, superscripts s and m denote the stationary and the mobile phase, respectively. Eq. (1) becomes useful only if we replace the number of moles with mol fractions or surface coverage. However, this replacement depends to some extent on the model of the retention mechanism. Thus we have a partition model and an adsorption model.

#### 3.1. Partition model

Since in this model only solute molecules can penetrate the interface region of the hydrocarbon chains,  $n_A^s$  may be expressed as  $n_A^s = x_A^s n_{A,\max}^s$ ,

where  $n_{A,\max}^s$  is the maximum number of moles of A in the stationary phase, and Eq. (1) yields:

$$\begin{aligned} \ln k' &= \lim_{n_A^m \rightarrow 0} \ln \frac{x_A^s n_{A,\max}^s}{x_A^m (n_A^m + n_B^m + n_S^m)} \\ &= \ln \frac{n_{A,\max}^s}{n_B^m + n_S^m} + \lim_{x_A^m \rightarrow 0} \ln \frac{x_A^s}{x_A^m} \end{aligned} \quad (2)$$

where  $x_A^m$  is the mol fraction of A in the mobile phase.

If we adopt the lattice model approximation to describe the properties of the mobile phase, i.e. if we assume that the mobile phase has a lattice structure where each molecule occupies one site, then we have  $n_B^m + n_S^m = M/L$ , since  $n_A^m \rightarrow 0$ . Here,  $L$  is Avogadro's number and  $M$  is the total number of lattice sites of the mobile phase. Consequently, under the lattice model approximation Eq. (2) results in:

$$\ln k' = \ln k^\# + \lim_{x_A^m \rightarrow 0} \ln \frac{x_A^s}{x_A^m} \quad (3)$$

where  $\ln k^\# = \ln(n_{A,\max}^s L/M)$ . Note that the lattice model approximation is widely adopted, either directly or indirectly, in theoretical studies on the effect of modifier concentration on the retention factor [1,2,5,12,14,27–30]. The effect of this approximation is discussed in Section 11.

However, a strict approach is to assume that it is not the number of lattice sites of the mobile phase that remain constant and independent of the modifier concentration but the volume  $V$  of this phase. Let  $\varphi$  be the concentration of the modifier B in the mobile phase expressed as the ratio of its volume to the total volume of the mobile phase. Therefore, if  $V$  ml is the volume of the mobile phase, then the number of moles of B is given by:

$$n_B^m = \frac{V\varphi\rho_B}{M_B} \quad (4)$$

where  $\rho_B$  is the density of the pure B and  $M_B$  is its molecular mass.

When the solvent S and the modifier B are polar compounds, their mixing is associated with a decrease in the total volume of the mixture. Let  $\delta$  be this decrease that is referred to 1 ml of solution. Then the number of moles of solvent S in 1 ml of solution is equal to  $(1 + \delta - \varphi)\rho_S/M_S$  and therefore

the number of moles of S in volume  $V$  of the mobile phase is given by:

$$n_S^m = \frac{V(1 + \delta - \varphi)\rho_S}{M_S} \quad (5)$$

Eqs. (4) and (5) yield:

$$n_B^m + n_S^m = \frac{V\rho_S}{M_S}(1 + \delta - \alpha\varphi) \quad (6)$$

where

$$a = 1 - \frac{\rho_B/M_B}{\rho_S/M_S} \quad (7)$$

In addition, the following relationships are valid:

$$x_B^m = \frac{n_B^m}{n_B^m + n_S^m} = \frac{\varphi(1 - \alpha)}{1 + \delta - \varphi\alpha},$$

$$x_S^m = \frac{1 + \delta - \varphi}{1 + \delta - \varphi\alpha} \quad (8)$$

yielding

$$\varphi = \frac{x_B^m(1 + \delta)}{1 - \alpha + \alpha x_B^m} \quad (9)$$

If Eq. (9) is introduced into Eq. (6), we obtain:

$$n_B^m + n_S^m = \frac{V\rho_B/M_B}{1 - \alpha + \alpha x_B^m}(1 + \delta) \quad (10)$$

Substitution of Eq. (10) into Eq. (2) results in

$$\ln k' = \ln k + \lim_{x_A^m \rightarrow 0} \ln \frac{x_A^s}{x_A^m} + \ln \frac{1 - \alpha + \alpha x_B^m}{(1 - \alpha)(1 + \delta)} \quad (11)$$

where  $k' = n_{A,\max}^s / (V\rho_S / M_S)$ .

### 3.2. Adsorption model

At the adsorbed layer the surface concentration of A is useful to be expressed in terms of the surface coverage  $\theta_A$ . In this case we have  $n_A^s = \theta_A n_{A,\max}^s$ , where  $n_{A,\max}^s$  is the maximum number of moles of A at the adsorbed layer. Following precisely the same approach as above we find that:

$$\ln k' = \ln k + \lim_{x_A^m \rightarrow 0} \ln \frac{\theta_A}{x_A^m} + \ln \frac{1 - \alpha + \alpha x_B^m}{(1 - \alpha)(1 + \delta)} \quad (12)$$

Note that if the lattice model approximation is

adopted, the last term of Eq. (12) does not appear and this equation is reduced to

$$\ln k' = \ln k^{\#} + \lim_{x_A^m \rightarrow 0} \ln \frac{\theta_A}{x_A^m} \quad (13)$$

It is seen that this equation is similar to the corresponding equation obtained for the partition model, i.e. to Eq. (3).

## 4. Equilibrium equations

In order to proceed further, we must find the dependence of  $x_A^s/x_A^m$  and  $\theta_A/x_A^m$  upon  $x_B^m$  based on the equilibrium equations valid in each case.

### 4.1. Partition model

In this model there is just one equilibrium process,  $A^s \Leftrightarrow A^m$ , which leads to the following equilibrium equation:

$$\ln \frac{x_A^s}{x_A^m} + \ln \frac{f_A^s}{f_A^m} = \ln \beta_A^p \quad (14)$$

where  $f_A^s$ ,  $f_A^m$  are activity coefficients and  $\beta_A^p$  is an equilibrium constant. We observe that the knowledge of the activity coefficients  $f_A^s$  and  $f_A^m$  is necessary for the determination of the ratio  $x_A^s/x_A^m$  as a function of  $x_B^m$ .

### 4.2. Adsorption model

Recent studies [31–34] have given rather strong indications that water molecules, and very likely the molecules of all polar solvents, form large clusters at adsorbed layers. These clusters have as a consequence the minimization of size effects in the mechanism of adsorption [31–34]. Therefore, at least as a first approximation we may completely ignore size effects. In this case, the adsorption process of solute A and modifier B from the polar solvent S may be expressed as



whereas the adsorption isotherms may be given by the following equations:

$$\ln \frac{\theta_A}{1 - \theta_A - \theta_B} + \ln \frac{f_A^s}{f_S^s} = \ln \beta_A + \ln \frac{f_A^m}{f_S^m} + \ln \frac{x_A^m}{x_S^m} \quad (17)$$

$$\ln \frac{\theta_B}{1 - \theta_A - \theta_B} + \ln \frac{f_B^s}{f_S^s} = \ln \beta_B + \ln \frac{f_B^m}{f_S^m} + \ln \frac{x_B^m}{x_S^m} \quad (18)$$

Again  $f_i$ ,  $i = A, B, S$  are activity coefficients and  $\beta_A$ ,  $\beta_B$  are equilibrium constants. However,  $x_A^m \rightarrow 0$ , which means  $\theta_A \rightarrow 0$  and therefore:

$$\ln \frac{\theta_A}{x_A^m} = \ln(1 - \theta_B) - \ln \frac{f_A^s}{f_S^s} + \ln \frac{f_A^m}{f_S^m} + \ln \frac{\beta_A}{x_S^m} \quad (19)$$

$$\ln \frac{\theta_B}{1 - \theta_B} + \ln \frac{f_B^s}{f_S^s} = \ln \beta_B + \ln \frac{f_B^m}{f_S^m} + \ln \frac{x_B^m}{x_S^m} \quad (20)$$

It is seen that the ratio  $\theta_A/x_A^m$  needed for the calculation of the retention factor from Eq. (12) can be obtained from the system of Eqs. (19) and (20) provided that the various activity coefficients are known.

## 5. Activity coefficients

We found above that the activity coefficients of the constituents of the mobile phase and those of the adsorbed or partition layer are necessary for the calculation of the retention factor from Eqs. (11) or 12, respectively. The activity coefficients of the constituents of the mobile phase can be determined by independent measurements. Indeed, limiting activity coefficients of solutes in the mobile phase may be determined by headspace gas chromatography [35]. In addition the activity coefficients of a liquid binary mixture of compounds B and S can be determined by vapour pressure measurements. Alternatively, for common modifiers, like methanol, ethanol, etc., there are tabulated data that allow the calculation of the activity coefficients, for example by means of Wilson equations [36,37]:

$$\ln f_B^m = -\ln(x_B^m + A_{BS}x_S^m) + x_S^m \left( \frac{A_{BS}}{x_B^m + A_{BS}x_S^m} - \frac{A_{SB}}{x_S^m + A_{SB}x_B^m} \right) \quad (21)$$

$$\ln f_S^m = -\ln(x_S^m + A_{SB}x_B^m) - x_B^m \left( \frac{A_{BS}}{x_B^m + A_{BS}x_S^m} - \frac{A_{SB}}{x_S^m + A_{SB}x_B^m} \right) \quad (22)$$

where  $A_{BS}$  and  $A_{SB}$  are not adjustable parameters but constants that can be calculated from the tabulated data. It is evident that Wilson's equations give approximate values for  $f_B^m$ ,  $f_S^m$ . However, this approximation is much better than ignoring the activity coefficients of the modifier and solvent in the mobile phase.

The activity coefficients of the constituents of the adsorbed and the hydrocarbon layer (partition layer) cannot be determined by independent measurements. In this case, as well as when the activity coefficients in the mobile phase are not available by independent measurements, we may proceed as follows:

A general and flexible but phenomenological approach to treat the activity coefficients is to consider the mobile phase and/or the adsorbed layer on the hydrocarbon chains of the stationary phase as independent phases. Then the excess free energy  $G^e$  of each of these phases may be expressed as [38–40]:

$$G^e/RT = \sum_i N_i \ln f_i, \quad i = A, B, S \quad (23)$$

Then making use of the thermodynamic relationship:

$$\sum_i N_i (\partial \ln f_i / \partial N_j) = 0 \quad (24)$$

we obtain

$$\ln f_j = \frac{\partial G^e/RT}{\partial N_j}, \quad j = A, B, S \quad (25)$$

The excess free energy  $G^e$  is a continuous function of the composition of the phase. Therefore, it can be expanded to a power series of the form [41–43]:

$$\frac{G^e}{RT} = M \sum_{q=0}^p \{ A_q x_A x_B (x_A - x_B)^q + B_q x_A x_S (x_A - x_S)^q + C_q x_B x_S (x_B - x_S)^q \} \quad (26)$$

where  $M=N_A+N_B+N_S$  is the total number of molecules in the system.

It is seen that the exact expression of the activity coefficients depends on the choice of  $p$ , that is on the number of terms in the expansion of  $G^E$ . It is evident that if we select high  $p$  values, the expressions of the activity coefficients obtained by means of Eqs. (25) and (26) can describe accurately every set of experimental data. However we found that the values  $p=0$  or  $p=1$  are enough in most cases. If we select  $p=1$  and take into account that  $x_A \rightarrow 0$ , we obtain:

$$\ln f_A = D_1 + D_2x_B + D_3x_B^2 + D_4x_B^3 \quad (27)$$

$$\ln f_B = C_0(1-x_B)^2 + C_1(1-x_B)^2(4x_B-1) \quad (28)$$

$$\ln f_S = C_0x_B^2 + C_1x_B^2(4x_B-3) \quad (29)$$

where  $D_1=B_0-B_1$ ,  $D_2=A_0-B_0-C_0+2C_1+2B_1$ ,  $D_3=-A_1-B_1-6C_1+C_0$  and  $D_4=4C_1$ . The above relationships for the activity coefficients are reduced to:

$$\ln f_A = D_1 + D_2x_B + D_3x_B^2 \quad (30)$$

$$\ln f_B = C_0(1-x_B)^2 \quad (31)$$

$$\ln f_S = C_0x_B^2 \quad (32)$$

when  $p=0$ . Here,  $D_1=B_0$ ,  $D_2=A_0-B_0-C_0$  and  $D_3=C_0$ . Note that in Eqs. (27)–(32) superscripts m or s are not used, since these equations can be used for the constituents of either the mobile phase or the adsorbed layer. It is evident that if these equations are used for the mobile phase, superscript m should be added to  $D_j$ ,  $C_j$  and  $x_B$ , whereas for the adsorbed layer, apart from the addition of superscript s to  $D_j$ ,  $C_j$ , the mol fraction  $x_B$  should be replaced by the surface coverage  $\theta_B$ .

It is worth noting that when  $p=0$ , the expressions for the activity coefficients, Eqs. (30)–(32), are identical to those obtained from lattice models under mean field approximation [43]. In this case, we have [43]:  $C_0=A^{BS}$ ,  $A_0=A^{AB}$  and  $B_0=A^{AS}$ , where  $A^{ij}=z[w_{ij}-(w_{ii}+w_{jj})/2]/kT$ ,  $w_{ij}$ ,  $w_{ii}$ ,  $w_{jj}$  are the interaction energies between  $i$ - $j$ ,  $i$ - $i$  and  $j$ - $j$  species, respectively, and  $z$  is the coordination number of the lattice structure of the mobile phase or the adsorbed layer. Therefore, we may conclude that Eqs. (27)–

(29) obtained with  $p=1$  reflect contributions from “local order” and non-nearest neighbouring interactions, i.e. contributions from effects that are neglected under mean field approximation.

A very interesting case arises when the excess free energy of the ternary mixture of A, B and S is described by Eq. (26) with a certain value of  $p$ , whereas the corresponding energy of the binary mixture of B and S is described by Eq. (26) with a lower value of  $p$ . In order to treat this case we first consider for generality a ternary mixture of A, B and S described by Eq. (26) with an arbitrary value of  $p$ . The limiting activity coefficient of A of this mixture is found if we differentiate Eq. (26) with respect to  $N_A$ , according to Eq. (25), and then take the limit  $x_A \rightarrow 0$ . We obtain:

$$\ln f_A = \sum_{q=0}^p \{(-1)^q A_q x_B^{q+1} + (-1)^q B_q (1-x_B)^{q+1} - C_q(1+q)x_B(1-x_B)(2x_B-1)^q\} \quad (33)$$

This equation can be readily written as a polynomial of the form:

$$\ln f_A = D_1 + D_2x_B + D_3x_B^2 + \dots + D_{p+3}x_B^{p+2} \quad (34)$$

where  $D_1, D_2, \dots$  are linear combinations of  $A_q, B_q$  and  $C_q$ , except  $D_{p+3}$  which is given by  $D_{p+3}=C_p(1+p)2^p$ .

It is seen that in general the limiting activity coefficients of a solute can be expressed as a polynomial of order  $p+2$ . However the order of this polynomial may be  $p+1$  if the value of  $p$  of the binary mixture of the modifier and solvent is smaller than the value of  $p$  of the ternary solution. To clarify this point we examine three examples:

- The ternary mixture of A, B, S is described by Eq. (26) with  $p=1$  and the binary mixture of B, S by the same equation with  $p=0$ . In this case the validity of Eqs. (31) and (32) necessarily entails that  $C_1=0$ . Thus Eq. (34) is reduced to Eq. (30) but with  $D_1, D_2, D_3$  given by  $D_1=B_0-B_1$ ,  $D_2=A_0-B_0-C_0+2B_1$  and  $D_3=C_0-A_1-B_1$ .
- The ternary mixture of A, B, S is described with  $p=2$  and the binary mixture of B, S with  $p=1$ . In this case, Eq. (34) is reduced to Eq. (27) with

different coefficients and in particular with  $D_1 = B_0 - B_1 + B_2$ ,  $D_2 = A_0 - B_0 - C_0 + 2B_1 + 2C_1 - 3B_2$ ,  $D_3 = C_0 - A_1 - B_1 - 6C_1 + 3B_2$  and  $D_4 = A_2 - B_2 + 4C_1$ , since  $C_2 = 0$ .

- (c) The ternary mixture of A, B, S is described by Eq. (26) with  $p=2$  and the binary mixture of B, S by the same equation with  $p=0$ . Now the activity coefficient of A is given by Eq. (27) with  $D_1 = B_0 - B_1 + B_2$ ,  $D_2 = A_0 - B_0 - C_0 + 2B_1 - 3B_2$ ,  $D_3 = C_0 - A_1 - B_1 + 3B_2$  and  $D_4 = A_2 - B_2$ , because the choice  $p=0$  for the mixture of B, S entails that  $C_2 = C_1 = 0$ .

It is seen that a certain expression of  $\ln f_A$  may express different models of the ternary mixture of A, B and S. Thus the validity of Eq. (30) shows that the ternary mixture of A, B, S may or may not be described by the regular solution theory under mean field approximation. When the mean field approximation holds, coefficients  $D_1, D_2, D_3$  are given by  $D_1 = B_0, D_2 = A_0 - B_0 - C_0$  and  $D_3 = C_0$ , otherwise we have  $D_1 = B_0 - B_1, D_2 = A_0 - B_0 - C_0 + 2B_1$  and  $D_3 = C_0 - A_1 - B_1$ . Note that  $D_3 = C_0$  when the mean field approximation is valid and  $D_3 = C_0 - A_1 - B_1$  when it does not. This difference can be used as a criterion for the validity of the mean field approximation, since  $C_0$  can be determined from measurements of the activity coefficients of the modifier and solvent in their binary mixture, as discussed in Section 10.2.

If the activity coefficients  $f_B^m, f_S^m$  are known from independent measurements or from Eqs. (21) and (22) using tabulated data, then by means of a proper least squares fitting we may: (a) conclude whether Eqs. (28) and (29) or Eqs. (31) and (32) describe the dependence of  $f_B^m, f_S^m$  upon  $x_B^m$ , (b) calculate the values of  $C_0, C_1$ , and (c) find which of the equations concerning the activity coefficient  $f_A^m$  is suitable for its representation. In the last case, we may determine the values of  $D_1^m, D_2^m, D_3^m, D_4^m$  by means of linear least squares provided that the dependence of  $f_A^m$  upon  $x_B$  is known from independent measurements. All these issues are further discussed below.

The equations derived above are valid for the species of the mobile phase and those of the adsorption layer. For the solute molecules that exist in the interface region of the hydrocarbon chains in the partition model we may write in general that  $\ln f_A = f(x_A^s)$ , where  $f(x_A^s)$  is an unknown but continuous

function of  $x_A^s$ . In addition, since every continuous function can be expanded in a series of powers of its independent variable, we may write:

$$\ln f_A^s = a_0 + a_1 x_A^s + a_2 (x_A^s)^2 + \dots \quad (35)$$

However,  $x_A^s \rightarrow 0$  and therefore:

$$\ln f_A^s = a_0 \quad (36)$$

## 6. Final expressions for the retention factor

The analytical expressions of the activity coefficients derived above in combination with the equilibrium Eq. (14) or Eqs. (19) and (20) allow the calculation of the retention factor  $k'$  of a solute as a function of the mol fraction of the modifier in the mobile phase,  $x = x_B^m$ . Note that the activity coefficients of the modifier and the solvent in the mobile phase were calculated by means of Wilson Eqs. (21) and (22), as described below. In what concerns the activity coefficient  $f_A^m$ , depending on the modifier, it was calculated by either Eq. (27) or Eq. (30). Thus the final expressions of the retention factor  $k'$  are the following:

### 6.1. Partition model

If  $f_A^m$  is given by Eq. (27), the retention factor arising from Eqs. (11), (14), (27) and (36) may be expressed as

$$\ln k' = \ln k^0 + D_2^m x + D_3^m x^2 + D_4^m x^3 + \ln \frac{1 - \alpha + \alpha x}{(1 - \alpha)(1 + \delta)} \quad (37)$$

whereas if  $f_A^m$  is given by Eq. (30), we have

$$\ln k' = \ln k^0 + D_2^m x + D_3^m x^2 + \ln \frac{1 - \alpha + \alpha x}{(1 - \alpha)(1 + \delta)} \quad (38)$$

where  $x = x_B^m$ . In both cases,  $\ln k^0 = \ln k' - a_0 + D_1^m + \ln \beta_A^p$ .

### 6.2. Adsorption model

Here for simplicity, we have adopted that the activity coefficients of the constituents of the ad-



sorbed layer are given by Eqs. (30)–(32), with  $\theta_B$  instead of  $x_B$ . This choice implies the acceptance of the mean field approximation for the adsorbed layer structure. Thus, by substitution of Eq. (27) or (30) for  $f_A^m$  and Eqs. (30) and (32) for the ratio  $\ln(f_A^s/f_S^s)$  into Eqs. (12) and (19), we obtain:

$$\begin{aligned} \ln k' = & \ln k^0 + \ln(1 - \theta_B) - D_2^s \theta_B + D_2^m x + D_3^m x^2 \\ & + D_4^m x^3 - \ln f_S^m - \ln(1 - x) \\ & + \ln \frac{1 - \alpha + \alpha x}{(1 - \alpha)(1 + \delta)} \end{aligned} \quad (39)$$

or

$$\begin{aligned} \ln k' = & \ln k^0 + \ln(1 - \theta_B) - D_2^s \theta_B + D_2^m x + D_3^m x^2 \\ & - \ln f_S^m - \ln(1 - x) \\ & + \ln \frac{1 - \alpha + \alpha x}{(1 - \alpha)(1 + \delta)} \end{aligned} \quad (40)$$

where again  $x = x_B^m$  and  $\ln k^0 = \ln k' - D_1^s + D_1^m + \ln \beta_A$ . The surface coverage  $\theta_B$  is calculated from the equilibrium Eq. (20), which may be written as:

$$\ln \frac{\theta_B}{1 - \theta_B} - 2C_0^s \theta_B = \ln \beta_B^* + \ln \frac{f_B^m x}{f_S^m (1 - x)} \quad (41)$$

where  $\ln \beta_B^* = \ln \beta_B - C_0^s$ . Note that the use of the mean field approximation in the adsorbed layer means that  $C_0^s$  is given by  $C_0^s = z[w_{BS} - (w_{BB} + w_{SS})/2]/kT$ , and therefore some molecular information about the intermolecular interactions at the adsorbed layer may be gained by the application of the above equations.

## 7. An alternative expression for $\ln k'$ arising from the adsorption model

If the activity coefficients  $f_B^m$ ,  $f_S^m$  are not known because of the lack of proper vapor pressure data, then we have to use either Eqs. (28) and (29) or Eqs. (31) and (32) for their calculation. Here, we adopted Eqs. (31) and (32) to keep the number of the adjustable parameters to a minimum. In this case, the expression of  $\ln k'$  may be written as follows:

$$\begin{aligned} \ln k' = & \ln k^0 + \ln(1 - \theta_B) - D_2^s \theta_B + D_2^m x \\ & - \ln(1 - x) + \ln \frac{1 - \alpha + \alpha x}{(1 - \alpha)(1 + \delta)} \end{aligned} \quad (42)$$

where  $\theta_B$  is calculated from the adsorption isotherm:

$$\ln \frac{\theta_B}{1 - \theta_B} - 2C_0^s \theta_B = \ln \beta_B^* - 2C_0^m x + \ln \frac{x}{1 - x} \quad (43)$$

Here,  $\ln \beta_B^*$  is given by  $\ln \beta_B^* = \ln \beta_B - C_0^s + C_0^m$ .

Eqs. (42) and (43) were not tested in the present paper, since we used mobile phases of known activity coefficients,  $f_B^m$ ,  $f_S^m$ . They are presented here to complete our treatment on the adsorption mechanism.

## 8. Data sets analyzed

In order to examine several possible cases of retention in reversed-phase columns, we used data taken from literature [44–46] and data obtained experimentally in our laboratory. All data grouped into 10 sets are shown in Table 1. Sets 1, 2 and 3 contain non-polar aromatic solutes with molecular masses ranging from 78 (benzene) to 134 (butylbenzene). Sets 4–7 contain solutes with relatively small molecules with dipole moments ranging from 1.3 D (2-chlorophenol) to 5 D (4-nitrophenol). All the above data sets were taken from literature [44–46]. Experimentally, we determined the retention factors of: (a) some catechol-related solutes in isopropanol (iPrOH)–aqueous buffer mixtures (*set 8*), and (b) the two macrolide antibiotics, clarithromycin (clari) and roxithromycin (roxi) in methanol–aqueous (*set 9*) and acetonitrile–aqueous (*set 10*) buffer mobile phases. The catechol-related solutes used in the present work were three amines: 3-methoxytyramine (3mt), 5-hydroxytryptamine (5ht) and *N* $\omega$ -methylserotonin (m5ht); two acids: 5-hydroxyindole-3-acetic acid (5hiaa) and homovanillic acid (hva); two amino acids: 5-hydroxytryptophan (5htp) and L-tryptophan (tryp); an alcohol, 5-hydroxytryptophol (5htoh) and a glycol, 4-hydroxy-3-methoxyphenyl-glycol (mhpg). The average molecular mass of the catechol-related compounds is about 200, while the macrolides clari and roxi are polar com-

Table 1  
Data sets examined in the present investigation

Set	Column	Modifier	Solutes	Reference
1	Lichrospher 100 RP-18	Methanol	Benzene, toluene, ethylbenzene, propylbenzene	[40]
2	Lichrospher 100 RP-18	Acetonitrile	As in set 1	[40]
3	Hypersil ODS	Isopropanol	Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene	[42]
4	Lichrospher 100 RP-18	Methanol	Nitrobenzene, phenol, 2-methylphenol, 2-, 3-, 4-nitrophenol, 2-, 3-chlorophenol, 2,4-dinitrophenol	[40]
5	Nucleosil 10 RP-18	Methanol	Phenol, 3-, 4-nitrophenol, benzyl alcohol, 4-hydroxybenzaldehyde, 4-nitrobenzaldehyde, 4-fluorophenol, 2-phenylethanol	[41]
6	Lichrospher 100 RP-18	Acetonitrile	As in set 4	[40]
7	Nucleosil 10 RP-18	Acetonitrile	As in set 5	[41]
8	Inertsil ODS-3	Isopropanol	3-Methoxytyramine, 5-hydroxytryptamine, <i>N</i> $\omega$ -methylserotonin, 5-hydroxyindole-3-acetic acid, homovanillic acid, 5-hydroxytryptophan, L-tryptophan, 4-hydroxy-3-methoxyphenylglycol, 5-hydroxytryptophol	Present work
9	Kromasil C <sub>18</sub>	Methanol	Clarithromycin, roxithromycin	Present work
10	Kromasil C <sub>18</sub>	Acetonitrile	As in set 6	Present work

pounds with molecular masses 748 and 837, respectively.

## 9. Experimental

Three experimental data sets were prepared here for the investigation of the effect of the organic modifier concentration on the retention of a variety of solutes on reversed-phase columns. All data sets were measured on a Shimadzu LC-9A HPLC system using a Gilson electrochemical (EC) detector. All separations were carried out isocratically at 25 °C, the injection volume was 50  $\mu$ l of working standard solutions (2  $\mu$ g/ml) and the elution was performed at a flow-rate of 1.0 ml/min. Different mobile phases consisting of an aqueous phosphate buffer (pH 7) and an organic modifier were used. The total ionic strength of the mobile phases was held constant at 0.02 M.

For the catechol-related compounds, nine different isopropanol concentrations were studied, spread over the range 0–20% v/v, as illustrated in Table 2.

Separations were made on a 250 $\times$ 4 mm (5  $\mu$ m Inertsil ODS-3) MZ-Analytical column. The hold-up time,  $t_0$ , was estimated as 1.88 min. The detection of the analytes was performed at 0.8 V versus the Ag/AgCl reference electrode.

For the two macrolide antibiotics, clarithromycin and roxithromycin, the EC response was monitored at 1.0 V versus the Ag/AgCl reference electrode. Separations were made on a MZ-Analysentechnik GMBH 250 $\times$ 4.6 mm I.D. (5  $\mu$ m Kromasil C<sub>18</sub>) column. The hold-up time of this column was found to be 2.26 min. The organic modifier was methanol for one of these data sets and acetonitrile for the other data set. The concentrations of the organic modifiers used are specified in Table 3.

All chemicals were used as received from commercial sources. Catechol-related compounds were available from Sigma or Aldrich. Clari and roxi were provided by Pharmanel, Pharma Industry (Athens, Greece). All other reagents were of analytical grade and solvents of HPLC grade. More experimental details were described in our previous works (e.g. [47,48]).

Table 2  
Retention values ( $\ln k'$ ) of solutes of set 8 in isopropanol–water mobile phase

$\varphi$	$\ln k'$								
	5hiaa	5ht	htoh	5htp	hva	mhpg	m5ht	3mt	tryp
0	2.272	2.926	4.388	2.416	2.361	2.667	3.316	2.929	3.314
0.004	1.814	2.385	3.857	1.944	1.878	2.169	2.693	2.293	2.844
0.01	1.430	1.878	3.371	1.501	1.442	1.716	2.102	1.688	2.421
0.02	0.943	1.273	2.800	0.961	0.935	1.212	1.437	1.019	1.941
0.03	0.571	0.816	2.348	0.550	0.522	0.797	0.944	0.535	1.571
0.05	−0.005	0.182	1.682	−0.050	−0.090	0.273	0.260	−0.120	1.037
0.10	−0.699	−0.565	0.777	−0.710	−0.779	−0.422	−0.546	−0.791	0.324
0.15	−0.994	−0.868	0.295	−0.922	−1.155	−0.908	−0.894	−0.950	−0.087
0.20	−1.211	−1.007	−0.145	−1.104	−1.421	−1.023	−1.055	−1.332	−0.472

## 10. Data analysis

### 10.1. Calculation of modifier and solvent activity coefficients in the mobile phase

The activity coefficients of the modifier B and the solvent S in the mobile phase were calculated by means of Eqs. (21) and (22). The constants  $A_{BS}$  and  $A_{SB}$  needed in these calculations are obtained from Refs. [36,37]

$$A_{ij} = (V_{m,j}/V_{m,i}) \exp(-A_{ij}/RT) \quad (44)$$

where  $V_{m,i}$ ,  $V_{m,j}$  are molar volumes and  $A_{BS}$ ,  $A_{SB}$  are Wilson's constants. These constants in  $\text{cal mol}^{-1}$  can be taken from literature. Thus, for the mobile phase methanol (B)–water (S) we used  $A_{BS} = -66.3736$ ,

$A_{SB} = 450.8741$  [49] and for the mixture isopropanol–water, we adopted the values  $A_{BS} = 736.6886$ ,  $A_{SB} = 1163.93$  [50]. To our knowledge for the mixture acetonitrile–water there is a lack of values of  $A_{BS}$ ,  $A_{SB}$  at 25 °C. This system has been studied at 50 and 60 °C [51,52]. For this reason, we adopted the values  $A_{BS} = 609.3806$ ,  $A_{SB} = 1507.466$  calculated from the data at 50 °C [51], taking into account that  $A_{BS}$ ,  $A_{SB}$  are in principle independent of temperature. This necessary choice shows that the calculated activity coefficients for the acetonitrile–water system are approximate.

Based on the above values of  $A_{ij}$ , the following values for  $A_{BS}$ ,  $A_{SB}$  were calculated: mobile phase methanol–water  $A_{BS} = 0.49627$ ,  $A_{SB} = 1.05303$ ; mobile phase acetonitrile–water  $A_{BS} = 0.12209$ ,  $A_{SB} = 0.22996$ ; and mobile phase isopropanol–water  $A_{BS} = 0.06772$ ,  $A_{SB} = 0.59719$ . The activity coefficients of the modifier B and solvent S calculated from Eqs. (21) and (22) are shown in Fig. 1 by points. The lines in the same figure have been calculated by means of Eqs. (28) and (29) or Eqs. (31) and (32) using a non-linear least squares fitting. In particular, we found that the methanol–water mixture can be satisfactorily described by Eqs. (31) and (32), whereas these equations are inappropriate for the acetonitrile–water and isopropanol–water mixtures. The two last mixtures can be described by Eqs. (28) and (29).

The whole data analysis has been carried out with Microsoft Excel spreadsheets using Solver for the curve fitting procedure. The results obtained are:

$$\text{MeOH-H}_2\text{O}: C_0^m = 0.5523, \text{SSR} = 0.033$$

Table 3  
Retention values ( $\ln k'$ ) of solutes of set 9 in methanol–water and of set 10 in acetonitrile–water mobile phase

$\varphi_{\text{MeOH}}$	$\ln k'$		$\varphi_{\text{ACN}}$	$\ln k'$	
	clari	roxi		clari	roxi
0.575	3.646	4.124	0.30	4.163	4.521
0.600	3.377	3.816	0.35	3.286	3.542
0.625	3.095	3.491	0.40	2.697	2.884
0.650	2.827	3.187	0.45	2.249	2.393
0.675	2.557	2.882	0.50	1.928	2.027
0.700	2.265	2.549	0.55	1.600	1.697
0.725	1.978	2.225	0.60	1.438	1.465
0.750	1.705	1.918			
0.775	1.435	1.613			
0.800	1.163	1.314			
0.825	0.904	1.030			

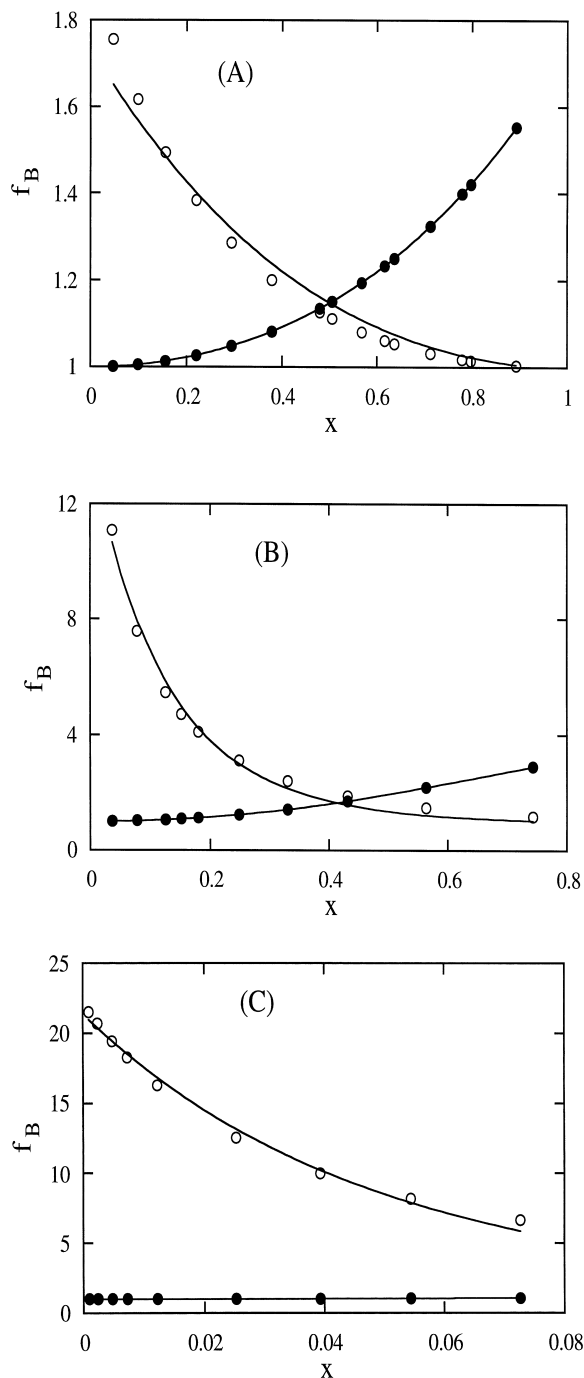


Fig. 1. Plots of the activity coefficient of modifier (○) and solvent (●) versus modifier molar fraction in (A) methanol–water, (B) acetonitrile–water and (C) isopropanol–water mobile phase. Points are experimental data obtained from Eqs. (21), (22) and (44). Curves have been calculated by means of Eqs. (31) and (32) (A) or Eqs. (28) and (29) (B, C) using a least squares fitting.

$$\text{ACN-H}_2\text{O}: C_0^m = 1.915, C_1^m = -0.7425,$$

$$\text{SSR} = 0.985$$

$$\text{iPrOH-H}_2\text{O}: C_0^m = -0.4034, C_1^m = -3.4717,$$

$$\text{SSR} = 1.853$$

where SSR is the sum of squares of residuals. Note that if Eqs. (28) and (29) are used for the mixture of methanol–water, the fit is much better ( $\text{SSR} = 0.0006$ ) and gives  $C_0^m = 0.53$  and  $C_1^m = -0.1$ .

It is worth noting that the activity coefficients of the constituents of the methanol–water mixture are satisfactorily described by Eqs. (31) and (32), which shows that for this mixture the random mixing approximation (mean field approximation) may be valid. However, the validity of this approximation and consequently the validity of the regular solution theory is likely to be phenomenological, because this theory under mean field approximation is strictly referred to mixtures of non-polar and non-associated molecules [38,39,53]. However, within the framework of this model, we can easily explain the sign of  $C_0^m$ . This parameter is given by  $C_0^m = z[w_{BS} - (w_{BB} + w_{SS})/2]/kT$ . In aqueous solutions the strongest interactions are those among the water molecules due to hydrogen bonds. Hence the sign of  $C_0^m$  should be determined mainly by the term  $w_{SS}$ , which is a negative number and consequently the sign of  $C_0^m$  is expected to be positive.

Unlike the methanol–water mixtures, the random mixing approximation does not hold at all for acetonitrile–water and isopropanol–water mixtures, since these mixtures are not described by Eqs. (31) and (32). This conclusion has a significant impact in Dill's model for the partition mechanism. Dill's model [2] is based on the random mixing approximation for the mobile phase. Moreover, Dill et al. [4] used a great number of experimental data to verify this model and in most cases these data concern aqueous solutions with modifiers methanol or acetonitrile. Consequently, their tests for the validity of the partition model are to some extent questionable, since the random mixing approximation is not valid at least for aqueous mobile phases modified with acetonitrile. In contrast, we should point out that the extension of the partition model presented here is not subject to the limitations of the random mixing

approximation and consequently it can be tested in every aqueous mobile phase.

### 10.2. Solute activity coefficients in the mobile phase

The test of the partition and adsorption models becomes significantly more reliable if the limiting activity coefficients of solutes in the mobile phase are known from independent measurements. Then using Eq. (27) or Eq. (30), the parameters  $D_2^m$ ,  $D_3^m$ ,  $D_4^m$  appearing in Eqs. (37)–(40) can be determined.

Cheong and Carr [46] have used headspace gas chromatography to determine the limiting activity coefficients of some alkylbenzenes in hydro-organic solvents. For the purposes of our work we focused our attention on the activity coefficients of benzene, toluene, ethylbenzene, propylbenzene and butylbenzene in methanol–water, acetonitrile–water and isopropanol–water mixtures. It is seen that these alkylbenzenes are the solutes of sets 1–3 studied in the present paper.

The activity coefficients of the above alkylben-

zenes were fitted to Eq. (27) or (30). In particular, Eq. (27) was used to fit  $\ln f_A$  values obtained in acetonitrile–water and isopropanol–water mixtures and Eq. (30) for data obtained in methanol–water mixtures. The results obtained are depicted in Table 4.

Plots of calculated and experimental  $\ln f_A$  versus  $x$  as well as the values of SSR of Table 4 show that, depending on the mobile phase, Eq. (27) or Eq. (30) describes satisfactorily the experimental data. However, the most interesting point is the following. As shown in Section 5, coefficient  $D_3^m$  of Eq. (30) is given either by  $D_3^m = C_0^m$  or  $D_3^m = C_0^m - A_1^m - B_1^m$ , depending on whether the ternary mixture of an alkylbenzene, methanol and water is subject to the mean field approximation or not. Since it was found that  $C_0^m = 0.55$  for the binary mixture of methanol–water, we conclude that  $D_3^m$  should be given by  $D_3^m = C_0^m - A_1^m - B_1^m$ . Therefore, while the mean field approximation seems to be valid in the mixture of methanol–water, the presence of alkylbenzenes makes this approximation invalid. Once more, we observe that the mean field approximation is margi-

Table 4  
Linear regression results for solute activity coefficients in three mobile phases

Solute <sup>a</sup>	$D_2^{mb}$	$D_3^{mb}$	$D_4^{mb}$	SSR <sup>b</sup>	$D_2^{mc}$	$D_3^{mc}$	$D_4^{mc}$	SSR <sup>c</sup>	SSR <sup>d</sup>
MeOH–H <sub>2</sub> O									
B	–11.88	5.54		0.018	–10.97	4.92		0.067	1.56
T	–13.71	6.48		0.023	–13.51	6.42		0.034	1.02
EB	–15.67	7.44		0.035	–15.39	7.29		0.046	1.01
PB	–19.42	10.14		0.018	–19.52	10.27		0.019	0.37
BB	–20.90	10.46		0.048	–	–		–	–
ACN–H <sub>2</sub> O									
B	–20.91	26.62	–12.98	0.021	–20.20	26.28	–13.19	0.068	1.39
T	–24.10	31.67	–15.79	0.048	–23.67	31.53	–16.00	0.067	1.08
EB	–27.75	37.29	–18.81	0.074	–27.56	37.52	–19.21	0.084	1.76
PB	–30.44	40.56	–20.30	0.132	–30.36	41.04	–20.87	0.142	1.58
BB	–34.96	48.42	–24.88	0.164	–	–	–	–	–
iPrOH–H <sub>2</sub> O									
B	–26.13	41.55	–23.54	0.107	–25.81	40.47	–22.74	0.114	1.00
T	–31.19	51.22	–29.53	0.188	–30.90	50.30	–28.91	0.195	1.34
EB	–35.75	59.96	–35.04	0.327	–35.61	59.48	–34.76	0.333	1.72
PB	–41.27	71.11	–42.36	0.464	–41.18	70.71	–42.18	0.474	2.78
BB	–45.16	78.06	–46.46	0.644	–45.42	78.53	–46.94	0.661	4.67

<sup>a</sup> B, benzene; T, toluene; EB, ethylbenzene; PB, propylbenzene; BB, butylbenzene.

<sup>b</sup> Results obtained from fitting  $\ln f_A$  data only.

<sup>c</sup> Results obtained from simultaneous fitting of  $\ln f_A$  and  $\ln k'$  data.

<sup>d</sup> Values concerning  $\ln f_A$  obtained from fitting  $\ln k'$  data as described in the text.

nally valid for aqueous mobile phases and this observation raises serious questions about the suitability of Dill's treatment of the partition model [2,4].

For the other two mobile phases we similarly observe the following. The last coefficient of Eq. (27) may be given either by  $D_4^m = 4C_1^m$  or  $D_4^m = 4C_1^m + A_2^m - B_2^m$ . In the first case the binary mixture of the modifier and solvent and the corresponding ternary mixture with the addition of an alkylbenzene are described by the same  $p=1$  value for Eq. (26), whereas in the second case the ternary mixture requires the use of  $p=2$ . The experimental data clearly show that the ternary mixtures, even in the limit of  $x_A \rightarrow 0$ , are described using a different value of  $p$  ( $p=2$ ) from that of the corresponding binary mixtures ( $p=1$ ), since  $D_4^m$  was found to be different from  $4C_1^m$  in all cases.

### 10.3. Calculation of the contraction of volume

Parameter  $\delta$ , which expresses the relative contraction of volume after mixing the solvent S with the modifier B, can be calculated from the following equation:

$$\delta = (V_{th} - V_{exp})/V_{th} \quad (45)$$

where

$$V_{th} = x_B M_B / d_B + (1 - x_B) M_S / d_S \quad (46)$$

and

$$V_{exp} = (x_B M_B + (1 - x_B) M_S) / d \quad (47)$$

$d$  being the density of solution. Thus from density measurements, the parameter  $\delta$  can readily be calculated. We found that for the systems we examined,  $\delta$  may be expressed by the following relationships, provided that the volume is expressed in  $\text{cm}^3$ :

$$\begin{aligned} \text{MeOH-H}_2\text{O}: \delta &= 0.1031x_B^3 - 0.3022x_B^2 \\ &+ 0.1999x_B - 0.0011 \end{aligned}$$

$$\begin{aligned} \text{ACN-H}_2\text{O}: \delta &= 0.3051x_B^3 - 0.4678x_B^2 + 0.1835x_B \\ &+ 0.0012 \end{aligned}$$

$$\text{iPrOH-H}_2\text{O}: \delta = -0.2437x_B^2 + 0.329x_B - 0.0005$$

Note that, in general, the effect of this parameter in

the calculated values of  $\ln k'$  from Eqs. (37)–(40) and (42) is very small.

### 10.4. Fitting experimental to calculated $\ln k'$ data

The theoretical expressions of  $\ln k'$  derived in the previous sections depend directly or not on a number of adjustable parameters ranging from one [ $\ln k^0$  in Eqs. (37) and (38) provided that  $D_2^m, D_3^m, D_4^m$  are known from independent measurements] to seven [ $\ln k^0, \ln \beta_B^*, D_2^m, D_3^m, D_4^m, D_2^s$  and  $C_0^s$  in Eqs. (39) and (41) provided that  $D_2^m, D_3^m, D_4^m$  are unknown].

The adjustable parameters that correspond to the partition model can be determined by means of a simple linear least squares fitting. In contrast, the determination of the adsorption model adjustable parameters requires the use of a non-linear least squares fitting of the experimental values of  $\ln k'$  to the corresponding calculated ones. Note that this fitting procedure requires the simultaneous solution of the non-linear Eq. (41) with respect to  $\theta_B$ . For the solution of this equation we adopted two techniques: the iterative method and the grand ensemble expression for  $\theta_B$ . The iterative relationship used arises directly from Eq. (41) and it was the following:

$$\theta_B^{i+1} = \frac{A \exp(2C_0^s \theta_B^i)}{1 + A \exp(2C_0^s \theta_B^i)} \quad (48)$$

where  $A = \beta_B^* f_B^m x / f_S^m (1 - x)$ . To start the iteration, we used  $\theta_B^0 = 0$ . Normally after 25 iterations, the values of  $\theta_B$  obtained from Eq. (48) converge to the equilibrium value of  $\theta_B$ . However, for values of  $C_0^s$  close to or higher than its critical value ( $C_{0,cr}^s = 2$ ), convergence may not be observed. In such cases,  $\theta_B$  was calculated from the following equation [54]:

$$\theta_B = \frac{\sum_{N=1}^M N A^N \exp(N^2 C_0^s / M) / \{N!(M-N)!\}}{M \sum_{N=1}^M A^N \exp(N^2 C_0^s / M) / \{N!(M-N)!\}} \quad (49)$$

where  $A$  is given by the same expression as in Eq. (48), and  $M$  is the maximum number of molecules in the system. Due to computational problems in  $(M-N)!$ , Eq. (49) has been applied using  $M$  values ranging from 100 to 1000 [55]. In the present paper,

we used  $M = 170$ , since Microsoft Excel 2000 cannot calculate  $M!$  values when  $M$  is higher than 170.

### 10.5. Calculation of parameters $\beta_B^*$ and $C_0^s$ of the adsorption model

The theoretical equations describing the retention within the framework of the adsorption model contain two adjustable parameters that characterize the behavior of modifier in the column. These parameters are the equilibrium constant  $\beta_B^*$  and the interaction parameter at the adsorbed layer  $C_0^s$ . Therefore, these parameters should be independent of the solute and they can be determined using the following strategy. At each data set, which corresponds to a certain column and mobile phase modified with a certain organic solvent, the experimental data were fitted to the relevant equations using all the unknown parameters as adjustable parameters. Then the average value and the standard deviation of  $\beta_B^*$  and  $C_0^s$  were determined. Any extreme values of these parameters were ignored in the calculation of their average values. The (average) values of  $\beta_B^*$  and  $C_0^s$  obtained in this way were used in order to refit the experimental data, using as adjustable parameters the rest of the unknown parameters.

## 11. Results and discussion

### 11.1. Partition model

We first examined the applicability of the partition model to the retention of benzene, toluene, ethylbenzene and propylbenzene from aqueous solutions of methanol, acetonitrile and isopropanol, as well as of butylbenzene from aqueous solutions of isopropanol, since for these systems the values of  $D_2^m$ ,  $D_3^m$ ,  $D_4^m$  are known, and are listed in Table 4. Therefore the fundamental equation of the partition model, that is Eq. (37) or (38), has just one adjustable parameter, parameter  $\ln k^0$ , which can be determined by a simple linear regression. Note that Eq. (37) has been used for aqueous mobile phases modified with acetonitrile and isopropanol (data sets 2 and 3) and Eq. (38) for the mobile phase using methanol as organic modifier (data set 1).

The results obtained concerning the quality of

Table 5

Linear regression results of SSR for fitting  $\ln k'$  of sets 1–3 to Eq. (37) or (38)

Solute <sup>a</sup>	MeOH–H <sub>2</sub> O		ACN–H <sub>2</sub> O		iPrOH–H <sub>2</sub> O	
	SSR <sup>b</sup>	SSR <sup>c</sup>	SSR <sup>b</sup>	SSR <sup>c</sup>	SSR <sup>b</sup>	SSR <sup>c</sup>
B	0.391	0.166	0.278	0.106	0.088	0.072
T	0.058	0.029	0.099	0.051	0.115	0.099
EB	0.074	0.048	0.071	0.048	0.160	0.146
PB	0.006	0.005	0.071	0.047	0.265	0.241
BB	–	–	–	–	0.419	0.382

<sup>a</sup> B, benzene; T, toluene; EB, ethylbenzene; PB, propylbenzene; BB, butylbenzene.

<sup>b</sup> Fitting  $\ln k'$  to Eq. (37) or (38) using  $D_2^m$ ,  $D_3^m$ ,  $D_4^m$  from Table 4.

<sup>c</sup> Simultaneous fitting of  $\ln k'$  and  $\ln f_A$  to Eqs. (27) and (37) or Eqs. (30) and (38).

fitting, i.e. the values of SSR, are shown in Table 5. A clearer picture is obtained if we plot experimental and calculated values of  $\ln k'$  versus  $x$ . Fig. 2 shows some of these plots. It is seen that the partition model describes satisfactorily the experimental data of sets 1 to 3. The deviations between experimental and calculated values of  $\ln k'$  are small and fall within the experimental error.

The quality of fitting can be improved if we proceed to a simultaneous fitting of the experimental  $\ln f_A$  and  $\ln k'$  values to Eqs. (27) and (37) or Eqs. (30) and (38). The results obtained are shown in Tables 4 and 5. Table 4 depicts the values of  $D_2^m$ ,  $D_3^m$ ,  $D_4^m$  and the quality of fitting of the  $\ln f_A$  data, and Table 5 shows the quality of fitting of the  $\ln k'$  data. Thus the SSR values of Table 4 are referred to  $\ln f_A$  values and those of Table 5 to  $\ln k'$  values. As expected, this procedure yields even better fittings for the  $\ln k'$  data.

It is surprising that the equations we propose for the partition model when we use  $D_2^m$ ,  $D_3^m$ ,  $D_4^m$  values taken from an independent experiment and adjust just one parameter ( $\ln k^0$ ), describe very satisfactorily the retention behavior of data sets 1–3. This is strong evidence or even a proof about the validity of the partition mechanism in the case of retention of small and non-polar molecules in reversed-phase liquid chromatographic columns.

At this point, it is interesting to examine the effect of the omission of the last term of Eq. (37) or (38), which corresponds to the use of the lattice model approximation for the mobile phase. This approxi-

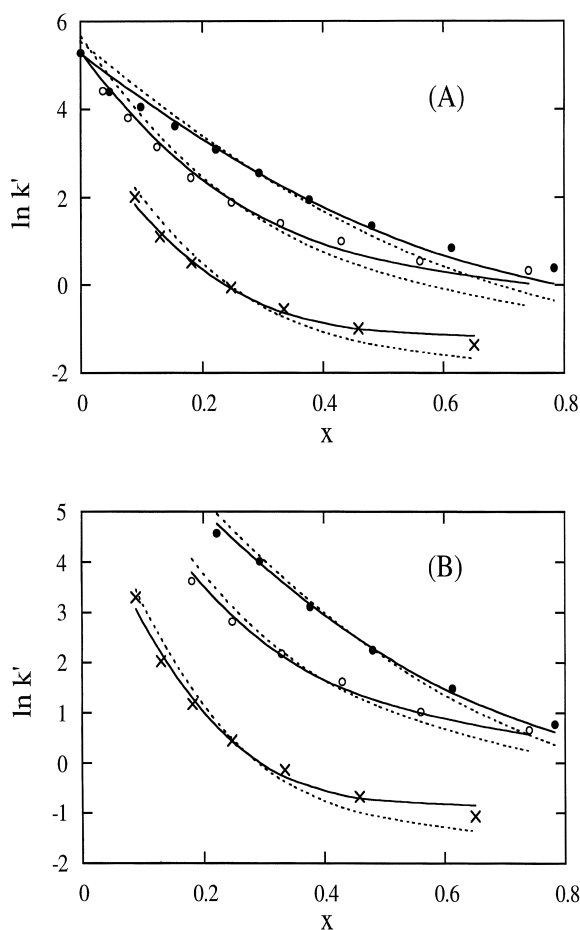


Fig. 2. Retention plots of benzene (A) and ethylbenzene (B) in aqueous mobile phases modified with methanol (●), acetonitrile (○) and isopropanol (×). Solid curves have been calculated from the partition model, Eq. (37) (○, ×) and Eq. (38) (●), using for  $D_2^m, D_3^m, D_4^m$  the relevant values of Table 4 indicated by “b” plus the following values for  $\ln k^0$ : (A) 5.27 (●), 5.31 (○), 3.62 (×); (B) 7.67 (●), 7.43 (○), 5.56 (×). Dotted curves have been calculated from the same equations by the omission of their last term using for  $D_2^m, D_3^m, D_4^m$  the same values as above and the following values for  $\ln k^0$ : (A) 5.55 (●), 5.68 (○), 4.23 (×); (B) 8.08 (●), 7.98 (○), 6.17 (×).

mation has been widely used in previous studies. Note that this omission reduces Eq. (38) to Dill’s equation [2]:

$$\ln k' = \ln k^0 + D_2^m x + D_3^m x^2 \quad (50)$$

The broken curves in Fig. 2 have been calculated from Eq. (37) or (38) by the omission of their last

term. It is seen that this term has a significant contribution to  $\ln k'$  when  $x$  varies over a wide range of values and its omission by itself introduces a considerable error in the results obtained. Note that in the absence of the last term the experimental values of  $\ln k'$  lie systematically below the theoretical curve at low  $x$  values and above that curve at high  $x$  values. Thus one could erroneously conclude that, due to the systematic deviations between experimental and calculated values of  $\ln k'$ , there must be some (unknown) factor which is responsible for these deviations and not taken into account in the partition model. Consequently, if we do not take into account this term, we cannot conclude about the validity of the partition mechanism.

Finally, we should point out that for sets 1–3, the values of  $D_2^m, D_3^m, D_4^m$  obtained from fitting  $\ln k'$  data to Eq. (37) or Eq. (38) can be used to calculate  $\ln f_A^m$  by means of Eq. (27) or (30). In fact for the calculation of  $\ln f_A^m$ , there is one unknown coefficient,  $D_1^m$ , which can be obtained from the minimization of the sum of squares of the residuals  $\ln f_A^m(\text{calc}) - \ln f_A^m(\text{exp})$ . This minimization process has been carried out using Solver and the values obtained for SSR are depicted in the last column of Table 4. It is seen that the calculated values of  $\ln f_A^m$  do not describe satisfactorily the experimental data, which means that the values of  $D_2^m, D_3^m, D_4^m$  obtained from the fitting of  $\ln k'$  data are not correct. Therefore, knowledge of the values of  $D_2^m, D_3^m, D_4^m$  by independent measurements is a necessary element to obtain a correct picture about the validity of the partition mechanism.

To complete the treatment of the partition mechanism we examined the applicability of Eq. (37) or (38) to all data sets using  $D_2^m, D_3^m$  and  $D_4^m$  as adjustable parameters. Selected results are shown in Tables 6–9. We found that, due to the simple shape of the  $\ln k'$  versus  $x$  curves, Eq. (37) or Eq. (38) describe satisfactorily all data sets except set 8 (see also Fig. 3). It is reasonable to accept that the abnormal shape of the calculated  $\ln k'$  versus  $x$  curves for the solutes of set 8 (Fig. 3) is due to the fact that the partition mechanism is not valid for the retention of the catechol-related compounds, since the size of the molecules of these compounds is comparable to that of the chains of the hydrocarbon layer and therefore it is impossible for these solutes



Table 6  
Fitted parameters of Eq. (37) for data set 6

Solute	$\ln k^0$	$D_2^m$	$D_3^m$	$D_4^m/4$	SSR
Phenol	3.53	-21.57	38.82	-6.16	0.095
2-Methylphenol	4.81	-27.44	49.88	-7.94	0.155
Nitrobenzene	5.00	-22.55	33.61	-4.65	0.028
2-Chlorophenol	4.99	-28.38	50.98	-7.98	0.089
3-Chlorophenol	5.04	-25.69	41.48	-6.02	0.039
2-Nitrophenol	4.53	-20.77	30.52	-4.19	0.008
3-Nitrophenol	4.66	-27.94	50.91	-8.06	0.122
4-Nitrophenol	4.56	-27.79	50.91	-8.09	0.126
2,4-Dinitrophenol	4.82	-25.95	42.96	-6.36	0.037

to occupy cavities inside this layer. For the same reason, we cannot accept that the retention of the solutes of sets 9 and 10 is governed by the partition mechanism. However, we observe (Table 9) that Eq. (37) or (38) of the partition model describe absolutely satisfactorily the experimental data of these sets. This is a clear indication that the validity of an equation does not necessarily entail the validity of the model that it is based on. For this reason we cannot verify if the partition model explains at a molecular level the retention of the solutes of sets 4–7. The fits concerning these sets are very good ( $SSR < 0.1$ ), but we have no other elements to verify the applicability of the partition model at a molecular level.

The discussion presented above reveals that the only secure conclusions about the validity of the partition mechanism should be based on the retention data of alkylbenzenes, since for these solutes the activity coefficients in the mobile phase are known from an independent source of data. For these solutes their retention in reversed-phase liquid chromatographic columns is very likely to be governed by the partition mechanism. The retention of large mole-

Table 8  
Fitted parameters of Eq. (37) for data set 8

Solute	$\ln k^0$	$D_2^m$	$D_3^m$	$D_4^m/4$	SSR
5hiia	2.08	-251.76	6894.55	-15 793.5	0.089
5ht	2.68	-307.01	8676.52	-19 949.6	0.170
5htoh	4.16	-297.32	8000.21	-18 602.4	0.137
5htp	2.22	-279.13	8002.26	-18 625.2	0.105
hva	2.15	-269.44	7446.20	-17 277.4	0.122
mhpq	2.42	-257.91	6873.12	-15 428.6	0.191
m5ht	3.02	-340.98	9728.58	-22 522.6	0.251
3mt	2.63	-353.49	10 908.90	-26 715.9	0.233
tryp	3.09	-250.88	6890.67	-16 285.9	0.124

cules, like those of the catechol-related solutes of set 8 and especially the retention of clarithromycin and roxithromycin of sets 9 and 10, cannot be described in principle by the partition mechanism. In this case the retention should be due to adsorption, as discussed below. The question is which is the predominant mechanism for the retention of small but polar molecules, like those of sets 4–7. The treatment presented above shows that the equations of the partition model describe satisfactorily the retention data of these sets. However, we have no additional evidence that this description entails the validity of the partition model.

### 11.2. Adsorption model

The major problem in testing the adsorption model is the great number of adjustable parameters. Thus, when  $D_2^m$ ,  $D_3^m$ ,  $D_4^m$  are known, there are four adjustable parameters, whereas the corresponding partition model has one adjustable parameter. This drawback, in combination with the use of non-linear least-squares fitting needed to test the adsorption model, is expected to make it difficult to answer the

Table 7  
Fitted parameters of Eq. (37) for data set 7

Solute	$\ln k^0$	$D_2^m$	$D_3^m$	$D_4^m/4$	SSR
Phenol	2.86	-19.32	26.30	-3.55	0.021
Benzylalcohol	2.75	-21.62	34.55	-5.12	0.058
4-Fluorophenol	3.41	-21.44	28.74	-3.79	0.027
4-Hydroxybenzaldehyde	2.75	-21.62	34.55	-5.12	0.058
2-Phenylethanol	3.30	-22.89	35.30	-4.99	0.012
3-Nitrophenol	4.06	-24.90	36.10	-5.10	0.032
4-Nitrophenol	3.73	-21.85	26.76	-3.17	0.107
4-Nitrobenzaldehyde	3.71	-22.97	34.68	-5.09	0.072

Table 9  
Fitted parameters of Eqs. (37) and (38) for data sets 9 and 10

Solute	$\ln k^0$	$D_2^m$	$D_3^m$	$D_4^m/4$	SSR
Clari (set 9)	9.03	-18.15	7.83	-	0.0003
Roxi (set 9)	10.39	-21.15	9.57	-	0.0005
Clari (set 10)	11.18	-86.60	261.29	-70.4	0.0031
Roxi (set 10)	12.69	-102.00	319.49	-89.5	0.0019

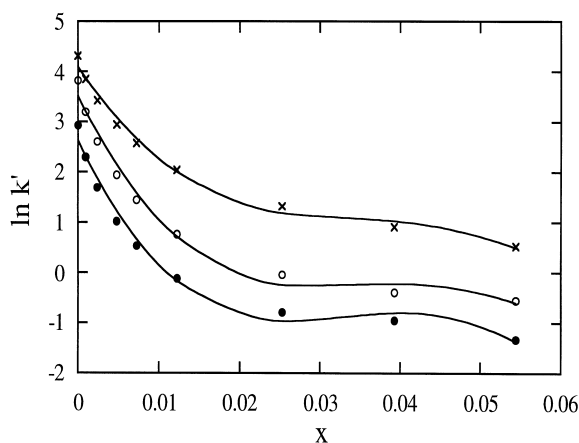


Fig. 3. Retention plots of 3mt (●), 5mht (○) and tryptophan (×) in isopropanol–water mobile phase. Curves have been calculated from partition model using Eq. (37) and the relevant parameters of Table 8. Data for 5mht and tryptophan have been shifted along the y-axis by +0.5 and +1, respectively.

question whether the description of a data set by the equations of the adsorption model entails undoubtedly the validity of the adsorption mechanism or not.

To test the adsorption model, the procedure described in Sections 10.4 and 10.5 was followed. Thus the (average) values of  $\beta_B^*$  and  $C_0^s$  were first determined for each data set. The results obtained, i.e. the fitted values of  $\beta_B^*$  and  $C_0^s$  as well as their standard deviations, are shown in Table 10. In respect to this table, the following points should be clarified:

- Data sets 1–3 were analyzed using for  $D_2^m$ ,  $D_3^m$ ,  $D_4^m$  the values in Table 4 obtained from fitting  $\ln f_A$  data.
- Data sets 2, 6, 7 and 10, i.e. the retention data obtained in aqueous mobile phases modified with acetonitrile, showed good numerical behavior and the values of  $\beta_B^*$  and  $C_0^s$  were determined without problems. It is interesting to point out the small

Table 10  
Fitted parameters  $\beta_B^*$  and  $C_0^s$  for all data sets according to the adsorption model

	Set 1	Set 4	Set 5	Set 9
MeOH–H <sub>2</sub> O				
$\beta_B^*$	0.80±0.5	(0.80)	1.05±0.11	0.80±0.02
$C_0^s$	(0.25)	(0.25)	0.25±0.13	(0.25)
	Set 2	Set 6	Set 7	Set 10
ACN–H <sub>2</sub> O				
$\beta_B^*$	0.17±0.05	0.18±0.3	0.18±0.3	0.22±0.3
$C_0^s$	1.88±0.20	1.9±0.05	1.86±0.05	1.84±0.05
	Set 3	Set 8		
iPrOH–H <sub>2</sub> O				
$\beta_B^*$	0.25±0.01	(a) 0.24±0.02, (b) 2.15±0.60		
$C_0^s$	2.03±0.10	(a) 2.20±0.10, (b) -1.20±0.40		

values of their standard deviations and the fact that the values of  $\beta_B^*$  and  $C_0^s$  are almost the same in the four data sets.

- (c) A similar behavior was found for sets 3 and 8, where the mobile phase is modified with isopropanol. However, for set 8, Solver converged to two solutions: (i)  $\beta_B^* = 0.24 \pm 0.02$ ,  $C_0^s = 2.20 \pm 0.10$  and (ii)  $\beta_B^* = 2.15 \pm 0.60$ ,  $C_0^s = -1.20 \pm 0.40$ . For reasons explained below, the second solution is rejected.
- (d) In contrast to the above sets, all retention data obtained in methanol–water mobile phases showed the worst numerical behavior. The fitting procedure could not determine the values of  $\beta_B^*$ ,  $C_0^s$  for sets 1, 4 and 9 under all circumstances. The values  $\beta_B^* = 1.05 \pm 0.11$  and  $C_0^s = 0.25 \pm 0.13$ , depicted in Table 10 for set 5, were determined only when we used as initial values for  $D_2^m$ ,  $D_3^m$  the values determined from the partition model. Since the adsorbed layer is formed on the same substrate, i.e. on hydrocarbon chains, and  $C_0^s$  is given by  $C_0^s = z[w_{BS} - (w_{BB} + w_{SS})/2]/kT$ , it is reasonable to assume that the value of  $C_0^s$  should be almost the same at sets 1, 4, 5 and 9. For this reason, we assumed that the value  $C_0^s = 0.25$  is also valid for sets 1, 4 and 9, where we could not determine the value of  $C_0^s$  by the fitting procedure. This value of  $C_0^s$  for sets 1, 4, 9 is enclosed within brackets to indicate that it does not arise from a certain statistical analysis. The value  $C_0^s = 0.25$  for sets 1, 4, 9 was further used for the determination of  $\beta_B^*$  following the same fitting procedure as that described in Section 10.5. We found that this procedure was effectively applied only to sets 1 and 9. The values of  $\beta_B^*$  determined in this way and their standard deviations are also listed in Table 10. Note that the value of  $\beta_B^*$  is the same for sets 1, 9 and close to that of set 5. For this reason the value  $\beta_B^* = 0.8$  was also adopted for set 4. The poor numerical behavior of sets 1, 4, 5 and 9 is attributed to the combination of two factors: (a) The sets have relatively few data points, and (b) the plots of  $\ln k'$  versus  $x$  exhibit the smallest curvature.

It is seen that for the acetonitrile–water mobile phases, where the fitting procedure was applied without problems,  $\beta_B^*$  varies in the very narrow region from 0.18 to 0.22 and  $C_0^s$  from 1.84 to 1.90.

Since these two parameters describe the adsorption behavior of acetonitrile on the stationary phase, we readily conclude that the adsorption isotherm of acetonitrile depends weakly upon the column. This is quite reasonable, since the columns are of the same type and therefore the adsorbed layer is not expected to differ significantly from one set to another. Another interesting point is the value of  $C_0^s$ , which is about 1.85. For the adsorbed layer, we have adopted the mean field approximation and hence  $C_0^s$  is given by  $C_0^s = z[w_{BS} - (w_{BB} + w_{SS})/2]/kT$ . Therefore, the value  $C_0^s \approx 1.85$  shows the existence of strong hydrophobic interactions at the adsorbed layer [38,39,53]. Note that similarly strong hydrophobic interactions determine the properties of the mobile phase causing the appearance of strong positive deviations from Raoult's law. These deviations are clearly shown if we construct the plot of  $a_{ACN}$  versus  $x_{ACN}$  from the activity coefficients data of acetonitrile and water calculated in Section 5.

A similar behavior is observed in the case of isopropanol–water mobile phase if we take into account the first solution of Solver for set 8. In fact the second solution, i.e. the values  $\beta_B^* = 2.15 \pm 0.60$ ,  $C_0^s = -1.20 \pm 0.40$ , can hardly be justified on the basis of molecular arguments. The value  $C_0^s = -1.20$  means negative deviations from Raoult's law at the adsorbed layer, whereas the same system exhibits strong positive deviations from Raoult's law at the mobile phase. The latter conclusion arises if we construct the  $a_{isopropanol}$  versus  $x_{isopropanol}$  plot. Negative deviations from Raoult's law mean that the B–S interactions are attractive and stronger than the interactions among the water molecules. Taking into account that the B–S interactions are hydrophobic and the water molecules form hydrogen bonds, we readily conclude that negative deviations cannot be observed at the adsorbed layer. We should also point out that the second solution of Solver is associated with positive values of  $D_2^s$ , higher than 8. However,  $D_2^s = A_0 - B_0 - C_0 = z(w_{AB} - w_{AS} - w_{BS} + w_{SS})/kT$  and therefore such positive values cannot be explained for the same reason that  $C_0^s$  cannot take negative values. For the above reasons as well as because the first solution of Solver, i.e. the values  $\beta_B^* = 0.24 \pm 0.02$ ,  $C_0^s = 2.2 \pm 0.1$  are in agreement with the corresponding solution of set 3, the solution of  $\beta_B^* = 2.15$ ,  $C_0^s = -1.2$  was rejected.

Table 11

Fitted parameters of Eqs. (39) and (40) for data sets 1–3 using  $\beta_B^*$  and  $C_0^s$  values from Table 10

Solute <sup>a</sup>	MeOH–H <sub>2</sub> O			ACN–H <sub>2</sub> O			iPrOH–H <sub>2</sub> O		
	ln $k^0$	$D_2^s$	SSR	ln $k^0$	$D_2^s$	SSR	ln $k^0$	$D_2^s$	SSR
B	5.05	–1.01	0.104	4.95	–1.74	0.239	6.37	0.45	0.006
T	6.36	–0.55	0.006	5.74	–2.09	0.026	4.60	–2.24	0.026
EB	7.43	–0.80	0.021	6.74	–2.15	0.011	5.67	–2.05	0.047
PB	9.30	–0.38	0.004	7.77	–2.15	0.011	6.61	–2.14	0.042
BB	–	–	–	–	–	–	7.56	–2.02	0.090

<sup>a</sup> B, benzene; T, toluene; EB, ethylbenzene; PB, propylbenzene; BB, butylbenzene.

The values  $C_0^s=2.04$  and  $2.20$  are above the critical value of  $C_0^s$ , which is equal to 2, and hence they show that the adsorption of isopropanol on the chains of the hydrocarbon layer is accompanied by a surface phase transition. This phenomenon may take place or these values may indicate that the adsorption isotherm (Eq. (41)) may not be the most suitable to describe the adsorption features of isopropanol. An obvious extension of Eq. (41) results from the use of Eqs. (28) and (29) instead of Eqs. (31) and (32). The resulting adsorption isotherm may be expressed as:

$$\ln \frac{\theta_B}{1 - \theta_B} - 2C_0^s \theta_B + 6C_1^s \theta_B (1 - \theta_B) = \ln \beta_B^* + \ln \frac{f_B^m x}{f_S^m (1 - x)} \quad (51)$$

However, we found that the iterative method for solving this equation does not converge for all possible values of  $C_0^s$  and  $C_1^s$  and therefore we cannot fully investigate the properties of the adsorbed layer using this isotherm. In any case the values  $C_0^s=2.04$  and  $2.20$  show that the properties of the adsorbed layer in the presence of isopropanol are

governed by the existence of very strong hydrophobic interactions stronger than those in the presence of acetonitrile.

It is reasonable to assume that the above observations about the adsorption isotherm of the modifier and the interactions at the adsorbed layer should also be valid when methanol–water mobile phases were used. The limited results we obtained from the fitting procedure when methanol is used as modifier seem to verify the above assumption. Thus the value  $C_0^s=0.25$  obtained for set 5 is comparable to the corresponding value at the mobile phase ( $C_0^m=0.55$ ). In addition, when we use this value of  $C_0^s$  to determine the equilibrium constant  $\beta_B^*$ , we found  $\beta_B^*=0.8$  for data sets 1 and 9. It is seen that the adsorption isotherms of methanol for sets 1, 5 and 9 are almost the same.

Using the values of  $\beta_B^*$ ,  $C_0^s$  of Table 10 we refitted the experimental data and selected results are shown in Tables 11–15. From the values of the sum of squares of residuals (SSR) and the plots of calculated and experimental ln  $k'$  versus  $x$ , it was found that the equations of the adsorption model describe all data sets absolutely satisfactory. This is shown in Figs. 4

Table 12

Fitted parameters of Eq. (39) for data set 6 using  $\beta_B^*=0.18$  and  $C_0^s=1.9$ 

Solute	ln $k^0$	$D_2^s$	$D_2^m$	$D_3^m$	$D_4^m/4$	SSR
Phenol	3.55	–5.92	–35.25	57.69	–8.36	0.054
2-Methylphenol	4.84	–7.31	–44.86	72.67	–10.45	0.071
Nitrobenzene	4.28	–4.54	–32.44	52.38	–7.62	0.013
2-Chlorophenol	5.01	–6.78	–44.36	72.24	–10.36	0.022
3-Chlorophenol	5.30	–6.17	–42.36	67.10	–9.41	0.003
2-Nitrophenol	4.62	–3.77	–29.45	45.42	–6.30	0.006
3-Nitrophenol	4.70	–6.92	–44.29	72.55	–10.47	0.054
4-Nitrophenol	4.58	–7.09	–44.61	73.04	–10.54	0.051
2,4-Dinitrophenol	3.96	–6.30	–38.84	61.28	–8.29	0.044

Table 13

Fitted parameters of Eq. (39) for data set 7 using  $\beta_B^* = 0.18$  and  $C_0^s = 1.86$ 

Solute	$\ln k^0$	$D_2^s$	$D_2^m$	$D_3^m$	$D_4^m/4$	SSR
Phenol	2.73	-5.26	-28.20	36.45	-4.58	0.019
Benzylalcohol	2.52	-8.46	-36.71	49.46	-6.40	0.046
4-Fluorophenol	3.18	-8.60	-36.82	43.88	-5.08	0.015
4-Hydroxybenzaldehyde	2.37	-9.82	-39.73	48.36	-5.58	0.098
2-Phenylethanol	3.18	-5.01	-31.28	45.08	-6.00	0.007
3-Nitrophenol	3.85	-7.90	-38.92	50.19	-6.33	0.022
4-Nitrophenol	3.83	2.86	-14.85	24.77	-3.57	0.103
4-Nitrobenzaldehyde	3.73	0.18	-21.25	36.73	-5.70	0.074

Table 14

Fitted parameters of Eq. (39) for data set 8 using  $\beta_B^* = 0.24$  and  $C_0^s = 2.2$ 

Solute	$\ln k^0$	$D_2^s$	$D_2^m$	$D_3^m$	$D_4^m/4$	SSR
5h1aa	2.118	-13.236	-349.15	10 114.25	-30 080.2	0.046
5ht	2.738	-18.929	-449.95	13 441.29	-41 144.2	0.075
5htoh	4.204	-16.076	-417.42	11 990.21	-36 333.7	0.072
5htp	2.262	-14.994	-390.60	11 699.52	-35 046.4	0.049
hva	2.198	-16.481	-392.81	11 547.19	-35 503.4	0.052
mhpg	2.480	-20.963	-417.15	12 191.03	-39 094.6	0.074
m5ht	3.087	-22.363	-511.43	15 427.15	-47 889.5	0.115
3mt	2.695	-20.451	-508.64	16 088.41	-49 762.4	0.122
tryp	3.141	-15.449	-365.98	10 711.13	-33 258.0	0.064

and 5, where selectively plots of  $\ln k'$  versus  $x$ , are presented. A simple comparison of Fig. 3 to Fig. 5 shows the superiority of the adsorption model for the description of the retention of the catechol-related solutes at least. It is also interesting to note that the values of  $\ln k^0$  for clari and roxi are independent of the modifier (Table 15), as is expected.

However, some inconsistencies should be pointed out and discussed. The retention of phenol, 3- and 4-nitrophenol in methanol- and acetonitrile-water mobile phases has been investigated in two independent studies. It is evident that the analysis of the retention data of these solutes by the adsorption model must yield comparable results. However, from Tables 12 and 13, we observe that the fitted values of

$D_2^s$ ,  $D_2^m$ ,  $D_3^m$  and  $D_4^m$  exhibit significant deviations between sets 6 and 7. A similar behavior is observed when methanol is used as modifier. In our opinion, the above differences should be attributed to the great number of adjustable parameters in combination with the rather limiting number of experimental data points in each set. Note that precisely the same behavior is also observed for the partition model (Tables 6 and 7). Hence, the above differences are not an indication of the weaknesses of the adsorption or partition model but a clear message that reliable conclusions about the validity of the partition or adsorption mechanism could be obtained if: (a) the number of adjustable parameters is reduced by determining the values of  $D_2^m$ ,  $D_3^m$  and  $D_4^m$  from

Table 15

Fitted parameters of Eqs. (39) and (40) for data sets 9 and 10

Solute	$\ln k^0$	$D_2^s$	$D_2^m$	$D_3^m$	$D_4^m/4$	SSR
Clari (set 9)	9.0	3.37	-14.63	7.47	-	0.004
Roxi (set 9)	10.0	1.96	-17.93	8.19	-	0.008
Clari (set 10)	9.0	2.80	-31.60	73.42	-15.48	0.0015
Roxi (set 10)	10.0	3.64	-34.25	83.10	-19.08	0.0002

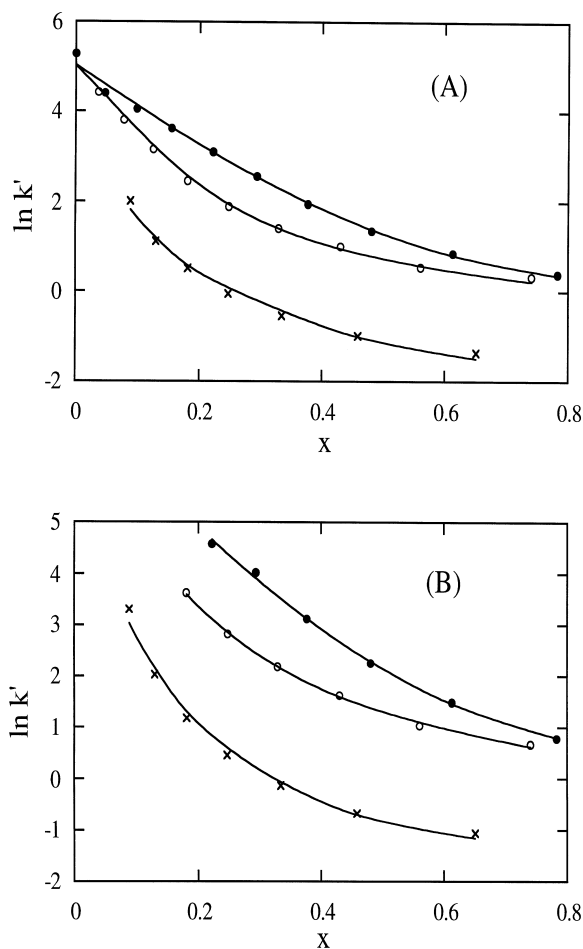


Fig. 4. Retention plots of benzene (A) and ethylbenzene (B) in aqueous mobile phases modified with methanol (●), acetonitrile (○) and isopropanol (×). Curves have been calculated from the adsorption model, Eqs. (39) and (41) (○, ×) and Eqs. (40) and (41) (●), using for the various parameters the relevant values of Tables 4, 10 and 11.

independent measurements, and (b) the number of data points used in the fitting procedure is increased.

Summarizing all the above results, we can conclude that the equations of the adsorption model describe all data sets absolutely satisfactorily and result in a physically reasonable picture about the behavior of modifier and solvent at the adsorbed layer. However, whether the high applicability of the adsorption model entails the validity of the adsorption mechanism at a molecular level cannot be safely concluded. The most puzzling point is the retention

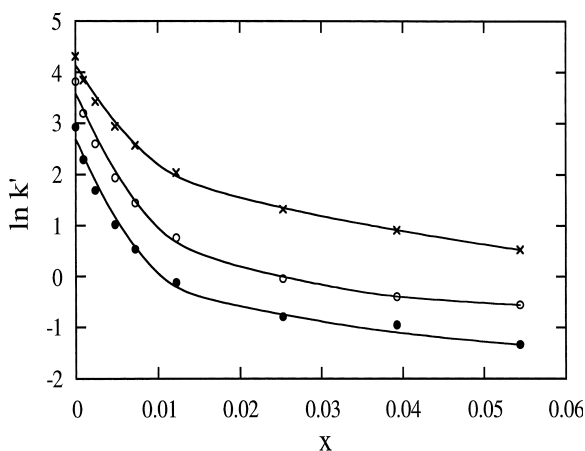


Fig. 5. Retention plots of 3mt (●), 5mht (○) and tryptophan (×) in isopropanol–water mobile phase. Curves have been calculated from the adsorption model using Eqs. (39) and (41) and the relevant parameters of Tables 10 and 14. Data for 5mht and tryptophan have been shifted along the y-axis by +0.5 and +1, respectively.

of solutes of sets 1–3, for which values of  $D_2^m$ ,  $D_3^m$  and  $D_4^m$  are known from independent measurements. We found that the retention of these solutes is described equally satisfactorily from both the adsorption and partition model. However, the fact that for the application of the partition model we use only one adjustable parameter is strong evidence for the validity of this model for the case of the retention of small and non-polar molecules. Thus, whereas the adsorption mechanism should govern the retention of solutes with large and polar molecules, the partition is very likely to describe the retention mechanism of solutes with small and non-polar molecules. Concerning the intermediate case of solutes with small but polar molecules, our results do not give a clear answer. Both models describe equally satisfactorily the experimental data and it would be rather arbitrary to choose one of them on the basis of the results of the present treatment.

It is seen that the present treatment could not unambiguously clarify whether the adsorption or the partition mechanism describes the retention in reversed-phase columns. Note that Carr et al. [7–9,56] in an interesting series of papers have shown that the type of retention mechanism, adsorption-like or partitioning-like, depends upon the solute polarity, the type of the bonded phase and the mobile phase composition. However, the following question is

raised: If there are solutes that follow the adsorption mechanism, could we exclude the possibility that this mechanism also occurs in the case of small and non-polar molecules? In fact even if the partition mechanism is valid when a solute has small and non-polar molecules, a solute molecule must replace solvent and/or modifier molecules adsorbed on the chain tips of the hydrocarbon layer before this molecule enters a cavity inside the hydrocarbon layer. However, this replacement procedure is in fact an adsorption process. Note that a combined mechanism has also been previously suggested [4,8]. In our opinion, the next steps towards the elucidation of the retention mechanism in reversed-phase chromatographic columns should be: (a) to develop the combined adsorption–partition model and (b) to test this model using data sets with a great number of data points, higher than 15, covering a wide range of  $x$  values. An additional useful prerequisite for the choice of a data set is the knowledge of the activity coefficients of solute, solvent and modifier in the mobile phase from independent measurements. In this way, the number of the adjustable parameters is reduced and the reliability of the fitting is increased. The above task has already been started within the framework of this series of publications.

## 12. Conclusions

From the study reported here, the following conclusions can be drawn:

(1) The lattice model approximation, i.e. the assumption that the mobile phase has a lattice structure where each molecule occupies one site and which is equivalent to the omission of the last term of the expressions of  $\ln k'$  developed in the present paper, is a poor approximation when the mol fraction of modifier varies in a wide range of values. Therefore, the use of this approximation may lead to erroneous results.

(2) The mean field approximation adopted in many previous studies for the structure of the mobile phase seems to be valid only for the binary mixture of methanol–water. It does not hold for binary mixtures of acetonitrile or isopropanol with water as well as for all ternary mixtures consisting of the above binary mixtures plus a solute.

(3) Conclusions (1) and (2) raise serious questions about the validity of Dill's treatment for the partition mechanism [2,4], since this treatment is based on both the mean field and the lattice model approximation. In contrast, the treatment of the partition model presented here is free from these approximations.

(4) The equilibrium equations of the adsorption model describe all data sets absolutely satisfactorily and result in a physically reasonable picture about the behavior of modifier and solvent at the adsorbed layer. However, we are sure about the validity of the adsorption mechanism only for the retention of solutes with large molecules. In contrast, the retention of solutes with small and non-polar molecules is likely to be governed by the partition mechanism. Thus, despite the high applicability of the adsorption model, there is a need of further studies before we conclude about the validity range of the adsorption mechanism at a molecular level.

(5) Although the present treatment undoubtedly does not clarify the retention mechanism of small molecules, it shows the next steps necessary for the final elucidation of this mechanism in reversed-phase chromatographic columns. These should be the development of the combined adsorption–partition model and testing of this model using data sets with a great number of data points and of known activity coefficients of solute, solvent and modifier in the mobile phase from independent measurements.

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