Theory of Retention in Reversed Phase Liquid Chromatography with Ternary Mobile Phases

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The partition model of retention is developed for reversed phase liquid chromatography with multicomponent mobile phases. Simple equations for the retention and selectivity in ternary mobile phases are derived. For the systems in which the ratio of volume fractions of organic modifiers remains fixed, new linear dependences for retention factor and selectivity are proposed. These equations are successfully used to describe experimental data found in the literature. An influence of the nature of organic solvents and proportion in which they are mixed on retention and selectivity is discussed.

1. Introduction

During the last two decades, reversed phase liquid chromatography (RPLC) has developed into a highly advantageous and successful technique. Nowadays, most modern liquid chromatographic analyses utilize the RPLC mode. For this reason, a great deal of effort has been devoted to explain the retention mechanism in RPLC systems. An understanding of the solute distribution is crucial for effective control separations. The main aim of many investigations was to describe how retention and selectivity depend on the various mobile and stationary phase variables.[1–3] A great number of different parameters have been taken into account, including the nature of the chemically bonded phase (CBP), the properties of solvents, and the type of solute.

In RPLC systems, the stationary phase is a thin polymeric film consisting of hydrocarbon chains terminally anchored at the solid surface. The structure and composition of the polymeric film have a direct effect on the solute distribution in the chromatographic system.[3–6] The behavior of the grafted layers depends on the chain length, surface coverage, intrinsic stiffness of the *n*-alkyl chains, and quality of the solvent in which they are embedded.[7,8] Two limiting models of the chemically bonded phase have been considered, namely, fully extended and completely collapsed chains. Nonpolar solvents presumably lead to a stationary phase having a brushlike structure, where the chains are stretched out in the direction normal to the surface. In this case, penetration by the solvent and the solute is possible. However, polar solvents tend to promote collapse of the chains and lead to a quasi-liquidlike layer of recumbent alkyl chains oriented in some manner. In such a stationary phase, the solvent penetration is negligible but the solute penetration is not denied. Apart from these extreme models of hydrocarbon films, various intermediate structures have been considered.[4–11]

A sophisticated theoretical description of the process can provide a powerful tool for searching the optimum separation conditions. The use of a mixed mobile phase gives an extra dimension for the control of separation because this allows a continuous change of retention time and selectivity. As the result of theoretical considerations, one obtains a functional dependence between the retention factor and the mobile phase composition. Each theoretical treatment is based on the assumed retention model. The retention mechanism in reversed phase liquid chromatography has been a topic of much study and discussion.[4,6,9–28] Two principal mechanisms have been proposed: adsorption of the solute and solvents on the tips or the stem of hydrocarbon chains and partition of the solute molecules between the mobile phase and cavities existing within the chains.[28] The general semi-thermodynamic treatment for adsorption or partition of a solute from a mobile phase has been recently formulated and tested for numerous experimental data.[4,28] This approach involves both adsorption and partition effects in chromatographic processes.[4,23,24] The lattice-based mean field models have given insight into driving forces of retention, but the physical assumptions inherent in them are restrictive.[4,17–19] The more realistic microscopic model was used in molecular dynamics simulations carried out by Klatt and Beck.[9–11] Their works have shed new light on solute transfer and showed that a number of specific interfacial effects figure prominently in retention.

Systems involving binary mobile phases have usually been studied. For such systems, numerous equations describing the influence of the mobile phase composition on retention have been discussed. Snyder and co-workers[3,29,30] have proposed a linear relationship between the logarithm of the retention factor and the volume fraction of organic modifier. The slope of the straight line was interpreted as the solvent strength parameter. In several works, the logarithm of the retention factor has been expressed as a quadratic function of the volume fraction of nonpolar solvent in the mobile phase.[16–19,31–33] A physical meaning of the coefficients of the considered parabolas was a subject of discussion.[5] A logarithmic dependence of retention to modifier concentration has also been proposed.[34] The comparison of selected equations describing retention in reversed phase liquid chromatography with binary mobile phases has recently been published.[35]

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Relatively little attention has been paid to retention in ternary mobile phases; only few papers have dealt with such systems.[36−41] However, for numerous separations, the utilization of ternary, or generally multicomponent, mobile phases is needed. This considerably increases the flexibility of the method. The choice of a mobile phase for a given separation is still a difficult task. Various systematic strategies have been proposed for the selection of suitable solvents.[40,42,43] The fundamental theoretical results have been published by Schoenmakers et al.[39] Certain aspects of retention in multicomponent mobile phases have been previously analyzed but without any concrete solutions and applications.[23] The equations describing retention in such systems are relatively complicated and may be difficult to use by a practicing chromatographer.

In this paper, equations for the retention and selectivity in multicomponent mobile phases are derived using methods of statistical thermodynamics. The case of ternary mobile phases is considered with all details. A very simple linear dependence is proposed for the systems in which the ratio of volume fractions of organic modifiers remains fixed. The study is based on the partition model of retention. We focus our attention on the consequences of the use of ternary mobile phases instead of the more popular binary mixtures. The usefulness of the derived equations is confirmed by experimental data found in the literature.

2. Theory

2.1. Multicomponent Mobile Phases. Here, we develop a simple model to obtain the relevant equations for treating solute retention in RPLC systems with multicomponent mobile phases. The mobile phase consists of a solute (s) and n solvents. The liquid mixture contains molecules of different sizes. We assume that a molecule of the ith component contains r_i segments. All species are nonelectrolytic. The stationary phase consists of long n-alkyl chains permanently attached to a solid surface. We assume that the chains of the chemically bonded phase are completely collapsed. Under this condition, unswollen CBP chains exhibit a quasi-liquidlike behavior. Solvents are immiscible with the grafted chains.

The solute retention is treated as ordinary partitioning between two liquid phases. The principal driving force for the transfer of the solute is its relative chemical affinity for mobile and stationary phase molecules. In this case, the retention factor is given by

$$k_s = q\gamma_s^\sigma/\gamma_s^l \tag{1}$$

where γ_s^σ and γ_s^l are the activity coefficients of the solute (s) in the stationary and mobile phases, respectively; $q = n^\sigma/n^l$ is the ratio of the number of moles in both phases. We assume that the parameter q is fixed for any composition of the mobile phase.

According to the Flory theory of polymers, the activity coefficient of an infinitely diluted solute in the mobile phase can be expressed as[23]

$$\ln \gamma_s^l = \sum_{i=1}^{n}(1 - r_{si})\varphi_i + r_s\sum_{i=1}^{n}\omega_{si}\varphi_i - r_s\sum_{\substack{i=1 \\ j>i}}^{n}\sum_{j=1}^{n}\omega_{ij}\varphi_i\varphi_j \tag{2}$$

where φ_i is the volume fraction of the ith component in the mobile phase, $r_{si} = r_s/r_i$ characterizes the difference of molecular

sizes, and ω_{ij} is the Flory parameter defined by the following equation

$$\omega_{ij} = (z/k_B T)[u_{ij} - (^1/_2)(u_{ii} + u_{jj})] \tag{3}$$

In the above, the symbol u_{ij} denotes the interaction energy between two segments of the ith and jth molecules, z is the lattice coordination number, k_B denotes the Boltzmann constant, and T is the absolute temperature.

It should be pointed out that the above expression for the activity coefficient has been derived using the random mixing (mean field) approximation. In the case of aqueous solutions, the approach may be treated as a starting point for more advanced theoretical considerations.

On the other hand, the activity coefficient of a solute in the stationary phase can be written as follows

$$\ln \gamma_s^\sigma = (1 - r_{sc}) + r_s\omega_{sc} + \Delta_{sc} = \overline{\omega_{sc}} \tag{4}$$

where the index "c" denotes the chemically bonded phase and Δ_{sc} is a correction term, which describes the difference between free and grafted chains.[23]

Substitution of eqs 2 and 4 into eq 1 yields

$$\ln k_s = \sum_{i=1}^{n}\varphi_i \ln k_{s(i)} - r_s\sum_{\substack{i=1 \\ j>i}}^{n}\sum_{j=1}^{n}\omega_{ij}\varphi_i\varphi_j \tag{5}$$

where $k_{s(i)}$ is the retention factor for the pure ith solvent

$$\ln k_{s(i)} = r_s(r_i^{-1} - r_c^{-1} + \overline{\omega_{sc}} - \omega_{si}) + \ln q \tag{6}$$

Equation 5 describes the dependence of the retention factor on the composition of the multicomponent phase. It was originally presented by Martire and Boehm.[4] If a mixture of the solvents is ideal ($\omega_{ij} = 0$; $i, j = 1,2, ... , n$), then eq 5 becomes a very simple form

$$\ln k_s = \sum_{i=1}^{n}\varphi_i \ln k_{s(i)} \tag{7}$$

One of the most important parameters characterizing the chromatographic system is the selectivity defined as the ratio of the retention factors of two solutes

$$\alpha_{sp} = k_s/k_p \tag{8}$$

Using eq 5, we obtain

$$\ln \alpha_{sp} = \sum_{i=1}^{n}\varphi_i \ln \alpha_{sp(i)} - \Delta r_{sp}\sum_{\substack{i=1 \\ j>i}}^{n}\sum_{j=1}^{n}\omega_{ij}\varphi_i\varphi_j \tag{9}$$

where $\Delta r_{sp} = r_s - r_p$ and $\alpha_{sp(i)}$ is the selectivity in the pure solvent "i". As one can see, the selectivity is a complex function of the mobile phase composition. However, if the molecular sizes of the solutes are similar ($\Delta r_{sp} = 0$), the following relationship holds

$$\ln \alpha_{sp} = \sum_{i=1}^{n}\varphi_i \ln \alpha_{sp(i)} \tag{10}$$

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2.2. Ternary Mobile Phases. In the case of ternary mobile phases, eq 5 can be written in the following form

$$\ln k_s = A_1\varphi_1^2 + A_2\varphi_2^2 + B_1\varphi_1 + B_2\varphi_2 + C + D\varphi_1\varphi_2 \quad (11)$$

where

$$A_i = r_s\omega_{i3}, \quad i = 1, 2 \quad (12)$$

$$B_i = \ln(k_{s(i)}/k_{s(3)}) - r_s\omega_{i3}, \quad i = 1, 2 \quad (13)$$

$$C = \ln k_{s(3)} \quad (14)$$

$$D = r_s(\omega_{13} + \omega_{23} - \omega_{12}) \quad (15)$$

From now on, we assign the subscripts "1" and "2" to organic solvents (methanol, acetonitrile, tetrahydrofuran, etc.) and "3" to water. The logarithm of the retention factor is a quadratic function of the concentrations of organic modifiers. The same equation has already been proposed by Schoenmakers et al.[39] However, their parameters A_i, B_i, C, and D are different from those predicted by eqs 12–15. They used the solubility parameter concept to describe molecular interactions in a solution. Nowadays, it is obvious that the Hildebrand approach is a poor approximation for the most real systems. A principal weakness arises in representing the pair interaction $i-j$ as the geometric mean of $i-i$ and $j-j$ interactions. This simplification leads to certain special relations between the coefficients A_i, B_i, C, and D. In particular, according to Schoenmakers el al.,[39] the value of D is the geometric mean of A_1 and A_2. Equations 12–15 provide a more sophisticated and more general physical interpretation of these parameters on the molecular level. According to eq 15, D depends on three exchange parameters— ω_{12}, ω_{13}, and ω_{23}—which characterize molecular interactions in the suitable binary solvents. This makes possible a more realistic description of the behavior of complicated systems.

Our model predicts other iso-eluotropic diagrams than the theory of Schoenmakers et al.[39] The iso-eluotropic line is defined as a line connecting points on the Gibbs triangle at which the retention factor k_s has the same value. As follows from analysis of eq 11, in a general case, iso-eluotropic lines are not straight lines. This conclusion was confirmed by experimental data.[39] The experimental curves are usually slightly concave. However, under certain conditions, the iso-eluotropic diagrams consist exclusively of straight lines. Such a behavior is observed in two cases: for ideal mixtures of the solvents (see eq 7) and for systems satisfying relations between coefficients of eq 11 described by Schoenmaker and co-workers.[39] Their theory predicts only linear iso-eluotropic diagrams.

2.3. Special Conditions. The experimental study of mobile phase effects in the whole region of solvent concentrations is a very cumbersome and time-consuming task. For a ternary mobile phase, the retention factor depends on two variables: φ_1 and φ_2. However, under certain conditions, it is possible to express the retention factor by means of one variable only.

Let us consider a chromatographic system for which

$$\varphi_1/\varphi_2 = w = \text{const} \quad (16)$$

The points corresponding to mobile phases that satisfy eq 16 lie on the straight line which begins at the apex assigned to solvent "3" in the Gibbs triangle.

In this case, one can write

$$\varphi_i = p_i\varphi, \quad i = 1, 2 \quad (17)$$

where φ is the total volume fraction of organic solvents

$$\varphi = \varphi_1 + \varphi_2 = 1 - \varphi_3 \quad (18)$$

and

$$p_1 = \frac{w}{1 + w}, \quad p_2 = \frac{w}{1 + w} \quad (19)$$

In the above, p_1 and p_2 are equal to the volume fractions of the solvents "1" and "2" in the two-component mobile phase (1,2) that fulfils eq 16.

Combination of eqs 11 and 17 leads to the following relationship

$$\ln k_s = A\varphi^2 + B\varphi + C \quad (20)$$

with

$$A = A_1p_1^2 + A_2p_2^2 + Dp_1p_2 = r_s\Delta\omega \quad (21)$$

$$B = B_1p_1 + B_2p_2 =$$
$$p_1 \ln k_{s(1)} + p_2 \ln k_{s(2)} - \ln k_{s(3)} - r_s(p_1\omega_{13} + p_2\omega_{23}) \quad (22)$$

where $\Delta\omega$ is the parameter characterizing only the ternary mobile phase and is independent of the solute (s)

$$\Delta\omega = p_1\omega_{13} + p_2\omega_{23} - p_1p_2\omega_{12} \quad (23)$$

After rearrangement, eq 20 becomes the linear form

$$g(\varphi) = \frac{1}{1 - \varphi} \ln(k_s/k_{s(1,2)}) = \alpha\varphi + \beta \quad (24)$$

with

$$\alpha = -A \quad (25)$$

$$\beta = -(A + B) \quad (26)$$

where $k_{s(1,2)}$ is the retention factor in the binary mobile phase consisting of organic solvents that fulfills eq 16. Equations 20 and 24 are analogous to those derived for the binary mobile phase.[17] The slope (A) depends on the proportion of volume fractions of organic solvents in the mobile phase (w), the parameters characterizing molecular interactions in suitable binary mixtures of solvents (ω_{12}, ω_{13}, and ω_{23}), and the molecular size of the solute (r_s) (see eqs 21, 24, and 25).

Now we consider the selectivity in the studied systems. Along the line described by eq 16, the logarithm of the selectivity is a quadratic function of the mole fraction of the total concentration of organic solvents. From eq 20, we obtain

$$\ln \alpha_{sp} = \Delta A\varphi^2 + \Delta B\varphi + \Delta C \quad (27)$$

where ΔA and ΔB are the differences of the parameters A and B estimated for the solutes "s" and "p". If $\Delta r_{sp} = 0$, the coefficient ΔA vanishes and the logarithm of the selectivity is a linear function of the volume fraction (φ).

Similar to the case of eq 20, the linearization of eq 27 leads to

$$h(\varphi) = \frac{1}{1 - \varphi} \ln(\alpha_{sp}/\alpha_{sp(1,2)}) = \Delta\alpha\varphi + \Delta\beta \quad (28)$$

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where $\alpha_{sp(1,2)}$ is the separation factor in the mixture of organic solvents, $\Delta\alpha = -\Delta A$, and $\Delta\beta = -(\Delta A + \Delta B)$.

It may be convenient to use another variable to characterize the composition of the mobile phase for which condition 16 is fulfilled, for example, the volume fraction of water $\varphi_3 = 1 - \varphi$. Introducing this variable into eqs 20 and 24 leads to the analogous quadratic functions of φ_3.

A pair of analytes can be effectively separated when their retention factors and the selectivity become certain optimal values. These quantities can be calculated using eqs 5 and 9. In the case of a ternary mobile phase satisfying condition 16, the simplified dependences 24 and 28 can be applied. The following optimization procedure may be used. First, one should measure retention factors in the mixed organic solvent and few retention factors in ternary mobile phases of the suitable compositions. Second, the coefficients α and β should be found by fitting eq 24 to the experimental data. Then, retention factors and selectivity can be estimated for any ternary mobile phase, which fulfills eq 16. Finally, one can choose a ternary mobile phase composition corresponding to the needed values of the retention parameters.

3. Results and Discussion

Schoenmakers et al.[39] presented an extensive experimental survey of the retention behavior of numerous solutes in the ternary mobile phases: methanol (1)−tetrahydrofuran (2)−water (3) and methanol (1)−acetonitrile (2)−water (3). The columns were packed with Nucleosil 10-RP18. The stationary phase was the porous silica gel covered by relatively long alkyl chains (containing 18 C-atoms). In this case, the partition mechanism of retention was expected. These retention data were fitted to second-order eq 11 with very good accuracy. The estimated coefficients A_1, A_2, B_1, B_2, and C are consistent with the theory. However, in contradiction to their theoretical predictions, the values of D are larger than the geometric mean of A_1 and A_2. The theory presented in this paper allows us to explain this inconsistency (see eq 15).

Schoenmakers and co-workers[39] used the mobile phases, which satisfied the condition described by eq 16 with $w = 0$ (a binary mixture (2,3)), 1, 3, and ∞ (the binary mixture of organic solvents (1,2)). We have applied their data to test the special eqs 20, 24, 27, and 28 derived in the previous section.

We have fitted the retention data to the linear dependence eq 24 for different values of the ratio of volume fractions of organic solvents, $w = \varphi_1/\varphi_2$. The accuracy of approximation was satisfactory. The illustrative examples of the results are presented in Figures 1−5.

Figures 1 and 2 show the logarithms of experimental retention factors as functions of the concentration of organic solvents (the left part) and the corresponding linear dependence eq 24 (the right part). These data are plotted for a chosen solute and different values of w. The results for methanol−tetrahydrofuran (THF) mixtures are drawn in Figure 1, whereas Figure 2 presents data for methanol−acetonitrile (ACN) solutions. These figures show in such a way the nature of organic solvents and quantitative proportion between them in a ternary mobile phase can affect the solute retention. In the considered systems, the curves log k_s versus φ plotted for ternary mobile phases lie between the lines corresponding to binary mobile phases (1,3) and (2,3). Obviously, the curves intersect at the point $\varphi = 1$. One can see that for fixed total volume fraction of organic solvents (φ), the retention factors decrease with increasing concentration of THF. This effect is less pronounced for mobile phases containing ACN.
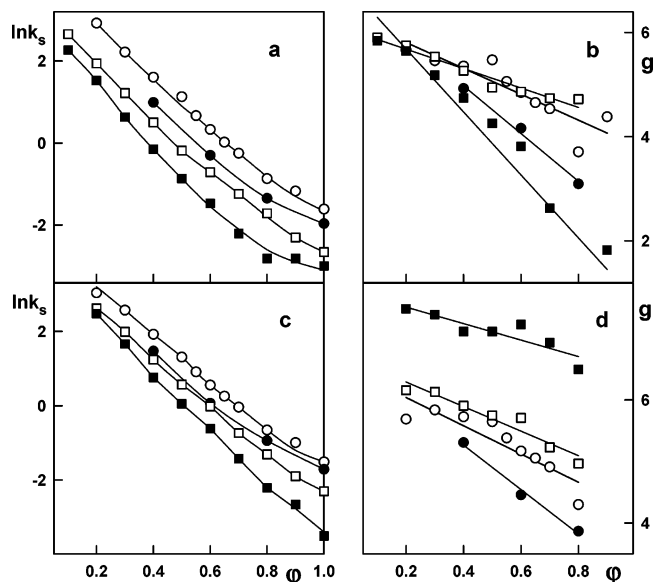

Figure 1. Dependences ln k_s vs φ (a, c) and $g(\varphi)$ vs φ (b, d) for 2-phenylethanol (a, b) and *p*-nitroacetophenone (c, d) and the mobile phase consisting of methanol (1)−tetrahydrofuran (2)−water (3) for different values of the ratio w: (methanol−water) (○); 3 (●); 1 (□); 0 (THF−water) (■).
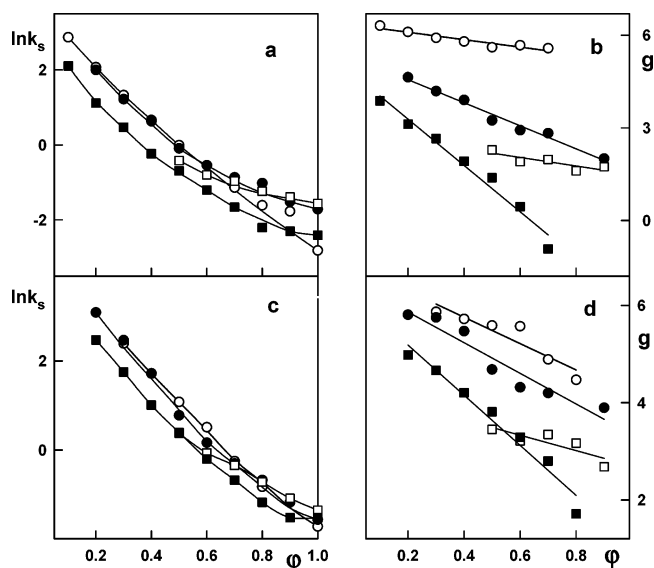

Figure 2. Dependences ln k_s vs φ (a, c) and $g(\varphi)$ vs φ (b, d) for *p*-hydroxybenzaldehyde (a, b) and *o*-cresol (c, d) and the mobile phase consisting of methanol (1)−acetonitrile (2)−water (3) for different values of the ratio w: (methanol−water) (○); 3 (●); 1 (□); 0 (ACN−water) (■).

Let us analyze eq 24 for the boundary values of φ. The function $g(\varphi)$ is defined exclusively for $\varphi \neq 1$. However, if $\varphi = 0$, then $g(0) = \ln k_s - \ln k_{s(1,2)}$, and it depends on the ratio w. Thus, the eq 24 lines plotted for different w do not cross the line $\varphi = 1$ at the same point. Moreover, the slopes (α) also vary when w is changed. This is a reason for an apparently chaotic sequence of the straight lines for a given solute and different values of the ratio w.

In Figures 3 and 4, the linear relationships (eq 24) are plotted for the series of solutes and gradually changing mixtures of organic solvents. According to the theory, the slopes (A) are the same for fixed w and the solutes of the same molecular sizes (r_s). Then, the considered straight lines should be parallel. The influence of the nature of the second organic solvent on
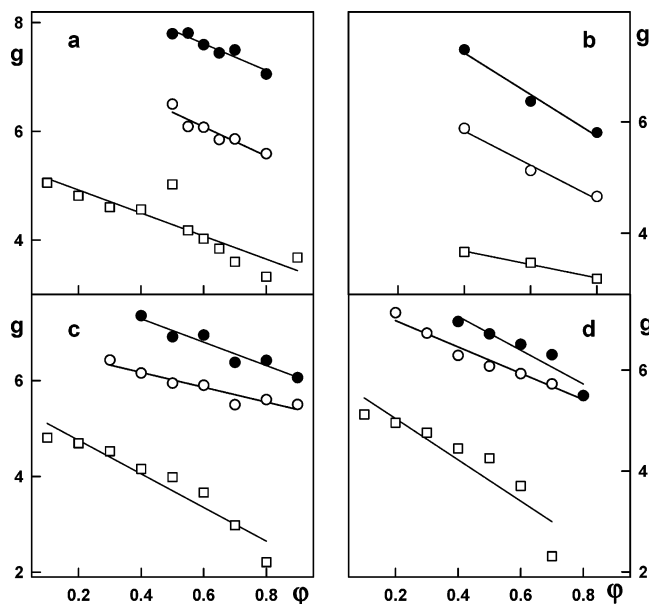
Figure 3. Linear dependences $g(\varphi)$ vs φ for anisole (○), chlorobenzene (●), and benzyl alcohol (□) in the mobile phase consisting of methanol (1)−tetrahydrofuran (2)−water (3) for different values of the ratio w: (methanol−water) (a); 3 (b); 1 (c); 0 (THF−water) (d).
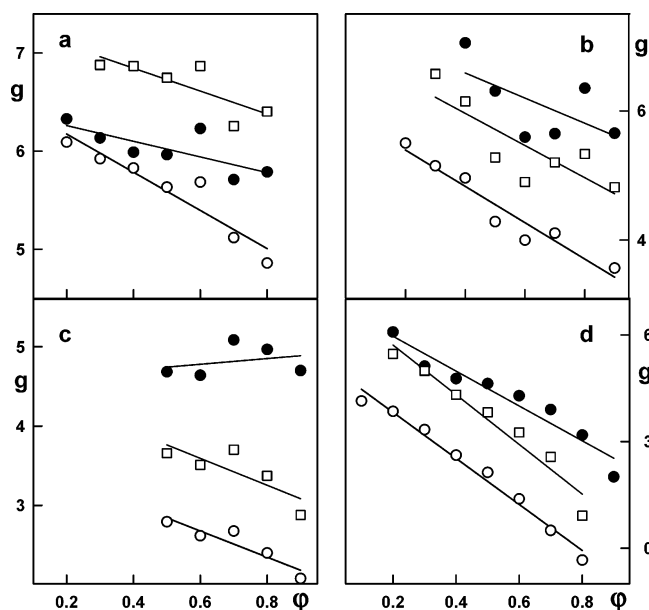

Figure 5. Dependences ln k_s vs φ (a, c) and $g(\varphi)$ vs φ (b, d) for phenol and the mobile phases consisting of methanol (1)−acetonitrile (2)−water (3) (a, b) and methanol (1)−tetrahydrofuran (2)−water (3) (c, d) for different values of the ratio w: (methanol−water) (○); 3 (●); 1 (□); 0 (THF−water or ACN−water) (■).

Figure 4. Linear dependences $g(\varphi)$ vs φ for 2-phenylethanol (○), p-nitrobenzaldehyde (●), and p-chlorophenol (□) in the mobile phase consisting of methanol (1)−acetonitrile (2)−water (3) for different values of the ratio w: (methanol−water) (a); 3 (b); 1 (c); 0 (ACN−water) (d).

Figure 6. Linear dependences $h(\varphi)$ vs φ for the pair nitrobenzene/phenol in the mobile phase consisting of methanol (1)−tetrahydrofuran (2)−water (3) for different values of the ratio w: (methanol−water) (○); 3 (●); 1 (□).

the retention may be illustrated by comparing the results obtained for phenol chromatographed in different mobile phases consisting, apart from methanol and water, of THF or ACN (see Figure 5). The retention factors in mobile phases containing THF are smaller than those for ACN mixtures. An influence of the parameter w on the retention from ternary mobile phases is better visible for mixtures with THF. In all studied systems, the linear dependence eq 24 approximated experimental data with satisfactory accuracy. This means that this simplified equation can be used to interpret experimental data measured under assumed conditions.

The examples of relationships between the logarithm of selectivity and the total concentration of organic solvents are shown in Figures 6−8. According to eq 27, for fixed proportion
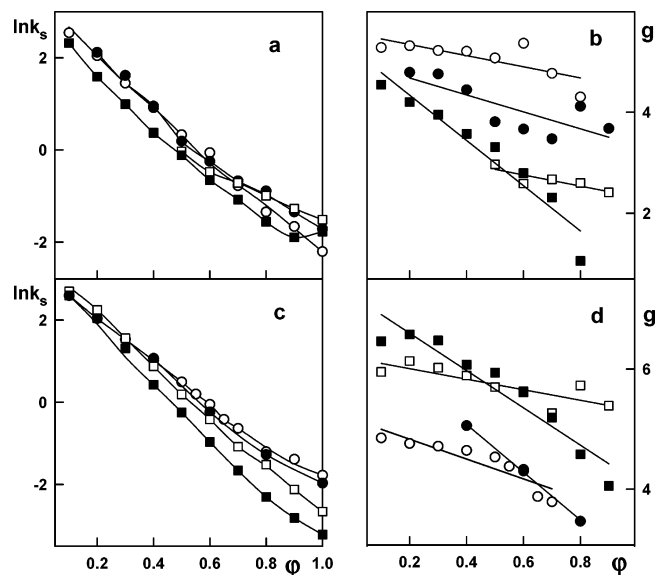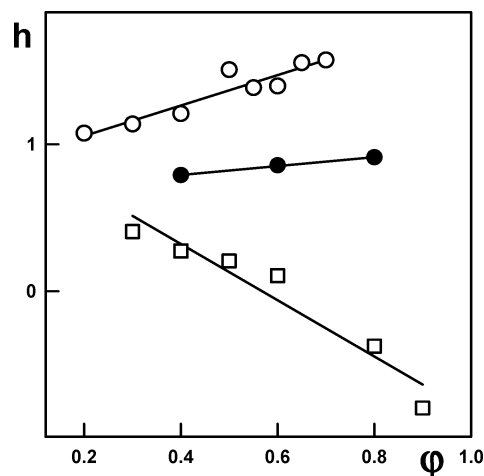
between organic solvents, the logarithm of the selectivity should be a quadratic function of the total volume fraction of organic solvents. As an example, Figure 6 shows the linearized form of eq 28 drawn for the pair nitrobenzene/phenol and different mobile phases. Unfortunately, the deviations from linearity are considerable. Likely, the errors following from approximation of the retention factors for both solutes cumulate, and as a consequence, fitting of their ratio is not satisfactory. On the other hand, as has already been mentioned, if two solutes have similar molecular sizes ($r_s = r_p$), the logarithm of the selectivity is a linear function of the total fraction of organic solvents. In this case, we have

$$\ln \alpha_{sp} = a\varphi + b \qquad (29)$$

where

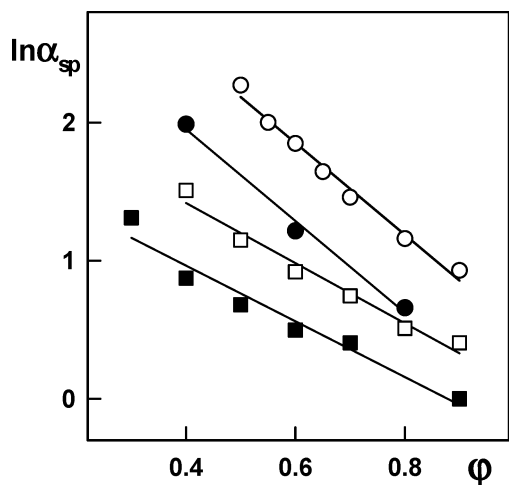$$a = \Delta B = p_1 \ln \alpha_{sp(1)} + p_2 \ln \alpha_{sp(2)} - \ln \alpha_{sp(3)} \qquad (30)$$

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Figure 7. Logarithm of selectivity ln α_{sp} vs φ for the pair benzophenone/benzonitrile in the mobile phase consisting of methanol (1)−tetrahydrofuran (2)−water (3) for different values of the ratio w: (methanol−water) (○); 3 (●); 1 (□); 0 (THF−water) (■).
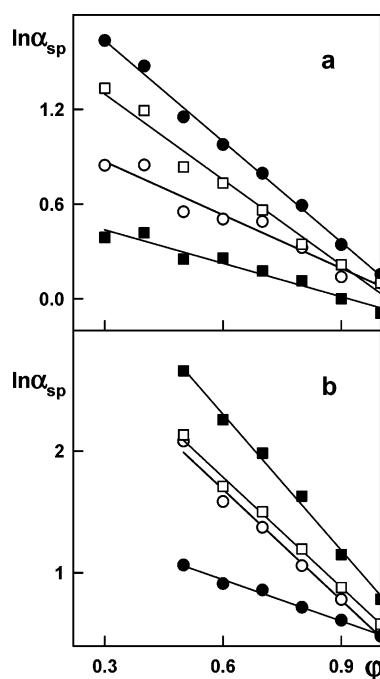

Figure 8. Logarithm of selectivity ln α_{sp} vs φ in methanol (1)−acetonitrile (2)−water (3) with $w = 3$ (a) for *m*-dinitrobenzene/*p*-fluorophenol (○), *m*-dinitrobenzene/*p*-hydroxybenzaldehyde (●), *m*-dinitrobenzene/benzyl alcohol (□), and *m*-dinitrobenzene/benzonitrile (■) and with $w = 1$ (b) for diphenyl ether/*m*-fluoronitrobenzene (○), *p*-chlorobenzaldehyde/*o*-fluoronitrobenzene (●), biphenyl/*o*-nitrophenol (□), and ethylbenzene/*p*-hydroxybenzaldehyde (■).

The sign of the slope (*a*) depends on the relations between the values of selectivities in the pure solvents and the ratio w. In Figure 7, the functions ln α_{sp} versus φ are shown for the pair benzophenone/benzonitrile chromatographed in mobile phases with different values of w. The influence of this parameter on the selectivity is obvious. The selectivity increases with an increase in the concentration of methanol. Figure 8 displays additional examples of linear dependences ln α_{sp} versus φ. The agreement of the experimental data and theoretical eq 29 is quite good. In numerous analyses, the solutes have comparable molecular sizes and thus eq 28 may be applied to predict the system selectivity.

4. Conclusions

The partition model of retention has been developed for reversed phase liquid chromatography with multicomponent mobile phases. The equations for the retention and selectivity in ternary mobile phases are derived. The mobile phases consisting of two organic solvents and water were considered. The model predicts more general behavior of the ternary systems than the previously formulated theories. For the systems in which the ratio of volume fractions of organic modifiers remains fixed, the retention factor was expressed as a function of the total volume fractions of organic solvents. Moreover, new linear dependences for retention factors and selectivities are proposed. These equations were successfully used to describe experimental data found in the literature. The theory allows us a systematic study of mobile phase composition and retention. It has been shown that the properties of all solvents affect either the retention or the selectivity. We confirmed the opinion that the application of a ternary mobile phase augments considerably a chance to achieve the optimal values of retention and selectivity. The proposed equations can be use to continuously control these factors.

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