

# Adsorption-Driven Retention in Normal-Phase Chromatography with Ternary Mobile Phases

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## Abstract

The model for retention in chromatographic systems using ternary mobile phases is considered. The driving force for the separation is assumed to be only a difference in adsorptive properties of a solute and all solvents. The effects of mobile phase composition are discussed. A series of very simple dependences between parameters characterizing retention in ternary and binary solvents is presented. The linear relationship is proposed to predict the capacity factor in the ternary mobile phase, for which the ratio of mole fractions of two less-polar solvents remains fixed. Theoretical concepts are verified by comparing the calculated values with experimental data measured in the entire concentration region.

## Introduction

Liquid–solid chromatography is a powerful and often-used analytical technique. On the other hand, one can say that the most significant contribution of liquid chromatography (LC) is to reinforce the link between analytical and physical chemistry. Through the years, a large amount of effort has been made to explain the retention mechanism in liquid–solid chromatography. With the refinements of chromatographic techniques, combined with new, theoretical concepts, it was possible to better understand the broad principles governing the process. This bridges the gulf between the thermodynamic aspects of interfacial phenomena and practical applications of chromatography in analytical chemistry.

Using isocratic and gradient-elution normal-phase chromatography with mixed mobile phases, complex biological or industrial materials can be effectively separated. The use of binary (generally multicomponent) mobile phases provides an extra dimension of control of the chromatographic separation. Such mixed solvents allow continuous control of the elution strength of the mobile phase and the selectivity of the chromatographic system. This suggests a need for functional relationships between retention factors and the mobile phase composition. While the systems composed of mixed mobile phases are functional, they are also very complex and often

intractable to exact theoretical modeling. Nevertheless, numerous thermodynamic models have been proposed and effectively solved (1–13). The considerable contribution originated from the theory of adsorption from multicomponent solutions (3,4). Currently, it is clear that the retention is simultaneously controlled by the nature of a solute, properties of all solvents, and the adsorbent characteristics. Modern theories of retention involve such physical factors as competitive adsorption of solvents, nonspecific interactions in a mobile phase, association effects, geometry of solute molecules and their orientation on the surface, energetic heterogeneity of the adsorbent, etc. (5–11). The results were summarized in several reviews (1–4,14).

Despite intensive research, a lot of problems remain to be investigated. Most of the studies were devoted to chromatographic systems with binary mobile phases. Relatively little attention has been paid to the retention in multicomponent mobile phases (15–22). In most cases, the aim of the research was to propose a strategy for selecting an appropriate solvent, which yields a good separation of solutes (15). Solvent selection reliant upon on trial-and-error can be very time-consuming when a ternary or quaternary mobile phase is used. For this reason, various systematic strategies have been proposed; for example, the sequential simplex method (16), mixture design statistical approach (17), and PRISMA model (18). Another class of treatments leads to functional relationships between the retention factors and the mobile phase composition. Jandera et al. (12,13) have presented the three-parameter equation to describe retention behavior in ternary mobile phases at either a constant sum or constant ratio of mole fractions of two stronger solvents. They suggested the method for predicting retention in three-component mobile phases with varying concentration ratios of two polar solvents from the parameters of best-fit equations in binary mobile phases and a single experimental capacity factor in a ternary mobile phase. The approach, although very interesting and useful, does not shed light on the mechanism of retention. The estimated parameters do not have a clear physicochemical interpretation.

The quasicheical theory of liquid chromatography with multicomponent phases (3,4) provides good theoretical grounds for investigating retention on a molecular level. In this treatment, the retention ratios are expressed by the suitable

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thermodynamic constants and strictly defined molecular factors. Unfortunately, the theory leads to rather complicated equations, which involve many parameters and may be difficult to use by a practicing chromatographer. However, for special conditions, such general equations can be reduced to surprisingly simple relationships (19,20).

Herein, the model for retention in a ternary mobile phase that involves only adsorption effects in the chromatographic processes has been considered. Many features of retention are well predicted by such an elementary model. The capacity ratio in a ternary mobile phase is expressed by means of the retention parameters estimated for the pure solvents or their binary mixtures. Simple, linear dependence was used to predict the retention for a ternary mobile phase in which the ratio of less polar solvents remains fixed. Correlations between the retention parameters are explained on theoretical grounds.

### Theory

The goal of this research was to show how the retention in ternary mobile phases may be predicted from the molecular parameters of solutes, solvents, and adsorbents. The simplest possible model of retention was introduced. It is assumed that the system consists of a solute (*s*), ternary mobile phase (1 + 2 + 3), and adsorbent; the molecular sizes of all solutes and solvents are the same; the liquid mixture is ideal; the adsorbent is energetically homogeneous; and the monomolecular layer of liquid immediately adjacent to the adsorbent surface (adsorbed phase) is treated as the stationary phase.

The driving force for the transfer of solute from the mobile to the stationary phase is adsorption on the solid surface. Adsorption from solutions has a competitive character, and the process may be described using the following series of quasichemical reactions (5,6):



where the symbol  $(j)^\rho$  denotes the molecule *j* (*j* = *s*, 1, 2, 3) in the  $\rho$ -th phase, and *l* and  $\sigma$  refer to the mobile and stationary phases, respectively.

The capacity ratio is defined as:

$$k_s = q(y_s/x_s) \quad \text{Eq. 2}$$

where *q* is the constant independent of a solute (1), whereas *y<sub>s</sub>* and *x<sub>s</sub>* denote mole fractions of the substance in the stationary and mobile phases, respectively.

On the other hand, the capacity ratio may be expressed from the mass action law as:

$$k_s = qK_{si}(y_i/x_i) \quad \text{Eq. 3}$$

where  $K_{si}$  is the thermodynamic constant of the first reaction (equation 1) that characterizes adsorption in the binary mixture (*s*, *i*). Thus, the capacity ratio for infinitely low concentration of the solute in a pure solvent is equal:

$$k_{s(i)} = qK_{si} \quad \text{Eq. 4}$$

Considering that  $x_s \rightarrow 0$ , the Langmuir-like equation for the real adsorption of the *i*-th solvent is obtained by:

$$y_i = \frac{K_{i3}x_i}{\sum_{j=1}^3 K_{j3}x_j} \quad \text{Eq. 5}$$

where  $K_{ij}$  describes adsorption of the *i*-th solvent from the binary solution (*I* + *j*);  $K_{ij} = 1/K_{ji}$  and  $K_{33} = 1$ . These parameters can be estimated from adsorption data.

In the model, the following relationship bridges adsorption constants and chromatographic factors:

$$K_{ij} = K_{sj} / K_{si} = k_{s(j)} / k_{s(i)} \quad \text{Eq. 6}$$

Combining equations 3 and 5 yields (6):

$$\frac{1}{k_s} = \sum_{j=1}^3 \frac{x_j}{k_{s(j)}} \quad \text{Eq. 7}$$

Notice that by using equation 7, the solute retention for any composition of the ternary mobile phase can be predicted if the capacity factors in pure solvents are known. The capacity ratio can also be expressed in terms of mole fractions of solvents in the stationary phase (*y<sub>s</sub>*):

$$k_s = \sum_{j=1}^3 y_j k_{s(j)} \quad \text{Eq. 8}$$

Snyder (1) proposed the analogous equation for binary mobile phases. Assume that elution strengths of the solvents satisfy the following inequality:  $\epsilon_1 > \epsilon_2 > \epsilon_3$ . The very simple relationships can be derived for a ternary mobile phase if the ratio of mole fractions of both less polar solvents is fixed:

$$x_2 / x_3 = r \quad \text{Eq. 9}$$

Various data characterizing 3-component mixtures are often presented in the Gibbs triangle. The points corresponding to mobile phases that satisfy equation 9 lie on the straight line that begins at the apex corresponding to the first solvent. For such ternary mobile phases, adsorption and capacity ratio are functions of one variable: *x<sub>1</sub>* (because  $x_3 = (1 - x_1)/(r + 1)$ ,  $x_2 = rx_3$ ). Furthermore, these quantities can be expressed by means of equations analogous to those used for a binary mobile phase. In particular, the adsorption isotherm of the most polar solvent is given by the Everett-type equation (23):

$$y_1 = x_1 / [(1 - K)x_1 + K] \quad \text{Eq. 10}$$

in which adsorption constant is given by the weighted average of the constants characterizing adsorption in the binary solutions (1 + 3) and (2 + 3):

$$K = \frac{r}{r + 1} K_{21} + \frac{1}{r + 1} K_{31} \quad \text{Eq. 11}$$

The coefficients before the adsorption constants are equal to mole fractions  $x_2 = r/(r + 1)$  and  $x_3 = 1/(1 + r)$  in the binary mixture (2 + 3) satisfying equation 9. In this case, the capacity ratio can be expressed as:

$$\frac{1}{k_s} = \frac{x_1}{k_{s(1)}} + \frac{(1-x_1)}{k_{s(2,3)}} \quad \text{Eq. 12}$$

where  $k_{s(2,3)}$  is the capacity ratio in the binary mobile phase (2 + 3) for which  $x_2/x_3 = r$ , and

$$\frac{1}{k_{s(2,3)}} = \frac{r}{r+1} \frac{1}{k_{s(2)}} + \frac{1}{r+1} \frac{1}{k_{s(3)}} \quad \text{Eq. 13}$$

It is worth noting that the capacity ratio in a ternary mobile phase can be expressed by means of capacity ratios estimated for binary solvents (1 + 2) and (1 + 3) containing the same mole fraction of the first solvent:

$$\frac{1}{k} = \frac{r}{r+1} \frac{1}{k_{s(1,2)}} + \frac{1}{r+1} \frac{1}{k_{s(3)}} \quad \text{Eq. 14}$$

Moreover, for such a class of mobile phases, equation 8 may be written as:

$$k_s = y_1 k_{s(1)} + (1 - y_1) k_{s(2,3)} \quad \text{Eq. 15}$$

where  $(1 - y_1)$  characterizes the total adsorption of less polar solvents:

$$1 - y_1 = y_2 + y_3 \quad \text{Eq. 16}$$

Similarly, as for binary mobile phases (24), equation 12 can be rewritten in a linear form:

$$\frac{1}{k_s} = ax + b \quad \text{Eq. 17}$$

where

$$a = (1/k_{s(1)}) - (1/k_{s(2,3)}) \quad \text{Eq. 18A}$$

$$b = 1/k_{s(2,3)} \quad \text{Eq. 18B}$$

Using equations 13 and 18, the theoretical values of the parameters  $a$  and  $b$  can be found. However, this requires knowledge of the capacity factors in all pure solvents. Unfortunately, estimating the capacity ratio in the weakest solvent (solvent 3) may be difficult because of its long retention time. In such a situation, retention can be measured in the mobile phases of high concentrations of the polar solvent (solvent 1), and the parameters  $a$  and  $b$  can be extracted by fitting them to the experimental data.

Several simple relationships between retention parameters characterizing chromatographic systems with ternary and binary mobile phases are shown, which may be used to validate our theoretical concepts. It is easy to show that there is the fol-

lowing relationship between the coefficients  $a$  and  $b$  for a ternary mobile phase and the coefficients estimated for binary solvents (1 + 2) and (1 + 3):

$$a = \frac{r}{r+1} a_{(1,2)} + \frac{1}{r+1} a_{(1,3)} \quad \text{Eq. 19A}$$

$$b = \frac{r}{r+1} b_{(1,2)} + \frac{1}{r+1} b_{(1,3)} \quad \text{Eq. 19B}$$

In the considered case of liquid–solid adsorption chromatography with an ideal mobile phase, exceptionally simple relationships between retention parameters in different solvents have been found. These dependences may be used in the mathematical analysis of the results.

From equations 11–13 we find:

$$k_s = \alpha k_{s(1)} \quad \text{Eq. 20}$$

where

$$\alpha = [x_1(1 - K) + K]^{-1} \quad \text{Eq. 21}$$

This means that for a given value of the mole fraction  $x_1$ , the capacity factor in any mobile phase is strictly proportional to the capacity ratio of the solute in a reference solvent, (e.g., in the most polar first solvent). The proportionality coefficient  $\alpha$  depends on the nature of the mobile phase. In this way, the separation of the solute and solvent effects is possible. Moreover, the parameters  $a$  and  $b$  are associated in the following way:

$$a = \beta \cdot b \quad \text{Eq. 22}$$

where

$$\beta = (1/K) - 1 \quad \text{Eq. 23}$$

It should be pointed out that equations 20–23 hold only when the solute and solvent molecules have similar sizes. This is the major limitation of the used model. The equations 4, 21, and 23 give links between chromatographic factors and purely adsorption parameters.

## Experimental

The chromatographic measurements were made using thin-layer adsorption chromatography. The chromatograms were developed on 10- × 10-cm precoated SiO<sub>2</sub>-60 plates (Merck, Darmstadt, Germany) in saturated DS horizontal sandwich chambers (Chromdes, Lublin, Poland) at 293 K. Solutes were automatically applied to the plates by means of Camag Linomat IV applicator (Muttenez, Switzerland). The spots were visualized in iodine vapor and detection was performed by means of a Shimadzu CS-9000 dual-wavelength scanner (Kyoto, Japan) and Camag Reprostar 3 video camera. The components of mobile

phase were cyclohexane, benzene, toluene, and ethylene chloride. In mixed mobile phases (binary and ternary), the concentration of the most polar solvent (solvent 1) was equal to 0.1, 0.3, 0.5, 0.7, 0.9, and 1.0 of mole fractions. In the preparation of the ternary mobile phases, the concentration of the binary phase (2 + 3) was first defined [i.e., the mole ratio ( $r = x_2/x_3$ ) being equal to  $1/3$ , 1, and 3]. Then the most polar solvent was added in concentrations equaling 0.1, 0.3, 0.5, 0.7, and 0.9 of the mole fraction value. Additionally, in order to eliminate autochromatography of mobile phase components, the plates were preconditioned, before developing, in the vapor of the most polar solvent for 5 min.

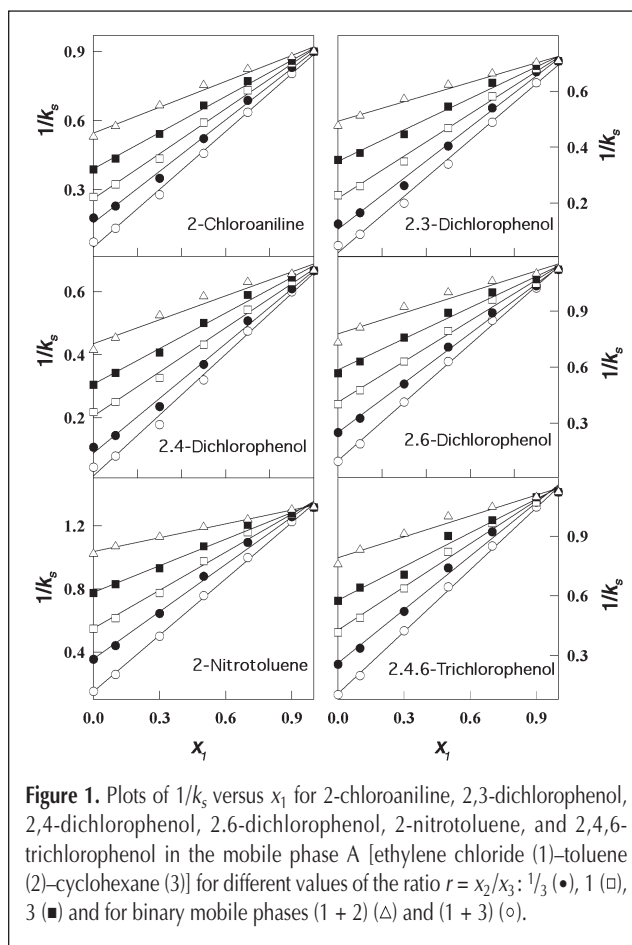
## Results and Discussion

The equations presented in the previous section have been used for the interpretation of the retention data measured for chosen solutes and the two ternary mobile phases, (A) [ethylene chloride (1)–toluene (2)–cyclohexane (3)] and (B) [ethylene chloride (1)–benzene (2)–cyclohexane (3)]. The following values of the ratio  $r = x_2/x_3$  were considered:  $1/3$ , 1, and 3. The data measured for the binary mobile phases (1 + 2) and (1 + 3) have also been analyzed. The use of thin-layer chromatography permits quick measurements for the whole concentration range.

The elution properties of the mobile phases used are discussed. A ternary mobile phase can be considered as a mixture of the polar component 1 and a binary mixed solvent (2 + 3). Both systems consist of the same strong solvent (1-ethylene chloride) and different binary mixtures of the weaker solvents. On the other hand, the considered mobile phases are composed of two relatively strong solvents (1 and 2), whereas the third component is a very low-strength solvent.

Attention was focused on the results obtained for the mobile

phase A. The capacity factors in the pure solvents  $k_{s(i)}$  ( $i = 1, 2$ , and 3) and the ratios  $k_{s(1)}/k_{s(i)}$  are listed in Table I. For all considered solutes the ratios of retention factors  $k_{s(1)}/k_{s(2)}$  have similar values, whereas the ratios  $k_{s(1)}/k_{s(3)}$  (Table I) are of the same order. This suggests that differences in molecular sizes of solutes and solvents may be neglected, and the theory dis-



**Figure 1.** Plots of  $1/k_s$  versus  $x_1$  for 2-chloroaniline, 2,3-dichlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 2-nitrotoluene, and 2,4,6-trichlorophenol in the mobile phase A [ethylene chloride (1)–toluene (2)–cyclohexane (3)] for different values of the ratio  $r = x_2/x_3$ :  $1/3$  (●), 1 (□), 3 (■) and for binary mobile phases (1 + 2) (Δ) and (1 + 3) (○).

**Table I. Results for the Mobile Phase A [Ethylene Chloride (1)–Cyclohexane (2)–Hexane (3)]\***

Solute	$k_1$	$k_2$	$k_3$	$k_{s(1)}/k_{s(3)}$		$k_{s(1)}/k_{s(2,3)}$		$k_{s(1)}/k_{s(2)}$
				$r = 0$	$r = 1/3$	$r = 0$	$r = 3$	
2-nitrotoluene	0.76	0.98	6.61	0.11	0.27	0.42	0.59	0.78
2-chloroaniline	1.11	1.88	13.71	0.08	0.20	0.30	0.43	$\infty/0.59$
2-nitrophenol	0.79	1.05	7.62	0.10	0.25	0.40	0.54	0.75
2,3-dichlorophenol	1.41	2.09	20.28	0.07	0.18	0.32	0.50	0.67
2,4-dichlorophenol	1.50	2.41	22.80	0.07	0.16	0.33	0.46	0.62
2,6-dichlorophenol	0.89	1.36	10.23	0.09	0.22	0.36	0.51	0.65
2,4,6-trichlorophenol	0.89	1.32	9.64	0.09	0.23	0.37	0.51	0.67
$K$				0.09	0.22	0.36	0.51	0.68
$\alpha$				21.00	8.01	3.73	2.42	1.82
$\beta$				4.3	1.68	0.93	0.41	0.08

\* The capacity factors in the pure solvents ( $k_{s(i)}$ ), the ratios of these factors: ( $k_{s(1)}/k_{s(2)}$ ), ( $k_{s(1)}/k_{s(3)}$ ) and the ratios ( $k_{s(1)}/k_{s(2,3)}$ ) for the binary mobile phase (2 + 3) and different values of the ratio  $r = x_2/x_3$ :  $1/3$ , 1, 3. The adsorption constants  $K$  (equation 11), the proportionality factors  $\alpha$  (equation 20) and  $\beta$  (equation 22).

Solute	$r = 0 (1 + 3)$				$r = 1/3$				$r = 1$				$r = 3$				$r = \infty (1 + 2)$			
	$a_{exp}$	$a_{th}$	$b_{exp}$	$b_{th}$	$a_{exp}$	$a_{th}$	$b_{exp}$	$b_{th}$	$a_{exp}$	$a_{th}$	$b_{exp}$	$b_{th}$	$a_{exp}$	$a_{th}$	$b_{exp}$	$b_{th}$	$a_{exp}$	$a_{th}$	$b_{exp}$	$b_{th}$
2-nitrotoluene	1.19	1.17	0.15	0.15	1.00	0.95	0.36	0.37	0.80	0.73	0.55	0.59	0.56	0.52	0.78	0.80	0.29	0.30	1.04	1.02
2-chloroaniline	0.84	0.83	0.05	0.07	0.75	0.71	0.16	0.19	0.65	0.60	0.26	0.30	0.52	0.48	0.39	0.42	0.37	0.37	0.55	0.53
2-nitrophenol	1.14	1.14	0.09	0.13	0.97	0.93	0.28	0.34	0.79	0.73	0.48	0.54	0.58	0.52	0.69	0.75	0.30	0.32	0.98	0.95
2,3-dichlorophenol	0.67	0.66	0.02	0.05	0.61	0.55	0.11	0.16	0.50	0.44	0.22	0.27	0.38	0.34	0.35	0.37	0.23	0.23	0.49	0.48
2,4-dichlorophenol	0.64	0.62	0.02	0.04	0.58	0.53	0.09	0.14	0.47	0.44	0.21	0.23	0.38	0.35	0.31	0.32	0.24	0.26	0.44	0.41
2,6-dichlorophenol	1.04	1.03	0.10	0.10	0.88	0.86	0.25	0.26	0.73	0.70	0.41	0.42	0.56	0.54	0.59	0.58	0.38	0.38	0.78	0.74
2,4,6-trichlorophenol	1.04	1.02	0.11	0.10	0.90	0.85	0.26	0.27	0.72	0.69	0.43	0.43	0.57	0.52	0.58	0.60	0.35	0.36	0.79	0.76

\* The coefficients  $a_{exp}$ ,  $b_{exp}$  estimated from experimental data and the theoretical coefficients  $a_{th}$  and  $b_{th}$  calculated from equation 18 for the ternary mobile phases with different values of the ratio  $r = x_2/x_3$ :  $1/3$ , 1, 3 and for the binary mobile phases (1 + 2) and (1 + 3).

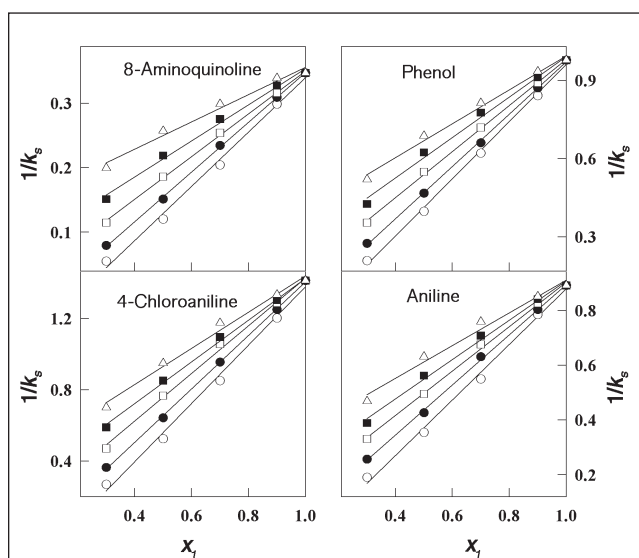


Figure 2. Plots of  $1/k_s$  versus  $x_1$  for 8-aminoquinoline, phenol, 4-chloroaniline, aniline, and phenol in the mobile phase B [ethylene chloride (1)–benzene (2)–cyclohexane (3)] for different values of the ratio  $r = x_2/x_3$ :  $1/3$  (●), 1 (□), 3 (■) and for binary mobile phases (1 + 2) (△) and (1 + 3) (○).

Solute	1 + 3	$r = 1/3$		$r = 1$		$r = 3$		1 + 2
	$1/k_{exp}$	$1/k_{exp}$	$1/k_{th}$	$1/k_{exp}$	$1/k_{th}$	$1/k_{exp}$	$1/k_{th}$	$1/k_{exp}$
8-aminoquinoline	0.12	0.15	0.15	0.18	0.18	0.21	0.21	0.24
8-hydroxyquinoline	0.62	0.72	0.74	0.85	0.86	0.98	0.98	1.10
phenol	0.40	0.47	0.47	0.55	0.55	0.62	0.62	0.69
aniline	0.36	0.43	0.43	0.50	0.50	0.56	0.56	0.63
4-chloroaniline	0.52	0.64	0.63	0.77	0.74	0.85	0.84	0.95

\* The reciprocals of retention factors for the binary mobile phases (1 + 3) and (1 + 2) measured at  $x_1 = 0.5$ . The experimental values of reciprocals of retention factors and these quantities calculated from equation 14 for the ternary mobile phases with different values of the ratio  $r = x_2/x_3$ :  $1/3$ , 1, 3 at  $x_1 = 0.5$ .

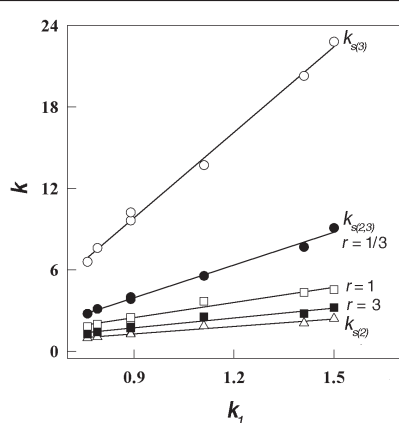
cussed in the previous section can be used. The deviations observed for the third solvent may be caused by errors connected with the experimental estimation of the retention factors for such a weak solvent. The adsorption constants  $K_{21}$  and  $K_{31}$  have been calculated using equation 6. Moreover, the ratios of the retention factors in the first solvent and the retention factors in the binary solvent (2 + 3) have been estimated, for which  $x_2/x_3 = r$  that is, the quantities:  $k_{s(1)}/k_{s(2,3)}$ . In accordance with equations 11 and 13, these ratios should be equal to the constant  $K$  that characterizes adsorption of solvents 2 and 3 in their mixture with the polar solvent 1. As follows from Table I, the suitable values obtained for different solutes are close. The average values of adsorption constants are presented in Table I. The constant  $K$  increases with increasing value of the ratio  $r$  and changes from  $K = K_{31} = 0.09$  for the binary mobile phase (1 + 3) to  $K = K_{21} = 0.68$  for the binary mobile phase (1 + 2). In our model, the retention depends on competitive adsorption of the solute and all solvents. The solvent with the strongest adsorption was solvent 1, whereas adsorption of the third solvent was weakest. Indeed, we have found that  $K_{31} < K_{21} < 1$ .

Figure 1 illustrates the influence of the composition of a ternary mobile phase on the capacity factors of the selected solutes. The plots  $1/k_s$  versus  $x_1$  for different values of the ratio of concentrations of the less-polar solvent fans out between the straight lines drawn for the binary mobile phases (1 + 3) and (1 + 2). The slopes of the plots depend considerably on the relative concentrations of solvents 2 and 3 in the mobile phase. This means that adsorption of all solvents plays an important role in the retention process. Figure 1 gives evidence that equations 17 and 18 can be used to predict the capacity ratio in ternary mobile phases. The theoretical parameters  $a$  and  $b$  have been calculated from equation 18, using solely the capacity ratios in pure solvents. These constants are compared with the best-fit parameters estimated for

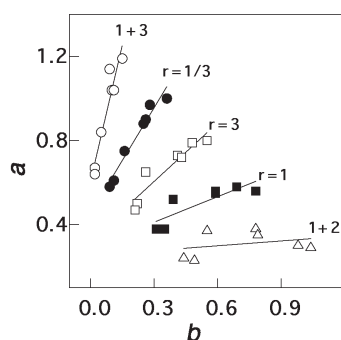
experimental linear dependences  $1/k_s$  versus  $x_1$  in Table II. Agreement between these values is surprisingly good.

The illustrative examples of the results obtained for several solutes and the mobile phase B are presented in Figure 2. According to equation 14, for a given concentration of the most polar component ( $x_1$ ), the capacity ratio in the ternary solvent may be expressed by means of the suitable retention factors for binary mobile phases (1 + 2) and (1 + 3). In Table III, the experimental values ( $1/k_{s(exp)}$ ) are collected together with those obtained from equation 14 ( $1/k_{s(th)}$ ) for  $x_1 = 0.5$  and different values of the ratio  $r$ . Also, in this case, the accuracy of theoretical predictions is satisfactory.

Lastly, two interesting correlations between parameters measured for various mobile phases are presented. The relationships between the capacity factors for different mobile phases and the capacity ratio in the reference solvent 1 are shown in Figure 3. The points for different solutes lie on the straight line corresponding to a given binary mobile phase (2 + 3), for which the ratio  $x_2/x_3 = r$  and on the lines plotted for the pure solvents 2 and 3. This is consistent with the theory. The slope



**Figure 3.** The data for the mobile phase A. Plots of the relationships equation 20 for binary mobile phases (2 + 3) with different values of the ratio  $r = x_2/x_3$ :  $1/3$  (•), 1 (□), 3 (■), and for the pure solvents (solvent 2) (Δ) and (solvent 3) (○). The solutes are collected in Table I.



**Figure 4.** The correlations between the parameters  $a$  and  $b$  (the linear dependence equation 17) for the ternary mobile phase A and different values of the ratio  $r = x_2/x_3$ :  $1/3$  (•), 1 (□), 3 (■), and for binary mobile phases (1 + 2) (Δ) and (1 + 3) (○). The solutes are collected in Table II.

of straight line (equation 20) is connected with the constant characterizing adsorption in a given mobile phase ( $K$ ) and decreases when the relative concentration of the second solvent is increased.

In Figure 4, the correlations between the coefficients  $a$  and  $b$  are shown for the ternary mobile phase A and the suitable binary solvents. As in Figure 3, the points for different solutes are grouped along the straight line corresponding to a given value of the ratio  $r$ . This confirms the theoretical equation 22, and it may be treated as an extra verification of the model used.

To sum up, the adsorption-based model of retention presented here may be used to interpret experimental data measured for ternary mobile phases. Agreement between the theoretical predictions and the results of experiments was very good in all investigated systems.

## Conclusion

The adsorption model of retention has been developed for chromatographic systems with ternary mobile phases. The approach leads to equations that permit the calculation of the capacity factors in ternary mobile phases by means of the capacity factors estimated for pure solvents or their binary mixtures. It has been shown that if the ratio of mole fractions of the less-polar solvents remain fixed, the very simple and linear dependence of  $1/k_s$  versus  $x_1$  is fulfilled. Such a relationship has been successfully used to describe experimental data. The theory allows for a deeper physicochemical interpretation of best-fit parameters and bridges the gap between the retention and adsorption data. The interesting correlations of retention parameters estimated for different mobile phases have been found.

The model permits greater insight into possible mechanisms of the process and its evolution with the change of mobile phase composition. It describes chromatographic systems in which the retention is strongly dominated by interactions within the solid surface, and the competitive adsorption of all solvents dictates the mode of the process. However, the assumption of the solvent's ideality is rather unrealistic for many popular mobile phases. This factor should be taken into account in more sophisticated theoretical treatments. Nevertheless, it can qualitatively describe the most significant features of numerous chromatographic systems.

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## References

1. L.R. Snyder. *Principles of Adsorption Chromatography*. Marcel Dekker, New York, NY, 1968.

2. E. Soczewiński. Solvent composition effects in thin-layer chromatography. Systems of the type silica gel-electron donor solvent. *Anal. Chem.* **41**: 179–82 (1969).
3. M. Borówko and M. Jaroniec. Current state in adsorption from multicomponent solutions of nonelectrolytes on solids. *Adv. Colloid Interface Sci.* **19**: 137–77 (1983).
4. M. Jaroniec, D.E. Martire, and M. Borówko. Theoretical basis of liquid adsorption chromatography with mixed mobile phases and its connection with the theory of adsorption from multicomponent solutions. *Adv. Colloid Interface Sci.* **22**: 177–227 (1985).
5. M. Borówko. "Adsorption on heterogeneous surfaces". In *Adsorption: Theory, Modeling and Analysis, Surfactant Science Series*". J. Tóth, Ed. Vol. 107. Marcel Dekker, New York, NY, pp.105–73 2002.
6. M. Jaroniec, J. Narkiewicz, and M. Borówko. Dependence of the capacity ratio on mobile phase composition in liquid adsorption chromatography. *Chromatographia* **11**: 581–85 (1978).
7. M. Borówko. Liquid adsorption chromatography with multicomponent mobile phase. *J. Colloid Interface Sci.* **102**: 519–26 (1984).
8. M. Borówko and M. Jaroniec. Association effects in adsorption from multicomponent solutions on solids and liquid adsorption chromatography. *J. Chem. Soc. Faraday Trans.1* **79**: 363–72 (1983).
9. M. Jaroniec and B. Ościk-Mendyk. Application of excess adsorption data measured for components of the mobile phase for characterizing chromatographic systems. *J. Chem. Soc. Faraday Trans.1* **77**: 1277–84 (1981).
10. R.E. Boehm and D.E. Martire. A unified theory of retention and selectivity in liquid chromatography. 1. Liquid-solid (adsorption) chromatography. *J. Phys. Chem.* **84**: 3620–30 (1980).
11. P. Jandera, M. Škavrada, L. Andel, D. Komers, and G. Guichon. Description of adsorption equilibrium in liquid chromatographic systems with binary mobile phases. *J. Chromatogr. A* **908**: 3–17 (2001).
12. P. Jandera, M. Kočerová, and J. Holíková. Description and prediction of retention in normal-phase high performance liquid chromatography with binary and ternary mobile phases. *J. Chromatogr. A* **762**: 15–26 (1997).
13. P. Jandera, L. Petránek, and M. Kočerová. Characterisation and prediction of retention in isocratic and gradient-elution normal-phase high-performance liquid chromatography on polar bonded stationary phases with binary and ternary solvent systems. *J. Chromatogr. A* **791**: 1–19 (1997).
14. E. Soczewiński. Mechanistic molecular model of liquid-solid chromatography. Retention-eluent composition relationships. *J. Chromatogr. A* **965**: 109–16 (2002).
15. D. Nurok. Strategies for optimizing the mobile phase in planar chromatography. *Chem. Rev.* **89**: 363–75 (1989).
16. B.M.J de Spiegeleer, P.H.M. de Moerloose, and G.A.S. Slegers. Criterion for evaluation and optimization in thin-layer chromatography. *Anal. Chem.* **59**: 62–64 (1987).
17. J.L. Glajch, J.J. Kirkland, K.M. Squire, and J.M. Minor. Optimization of solvent strength and selectivity for reversed-phase liquid chromatography using an interactive mixture-design statistical technique. *J. Chromatogr.* **199**: 57–79 (1980).
18. Sz. Nyiredy, K. Dallenbach-Toelke, and O. Sticher. Correlation and prediction of the k values for mobile phase optimisation in HPLC. *J. Liq. Chromatogr.* **12**: 95–116 (1989).
19. M. Borówko. On application of the Snyder-Soczewiński equation for describing of LSC process with a ternary mobile phase. *J. Liq. Chromatogr.* **14**: 1247–56 (1991).
20. M. Borówko and B. Ościk-Mendyk. A simple procedure of calculating the capacity ratio in liquid adsorption chromatography with ternary mobile phase. *J. Liq. Chromatogr.* **12**: 2601–2607 (1989).
21. B. Ościk-Mendyk. Mechanism of liquid adsorption chromatography in thin-layer chromatography with ternary mobile phases. *J. Chromatogr.* **550**: 645–56 (1991).
22. V. Ya. Davydov. "Study of adsorption from solutions by chromatography". In *Adsorption on New and Modified Inorganic Sorbents. Studies in Surface, Science and Catalysis*. A. Dąbrowski and V.A. Tertych, Eds. Elsevier Science B.V. Radarweg, Amsterdam, the Netherlands, 1996, p. 99.
23. D.H. Everett. Thermodynamics of adsorption from solution. *J. Chem. Soc., Faraday Trans. 1* **60**: 1803–13 (1964).
24. R.P.W. Scott and P. Kučera. Solute interactions with the mobile and stationary phases in liquid-solid chromatography. *J. Chromatogr.* **112**: 425–42 (1975).

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