

**COMPETITIVE ADSORPTION OF SOLUTE
AND MODIFIER IN A MIXED
PARTITION/DISPLACEMENT MECHANISM
IN REVERSED-PHASE LIQUID CHROMATOGRAPHY**

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SUMMARY

Competitive adsorption of the solute and the polar component of the mobile phase (the modifier) in reversed-phase liquid chromatography is discussed. Retention properties on surfaces differing in alkyl chain length (RP-18 compared with RP-8e) have been investigated. Typical solvent mixtures were used as mobile phases—methanol–water and acetonitrile–water. Three different types of compound were selected as solutes—two homologous cycloketones, cyclopentanone and cyclohexanone, and phenol. For all these solutes the adsorption mechanism was heterogeneous – the compounds had affinity for the polar uncovered surface and for the alkyl chains. Adsorption of organic solvents on the RP-8e (endcapped) adsorbent was measured and compared with that on RP-18e and RP-18 (non-endcapped) measured in previous studies. The adsorption equilibrium of solvents was strongly affected by the structure of the adsorbent surface and the polarity of the mobile phase.

Variation of separation selectivity as a function of surface properties and mobile-phase composition was also investigated, and is discussed. The model of competitive adsorption proposed in our previous study has been used for analysis of the retention behaviour of the solutes and for prediction of non-linear chromatographic band profiles.

INTRODUCTION

The effect of mobile phase modifiers on mechanisms of retention in chromatography columns has attracted attention for a long time. It is well known that the adsorption behaviour of a system can be affected by changes of mobile-phase composition, and this is often manipulated to improve

separation performance in chromatography. To predict and optimize the retention properties of the components to be separated as a function of the mobile-phase composition several retention models have been developed. This is particularly true for reversed-phase adsorbents, which are widely used in chromatography. Retention models for reversed-phase chromatography of non-ionizable compounds take two mechanisms into account. The first is partitioning of solutes between the mobile phase and the alkyl chains; the second, known as “adsorption” or “displacement”, assumes that molecules of solute and organic solvent are co-adsorbed on the tips of the hydrocarbon chains. Displacement is followed by removal of solvent molecules whereas in partitioning the solute is distributed between two phases. In this work the name “displacement” is adopted and the term “adsorption” is understood to be a general phenomenon of transfer of the solute between the mobile and stationary phases.

The first retention models to consider partition were developed by Martire and Boehm [1] and, later, by Dill [2] who predicted the retention of different solutes in typical reversed-phase systems by use of a partition and displacement model. Comparison revealed that partitioning dominates the retention mechanism. Although the validity of the model was confirmed for RP adsorbents with long carbon chains (i.e. C₁₈) for many solutes [3], substantial deviations were observed for compounds with affinity for polar exposed silanols. For these compounds a mixed mechanism of displacement on silanols and partition on alkyl chains could be expected [3]. Both partition and displacement mechanisms have been studied in detail [4–8].

To predict and optimize solute retention properties as a function of mobile-phase composition several simplified retention models have been suggested. The most frequently used is the linear solvent-strength theory (LSST) [9,10], which assumes that the retention of a solute is related to the concentration of the organic solvent by a logarithmic relationship. The coefficients of this relationship are determined empirically by analysing retention of pulses of the solute under investigation with different amounts of the organic component of the mobile phase. These retention data are then correlated with mobile-phase composition. To model the retention of structurally diverse solutes more detailed models can be used, e.g. a linear solvation energy relationship LSER. The LSER and LSST models, and their modifications, have been compared [11]. Also widely used are quadratic models [12] which correlate the logarithm of retention factors with organic solvent content by use of a quadratic equation. Curvature in plots

of logarithms of retention factors against mobile phase modifier content has also been suggested as resulting from mixed displacement/partition processes [3]. Several physicochemically-based retention models have also been proposed [13,14].

All the models mentioned were developed for linear chromatography and are usually verified on the basis of retention data of solute pulses recorded as the composition of the mobile phase is varied. Pulse experiments are not suitable for predicting the effect of mobile-phase composition in non-linear chromatography. Information about competitive non-linear equilibria can be gathered by analysis of the shapes of chromatographic bands. Changes in retention and peak shape can be correlated with the concentrations of the organic modifier in the mobile and stationary phases. Semi-empirical approaches dominate the modelling of chromatographic band profiles under non-linear conditions in the presence of multicomponent mobile phases. These approaches entail measurement of the adsorption equilibrium of the solute for different amounts of organic solvent in the mobile phase and correlation of the isotherm coefficients obtained with modifier concentration by use of theoretical models developed for linear chromatography, e.g. the LSST model. Such an approach has been used to indicate general trends in equilibrium changes, as a result of the presence of the organic solvent [15–17], and for optimization of mobile-phase composition [15,18]. This simplified approach has been extended by taking into account adsorption of the modifier [19].

The simplified models mentioned above do not take into account competitive adsorption equilibria of solute and solvents contained in the mobile phase and, therefore, cannot explain the mechanism of retention under non-linear conditions. We have recently developed a competitive adsorption model for prediction of the effects of mobile-phase composition and/or temperature on the retention of solutes on RP adsorbents [20, 21]. The model takes into account the partition and displacement mechanisms of solute retention and relates these to competitive adsorption of the organic solvent. A mixed displacement/partition mechanism was observed for the solutes selected for the study. To reproduce a simple analytical pulse experiment, all the models mentioned could be successfully used with empirical model terms properly adjusted. They failed when used to reproduce non-linear band profiles, however; under these conditions all the terms of the model were found to depend on modifier concentration. This reduced the theoretical model to the purely empirical approach discussed above.

The work reported below completes the studies reported elsewhere [20,21], i.e. the retention model suggested in those studies has been used to compare competitive adsorption of a solute and an organic solvent on different columns. Series of experiments were conducted under linear and non-linear isotherm conditions to investigate:

- (a) the effect of carbon chain length on adsorption equilibria in RP systems (RP-8e and RP-18);
- (b) the retention properties of different solutes, i.e. two homologous cycloketones and phenol, all of which had affinity for the polar exposed silanols present on typical RP surfaces; and
- (c) the dependence of separation selectivity for the two homologous compounds on the structure of the surface and on mobile-phase composition.

The experimental results reported in Ref. [20] have also been used in this work.

THEORY

Model of the Chromatographic Column

The equilibrium-dispersive model [22, 23] was used to model column dynamics. If the mole fraction is used as the unit of concentration the partial differential mass balance equation can be expressed in the form [20]:

$$\frac{\partial x_i^m}{\partial t} + F \frac{M^m}{\rho^m} \frac{\partial \Gamma_i^*}{\partial t} + w \frac{\partial x_i^m}{\partial z} = D_a \frac{\partial^2 x_i^m}{\partial z^2} \quad (1)$$

where $w = u / \varepsilon_t$ is the interstitial velocity, ε_t is the total column porosity, $F = (1 - \varepsilon_t) / \varepsilon_t$ is the phase ratio, D_a is the apparent dispersion coefficient related to the column efficiency N (number of theoretical plates), as $N = wL / 2D_a$, x_i^m is the mole fraction of the component in the mobile phase, t and z are the time and space co-ordinates, and the equilibrium excess adsorption Γ_i^* is correlated with x_i^m , i.e., $\Gamma_i^* = f(x_i)$. M^m and ρ^m are the molar mass and the density of the mobile phase, where $M^m = f(x_i^m)$ and $\rho^m = f(x_i^m)$.

The model has to be coupled with proper boundary and initial conditions.

To solve eq. (1) the equilibrium equation $\Gamma_i^* = f(x_i^m)$ must be specified.

The excess adsorption of component i of a multicomponent mixture can be calculated by use of eq. (2) [24–26]:

$$\Gamma_i^* = q_i^* - x_i^m \sum_{i=1}^{NC} q_i^* \quad (2)$$

where q_i^* is the concentration in the adsorbed phase, which is expressed by the isotherm equation. To quantify adsorption equilibrium the isotherm equation must be determined.

Model of Adsorption Equilibria

Adsorption Equilibria for the Binary System Organic Solvent ($i = A$)–Weak Solvent ($i = B$)

In this work the model of excess adsorption of an organic solvent from binary mixtures proposed elsewhere [20] has been used. This model takes into account non-ideality of the mobile and adsorbed phases. The non-ideality of the mobile phase is taken into account by use of activity coefficients and non-idealities of the adsorbed phase are suggested to originate from multilayer adsorption, whereby molecules can interact with already adsorbed layers [20]. A dual retention mechanism has been suggested:

- (a) displacement from a water-rich adsorbed phase; this phase is concentrated in the neighbourhood of the polar surface [27]; and
- (b) partition on non-polar alkyl chains.

It is assumed that molecules of the adsorbed organic solvent mutually interact creating clusters around the alkyl chains. This explains the phenomenon of extension of alkyl chains in the presence of organic solvents suggested elsewhere [28].

A detailed discussion of the model can be found elsewhere [20]. The same model has been used to predict the mixed effect of mobile-phase composition and temperature on the adsorption properties of a solute in RP systems [21]. In the following discussion only final isotherm equations are presented.

The total amount of organic solvent A adsorbed through both the mechanisms is expressed as:

$$\begin{aligned}
(q_A^*)_t &= (q_A^*)_d + (q_A^*)_a = \\
&= (q_A^*)_{d1} \cdot \frac{1 - \left(\frac{K_{dn} \cdot x_A^m \gamma_A^m}{(1-x_A^m)\gamma_B^m + K_{dn} \cdot x_A^m \gamma_A^m} \right)^{n_d}}{1 - \left(\frac{K_{dn} \cdot x_A^m \gamma_A^m}{(1-x_A^m)\gamma_B^m + K_{dn} \cdot x_A^m \gamma_A^m} \right)} + (q_A^*)_{a1} \cdot \frac{1 - \left(\frac{K_{an} \cdot x_A^m \gamma_A^m}{1 + K_{an} \cdot x_A^m \gamma_A^m} \right)^{n_a}}{1 - \left(\frac{K_{an} \cdot x_A^m \gamma_A^m}{1 + K_{an} \cdot x_A^m \gamma_A^m} \right)} \quad (3)
\end{aligned}$$

where: $(q_A^*)_{d1} = \frac{(q_A^\infty)_d K_{d1} \cdot x_A^m \gamma_A^m}{(1-x_A^m)\gamma_B^m + K_{d1} \cdot x_A^m \gamma_A^m}$ and $(q_A^*)_{a1} = \frac{(q_A^\infty)_a K_{a1} \cdot x_A^m \gamma_A^m}{1 + K_{a1} \cdot x_A^m \gamma_A^m}$

are the amounts of organic solvent A adsorbed in the first layer in the displacement, d, and the partition, a, mechanisms, respectively, $(q_A^\infty)_d$ and $(q_A^\infty)_a$ are the saturation capacity in each separate layer for both mechanisms, K_{d1} and K_{a1} are the equilibrium constants in the first (closest to the surface) layer, K_{dn} and K_{an} are the equilibrium constants in subsequent layers, n_d and n_a are the numbers of layers for both mechanisms, and γ_A^m is the activity coefficient in the mobile phase. Because non-ideality of the adsorbed phase was assumed to originate from multilayer adsorption, activity coefficients of solvents in the solid phase are not included.

When the organic solvent A is not retained or is weakly retained by the alkyl chains, eq. (3) becomes the homogenous model with $(q_A^*)_a = 0$ [20,21]. The excess adsorption of an organic solvent A, Γ_A^* , from binary mixtures with water, B, is related with the total amount adsorbed, $(q_A^*)_t$, by the equation [20,21]:

$$\Gamma_A^* = (q_A^*)_t - [(q_A^*)_t + (q_B^*)_d] x_A^m \quad (4)$$

where $(q_B^*)_d$ is adsorption of water which, according to the model, can occur exclusively by displacement on the polar surface [20,21].

Adsorption Equilibria for the Binary System Solute (i = P)–Weak Solvent (i = B)

The same dual retention mechanism was assumed for solute P. Because of its low concentration, however, the solute (typically) forms a single layer only. The total amount of P adsorbed in the presence of pure weak solvent B is expressed as:

$$(q_p^*)_{tot} = (q_p^*)_a + (q_p^*)_d = \frac{(q_p^\infty)_d K_{Pd} \cdot x_p^m \gamma_p^m}{(1-x_p^m)\gamma_B^m + K_{Pd} \cdot x_p^m \gamma_p^m} + \frac{(q_p^\infty)_a K_{Pa} \cdot x_p^m \gamma_p^m}{1 + K_{Pa} \cdot x_p^m \gamma_p^m} \quad (5)$$

Typically, for the solute (because of its low concentration) excess adsorption is equivalent to the concentration in the adsorbed phase:

$$\Gamma_p^* \cong (q_p^*) \quad (6)$$

Adsorption Equilibria for the System Solute P–Weak Solvent B–Organic Solvent A

In the presence of organic solvent the solute participates in:

- (a) Displacement of water from the polar surface free from adsorbed organic solvent. The amount adsorbed in the first layer on the polar surface, i.e. $(q_A^*)_{d1}$, determines competitive adsorption of the solute on this surface [20,21]. If the saturation capacities are assumed to be the same for all the components, the adsorption surface available for the solute can be calculated by simple subtraction, i.e. $[(q_p^\infty)_d - (q_A^*)_{d1}]$. Hence, the equation for competitive equilibrium can be expressed as:

$$(q_p^*)_d = \frac{K_{Pd} \cdot x_p^m \gamma_p^m \cdot [(q_p^\infty)_d - (q_A^*)_{d1}]}{(1-x_p^m - x_A^m) \cdot \gamma_B^m + K_{Pd} \cdot x_p^m \gamma_p^m} \quad (7)$$

If saturation capacities are different a more complicated competition model should be used for each retention mechanism (e.g. adsorbed solution theory – an example is given elsewhere [29] for a normal-phase system).

- (b) Partition on alkyl chains free from adsorbed organic solvent. Because the adsorbed molecules of organic solvent are assumed to interact along the alkyl chains, the total concentration of organic solvent retained on alkyl chains, i.e. $(q_A^*)_a$, is assumed to determine competitive adsorption. Thus, the equation of competitive equilibrium of the solute on alkyl chains can be written:

$$(q_p^*)_a = \frac{K_{Pa} \cdot x_p^m \gamma_p^m \cdot [(q_p^\infty)_a - (q_A^*)_a]}{1 + K_{Pa} \cdot x_p^m \gamma_p^m} \quad (8)$$

- (c) Displacement of organic solvent adsorbed in layers, for both mechanisms, creates additional adsorption surface $(q_A^*)_l$:

$$(q_P^*)'_d = \frac{K'_P \cdot (q_A^*)_t \cdot x_P^m \cdot \gamma_P^m}{x_A^m \cdot \gamma_A^m + x_P^m \cdot \gamma_P^m \cdot K'_P} \quad (9)$$

The total solid phase concentration is the sum of these terms:

$$q_{pt}^* = (q_P^*)'_d + (q_P^*)_a + (q_P^*)'_d \quad (10)$$

The terms of the competitive adsorption model (eqs 7–10), K_{pa} and K_{pd} , must be determined independently for the system solute–weak solvent (i.e. P–B) and the amounts adsorbed $(q_A^*)_a$, $(q_A^*)_{d1}$, and $(q_A^*)_{tot}$ are calculated independently in accordance with the adsorption equilibrium of the system organic solvent–weak solvent (i.e. A–B).

The coefficient K'_P in eq. (9) is the only adjustable term and must be determined from experimental data for competitive adsorption in the system P–A–B.

EXPERIMENTAL

All experiments were performed at ambient temperature.

Chemicals

The compounds investigated were two homologous cycloketones, cyclopentanone (C5) and cyclohexanone (C6) (Sigma–Aldrich), which are liquid under ambient conditions, and phenol (Sigma–Aldrich), which is solid under ambient conditions. The mobile phases were prepared from methanol, water, and acetonitrile for HPLC (Merck, Darmstadt, Germany).

Columns

All columns were 4 mm i.d. packed with bonded LiChrospher silica, pore volume $1.25 \text{ cm}^3 \text{ g}^{-1}$, pore diameter 100 \AA , particle size 5 \mu m from (Merck, Darmstadt, Germany). The column lengths and reversed-phase column types were:

- I RP-18, column length $L = 250 \text{ mm}$;
- II RP-18e, column length $L = 250 \text{ mm}$;
- III RP-18e, column length $L = 125 \text{ mm}$; and
- IV RP-8e, column length $L = 250 \text{ mm}$.

To determine the effect of surface structure on adsorption equilibria column IV was used. The retention properties of RP-8e were compared

with those of RP-18 and RP-18e investigated in other work [20]. Column III was used to compare the adsorption properties of the different solutes. All the columns were used to investigate the selectivity of separation of the two cycloketones. The properties of the adsorbents are summarized in Table I.

Table I

Properties of LiChrospher RP-18, RP-18e, and RP-8e

Adsorbent	LiChrospher RP-18 (column I)	LiChrospher RP-18e (columns II and III)	LiChrospher RP-8e (column IV)
Characteristics	Octadecyl derivative of spherical silica particles	Octadecyl derivative of spherical silica particles, endcapped	Octyl derivative of spherical silica particles, endcapped
Specific surface area ($\text{m}^2 \text{g}^{-1}$)	350	350	350
Carbon loading (%)	21.0	21.6	13
Bonding density ($\mu\text{mol m}^{-2}$)	3.17	3.38	3.56
Pore diameter (\AA)	100	100	100
Column void volume	0.63	0.63(column II), 0.65 (column III)	0.73

Instrumentation

HPLC was performed with an LaChrom chromatograph with UV and RI detectors, an autosampler, and a data station (Merck).

Determination of the Adsorption Isotherm

The coefficients of the adsorption isotherm for the organic solvent (eq. 3) were determined on the basis of experimental data for excess adsorption obtained by the perturbation method. The general principles of this method can be found elsewhere [22,30,31] and a detailed discussion is also available [20]. The coefficients of the isotherm equation for the solute in pure water as mobile phase (eq. 5) were determined by use of the inverse method (peak fitting) [22] and by the perturbation method. Values of the adjustable term K_P for the mechanism of competitive adsorption (eq. 9) were obtained by fitting the model simulations to the retention times of solute pulses recorded for different amounts of the organic solvent in the mobile phase. All these procedures have been described in detail elsewhere [20].

Activity coefficients, P , of the solutes and organic solvents in the mobile phase were determined by use of the UNIFAC method [32,33]. The detailed procedure is described elsewhere [20].

Column porosity was determined by integrating the set of data from determination of pulse retention of the organic solvent, acquired over the whole concentration range, i.e. 0–100%. The results obtained are listed in Table I. The procedure is described elsewhere [20].

The Selectivity of the Separation

Selectivity was defined as the ratio of the retention factors k of the two separated components:

$$\alpha = \frac{k_2}{k_1} \quad (14)$$

If the isotherm slope is expressed in mole fractions, then:

$$k_{i=1,2} = F \frac{M^m}{\rho^m} \left. \frac{d\Gamma_i^*}{dx_i^m} \right|_{x_i^m \rightarrow 0} \quad (15)$$

To determine selectivity, retention times of pulses of C5 and C6 in mixtures were measured for mobile phases of different composition. The selectivity was calculated (in accordance with eq. 14) as:

$$\alpha = \frac{t_{r2} - t_{r0}}{t_{r1} - t_{r0}} \quad (16)$$

where: t_{r1} and t_{r2} are the retention times of the solutes pulses in mixtures recorded under linear isotherm conditions.

RESULTS AND DISCUSSION

Retention for Pure Weak Solvent

Retention on Different Stationary Phases

The experimental part of the work started with investigation of the adsorption equilibria of solute C5 with pure water as mobile phase on RP-8e as stationary phase (column IV). For this purpose the perturbation method and peak fitting were used. First, the isotherm terms $(q_P^\infty)_d$, K_{Pd} , $(q_P^\infty)_a$, K_{Pa} of the heterogeneous isotherm model (eq. 5) were determined by use of

the perturbation method. The isotherm equation was coupled with the dynamic model (eq. 1) to simulate non-linear band profiles. The number of theoretical plates, N , was determined by fitting the model simulations to the experimental profiles. Because of severe peak tailing, standard methods for evaluation of column efficiency were not accurate. Low system efficiency was apparent when pure water was used as mobile phase. As discussed elsewhere [20], column efficiency is lowest for pure water as mobile phase and increases with increasing content of organic solvent. Because the simulations did not reproduce experimental peak shapes perfectly the isotherm variables were further tuned by peak fitting. Five variables were estimated – four isotherm variables and the number of theoretical plates, N . The results from the perturbation method were used as the starting values for the estimation,. The numerical procedure implemented for this purpose was based on a random search algorithm described elsewhere [20].

The results from peak fitting, i.e. the isotherm variables obtained, were compared with those obtained for RP-18 stationary phases [20]. All the results are summarized in Table II. It is evident that the solute was

Table II

Isotherm terms for C5 as a solute and pure water as mobile phase

$L = 25$	$(q_p^\infty)_d \times 10^4$	K_{Pd}	$(q_p^\infty)_a \times 10^3$	K_{Pa}	N (rounded values)
Column I (RP-18)	3.78	62.8	1.9	1.00	400
Column II (RP-18e)	3.07	46.7	1.4	1.20	800
Column IV (RP-8e)	5.05	36.7	4.2	0.44	1100

strongly retained on the polar surface and relatively weakly on phases with alkyl chains. Note that the values of the equilibrium constants K_{Pd} relate to displacement of water molecules from the water multilayer and are affected by the energy of interaction with the surface, access of water to the surface, and the structure of water multilayer. To compare the general trend of retention behaviour the products of the activity coefficients in the mobile phase, saturation capacities, and equilibrium constants for both mechanisms should be considered rather than single values of the equilibrium constants. It is evident that retention was greatest on the non-encapped adsorbent RP-18, for which the contribution of high-energy polar sites to the retention mechanism is high (as is apparent from the values of $(q_p^\infty)_d$ and K_{Pd}). Strong retention is also observed for the RP-8e

adsorbent, for which, because of the high accessibility of exposed silanol groups in the presence of short alkyl chains, the saturation capacity for both mechanisms is highest.

Comparing Retention Properties for Different Solutes

To investigate the retention properties of different solutes, series of experiments analogous with those described above were repeated for column III (the properties of which are given in Table I) and pure water as mobile phase for C5, C6, and phenol. The results of peak fitting (i.e. isotherm terms) for column III are shown in Table III. In the first stage of the peak-fitting procedure the saturation capacities for the polar and non-polar surfaces were found to be similar for both C5 and C6; this is to be expected for homologous compounds. In the next stage, therefore, the saturation capacity for C6 was set the same as that obtained for C5, i.e. only two isotherm coefficients, K_{Pd} and K_{Pa} , were determined for C6. As reported in Table III, values of the equilibrium constants for both the solutes were also very similar. Thus, the different retention can be attributed mainly to the different activity coefficients in the mobile phase.

Table III

Comparison of results from the inverse method with pure water and C5 and C6 as solutes (Column III)

	γ_p^m	$(q_p^\infty)_d \times 10^4$	K_{Pd}	$(q_p^\infty)_a \times 10^3$	K_{Pa}	N (rounded values)
C5	109	4.28	33.0	2.22	0.69	174
C6	324	4.28	31.9	2.22	0.70	100
Phenol	9.68	7.22	392	7.67	0.95	1100

The results obtained for phenol differ markedly from those for C5 and C6. The adsorbent surface is much more accessible to molecules of phenol; both saturation capacities are much higher than those for the cycloketones. The equilibrium constant K_{Pd} is also very high, indicating strong interaction between the phenyl group and the polar adsorbent surface. To compare retention properties the product of activity coefficients, equilibrium constant, and the saturation capacity, i.e. $q_p^\infty \gamma_p^m K_p$, should, nevertheless, be considered, instead of the single values of the equilibrium constant.

Note that the column efficiency N (definition given below eq. 1) for phenol is much higher, indicating significant resistance to mass transport for C5 and C6.

Adsorption Equilibria of Organic Solvents

The excess adsorption of methanol and acetonitrile on RP-8e were measured and compared with that obtained elsewhere [20] for long-chain RP-18 surfaces. Excess isotherms for methanol measured on three types of adsorbent differing in carbon chain length (RP-18e compared with RP-8e) and surface heterogeneity (RP-18e compared with RP-18) are depicted in Fig. 1a.

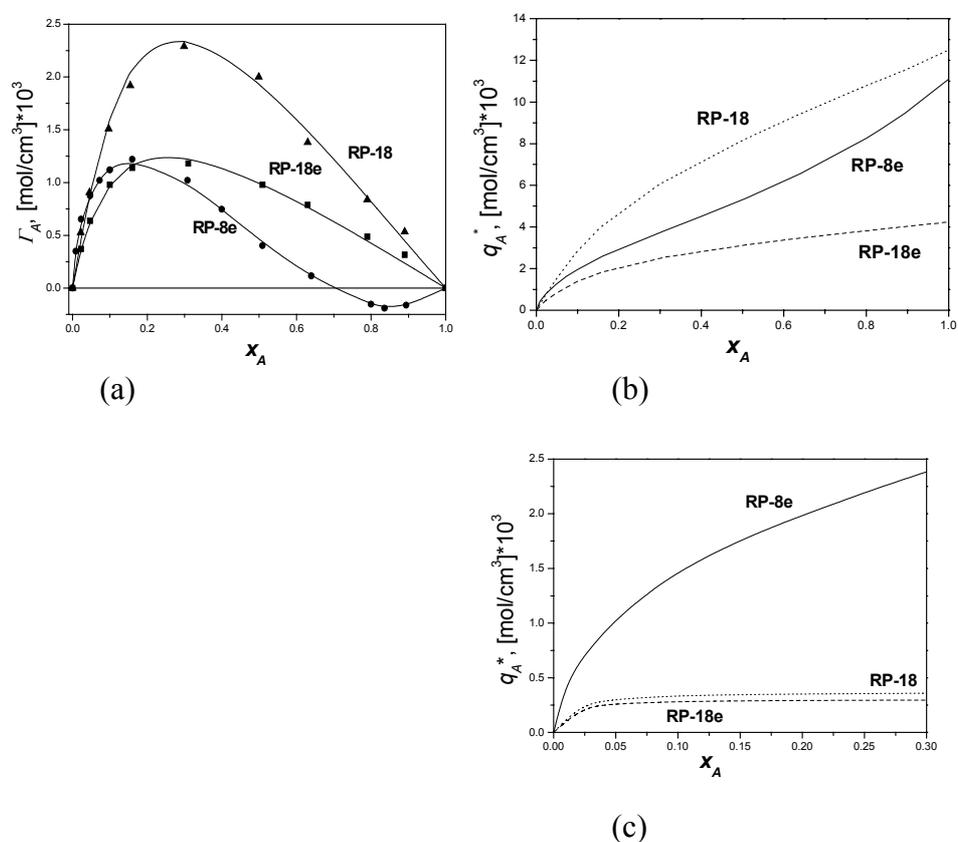


Fig. 1

(a) Comparison of the excess adsorption of methanol for RP-18, RP-18e, and RP-8e (columns I, II, and IV). Symbols denote experimental results and lines denote simulations by use of eqs (3) and (4); for RP-18 and RP-18e $(q_A^*)_a = 0$. (b) Isotherms corresponding to the excess adsorption presented in (a); simulations with eq. (3). (c) Adsorption of methanol in the first layer corresponding to the excess adsorption presented in (a). Simulations of $(q_A^*)_{d1}$ for RP-18 and $(q_A^*)_{d1} + (q_A^*)_a$ for RP-8e were performed with eq. (3)

It is evident that the mechanism of retention of methanol on adsorbent covered by alkyl chains of the same length (C₁₈) is similar – the adsorption data are not indicative of a negative adsorption excess, which originates from:

- (a) a displacement mechanism in which molecules of solvents replace each other on the adsorbent; and
- (b) asymmetric solvent adsorption in which water molecules participate solely in adsorption on exposed silanols whereas the organic solvent can penetrate both the polar and alkyl surface.

It was therefore suggested that the shape of negative excess on RP-8e surfaces originated from heterogeneity of the retention mechanism – the possibility of retention by both partition and displacement [20]. For the RP-18 adsorbents analysed in this study only the displacement mechanism was active, partition on alkyl chains was assumed to be weak and was neglected. This assumption is in agreement with microscopic simulations by Klätte and Beck [27], who indicated that molecules of methanol (and water) wet the RP-18 surface, creating layers, but did not alter the structure of the alkyl chains.

For the RP-8e adsorbent coated with short C₈ carbon chains the negative excess is distinct and both mechanisms can be expected to contribute to retention. Methanol molecules are not repelled by the less hydrophobic surface of RP-8e and can associate with the shorter chains; the contribution of partition to overall retention is therefore higher than for RP-18. This has a strong effect on the retention of the solutes in the presence of organic solvents. As is shown below, because of coverage of both surfaces by the organic solvent, retention of the solute on the RP-8e surface decreases rapidly from very high for pure water to low in the presence of the organic solvent.

The isotherm coefficients were determined on the basis of the excess adsorption data; in the presence of a negative excess a heterogeneous mechanism was assumed, i.e. eq. (3) with $(q_A^*)_a \neq 0$, otherwise a homogenous model was used, i.e. eq. (3) with $(q_A^*)_a = 0$.

The adsorption equation was combined with eq. (4). The positive excess adsorption measured over the range of low concentration of the organic solvent determines the amount adsorbed in the first layer on the polar surface whereas the curvature of the negative excess measured over the range of high concentration determines the total amount adsorbed in the partition mechanism not accessible for water. The results from estimation, i.e. the coefficients of the isotherm equation, are summarized in Table IV.

Table IV

Isotherm terms for methanol

	Column I (RP-18)	Column II (RP-18e)	Column IV (RP-8e)
K_d	42.7	60.0	63.1
K_{dn}	39.6	21.8	5.5
n_d (rounded)	33	14	14
K_a	–	–	1.6
K_{an}	–	–	0.5
n_a	–	–	5.3

To illustrate the different properties of the adsorption surfaces the overall isotherms corresponding to the excess adsorption (Fig. 1a) are presented in Fig. 1b. It is evident that the lowest retention was on RP-18e. The curvature of the isotherm is different for homogenous and heterogeneous mechanisms (compare RP-18 and RP-8e). To illustrate competitive adsorption of the organic solvent the amount of methanol adsorbed in the first layer, where competition occurs, is depicted in Fig. 1c. It is apparent the highest coverage in the first layer can be expected for RP-8e; it is much lower (and comparable) for both RP-18 adsorbents, which has a strong effect on solute retention, as indicated below.

It is important to note that the excess adsorption measured for different columns characterized by different void volumes cannot be used for comparison of the strength of retention on different surfaces (for example, compare the sequence of curves in Figs 1a and 1b). To compare retention properties the product of the activity coefficients, equilibrium constant, and saturation capacity should, again, be used.

Note that adsorption of the solvents is usually weak compared with that of the solutes.

The adsorption excess for acetonitrile for all the adsorbents investigated is shown in Fig. 2a. For all the adsorbents the negative excess of adsorption is evident, which suggests that both the displacement and partition mechanisms are active. Acetonitrile is more hydrophobic than methanol and can, therefore, participate in partition on alkyl chains. Thus, in contrast with methanol, the amount of solvent retained on a surface by partition is greater for longer C₁₈ chains than for C₈ (compare the number of layers in Table V). The possibility of association of acetonitrile with the chains has been suggested [28]. From the results depicted in Figs 1 and 2 it is evident that the behaviour of acetonitrile on each adsorbent investigated and that of methanol on the RP-8e surface is similar.

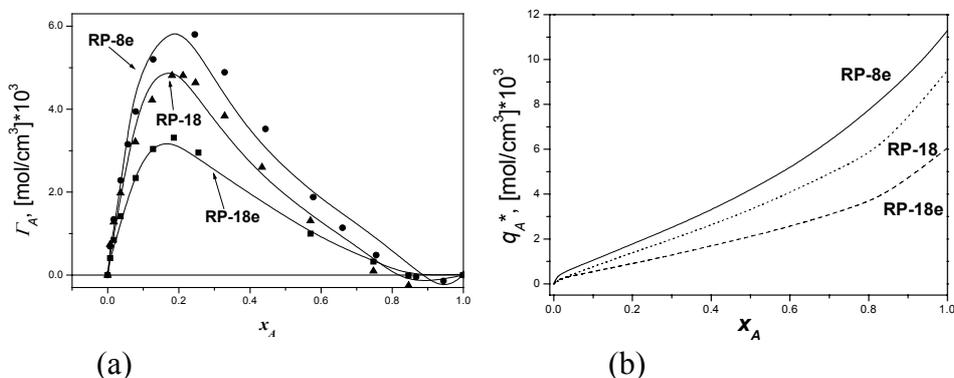


Fig. 2

(a) Comparison of the excess adsorption of acetonitrile for RP-18, RP-18e, and RP-8e (columns I, II, and IV). Symbols denote experimental results and lines denote simulations by use of eqs (3) and (4). (b) Isotherms corresponding to the excess adsorption presented in (a); simulations were performed with eq. (3)

Table V

Isotherm terms for acetonitrile

	Column I (RP-18)	Column II (RP-18e)	Column IV (RP-8e)
K_d	167.9	427.3	673.5
K_{dn}	0.012	0.086	0.9
n_d (rounded)	5	5	5
K_a	0.58	0.44	0.30
K_{an}	9.8	10.8	8.10
n_a	>30	>30	22

The isotherm coefficients were estimated on the basis of excess adsorption data, i.e. $\Gamma_A^* = f(x_A^m)$ (plot in Fig. 2a) and used to simulate the adsorption isotherm, i.e. $q_A^* = f(x_A^m)$ (Fig. 2b). For all the adsorbents the heterogeneous retention model was used. The results obtained are presented in Table V. Because of several model simplifications the values illustrate rather general trends; they should not be regarded as accurate isotherm coefficients. The model nevertheless enables calculation of the overall amount of acetonitrile retained on surfaces by the partition mechanism in which water does not contribute. This amount determines competition for alkyl chains. The model predictions were verified by analysis of changes

of retention and peak shape for mobile phases of different composition, as described below.

Competitive Adsorption – Chromatographic Elution of Solutes in the Presence of an Organic Solvent

The competitive adsorption model for the system P–A–B has been coupled with the model of column dynamics (eq. 1) and used to simulate band profiles for mobile phases of different composition. In the presence of methanol and water the competitive adsorption mechanism for the RP-18 surface consisted of eqs (7)–(10) for $(q_A^*)_a = 0$, which accounted for competitive adsorption of the organic solvent on the polar surface only. For acetonitrile–water on all the surfaces investigated and for methanol–water on the RP-8e adsorbent the heterogeneous retention model was used, i.e. $(q_A^*)_a \neq 0$.

The competitive amounts of adsorbed organic solvent $(q_A^*)_{d1}$ and $(q_A^*)_a$ were calculated in accordance with the adsorption isotherm for organic solvent–water (eq. 3; see also Ref. [20]).

The only adjustable term was K'_p (eq. 9), which was determined by fitting the model simulations to the retention times of solute pulses for the mobile-phase composition investigated. The values of K'_p for selected chromatograms are summarized in Table VI for methanol and Table VII for acetonitrile. Note that despite marked differences between the retention properties of the different types of adsorbent the adjusted values of K'_p are very similar. K'_p should be regarded as a model coefficient which includes all the inaccuracies of the model. It is evident that these values are

Table VI

Values of the adjustable term K'_p for C5 and selected methanol mobile-phase content

x_A^m	K'_p			
	γ_{C5}^m	Column I (RP-18)	Column II (RP-18e)	Column IV (RP-8se)
0.500 (70% v/v)	6.4	0.14	–	0.34
0.299 (50% v/v)	13.9	0.20	0.20	0.40
0.160 (30% v/v)	31.4	0.22	0.27	0.40
0.047 (10% v/v)	71.8	0.28	0.25	0.39

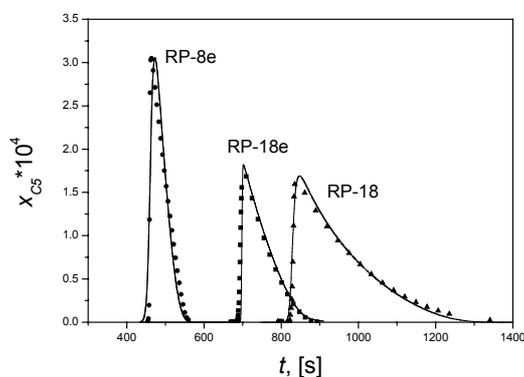
Table VII

Values of the adjustable term K'_p for C5 and selected acetonitrile mobile-phase content

x_A^m	K'_p			
	γ_{C5}^m	Column I (RP-18)	Column II (RP-18e)	Column IV (RP-8se)
0.075 (20% v/v)	48.5	0.52	0.60	0.53
0.036 (10% v/v)	73.1	0.83	0.84	0.77
0.018 (5% v/v)	89.5	1.10	1.05	1.20
0.007 (2% v/v)	100.5	1.40	1.10	1.51

nearly constant for water-rich mobile phases and decrease slowly for high organic solvent content. This indicates that “solute–organic solvent” interactions weaken with increasing number of layers because of weakening “organic solvent–surface” interactions.

Band profiles recorded for RP-8e and RP-18 surfaces and the same mobile phase methanol content are compared in Fig. 3.

**Fig. 3**

Comparison of selected experimental and simulated profiles on RP-18, RP-18e, and RP-8e for 10% (v/v) methanol in the mobile phase. The feed concentration of the sample, X_{pF} , was approximately 0.01 (5% w/w). The feed was always dissolved in the solvent used as the mobile phase. The flow rate was 1 mL min^{-1} and the injection volume $20 \mu\text{L}$.

The retention sequence results from two effects:

- (a) the competition mechanism, i.e. the greater the adsorption of organic solvent the less adsorption surface is available for the solute and the lower its retention; and

- (b) the adsorption properties of the solute, i.e. the higher the equilibrium constant and saturation capacity the higher the adsorption and retention of the solute.

It is evident the lowest retention was recorded for RP-8e, the polar surface of which is readily covered by methanol and for which competitive adsorption of organic solvent results in a decrease of surface accessible for adsorption (Fig. 1c). Retention was greatest on the RP-18 non-encapped column, because of the large contribution of displacement on the polar surface to the overall retention mechanism (high equilibrium constant K_{pD} ; Table II). Detailed discussion on the effect of surface heterogeneity on adsorption properties can be found elsewhere [20].

A similar comparison for the column saturated with acetonitrile is presented in Fig. 4. The retention sequence is similar to that reported for

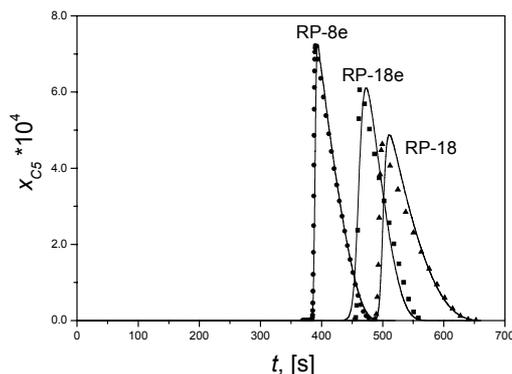


Fig. 4

Comparison of selected experimental and simulated profiles for RP-18, RP-18e, and RP-8e for 10% (v/v) acetonitrile in the mobile phase. The feed concentration of the sample, x_{pF} , was approximately 0.01 (5% w/w). The flow rate was 1 mL min^{-1} and the injection volume $20 \text{ }\mu\text{L}$.

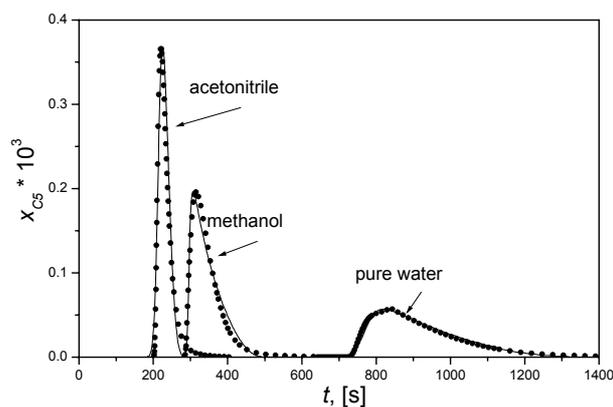
methanol and results from the reasons discussed above. Note that changes of peak shape are reproduced correctly, which validates the assumed mechanism of competitive adsorption. For comparison, the same calculation was performed for phenol. As reported above, the saturation capacities of organic solvents were assumed to be the same as those for the solute. The values of K'_p were adjusted as described above; they are summarized in Table VIII. Activity coefficients are also included in the same table. Note

Table VIII

Values of the adjustable term K'_p for phenol and selected mobile-phase methanol and acetonitrile content (Column III)

$x_{methanol}^m$	γ_{phenol}^m	K'_p	$x_{acetonitrile}^m$	γ_{phenol}^m	K'_p
0.500 (70% v/v)	0.66	5.60	0.255 (50%)	0.25	18
0.299 (50% v/v)	0.95	13.6	0.075 (20%)	0.94	48
0.047 (10% v/v)	4.48	16.5	0.036 (10%)	2.45	65
0.023 (5% v/v)	6.37	14.3	0.018 (5%)	4.58	58

that these coefficients take values below unity, particularly for acetonitrile, which indicates the possibility of clustering of the acetonitrile molecules around nonpolar solutes in the mobile phase. A similar mechanism of retention has been already postulated elsewhere [34]. The values of K'_p reported in Table VIII are higher than those obtained for cycloketones; this can be attributed to the high value of the equilibrium constant, K_{Pd} , on the polar surface. The products $\gamma_p^m K'_p$ which determine retention are, however, a similar order of magnitude for all the solutes, indicating comparable (i.e. same order of magnitude) retention by displacement in the adsorbed layer of organic solvent.

**Fig. 5**

Comparison of selected experimental and simulated profiles for phenol on RP-18e (column III) with pure water, 10% (v/v) methanol, and 10% (v/v) acetonitrile as mobile phase. The feed concentration of the sample, x_{pF} , was approximately 0.001 (0.5% w/w). The flow rate was 1 mL min^{-1} and the injection volume 10 μL

Experimental and simulated band profiles for phenol with pure water, 10% methanol, and 10% acetonitrile mobile phases are depicted in Fig. 5. As might be expected, the retention sequence is the same as for the cycloketones. Despite differences in the adsorption properties of phenol and the cycloketones the same model was capable of reproducing retention and changes of peak shape.

Effect of the Organic Solvent on the Selectivity of the Chromatographic Process

To investigate the effect of competitive adsorption of the organic solvent on the selectivity of separation, retention times of pulses of C5 and C6 in a mixture were measured on all the adsorbents investigated. Measurements were performed under linear isotherm conditions. The results obtained for different mobile-phase compositions are summarized in Table IX for methanol–water and in Table X for acetonitrile–water. The results clearly indicate that separation selectivity for the two homologous compounds investigated is independent of the type of RP packing material – for all columns it is almost the same. The same trend is apparent from retention data reported for different compounds on different columns by Wilson et al. [35]. The selectivity for pairs of homologous compounds is similar for all types of stationary phase.

Table IX

Separation selectivity for selected mobile-phase methanol content

x_A^m	γ_{C6}/γ_{C5}	α		
		Column I (RP-18)	Column II (RP-18e)	Column IV (RP-8se)
0.047 (10%)	2.74	2.80	2.83	2.67
0.160 (30%)	2.29	2.24	2.26	2.26
0.299 (50%)	1.90	1.90	1.90	1.88

Table X

Separation selectivity for selected mobile-phase acetonitrile content

x_A^m	γ_{C6}/γ_{C5}	α		
		Column I (RP-18)	Column II (RP-18e)	Column IV (RP-8se)
0.0069 (2%)	2.94	2.76	2.75	2.81
0.0177 (5%)	2.86	2.60	2.64	2.59
0.0364 (10%)	2.75	2.42	2.44	2.50
0.0747 (20%)	2.53	2.14	2.17	2.12

As mentioned above, the adsorption isotherms for both homologous solutes for pure water as mobile phase were almost identical. The retention differences were determined by activity coefficients in the mobile phases. Hence, in the presence of the modifier competitive adsorption for both the solutes must also be the same, i.e. the contribution of the uncovered polar and non-polar surface is the same. Differences appear only in the additional retention mechanism in the adsorbed layer of the organic solvent (eq. 9). To illustrate this trend K'_p values for both C5 and C6 are compared in Table XI. For all conditions (and all stationary phases), but particularly for acetonitrile, values of K'_p for the solute with the larger molecule, i.e. C6, are slightly lower than those for C5. This can result in worsening of separation selectivity compared with that predicted by the ratio of the activity coefficients, $\gamma_{C6}^m / \gamma_{C5}^m$.

Table XI

Values of the adjustable term K'_p for C5 and C6 and selected mobile-phase methanol and acetonitrile content (Column IV, RP-8e)

$x_{methanol}^m$	K'_p		$x_{acetonitrile}^m$	K'_p	
	C5	C6		C5	C6
0.299 (50%)	0.34	0.34	0.075 (20%)	0.53	0.45
0.160 (30%)	0.40	0.39	0.036 (10%)	0.77	0.67
0.100 (20%)	0.40	0.38	0.018 (5%)	1.20	1.02
0.047 (10%)	0.39	0.34	0.007 (2%)	1.51	1.36

In the presence of methanol the adsorption surface is covered rather slowly and the selectivity is determined by the contribution of competitive adsorption on the polar adsorbent surface and partition on alkyl chains uncovered by methanol. Because these contributions are the same for both the homologous compounds the selectivity is determined by the ratio of the activity coefficients in the mobile phase (compare data in Table IX). The contribution of the third mechanism is relatively small; note that the values of K'_p are also low.

In the presence of strongly adsorbed acetonitrile the adsorption surface is rapidly covered and retention of the solute on both polar and non-polar sites vanishes. Retention of the solute is governed by the third additional mechanism, which reduces the selectivity of the separation.

Hence, the selectivity is lower than that determined by the ratio of the activity coefficients (Table X).

Note that all the components have affinity for the polar and non-polar sites on the surface. They penetrate the alkyl chains well and compete with the adsorbed organic solvent. Strongly hydrophobic components can, to a large extent, be repelled from the polar surface, i.e. they penetrate the alkyl chains weakly. Such components are adsorbed strongly on the tips of alkyl chains, because of the partition mechanism, which becomes dominating (but not exclusive) in retention, as reported elsewhere [2,3].

CONCLUSIONS

Previous analysis of competitive adsorption of a solute and an organic solvent in a reverse-phase system has been extended. To investigate the effect of the structure of the adsorption surface on the retention properties of a solute a short-chain RP-8e adsorbent has been investigated and compared with RP-18 and RP-18e, investigated previously. Two typical solvent mixtures, methanol–water and acetonitrile–water were selected. The excess adsorption of the organic solvents was measured and the adsorption isotherms for the RP-8e surface were determined and compared with those determined for RP-18 and RP-18e. The strongest retention was on the non-encapped RP-18 adsorbent, for which contribution of polar sites to the overall retention mechanism was high. The best access to the adsorption surface was, however, recorded for the short chain RP-8e adsorbent. For this adsorbent retention was assumed to occur as a result of two mechanisms – displacement on the polar surface and partition on alkyl chains in the presence of both methanol and acetonitrile, whereas for RP-18 both mechanisms were active only in the presence of acetonitrile. Strong competitive adsorption of organic solvents reduced the accessible adsorption surface, and as a result retention of solutes on RP-8e in the presence of an organic solvent was low. Retention was greatest on the RP-18 non-encapped column, because of the large contribution to overall retention of the displacement mechanism on the polar surface.

The type of organic solvent was found to have significant effect on retention of the solutes. Because adsorption of acetonitrile was much stronger than that of methanol, retention of the solutes in the presence of acetonitrile in the mobile phase was much less than that in the presence of methanol, for all the adsorbents studied.

The effect of mobile-phase composition and surface structure on separation selectivity was also studied. In the presence of methanol selectivity for the two homologous compounds investigated was determined by their activity coefficients in the mobile phase; the presence of acetonitrile resulted in unfavourable changes of selectivity compared with those predicted from the activity coefficients.

To compare the retention behaviour of structurally different solutes, retention of phenol and cycloketones was studied. Despite the different physicochemical properties of these compounds the same trends were observed and the same retention model was able to reproduce their behaviour. Note that all these solutes behaved similarly – they had affinity for the polar surface and the alkyl chains. Despite all the simplifications and approximations the basic assumptions of the model with regard to multilayer adsorption of organic solvents and competition adsorption of the solutes seem to be valid.

The work illustrates the strong relationship between adsorption of organic solvent and the adsorption properties of solutes. The use of the excess adsorption can be regarded as a powerful tool for study of competitive adsorption. It has been proved that retention of the solute cannot be predicted or explained without predicting the adsorption of the organic solvent.

NOMENCLATURE

Symbol	Unit
D_a = Apparent diffusion coefficient	($\text{cm}^2 \text{s}^{-1}$)
$F = (1 - \varepsilon_t) / \varepsilon_t$ = phase ratio	(–)
K = Equilibrium constant	(–)
L = Column length	(cm)
M = Molar mass	(g mol^{-1})
N = Column efficiency	
n = Number of layers	
q = Amount adsorbed	(mol cm^{-3})
q^∞ = Saturation capacity	(mol cm^{-3})
t = Time coordinate	(s)
u = Superficial velocity	(cm s^{-1})
x = Mole fraction	(–)
z = Space coordinate	(cm)
Greek letters	
Γ = Excess adsorption	(mol cm^{-3})

γ = Activity coefficient
 ε_t = Total void fraction of the column (-)
 ρ = Density (g cm⁻³)

Subscripts

A = Organic solvent
a = Partition-adsorption mechanism
d = Displacement-adsorption mechanism
F = Feed
I = Component index
P = Sample

Superscripts

* = Equilibrium conditions
M = Mobile phase
s = Adsorbed phase

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