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Description of adsorption equilibria in liquid chromatography systems with binary mobile phases

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Abstract

Adsorption of some simple compounds from pure and mixed solvents and of some solvents from mixed binary solvent mixtures on columns used in normal-phase, aqueous–organic and non-aqueous liquid chromatography was investigated. The distribution of the compounds between the liquid and the stationary phase is affected by the composition of the solvent mixture. Although preferential sorption of stronger solvents can often be described by the Langmuir isotherm, significant deviations are observed in some systems. A model was suggested accounting for the deviations from Langmuir isotherm by association on already adsorbed molecules. In most systems studied in this work, a simple competitive Langmuir isotherm did not provide a good fit to the experimental distribution data from mixed solvents when competition between the solute and the strong solvent for the adsorption sites was considered. A competitive isotherm taking into account possible association of solute on its own already adsorbed molecules and on the adsorbed molecules of strong solvent improves the fit to the experimental distribution data and enables description of the distribution data in dependence on the concentration of the strong solvent in mixed solvents in normal-phase, aqueous–organic and non-aqueous reversed-phase systems. Even though we need more data to prove the validity of the present model, we observe a good qualitative agreement of the experimental isotherm coefficients with the relative strength of the interactions expected in various chromatographic systems employing mixed solvents. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Adsorption isotherms; Mobile phase composition; Phenols; Benzophenone; Cholesterol

1. Introduction

Adjusting the composition of the mobile phase is the most widely used tool for controlling the separation of two components in analytical high-performance liquid chromatography (HPLC). This approach is also applied to optimize separations in preparative liquid chromatography (LC). The effect of the mobile phase composition on the distribution

isotherms should be quantitatively described to allow numerical calculations of peak profiles and optimization of the effects of the mobile phase composition on the yield, purity and production rate under overloaded isocratic [1] and gradient elution [2,3] conditions. The simplest approach is to use suitable equations to describe the dependencies of the coefficients of the isotherms on the composition of mixed liquid phases. Recently, we investigated the effect of the concentration of the organic modifier (methanol) in aqueous–organic mobile phases on the distribution of phenol, *o*-cresol and resorcinol between the stationary and the mobile phase and on the

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coefficients of single- and two-component isotherm equations [4,5]. We used this approach for the prediction and optimization of the effect of the experimental parameters on the production rate and recovery yield in overloaded reversed-phase isocratic and gradient-elution chromatography.

Any rigorous approach used to describe the distribution between a mobile and a stationary phase should take into account the competitive sorption of all the solutes and the components of the mixed mobile phase as well as the different solute–solvent interactions taking place in both phases [6]. The components of a binary solvent system are distributed between the phases of a particular LC system so that the “strong” solvent (i.e., the more efficient eluent in a binary mobile phase) is usually preferentially adsorbed onto the adsorbent surface, except for solvent systems with very low concentrations of the “weak” solvent, in which case the weak solvent may be slightly adsorbed (e.g., water on a reversed-phase material). The distribution can be often described by the simple Everett equation [7], which is equivalent to the Langmuir isotherm if one solvent is strongly adsorbed.

Many authors argued regarding the most appropriate model of the molecular processes representing the solute distribution in HPLC, e.g., Refs. [8,9]. *Displacement*, a surface process that takes place at the solid–liquid interface, and *partition*, a volume process that may occur when the stationary phase is “thick enough” to accommodate solute molecules in its volume (a liquid–liquid chromatography process) were advocated. However, molecular models of thermodynamics strongly suggest that a liquid–liquid equilibrium model is inadequate [10]. In liquid–solid chromatography with chemically bonded phases which are only a monolayer thick, the actual mechanism seems rather to be an intermediate between these two extremes and to include the formation of a combined solvent–surface stationary phase and the distribution of the solute between this stationary phase and the bulk mobile phase [11].

A fundamental problem in any description of distribution data in HPLC is the definition of the stationary and of the mobile phase, with corresponding determination of the volume of each phase. In mixed solvents, the amount of strong solvent

preferentially adsorbed depends on the composition of the bulk liquid phase. The adsorbed layer of strong solvent changes the properties of the adsorbent surface and it becomes a part of the stationary phase. Since the components of the mobile phase are miscible, the adsorbed layer has no fixed boundary, a concentration gradient exists from the surface of the solid-phase to the bulk liquid, and it is impossible to fix a boundary between a homogeneous stationary and a homogeneous liquid phase. This problem was discussed by Riedo and Kováts [12], who introduced the hypothesis that the part of the solution that has a composition different from that of the bulk mobile phase remains stationary. Then, the Gibbs’ dividing plane between the phases is fixed by convention. In our treatment of the distribution data we accepted their convention of “nothing is adsorbed” in terms of the phase volumes, which means that the column hold-up volume is independent of the adsorbed amount of strong solvent and corresponds to the whole volume occupied by the liquid phase in the column while the volume of the stationary phase is by definition identical to the volume of the solid adsorbent matrix.

Recently, we investigated the possibility of fitting to isotherm models the distribution data of single- or two-component samples from two-component solvent mixtures in some normal-phase systems. As the competitive Langmuir isotherm failed to describe adequately the distribution data obtained for mixed mobile phases, we attempted using competitive quadratic isotherms for this purpose [13]. The quadratic isotherms showed improved fit to the experimental data, but the improvement was only moderate in some cases and the physical meaning of the isotherm coefficients obtained was unclear. In the present work we attempt to describe the distribution behavior of several solutes by taking into account the simultaneous effects of a competitive adsorption and of a possible multi-layer association of the already adsorbed molecules. We compare the adsorption of simple compounds from pure solvents, of individual solvents from binary solvent mixtures and of simple compounds from binary solvent mixtures in normal-phase systems with various types of solvents and from aqueous–organic and non-aqueous solutions in reversed-phase chromatography.

2. Theoretical

Several models have been suggested to describe the distribution of one sample component between the stationary and the mobile phases. The most common and the simplest nonlinear isotherm model is the two-parameter Langmuir model [14]:

$$q = \frac{q_s b C}{(1 + b C)} = \frac{a C}{(1 + b C)} \quad (1)$$

where q and C are the concentrations of the sample compound in the stationary and the mobile phase, respectively, and a , b are numerical coefficients, with $a = k_0 / \Phi$, where k_0 is the retention factor of the compound at infinite dilution, i.e., in analytical chromatography and $b = a / q_s = k_A / k_D$ (see later, Eq. (2)) is the ratio of the sorption and desorption rate constants, and q_s is the column saturation capacity. If the experimental data do not fit well to the Langmuir model, more complex isotherms can be used such as those based on the bi-Langmuir [15], the quadratic [16–18], the Fowler [19] or the Jovanovic [20] models.

The original Langmuir isotherm model accounts essentially for the limited capacity of the monolayer to accommodate adsorbate molecules. To improve this model, additional physical phenomena can be introduced in the adsorption model, such as the possibility of multi-layer adsorption on the adsorbent surface. Multi-layer adsorption from the gas phase can be described by the well-known BET theory, which is often applied to evaluate the monolayer adsorbent capacity from the multilayer region of the isotherm. However, this theory is less suitable for the description of distribution equilibria between the liquid and the solid phase.

In an earlier attempt to describe the liquid–solid equilibria where the Langmuir model is not fully satisfactory, we suggested that adsorption could proceed in two layers [21]. In the present work, we used this earlier approach and describe adsorption by taking into account a possible multi-layer association of the sorbed molecules. The description of the solute distribution under these conditions is derived using kinetic arguments similar to those used by Langmuir in his original derivation.

Like in the original Langmuir isotherm, the first-layer adsorption is described by equating the adsorption and desorption rates:

$$\frac{dq_1}{dt} = k_{1A} C (q_{1s} - q_1) = -\frac{dq_1}{dt} = k_{1D} q_1 \quad (2)$$

where C is the concentration of the compound in the mobile phase, q_1 is the concentration of the solute adsorbed in the first layer, k_{1A} and k_{1D} are the first-layer adsorption and desorption rate constants, respectively, and q_{1s} is the specific saturation first-layer capacity of the adsorbent (both first-layer saturation capacity and first-layer adsorbed concentration are per unit volume of the adsorbent matrix). The solution of this equation leads to the classical Langmuir isotherm (Eq. (1)). Multi-layer association can be accounted for by introducing similar equations describing adsorption (association) of solute molecules in the i -th layer onto the already adsorbed $(i-1)$ th layer:

$$\frac{dq_i}{dt} = k_{iA} C q_i = -\frac{dq_i}{dt} = k_{iD} q_{(i-1)} \quad (3)$$

where q_i and $q_{(i-1)}$ are the concentrations of the adsorbate in the i -th layer and the $(i-1)$ th layer, respectively, k_{iA} and k_{iD} are the adsorption and the desorption rate constants, respectively, into the i -th layer of solute molecules already adsorbed in the $(i-1)$ th layer. They differ from the rate constants for the first layer because the molecules there are in direct contact with the adsorbent surface. Assuming equal values of the rate constants for all associated layers 2 to n , the solution of Eqs. (2) and (3) leads to Eq. (4) describing adsorption in n layers:

$$q = \frac{q_{1s} b_1 C}{(1 + b_1 C)} \cdot \frac{[(b_2 C)^n - 1]}{(b_2 C - 1)} \quad (4)$$

where: $b_1 = k_{1A} / k_{1D}$ and $b_2 = k_{iA} / k_{iD}$

For $n=1$, Eqs. (1) and (4) are identical and we have the Langmuir isotherm. For $n=2$, the isotherm equation becomes:

$$q = \frac{q_{1s} b_1 C}{(1 + b_1 C)} \cdot (1 + b_2 C) = \frac{A_1 C}{1 + B_1 C} + A_2 C \quad (5)$$

This equation is formally identical with the empirical combination of a Langmuir and a linear

isotherm used occasionally by earlier workers [21,22]. For $n=3$, the isotherm equation becomes:

$$q = \frac{q_{1s} b_1 C}{(1 + b_1 C)} \cdot [1 + b_2 C + (b_2 C^2)] \quad (6)$$

The adsorption behavior of a mixture is more complex than that of a single component from a pure solvent. This applies as well to the adsorption of two compounds from a pure solvent or to the adsorption of a single compound from a binary solvent. Because there is competition between all the components of the liquid phase for the adsorption sites, the achievement of full saturation of the adsorbent by one of these compounds is more difficult and is possible only if its adsorption energy is much higher than that of the other components of the system.

Numerous and often complex models were suggested to describe competitive equilibria between the components i , j of a binary mixture and an adsorbent, yielding various competitive isotherm equations [23]. The competitive Langmuir isotherm [24] is often used, but it is thermodynamically consistent only if the saturation capacities of all the components are the same [25]. The distribution of compounds i and j is described by Eqs. (7a) and (7b):

$$q_i = \frac{q_s b_i C_i}{1 + b_i C_i + b_j C_j} \quad (7a)$$

$$q_j = \frac{q_s b_j C_j}{1 + b_i C_i + b_j C_j} \quad (7b)$$

where the parameters b_i , b_j are the same as the Langmuir single-component coefficients for solutes i , j .

More complex models were suggested to account for the experimental deviations from the competitive Langmuir model, such as for example the competitive Fowler isotherm [17,18], the competitive quadratic isotherm based on the statistical thermodynamics model [26], or the thermodynamically consistent LeVan–Vermeulen competitive isotherm [27].

The same approach as used for the derivation of a single-compound associative isotherm described by Eqs. (4)–(6) can be applied to model the adsorption of a single compound from a binary solution containing a weakly and a strongly adsorbed solvent. Then, the competition of the solute and the strong

solvent for the adsorption sites and the association of the adsorbed molecules of both the solute (i) and the strong solvent (j) should be taken into account. If we limit the model to two-layer adsorption, we can describe the basic phenomena by the following Eqs. (8)–(17).

(1) First-layer adsorption of the solute on the adsorbent:

$$\begin{aligned} \frac{dq_{i1}}{dt} &= k_{i1A} C_i (q_{1s} - q_{i1} - q_{j1}) = - \frac{dq_{i1}}{dt} \\ &= k_{i1D} q_{i1} \end{aligned} \quad (8)$$

$$q_{i1} = b_{i1} C_i (q_{1s} - q_{i1} - q_{j1}) \quad (9a)$$

$$b_{i1} = \frac{k_{i1A}}{k_{i1D}} \quad (9b)$$

(2) First-layer adsorption of the strong solvent on the adsorbent:

$$\begin{aligned} \frac{dq_{j1}}{dt} &= k_{j1A} C_j (q_{1s} - q_{i1} - q_{j1}) = - \frac{dq_{j1}}{dt} \\ &= k_{j1D} q_{j1} \end{aligned} \quad (10)$$

$$q_{j1} = b_{j1} C_j (q_{1s} - q_{i1} - q_{j1}) \quad (11a)$$

$$b_{j1} = \frac{k_{j1A}}{k_{j1D}} \quad (11b)$$

(3) Association of the molecules of solute with the molecules of strong solvent in the first layer:

$$\frac{dq_{i2}}{dt} = k_{i2A} C_i q_{j1} = - \frac{dq_{i2}}{dt} = k_{i2D} q_{i2} \quad (12)$$

$$q_{i2} = b_{i2} C_i q_{j1} \quad (13a)$$

$$b_{i2} = \frac{k_{i2A}}{k_{i2D}} \quad (13b)$$

(4) Association of the molecules of solute with the solute adsorbed in the first layer:

$$\frac{dq_{i3}}{dt} = k_{i3A} C_i q_{i1} = - \frac{dq_{i3}}{dt} = k_{i3D} q_{i3} \quad (14)$$

$$q_{i3} = b_{i3} C_i q_{i1} \quad (15a)$$

$$b_{i3} = \frac{k_{i3A}}{k_{i3D}} \quad (15b)$$

(5), (6) Association of the molecules of strong

solvent with the molecules of strong solvent (5) and with the molecules of solute adsorbed in the first layer (6), characterized by the rate constants b_{j2} and b_{j3} :

$$q_{j2} = b_{j2} C_j q_{i1} \quad (16a)$$

$$b_{j2} = \frac{k_{j2A}}{k_{j2D}} \quad (16b)$$

$$q_{j3} = b_{j3} C_j q_{i1} \quad (17a)$$

$$b_{j3} = \frac{k_{j3A}}{k_{j3D}} \quad (17b)$$

Combination of Eqs. (8)–(17) results in Eq. (18) describing the competitive two-layer association isotherm:

$$q_i = \frac{A_1 c_i + A_2 C_i C_j}{(1 + B_1 C_i + B_2 C_j + B_3 C_i C_j)} \quad (18)$$

with the coefficients A_1 , A_2 , B_1 , B_2 , B_3 defined as follows:

$$A_1 = q_{i3} b_{i1} \quad (19a)$$

$$B_1 = b_{i1} - b_{i3} \quad (19b)$$

$$B_2 = b_{j1} - b_{j3} \quad (19c)$$

$$A_2 = b_{i2} b_{j1} - b_{i1} b_{j3} \quad (19d)$$

$$B_3 = (b_{j1} - b_{j3}) \cdot (b_{i1} - b_{i3}) + (b_{i1} - b_{i2}) \cdot (b_{j2} - b_{j1}) \quad (19e)$$

This system of equations defines the competitive isotherm model used in this study.

3. Experimental

3.1. Chemicals

Cholesterol, tridecylbenzene, benzophenone and coumarin (all 99+ % grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA), and were used as obtained; phenol and *o*-cresol (all reagent grade) were from Lachema (Brno, Czech Republic). Before use, phenol was purified by distillation and

o-cresol by crystallization from methanol. Methanol, 2-propanol, *n*-heptane, *n*-hexane, dioxane, acetonitrile and dichloromethane (all HPLC grade) were purchased from Baker (Deventer, The Netherlands). Except for methanol, all organic solvents were kept dry in tightly closed dark bottles over molecular sieve beads Dusimo 5 Å (Lachema) previously activated at 300°C (ca. 30–40 g/l), to eliminate the effect of water on adsorption. Water was doubly distilled in glass with addition of potassium permanganate. Before their use, all solvents were filtered using a Millipore 0.45-µm filter and degassed by ultrasonication immediately before the use. Mixed solvents were prepared by exact mixing of the appropriate volumes of the components. Solutions of solid compounds for the determination of the distribution data were prepared by weighing the required amounts of the solutes in pure solvent or in a binary solvent mixture.

3.2. Instrumentation and methods

To acquire the data necessary for the determination of the equilibrium isotherms by frontal analysis [28], a HP 1090M liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) was used, equipped with a 3 DR gradient solvent delivery system and solvent reservoirs continuously stripped with helium to degas the mobile phase (placed in one reservoir flask) and the sample solution (in the other one), an automatic sample injector, a column switching valve, a temperature-controlled column compartment, a diode-array UV detector and a data workstation.

The columns for measuring the isotherm data were glass-cartridge, 150 mm×3.3 mm I.D., packed with Separon SGX silica gel ($V_M=0.94$ ml), Separon SGX Nitrile ($V_M=0.96$ ml) and with Separon SGX C₁₈ ($V_M=0.87$ ml), all 7 µm particle size (ECOM, Prague, Czech Republic) and stainless steel, 250 mm×4.6 mm I.D., packed with Impaq RG 2010 silica gel ($V_M=3.55$ ml) and with Impaq RG 2010-C₁₈ ($V_M=3.12$ ml), all 10 µm particle size (PQ Corp.). The column hold-up volumes, V_M , were evaluated from the record of the detector set to 200 nm after injection of pure *n*-hexane (with silica gel columns) or pure water (with C₁₈ columns) into mixed mobile phases. The volumes of stationary

phases were calculated as the differences between the geometrical volume of the column and its hold-up volume.

In each experiment, the mobile phase composition was changed by adjusting the ratio of the flow-rates of the two solutions between 0 and 100%, in successive 10% or 5% steps. Time was allowed for the stabilization of the detector signal after each concentration change. The flow-rate (1 ml/min) and the column temperature (40°C) were kept constant during all the experiments. The solute concentration in the stationary phase was determined from the appropriate integral mass balance equation [28], using (1) the experimental concentrations of the sample components at the plateaus of the frontal analysis curve and (2) the retention volumes corresponding to the inflection points of the breakthrough curve, corrected for the volume of the tubing between the mixing point of the liquids pumped in each channel and the column top (0.31 ml). All experiments were repeated at least twice.

4. Results and discussion

4.1. Solvent isotherms

To investigate the effect of a strong solvent on the adsorption of model solutes, the adsorption isotherms of several strong solvents were measured for some binary mobile phases common in normal-phase and in reversed-phase (RP) LC with aqueous–organic and non-aqueous mobile phases. Frontal analysis with refractometric detection was used. The best values of the coefficients of the Langmuir isotherm (Eq. (1)) and of the other isotherm models described earlier were derived for each experimental distribution data set (11–20 data points), using non-linear regression. The numerical results are reported in Table 1. With the multilayer associative isotherm (Eq. (4)), the number of layers providing the best fit of the isotherm equation to the experimental data was determined first. The optimum values of n are given in the legend of Table 1. $n(\text{opt})$ were close to one for 2-propanol in n -hexane on the Separon silica gel and nitrile columns and for acetonitrile in dichloromethane on the Impaq silica gel column. It was close to 2 for propanol in dichloromethane and

methanol in water, close to 3 for acetonitrile in dichloromethane on an Impaq C₁₈ column and it was between 1 and 2 for the other chromatographic systems tested. Depending on the $n(\text{opt})$ values, the isotherms with $n=2$ (Eq. (5)) or $n=3$ (Eq. (6)) were employed for further calculations with the associative isotherm. The coefficients of the isotherms are listed in Table 1. To allow a comparison of the fit quality of the two isotherms, the values of the coefficients of determination, C.D.s, and of the residual sums of squares, RSSs, are also shown.

Figs. 1 and 2 show the isotherms of strong solvents in normal-phase systems. The isotherms of 2-propanol in n -hexane on a Separon silica gel and on a bonded nitrile column (Fig. 1, curves 1 and 2) are strongly curved and show excellent fit to the Langmuir model. The associative isotherm does not yield any improvement in the values of either C.D. or RSS; the constant b_2 characterizing the association to the already adsorbed molecules is very close to zero and the values of the saturation capacities (q_s and q_{1s}) and of the constants of adsorption (b and b_1) determined with the two isotherm equations are close to each other. As could be expected, the saturation capacity for propanol is larger on the silica gel than on the bonded nitrile column. The experimental distribution of acetonitrile in dichloromethane on an Impaq silica gel column (Fig. 2, curve 1) is fit only marginally better by the associative two-layer isotherm than by the Langmuir model equation. This means that the effect of self-association in the second layer is relatively unimportant in these normal-phase chromatographic systems.

On the other hand, the experimental isotherms of dichloromethane in n -heptane (not shown), of 2-propanol in dichloromethane (Fig. 1, curve 3), and of dioxane in n -heptane (Fig. 1, curve 4) on the silica gel column are not appropriately described by the Langmuir model (dashed lines). The fit is significantly improved for the associative two-layer isotherm (full lines). The poor suitability of the Langmuir isotherm to describe the distribution data is further demonstrated by the unrealistically high values of the Langmuir saturation capacities, especially for dichloromethane in n -heptane on the silica gel column and for dioxane in n -hexane on a bonded nitrile column, corresponding approximately to 30% and 76% of the total column volume in the first and

Table 1

Coefficients q_s (mol/l), b (1/mol) of the Langmuir isotherm (Eq. (1)) and parameters n , q_{1s} (mol/l), b_1 (1/mol), b_2 (1/mol) of the associative multilayer isotherm (Eq. (4))^a

Langmuir isotherm						Associative multilayer isotherm					
C	S	q_s	b	C.D.	RSS	n	q_{1s}	b_1	b_2	C.D.	RSS
<i>Solvents</i>											
a	P/DC	4.84	0.971	0.9860	$7.2 \cdot 10^{-4}$	2	1.02	11.25	1.51	0.9998	$7.6 \cdot 10^{-6}$
a	DC/HP	26.96	0.162	0.9919	$9.2 \cdot 10^{-3}$	2	3.18	2.33	0.667	0.9998	$3.8 \cdot 10^{-5}$
a	DX/HP	6.16	1.008	0.9822	0.48	2	2.76	4.47	0.269	0.9993	$1.8 \cdot 10^{-2}$
a	P/HX	3.35	202	0.9985	$8 \cdot 10^{-5}$	2	3.35	202	0	0.9985	$8 \cdot 10^{-5}$
b	P/HX	2.38	7.13	0.9991	$2.1 \cdot 10^{-5}$	2	2.33	7.50	0.016	0.9992	$2.1 \cdot 10^{-5}$
b	DX/HX	48.97	0.106	0.9999	$2 \cdot 10^{-5}$	2	0.474	7.01	18.01	0.9999	$1.5 \cdot 10^{-5}$
c	A/DC	4.89	0.456	0.9981	$2 \cdot 10^{-5}$	2	4.425	0.578	0.005	0.9999	$1.3 \cdot 10^{-6}$
d	DC/A	5.02	0.080	0.9995	$1.6 \cdot 10^{-5}$	2	3.430	0.128	0.015	0.9999	$3.6 \cdot 10^{-6}$
d	A/DC	<0	<0	–	–	3	0.513	0.730	0.108	0.9998	$6.6 \cdot 10^{-6}$
e	M/W	81.80	0.053	0.9988	0.514	2	9.236	0.593	0.283	0.9996	0.195
<i>Other compounds</i>											
a	B/DC	1.526	4.31	0.9999	$2.6 \cdot 10^{-5}$	2	0.679	10.25	3.45	0.9998	$7.6 \cdot 10^{-6}$
a	CM/DC	1.419	13.78	0.9988	$8.4 \cdot 10^{-4}$	2	0.808	28.66	3.96	0.9999	$2.2 \cdot 10^{-5}$
c	CH/DC	1.942	30.33	0.9932	9.56	2	2.91	17.82	–2.22	0.9982	2.59
d	CH/DC	0.047	5.30	0.9996	$5.6 \cdot 10^{-5}$	2	0.028	9.44	1.95	0.9997	$3.6 \cdot 10^{-5}$
d	CH/A	0.782	35.17	0.9998	$2.2 \cdot 10^{-4}$	2	0.004	8700	5750	1.0000	$2.0 \cdot 10^{-6}$
d	CH/HX	0.020	100.1	0.9978	$9.3 \cdot 10^{-4}$	2	0.007	361.7	77.69	0.9999	$3.4 \cdot 10^{-6}$
d	TB/A	6335	0.001	0.9982	0.26	2	125.3	0.061	1.31	0.9995	$2.2 \cdot 10^{-4}$
e	P/W	1.33	9.69	0.9986	$1.4 \cdot 10^{-3}$	2	0.550	28.86	6.34	1.0000	$2 \cdot 10^{-5}$
e	CR/W	1.57	16.70	0.9932	$1.3 \cdot 10^{-2}$	3	0.639	64.00	8.41	0.9990	$2 \cdot 10^{-3}$

^a 1: More strongly adsorbed solvents from binary mobile phase systems (S): P/DC = 2-propanol–dichloromethane [$n(\text{opt})=2$], DC/HP = dichloromethane–*n*-heptane [$n(\text{opt})=1.7$], DX/HP = dioxane–*n*-heptane [$n(\text{opt})=1.5$], P/HX = 2-propanol–*n*-hexane [$n(\text{opt})=1$], DX/HX = dioxane–*n*-hexane [$n(\text{opt})=1.8$], A/DC = acetonitrile–dichloromethane [$n(\text{opt})=1.1$ for Impaq silica and $n(\text{opt})=3.4$ for Impaq C₁₈ columns], DC/A = dichloromethane–acetonitrile [$n(\text{opt})=1.5$], M/W = methanol–water [$n(\text{opt})=2$]. 2: Other compounds (B = benzophenone, CM = coumarin, CH = cholesterol, TB = tridecylbenzene, P = phenol, CR = *o*-cresol) from a single-component mobile phase (DC = dichloromethane, A = acetonitrile, HX = *n*-hexane, W = water), on various columns (C): a – Separon SGX (silica), b – Separon SGX Nitrile, c – Impaq RG 2010 (silica), d – Impaq RG 2010-C₁₈, e Separon SGX C₁₈. C.D. – Coefficient of determination, RSS – residual sum of squares.

second case, respectively. Formally, the Langmuir isotherm describes well the adsorption of dioxane in *n*-hexane on a bonded nitrile column (Fig. 1, curve 5) because this isotherm is almost linear. However, the values of q_s obtained are much larger and do not correspond to monolayer adsorption as assumed by the Langmuir model. The values of the first-layer saturation capacities, q_{1s} , calculated with the associative isotherm model are more realistic. In these systems, the values of the coefficient b_2 characterizing the strength of the association of the molecules to those in the adsorbed layer are much higher than for the other normal-phase systems investigated.

The experimental data for methanol in water on a C₁₈ column (not shown) fit only slightly better to the associative two-layer isotherm (Eq. (5)) than to the

Langmuir isotherm in the lower concentration range. However, here again, the Langmuir saturation capacity is unrealistically high and corresponds to an adsorbed volume of methanol that is larger than the column hold-up volume. The coefficients of the associative two-layer isotherms in Table 1 suggest an almost 10-times lower first-layer saturation capacity and a relatively strong association of the molecules of methanol to those in the already adsorbed layer.

A C₁₈ column adsorbs either solvent from a non-aqueous solution of acetonitrile and dichloromethane. This is illustrated by the adsorption isotherms of acetonitrile (Fig. 2, curve 2) and of dichloromethane (Fig. 2, curve 3) from their solutions on an Impaq C₁₈ column. The octadecyl silica has a larger affinity for the less polar dichloromethane and its

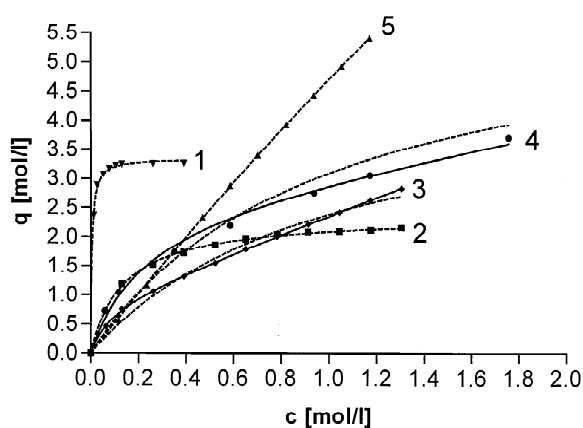


Fig. 1. Adsorption isotherms of strong solvents from binary solvent mixtures. 1=2-Propanol from *n*-hexane on Separon SGX silica gel; 2=2-propanol from *n*-hexane on Separon SGX Nitrile; 3=2-propanol from dichloromethane on Separon SGX silica gel; 4=dioxane from *n*-heptane on Separon SGX silica gel; 5=dioxane from *n*-hexane on Separon SGX Nitrile. Dashed lines: Langmuir isotherm (Eq. (1)), full lines: two-layer associative isotherm (Eq. (5)). q = Concentration of adsorbed strong solvent in the stationary phase (per volume unit of solid adsorbent matrix), c = concentration of strong solvent in the liquid binary solvent mixture.

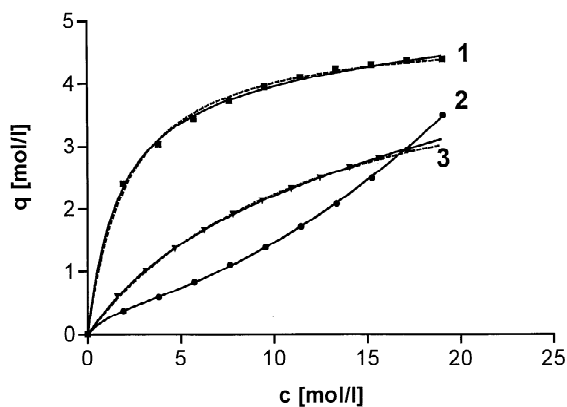


Fig. 2. Adsorption isotherms of strong solvents from binary solvent mixtures. 1=Acetonitrile from dichloromethane on Impaq RG 2010 silica gel; 2=acetonitrile from dichloromethane on Impaq RG 2010-C₁₈; 3=dichloromethane from acetonitrile on Impaq RG 2010-C₁₈. Dashed lines: Langmuir isotherm (Eq. (1)), full lines: two-layer associative isotherm (curves 1 and 3, Eq. (5)) and three-layer associative isotherm (curve 2, Eq. (6)). q = Concentration of adsorbed strong solvent in the stationary phase (per volume unit of solid adsorbent matrix), c = concentration of strong solvent in the liquid binary solvent mixture.

isotherm from acetonitrile is well described by the Langmuir model. The improvement of the data fit given by the associative two-layer isotherm is minute and the value of the coefficient b_2 characterizing the strength of association to the adsorbed layer is very low.

However, the adsorption behavior of acetonitrile from dichloromethane is different. It is characterized by an S-shape isotherm (Fig. 2, curve 2) that cannot be accounted for by the Langmuir model but is adequately described by the three-layer associative isotherm model. The coefficients of this isotherm (Table 1) suggest a very low first-layer saturation capacity and a much stronger first-layer adsorption (the coefficient b_1 is close to the values measured for the adsorption of acetonitrile from dichloromethane on an unmodified silica gel Impaq column). The association to already adsorbed molecules as characterized by the value of the coefficient b_2 in Table 1 is relatively strong. This behavior can possibly be explained by the adsorption of polar acetonitrile onto residual unmodified silanol groups on the C₁₈ silica, the density of which is lower than on the surface of bare silica.

4.2. Isotherms of other compounds in pure solvents

The distribution data of several model compounds from pure solvents in normal-phase, aqueous–organic and non-aqueous reversed-phase systems were processed as the distribution data of the solvents. The best coefficients of the Langmuir and associative multilayer isotherms are given in Table 1. The adsorption of benzophenone, coumarine and cholesterol from pure dichloromethane on silica gel columns is adequately characterized by the Langmuir isotherm with realistic values of coefficients (Table 1). The use of the associative two-layer isotherm improves only marginally the fit of the experimental distribution data. Moreover, the coefficient b_2 for cholesterol is negative, which would suggest repulsion between the already adsorbed molecules and the molecules in the second layer, a result which makes no physical sense. The adsorption of phenol and *o*-cresol from water on a C₁₈ column are also adequately described by the Langmuir isotherm with reasonable values of the coefficients, even though the application of the two-layer (for phenol) and three-

layer (for cresol) associative isotherm models improves the fit of the experimental data (Table 1).

The adsorption of cholesterol on a C_{18} column from dichloromethane, acetonitrile and *n*-hexane was compared (Fig. 3). The Langmuir isotherm fits well the distribution data of cholesterol between dichloromethane and a C_{18} column (Fig. 3, curve 1) and the two-layer associative isotherm does not bring any improvement. However, the distribution data of cholesterol in acetonitrile (Fig. 3, curve 2) and in *n*-hexane (Fig. 3, curve 3) fit this model significantly better than the Langmuir model (see C.D. and RSS values in Table 1). Acetonitrile is a poor solvent of cholesterol, which probably explains the much steeper isotherm than in the other two solvents. The solubility of cholesterol in *n*-hexane is approximately five-times higher and in dichloromethane approximately 60-times higher than in acetonitrile. The adsorption and self-association of cholesterol in different solvents can be expected to increase with decreasing solubility. Accordingly, the values of the coefficients b_1 and b_2 of the associative isotherm in the three solvents increase with decreasing solubility (Table 1). The distribution isotherm of tridecylbenzene between acetonitrile and a C_{18} column (Fig. 3, curve 4) is almost linear up to the concentration 0.1

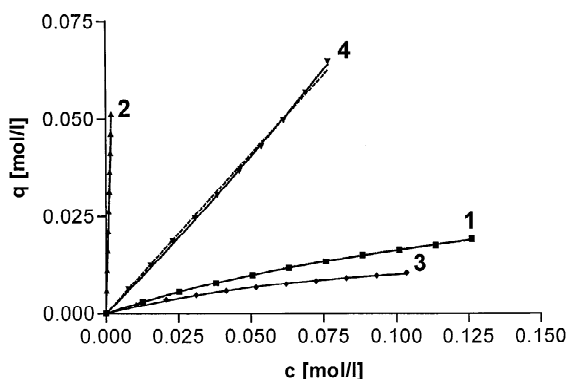


Fig. 3. Adsorption isotherms on Impaq RG 2010- C_{18} from non-aqueous solvent mixtures. 1=Cholesterol from dichloromethane; 2=cholesterol from acetonitrile; 3=cholesterol from *n*-hexane; 4=tridecylbenzene from acetonitrile (*x*-axis scale $\times 0.1$). Dashed lines: Langmuir isotherm (Eq. (1)), full lines: two-layer associative isotherm (Eq. (5)). q =Concentration of adsorbed strong solvent in the stationary phase (per volume unit of solid adsorbent matrix), c =concentration of strong solvent in the liquid binary solvent mixture.

mol/l, which may explain why the data fit equally well to the Langmuir and the associative isotherm models. However, the coefficients of the Langmuir isotherm found by regression have no actual physical meaning and the saturation capacity given by the associative isotherm is unrealistically high.

4.3. Effects of the strong solvent on the isotherms in binary mobile phases

Adequate retention in various HPLC modes can usually be achieved only in mixed mobile phases. To optimize the experimental conditions of preparative separations, either in isocratic or gradient elution, the effect of the mobile phase components on the distribution equilibria should be known. This problem is most often approached by using the same form of isotherm equation as for the single-component mobile phase and the effect of varying the mobile phase composition on the distribution equilibrium is taken into account in the numerical values of the isotherm coefficients. This is justified only if all the mobile phase components are only weakly adsorbed by the stationary phase, which is not true in many chromatographic systems, especially in normal-phase chromatography, where the polar solvent in the mobile phase competes with the sample solutes for the adsorption sites on the surface of the adsorbent. A rigorous description of the distribution process should take this competition into account.

To compare the effect of different solvents on the distribution isotherms in various chromatographic systems, we measured the distribution data of several compounds in normal-phase, aqueous–organic and non-aqueous reversed-phase chromatographic systems, with different binary mobile phases. We used frontal analysis and varied the mobile phase composition. The systems studied were (1) benzophenone and coumarin in dioxane–*n*-heptane, 2-propanol–dichloromethane, dichloromethane–*n*-heptane and 2-propanol–*n*-hexane on a silica gel column; (2) phenol and *o*-cresol in methanol–water; and (3) cholesterol in dichloromethane–acetonitrile on bonded octadecyl silica columns. The simplest model for these data is the competitive Langmuir isotherm (Eq. (7)) with the strong solvent competing with the solute. The coefficients of this isotherm for the systems studied are listed in Table 2. We

Table 2
Coefficients of the competitive Langmuir isotherm (Eq. (7))^a

S	C	q_s (mol/l)	b_1 (l/mol)	b_s (l/mol)	C.D.	RSS
a	BP	2.09	3.83	0.967	0.9932	$9.8 \cdot 10^{-4}$
a	CM	-8.95	-3.55	1.493	0.9739	$5.3 \cdot 10^{-2}$, N
b	BP	1.38	4.87	11.31	0.9806	$2.3 \cdot 10^{-2}$
b	CM	1.41	13.64	23.16	0.9918	$3.4 \cdot 10^{-2}$
d	BP	0.282	6.82	1.132	0.9558	$4.2 \cdot 10^{-5}$
d	CM	3.39	11.00	6.35	0.9867	$1.6 \cdot 10^{-3}$
e	PH	1.446	8.69	0.256	0.9943	$1.6 \cdot 10^{-2}$
e	CR	1.562	17.41	0.399	0.9901	$6 \cdot 10^{-2}$

^a S: Chromatographic system: a=Separon SGX silica/dioxane-*n*-heptane; b=Separon SGX silica/2-propanol-dichloromethane; c=Separon SGX silica/dichloromethane-*n*-heptane; d=Separon SGX silica/2-propanol-*n*-hexane; e=Separon SGX C₁₈/methanol-water; f=Impaq C₁₈/dichloromethane-acetonitrile. C: Sample compound: BP=benzophenone; CM=coumarine; PH=phenol; CR=*o*-cresol. b_1 – relates to the sample compound, b_s – relates to the strong solvent, C.D. – Coefficient of determination, RSS – residual sum of squares. N – The isotherm does not fit the data.

obtained reasonable numerical values of the coefficients of the competitive Langmuir isotherm for the distribution of benzophenone and coumarine between silica gel and mobile phases containing 2-propanol, of benzophenone between silica gel and mobile phases containing dioxane in *n*-heptane, and of phenol and *o*-cresol between a bonded C₁₈ column and methanol-water mobile phases. The fit of the experimental data to this model was only fair in most cases, however, as demonstrated by the values of the coefficients of determination, between 0.95 and 0.99. Note that, in these systems, the associative isotherm (Eq. (4)) brings a less significant improvement in the description of the single-component distribution data of the strong solvent with respect to the single-component Langmuir isotherm (Eq. (1)) than it does in the other systems tested, see Section 4.2.

The competitive Langmuir isotherm equation completely failed to describe the distribution of benzophenone and coumarine between dichloromethane-*n*-heptane and silica gel and of cholesterol between dichloromethane-acetonitrile and a bonded C₁₈ silica. In these cases, the non-linear regression fitting procedure did not converge. Poor fit of the data to the competitive Langmuir isotherm was observed for coumarine between dioxane-*n*-heptane and silica gel. Moreover, the negative values of the column saturation capacity and of the coefficient b_1 do not have any physical meaning (Table 2). One of the basic assumptions of the competitive Langmuir model, an equal saturation capacity for the solute and

the solvent, probably does not apply to the systems studied. Our results indicate that the competitive Langmuir model is suitable to describe distribution from binary solvents only in separation systems with high polarity differences between the adsorbent and the weak solvent. Hence, this model provides better results in normal-phase systems with binary solvents containing *n*-heptane rather than dichloromethane as the weak solvent and in reversed-phase systems with water rather than with acetonitrile as the weak solvent.

The best values of the coefficients of the associative competitive isotherm (Eq. (18)) are given in Table 3. The distribution data of all systems studied, including those for which the competitive Langmuir isotherm failed, could be fitted to this isotherm equation. All the distribution data (except for benzophenone on silica in dioxane-*n*-heptane), showed improved coefficients of determination and lower residuals for the competitive association isotherm compared to the competitive Langmuir isotherm. By itself, the better fit does not prove the validity of the associative model since it can originate from a higher number of coefficients (five versus three). However, fitting the same data to a six-parameter competitive statistical isotherm gave similar or even worse results.

The coefficients A_2 , B_1 and B_3 of Eq. (18) have either positive or negative values, depending on the compound, the stationary and the mobile phase. As each coefficient results from a combination of con-

Table 3

Coefficients of the competitive associative isotherm accounting for the effect of the strong solvent in the mobile phase (Eq. (18))^a

S	C	A_1	A_2 (l/mol)	B_1 (l/mol)	B_2 (l/mol)	B_3 (l ² /mol ²)	C.D.	RSS
a	BP	6.91	-0.218	1.54	7.71	1.42	0.9934	$9.6 \cdot 10^{-4}$
a	CM	18.80	-3.317	-2.60	2.45	0.816	0.9816	$3.8 \cdot 10^{-2}$
b	BP	7.00	42.9	5.21	397.7	3.64	0.9976	$2.8 \cdot 10^{-3}$
b	CM	19.49	65.7	13.69	501.6	30.8	0.9995	$2.0 \cdot 10^{-3}$
c	BP	91.81	-2.60	84.02	7.41	-3.36	0.9996	$4.1 \cdot 10^{-3}$
c	CM	457.3	-16.5	261.5	10.94	-1.29	0.9981	$8.9 \cdot 10^{-3}$
d	BP	42.92	12.2	-5.47	225.0	139	0.9991	$8.9 \cdot 10^{-7}$
d	CM	73.31	58.1	13.41	288.3	64.7	0.9980	$2.4 \cdot 10^{-4}$
e	PH	12.81	-0.587	9.39	4.43	-0.849	0.9979	$8.4 \cdot 10^{-3}$
e	CR	26.43	-1.57	16.87	4.95	-1.013	0.9972	$1.7 \cdot 10^{-2}$
f	CH	39.73	-2.08	-147.3	20.15	31.8	0.984	$5.3 \cdot 10^{-4}$

^a S: Chromatographic system: a=Separon SGX silica/dioxane-*n*-heptane; b=Separon SGX silica/2-propanol-dichloromethane; c=Separon SGX silica/dichloromethane-*n*-heptane; d=Separon SGX silica/2-propanol-*n*-hexane; e=Separon SGX C₁₈/methanol-water; f=Impaq C₁₈/dichloromethane-acetonitrile. C: Sample compound: BP=benzophenone; CM=coumarine; PH=phenol; CR=*o*-cresol; CH=cholesterol. C.D. - Coefficient of determination, RSS - residual sum of squares.

stants characterizing the adsorption of the solute and the strong solvent and various possible associations between the molecules of solute and strong solvent, negative values of these coefficients may have actual physical meaning.

The coefficient A_1 should always be positive and this was observed for all solutes and systems investigated (Table 3). The coefficient B_1 should be positive if the first-layer adsorption equilibrium constant of the solute is stronger than the constant characterizing the association on the adsorbed solute layer. This was observed in all cases except for the silica gel adsorption of coumarine in dioxane-heptane and of benzophenone in propanol-hexane mobile phases. We have no explanations for these exceptions. On the other hand, strongly negative value of B_1 in non-aqueous RP systems can be more easily understood, as the result of a stronger affinity of cholesterol to its own molecules than to the C₁₈ surface. The relationship of the coefficient B_2 to the sorption behavior of the strong solvent is similar. Positive values obtained for all systems studied can be interpreted as meaning that the first-layer adsorption of all strong solvents tested is stronger than their self-association.

The coefficient A_2 is expected to be positive if the combined effect of the first-layer sorption of a compound and of its self-association is stronger for the strong solvent than for the solute. Positive values

of A_2 were obtained for the adsorption on silica gel from mobile phases containing strongly polar 2-propanol, which obviously has a significantly greater affinity for the adsorbent than the other strong solvents used. Negative values of A_2 were measured for these other solvents. Finally, the coefficient B_3 can be negative if the association between the molecules of the solute and of the strong solvent has a greater effect than the first-layer adsorption of either the solute or the solvent (i.e., provided that both B_1 and B_2 are positive). Negative B_3 were determined for the sorption of the solutes studied on silica gel from dichloromethane-*n*-heptane and on a C₁₈ column from aqueous methanol, for which such a behavior is more likely than for other systems.

Positive or negative values of the coefficients of the associative isotherm in agreement with our understanding of the relative strength of various interactions do not prove the validity of the model, although it is a necessary condition of its soundness. Unfortunately, the equation of the isotherm containing the coefficients related to the individual effects postulated, q_{1s} , $b_{i1}-b_{i3}$, $b_{j1}-b_{j3}$, in an explicit form was too complex to be directly processed by the statistical software and we could not derive the best values of these parameters. More work in this area would be necessary.

Various effects of binary solvent systems result in different profiles of the adsorption isotherm char-

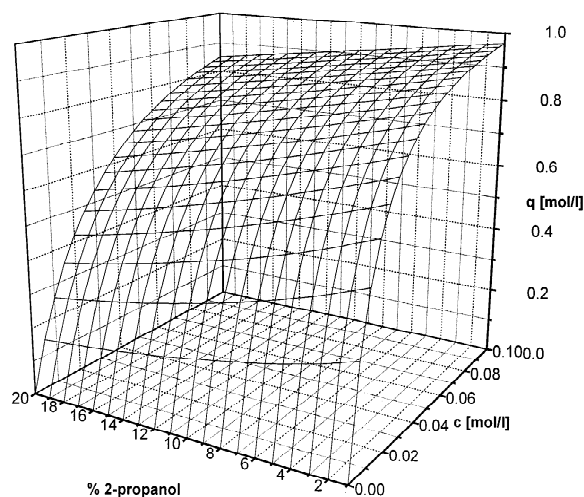


Fig. 4. Competitive two-layer associative isotherm (Eq. (18)) of benzophenone on Separon SGX silica gel from 2-propanol–dichloromethane solvent mixtures. q = Concentration of adsorbed strong solvent in the stationary phase (per volume unit of solid adsorbent matrix), c = concentration of strong solvent in the liquid binary solvent mixture.

acterized by Eq. (18), as is illustrated by several examples of three-dimensional plots showing competitive isotherms as functions of the concentration of the strong solvent in the binary systems. Figs. 4–6

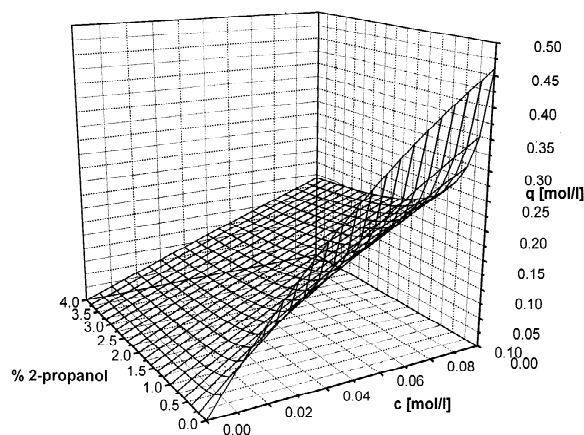


Fig. 5. Competitive two-layer associative isotherm (Eq. (18)) of benzophenone on Separon SGX silica gel from 2-propanol–*n*-hexane solvent mixtures. q = Concentration of adsorbed strong solvent in the stationary phase (per volume unit of solid adsorbent matrix), c = concentration of strong solvent in the liquid binary solvent mixture.

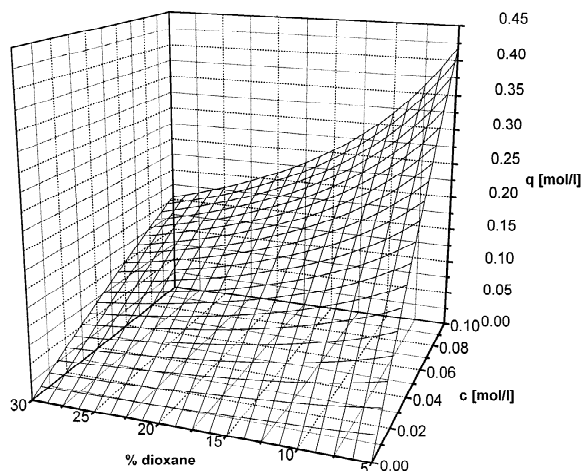


Fig. 6. Competitive two-layer associative isotherm (Eq. (18)) of benzophenone on Separon SGX silica gel from dioxane–*n*-heptane solvent mixtures. q = Concentration of adsorbed strong solvent in the stationary phase (per volume unit of solid adsorbent matrix), c = concentration of strong solvent in the liquid binary solvent mixture.

show distribution isotherms of benzophenone on silica gel. These surfaces strongly depend on the nature of the binary solvent: 2-propanol–dichloromethane (Fig. 4), 2-propanol–*n*-hexane (Fig. 5) and dioxane–*n*-heptane (Fig. 6). Fig. 7 shows the competitive isotherm of phenol on a C_{18} column in aqueous methanol and Fig. 8 the competitive isotherms of cholesterol on a C_{18} column in binary mixtures of dichloromethane and acetonitrile.

These three-dimensional isotherms illustrate the different effects of mixed solvents on the solute distribution in various chromatographic systems. The isotherm of benzophenone on silica gel is strongly convex in 0–20% solutions of 2-propanol in dichloromethane. Increasing concentrations of 2-propanol have only a minor effect on the isotherm shape and on the adsorbed amount of benzophenone (Fig. 4). The isotherms of benzophenone in 2-propanol–*n*-heptane (Fig. 5) and in dioxane–*n*-heptane (Fig. 6) on silica gel are less curved when the concentration of the strong solvent in the binary mixture increases. Also, the adsorbed amount of benzophenone decreases more steeply with increasing concentration of strong solvent than in 2-propanol–dichloromethane solutions. The effect of the addition of 2-propanol to *n*-heptane is greater than that of the addition of the

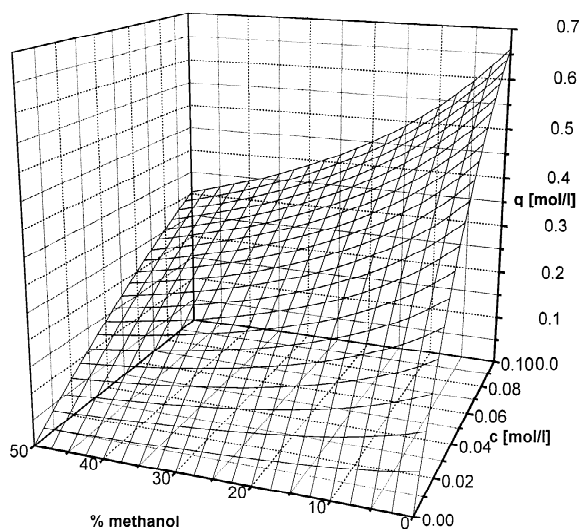


Fig. 7. Competitive two-layer associative isotherm (Eq. (18)) of phenol on Separon SGX C_{18} from methanol–water solvent mixtures. q = Concentration of adsorbed strong solvent in the stationary phase (per volume unit of solid adsorbent matrix), c = concentration of strong solvent in the liquid binary solvent mixture.

same concentration of dioxane, because of the stronger polarity of 2-propanol.

The effect of methanol on the adsorption of phenol in aqueous–organic RPLC systems (Fig. 7) is similar

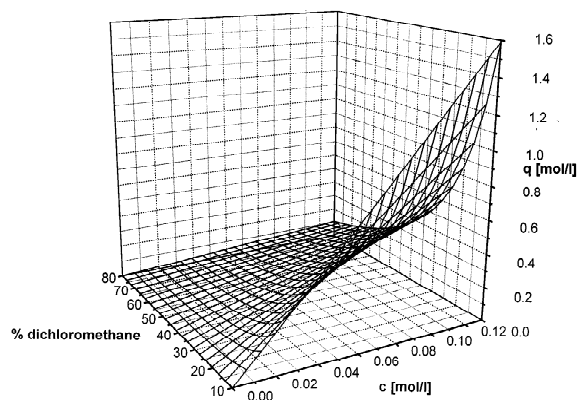


Fig. 8. Competitive two-layer associative isotherm (Eq. (18)) of cholesterol on Impaq RG 2010- C_{18} from dichloromethane–acetonitrile solvent mixtures. q = Concentration of adsorbed strong solvent in the stationary phase (per volume unit of solid adsorbent matrix), c = concentration of strong solvent in the liquid binary solvent mixture.

to that of 2-propanol in *n*-heptane on the adsorption of benzophenone on silica gel. However, the decrease of the adsorbed amount with increasing concentration of methanol is less steep. The isotherm of cholesterol in a non-aqueous reversed phase (NARP) system is almost linear (Fig. 8) and the effect of increasing concentration of dichloromethane in acetonitrile on the adsorbed amount of cholesterol is greater than the effect of 2-propanol in *n*-heptane on the adsorption of benzophenone on silica gel (Fig. 5).

The composition of a binary solution affects also the saturation capacities, q_s , of the stationary phase for various solutes. The knowledge of q_s is important for the calculation of the column loading factor in preparative separations. In systems that can be described by a competitive Langmuir isotherm (Eq. (7)), the part of the column capacity available for sorption of a solute decreases with increasing adsorbed amount of strong solvent. If the molecules of a solute associate on already adsorbed molecules, the determination of saturation capacity is less straightforward because the plateau of the isotherm is often far outside the range of useful concentrations. In this case, the maximum adsorbed amount of solute is limited by the concentration of its saturated solution rather than by the column capacity. The solubility in mixed solvents usually improves with increasing concentration of strong solvent. Thus, at low strong-solvent concentrations, the amount of product which can be injected and purified increases with increasing concentration of strong solvent because of the improving solubility. On the other hand, this amount decreases at high concentrations of strong solvent because the retention decreases. Accordingly, there is an optimum concentration of strong solvent, often allowing the use of relatively high solute concentrations. This is illustrated in Fig. 9, showing the dependence of the adsorbed concentration of cholesterol on a C_{18} silica in equilibrium with saturated solutions of cholesterol in mixed solvents with various concentrations of dichloromethane in acetonitrile. In this case, a maximum adsorbed concentration of cholesterol is found at approximately 40% dichloromethane but this concentration remains almost constant between 20 and 60% dichloromethane. This broad solvent composition range allows an easy adjustment of the optimum concentration of

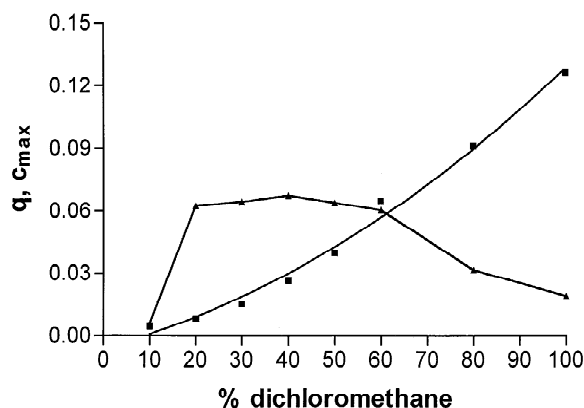


Fig. 9. Dependencies of (a) maximum solubility of cholesterol in dichloromethane–acetonitrile mixed mobile phases (C_{\max} , in mol/l, squares) and (b) corresponding adsorbed concentration of cholesterol per volume unit of the solid matrix of Impaq RG 2010- C_{18} (q , in mol/l, triangles) on the concentration of dichloromethane in the liquid phase.

dichloromethane for maximum production rate at a required purity.

5. Conclusions

The adsorption from mixed solvents is affected by the composition of the solvent mixture. The strong solvent is adsorbed preferentially and this adsorption can often be adequately described by the simple Langmuir isotherm. For some chromatographic systems, however, the Langmuir isotherm fails to describe adequately the solvent adsorption. Deviations from the Langmuir model can possibly be accounted for by multi-layer association on already adsorbed strong solvent layers. A modification of the Langmuir isotherm equation may account for this effect. For most systems studied (solvents and simple compounds adsorbed from a pure solvent), a two- or three-layer adsorption model gives a better fit of the adsorption data than the simple Langmuir model.

As retention and separation considerations require that two- or multi-component mobile phases should be used for most chromatographic separations, both at analytical and preparative scale, the effect of the composition of mixed solvents on the adsorption isotherms should be better understood. In the systems studied, the experimental data rarely fit well to

the competitive Langmuir isotherm. For this purpose, we suggested a competitive associative isotherm taking into account a possible association of the solute with its own already adsorbed molecules and with the adsorbed molecules of strong solvent. This isotherm model is simpler than the statistical quadratic isotherm introduced earlier. It enables a description of the dependence of the adsorption data on the concentration of strong solvent in the mixed solvent. We found this type of isotherm to be more suitable than the Langmuir isotherm to describe the sorption behavior of various solutes in normal-phase, aqueous–organic and non-aqueous reversed-phase systems. Even though more data are necessary to prove the validity of this model, it should be noted that the isotherm coefficients derived from experimental data agree qualitatively well with the nature and relative intensity expected for molecular interactions in various chromatographic systems employing mixed solvents. It was also shown that the profile of the isotherms is strongly affected by the concentration and the nature of the solvents used as the mobile phase.

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References

- [1] P. Jandera, D. Komers, G. Guiochon, J. Chromatogr. A 787 (1997) 13.
- [2] P. Jandera, D. Komers, G. Guiochon, J. Chromatogr. A 760 (1997) 25.
- [3] P. Jandera, D. Komers, G. Guiochon, J. Chromatogr. A 796 (1998) 115.
- [4] P. Jandera, Z. Pošvec, P. Vraspír, J. Chromatogr. A 734 (1996) 125.
- [5] P. Jandera, D. Komers, J. Chromatogr. A 762 (1997) 3.

- [6] M. Jaroniec, D.E. Martire, J. Chromatogr. 387 (1987) 55.
- [7] D.H. Everett, Trans. Faraday Soc. 60 (1964) 1803.
- [8] L.R. Snyder, H. Poppe, J. Chromatogr. 184 (1980) 363.
- [9] L.C. Sander, A.A. Wise, CRC Crit. Rev. Anal. Chem. 18 (1987) 417.
- [10] I. Quiñones, J.C. Ford, G. Guiochon, Chem. Eng. Sci. 55 (2000) 909.
- [11] M. Jaroniec, J. Chromatogr. A 656 (1993) 37.
- [12] F. Riedo, E. Kováts, J. Chromatogr. 239 (1982) 1.
- [13] P. Jandera, D. Komers, L. Anděl, L. Prokeš, J. Chromatogr. A 831 (1999) 131.
- [14] I. Langmuir, J. Am. Chem. Soc. 38 (1916) 2221.
- [15] D. Graham, J. Phys. Chem. 57 (1953) 665.
- [16] H. Poppe, J. Chromatogr. 556 (1991) 95.
- [17] B. Lin, Z. Ma, S. Golshan-Shirazi, G. Guiochon, J. Chromatogr. 475 (1989) 1.
- [18] J. Zhu, M. Katti, G. Guiochon, J. Chromatogr. 552 (1991) 71.
- [19] R.H. Fowler, E.A. Guggenheim, Statistical Thermodynamics, Cambridge University Press, Cambridge, 1939.
- [20] D.S. Jovanovic, Kolloid Z. 235 (1969) 1203.
- [21] P. Jandera, G. Guiochon, J. Chromatogr. 605 (1992) 1.
- [22] M.Z. El Fallah, G. Guiochon, Anal. Chem. 63 (1991) 2244.
- [23] A.M. Katti, G. Guiochon, Adv. Chromatogr. 31 (1992) 1.
- [24] G.M. Schwab, in: Ergebnisse der Exakten Naturwissenschaften, Vol. 7, Springer, Berlin, 1928, p. 276.
- [25] J.-X. Huang, G. Guiochon, J. Colloid Interface Sci. 128 (1989) 577.
- [26] D.M. Ruthven, M. Goddard, Zeolites 6 (1986) 276.
- [27] M.D. LeVan, T. Vermeulen, J. Phys. Chem. 85 (1981) 3274.
- [28] S. Golshan-Shirazi, S. Ghodbane, G. Guiochon, Anal. Chem. 60 (1988) 2630.