Fitting competitive adsorption isotherms to the distribution data in normal phase systems with binary mobile phases

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Abstract

To investigate the effects of the solvents on the overloaded separations in normal-phase systems, the distribution isotherms of benzophenone and of coumarin as model compounds were determined between a silica gel column and binary solvents containing various concentrations of 2-propanol in *n*-hexane and in dichloromethane and of dichloromethane in *n*-heptane. The distribution data in the individual solvent systems can be formally described by the Langmuir adsorption isotherm, but the quadratic isotherm generally improves the fit to the experimental data. Various underlying adsorption models yielding the three-parameter quadratic isotherm are compared. The distribution data of mixed samples in binary solvent systems can be described by a two-component quadratic isotherm with six parameters, of which four are single-component isotherm coefficients. A six-parameter or a five-parameter two-component quadratic isotherm should be used to describe adequately the distribution of a single sample compound over a wide composition range of mixed binary solvents, taking into account the competition of the polar solvent for the adsorption sites. The description of the distribution of a two-component sample mixture by a three-component quadratic isotherm requires three additional coefficients to respect the competitive adsorption of the sample compounds. The validity of the equations proposed to describe the distribution data in normal-phase systems was verified by comparison of the numerically calculated and the experimental band profiles. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Few experimental distribution data of multicomponent samples between the liquid mobile phase and the solid column packing materials used in contemporary liquid chromatography have been published so far, but difficulties in selecting an adequate model for competitive adsorption equilibria have been clearly demonstrated [1].

The composition of the mobile phase is the most

widely used tool for controlling the separation in liquid chromatography. Recently, we have investigated the effect of the concentration of the organic modifier (methanol) in aqueous–organic mobile phases on the distribution of phenol and resorcinol between the stationary and the mobile phases and on the coefficients of some single- and two- component isotherm equations [2,3]. The effect of the mobile phase on the distribution isotherms should be quantitatively described to allow numerical calculations of the peak profiles and optimization of the effects of the mobile phase on the yield, purity and production

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rate under overloaded isocratic [4] and gradient elution [5,6] conditions. For this purpose, suitable equations can be used for the dependencies of the coefficients of the isotherms on the composition of the mobile phase. We used this approach for the prediction and optimization of the effect of experimental parameters on the production rate and on the recovery yield in overloaded reversed-phase isocratic and gradient-elution chromatography. A single isotherm equation, taking into account the competing effect of the stronger solvent in the mobile phase for the adsorption sites of the adsorbent, can describe the dependence of the distribution data on the composition of a binary mobile phases in a theoretically more rigorous way. Presently, we are investigating this approach in reversed-phase overloaded HPLC.

Normal-phase chromatography has some advantages over reversed-phase chromatography for preparative HPLC separations. First, many organic compounds are more soluble in normal-phase solvents. Second, silica gel, the most often used column packing material in normal-phase chromatography, offers lower cost, greater stability, higher loadability and less danger of contaminating collected sample fractions than bonded phase materials commonly used in reversed-phase systems.

In the present work, possibilities of description of the distribution data that depend on the composition of binary mobile phases in normal phase systems, are studied. For this purpose, we selected benzophenone and coumarin as model compounds. The molecule of benzophenone possess one keto group and the molecule of coumarin one keto and one ether group. Hence, benzophenone is a weak and coumarin a stronger proton acceptor and significant differences can be expected in the adsorption behaviour of the two compounds as a function of the composition of the sample solvent. The binary solvent systems tested involved various mixtures of 2-propanol (a polar localising solvent with both proton-donor and proton-acceptor properties), *n*-hexane (*n*-heptane) and dichloromethane as a non-polar and a moderately polar non-localising solvents, to study the effects of various types of interactions on the sorption behaviour on unmodified silica gel. It is believed that the information obtained in this investigation could be more generally useful for the selection of mobile phases for normal-phase preparative separations. Further scope of this work was to find out an appropriate description of the distribution isotherms in dependence on the composition of mobile phases in normal-phase systems making possible numerical calculations of experimental band profiles under overloaded separation conditions. This would make possible a prediction of the quality of separation as a function of changing operation parameters and could be used for the optimization of preparative separations on polar adsorbents.

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 most simple nonlinear isotherm is the two-parameter Langmuir one [7]:

$$q = \frac{a \cdot c}{1 + b \cdot c} \tag{1}$$

Here, q is the concentration of the sample compound in the stationary and c that in the mobile phases and a, b are the coefficients of the isotherm ($a = k_0/\Phi$, where k_0 is the limit retention factor of the sample compound at infinite dilution, i.e., in analytical chromatography, $\Phi = V_S/V_M$ is the ratio of the volumes of the stationary (solid) and of the mobile (liquid) phases in the column (i.e., the phase ratio) and $b = a/q_s$, q_s is the column saturation capacity). From the kinetic derivation of the Langmuir model it follows that b is the ratio of the adsorption (k_a) and desorption (k_d) rate constants [8]. If the Langmuir model does not fit well the experimental data, more complex isotherms can be used for this purpose, such as those based on bi-Langmuir [9], quadratic [10–12], Fowler [13] or Jovanovic [14] models. The single-component quadratic isotherm is described by Eq. (2):

$$q = \frac{A_1 \cdot c + A_2 \cdot c^2}{1 + B_1 \cdot c + B_2 \cdot c^2} \tag{2}$$

The quadratic isotherm can be derived on the basis of different models of sorption behaviour and ac-

cordingly, the parameters A_1, A_2, B_1, B_2 may have various physical meanings.

1. Statistical thermodynamics leads to the derivation of the equilibrium isotherm written as the ratio of two polynomials of the same degree. The first order isotherm derived in this way is identical with the Langmuir isotherm. Higher order statistical isotherms employ a model assuming effects of the interactions between the two molecules adsorbed on adjacent adsorption sites. Practically important is only the second degree, i.e., the quadratic isotherm equation, where the parameters q_s and b_1 have the same meaning as in the Langmuir isotherm Eq. (1) and the parameter b_2 indicates the effect of interactions between the adjacent adsorbed molecules, not accounted for in the Langmuir model [15]:

$$q_i = \frac{q_s \cdot (b_1 \cdot c + 2 \cdot b_2 \cdot c^2)}{1 + b_1 \cdot c + b_2 \cdot c^2} \tag{3}$$

Eq. (3) is formally identical with Eq. (2) for $A_1 = q_s.b_1$ and $a_2 = 2b_2$. However, it should be noted that the statistical thermodynamics isotherm equation constitutes a Pade approximation that is able to mimic almost any mathematical function. Thus, the physical meaning of the experimentally determined coefficients of this equation may be doubtful.

2. Eq. (2) formally describes also the bi-Langmuir model assuming adsorption on two different independent sites of adsorption on the adsorbent surface, where the molecules occupying a site of one type do not interact with neighbour molecules and the adsorption on each type of site follows the Langmuir adsorption model. This is in fact a special case of quadratic isotherm with:

$A_1 = (q_{s1}.b_1 + q_{s2}.b_2)$, $A_2 = (q_{s1} + q_{s2}).b_1.b_2$,

$B_1 = (b_1 + b_2)$ and $B_2 = b_1.b_2$,

where the indices 1 and 2 relate to the Langmuir parameters for the individual adsorption sites. If the saturation capacities on the two sites are equal, $q_{s1} = q_{s2} = q_s$, $A_1 = q_s.(b_1 + b_2)$ and $A_2 = 2q_s.b_1.b_2$, which means that the same relations apply between the parameters A_1, $B_1(A_1 = q_s.B_1)$ and A_2, $B_2(A_2 = 2q_s.B_2)$ as with the statistical quadratic isotherm (3) [16].

3. In the bilayer adsorption model, where the adsorption occurs in two adsorption layers with different ratios of the adsorption and desorption rate constants for the adsorption in the first (b_1) and in the second (b_2) layers, we obtain again the formal quadratic isotherm Eq. (2), with $A_1 = q_s.b_1$, $A_2 = 2q_s.b_1.b_2$, $B_1 = (b_1 + b_2)$ and $B_2 = b_1.b_2$, where q_s is the total bilayer saturation capacity of the adsorbent [17].

4. Finally, a model assuming multilayer adsorption, which is not limited by the number of molecules adsorbed on the first layer, leads to the derivation of quadratic isotherm Eq. (2) with $A_1 = q_s.b_1$, $A_2 = q_s.b_1.b_2$, $B_1 = b_1$ and $B_2 = 0$, where b_2 is a measure of the adsorption rate in the second and subsequent layers [16].

To summarize, various quadratic isotherm models are more adequate than the Langmuir model to describe the single-component distribution in the systems where, for various reasons, the plateau of the adsorption isotherm corresponding to the full saturation of the adsorbent cannot be achieved within the concentration range of the solute in the liquid phase (usually because of solubility limitations). The isotherm equation derived either from the statistical thermodynamic or from the bi-Langmuir model is described by three-parameter isotherms with the same relationships between the parameters A_1, B_1 and A_2, B_2, so that they can not be distinguished from one another. The bilayer model yields also a three-parameter isotherm equation, but with a different relationship between the parameters A_1, B_1 and the multi-layer model yields a three-parameter isotherm equation with $B_2 = 0$. These differences offer a means for distinguishing between various models on the basis of goodness of their fits to the experimental data. However, a good fit alone does not prove the validity of a physical model.

The adsorption behaviour of mixtures (such as of one sample compound from a mixed binary solvent or of two sample compounds from a pure solvent or from a solvent mixture) is much more complicated than the adsorption of a single component from a pure solvent. Because of the competition of all the compounds in the liquid phase for the adsorption sites, the achievement of full saturation of the sorbent by one compound is more difficult and is possible only if the adsorption energy of such a compound is much higher than the adsorption ener-

gies of the remaining compounds in the liquid phase. Numerous and often complicated models have been suggested to describe the competitive equilibria involved between the components i, j of a binary sample mixture and the adsorbent, yielding various competitive isotherm equations [18]. The competitive Langmuir isotherm [19] is often used, but it is thermodynamically justified only if the column has the same saturation capacities for all sample components [1]:

$$q_i = \frac{a_i \cdot c_i}{1 + b_i \cdot c_i + b_j \cdot c_j} \tag{4}$$

The parameters a_i, b_i, b_j are the Langmuir single-component coefficients for solutes i, j.

More complex models have been suggested to account for the experimental deviations from the competitive Langmuir model, such as for example the competitive Fowler isotherm [10,11], the competitive quadratic isotherm based on the statistical thermodynamics model [20]:

$$q_i =$$

$$\frac{q_s \cdot (b_{1,i} \cdot c_i + 2b_{2,i} \cdot c_i^2 + b_{3,ij} \cdot c_i \cdot c_j)}{1 + b_{1,i} \cdot c_i + b_{2,i} \cdot c_i^2 + b_{1,j} \cdot c_j + b_{2,j} \cdot c_j^2 + b_{4,ij} \cdot c_i \cdot c_j} \tag{5}$$

or the thermodynamically more consistent LeVan–Vermeulen competitive isotherm [21].

In the competitive isotherm Eq. (5) q_s, b_1, b_2 are the single-component quadratic isotherm coefficients of the sample components i, j, whereas the coefficients $b_{3,ij}$ and $b_{4,ij}$ account for competitive sorption behaviour. Unfortunately, if the single-component Langmuir coefficients are used in the isotherm equations (4) or (5), poor fit to the experimental competitive distribution data is often obtained [2,10,22–25]. To overcome this problem, the coefficients evaluated from the competitive data have been used [2,9,10,26–28], which often differ significantly from the single-component isotherm coefficients and their values depend on the concentration ratio of the sample compounds, in contrast to their definition. Such isotherm equations are only empirical and do not allow fundamental interpretation of the distribution data.

As the retention behaviour can be most conveni-

ently controlled by the composition of binary (or more complex) solvent mixtures, both in analytical and in preparative separations, the optimisation of preparative separations requires that the effect of the solvent system on the adsorption isotherms be known. In normal phase systems, the competition of polar solvents for the adsorption sites on the surface of a polar adsorbent can be very significant. The most simple (although not rigorous) description of this effect can be obtained using dependencies of the isotherm coefficients on the composition of a two-component (binary) mobile phase analogous to the dependencies of the retention factors under linear isotherm conditions obtained using theoretical models of adsorption. Based on the original Snyder concept of adsorption as a competitive phenomenon, a three-parameter equation was derived to describe the dependence of the retention factor, k, of a sample compound (at concentrations close to infinite dilution) on the concentration of the stronger (more polar) solvent, φ, in a binary organic mobile phase [29–31]. As the coefficient a of the Langmuir isotherm is directly proportional to the retention factor, the same form of equation could possibly describe the dependencies of the Langmuir parameters a and b on φ, assuming that the column saturation capacity, q_s, does not depend significantly on the composition of the binary mobile phase:

$$a = (s_a + p_a \cdot \varphi)^{-m_a}; \ b = (s_b + p_b \cdot \varphi)^{-m_b} \tag{6}$$

Here, s_a, p_a and m_a, s_b, p_b and m_b are experimental constants depending on the solute and on the chromatographic system. If the retention in pure nonpolar solvent is very high, the terms s_a, s_b in Eq. (6) can be neglected.

However, this description does not take into account the competition of the more polar solvent in the binary solvent mixture with sample compounds for the adsorption sites on the surface of a polar adsorbent. A more rigorous description of the distribution of a single sample compound between the stationary and the binary solvent systems should be based on a two-component isotherm with the sample compound as the first and the more polar solvent as the second component of the distribution system. A single isotherm equation of this type could be used to describe the distribution of a sample compound over a wide composition range of a binary solvent mixture. For this purpose, the most simple is the

equation describing the Langmuir competitive iso-therm (Eq. (4)) with the concentration of the polar solvent, φ, used instead of c_j:

$$q_i = \frac{a_i \cdot c_i}{1 + b_i \cdot c_i + p \cdot \varphi} \tag{7}$$

It may be interesting that Eq. (7) leads to Eq. (6) for a with $m_a = 1$ at very low concentrations of sample compounds, c_i.

In the case of non-ideal behaviour, a better description of the experimental distribution can be expected with a statistical thermodynamic two-component quadratic isotherm equation (Eq. (5) with φ instead of c_j), in respect of the competitive effect of the polar solvent:

$$q_i =$$
$$\frac{q_s \cdot (b_1 \cdot c_i + 2b_2 \cdot c_i^2 + b_{5,s} \cdot c_i \cdot \varphi)}{1 + b_1 \cdot c_i + b_2 \cdot c_i^2 + b_{5,s} \cdot c_i \cdot \varphi + b_{4,s} \cdot \varphi + b_{6,s} \cdot \varphi^2} \tag{8}$$

The terms b_{4s}, b_{5s}, b_{6s} theoretically have the meaning of the rate constant of adsorption of the polar solvent, of the energy of interactions between the adsorbed molecules of the solute and of the polar solvent and between the adjacent adsorbed molecules of the polar solvent, respectively. However, different saturation capacities for the solute and for the polar solvent may affect significantly the values of these coefficients. If the term with the second power of φ can be neglected, Eq. (8) is simplified to Eq. (9):

$$q_i = \frac{q_s \cdot (b_i \cdot c_i + 2b_2 \cdot c_i^2 + b_{5,s} \cdot c_i \cdot \varphi)}{1 + b_1 \cdot c_i + b_2 \cdot c_i^2 + b_{5,s} \cdot c_i \cdot \varphi + b_{4,s} \cdot \varphi} \tag{9}$$

To describe the distribution of a two-component sample between the stationary and the binary mobile phase in this way, a three-component competitive adsorption isotherm equation is necessary. We suggested Eq. (10) for the description of such a competitive quadratic isotherm:

$$q_i =$$
$$\frac{q_s \cdot (b_{1i} \cdot c_i + 2b_{2i} \cdot c_i^2 + b_{3ij} \cdot c_i \cdot c_j + b_{5is} \cdot c_i \cdot \varphi)}{1 + b_{1i} \cdot c_i + b_{1j} \cdot c_j + b_{2i} \cdot c_i^2 + b_{2j} \cdot c_j^2 + b_{7ij} \cdot c_i \cdot c_j + b_{5is} \cdot c_i \cdot \varphi + b_{5js} \cdot c_j \cdot \varphi + b_{4s} \cdot \varphi + b_{8s} \cdot \varphi^2} \tag{10}$$

Here, the coefficients q_s, b_{1i}, b_{1j}, b_{2i}, b_{2j}, b_{5is}, b_{5js}, b_{4s} are the coefficients of Eq. (8) for the competitive

isotherms of single compounds i and j, respectively, accounting for the effect of a polar solvent. Only b_{3ij}, b_{7ij}, b_{8s} are additional free floating coefficients accounting for the competitive effects of the compound j. Theoretically, $b_{3ij} = b_{7ij}$ and the coefficient b_{8s} should be the same as in the two-component competitive isotherm Eq. (8), but because of different saturation capacities of the adsorbent for sample compounds and for the polar solvents, the statistical thermodynamics model is not expected to be exactly valid and more than one independent parameter is required to describe adequately the experimental data. If the term with the second power of φ can be neglected, Eq. (10) is simplified to:

$$q_i =$$
$$\frac{q_s \cdot (b_{1i} \cdot c_i + 2b_{2i} \cdot c_i^2 + b_{3ij} \cdot c_i \cdot c_j + b_{5is} \cdot c_i \cdot \varphi)}{1 + b_{1i} \cdot c_i + b_{1j} \cdot c_j + b_{2i} \cdot c_i^2 + b_{2j} \cdot c_j^2 + b_{7ij} \cdot c_i \cdot c_j + b_{5is} \cdot c_i \cdot \varphi + b_{5js} \cdot c_j \cdot \varphi + b_{4s} \cdot \varphi} \tag{11}$$

The meaning of the coefficients is the same as in Eq. (10).

3. Experimental

3.1. Chemicals

Benzophenone and coumarin, all reagent grade, were purchased from Sigma–Aldrich, Prague, Czech Republic and were used as obtained. 2-Propanol, *n*-heptane, *n*-hexane and dichloromethane, all of HPLC grade, were purchased from Baker, (Deventer, The Netherlands). To eliminate the effect of water on the adsorption, dry solvents were kept in tightly closed dark bottles over molecular sieve beads Dusimo 5 A (Lachema, Brno, Czech Republic), previously activated at 300°C (ca. 30–40 g/l), filtered using a Millipore 0.45 μm filter and degassed by ultrasonication immediately before the use. Mobile phases were prepared by exact mixing of the appropriate volumes of the components.

3.2. Instrumentation and methods

To acquire the data necessary for the determination of the equilibrium isotherms by the frontal analysis method [32], an 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 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 ratio of the flow-rates of the two solutions was adjusted from 0 to 100% in successive 10% 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 eluate from the column used to measure the isotherm passed through an external LCD 2563 UV detector (Laboratory Instruments Works, Prague, Czech Republic) working at 348 nm and via a six-port column-switching valve to an analytical column the outlet of which was connected to the diode-array detector set at 348 nm. In this way, automated collection and analysis of the fractions was performed using a pre-set switching-valve program to determine the concentrations of the two components in the eluate from the main column. The output signal from the external detector was acquired via an analog–digital convertor (760 Series Interface, Hewlett-Packard) by the data station of the chromatograph, so that the signals from both the diode-array and the external UV detectors were simultaneously processed. The band profiles of the sample compounds in overloaded separations were measured using the HP 1090 M liquid chromatograph in the standard setup, with the column coupled directly to the diode-array UV detector and samples were introduced by directly pumping larger sample volumes from the reservoir before the start of the separation. For the calculation of band profiles, the equations of the equilibrium- dispersive model of chromatography were solved using the finite difference scheme based on the forward–backward ''Rouchon'' algorithm [33]. Competitive quadratic three-component isotherms taking into account the effect of the 2-propanol concentration in the mobile phase (Eq. (11)) and the column efficiency of approximately 3 000 theoretical plates were employed in the numerical calculations.

The column for measuring the isotherm data and the band profiles of sample solutes – glass-cartridge,

150 mm long, 3.3 mm in diameter, packed with Separon SGX silica gel, 7 μm particle size – was purchased from ECOM, Prague, Czech Republic. The column dead volume (0.94 ml) was evaluated from the record of the detector set to 200 nm after injection of pure *n*-heptane in mixed mobile phases. The volume of the stationary phase, 0.34 ml, was calculated as the difference between the volume of the void column and the column dead volume. Another silica gel column of the same type was used for the analysis of the fractions of the eluate.

In each experiment the solute concentration in the stationary phase was determined from the appropriate integral mass balance equation [27], using (A) the concentrations of the sample components at the plateaus of the frontal analysis curve, corresponding either to the dilution of the sample after each step or determined by the external UV detector using the calibration curve method (in case of competitive isotherms) and (B) the retention volumes corresponding to the inflection points on 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 [27] (0.31 ml). Solutions of the individual compounds (0.01 mol/l or 0.1 mol/l) were used for the determination of single-component isotherms and sample mixtures containing benzophenone and coumarin in concentration ratios 0.1: 0.1, 0.1: 0.02 and 0.02: 0.1 mol/l for the determination of competitive isotherms. All experiments were repeated at least twice.

The Adstat statistical software (Trilobyte, Prague, Czech Republic) was used to fit isotherms to the experimental data sets using non-linear regression. Non-linear regression was used also to fit the Langmuir isotherm instead of fitting the linearised form of Eq. (1) by linear regression (as it is often practised), because of better fit to the experimental data.

4. Results and discussion

4.1. Adsorption isotherms of more polar solvents on a silica gel column.

As the distribution data in normal-phase systems can be affected by the competition with the more

polar solvent of a binary mobile phase for the adsorption sites, we measured first the distribution isotherms of the components of the mobile phases used, i.e., of 2-propanol in n-hexane and in dichloromethane and of dichloromethane in n-heptane on a Separon SGX silica gel column (Fig. 1 and Table 1). Special care was taken to use freshly dried solvents in all isotherm measurements to eliminate possible effects of trace concentrations of water on the adsorption. The experimental distribution data of 2-propanol in n-hexane (Fig. 1A) are well described by the Langmuir isotherm (Eq. (1)). The column saturation capacity for 2-propanol is almost achieved in mobile phases containing 2% 2-propanol and a further increase in the concentration of 2-propanol in the mobile phase does not significantly affect its concentration in the stationary phase.

The distribution of 2-propanol between the stationary and the mobile phases in binary mixtures with dichloromethane is different (Fig. 1B), the saturation capacity of 2-propanol predicted from the Langmuir model is much higher than in n-hexane and the data of Table 1 suggest that the saturation capacity would not be approached even at concentrations of 2-propanol much higher than 10%. Both the distribution constant at infinite dilution, a, and the sorption rate constant, b, of 2-propanol predicted from the Langmuir model are significantly lower in dichloromethane than in n-hexane. The higher-concentration part of the isotherm is steeper than predicted from the Langmuir isotherm, which shows significant deviations from the experimental data (Fig. 1B).

The distribution data of 2-propanol in dichloromethane can be adequately described by three-parameter quadratic isotherm (Eq. (2)). The best-fit coefficients of this isotherm, determined assuming various models discussed in the theoretical part, are listed in Table 1. The coefficients determined for the statistical thermodynamics, the bi-Langmuir and the bilayer models differ little from each other and provide very similar fits to the experimental data. All models suggested the significant role of additional interactions of the adsorbed molecules described by the coefficient b_2, but the results do not allow to distinguish whether these interactions occur between adsorbed molecules on adjacent adsorption sites, or between molecules in two adsorbed layers or whether there are two different types of adsorption
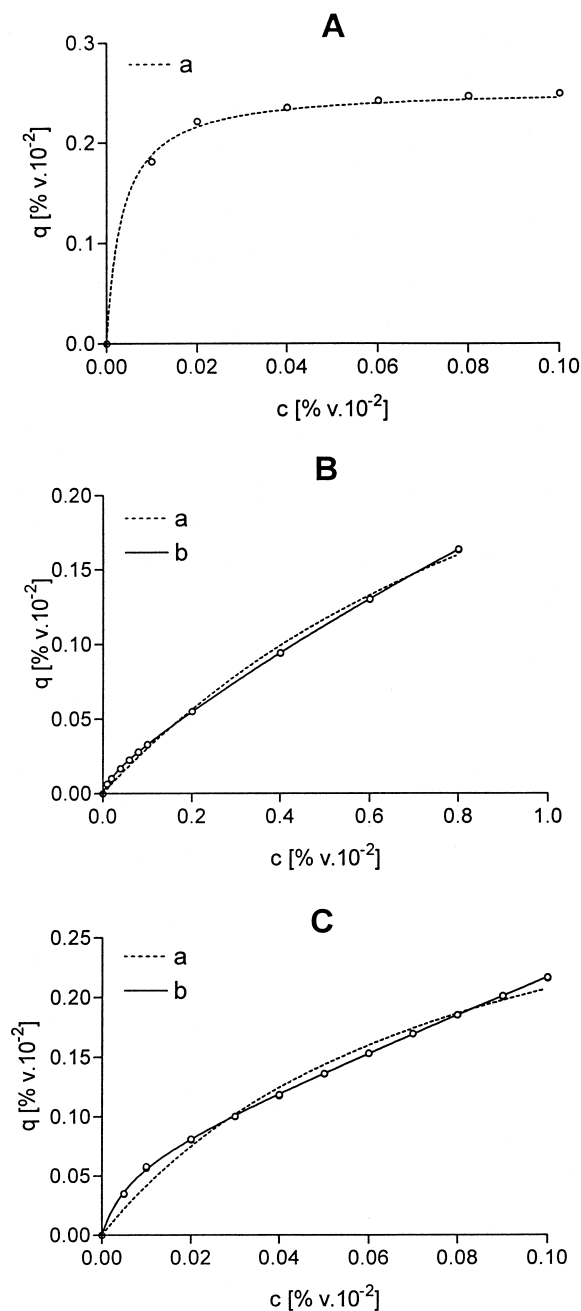

Fig. 1. Adsorption isotherms of 2-propanol in n-hexane (A) and in dichloromethane (B) and of dichloromethane in n-heptane (C) on a Separon SGX silica gel column. (a) Langmuir isotherms (Eq. (1), dotted lines) and (b) quadratic isotherm (Eq. (2), solid line) fitted to the experimental data (points). Parameters of the isotherms are in Table 1. c, q = concentrations of the more polar solvent in the mobile and in the stationary phases, respectively, in % vol.10^{-2}.

Table 1
Coefficients of the distribution isotherms of 2-propanol in *n*-hexane (A), 2-propanol in dichloromethane (B) and dichloromethane in *n*-heptane (C) on a Silica gel Separon SGX column

Eq. No.	Coefficient	Solvent system A	Solvent system B	Solvent system C
1	a	730	5.74	0.435
	b [l mol^{-1}]	4674	2.78	0.015
	q_s [mol l^{-1}]	0.156	2.07	27.86
2 (3)	q_s [mol l^{-1}]	–	3.53	4.15
statistical	b_1 [l mol^{-1}]	–	1.77	0.080
thermodynamics	b_2 [l^2 mol^{-2}]	–	0.37	0.001
2	q_s [mol l^{-1}]	–	3.17	4.10
bi-Langmuir	b_1 [l mol^{-1}]	–	1.33	0.068
	b_2 [l mol^{-1}]	–	0.22	0.014
2	q_s [mol l^{-1}]	–	3.09	4.10
bilayer	b_1 [l mol^{-1}]	–	1.64	0.082
	b_2 [l mol^{-1}]	–	0.30	0.020
2	q_s [mol l^{-1}]	–	0.88	5.73
multilayer	b_1 [l mol^{-1}]	–	11.29	0.78
	b_2 [l mol^{-1}]	–	1.74	0.01

a,b coefficients of the Langmuir isotherm (Eq. (1)); $q_s = a/b$ saturation capacity of the column; q_s, b_1, b_2 coefficients of the quadratic isotherm (Eq. (2)) based on various models (the physical meaning of the individual parameters with different models is explained in the theoretical part).

sites (this possibility is not likely with the silica gel adsorbent).

The isotherm based on the multilayer adsorption model (described by Eq. (2) with $B_2 = 0$) provided the fit to the experimental data with the residual sum of squares two orders of magnitude lower than the other quadratic isotherm models, which may indicate better suitability of this model to describe the experimental sorption behaviour. Further, the multilayer adsorption seems to be more likely than the bilayer adsorption, because – as Snyder and Poppe pointed out – there is no reason why the build-up of multilayers should stop with the second layer and the multilayer build-up should be facilitated by the possibility of strong hydrogen bonding between the subsequent adsorbed layers [34]. Such interactions and multilayer adsorption obviously could occur also when 2-propanol is adsorbed from *n*-hexane, but because of a several orders of magnitude higher rate constant of adsorption (b and b_1, respectively in Table 1), it has a relatively less important role and the coefficient b_2 cannot be evaluated accurately from the experimental distribution data.

The distribution data of dichloromethane in *n*-heptane also can be described much better by the quadratic than by the Langmuir isotherm (Fig. 1C). The Langmuir model predicts an unrealistically high saturation capacity for dichloromethane. The quadratic model describes the adsorption adequately, with best fit obtained for the multilayer model (the residual sum of squares one order of magnitude lower) than with other models. All models predict significantly (one to two orders of magnitude) lower energy of interaction (b_2) between the adsorbed molecules of less polar dichloromethane than between the adsorbed molecules of 2-propanol, but the rate constant of adsorption of dichloromethane from *n*-hexane (b and b_1, respectively) is approximately four to five orders of magnitude lower than that of 2-propanol, which may explain the relatively significant effect of the multilayer interactions on the shape of the adsorption isotherm.

4.2. Adsorption isotherms of pure benzophenone and of coumarin in various solvents

Because of poor solubility of sample compounds in solvent mixtures containing low concentrations of 2-propanol in *n*-hexane, their distribution was mea-

sured in the concentration range 0.001–0.01 mol/l. The solubility is significantly improved in dichloromethane-containing solvent mixtures, where the experimental concentration range of sample compounds was ten times higher, 0.01–0.1 mol/l. From all mixed solvents, benzophenone is adsorbed less strongly than coumarin.

The experimental data in all mobile phases containing 2-propanol are formally well described by the Langmuir isotherm (Eq. (1)). Somewhat better fit to the experimental data measured in 2-propanol-*n*-hexane mixed solvents is obtained for a three-parameter quadratic isotherm (Eq. (2)). The quadratic isotherm fits significantly better the experimental distribution data in dichloromethane-*n*-heptane mixed solvents (Fig. 2). The coefficients of the isotherms for pure sample solutes are listed in Table 2. As expected, the coefficients a, b of the Langmuir isotherm generally decrease as the concentrations of more polar solvents increase. The exception to this rule is the dependence of the coefficient b of benzophenone on the concentration of 2-propanol in *n*-hexane.

The saturation capacity of the column for the sample compounds is little affected by the composition of the mobile phases containing dichloromethane, but its decrease with increasing concentration of 2-propanol in *n*-hexane is significant. This behaviour can be possibly caused by a very strong affinity of 2-propanol to silica gel in these mixed solvents, which diminishes the capacity of the column available for the adsorption of sample compounds. From the experimental values in Tables 1 and 2 it can be seen that the Langmuir constant a (i.e., the limit distribution constant at low concentrations of the solute) for 2-propanol is approximately two orders of magnitude higher than the constants a of benzophenone and of coumarin. This means that the adsorption energies of sample compounds are two orders of magnitude lower than the adsorption energy of 2-propanol, so that the two sample compounds, in the concentrations used in the experiments, cannot efficiently displace 2-propanol from the silica gel surface. This explanation is further supported by comparison of the saturation capacities of the column for the two sample compounds, which are similar in mobile phases containing dichloromethane, but significantly higher for more strongly
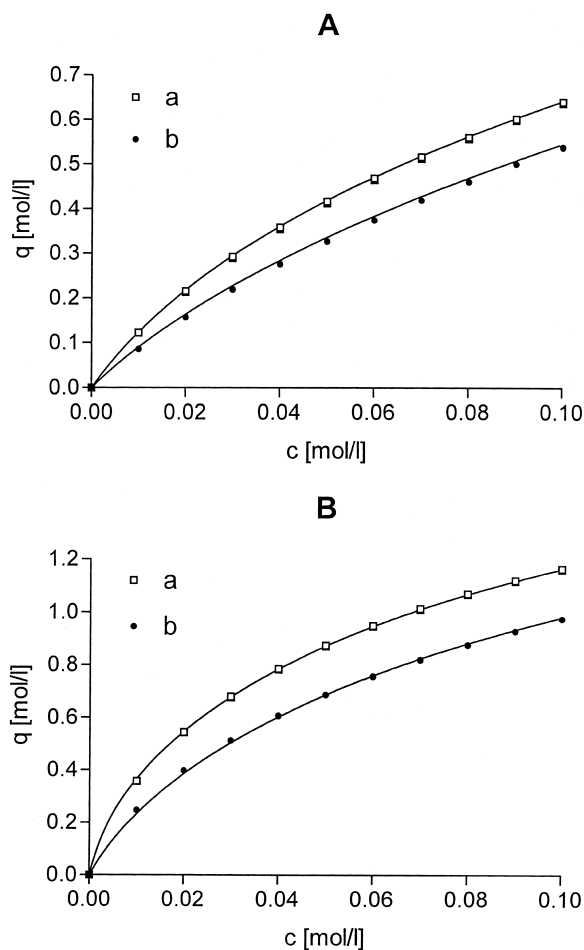

Fig. 2. Single-component quadratic isotherms (Eq. (2)) of benzophenone (A) and of coumarin (B) on a Separon SGX silica gel column (solid lines) fitted to the experimental data (points). Mobile phase: 60% (a) and 80% (b) dichloromethane in *n*-heptane. Parameters of the isotherms are in Table 2. c, q = concentrations of sample compounds in the mobile and in the stationary phases, respectively, in mol/l.

retained coumarin than for benzophenone in 2-propanol-*n*-hexane binary mixtures.

Various adsorption models, including the multilayer one, provided approximately equal fit (residual sum of squares) of three-parameter quadratic isotherms. The statistical thermodynamics and the bilayer models indicate a slight increase of the saturation capacities and a decrease of the rate constants of the sorption, b_1, and of the interactions with adsorbed molecules, b_2, with increasing

Table 2

Coefficients of single-component isotherms of benzophenone (1) and of coumarin (2) on a Silica gel Separon SGX column in solvent systems A–C

Model	Solvent system	Benzophenone			Coumarin		
		a	b	q_s	a	b	q_s
I – Langmuir model							
	A; 1%P	1.82	4.86	0.37	20.31	5.75	3.64
	A; 2%P	1.39	6.27	0.22	12.72	5.43	2.34
	A; 4%P	1.03	7.56	0.14	8.84	4.80	1.84
	A; 8%P	0.92	6.80	0.14	5.80	3.43	1.69
	A; 16%P	0.80	8.20	0.10	3.73	3.04	1.23
	B; 0%P	6.74	4.75	1.42	20.34	14.89	1.37
	B; 0.5%P	3.33	1.98	1.68	7.04	5.13	1.37
	B; 1%P	2.52	1.35	1.87	4.70	3.18	1.48
	B; 2%P	2.12	1.16	1.83	3.35	2.03	1.65
	C; 80%D	8.85	6.70	1.32	26.89	18.21	1.48
	C; 60%D	12.85	10.51	1.22	40.96	25.90	1.58

		Benzophene			Coumarin		
		q_s	b_1	b_2	q_s	b_1	b_2
Quadratic isotherm, A: 2-propanol/n-hexane							
II	1% P	0.284	6.39	6.72	2.49	8.41	11.49
II	2% P	0.158	8.74	12.52	1.72	7.37	8.89
II	4% P	0.093	11.06	20.11	1.41	6.22	6.35
II	8% P	–	–	–	1.26	4.61	3.51
II	16% P	0.046	17.66	49.67	1.01	3.66	2.28
III	1% P	0.277	6.54	2.13	2.48	8.47	2.71
III	2% P	0.159	8.71	2.79	1.72	7.37	2.37
III	4% P	0.094	10.95	3.45	1.41	6.23	2.02
III	8% P	–	–	–	1.23	4.72	1.55
III	16% P	0.068	11.79	3.70	0.83	4.49	1.46
IV	1% P	0.0035	65.2	473	0.621	14.5	19.23
IV	2% P	0.0043	55.9	267	0.021	77.8	531
IV	4% P	0.0041	13.8	112	0.0073	121	1104
IV	8% P	–	–	–	0.014	45.5	384
IV	16% P	0.0350	13.5	9.26	0.0041	71.6	850
Quadratic isotherm, B: 2-propanol/dichloromethane							
II	0% PA,	1.003	6.63	6.46	0.936	21.92	56.13
II	0.5% P	1.333	2.45	0.95	0.974	7.11	7.36
II	1% P	1.489	1.67	0.45	1.075	4.31	2.84
II	2% P	1.595	1.31	0.28	1.188	2.79	1.24
III	0% P	1.004	6.65	1.73	0.927	22.74	3.99
III	0.5% P	1.324	2.47	0.75	0.970	7.18	1.85
III	1% P	1.315	1.91	0.59	1.067	4.35	1.23
III	2% P	1.580	1.32	0.42	1.189	2.79	0.83
IV	0% P	0.447	10.31	5.27	0.684	29.13	4.80
IV	0.5% P	0.081	13.57	28.82	0.440	11.08	5.40
IV	1% P	0.048	14.34	40.46	0.355	7.66	5.80
IV	2% P	0.036	11.67	47.81	0.192	7.36	10.32
Quadratic isotherm, C: dichloromethane/n-heptane							
II	80% D	0.932	9.33	12.22	0.996	27.92	84.68
II	60% D	0.845	15.17	29.34	1.038	43.47	178.7
III	80% D	0.929	9.45	2.26	0.99	28.96	4.54
III	60% D	0.841	15.47	3.16	1.03	45.62	5.78
IV	80% D	0.470	14.15	5.50	0.799	34.93	4.45
IV	60% D	0.580	19.58	4.39	1.006	45.52	3.41

Solvent systems: A, 2-propanol (P)-*n*-hexane (HX); B, 2-propanol (P)-dichloromethane (D); and C, dichloromethane (D) *n*-heptane (HP). I, Langmuir model; II, statistical thermodynamics model; III, bilayer model; IV, multilayer model.

a, b, coefficients of the Langmuir isotherm (Eq. (1)), $q_s = a/b$, saturation capacity of the column. q_s, b_1, b_2, coefficients of the quadratic isotherm (Eq. (2)) based on various models (the physical meaning of the individual parameters with different models is explained in the theoretical part).

Concentrations of the components 1 and 2 in mol/l [0.001–0.01 *M* in (A); 0.01-0.1 *M* in (B) and (C)]. b, b_1, b_2 in l mol^{-1} (b_2 in l^2 mol^{-2} for the statistical thermodynamics model Eq. (3)), q_s in mol l^{-1}.

polarities of mobile phases containing dichloromethane. An increasing concentration of 2-propanol in *n*-hexane caused a significant decrease of q_s and increase of b_1 and b_2 of benzophenone, but decreased the rate constants of coumarin. The low saturation capacity available suggests that the sample solutes, especially benzophenone with only one carbonyl functional group, can not compete very efficiently with 2-propanol for the adsorption sites in mixed solvents containing *n*-hexane. Increase of the sorption rate constants b_1 with increasing concentration of 2-propanol could possibly indicate that the sorption may occur on the previously adsorbed molecules of 2-propanol, in addition to the sorption on the silanol groups, and increasing b_2 could mean increasing strength of interactions with adjacent molecules in systems with higher concentrations of 2-propanol. Very low values of q_s were found with the multilayer model, but the effect of the composition of mixed solvents on b_1 and b_2 was difficult to interpret (Table 2), which indicates that the multilayer model is probably not appropriate to describe the adsorption of solutes from mixed solvents.

The competition between the sample compounds and the more polar solvents for the adsorption sites makes the single-component Langmuir model unsuitable to describe correctly the distribution between the mixed solvents and silica gel. For the same reason, the interpretation of the quadratic isotherm models may be subject to limitations and the conclusions are rather qualitative.

4.3. Adsorption isotherms of benzophenone and of coumarin in mixed samples

The two-component distribution data were measured for the mixtures of the sample compounds in the same concentration range as the distribution data of single-compound samples. In the low concentration range of sample compounds that was dictated by low solubility in 2-propanol-*n*-hexane mixtures, the concentrations of the compounds in the stationary phase were very close to the single-component data (within the experimental error in 8% and in 16% 2-propanol), which means that the competition effect is weak there, especially for the more strongly retained coumarin.

The competitive Langmuir isotherm (Eq. (4)) provides poor fit to the experimental distribution data. The single-component thermodynamic statistics quadratic isotherm coefficients q_s, b_1 and b_2 of benzophenone and of coumarin given in Table 2 were used when fitting the competitive isotherm described by Eq. (5) to the experimental data, and only two additional free floating coefficients, $b_{3,ij}$ and $b_{4,ij}$ were determined by nonlinear regression (Table 3). The competitive quadratic isotherm determined in this way fits the experimental data very well, with differences of 0.5% or less, which is within the experimental error of the determination. The fit of the two-component quadratic isotherm is illustrated in Fig. 3 for the data measured in dichloromethane-*n*-heptane mixed solvents. No systematic dependence of the coefficients $b_{3,ij}$ and $b_{4,ij}$ on the composition of the solvent mixtures is apparent from the data in Table 3. If the saturation capacities of the adsorbent for the two sample compounds were equal, the coefficients $b_{3,ij}$ and $b_{4,ij}$ should be equal to each other. However, this was not found experimentally. Further, the two-component competitive isotherm can provide only a formal description of the distribution data in a given binary

Table 3
Coefficients of quadratic competitive isotherms (Eq. (5)) of benzophenone (1) and of coumarin (2) on a silica gel Separon SGX column in mobile phases A–C

Solvent system	Benzophenone		Coumarin	
	$b_{3,ij}$	$b_{4,ij}$	$b_{3,ij}$	$b_{4,ij}$
A, 1% P	-28.62	-395.9	24.34	1310
A, 2% P/H	140.7	2492	90.69	882
A, 4% P/H	43.70	1413	11.39	-307
B, 0% P	59.58	43.91	-88.79	-62.15
B, 0.5% P	1.94	-32.31	-12.18	-24.44
B, 1% P	8.48	5.06	1.97	0.58
C, 80% D	34.14	-119.2	200.3	59.62
C, 60% D	54.77	-195.2	290.4	42.07

Mobile phases: A, 2-propanol (P)-*n*-hexane (HX); B, 2-propanol (P)-dichloromethane (D); C, dichloromethane (D)-*n*-heptane (HP).
Concentrations of the components 1 and 2 in mol/l [0.001–0.1 M in (A); 0.01–0.1 M in (B) and (C)].
Coefficients $b_{3,ij}$, $b_{4,ij}$ in l^2 mol^{-2}, coefficients q_s, b_1, b_2, are given in Table 3 (quadratic isotherm, statistical thermodynamics model). Indices i and j relate to the compound of interest and to the competing compound in tested mixtures, respectively.
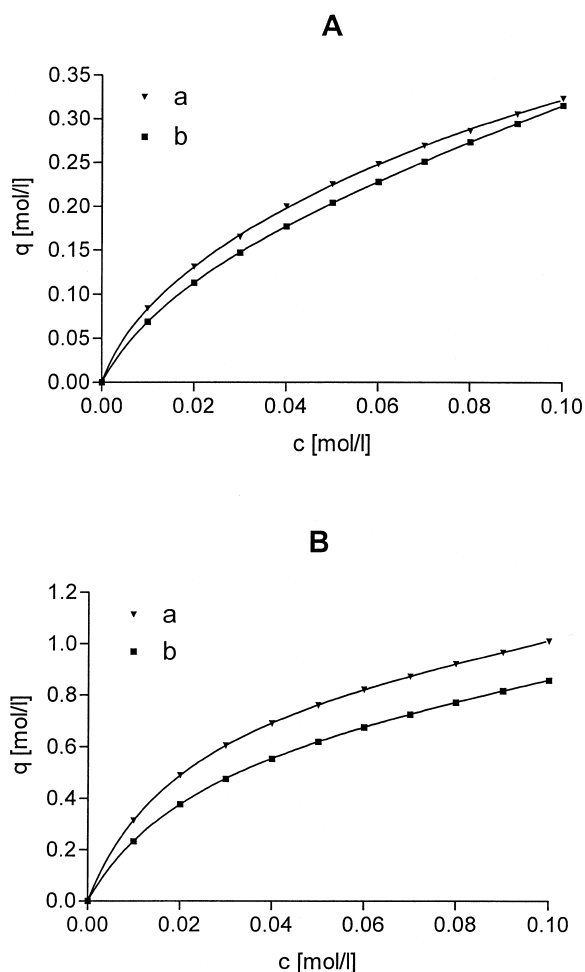
A

B

Fig. 3. Two-component quadratic isotherms (Eq. (5)) of ben-zophenone (A) and of coumarin (B) on a Separon SGX silica gel column (solid lines) fitted to the experimental data (points) measured for mixtures of sample compounds in concentration ratios 1:1. Mobile phase: 60% (a) and 80% (b) dichloromethane in *n*-heptane. Parameters of the isotherms are in Table 3. c, q = concentrations of sample compounds in the mobile and in the stationary phases, respectively, in mol/l.

A

B

C

Fig. 4. Experimental (a: solid line, calculated from the calibrated detector response) and numerically calculated using a two-com-ponent quadratic isotherm (Eq. (5), parameters in Table 2; b: dotted line) band profiles of benzophenone and of coumarin in overloaded chromatography on a Separon SGX silica gel column with 80% dichloromethane in *n*-heptane as the mobile phase. Sample volume: 0.5 ml (A), 1 ml (B) and 2 ml (C), concentration of each compound 0.1 mol/l. Flow-rate: 1 ml/min, temperature: 40°C.

mixed solvent, as it does not take into account the competition of the polar solvent for the adsorption sites.

Adequate description of two-component distribu-tion data by an isotherm that takes into account the competition between the sample compounds for the adsorption sites is essential for correct numerical calculations of overloaded chromatographic band profiles. Fig. 4 compares the experimental band
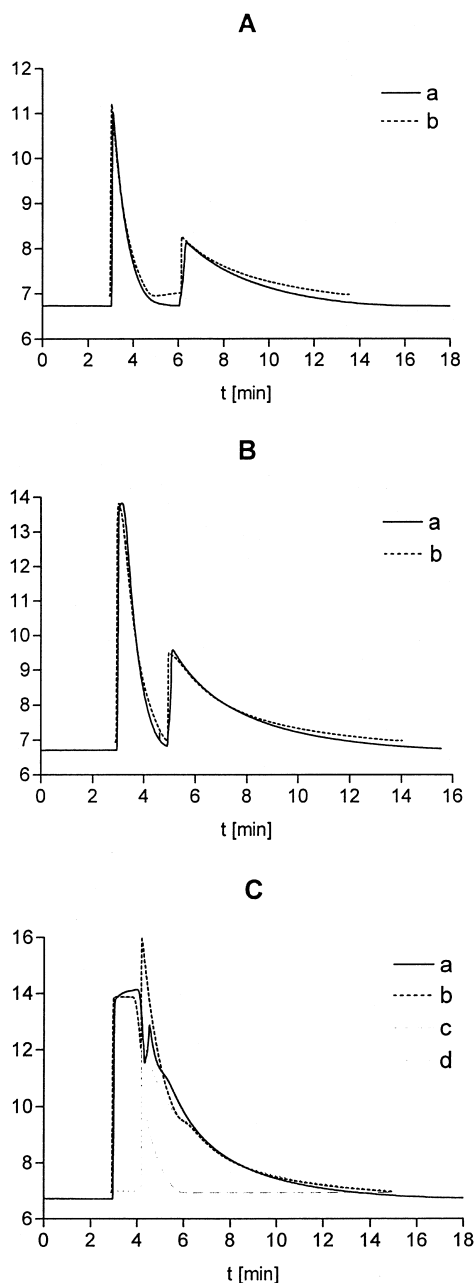
profiles in overloaded separation of the sample compounds in different feed volumes using 80% dichloromethane in *n*-heptane as the mobile phase with the profiles calculated numerically using the coefficients of the two-component quadratic isotherm that are valid in the mobile phase used (Eq. (5)) in Table 3. The agreement between the experimental and the calculated profiles demonstrates suitability of the quadratic competitive isotherms to describe the distribution data in the systems studied. Although the calculations using the isotherms determined for a three-component system would be more correct, it can be seen that a possible decrease of concentration of dichloromethane in the sample zones due to the competition with sample compounds is probably negligible and has no significant effect on the results of band profile calculations.

4.4. Effect of the mobile phase on the distribution data.

A description of the adsorption in binary solvent mixtures of different composition would offer an efficient tool for optimising the experimental conditions in overloaded HPLC. We investigated first the effect of the mobile phase on the coefficients of the Langmuir and of the quadratic isotherms. Eq. (6) can be used to describe the effect of the concentration of 2-propanol in dichloromethane on both Langmuir coefficients *a* and *b* (Table 4), but it fails with the coefficient *b* in 2-propanol–*n*-hexane mixed solvents. No suitable equation was found to describe the dependence of the coefficients of quadratic isotherms on the composition of binary solvent mixtures. This means that the possibilities of this approach are limited. Hence, we investigated possibilities of characterising the experimental data in various solvent systems using a more rigorous approach with a single equation for a competitive isotherm taking into account the more polar solvent in a binary solvent mixture as a second compound competing for adsorption sites.

Fitting the competitive Langmuir isotherm with the coefficient *p* accounting for the effect of the polar solvent (Eq. (7)) to the experimental data resulted in large differences between the experimental data and the adsorbed concentrations calculated using Eq. (7) with the best fit parameters of Table 4.

The failure of Eq. (7) to describe the experimental behaviour can be possibly explained by large differences between the saturation capacities of silica gel for 2-propanol and for the sample compounds. This means that a more complex isotherm equation should be used. Table 4 lists the coefficients of a full two-component six-parameter quadratic isotherm (Eq. (8)) and of the quadratic isotherm with neglected second power term of the concentration of 2-propanol (Eq. (9)). Figs. 5 and 6 illustrate the fit of these equations to the distribution data of each sample compound in 2-propanol-*n*-hexane and in 2-propanol–dichloromethane binary solvent mixtures. It should be noted that six parameters used in the competitive adsorption isotherm are required by the theoretical statistical thermodynamics model. Five is the minimum number of independent coefficients necessary in an equation describing any non-linear dependence on two independent variables and this is the number of coefficients in the isotherm described by Eq. (9). All the distribution data, except for those of benzophenone in pure dichloromethane, show negligible differences between the quality of the fit of the full and of the simplified two-component quadratic equations, which means that a single equation (9) can be used to describe the distribution data of a single sample compound in binary mobile phases with different proportions of their components.

The coefficients q_s, b_1 and b_2 of Eq. (8) or Eq. (9) are the saturation capacity, the rate constant of adsorption and the energy of interactions with adjacent solute molecules in pure less polar solvent. The coefficients $b_{4,s}$ and $b_{6,s}$ relate to the adsorption of the polar solvent and the coefficient $b_{5,s}$ characterizes the energy of interactions between the adsorbed molecules of the solute and of the polar solvent (Table 4). The values of the coefficients q_s, b_1 and b_2 of these isotherms for 2-propanol-dichloromethane mixed solvents in Table 4 are not very different from the values measured in pure dichloromethane (Table 2).

Finally, we attempted to provide a description of the distribution data of a binary sample mixture in various binary mixed solvents using a single-equation isotherm over a wide composition range of the solvent mixtures. A three-component Langmuir isotherm cannot be expected to provide good fit to such

Table 4

Coefficients of the equations describing the effect of the composition of the mixed solvent on the distribution isotherms of benzophenone (1) and of coumarin (2) on a Silica gel Separon SGX column

Compound		1: Benzophenone		2: Coumarin	
Solvent system		P-HX	P-D	P-HX	P-D
Eq. No.	Coefficient				
6	s_a	−0.127	0.0051	−0.0015	0.0073
Langmuir	p_a	0.199	0.0633	0.0049	0.0688
parameter	m_a	0.229	0.362	0.533	0.612
a	R	0.9906	0.9995	0.999	0.9999
6	s_b	–	0.0198	–	0.0368
Langmuir	p_b	–	0.304	–	0.199
parameter	m_b	–	0.398	–	0.818
b	R	–	0.9988	–	0.9999
7	a	1.922	6.363	37.264	19.278
	b [l mol^{-1}]	6.817	4.079	10.997	13.641
	p [l mol^{-1}]	313.79	2916	1758	6414
	R	0.9558	0.9833	0.9867	0.9918
8	q_s [l mol^{-1}]	0.0739	1.113	517.1	1.188
	b_1 [l^2 mol^{-2}]	80.20	5.956	1.34	16.94
	b_2 [l^2 mol^{-2}]	1204	4.045	−4.97	13.31
	$b_{4,s}$ [l^2 mol^{-2}]	27.89	20.791	325.7	41.12
	$b_{5,s}$ [l^2 mol^{-2}]	26.35	9.6	3.95	54.03
	$b_{6,s}$ [l^2 mol^{-2}]	−0.317	−28.18	139	−8.69
	R	0.9990	0.9999	0.9993	0.9996
9	q_s [mol l^{-1}]	0.0714	0.894	14.821	1.171
	b_1 [l mol^{-1}]]	90.27	7.38	4.80	17.24
	b_2 [l^2 mol^{-2}]	1475	10.60	−10.36	14.94
	$b_{4,s}$ [l^2 mol^{-2}]	32.09	30.4	21.16	47.62
	$b_{5,s}$ [l^2 mol^{-2}]	324	53.27	3.68	65.46
	R	0.9990	0.9997	0.9980	0.9996
10[a]	$b_{3,ij}$ [l^2 mol^{-2}]	–	20.38	–	5.36
	$b_{7,ij}$ [l mol^{-1}]	–	−49.72	–	−17.84
	$b_{8,s}$ [l^2 mol^{-2}]	–	75.65	–	87.12
	R	–	0.9864	–	0.9932
11[b]	$b_{3,ij}$ [l^2 mol^{-2}]	−3170	57.95	166.45	19.30
	$b_{7,ij}$ [l^2 mol^{-2}]	−10530	52.91	3965.6	−16.77
	R	0.9939	0.9820	0.9990	0.9930

φ = concentration of 2-propanol (P) in A: n-hexane (HX) and in B: dichloromethane (D).

R = correlation coefficient. All concentrations in mol/l.

[a] Parameters q_s, b_1, b_2, $b_{4,s}$, $b_{5,s}$, $b_{6,s}$ as in Eq. (8), this table.

[b] Parameters q_s, b_1, b_2, $b_{4,s}$, $b_{5,s}$ as in Eq. (8), this table.

Indices i, j relate to the compound of interest and to the competing compound, respectively.

a complex data set, so that we fitted a full (Eq. (10)) and a simplified (neglecting the term with the second power of the concentration of 2-propanol, Eq. (11)) three-component quadratic isotherm. The values of all coefficients of the isotherm Eq. (8) from Table 4 (except for the coefficient $b_{6,s}$) were used in fitting Eq. (10) or Eq. (11) to the experimental data set to find only three or two floating parameters ($b_{3,ij}$, $b_{7,ij}$ and $b_{8,s}$) accounting for the competitive adsorption

of the second sample component. The values of these coefficients are listed in Table 4. The full adsorption isotherm Eq. (10) failed to converge in 2-propanol–n-hexane solvent mixtures, but the simplified Eq. (11) was found to be adequate to describe the whole set of the experimental data, with slightly higher deviations from the experimental data than with the isotherms (8) or (9), describing the distribution of a single sample compound over the whole composition
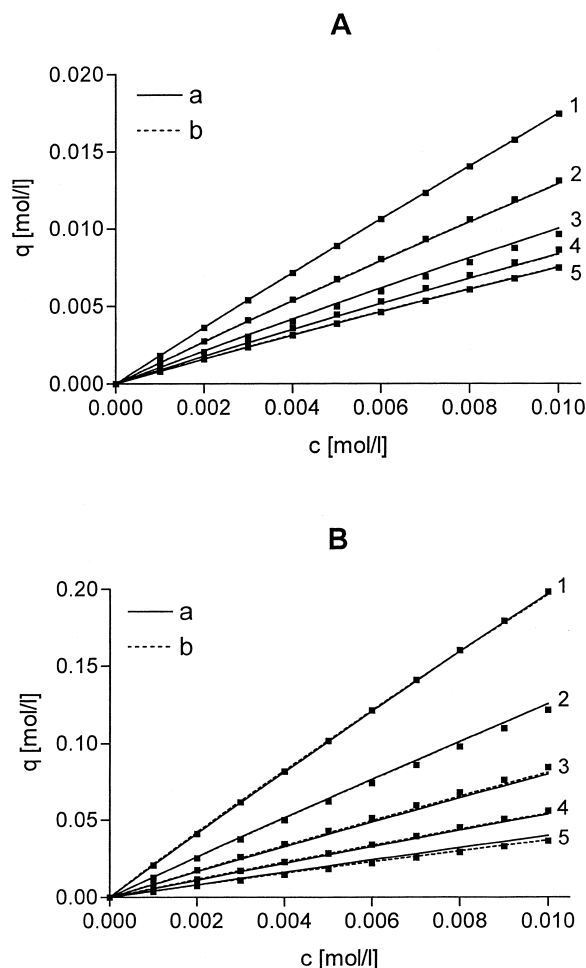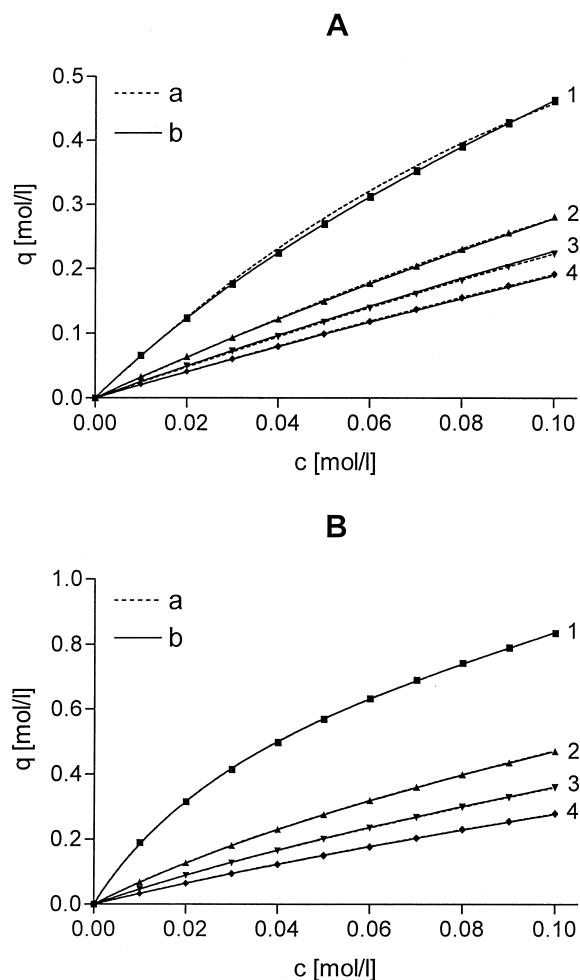
Fig. 5. Two-component quadratic isotherms of benzophenone (A) and of coumarin (B) on a Separon SGX silica gel column (Eq. (8) – solid lines and Eq. (9) – dotted lines) fitted to the experimental data (points). A single isotherm equation taking into account the competitive effect of 2-propanol is used for all mobile phases: 1% (1), 2% (2), 4% (3), 8% (4) and 16% (5) 2-propanol in *n*-hexane. Parameters of the isotherms are in Table 4. *c*, *q* = concentrations of sample compounds in the mobile and in the stationary phases, respectively, in mol/l.

Fig. 6. Two-component quadratic isotherms of benzophenone (A) and of coumarin (B) on a Separon SGX silica gel column (Eq. (8) – solid lines and Eq. (9) – dotted lines) fitted to the experimental data (points). A single isotherm equation taking into account the competitive effect of 2-propanol is used for all mobile phases: 0% (1), 0.5% (2), 1% (3) and 2% (4) 2-propanol in dichloromethane. Parameters of the isotherms are in Table 4. *c*, *q* = concentrations of sample compounds in the mobile and in the stationary phases, respectively, in mol/l.

of the binary solvent mixtures tested. Figs. 7 and 8 illustrate the fit of the three-component quadratic isotherms to the experimental data in 2-propanol–*n*-hexane and in 2-propanol–dichloromethane solvent mixtures. It is interesting to note that increasing the concentration of 2-propanol in dichloromethane from 0.5 to 1% has little effect on the distribution of benzophenone from mixed samples.

To provide further verification of the validity of the three-component isotherms in normal-phase systems, the isotherm Eq. (11) with the coefficients of Table 4 was used to calculate the band profiles in overloaded separations of a 0.5-ml sample containing 0.1 mol/l of each of benzophenone and coumarin, with 0.5% and 1% 2-propanol in dichloromethane as the mobile phase. The band profiles determined by
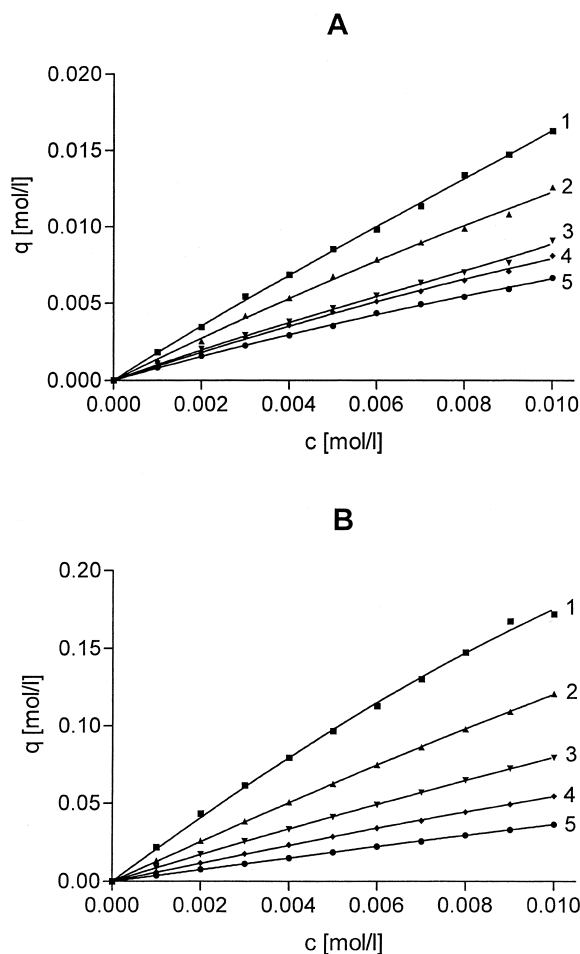
Fig. 7. Three-component quadratic isotherms of benzophenone (A) and of coumarin (B) on a Separon SGX silica gel column (Eq. (11)) fitted to the experimental data (points) measured for mixtures of sample compounds in concentration ratios 1:1. A single isotherm equation taking into account the competitive effect of 2-propanol is used for all mobile phases: 1% (1), 2% (2), 4% (3), 8% (4) and 16% (5) 2-propanol in *n*-hexane. Parameters of the isotherms are in Table 4. *c*, *q* = concentrations of sample compounds in the mobile and in the stationary phases, respectively, in mol/l.
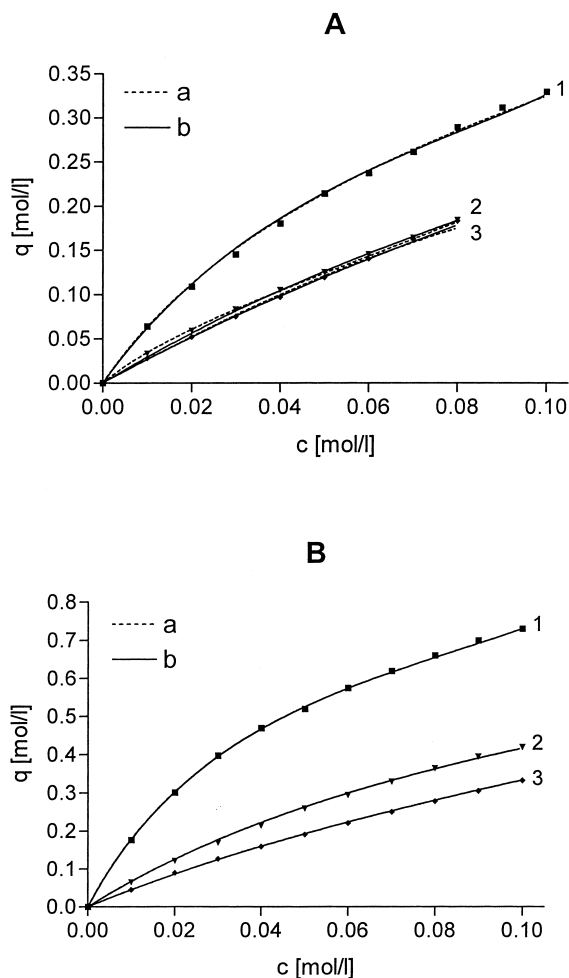
Fig. 8. Three-component quadratic isotherms of benzophenone (A) and of coumarin (B) on a Separon SGX silica gel column (Eq. (10) – solid lines and Eq. (11) – dotted lines) fitted to the experimental data (points) measured for mixtures of sample compounds in concentration ratios 1:1. A single isotherm equation taking into account the competitive effect of 2-propanol is used for all mobile phases: 0% (1), 0.5% (2) and 1% (3) 2-propanol in dichloromethane. Parameters of the isotherms are in Table 4. *c*, *q* = concentrations of sample compounds in the mobile and in the stationary phases, respectively, in mol/l.

numerical calculations using a three-component iso-therm with 2-propanol as one of the components in the system are compared with the experimental chromatograms in Fig. 9. Approximate agreement between the three-component calculation results and the experiment was found, except for the high-con-centration range where the detector calibration curve

is non-linear because of too high absorbance and where the concentration measurement is subject to large errors. On the other hand, some disagreement between the top parts of the bands and the degree of tailing (especially in Fig. 9B) can possibly indicate that the lumping of all dispersion terms into a single
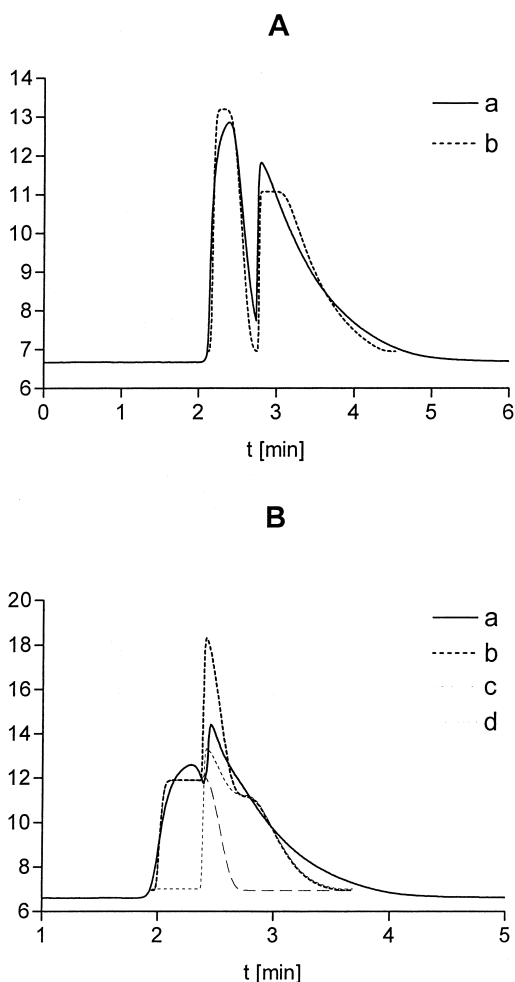
A

B

Fig. 9. Experimental (a) – solid line, calculated from the calibrated detector response and numerically calculated using a three-component quadratic isotherm (Eq. (11), parameters in Table 2; (b) – dotted line) band profiles of benzophenone and of coumarin in overloaded chromatography on a Separon SGX silica gel column with 0.5% (A) and 1% (B) 2-propanol in dichloromethane as the mobile phase. Sample volume: 0.5 ml, concentration of each compound 0.1 mol/l. Flow-rate: 1 ml/min, temperature: 40°C.

one, adopted in the Rouchon algorithm used for the calculation of band profiles, may not be fully justified for the chromatographic system studied.

5. Conclusions

The adsorption isotherm of 2-propanol on silica

gel is strongly affected by the polarity of the second solvent in a binary solvent mixture and the plateau of the isotherm corresponding to the saturation capacity of the adsorbent for 2-propanol is reached at significantly lower concentrations in non-polar *n*-hexane than in medium-polarity dichloromethane. The adsorption of 2-propanol from dichloromethane and of dichloromethane from *n*-heptane is more adequately described by a three-parameter quadratic isotherm than by the Langmuir equation, probably because of the effect of multi-layer adsorption.

The adsorption of benzophenone or of coumarin on silica gel from a binary solvent mixture containing 2-propanol can be formally described by the Langmuir isotherm regardless of the polarity of the weaker solvent. However, the quadratic isotherm derived on the basis of the statistical thermodynamics model is more adequate for the description of the distribution of single-component samples than the Langmuir isotherm, especially in binary mobile phases with a less polar strong solvent such as in mixtures of dichloromethane and *n*-heptane. This is possibly caused by the interactions between the molecules adsorbed on adjacent adsorption sites. The distribution of two-component sample mixtures cannot be adequately characterised by the competitive Langmuir isotherm, but the competitive quadratic isotherm is suitable for this purpose.

The competitive quadratic isotherm equation is also well suited to describe the complete data set of sample distribution between silica gel and binary mobile phases with different concentration ratios of mobile phase components, taking into account the competition of the sample compounds with the more polar solvent in the mobile phase (2-propanol) for the adsorption sites. The two-component quadratic isotherm should be used to describe the distribution of a single sample compound and the three-component quadratic isotherm is necessary for adequate description of the distribution of the components of a two-component sample mixture. In these equations, the term with the second power of the concentration of 2-propanol can be neglected. Most coefficients of the simplified three-component isotherm are identical with the coefficients of the two-component isotherm and can be determined from the distribution concentrations of single-component samples, with only two or three additional coefficients that should be

determined from the two-component sample distribution data. The isotherm equations can be used in numerical calculations of band profiles in the overloaded column separations and could be potentially useful in the optimisation of preparative isocratic and gradient-elution separations. This topic is the subject of our present research. However, additional experimental data would be necessary to verify the more general validity of the current results in normal-phase liquid chromatography systems.

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