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Mobile phase effects on single-component and competitive adsorption isotherms in reversed-phase systems

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Abstract

The distribution of phenol and resorcinol as the model compounds between octadecyl silica adsorbents and water or aqueous methanol as the mobile phases can be described by the Langmuir adsorption isotherms. The dependence of the coefficients of the isotherms on the composition of the mobile phase can be described by three-parameter equations over the range 0–40% methanol in water. Various approaches for processing the data acquired were investigated to obtain an adequate description of the two-component experimental distribution data. Best fit was obtained using Langmuir isotherms with the coefficients determined at a constant concentration ratio of the two sample compounds. For the data acquired at different concentration ratios, the competitive Langmuir isotherm with the coefficients b determined by multi-linear regression and with forced single-component Langmuir coefficient a fits the experimental distribution data of the sample solutes better than the competitive isotherm with single-component coefficients. The agreement between the experimental distribution of sample compounds and the data calculated from the quadratic isotherm for more strongly retained compounds was better than with the data calculated from the Langmuir competitive isotherm, while the opposite behaviour was observed for the earlier eluted solutes. The Le Van–Vermeulen isotherm with single-component Langmuir coefficients did not fit the data.

Keywords: Adsorption isotherms; Mobile phase composition; Phenol; Resorcinol

1. Introduction

The isotherm is the fundamental thermodynamic property of importance in theoretical and experimental studies of the separation processes based on the distribution of solutes between two phases. It describes the distribution between the mobile and the stationary phases in a functional form usually derived on the basis of a theoretical model. The parameters of the distribution equation can be determined by fitting the model to the experimental data. If the model is adequate and the form and the coefficients of the isotherm equation are known, it is possible to predict the individual band profiles of separated sample components under various working conditions and to optimize the separation. In analytical chromatography, the concentrations of the

sample components are so low that linear isotherm may be used, which means that the capacity factors of the analytes are independent of their concentrations in the sample and the retention data can be calculated in a straightforward manner. However, in preparative chromatography high production rates usually dictate that high concentrations of the components in the sample should be used and the separation is usually run under overloaded column conditions controlled by a non-linear isotherm, so that theoretical background of linear chromatography cannot be used here and the separation can be modelled only by using simulation calculations of the whole band profiles of the sample components.

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

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for competitive adsorption equilibria have been clearly demonstrated [1]. The objective of the present work is to investigate possibilities of fitting various isotherms to the experimental single- and two-component data in reversed-phase liquid chromatography systems. For this purpose, we selected simple model compounds, namely phenol and resorcinol.

The composition of the mobile phase is the most often used tool for controlling the separation in liquid chromatography. This was the reason why we have investigated the effect of the concentration of the organic modifier (methanol) in aqueous–organic mobile phases on the distribution of the sample compounds between the stationary and the mobile phases and on the coefficients of single- and two-component isotherm equations. These dependences should be known to allow numerical simulations of the peak profiles under overloaded gradient elution conditions and optimization of the effects of the mobile phase composition on the yield, purity and production rate under isocratic conditions, which is the subject of our present investigation.

2. Theoretical

Several models have been suggested to fit non-linear isotherms to single-component data describing the distribution of one sample component between the stationary and the mobile phases. The most common and the most simple is the two-parameter Langmuir isotherm [2]:

$$q = \frac{a \cdot c}{1 + b \cdot c} \quad (1)$$

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 capacity factor of the sample compound at infinite dilution, i.e., in analytical chromatography and $b = a/q_s$, where q_s is the column saturation capacity). If the Langmuir model does not fit the experimental data well, more complex isotherms can be used for this purpose, such as those based on bi-Langmuir [3], quadratic [4–6], Fowler [7] or Jovanovic [8] models.

Numerous and often complicated models have

been suggested to describe the competitive equilibria involved between the components of a mixed sample and the adsorbent, yielding various competitive isotherm equations [9]. The competitive Langmuir isotherm is often used because of its simplicity, however it is thermodynamically justified only if the column has the same saturation capacities for all sample components (which is rarely found in the real world), otherwise the Gibbs–Duhem relationship is violated [1,3]. For two-component samples with compounds i , j , the isotherm acquires the form [10]:

$$q_i = \frac{a_i \cdot c_i}{1 + b_i \cdot c_i + b_j \cdot c_j} \quad (2)$$

Here, the subscripts i , j relate to the concentrations and the Langmuir single-component coefficients of the individual compounds i , j . A numerical approximation of the two-component competitive Langmuir isotherm was suggested to eliminate time-consuming simulation calculations of band profiles using the Craig model [11,12].

The single-component coefficients when used in the competitive Langmuir equation often do not fit well to the multicomponent experimental data and do not give accurate prediction of overloaded band profiles [1,6,13]. Instead of the competitive Langmuir isotherm, more complex models have been suggested to account for the differences in column saturation capacities of the sample components, such as for example the competitive Fowler isotherm [6,7], the competitive quadratic isotherm which can be simplified by neglecting the terms with c_i^2 to: [6]

$$q_i = \frac{a_i \cdot c_i + a_{ij} \cdot c_i \cdot c_j}{1 + b_i \cdot c_i + b_j \cdot c_j + b_{ij} \cdot c_i \cdot c_j} \quad (3)$$

or a thermodynamically more consistent Le Van–Vermeulen competitive isotherm [14] derived from the ideal adsorption solution (IS) theory [15] the second-order approximation of which may be written as [6]:

$$q_i = \frac{a_i \cdot c_i + a_j \cdot c_j}{b_i \cdot c_i + b_j \cdot c_j} \cdot \frac{b_i \cdot c_i}{1 + b_i \cdot c_i + b_j \cdot c_j} + \left(\frac{a_i}{b_i} - \frac{a_j}{b_j} \right) \cdot \frac{b_i \cdot b_j \cdot c_i \cdot c_j}{(b_i \cdot c_i + b_j \cdot c_j)^2} \cdot \ln(1 + b_i \cdot c_i + b_j \cdot c_j) \quad (4)$$

In quadratic (Eq. (3)) and in Le Van–Vermeulen (Eq. 4) competitive isotherms, the coefficients a_i , b_j , a_j , b_i are the single-component Langmuir coefficients of the sample components i , j , whereas the coefficients a_{ij} , b_{ij} in the quadratic isotherm account for the competitive behaviour of the sample compounds. Unfortunately, if the single-component Langmuir parameters are used in the isotherm Eq. (3) or Eq. (4), poor fit to the experimental competitive distribution data is often obtained [6,16–19]. To overcome this problem, empirical correction coefficients have been introduced into the isotherm equations [19] or the coefficients evaluated from the competitive data have been used [5,6,20–22]. However, these parameters are often significantly different from the single-component isotherm parameters and depend on the concentration ratio of the sample compounds or acquire negative instead positive values, which means that the underlying theoretical model is inconsistent. Such isotherm equations can be considered only as empirical ones and do not allow fundamental interpretation of the distribution data. From the practical point of view, physico-chemical interpretation of the isotherm is irrelevant with respect to the simulation of the band profiles under overloaded column conditions and any mathematical function can be used as long as it correlates the experimental results [1].

The variation of the retention volume (or of the capacity factor, k) of a solute as a function of the concentration of the organic solvent (modifier), φ , in aqueous–organic mobile phases in analytical reversed-phase chromatography is most often described by a simple two-parameter equation [23–25]:

$$\log k = \log k_w - m \cdot \varphi \quad (5)$$

where k_w is the capacity factor extrapolated to pure water as the mobile phase. Numerous experimental observations revealed significant deviations from the linearity of the plots of $\log k$ versus φ , especially in the mobile phases with low concentrations of the organic modifier, the curvature being usually more significant with less polar organic solvents. To account for this behaviour, a second-degree polynomial equation can be used which was derived on the basis of the solubility parameter [23,26] and interaction indices [27] concepts:

$$\log k = \log k_w - m \cdot \varphi + d \cdot \varphi^2 \quad (6)$$

Only a few experimental studies have been reported so far regarding the dependence of the isotherm parameters on the concentration of the organic modifier in the mobile phase in reversed-phase systems and these studies have been mostly limited to the single-component Langmuir isotherm. The coefficient a of the Langmuir isotherm is directly proportional to the capacity factor under analytical conditions, so that the dependences of a on φ can be expected to have the form of Eq. 5 or Eq. 6, with different meanings of the intercept. If the saturation capacity q_s of the column for a sample solute is independent of the concentration of the organic modifier in the mobile phase, the same dependence of the coefficient b as of the coefficient a should apply, except for the value of the intercept [28]. This has been found experimentally for 2-phenylethanol in acetonitrile–water mobile phases, where the dependences of $\log a$ and $\log b$ on φ were slightly curved [29].

Similar results were found also for cholesterol in non-aqueous reversed-phase chromatography with mobile phases comprised of dichloromethane and acetonitrile, where a semi-empirical equation in the form of a combination of Langmuir and linear isotherms described the experimental distribution behaviour adequately [30,31].

3. Experimental

3.1. Instrumentation

Two instrumental set-ups were used to acquire the data necessary for the determination of the equilibrium isotherms: (1) Two M 6000A pumps with an M 660 gradient controller and an M 440 UV (280 nm) or an R 401 refractometric detector (all from Waters-Millipore, Milford, MA, USA) and a TZ line recorder (Laboratory Instruments Works, Prague, Czech Republic), with the column placed in a glass jacket connected to an external water-bath thermostat. (2) An HP 1090M liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a 3 DR solvent delivery system and solvent reservoirs continuously stripped with helium to degas the mobile

phase and the sample solution, an automatic sample injector, a column-switching valve, a temperature-controlled column compartment, a diode-array UV detector and a data workstation. The outlet from the column used to measure the isotherm was connected to an external LCD 2563 UV detector (Laboratory Instruments Works), set to 289 nm and connected via a six-port column-switching valve to a high-speed analytical column the outlet of which was connected to the diode-array detector (set to 254 nm), to make possible automated collection and analysis of the fractions in the eluate from the main column. The signal of the external detector was connected via an analog-digital convertor (760 Series Interface, Hewlett-Packard) so that the signals from both the diode-array and the external UV detectors were simultaneously processed by the data station of the chromatograph.

The columns for measuring the isotherm data were glass-cartridge, 150×3.3 mm I.D., packed with Separon SGX C₁₈ (monomer bonded phase) and Separon SGX RPS (polymer bonded phase), both 7- μ m particle size, obtained from Tessek (Prague, Czech Republic). The column dead volumes were evaluated from the record of the detector set to 200 nm after injection of pure methanol. The characteristics of the columns are given in Table 1. A Hypersil ODS, 3 μ m, 60×4.6 mm I.D., high-speed column was used for the analysis of the fractions of the eluate.

3.2. Chemicals

Phenol and resorcinol, both analytical grade, were obtained from Lachema (Brno, Czech Republic). Before use, phenol was purified by distillation and resorcinol by crystallization from water.

Methanol of spectroscopic grade was obtained from Lachema. Water was doubly distilled in glass with the addition of potassium permanganate. The solvents were filtered using a Millipore 0.45- μ m filter and the mobile phases were prepared by mixing the components in the required ratios and degassed by ultrasonication before use. The sample solutions used for the determination of the distribution data were prepared by weighing the required amounts of the sample solutes and dissolving in the mobile phase.

3.3. Determination of distribution isotherms by frontal analysis

The equilibrium isotherms were measured using the frontal analysis method as described previously [30]. The mobile phase was stored in one of the solvent flasks of the solvent delivery system, the solution of sample solutes in a solvent of the same composition as the mobile phase in another flask. The gradient-delivery system was used to pump and mix the solutions needed for the frontal analysis experiments.

The ratio of the flow-rates of the two solutions controls the concentration of the solute delivered continuously to the column. It 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.

In each experiment the solute concentration in the stationary phase was determined from the integral mass balance equation, using the experimental retention volume (inflection point of the break-through curve), corrected for the volume of the tubing

Table 1
Properties of the columns used

	A	B
Column packing	Separon SGX C ₁₈	Separon SGX RPS
Bonded phase	Monomeric	Polymeric
Particle size	7 μ m	7 μ m
Volume of the mobile phase, V_m	0.91 ml	0.75 ml
Volume of the stationary phase, V_s	0.37 ml	0.53 ml
Phase ratio, $\Phi = V_s/V_m$	0.41	0.71

Column dimensions: 150×3.3 mm I.D.

between the mixing point of the liquids pumped in each channel and the column top [32] (0.35 ml for Hewlett-Packard and 0.02 ml for Waters Instruments). Solutions containing 0.1 mol/l of the individual compounds in the mobile phase were used for measuring the single-component isotherms and the competitive isotherms were determined with sample mixtures containing phenol and resorcinol in concentration ratios 0.1:0.1, 0.1:0.02 and 0.02:0.1 mol/l, where the concentrations of the individual sample compounds corresponding to the two plateaus on the detector response record of the frontal analysis curve were measured by the analysis of the fractions of the eluate. The data obtained were used in the appropriate mass-balance equation [20]. All the experiments were repeated at least twice.

The isotherm distribution was determined by solving the mass-balance equations for single- or for two-component data [20,32] using a spread-sheet program run on a 486 IBM compatible personal computer. The spread-sheet program was used also to fit the isotherm data. Non-linear and multilinear regression analysis of the data was performed using the ADSTAT software (Trilobyte, Prague).

4. Results and discussion

4.1. Single-component distribution data

The Langmuir isotherm fits well the single-component experimental dependences of the adsorbed concentrations q on the concentrations c_m of phenol and resorcinol in the mobile phase. Using the quadratic isotherm did not bring any significant

improvement of the fit. Table 2 surveys the coefficients a and b of the Langmuir isotherm and the column saturation capacities, q_s , calculated as the ratio of the coefficients a and b of phenol and resorcinol on the two columns used. As expected, the retention and the coefficients a of the Langmuir isotherm are higher on the polymeric Separon SGX RPS column than on the monomeric Separon SGX C₁₈ column. This applies also for the coefficient b , however, to a different extent for the two sample compounds, so that the saturation capacity is higher on the RPS than on the C₁₈ column for phenol, but it is lower for the less retained resorcinol.

Plots of the logarithms of the coefficients of the single-component Langmuir isotherm versus the concentration of methanol in the mobile phase are shown in Figs. 1A, 1B, 2A and 2B. The plots are curved both for phenol and for resorcinol and for the two types of column tested (polymeric and monomeric) so that first degree semilogarithmic Eq. 7 and Eq. 8 can be used to fit the experimental data only over a limited range of methanol concentrations in the mobile phase.

$$\log a = \log a_0 - m_a \cdot \varphi \quad (7)$$

$$\log b = \log b_0 - m_b \cdot \varphi \quad (8)$$

The data are adequately described by a second degree semilogarithmic Eq. 9 and Eq. 10 in mobile phases containing 0–40% methanol, as it is demonstrated by the plots in Fig. 1 and Fig. 2 and by the values of the correlation coefficients in Table 3.

$$\log a = \log a_0 - m_a \cdot \varphi + d_a \cdot \varphi^2 \quad (9)$$

Table 2

Coefficients a , b (l/mol) and saturation capacities, q_s (mol/l) of the single-component Langmuir isotherms of resorcinol and phenol in mobile phases with various concentrations of methanol on Separon C₁₈ and Separon RPS columns

Methanol (%)	Phenol						Resorcinol					
	C ₁₈			RPS			C ₁₈			RPS		
	a	b	q_s	a	b	q_s	a	b	q_s	a	b	q_s
0	20.32	17.23	1.18	33.97	23.79	1.43	6.32	13.00	0.49	15.32	44.81	0.34
10	7.05	6.72	1.05	17.34	13.04	1.33	2.60	7.74	0.34	5.60	16.94	0.33
20	4.35	4.32	1.01	10.91	8.68	1.26	1.55	5.19	0.49	3.17	11.64	0.27
30	2.85	3.20	0.89	7.12	6.26	1.14	0.92	3.21	0.29	1.78	7.20	0.25
40	1.78	2.05	0.87	5.26	7.27	0.72	0.60	2.33	0.26	1.02	6.35	0.16

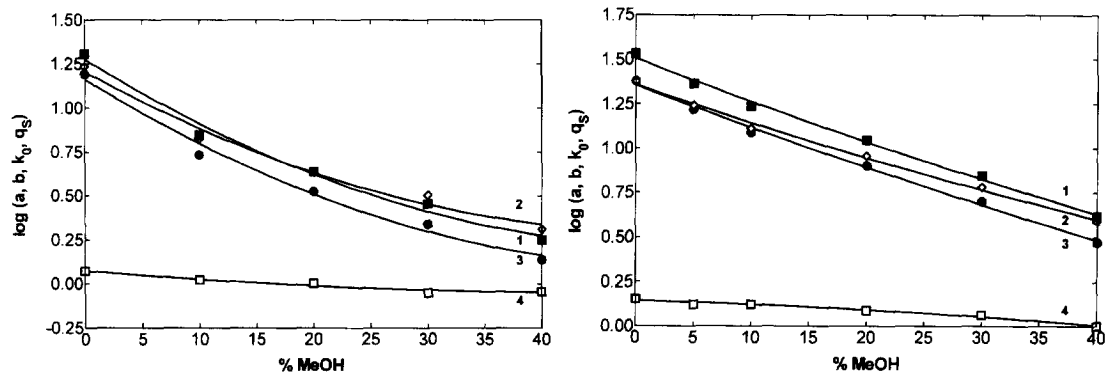


Fig. 1. Coefficients a (1), b (2) of the single-component Langmuir isotherms, capacity factors at infinite dilution, k_0 (3) and column saturation capacities q_s (4) of phenol in mobile phases with different concentrations of methanol in water, (A, left) on a Separon SGX C₁₈ column and (B, right) on a Separon SGX RPS column.

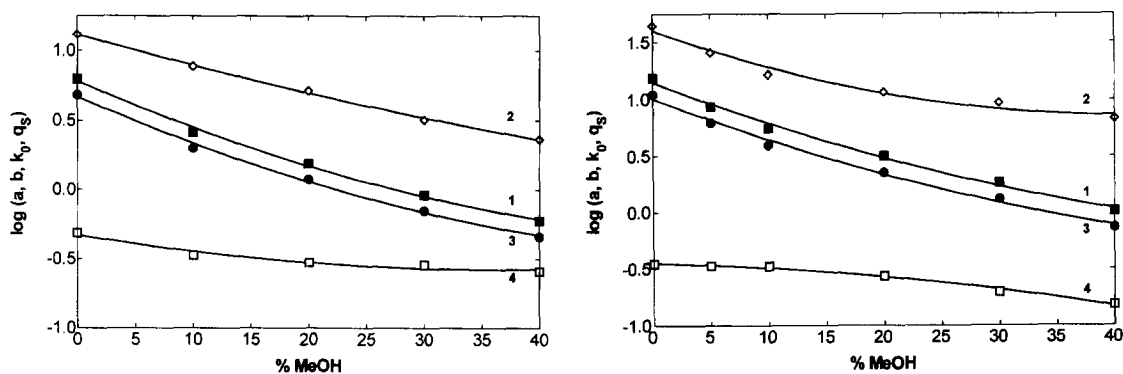


Fig. 2. Coefficients a (1), b (2) of the single-component Langmuir isotherms, capacity factors at infinite dilution, k_0 (3) and column saturation capacities q_s (4) of resorcinol in mobile phases with different concentrations of methanol in water, (A, left) on a Separon SGX C₁₈ column and (B, right) on a Separon SGX RPS column.

Table 3

Parameters of the second degree polynomial equations describing the dependences of the coefficients of single-component Langmuir isotherms on the concentration of methanol, φ (% vol·10⁻²), in the mobile phase

Compound	Column	Equation	R
Phenol	Separon SGX C ₁₈	$\log a = 1.278 - 4.041\varphi + 3.833\varphi^2$	0.985
		$\log b = 1.208 - 3.579\varphi + 3.519\varphi^2$	0.992
	Separon SGX RPS	$\log a = 1.507 - 2.510\varphi + 0.805\varphi^2$	0.998
		$\log b = 1.360 - 2.238\varphi + 0.860\varphi^2$	0.998
Resorcinol	Separon SGX C ₁₈	$\log a = 0.798 - 4.250\varphi + 5.464\varphi^2$	0.999
		$\log b = 1.113 - 2.267\varphi + 0.978\varphi^2$	0.999
	Separon SGX RPS	$\log a = 1.153 - 3.909\varphi + 2.773\varphi^2$	0.997
		$\log b = 1.603 - 3.637\varphi + 4.451\varphi^2$	0.988

R = correlation coefficient.

$$\log b = \log b_0 - m_b \cdot \varphi + d_b \cdot \varphi^2 \quad (10)$$

This table lists the parameters of the Eq. 9 and Eq. 10 describing the plots of $\log a$ and $\log b$ versus the concentration of methanol in the mobile phase, φ (in % vol·10⁻² units).

Figs. 1 and 2 and Table 2 show that the column saturation capacities for phenol and resorcinol decrease as the concentration of methanol in the mobile phase is increased, in contrast to the assumptions employed by previous workers [28,29].

4.2. Two-component distribution data

Various types of isotherms were fitted to the distribution data measured for sample mixtures containing phenol and resorcinol on the two reversed-phase columns in mobile phases with various concentrations of methanol in water. The most simple is the competitive Langmuir isotherm (Eq. 2). When we used this isotherm with the coefficients a , b of the individual components determined from the single-component data, the calculated isotherms did not fit the experiment, probably because of three to four times higher saturation capacities of the two columns for phenol than for resorcinol (Table 2).

We tried to fit the Langmuir isotherm to the experimental data acquired at different constant concentration ratios (r) of phenol and resorcinol in the mobile phase used in the frontal analysis, $r = c_i:c_j$. If r is constant, the denominator of the right-hand side of Eq. (2) depends only on c_i :

$$\begin{aligned} 1 + c_i \cdot b_i + c_j \cdot b_j &= 1 + c_i \cdot \left(b_i + \frac{b_j}{r} \right) \\ &= 1 + c_i \cdot b \end{aligned} \quad (11)$$

This single-component Langmuir isotherm fits reasonably well the two-component experimental data at lower concentrations, however, significant deviations are observed at the two highest concentrations of the sample compounds. The practical value of such an isotherm is of course limited.

To find a common isotherm with the coefficients fitting better the two-component distribution data acquired at various concentration ratios of phenol and of resorcinol, we introduced the dependence of the coefficient b of the Langmuir isotherm determined from the two-component distribution data on

the concentration ratio r into the isotherm equation, but this approach was not successful. The differences between the coefficients b of the Langmuir isotherm determined at different concentration ratios of phenol and resorcinol are far more significant than between the coefficients a , which indicates that a single value of a could possibly be used to suit the data at different concentration ratios r . If the coefficient a is known, the competitive Langmuir isotherm (Eq. 2) can be rewritten as follows:

$$q_i - a_i \cdot c_i = -b_i \cdot c_i \cdot q_i - b_j \cdot c_j \cdot q_i + \Delta \quad (12)$$

The left-hand side of Eq. 12 can be calculated using the known value of a single-component Langmuir isotherm parameter a for all the experimental data acquired at various concentration ratios of the two sample components in a mobile phase of given composition and can be subject to multilinear regression in dependence on the products $c_i \cdot q_i$, $c_j \cdot q_i$, to find the coefficients b_i and b_j . The intercept Δ of the regression Eq. 12 should theoretically be zero and is a measure of the error of calculation. The values of the coefficients b_i and b_j and of Δ determined by this regression with the forced parameter a are given in Table 4. Statistical tests show that the value of the intercept Δ is close to zero within the limits of the error of determination in all the systems tested.

Fig. 3 compares the plots of the logarithms of the coefficients b_i and b_j determined in this way versus the concentration of methanol in the mobile phase (full points, dashed lines) with analogous dependences for the single-component Langmuir coefficients b of phenol and resorcinol (squares, full lines). Except for one outlying point, the coefficients are close to each other at the concentrations of methanol up to 20%, in more concentrated aqueous methanol the deviations from the single-component data increase as the concentration of methanol in the mobile phase is increased. Low values of the intercept Δ and fair agreement between the coefficients b of single- and multi-component isotherms indicate that this approach to the data analysis may be reasonable, but they do not prove suitability of the isotherms with these coefficients, which can be verified only by comparing the fitted and the calculated isotherm data, which will be discussed later.

We tested some more complex isotherms described by Eq. 3 and Eq. 4 which make use of

Table 4

Coefficients b_p (phenol), b_R (resorcinol) of the competitive Langmuir isotherm determined from all experimental data at different concentration ratios of phenol to resorcinol by regression with forced single-component Langmuir coefficient a of the individual sample compounds

Column	Methanol(%)	b_p	b_R	Δ
C ₁₈	0	10.12	13.55	0.008
	10	6.13	6.72	0.004
	20	4.42	4.32	0.001
	30	4.38	3.20	0.001
	40	4.86	2.05	0.001
RPS	20	8.62	11.90	-0.04

Δ (mol/l) is the error of calculation (Eq. 12).

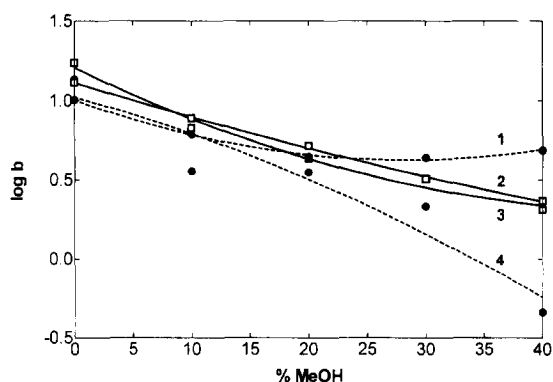


Fig. 3. Coefficients b of the Langmuir isotherms of phenol (1, 3) and resorcinol (2, 4) on a Separon SGX C₁₈ column in mobile phases with different concentrations of methanol in water. Solid lines (2, 3) and squared points correspond to the single-component isotherm coefficients and dashed lines (1, 4) and full circle points to the coefficients determined by multilinear regression analysis of all the experimental two-component data at various concentration ratios of phenol and resorcinol with forced single-component Langmuir coefficient a , using Eq. 12.

single-component Langmuir coefficients for suitability to describe the whole set of the experimental data measured at different concentration ratios of phenol and resorcinol. The coefficients a_{ij} , a_{ji} , b_{ij} , b_{ji} of the quadratic isotherm (Eq. 3) were determined by non-linear regression of all the experimental data at a given composition of the mobile phase, with coefficients a_i , a_j , b_i , b_j determined using the single-component data of the individual sample components and are given in Table 5. (The subscript i relates to phenol and j to resorcinol.) The plots of the coefficients of the quadratic isotherm versus the concentration of methanol in the mobile phase are shown in Fig. 4. The coefficients a_{ij} determined from the data for phenol (line 4) and for resorcinol (line 3) should be theoretically equal each to the other. They are close to zero and independent of the composition of the mobile phase. Also the coefficient b_{ij} determined from the data for phenol (line 2) is almost independent of the concentration of methanol

Table 5

Coefficients a_{ij} , b_{ij} of the competitive quadratic isotherms (Eq. 3) of phenol and resorcinol on Separon SGX C₁₈ and Separon SGX RPS column from all experimental data at different concentration ratios of phenol to resorcinol by regression with forced parameters a_i , b_i , a_j , b_j of single-component Langmuir isotherms

Column	Methanol (%)	Compound			
		Phenol		Resorcinol	
		a_{ij}	b_{ij}	a_{ij}	b_{ij}
C ₁₈	0	0.004	1.78	2.022	24.64
	10	-0.005	1.09	0.010	1.22
	20	0.000	1.17	0.397	9.05
	30	0.002	1.24	0.221	7.01
	40	0.003	1.30	0.000	0.63
RPS	20	0.023	1.27	0.002	1.08

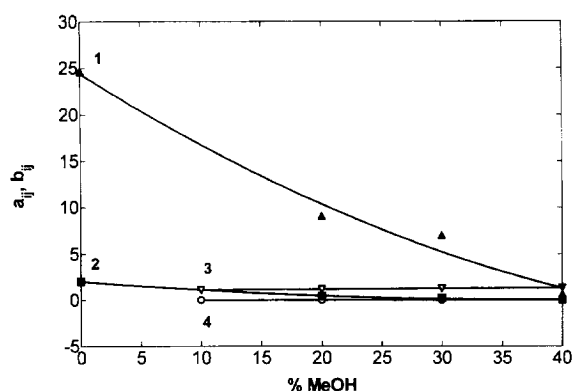


Fig. 4. Coefficients a_{ij} (2, 4) and b_{ij} (1, 3) of quadratic isotherms (Eq. 3) of phenol (3, 4) and of resorcinol (1, 2) on a Separon SGX C_{18} column in mobile phases with different concentrations of methanol in water. The coefficients were determined by non-linear regression of all the experimental data at various concentration ratios of phenol and resorcinol with forced single-component Langmuir coefficients a_i , a_j , b_i , b_j .

in the mobile phase, but contrary to the theory, it differs very significantly from the coefficient b_{ij} determined from the data for resorcinol, which strongly decreases as the concentration of methanol in the mobile phase is increased (line 1). This means that the coefficient a_{ij} does not affect significantly the calculated isotherm data and consequently the regression of the two-component data yields in

practice only one independent coefficient, b_{ij} , for each compound of the two-component sample. Different competitive isotherm coefficients b_{ij} should be used for phenol and for resorcinol.

Finally, the fit of the Le Van–Vermeulen isotherm (Eq. 4) to the experimental two-component distribution data determined at various concentration ratios of phenol and resorcinol was tested. This isotherm contains only the single-component Langmuir coefficients determined independently of the two-component experimental data, so that the fit of this isotherm to the experimental data would prove the consistency of the model.

Typical examples of the results of the fit of competitive isotherms to the experimental data points are shown in Figs. 5–7 for different concentration ratios of phenol and resorcinol (0.1:0.1 mol/l, 0.02:0.1 mol/l and 0.1:0.02 mol/l) on the Separon SGX C_{18} column in 20% methanol as the mobile phase. The Langmuir isotherms determined for the individual data sets measured at various constant ratios of the concentrations of phenol and resorcinol (Eq. 11, full lines) show the best fit of all the isotherms tested, with some deviations at the highest concentrations. The competitive Langmuir isotherms with the coefficients b_i and b_j found by multilinear regression (Eq. 12, dotted lines) fit the experimental

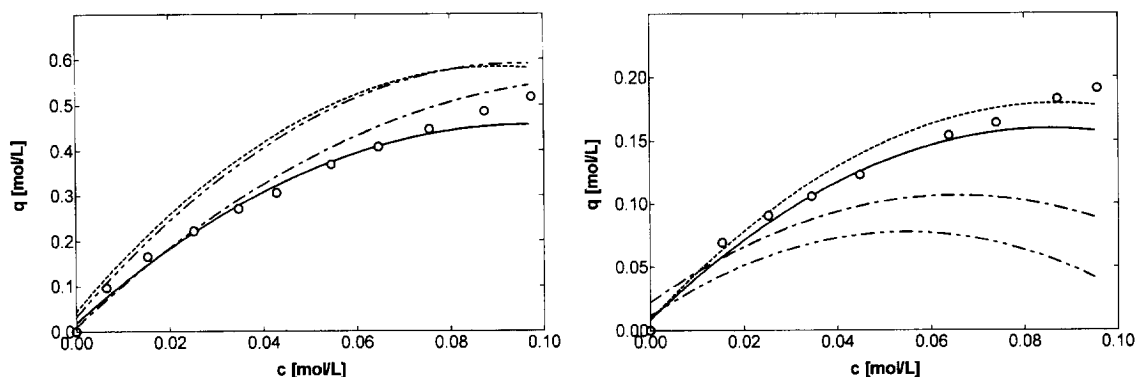


Fig. 5. Distribution isotherms fitted to the two-component experimental data (points) of phenol (A, left) and resorcinol (B, right) on a Separon SGX C_{18} column in 20% methanol as the mobile phase at the concentration ratio of phenol and resorcinol 0.1:0.1 mol/l. Full lines: Langmuir isotherms (Eq. 1) determined at a constant concentration ratio of phenol and resorcinol. Dotted lines: Competitive Langmuir isotherms (Eq. 2) with forced single-component coefficients a_i , a_j and coefficients b_i , b_j determined by multilinear regression of all the experimental data at various concentration ratios of phenol and resorcinol. Dash-dotted lines: Competitive quadratic isotherms (Eq. 3) with forced single-component Langmuir coefficients a_i , a_j , b_i , b_j and coefficients a_{ij} , b_{ij} determined by non-linear regression of all the experimental data at various concentration ratios of phenol and resorcinol. Dash-double dotted lines: Competitive LeVan–Vermeulen isotherm (Eq. 4) with single-component Langmuir coefficients.

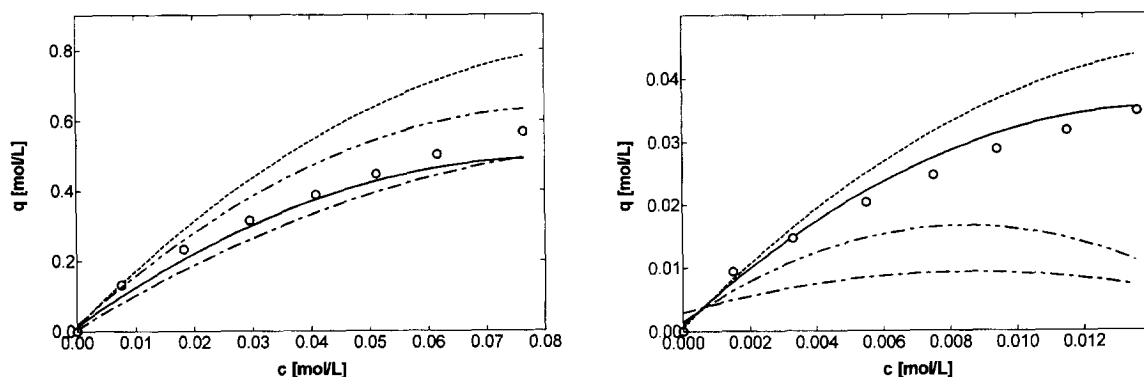


Fig. 6. Distribution isotherms fitted to the two-component experimental data (points) of phenol (A, left) and resorcinol (B, right) on a Separon SGX C_{18} column in 20% methanol as the mobile phase at the concentration ratio of phenol and resorcinol 0.1:0.02 mol/l. Lines as in Fig. 5.

data better than the competitive Langmuir isotherm with single-component coefficients and the fit to the data of resorcinol is better than that of the quadratic competitive isotherms with single-component coefficients a_i , b_i or the Le Van–Vermeulen competitive isotherms with all the coefficients of single-component Langmuir isotherms. As shown in Fig. 3, the differences between the coefficients b determined by multilinear regression of two-component data with forced coefficient a and the single-component coefficients b are lower in mobile phases with lower contents of methanol.

The quadratic isotherm fits the data of phenol better than the competitive Langmuir isotherm with the single-component coefficient a , but it completely

fails to describe the experimental data of resorcinol (dash-dotted lines in Figs. 5–7). The fit of the quadratic competitive isotherm to the whole set of the experimental data is poorer than that of the competitive Langmuir isotherm using the coefficients b_i , b_j determined by multilinear regression with forced single-component coefficients a_i , a_j . This may seem quaint at a first glance because generally the more coefficients the better an equation fits to the data set and the competitive quadratic isotherm Eq. 3 has five coefficients whereas the competitive Langmuir isotherm Eq. 2 only three. However, of the five coefficients used in the quadratic isotherm three were the forced single-component Langmuir coefficients and the coefficient a_{ij} was close to zero, so that in

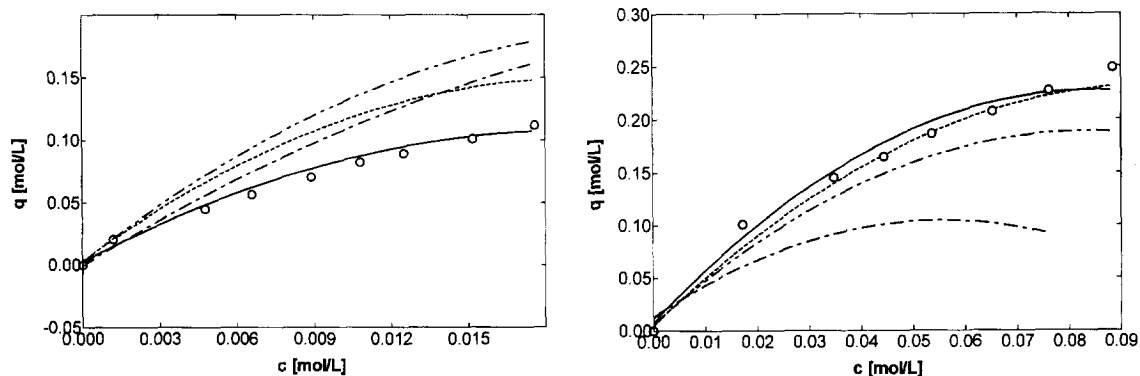


Fig. 7. Distribution isotherms fitted to the two-component experimental data (points) of phenol (A, left) and resorcinol (B, right) on a Separon SGX C_{18} column in 20% methanol as the mobile phase at the concentration ratio of phenol and resorcinol 0.02:0.1 mol/l. Lines as in Fig. 5.

practice only single coefficient b_{ij} characterized the two-component data set at various concentration ratios of resorcinol and phenol, whereas two coefficients (b_i, b_j) were determined by fitting the Langmuir competitive isotherm Eq. 2 with forced coefficient a to the two-component data set.

The second-order approximation of the Le Van–Vermeulen isotherm with the coefficients of the individual Langmuir isotherms (dash-double dotted curves in Figs. 5–7) did not fit the experimental data. Because this isotherm makes use only of single-component Langmuir isotherm coefficients, the failure of the fit to the competitive data proves that this model is inconsistent with the sorption behaviour studied here.

5. Conclusions

To describe adequately the experimental dependence of the coefficients of single-component and competitive isotherms of phenol and resorcinol on the concentration of the organic solvent in aqueous–organic mobile phases in reversed-phase chromatography, a second-degree semilogarithmic equation is required. We did not completely succeed in fitting various competitive isotherms to the experimental data set at different concentration ratios of the sample compounds, especially for the less retained component, resorcinol. The results show that none of the theoretical models used is consistent with the experimental data set of phenol and resorcinol in the reversed-phase systems tested. This can be possibly attributed to different interactions of mono- and dihydroxybenzenes with the surface of the column packing material. We are presently studying other sample mixtures where the saturation capacities of the sample components are significantly closer each to the other and preliminary results indicate significantly better fit of competitive isotherms to the experimental data set than in the present study.

The competitive Langmuir isotherm with the coefficient b calculated by multiparameter linear regression using the single-component Langmuir coefficient a showed best overall fit to the experimental data at various concentration ratios of the two compounds tested of all the competitive isotherms tested, but the deviations from the experiment

are too large for this isotherm to be useful in simulation of the band profiles. For this purpose, only the Langmuir isotherm fitted to the data at a constant concentration ratio of phenol and resorcinol shows sufficiently close fit to the experimental data, but the limitation to a single concentration ratio of the two sample components is a serious practical handicap.

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