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Effects of the composition of the mobile phase on the production rate in reversed-phase overloaded chromatography

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Abstract

Band profiles in overloaded reversed-phase chromatography on octadecyl silica columns were calculated using the experimental parameters of the distribution isotherms of binary mixtures of (1) resorcinol and phenol and (2) phenol and *o*-cresol determined by binary frontal analysis with automatic, on-line analysis of the composition of the breakthrough curve plateaus. The dependencies of the isotherm coefficients on the concentration of methanol in aqueous–organic mobile phases were described by three-parameter equations and used in the numerical calculations. Calculated band profiles of the sample components allowed determination of the fraction range, recovery yield and production rate necessary to obtain the sample components in required purity with various loading factors and concentration ratios of sample components in dependence on the concentration of methanol in the mobile phase. It was found that there is an optimum composition of the mobile phase, at which the optimum production rate can be achieved, which depends on the isotherms and on the concentration ratio of components in the sample feed. © 1997 Elsevier Science B.V.

Keywords: Mobile phase composition; Preparative chromatography; Overloaded chromatography; Production rate; Band profiles; Resorcinol; Phenol; Cresols

1. Introduction

In preparative chromatography sample mixtures are separated to obtain individual components at the required purity and (or) recovery yield. The requirements of the economy of the preparative process dictate that the separation should be performed on overloaded columns, to maximize the production rate and to minimize the solvent consumption and labor costs. Unlike in analytical chromatography, where the optimization of the chromatographic process usually means obtaining the necessary resolution of a sample mixture in minimum time, the optimization of the experimental conditions for economic production usually means maximizing the yield and the production rate or minimizing the solvent consumption to get minimum separation cost. The optimization of preparative separations can be empirical, it can be based on the solution of the ideal model of chromatography with necessary corrections for band dispersion, or it can use numerical solutions of a non-ideal model of chromatography to calculate the

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band profiles of the individual components of the feed at the outlet of the separation column [1].

Knox and Pyper [2] used a simple chromatographic model to optimize the experimental conditions, such as the inlet pressure, column plate number, diameter and length, in preparative chromatography. Later, this approach was elaborated and refined to describe overlapping bands and to cover a broad variety of experimental parameters in addition to the column plate number, the flow velocity and the inlet pressure, such as the adsorbent particle size, the separation factor and the loading factor (defined as the fraction of the column saturation capacity corresponding to the amount of feed injected) [3–7].

Numerical methods of optimization of the sample size and the column efficiency for maximum production rate of a compound of required purity based on the Craig model were suggested by Snyder and Dolan for both isocratic [8] and gradient [9] elution conditions. Ghodbane and Guiochon [10] used numerical methods to optimize simultaneously the sample size and the flow-rate of the mobile phase or the particle size and the column length. Later Katti and Guiochon [11] optimized simultaneously the sample volume and the sample size. Felinger and Guiochon [12] used a modified simplex method to optimize simultaneously the column length, the particle diameter, the flow-rate and the sample size to obtain the maximum production rate and minimum production cost [13]. Experimental results were found in agreement with the calculated optimum band profiles [14]. Newburger and Guiochon [15] studied the effects of the mobile phase composition and the flow-rate on the production rate in normal-phase chromatography.

The composition of the mobile phase is the most widely used tool for controlling the separation in analytical liquid chromatography. The objective of the present work is the optimization of the composition of the mobile phase in overloaded reversed-phase chromatography. The dependencies of the coefficients of the isotherms should be known to allow numerical calculations of the peak profiles and optimization of the effects of the mobile phase on the yield, purity and production rate under overloaded separation conditions.

We selected binary mixtures of resorcinol and phenol and of phenol and *o*-cresol as model samples. The individual bad profiles of sample components were calculated using numerical methods. From these profiles, the production rate, the recovery yield and the fraction purity were derived as a function of the concentration of methanol in aqueous–organic mobile phases.

2. Theoretical

The production rate, P_r, is the amount of feed purified at the required degree of purity per unit time. To allow general comparisons, P_r is normalized to the column cross-section area

$$P_r = \frac{V_{inj} c_0 R_i}{t_c S} = \frac{V_{inj} c_0 R_i}{t_c \pi r^2 L \epsilon_T} \quad (1)$$

where V_{inj} is the feed volume injected, c_0 the concentration of the compound of interest in the sample, R_i the recovery yield, t_c the cycle time or the end-cut time of the last fraction, r, L, S and ϵ_T the radius, length, cross-section area and total porosity of the column, respectively.

In numerical calculations of band profiles, any isotherm equation can be used. The one relating best the experimental values of the equilibrium concentrations measured in the stationary phase as a function of the mobile phase concentration is selected. The most common and the most simple non-linear isotherm is the two-parameter Langmuir one [16].

$$q = \frac{ac}{1 + bc} \quad (2)$$

Here, q is the concentration of the sample compound in the stationary and c is 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, $\phi = V_S/V_M$ is the phase ratio, i.e., the ratio of the volumes of the stationary, V_S, and of the mobile, V_M, phases in the column). If the Langmuir model does not fit well the experimental data, mare complex isotherms can be used for this

purpose. 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 [17]. 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]. Competitive Langmuir isotherm with single-component Langmuir coefficients [19] is often used, but it is thermodynamically justified only if the column has the same saturation capacities for all sample components [16,17]:

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

More complex models have been suggested to account for the differences in column saturation capacities of the sample components [20].

In the competitive isotherm Eq. (3) a_i, a_j, b_i, b_j are the single-component Langmuir coefficients of the sample components i, j. Unfortunately, if the single-component Langmuir coefficients are used in the isotherm Eq. (3), poor fit to the experimental competitive distribution data is often obtained [21–24]. To overcome this problem, the coefficients evaluated from the competitive data have been used [25–27], which often differ significantly from the single-component isotherm coefficients and their values depend on the concentration ratio of the sample compounds.

In analytical liquid chromatography, retention data can be calculated using a wide variety of equations relating the retention (capacity) factor k and the concentration of the organic solvent in the mobile phase, φ [28–30]. The most widely used is a simple two-term equation:

$$\log k = \log k_0 - m\varphi \quad (4)$$

where k_0 is k in pure water. In some cases, deviations from linearity are observed and a more complex equation should be used:

$$\log k - \log k_0 - m\varphi + d\varphi^2 \quad (5)$$

In Eqs. (4) and (5), m and d are experimental constants which depend on the solute and on the chromatographic system.

Relationships similar to Eq. (4) were assumed to apply also for the dependence of the coefficients of the Langmuir isotherm on the concentration of the organic solvent φ and were introduced into the algorithms for the calculation of overloaded band profiles. The column saturation capacity was assumed to be independent of φ. These assumptions may be justified in many chromatographic systems, but very often significant deviations from the validity of Eq. (4) are observed, especially at low φ, where k is high. Here, more sophisticated equations should be used.

Recently, we have investigated the effect of the concentration of the organic modifier (methanol) in aqueous–organic mobile phases on the distribution of phenol, *o*-cresol and resorcinol between the stationary and the mobile phases and on the coefficients of some single- and two-component isotherm equations [31,32]. The results have shown that the plots of the logarithms of the Langmuir coefficients a, b versus the concentration of methanol in the mobile phase deviate significantly from straight lines. Second-degree polynomial equations can account better for this behavior.

$$\log a = \log a_0 - m_a\varphi + d_a\varphi^2 \quad (6)$$

$$\log b = \log b_0 - m_b\varphi + d_b\varphi^2 \quad (7)$$

These equations were used in present work.

3. Experimental

3.1. Chemicals

Phenol, resorcinol and *o*-cresol, all analytical grade, were obtained from Lachema (Brno, Czech Republic). Before the use, phenol was purified by distillation and *o*-cresol and resorcinol by crystallization from water and methanol. Spectroscopic grade methanol was obtained from Lachema. Water was doubly distilled in a glass vessel, with addition of potassium permanganate. The solvents were filtered on a Millipore 0.45-μm filter. The mobile phases

were prepared by mixing their 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 solutes and dissolving them in the mobile phase.

Methanol of spectroscopic grade was obtained from Lachema. Water was doubly distilled in glass with addition of potassium permanganate. The solvents were filtered using a Millipore 0.45-μm filter, mixed in the required ratios and degassed by ultrasonication before the 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.

3.2. Instrumentation

The data needed for the determination of the equilibrium isotherms and the experimental overloaded band profiles were acquired with 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 solutions, an automatic sample injector, a column switching valve, a temperature-controlled column compartment, a diode-array UV detector and a data workstation.

For the determination of the competitive isotherms, the eluate from the column was directed to an external LCD 2563 UV detector (Laboratory Instruments works, Prague, Czech Republic) working at 289 nm and then, via a six-port column-switching value, to a high-speed analytical column the outlet of which was connected to the diode-array detector set at 254 nm. In this way, automated collection and analysis of the fractions in the eluate from the main column could be performed using a pre-set switching-valve program. The electrical output from the external detector was connected via an analog–digital convertor (760 Series Interface, Hewlett–Packard) to the data station of the chromatograph, so that the signals from both the diode-array and the external UV detector were simultaneously processed.

The columns used for measuring the isotherm data and for experiments with large samples were 150× 3.3 mm glass cartridges, packed with two batches of Separon SGX C_{18} material, 7-μm particle size, obtained from Tessek (Prague, Czech Republic). The column hold-up (dead) volumes were derived from the retention time of pure methanol (recorded at 200 nm) and were 0.97 (column 1) and 0.87 ml (column 2), corresponding to column total porosities $\epsilon_T = 0.71$ and 0.68. A Hypersil ODS, 3 μm, 60×4.6 mm high-speed column was used for the analysis of the eluate fractions in the determination of isotherms by binary frontal analysis.

3.3. Procedures

The equilibrium isotherms have been measured using binary frontal analysis [33]. 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 solute concentration in the stationary phase was determined from the integral mass balance equation using the experimental concentrations of the sample components at the plateaus of the frontal analysis curve and the retention (breakthrough) 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 (0.35 ml). The steps on the frontal analysis curve were steep and the inflection points were close enough to the half-heights of the steps so that possible errors in the breakthrough volumes were lower than 0.01 ml, which was within the limits of the accuracy of the instrumental determination of the elution volumes. The details of the method are described elsewhere [32].

The band profiles were measured using the HP 1090M liquid chromatograph in standard set-up, but equipped with a 250-μl injector sample valve. For larger injection volumes, the column switching valve of the instrument was equipped with a loop of appropriate volume, instead of the standard injector. The diode-array detector wavelength was set at 289 nm, the flow-rate (1 ml/min or 0.5 ml/min) and the column temperature (40°C) were kept constant in all the experiments, which were all repeated at least twice. Water and methanol in the glass flask reser-

Table 1
Coefficients of competitive Langmuir isotherms (Eq. (3)) of resorcinol (R) and phenol (P) on column 1 (A) and of phenol (P) and *o*-cresol (C) on column 2 (B) in mobile phase with various concentrations of methanol in water, φ (% v/v·10^{-2}) and coefficients of the dependencies of Langmuir parameters a and b on the concentration of methanol (Eqs. (6) and (7))

	CH_3OH (%)			
	10	20	30	40
(A) V_S = 0.37 ml and V_M = 0.91 ml in the column Separon SGX C_{18} 150×3.3 mm I.D. Phase ratio = V_S/V_M = 0.41				
Resorcinol				
a	2.55	1.56	0.95	0.58
b (l/mol)	7.68	5.11	3.39	2.26
q_s (M)	0.33	0.31	0.28	0.26
k	1.04	0.64	0.39	0.24
$\log a = 0.798 - 4.25\varphi + 5.464\varphi^2$				
$\log b = 1.113 - 2.267\varphi + 0.978\varphi^2$				
Phenol				
a	6.99	4.43	2.81	1.78
b (l/mol)	6.62	4.50	3.06	2.08
q_s (M)	1.06	0.98	0.92	0.86
k	3.35	1.72	1.06	0.77
$\log a = 1.278 - 4.041\varphi + 3.833\varphi^2$				
$\log b = 1.208 - 3.579\varphi + 3.519\varphi^2$				
α (P/R)	2.74	2.84	2.96	3.07
R_S	7.95	5.97	4.86	4.52
R_S/t_2	1.82	2.19	2.36	2.55
(B) V_S = 0.41 ml and V_M = 0.87 ml in the column Separon SGX C_{18} 150×3.3 mm I.D. Phase ratio = V_S/V_M = 0.47				
Phenol				
a	14.84	8.68	5.08	2.97
b (l/mol)	8.63	5.42	3.41	2.09
q_s (M)	1.72	1.60	1.49	1.42
k	6.97	4.08	2.39	1.40
$\log a = 1.404 - 3.326\varphi - 0.006\varphi^2$				
$\log b = 1.125 - 1.847\varphi - 0.415\varphi^2$				
o-Cresol				
a	42.17	22.44	11.95	6.36
b (l/mol)	27.38	14.96	8.13	4.39
q_s (M)	1.54	1.50	1.47	1.45
k	19.82	10.55	5.62	2.99
$\log a = 1.899 - 2.739\varphi + 0.002\varphi^2$				
$\log b = 1.697 - 2.585\varphi - 0.128\varphi^2$				
α (C/P)	2.84	2.59	2.35	2.14
R_S	9.26	8.40	7.32	6.00
R_S/t_2	0.44	0.72	1.10	1.50

$q_s = a/b$ is the column saturation capacity, k = retention factor, R_S = resolution, t_2 is the end time of the chromatographic peak of the second eluted compound, all under linear (analytical) conditions.
Column efficiency, N = 3600 theoretical plates, flow-rate 1 ml/min assumed.
V_S and V_M are the volumes of the stationary and of the mobile phases, respectively.

voirs were continuously de-gassed by stripping with helium.

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 [34]. Competitive Langmuir isotherms with quadratic dependences of the coefficients on the methanol concentration in the aqueous mobile phase were employed in the calculations (Table 1). The column efficiency of approximately 3000 theoretical plates was found experimentally and this valve was used in the numerical calculations.

4. Results and discussion

The distribution of binary mixtures containing 0.1 M resorcinol and phenol or phenol and *o*-cresol was measured between the octadecyl silica column and aqueous–organic mobile phases containing various concentrations of methanol (0–40%). The distribution data for phenols in the concentration range 0.01–0.1 M strongly deviate from linear behavior and can be adequately described by competitive Langmuir isotherms. More details about isotherm fitting in this particular case can be found elsewhere [31,32]. The experimental parameters a, b of the competitive Langmuir isotherms of the compounds studied were taken from [32]. The dependences of these parameters on the concentration of methanol in the mobile phase follow Eqs. (6) and (7). In order to get smoothed dependences of the characteristics of the overloaded separation on the concentration of methanol in the mobile phases, the parameters a and b calculated from the regression Eqs. (6) and (7), which slightly differ from the experimental parameters in [32], were used in numerical calculations and are given in Table 1, together with the column saturation capacities, $q_S = a/b$.

The parameters of the Langmuir isotherms for phenol in Table 1A and B differ significantly from each other, as two different columns were used to measure the distribution of resorcinol and phenol and of phenol and *o*-cresol. Both columns were octadecylsilica and were produced by the same manufacturer, but using different technologies, so that the column referred to in Table 1B has higher saturation

capacity and provided larger retention factors at linear chromatography (analytical) conditions and consequently larger values of the Langmuir parameter a than the column of Table 1A.

The saturation capacity was found to decrease by approximately 20% for resorcinol and phenol and by approximately 7% for o-cresol when the methanol concentration increases from 10 to 40% (Table 1). This is possibly caused by the competition of methanol with phenols for the surface of the octadecyl silica material. The effect of methanol on the saturation capacity of o-cresol may be less significant because o-cresol is more strongly retained than phenol and resorcinol, but further experiments would be necessary to verify this hypothesis.

Table 1 also shows the coefficients a_0, m_a, d_a, b_0, m_b, and d_b found by fitting Eqs. (6) and (7) to the experimental data sets using non-linear regression. Using these coefficients and equations, band profiles of resorcinol and phenol and of phenol and o-cresol were determined by numerical calculation for overloaded separation of binary sample feeds in mobile phases with various concentrations of methanol.

Fig. 1 compares the calculated and experimental band profiles obtained with 0.55 ml of a 0.1 M solution of phenol and o-cresol in two mobile phases, 30% methanol (A) and 40% methanol (B), i.e. at the loading factors of 18% (A) and 19% (B). The calculated concentration profiles were transformed to the response of the UV detector (y, in milliabsorbance units) using non-linear calibration curves, as differences in calibration curves of sample components made transformation of detector signal to concentrations not feasible when the peaks were overlapping. The calibration curve is very flat in the range of higher y values, especially over 1300, so that the transformation of concentration into absorbance is not very accurate there. The experimental band profile of o-cresol in Fig. 1B shows more significant tailing than the profile predicted by

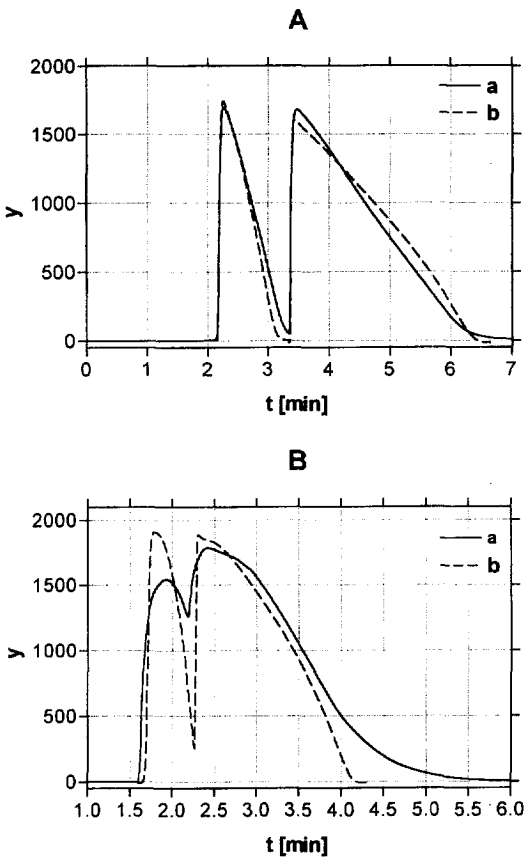

Fig. 1. Calculated (dashed lines) and experimental (full lines) band profiles of phenol (first peak) and o-cresol (second peak) in overloaded separation of 0.5-ml sample feed containing 0.1 M (0.05 mmol) of each component dissolved in the mobile phase, 30% methanol (A) and 40% methanol (B) in water. Column: Separon SGX C_{18}, 7 μm, 150×3.3 mm I.D., flow-rate 1 ml/min, y-response of the UV detector at 289 nm in millibsorbance units.

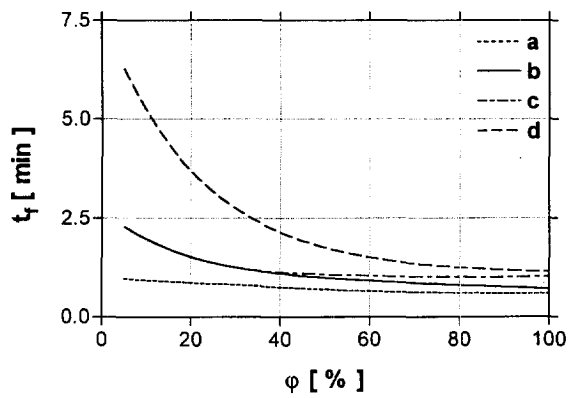

Fig. 2. Dependence of the start (a, c) and the end (b, d) cut-off times, t_r, of fractions of resorcinol (a, b) and phenol (c, d) on the concentration of methanol, φ (%, v/v). Sample feed: 1 ml, containing 0.1 M (0.1 mmol) of each compound. Column 1, 1 ml/min.

numerical calculation based on the 'Rouchon' algorithm. Whatever the reason may be (slower kinetics of the process, errors in the detector calibration, etc.), such a difference does not affect significantly the ratio of the areas of the bands of the sample solutes and the fraction cut-off volumes, which control the production rate and the recovery, so that it does not cause a significant error in the results shown in Figs. 6–11.

To investigate the effect of the mobile phase on the separation under overloaded conditions, band profiles were calculated for 0.5 ml and 1 − ml sample feeds containing mixtures of resorcinol and phenol and of phenol and *o*-cresol at several concentration ratios. From the calculated band profiles, the initial and the final cut-off times of the fractions of 99% pure sample components were determined. Dependencies of the cut-off times on the concentration of methanol are shown in Fig. 2 for 1-ml sample feed containing 0.1 *M* resorcinol and phenol concentrations (i.e., 0.1 mmol) and in Fig. 3A and B for 0.5-ml sample feeds containing phenol and *o*-cresol in concentrations of 0.1 and 0.02 *M* (0.05 or 0.01 mmol, respectively).

Because the saturation capacity decreases as the concentration of methanol in the mobile phase, φ, increases (Table 1), the loading factor L_f increases

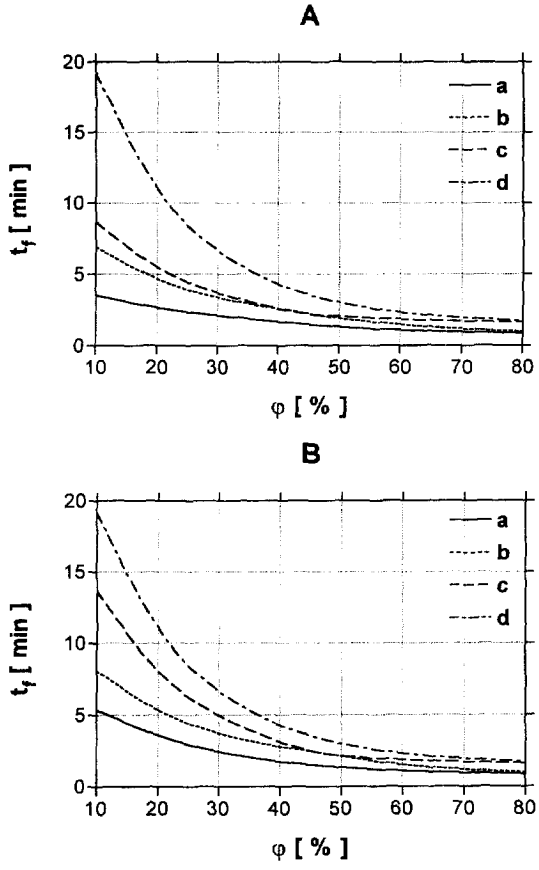

Fig. 3. Dependence of the start (a, c) and the end (b, d) cut-off times, t_f, of fractions of phenol (a, b) and *o*-cresol (c, d) on the concentration of methanol, φ (%, v/v). Sample feed: 0.5 ml, containing 0.1 *M* (0.05 mmol) (A) and 0.02 *M* (0.01 mmol) (B) of each compound. Column 2, 1 ml/min.

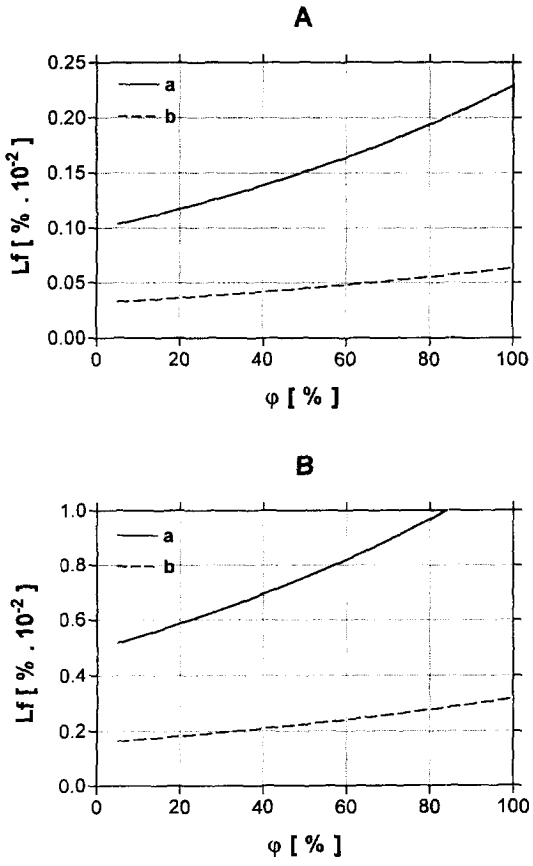

Fig. 4. Dependencies of loading factor, L_f, af resorcinol (a) and phenol (b) on the concentration of methanol, φ (%, v/v). Sample feed: 1 ml, containing 0.02 *M* (0.02 mmol) (A) and 0.1 *M* (0.1 mmol) (B) of each compound. Column 1, 1 ml/min.

with increasing. $\phi(L_f$ is defined as the ratio of the amount of sample component in the sample feed to the saturation capacity of the column for the sample component, $q_S V_S$, where $V_S = V_{col}(1-\epsilon)$ is the volume of the stationary phase in the column volume V_{col} and ϵ is the column porosity.) Figs. 4 and 5 illustrate the dependencies of the loading factors for 1 − ml volume of the sample feed containing 0.02 *M* (Fig. 4A) and 0.1 *M* (Fig. 4B) resorcinol and phenol and for a 0.5-ml volume of the sample feed containing 0.1 *M* (Fig. 5A) and 0.02 *M* (Fig. 5B) phenol and *o*-cresol on two C_{18} columns.

The recoveries of sample components in required purity (99%) in dependence on the concentration of methanol are 100% until the peak overlap becomes significant in mobile phases containing more than 20–40% methanol for samples of resorcinol and phenol (Fig. 6) or more than 40–45% methanol for

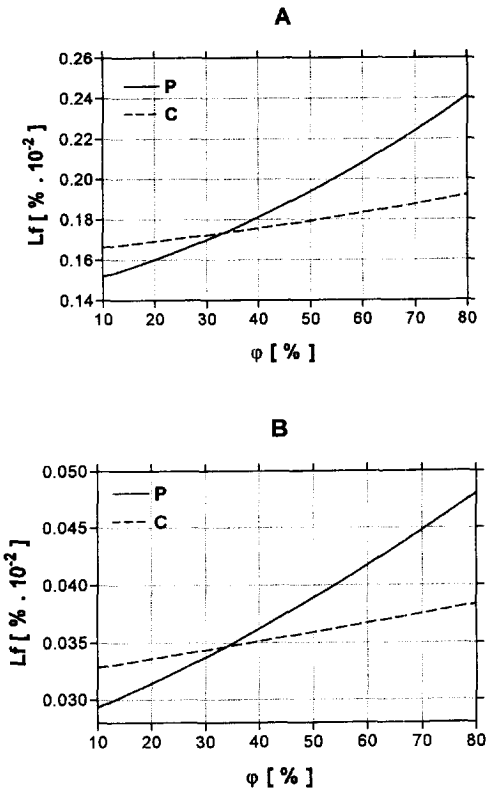

Fig. 5. Dependencies of loading factor, L_f, of phenol (a) and *o*-cresol (b) on the concentration of methanol, φ (%, v/v). Sample feed: 0.5 ml, containing 0.1 *M* (0.05 mmol) (A) and 0.02 *M* (0.01 mmol) (B) of each compound. Column 2, 1 ml/min.

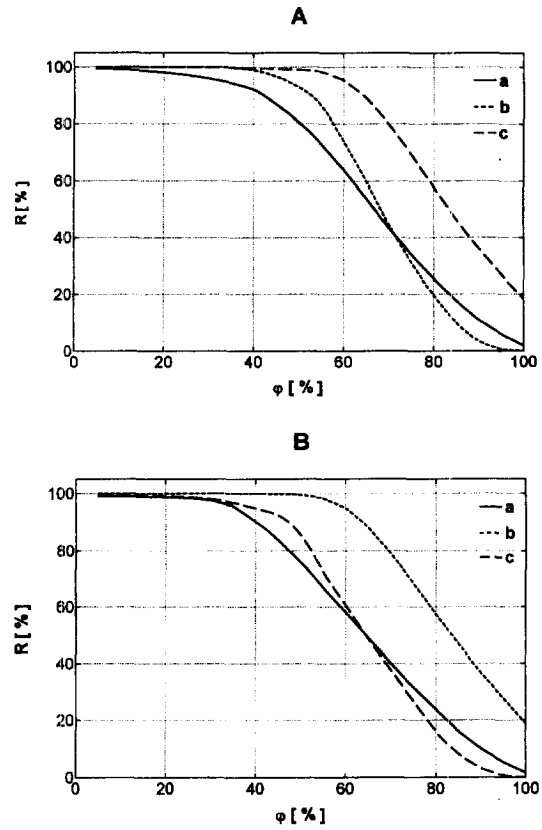

Fig. 6. Recovery yield *R* of resorcinol (A) and phenol (B) in dependence on the concentration of methanol, φ (%, v/v). Sample feed: 1 ml, containing 0.1 *M* (0.1 mmol) of each compound (a), 0.02 *M* (0.02 mmol) resorcinol and 0.1 *M* (0.1 mmol) phenol (b) and 0.1 *M* (0.1 mmol) resorcinol and 0.02 *M* (0.02 mmol) phenol (c). Column 1, 1 ml/min.

samples of phenol and *o*-cresol (Fig. 7). The concentration of methanol at which the recovery starts to decrease depends both on the loading factor (amount of the sample components in the feed) and on the concentration ratio of the sample components. As can be expected, the recovery of a sample component starts to decrease at lower concentrations of methanol if the loading factor increases (plot a in Fig. 6A and B). If the concentration ratio of sample components is different, the recovery starts to decrease at a lower concentration of methanol for the compound present at a lower concentration (plot b in Fig. 6A and plot c in Fig. 6B). Similar, but less pronounced effect of the loading factor and concentration ratio of components on the recovery is

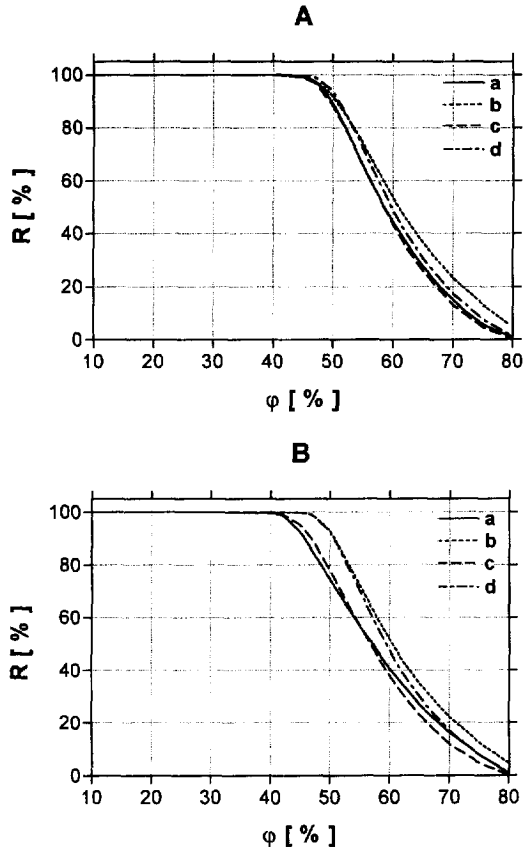

Fig. 7. Recovery yield R of phenol (A) and resorcinol (B) in dependence on the concentration of methanol, φ (%, v/v). Sample feed: 0.5 ml, containing 0.1 M (0.05 mmol) (a) or 0.02 M (0.01 mmol) (b) of each compound, 0.02 M (0.01 mmol) phenol and 0.1 (0.05 mmol) M o-cresol (c) and 0.1 M (0.05 mmol) phenol and 0.02 M (0.01 mmol) o-cresol (c). Column 1, 1 ml/min.

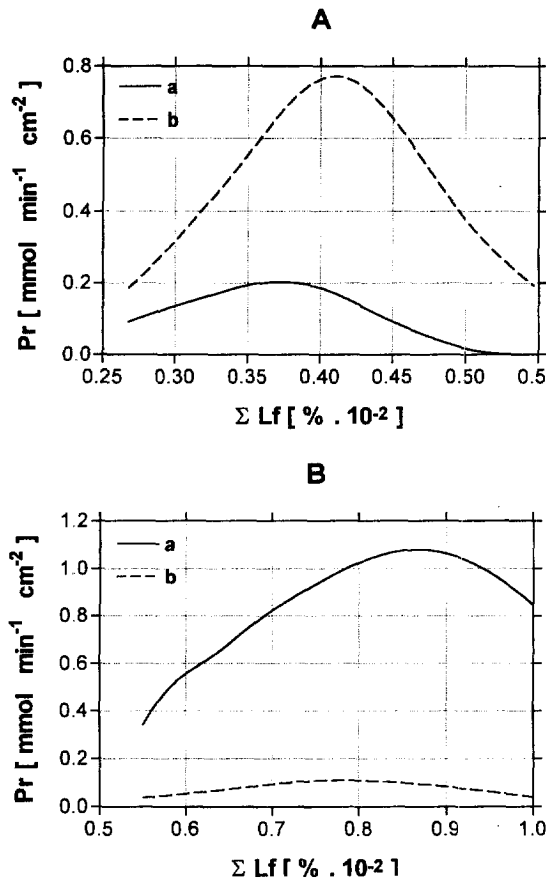

Fig. 8. Dependence of the production rate normalized to the column cross-section area, P_r, of resorcinol (A) and of phenol (B) on the sum of loading factors of the two sample components. Sample feed: 1 ml, containing 0.02 M, i.e., 0.02 mmol of resorcinol and 0.1 M, i.e., 0.1 mmol of phenol (a) and 0.1 M, i.e., 0.1 mmol of resorcinol and 0.02 M, i.e., 0.02 mmol of phenol (b). Column 1, 1 ml/min.

apparent with a smaller sample feed of the mixture of phenol and o-cresol (Fig. 7A and B).

Figs. 8 and 9 show plots of the production rate P_r of sample components in dependence on the sum of their loading factors, ΣL_f, at various concentrations and concentration ratios of the two components in the sample feed in mobile phases with varying concentration of methanol. The production rate first increases with increasing loading factor, but when certain column load is reached the separation starts to impair and this effect eventually causes production rate to drop as the loading factor is further increased. In all cases an optimum loading factor was found for which the highest production rate is obtained. It should be noted that the amount of solutes in the sample feed has much more significant effect on this behavior than a slight decrease in the column saturation capacity with increasing concentration of methanol in the mobile phase and that the same behavior would be observed even if the saturation capacity was independent of the composition of the mobile phase. The optimum amount of the sample in the feed (the loading factor) depends on the character (sorption isotherm) on the sample component and increases for each compound when the concentrations of the two components in the sample feed

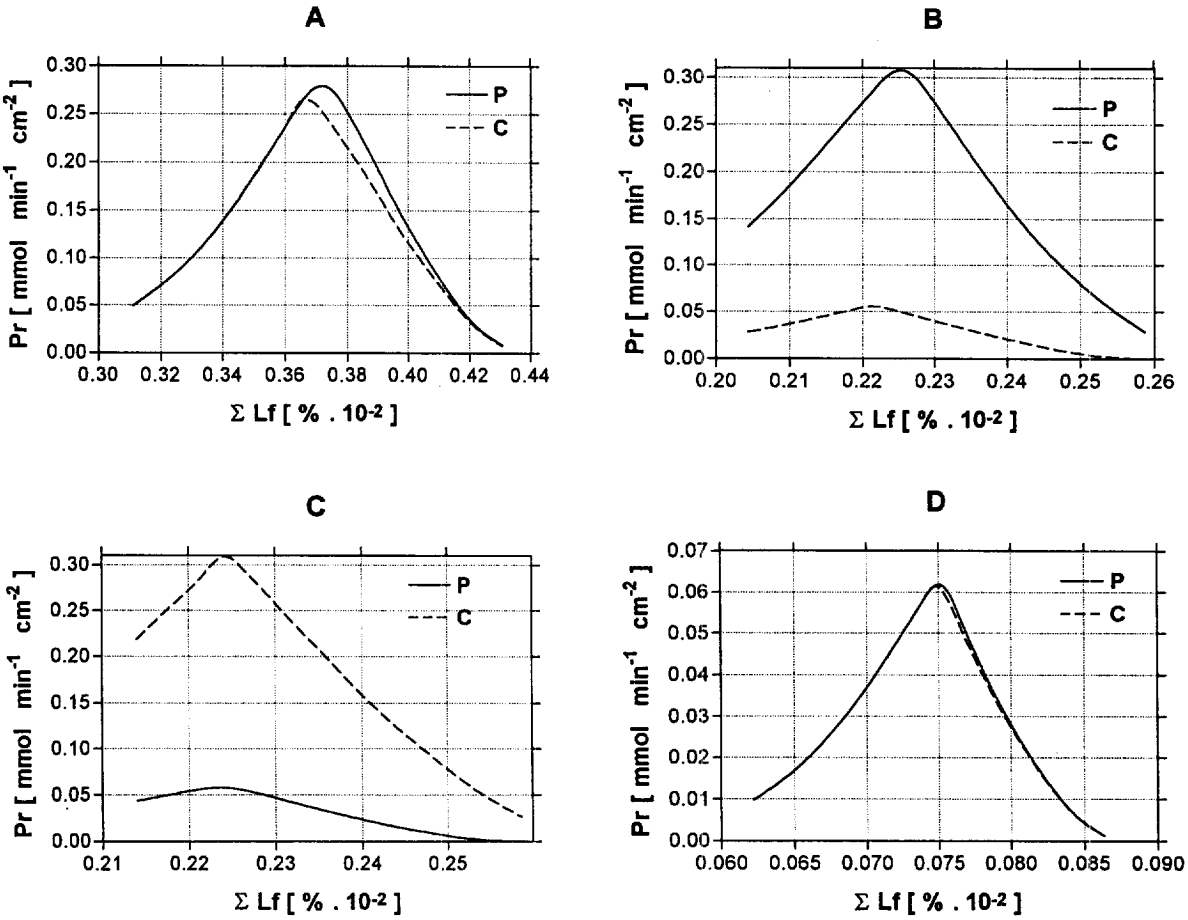

Fig. 9. Dependence of the production rate normalized to the column cross-section area, P_r, of phenol (P) and of *o*-cresol (C) on the sum of loading factors of the two sample compounds. Sample feed: 0.5 ml, containing 0.1 *M*, i.e. 0.05 mmol (A) or 0.02 *M*, i.e., 0.01 mmol (D) of each compound, 0.02 *M*, i.e., 0.01 mmol of phenol and 0.1 *M*, i.e., 0.05 mmol of *o*-cresol (C) and 0.1 *M*, i.e., 0.05 mmol of phenol and 0.02 *M*, i.e., 0.01 mmol of *o*-cresol (B). Column 2, 1 ml/min.

increase, as it is demonstrated for various concentration ratios of resorcinol and phenol in Fig. 8A and B and of phenol and *o*-cresol in Fig. 9A to D.

The dependencies of the production rate on the concentration of methanol in the mobile phase are similar to the dependencies of P_r on the loading factor. This is illustrated in Fig. 10 for samples of mixtures of resorcinol and phenol and in Fig. 11 for samples of mixtures of phenol and *o*-cresol. The occurrence of the maximum P_r can be understood as the result of different behavior of the recovery yield R_i (Figs. 6 and 7) and of the cycle time (the end time of the last fraction, t_c, see plots in Figs. 2 and 3) in dependence on the concentration of methanol in the mobile phase. The production rate P_r is proportional to R_i and to the reciprocal value of t_c (Eq. (1)). At lower concentrations of methanol, φ, the resolution of the sample bands is complete and R_i is independent of φ but t_c (the time of separation) decreases as φ increases and consequently P_r increases. At higher concentrations of methanol, R_i and recovery of purified sample solutes steeply decrease because of impaired separation of sample compounds, but the decrease of t_c is slow and consequently P_r decreases as the concentration of methanol increases. The two effects are opposite to

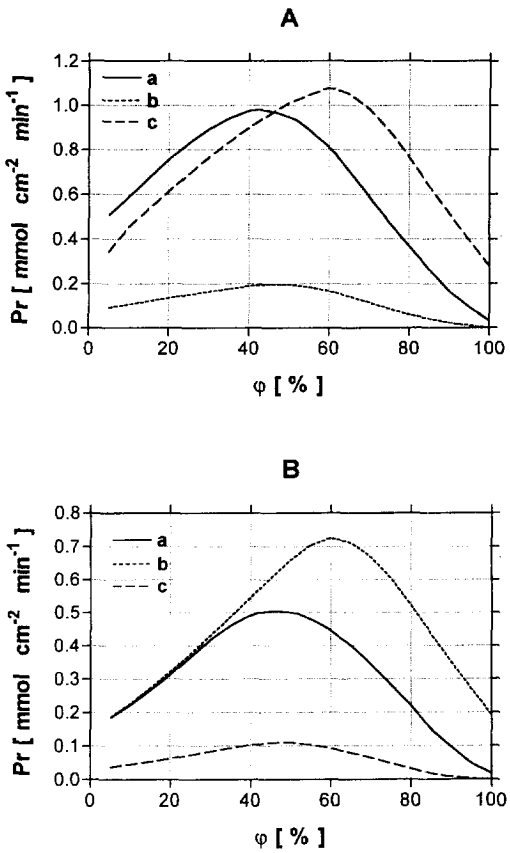

Fig. 10. Dependence of the production rate normalized to the column cross-section area, P_r, of resorcinol (A) and of phenol (B) on the concentration of methanol in the mobile phase, φ. Sample feed: 1 ml, containing 0.1 *M* (0.1 mmol) of both sample components (a), 0.02 *M* (0.02 mmol) resorcinol and 0.1 *M* (0.1 mmol) phenol (b) and 0.1 *M* (0.1 mmol) resorcinol and 0.02 *M* (0.02 mmol) phenol (c). Column 1, 1 ml/min.

each other and result in occurrence of maximum P_r as a function of loading factor or of the concentration of methanol in the mobile phase (Figs. 8–11).

The dependence of the production rate on the concentration of methanol in the mobile phase is also subject to the effects of the mobile phase on the separation factor, α, which is the ratio of the retention factors under non-overloaded, i.e., linear chromatography conditions (or of the Langmuir parameters *a*) of the two sample components (Table 1). These effects are different with the two sample mixtures studied. The separation factor of phenol/resorcinol increases from 2.74 to 3.07 when the methanol concentration increases from 10 to 40%, while in the same time, α for *o*-cresol/phenol decreases from 2.74 to 2.14 (Table 1). Increasing α should contribute to improving separation and to increasing production rate of resorcinol and phenol in the lower concentration range of methanol, until the effect of increasing retention factor on decreasing resolution prevails. On the other hand, the opposite effect observed with phenol and *o*-cresol contributes to impairing production rate as the concentration of methanol is increased. This could possibly explain why the maximum production rate of phenol/resorcinol (Fig. 10) is larger and is observed at a higher concentration of methanol than the maximum P_r of *o*-cresol/phenol (Fig. 11).

In the overloaded separation of resorcinol and phenol, the optimum concentration of methanol for maximum production rates is more significantly affected by the concentration of the second component in the sample feed than by the concentration of the compound to be isolated (resorcinol in Fig. 10A and phenol in Fig. 10B). The higher the concentration of the second compound, the lower is the optimum concentration of methanol and the maximum production rate.

Under the conditions of linear (analytical) chromatography, the separation is usually characterised by resolution, R_S. The resolution related to the time of elution of the end of the second band, R_S/t_2 ($t_2 = t_{R,2} + 0.5w_{t2}$, where $t_{R,2}$ is the elution time and w_{t2} the bandwidth at the baseline in time units of the later eluted sample compound) can be understood as the analogy of productivity in preparative chromatography. The values of R_S and of R_S/t_2 are given in Table 1, assuming column efficiency of 3600 theoretical plates, $V_0 = 1$ ml and flow-rate 1 ml/min. The resolution is proportional to the retention factors of sample components, *k*, and to their separation factor, α. In the particular case of the separation of resorcinol and phenol, the effects of the concentration of methanol on *k* and on α are opposite to each other (see Table 1) and consequently, minimum analytical resolution is observed in mobile phase with 40% methanol (where maximum preparative production rate is obtained). On the contrary, R_S/t_2 first increases as the concentration of methanol in the mobile phase is increased, until the maximum is

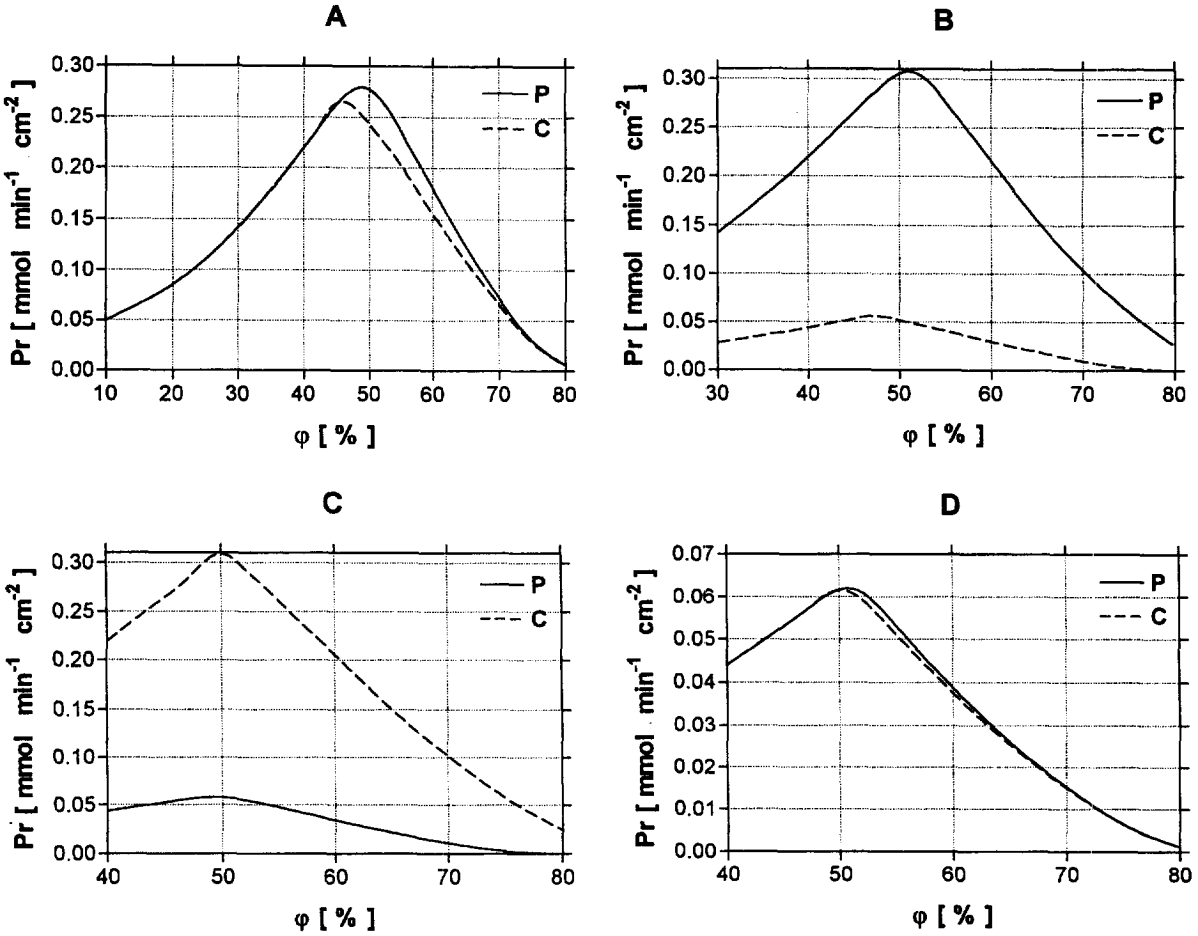

Fig. 11. Dependence of the production rate normalized to the column cross-section area, P_r, of phenol (P) and of *o*-cresol (C) on the concentration of methanol in the mobile phase, φ. Sample feed: 0.5 ml, containing 0.1 *M* (0.05 mmol) (A) or 0.02 *M* (0.01 mmol) (D) of each compound, 0.02 *M* (0.01 mmol) of phenol and 0.1 *M* (0.05 mmol) of *o*-cresol (C) and 0.1 *M* (0.05 mmol) of phenol and 0.02 *M* (0.01 mmol) of *o*-cresol (B). Column 2, 1 ml/min.

obtained at 75% methanol and than decreases. The maximum production rate of resorcinol and phenol corresponds to the analytical retention factors $k=$ 0.24 and 0.77, respectively, with the separation factor $\alpha=3$.

The effect of the concentration ratio of sample compounds on the optimum production rate was apparent, but much less significant also with the overloaded separation of phenol and *o*-cresol, where the sum of the loading factors was approximately three- to four-times lower than in the separation of resorcinol and phenol (compare Fig. 9A and 8B for phenol and Fig. 9A and C for *o*-cresol). Very similar optimum concentration of methanol was found to yield maximum production rate for both phenol and *o*-cresol (Fig. 11A to D). This optimum is at 50 and 45% methanol, respectively, for 0.2 ml of the solution containing 0.1 *M* phenol and 0.1 or 0.02 *M* *o*-cresol (Fig. 11A and B) and 50% for both phenol and *o*-cresol in the same volume of sample feed containing 0.1 *M* *o*-cresol and 0.1 or 0.02 *M* phenol (Fig. 11C and D).

In contrast to the behavior of the pair of resorcinol and phenol, the separation factor of *o*-cresol and phenol decreases as the concentration of methanol in the mobile phase increases and so does the analytical

resolution over the whole range of methanol concentrations in the mobile phase (Table 1B). Maximum R_S/t_2 is observed in 50% methanol as the mobile phase, which agrees with the conditions for maximum production rate in preparative chromatography (Fig. 11). The retention factors of dilute solutes under these conditions are 1.59 for *o*-cresol and 0.82 for phenol.

5. Conclusions

In overloaded reversed-phase separation of phenols, the saturation capacities of C_{18} columns decrease and consequently the loading factors increase as the concentration of methanol in aqueous–organic mobile phases increases. More important is the effect of the concentration of methanol on the recovery yield and on the cycle time. The first effect impairs, but the second effect enhances the production rate as the concentration of methanol increases, which leads to the occurrence of an optimum concentration of methanol for maximum production rate under isocratic conditions. The optimum composition of the mobile phase depends on the sample load and on the concentration ratio of the components in the sample feed. The optimum concentration of methanol in the mobile phase and the maximum production rate of isolated sample component decreases as the concentration of the second sample component increases, but the concentration of the isolated sample component has little effect on the optimum concentration of methanol. This behavior was observed for the overloaded separation of resorcinol from phenol and of phenol from *o*-cresol, but was more significant at higher sample loading factors employed in the first case.

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