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Effects of the gradient profile on the production rate in reversed-phase gradient elution overloaded chromatography

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Abstract

Band profiles in overloaded gradient elution reversed-phase chromatography were calculated using the experimental parameters of the distribution isotherms of binary mixtures of 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 composition of the mobile phase were described by three-parameter equations and used in the numerical calculations. Calculated band profiles of the sample components determined the fraction range, recovery yield and production rate necessary to obtain the rquired purity of the sample components with various loading factors and concentration ratios of sample components depending on the gradient profile. The production rate in preparative gradient elution HPLC depends more on the initial concentration of the stronger eluent than on the steepness of the gradient. The highest production rates can be achieved with steep gradients starting in pure water, where the sample-focusing effect is the strongest, provided feed solubility is adequate.

Keywords: Gradient elution; Preparative chromatography; Band profiles; Overloaded columns; 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 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 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 band profiles of the individual components of the feed at the outlet of the separation column.

Knox and Pyper [1] 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

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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) [2–6].

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 [7] and gradient [8] elution conditions. Ghodbane and Guiochon [9] 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 [10] simultaneously optimized the sample volume and the sample size. Felinger and Guiochon [11] used a modified simplex method to simultaneously optimize the column length, the particle diameter, the flow-rate and the sample size to obtain the maximum production rate. These authors also investigated theoretically the effect of the retention factor on the production rate [12]. Experimental results were found to be in agreement with the calculated optimum band profiles [13]. Newburger and Guiochon [14] studied the effects of the mobile phase composition and the flow-rate on the production rate in normal-phase chromatography.

Semiempirical modifications of the Craig model [15–17], numerical solutions of the mass balance equation using orthogonal collocation on finite elements [18], and a finite difference method with a backward-forward calculation scheme [19] were used to calculate overloaded band profiles in gradient elution chromatography. Felinger and Guiochon [20] studied the effects of the separation factor and the gradient steepness in overloaded gradient elution chromatography and found increased production rates with respect to isocratic separation, provided the retention of sample solutes is high at the start of the gradient.

The objective of the present work is the optimization of the experimental parameters in overloaded reversed-phase gradient elution chromatography. This requires an investigation of the simultaneous effects of the initial concentration and the slope of the gradient, the most important of these parameters, on the production rate, the recovery yield and the fraction purity. For this purpose, binary mixtures of phenol and *o*-cresol were used as model samples and their individual band profiles calculated using numerical methods. From these profiles, the performance characteristics listed above were derived as a function of the slope of the gradient and the initial 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 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.

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

Previously published work on overloaded gradient elution chromatography have used the Snyder model of linear gradient elution in reversed-phase systems, based on the assumption that the dependence of the capacity factor, $k' = t_R/t_0 - 1$, on the concentration of the organic solvent in aqueous–organic mobile phases can be described by a simple two-parameter equation:

$$\log k' = \log k'_w - S\varphi \quad (2)$$

where t_R is the retention time, t_0 the hold-up time of the column, k'_w the capacity factor in pure water, φ the concentration of the organic solvent in the aqueous organic mobile phase and S a so-called solvent strength parameter. If Eq. (2) applies, a linear change of φ with time t elapsed from the start of the gradient leads to a linear decrease of log k'

$$\log k' = \log k'_i - \beta \frac{t}{t_0} \quad (3)$$

Here, k'_i is the capacity factor at the start of a linear gradient and β the steepness of the gradient. Based on this equation, retention volumes in chroma-

tography with linear gradient elution can be calculated using a simple equation [21]. Relationships similar to Eq. (2) 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. (2) are observed. Eq. (2) does not apply at low φ, where k' is high, and more sophisticated equations should be used.

Even though the concept of linear gradient elution is very instructive and allows straightforward analogies to be made with isocratic elution, the introduction of k'_1 and β has the drawback of combining the thermodynamic properties of the solute in a given chromatographic system with parameters which are controlled experimentally in a simple manner, the initial concentration of the organic solvent and the slope of the concentration gradient. In analytical liquid chromatography, retention data can be calculated for a wide variety of equations relating k' and φ combined with equations describing the gradient profile [22–24]. In numerical calculations of band profiles, any isotherm and any equations relating isotherm coefficients and mobile phase composition can be introduced, independently of the equation describing the experimental gradient profile, which can be either linear (most often) or non-linear (when appropriate). This approach was used in the present work.

3. Experimental

3.1. 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 valve, 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 column used for measuring the isotherm data and for experiments with large samples was a 150× 3.3 mm glass cartridge packed with two batches of Separon SGX C_{18} material, 7 μm particle size, obtained from Tessek (Prague, Czech Republic). The column hold-up volume was derived from the retention time of pure methanol (recorded at 200 nm). 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.

3.2. Chemicals

Analytical grade phenol and *o*-cresol (Lachema, Brno, Czech Republic) were purified before use, phenol by distillation and *o*-cresol by crystallization from 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 were 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.

3.3. Procedures

The equilibrium isotherms were measured using binary frontal analysis [25]. 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 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 details of the method are described elsewhere [26].

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 gradient dwell volume was 500 μl and the column dead volume 0.87 ml, corresponding to a column total porosity $\varepsilon_T = 0.68$. The diode-array detector wavelength was set at 250 nm, the flow-rate (1 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 reservoirs 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 two finite difference schemes [27]: the backward–forward "Craig machine" and the forward–backward "Rouchon" algorithms. The latter was adapted for gradient elution, considering varying time increments during the gradient run in each length increment of the column so that the isotherm parameters corresponding to the actual composition of the mobile phase in each column length and time increments are used during the whole simulated gradient run [28]. 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).

Table 1
Coefficients a_i, b_i of competitive Langmuir isotherms of phenol (P) and *o*-cresol (C)

% Methanol	10	20	30	40
Phenol				
a_P	17.80	9.13	5.40	3.15
b_P, l/mol	9.11	4.92	3.13	1.62
q_s, mol/l	1.95	1.86	1.72	1.54
k'_0	8.34	4.28	2.53	1.48
o-Cresol				
a_C	38.66	21.56	12.48	6.78
b, l/mol	16.17	10.96	7.50	4.55
q_s, mol/l	2.39	1.97	1.67	1.49
k'_0	18.11	10.10	5.85	3.18

Phenol: $a_0 = 1.550$; $a_1 = 3.166$; $a_2 = 1.361$; $b_0 = 1.236$; $b_1 = 2.810$; $b_2 = 0.608$; $q_s = 1.993 - 0.2\varphi - 2.333\varphi^2$.
o-Cresol: $a_0 = 1.824$; $a_1 = 2.363$; $a_2 = -0.284$; $b_0 = 1.314$; $b_1 = 0.895$; $b_2 = -2.047$; $q_s = 3.027 - 7.235\varphi + 9.25\varphi^2$.

$$q_P = \frac{a_P c_P}{1 + b_P c_P + b_C c_C} \quad ; \quad q_C = \frac{a_C c_C}{1 + b_P c_P + b_C c_C}$$

c_i: concentration in the mobile phase; q_i: concentration in the stationary phase, all in mol/l. Subscripts relate to phenol (P) and *o*-cresol (C), respectively; $q_s = a_i/b_i$: column saturation capacity; k'_0: capacity factor at infinite dilution; a_0, a_1, a_2 and b_0, b_1, b_2 are the coefficients of the dependencies of the Langmuir coefficients a and b on the concentration of methanol (φ, %vol·10^{-2}) in the mobile phase (Eqs. (4) and (5)). Column: Separon SGX C_{18}, 150×3.3 mm I.D., $V_0 = 0.87$ ml.

4. Results and discussion

The distribution of phenol and *o*-cresol between the octadecyl silica column and water–methanol solutions with various concentrations of methanol can be adequately described by competitive Langmuir isotherms. More complex quadratic and Jovanovic isotherms did not improve significantly the fit to the experimental data. More details about isotherm fitting in this particular case can be found elsewhere [26]. The best-fit parameters a, b of the competitive Langmuir isotherms of phenol and *o*-cresol are given in Table 1, together with the column saturation capacities, $q_s = a/b$, and the capacity factors at infinite solute dilution, $k'_0 = a\phi = aV_s/V_m$ (ϕ is the phase ratio, i.e., the ratio of the volumes of the

stationary, V_s, and of the mobile phase, V_m, in the column). The saturation capacity of the column was found to decrease with increasing concentration of methanol.

Second degree polynomials must be used to describe adequately the dependence of the logarithms of the Langmuir coefficients a and b on the concentration of methanol, φ, because the deviations of the experimental values from the best-fit straight-line plots, especially those of log b versus φ are significant:

$$\log a = a_0 - a_1\varphi + a_2\varphi^2 \quad (4)$$

$$\log b = b_0 - b_1\varphi + b_2\varphi^2 \quad (5)$$

The coefficients a_0, a_1, a_2, b_0, b_1 and b_2 in Eqs. (4) and (5) are reported in Table 1. These coefficients were used to calculate the isotherms of phenol and of *o*-cresol as a function of the methanol concentration in the mobile phase during gradient elution during the numerical calculations of the band profiles of phenol (first peak) and *o*-cresol (second peak). From the calculated band profiles, the cut-times of the fractions collected, the fraction purities, the recovery yield and the production rate were calculated. In the calculations, the gradient dwell volume of 0.5 ml was taken into account.

Fig. 1 compares the calculated and experimental band profiles obtained with 0.25 ml of a 0.1 *M* solution of phenol and *o*-cresol in two different cases, with a 15 min gradient from 0 to 100% methanol (A) and with a 20 min gradient from 30 to 100% methanol (B). The response of the UV detector was transformed into a concentration profile using the nonlinear calibration curves. The absorbance of either compound above 0.018 mol/l is too high, and higher concentrations cannot be obtained from the response. The calculated chromatograms were slightly shifted to higher retention times (by approximately 0.1 min). The calculated band widths were nearly the same as those of the experimental peaks. These differences can be attributed to experimental errors in the determination of the parameters of Eqs. (4) and (5) describing the dependence of the coefficients of the competitive Langmuir isotherms on the actual composition of the mobile phase. The differences are small enough to allow using the calculated band profiles to investigate the effects of the gradient parameters on the performance of separation under overloaded gradient-elution conditions.

Because the column saturation capacity decreases with increasing methanol concentration (Table 1), the loading factor depends on the mobile phase composition. This is so even when a given sample amount is injected in mobile phases of different composition. Thus, the loading factor changes during gradient elution. A meaningful definition of the loading factor in gradient-elution chromatography would refer to the conditions at the start of the gradient, assuming that the sample solvent has the same composition as the initial mobile phase. Fig. 2 illustrates the dependence of the loading factor of phenol on the initial concentration of methanol at the start of the gradient, for the injection of different volumes of a 0.1 *M* solution. The loading factor is almost independent of the initial concentration of methanol up to 40–50%, but it significantly increases at higher methanol concentrations.

Fig. 3 illustrates the dependence of the initial and final cut-off times of the fractions of 99% pure phenol and *o*-cresol on the starting concentration of methanol for gradient times of 10 min, with 2 ml samples of 0.1 *M* solutions of both compounds. Up to a starting concentration of 10% methanol, complete resolution of the two compounds is obtained. With higher starting concentrations of methanol, the bands overlapped more and more and narrower fractions should be collected. This leads to decreasing recovery, when the starting concentration of methanol is increased. As illustrated in Fig. 4, which compares the results for 5 min and 15 min gradients, this effect is more significant for the last compound eluted (*o*-cresol) and the recovery of the first one is only slightly affected by the gradient time.

Fig. 5 shows the dependence of the production rate P_r of phenol on the initial loading factor L_f at the start of the gradient for gradient times of 5 (A) and 15 min (B) and various initial methanol concentrations. At low starting concentrations ($\leq$20% methanol), the production rate increases with increasing loading factor. At high starting concentrations ($\geq$40% methanol), there is an optimum loading factor for which the highest production rate is obtained. This corresponds to a loading factor of

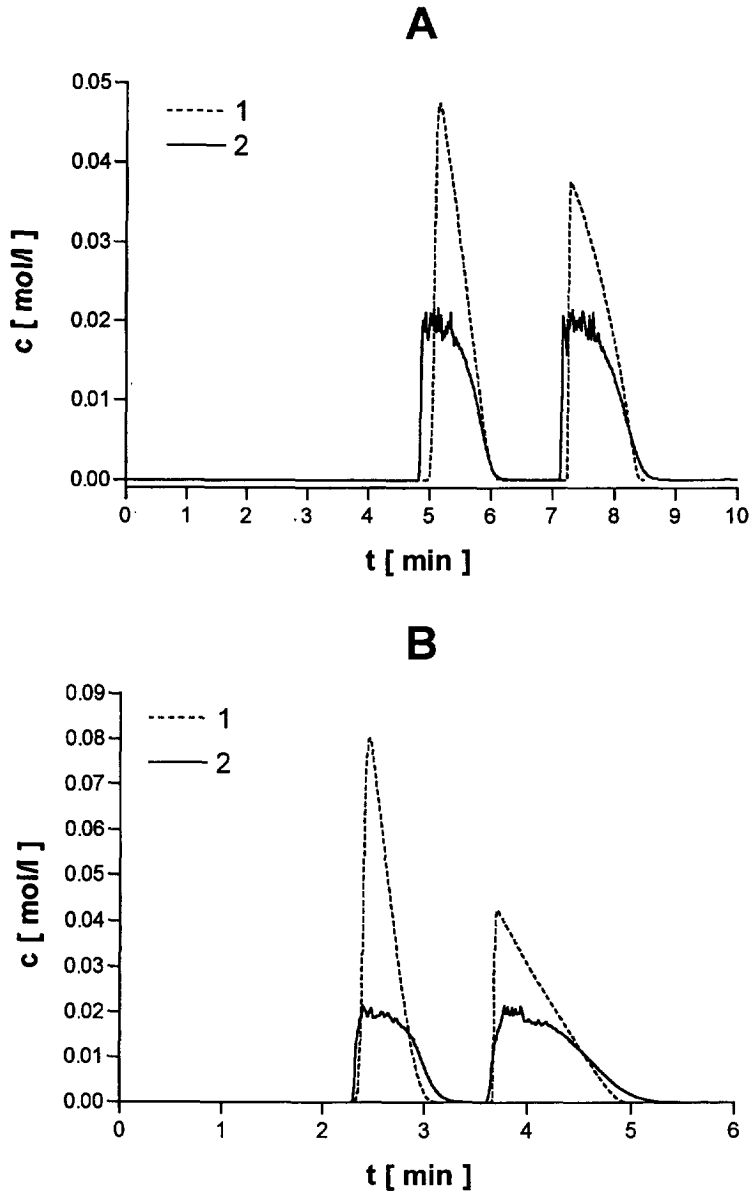

Fig. 1. Calculated (dashed lines) and experimental (full lines) band profiles of phenol (first peak) and *o*-cresol (second peak) in gradient elution on an octadecylsilica column (Separon SGX C_{18}, 7 μm, 150×3.3 mm I.D.), 1 ml/min. (A): gradient from 0 to 100% methanol in 15 min; (B): gradient from 30 to 100% methanol in 20 min. Sample: 0.25 ml containing 0.1 mol/l of both phenol and *o*-cresol dissolved in the solvent of the same composition as the mobile phase at the start of the gradient.

20%. The production rate increases with increasing steepness and with decreasing initial concentration of the gradient.

The effect of the gradient time, t_G, (proportional to the reverse of the gradient steepness) on the retention time is illustrated in Fig. 6 for the injection of 0.25 ml (A) and 2 ml (B) of 0.1 *M* solutions of phenol and *o*-cresol at various initial concentrations of methanol. The production rate decreases with increasing gradient time. At low loading factors (A) it

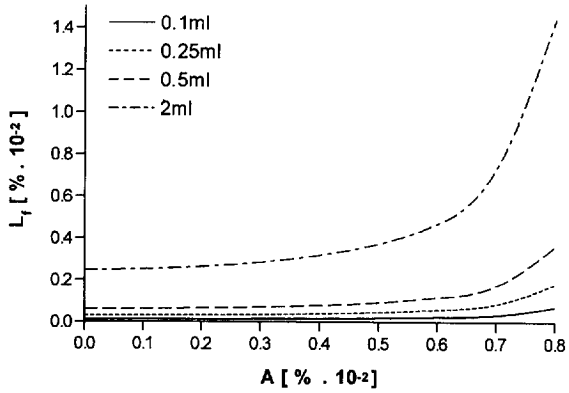

Fig. 2. Dependence of loading factor, L_f, of phenol on the initial concentration of methanol, A, in gradient elution for various sample volumes injected. Column as in Fig. 1, constant concentrations 0.1 mol/l of both compounds in the sample injected, 1 ml/min.

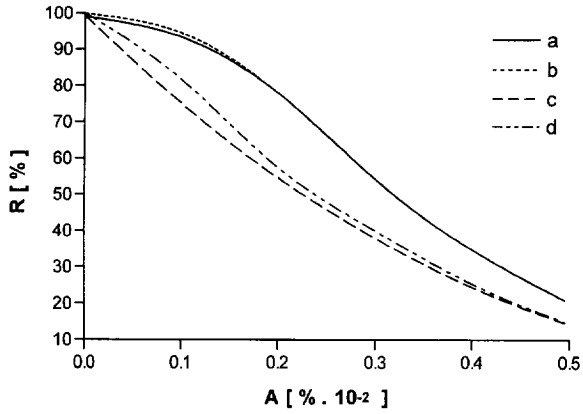

Fig. 4. Recovery yield R of phenol (a,b) and o-cresol (c, d) in gradient elution starting at various concentrations of methanol, A, and gradient times of 5 min (a, c) and 15 min (b, d) for 2 ml samples containing 0.1 mol/l of each compound. Column as in Fig. 1, 1 ml/min.

increases with increasing initial concentration of methanol, until a maximum is achieved with gradients starting at 40% methanol. The effect of the initial concentration of methanol is different at high loading factors (B). Then, similar production rates, P_r, are obtained when using gradients starting at 0–20% methanol, but P_r decreases significantly at higher methanol concentrations because of a reduced resolution in these gradient runs. This behavior is better demonstrated by the dependence of the production rate on the initial methanol concentration at various gradient times, t_G, shown in Fig. 7 for phenol (A) and for o-cresol (B), with 2 ml sample containing 0.1 mol/l of each sample component. Maximum production rate of phenol is achieved with gradients starting at 15–20% methanol, whereas gradients starting at 0% methanol provide maximum production rate for o-cresol. The production rate decreases as the gradient time increases.

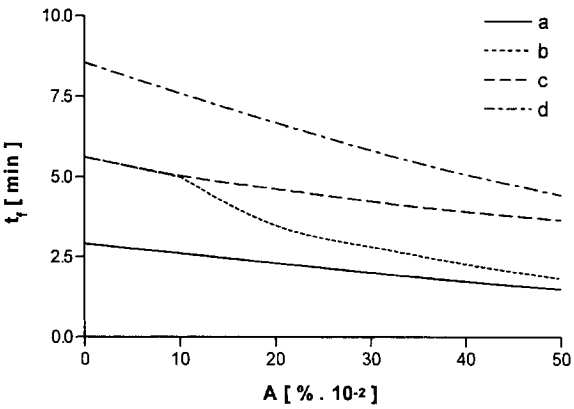

Fig. 3. Dependence of the start (a, c) and the end (b, d) cut-off times, t_f, of fractions of phenol (a, b) and o-cresol (c, d) for gradients starting at various initial concentrations of methanol, A, at the gradient time of 10 min and 2 ml samples containing 0.1 mol/l of each compound. Column as in Fig. 1, 1 ml/min.

This behavior differs from the one observed in overloaded isocratic elution chromatography (Fig. 8). In this case, the same optimum concentration of methanol was found to yield maximum production rates for both phenol (A) and for o-cresol (B). This optimum is 30 to 40% for 2 ml of 0.1 M solution (high loading factor) and 50% for a loading factor four times smaller.

Calculated isocratic band profiles of phenol and o-cresol with optimized methanol concentration are shown in Fig. 9 for a high and a low loading factors. With high loading factor, a strong displacement effect of o-cresol is apparent and a high production rate is traded for a low recovery yield. When the loading factor is four times lower, only a slight displacement effect is observed, the resolution and the recovery yields are significantly improved, at the cost of a lower production rate.

Fig. 10 shows calculated band profiles under

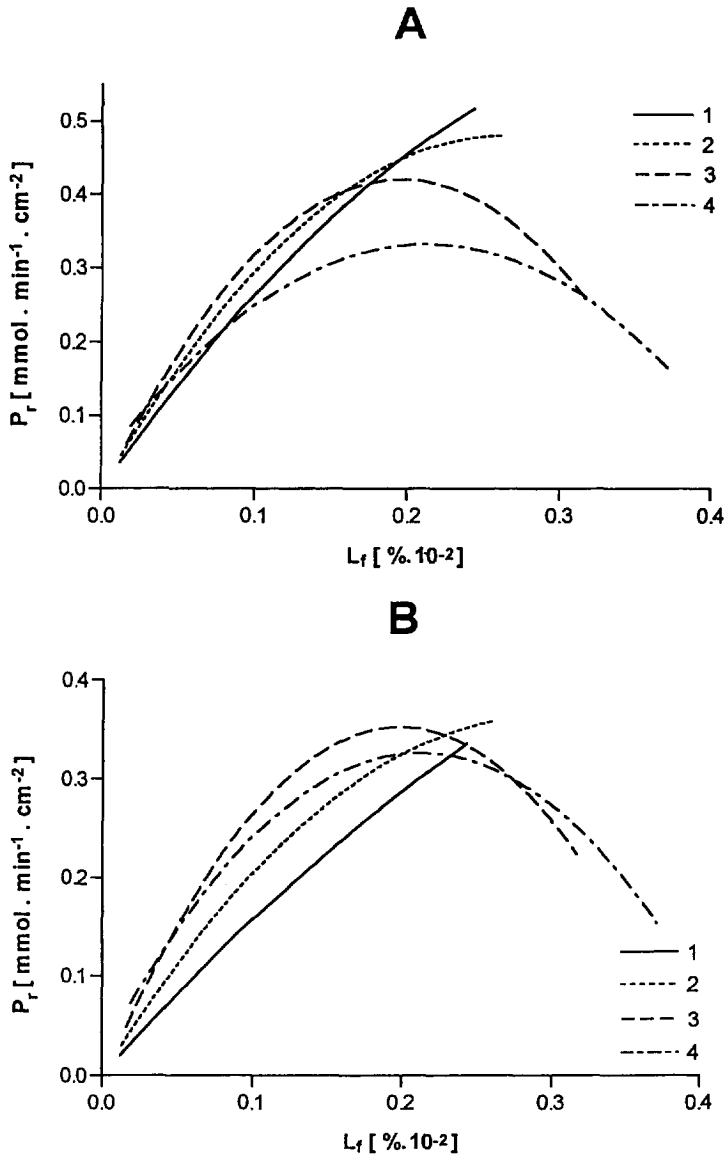

Fig. 5. Dependence of the production rate normalized to the column cross-section area, P_r, on the initial loading factor, L_f, for 5 min (A) and 15 min (B) gradients starting at 0 (1), 20 (2), 40 (3) and 50 (4)% methanol. Column as in Fig. 1, constant concentrations 0.1 mol/l of both compounds in the sample injected, 1 ml/min.

gradient-elution conditions for the same samples and loading factors as in Fig. 9. Here, similar band profiles are obtained for both high (A) and low (B) loading factors, with good recovery yields in both cases. This is explained by the combination of a sample self-displacement effect (the rear part of the peak migrates in a mobile phase which has a higher elution strength, i.e., at a higher velocity than the front part) and a sample focusing effect that takes place because the sample injected is dissolved in a solvent with a low elution strength (water in the separation shown in Fig. 10A). In this case, the

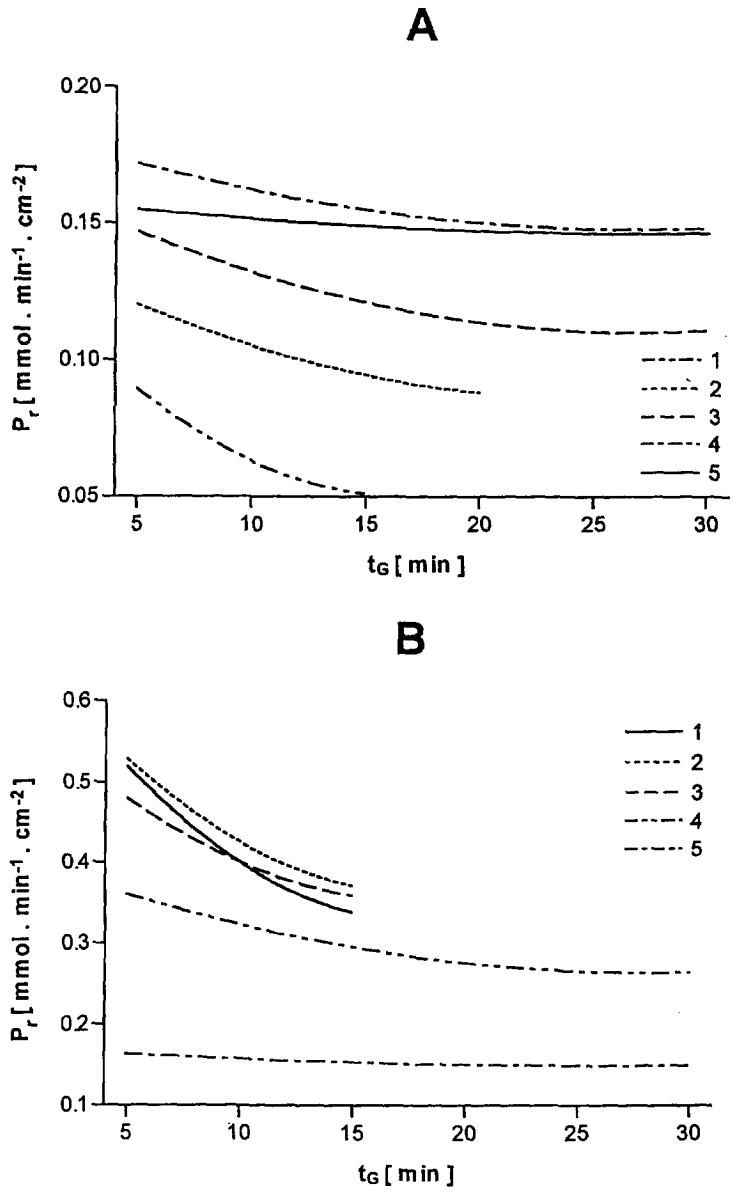

Fig. 6. Dependence of the production rate normalized to the column cross-scetion area, P_r, of phenol on the gradient time, t_G, for 0.25 ml (A) and 2 ml (B) samples containing 0.1 mol/l of each phenol and *o*-cresol for gradients starting at 0% (1A, B), 10% (2B), 20% (2A, 3B), 30% (3A, 4B), 40% (4A) and 50% (5A, B) methanol. Column as in Fig. 1, 1 ml/min.

distribution constant is high at injection and most of the sample components are adsorbed in a short section at the top of the column, even if large sample volumes are injected. The two effects lead to the elution of a part of the bands of the two sample compounds at a higher concentration than in the sample feed injected (case A). This effect is apparent only with the first compound eluted (phenol) in case B.

Fig. 11 shows calculated band profiles obtained

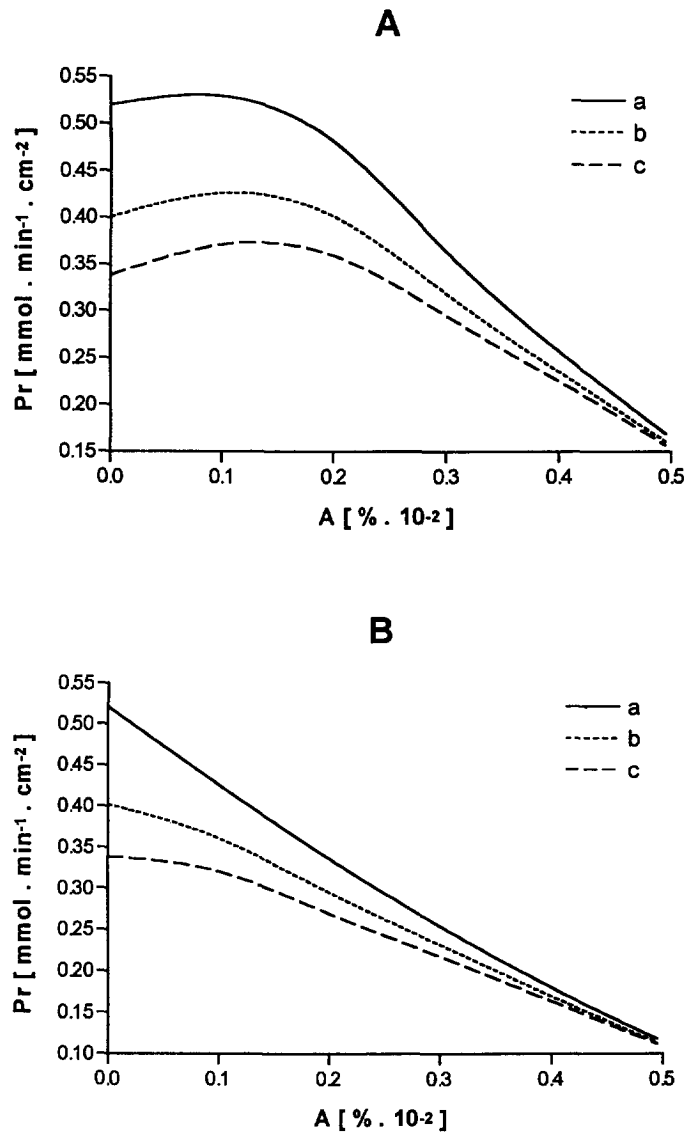

Fig. 7. Dependence of the production rate normalized to the column cross-section area, P_r, of phenol (A) and *o*-cresol (B) on the initial concentration of methanol in gradient with various gradient times: $t_G = 5$ min (a), 10 min (b) and 15 min (c). Column as in Fig. 1, 1 ml/min.

with a large volume (2 ml) of feed containing a concentration of either phenol (A) or *o*-cresol (B) which is five times higher than the concentration of the other component. The separations were done with a gradient starting at a zero concentration of methanol. Here again, an increase of the phenol concentration is observed in the front part of the first peak because of the sample-focusing and the self-displacement effects. This is even more apparent with the second peak (*o*-cresol) in Fig. 11B. In addition, some displacement of phenol by *o*-cresol takes place in case A.

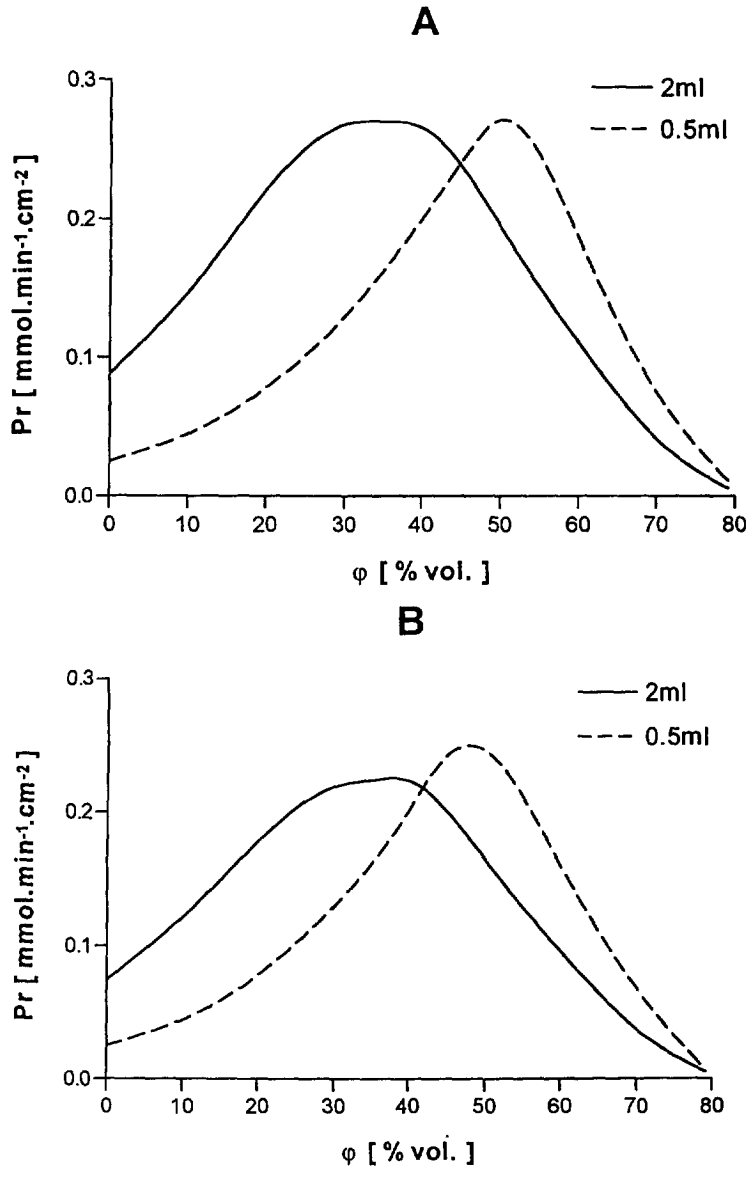

Fig. 8. Dependence of the production rate normalized to the column cross-section area, P_r, of phenol (A) and *o*-cresol (B) on the concentration of methanol, φ, in isocratic elution with mobile phases methanol–water for 2 ml and 0.5 ml samples containing 0.1 mol/l of each compound. Column as in Fig. 1, 1 ml/min.

5. Conclusions

In the separations investigated in this work, the initial concentration of the organic solvent (methanol) in the mobile phase at the beginning of the gradient run has a stronger effect on the production rate than the gradient time (or steepness of the gradient). At low loading factors, which are some-

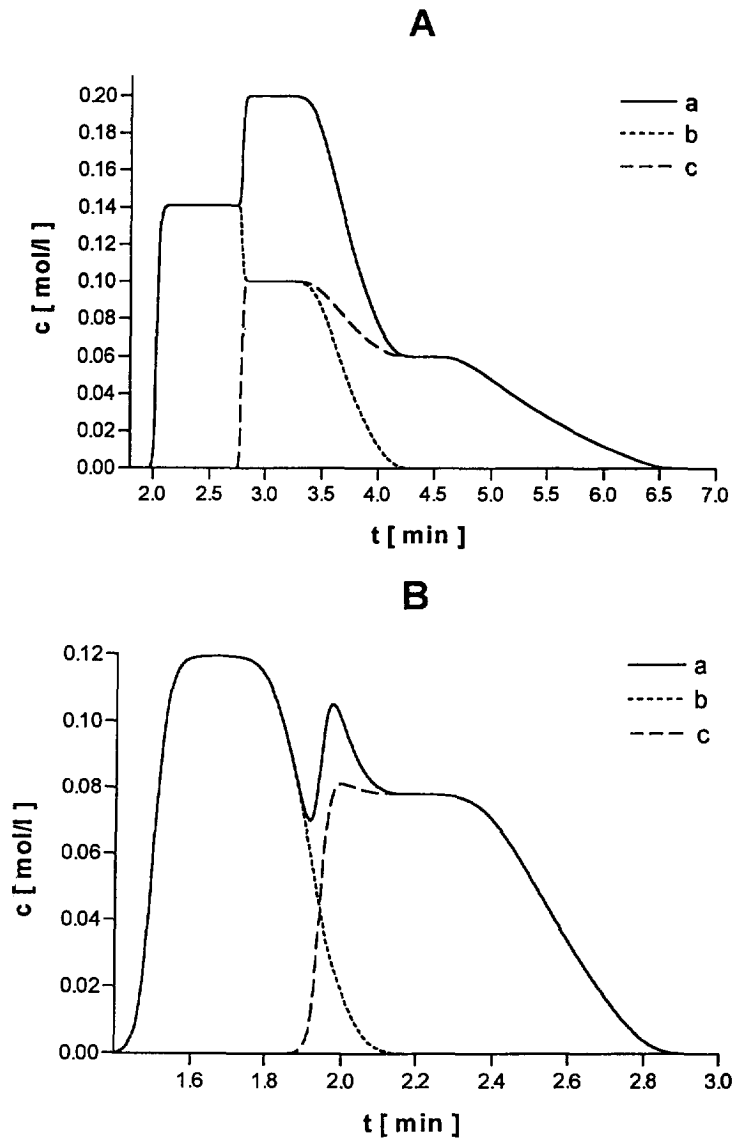

Fig. 9. Calculated band profiles of phenol (b) and cresol (c) in isocratic elution chromatography of a 2 ml sample in 35% methanol (A) and of a 0.5 ml sample in 50% methanol (B), corresponding to a maximised production rate, P_r, in Fig. 8. Concentration of sample compounds injected 0.1 mol/l. Column as in Fig. 1, 1 ml/min. Full line (a) corresponds to the sum of concentrations of phenol and *o*-cresol.

times dictated by a limited solubility of the feed components, there is an optimum initial concentration of the organic solvent at which the maximum production rate is obtained. This is similar to isocratic overloaded chromatography, where an optimum mobile phase composition can be found for maximum production rate.

If there are no solubility limitations regarding the sample injection, the gradient can be started at zero initial concentration of the organic solvent. This is beneficial for the separation, as sample focusing from the weak solvent into a narrow section of the adsorbent at the top of the column can be utilized together with the sample self- displacement taking

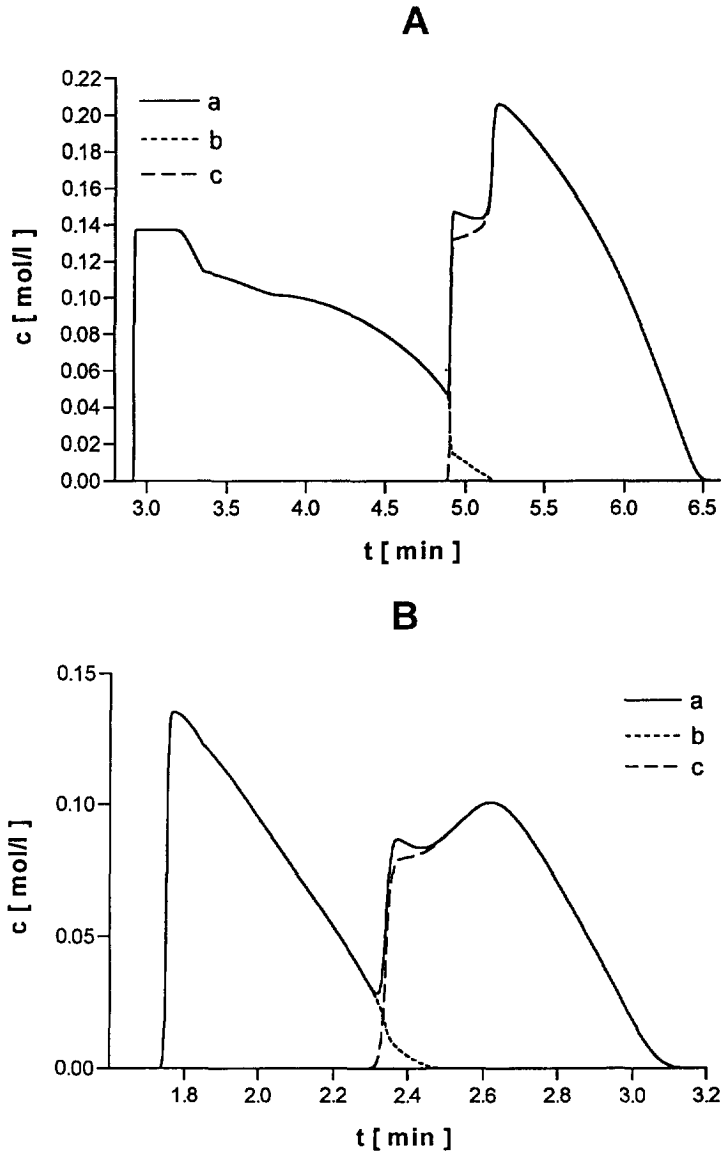

Fig. 10. Calculated band profiles of phenol (b) and *o*-cresol (c) in gradient-elution chromatography of a 2 ml sample in a 5 min gradient from 0 to 100% methanol (A)—maximised production rate and of a 0.5 ml sample in a 5 min gradient from 40 to 100% methanol (B). Concentration of sample compunds injected 0.1 mol/l. Column as in Fig. 1, 1 ml/min. Full line (a) corresponds to the sum of concentrations of phenol and *o*-cresol.

place during gradient elution to increase both the concentration of the sample components in the fractions collected and the production rate. Large feed amounts can be injected under these conditions, unlike in isocratic separations where poor recovery would occur if the same sample were injected under conditions optimized to achieve maximum production rate. Even if 6 column void volumes are allowed to regenerate the column after the end of the gradient, there still remains an approximate 10%

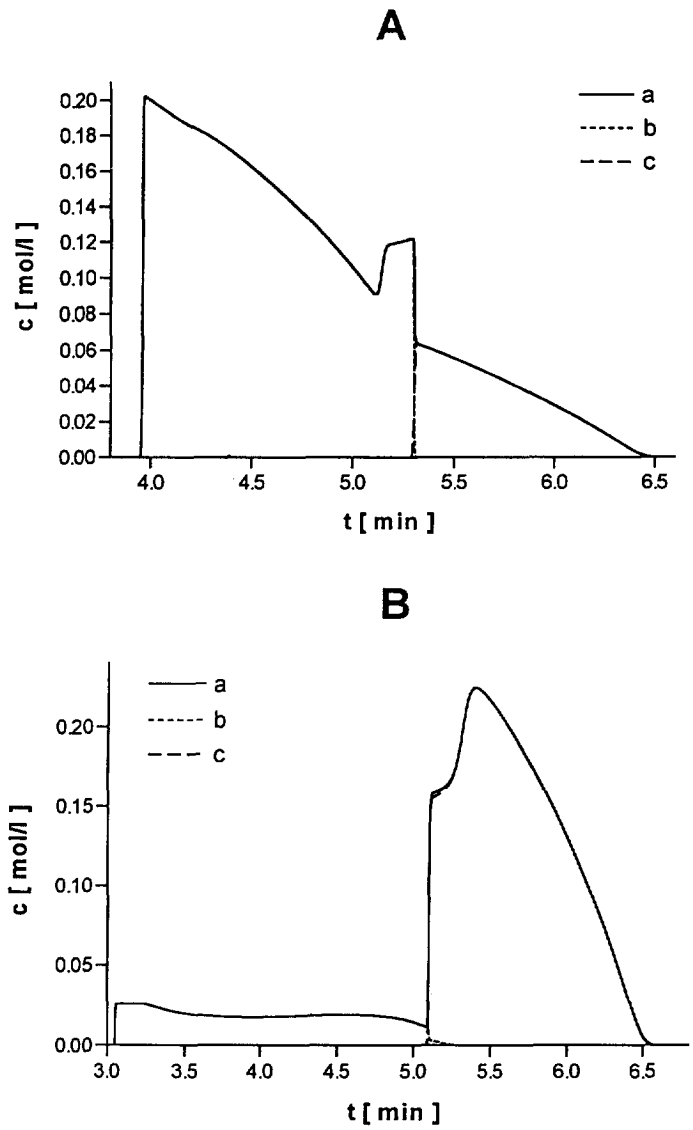

Fig. 11. Calculated band profiles of phenol (b) and *o*-cresol (c) in gradient-elution chromatography. Conditions as in Fig. 10A, except for different concentrations of sample components in the sample injected—0.1 mol/l phenol+0.02 mol/l *o*-cresol (A) and 0.02 mol/l phenol+0.1 mol/l *o*-cresol (B). Column as in Fig. 1, 1 ml/min. Full ine (a) corresponds to the sum of the concentrations of phenol and *o*-cresol.

gain in the production rate obtained in gradient elution with large sample volumes and gradient starting at zero initial concentration of methanol, as compared to the best isocratic separations of the test compounds.

Even though more data would be necessary before these conclusions can be generalized, the experimental conditions used in this work are believed to be typical for the separation of low molecular size compounds.

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References

[1] J.H. Knox and M. Pyper, J. Chromatogr., 363 (1986) 1.
[2] S. Golshan-Shirazi and G. Guiochon, J. Chromatogr., 517 (1990) 229.
[3] S. Golshan-Shirazi and G. Guiochon, Anal. Chem., 61 (1989) 1368.
[4] S. Golshan-Shirazi and G. Guiochon, J. Chromatogr., 536 (1991) 57.
[5] J. Newburger, L. Liebes, H. Colin and G. Guiochon, Separat. Sci. Technol., 22 (1987) 1933.
[6] J.A. Perry and T.J. Szczerba, J. Chromatogr., 484 (1989) 267.
[7] L.R. Snyder and J.W. Dolan, J. Chromatogr., 483 (1989) 63.
[8] L.R. Snyder and J.W. Dolan, J. Chromatogr., 540 (1991) 21.
[9] S. Ghodbane and G. Guiochon, Chromatographia, 26 (1989) 53.
[10] A. Katti and G. Guiochon, Anal. Chem., 61 (1989) 982.
[11] A. Felinger and G. Guiochon, J. Chromatogr., 591 (1992) 31.
[12] A. Felinger and G. Guiochon, Biotechnol. Bioeng., 41 (1993) 134.
[13] S. Jacobson, A. Felinger and G. Guiochon, Biotechnol. Progr., 8 (1992) 533.
[14] J. Newburger and G. Guiochon, J. Chromatogr., 523 (1990) 63.
[15] J.E. Eble, R.L. Grob, P.E. Antle and L.R. Snyder, J. Chromatogr., 405 (1987) 31.
[16] L.R. Snyder, G.B. Cox and P.E. Antle, J. Chromatogr., 444 (1988) 303.
[17] G.B. Cox, P.E. Antle and L.R. Snyder, J. Chromatogr., 444 (1988) 325.
[18] F.D. Antia and Cs. Horváth, J. Chromatogr., 484 (1989) 1.
[19] M.Z. El Fallah and G. Guiochon, Anal. Chem., 60 (1988) 2244, 2364.
[20] A. Felinger and G. Guiochon, J. Chromatogr. A, in press.
[21] L.R. Snyder, in Cs. Horváth (Editor), High-Performance Liquid Chromatography—Advances and Perspectives, Vol. 1, Academic Press, New York, 1980, p. 208.
[22] P. Jandera and J. Churáček, J. Chromatogr., 91 (1974) 223.
[23] P. Jandera and J. Churáček, Adv. Chromatogr., 19 (1981) 125.
[24] P. Jandera and J. Churáček, Gradient Elution in Liquid Chromatography, Elsevier, Amsterdam, 1985.
[25] S. Golshan-Shirazi, S. Ghodbane and G. Guiochon, Anal. Chem., 60 (1988) 2630.
[26] P. Jandera and D. Komers, J. Chromatogr. A, in press.
[27] G. Guiochon, S. Golshan-Shirazi and A.M. Katti, Fundamentals of Preparative and Nonlinear Chrumatography, Academic Press, New York, 1994, p. 384.
[28] P. Jandera et al., in preparation.