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# Optimization of the recovery yield and of the production rate in overloaded gradient-elution reversed-phase chromatography

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**Abstract**

To investigate the effects of various parameters on the production rate and on the recovery yield in overloaded reversed-phase gradient-elution chromatography, band profiles of binary mixtures of phenol and *o*-cresol were calculated using the experimental parameters of the distribution isotherms determined by binary frontal analysis. The effects of the feed volume and of the steepness of a continuous gradient (gradient time) of methanol in aqueous–organic mobile phases on the separation were studied. If the sample feed in a solvent with weak elution strength is used, combined effects of on-column enrichment, frontal chromatography and sharpening of the later eluted bands may enhance the production rate and the recovery yield. Steep continuous gradients offer better results than isocratic elution if the feed is dissolved in water and injected into the mobile phase with a higher elution strength, because larger volumes of dilute feed can be separated with high recovery yields. © 1998 Elsevier Science B.V.

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## 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.

The production rate, *Pr*, is the amount of feed purified at the required degree of purity per unit time. To allow general comparisons for columns of different diameters, *Pr* is normalized to the column cross-section area:

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

where $V_{\mathrm{inj}}$ 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, $r$, $S$ and $\epsilon_T$ are the radius, cross-section area, and total porosity of the column [1].

Unlike in analytical chromatography, where the optimization of the chromatographic process usually means obtaining the necessary resolution of a sample

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

A simple chromatographic model suggested by Knox and Pyper [2] to optimize the experimental conditions was later 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 the feed 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. Jandera et al. [16] found that there is an optimum composition of the mobile phase at which maximum production rate can be achieved, depending on the isotherms and on the concentration ratio of components in the sample feed.

In analytical gradient-elution liquid chromatography (LC), retention data can be calculated for a wide variety of equations relating the retention factor, $k$, and the concentration of the stronger solvent in a binary mobile phase, $\varphi$, combined with equations describing the gradient profile [17–19]. In overloaded gradient-elution chromatography, numerical calculations of band profiles should be used. For this purpose, any adequate equation describing the adsorption isotherm can be used, provided the mathematical form of the dependence of the isotherm coefficients on the mobile phase composition has been determined.

Semiempirical modifications of the Craig model [20–22], numerical solutions of the mass balance equation using orthogonal collocation on finite elements [23], and a finite difference method with a backward–forward calculation scheme [24] were used to calculate overloaded band profiles in gradient elution chromatography. Felinger and Guiochon [25] studied theoretically the effects of the separation factor and the gradient steepness in overloaded gradient-elution chromatography and found increased production rate with respect to isocratic separation, provided the retention of sample solutes is high at the start of the gradient.

In our recent work we found that the production rate in preparative gradient elution high-performance liquid chromatography (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 the feed solubility is adequate [26].

The objective of the present work was to investigate the combined effects of the volume and concentrations of the components in the feed and of the gradient steepness on the production rate and on the recovery yield in overloaded reversed-phase chromatography. We selected binary mixtures of phenol and *o*-cresol as model samples. The individual band profiles of sample components were calculated using numerical methods. From these profiles, the production rate, the recovery yield and the fraction purity were determined, as described earlier [26].

## 2. Experimental

### 2.1. Chemicals

Phenol and *o*-cresol, 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. Methanol, gradient elution grade, was obtained from Merck, Darmstadt, Germany. Water was doubly distilled in a glass vessel, with addition of potassium permanganate. The solvents were filtered on a 0.45-μm filter (Millipore, Bedford, MA, USA). For the determination of the distribution isotherm data, the sample solutions used were prepared by weighing the required amounts of solutes and dissolving them in the mobile phase and mobile phases were prepared by premixing their components in the required ratios and were degassed by ultrasonication before use.

### 2.2. Instrumentation

The experiments were performed using an HP 1090M liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA), equipped with a 3DR solvent-delivery system, 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. The gradient dwell volume of the instrument was 0.5 ml. A Separon SGX $C_{18}$ octadecyl silica (7 μm particle size) column glass cartridge, 15 cm×0.33 cm I.D. (Tessek, Prague, Czech Republic), was used to acquire the isotherm data and in the experiments with overloaded injection. The column hold-up volume (0.874 ml) was derived from the elution time of pure methanol recorded at 200 nm.

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 sixport column-switching valve, to a Hypersil ODS, 3 μm, 60× 4.6mm high-speed analytical column (Hewlett-Packard) 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 preset 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.

### 2.3. Procedures

The band profiles were measured using the HP 1090M liquid chromatograph in the standard set-up. The feed was introduced from one solvent reservoir using appropriate gradient program. The other two reservoirs were used to store water and methanol as the gradient components. The diode array detector wavelength was set at 289 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 degassed by stripping with helium.

The equilibrium isotherms have been measured using binary frontal analysis [27]. 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 isotherms were measured at four different concentrations of methanol in binary aqueous – organic mobile phases and competitive Langmuir isotherms were fitted to the experimental two-component distribution data [16]:

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

Here, $q_i$ is the concentration of the sample compound $i$ in the stationary and $c_i$ that in the mobile phase. The coefficients $a_i$, $a_j$, $b_i$, $b_j$ of the isotherm relate to the sample components $i$ (phenol) and $j$ (*o*-cresol). $a = k_0/\phi$, where $k_0$ is the retention factor of the sample compound at infinite dilution, i.e., in analytical chromatography and $b = a/q_s$, $q_s$ 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. As it has been found earlier, the dependence of the coefficients of the isotherm on the concentration of methanol in the mobile phase, $\varphi$, is adequately described by second-degree polynomial equations [28,29]:

$$\log (a) = \log (a_0) - m_a \varphi + d_a \varphi^2 \quad (3)$$

$$\log (b) = \log (b_0) - m_b \varphi + d_b \varphi^2 \quad (4)$$

The coefficients of Eqs. (3) and (4) determined by non-linear regression of the dependences of the isotherm parameters $a_i$, $a_j$, $b_i$, $b_j$ on the methanol concentration in the aqueous mobile phase (Table 1) were employed in the numerical calculations of the band profiles. A modified Rouchon finite difference algorithm was used in these calculations, which was found to provide the same results as the Craig computation scheme, but runs much faster so that an IBM-compatible personal computer can be used for computation instead of a big computer. The details on this calculation approach will be published elsewhere [30].

Table 1
Coefficients of the parameters of the dependencies of Langmuir parameters *a* and *b* on the concentration of methanol (Eqs. (3) and (4))

| Compound | Equation |
|---|---|
| Phenol | $\log a = 1.404 - 2.326\varphi$<br>$\log b = 1.125 - 1.847\varphi - 0.415\varphi^2$ |
| *o*-Cresol | $\log a = 1.899 - 2.739\varphi$<br>$\log b = 1.697 - 2.585\varphi - 0.128\varphi^2$ |

$V_S = 0.41$ ml and $V_M = 0.874$ ml are the volumes of the stationary and of the mobile phases, respectively, in the Separon SGX $C_{18}$, 150×3.3 mm I.D. column. Phase ratio $= V_S/V_M = 0.47$.

## 3. Results and discussion

To investigate the effect of the sample loading factor and of the feed volume on the recovery yield and on the production rate in gradient-elution and in isocratic overloaded chromatography, various gradients in combination with various sample feed volumes of phenol and *o*-cresol (0.5–10 ml) were compared. Water was used as the sample solvent with a low elution strength. The sample band profiles were calculated for different separation conditions using the constants of the competitive Langmuir isotherm (Table 1) and from the band profiles the start and end cut-off times and the recovery yield were determined at a constant fraction purity of 99%. Further, the production rate per column cross-section unit was calculated. The characteristics of the separation are listed in Table 2.

First, the effect of the sample loading factor on the gradient-elution separation was studied at a constant concentration ratio 1:1 of phenol to *o*-cresol in the 5-ml sample feed with 5 min gradients from 0% to 100% methanol in water at 1 ml/min (Fig. 1A and 1B). The starting value of the separation factor for diluted compounds ($\alpha = a2/a1$, $a$ = parameters of Eq. (2)) is 3.12 in pure water, but decreases during the gradient elution to 1.21 in 100% methanol.

At the concentrations of 0.1:0.1 and 0.08:0.08 mol/l in the sample feed, the sum of the loading factors is 141% and 113%, respectively, of the column saturation capacity. Consequently, the elution of phenol starts before the whole feed volume has passed through the column and large part of the feed is not separated. As the sample size, i.e., the concentrations of the sample components in the feed decrease, the end cut-off time of the fraction of phenol and the start cut-off time of the fraction of

Table 2
Characteristics of overloaded gradient-elution separations of phenol and *o*-cresol

| Gradient time (min) | $V_i$ (ml) | $c_i$ (P, C) (mol/l) | $t_c$ (min) | $R$ (P) (%) | $R$ (C) (%) | $Pr$ (P) | $Pr$ (C) |
|---|---|---|---|---|---|---|---|
| 5 | 5 | 0.01 | 9.38 | 99.99 | 100 | 0.092 | 0.092 |
| 5 | 5 | 0.02 | 9.37 | 99.98 | 100 | 0.183 | 0.183 |
| 5 | 5 | 0.03 | 9.37 | 99.98 | 100 | 0.275 | 0.275 |
| 5 | 5 | 0.04 | 9.37 | 98.21 | 94.24 | 0.360 | 0.346 |
| 5 | 5 | 0.05 | 9.37 | 93.73 | 78.64 | 0.430 | 0.361 |
| 5 | 5 | 0.08 | 9.36 | 73.16 | 53.33 | 0.538 | 0.392 |
| 5 | 5 | 0.1 | 9.36 | 59.24 | 42.64 | 0.544 | 0.392 |
| 5 | 0.5 | 0.4 | 4.87 | 99.83 | 100 | 0.705 | 0.706 |
| 10 | 0.5 | 0.4 | 6.77 | 99.97 | 100 | 0.508 | 0.508 |
| 15 | 0.5 | 0.4 | 8.30 | 99.99 | 100 | 0.414 | 0.414 |
| 20 | 0.5 | 0.4 | 9.58 | 99.99 | 100 | 0.360 | 0.360 |
| 5 | 1 | 0.2 | 5.37 | 99.95 | 100 | 0.640 | 0.640 |
| 10 | 1 | 0.2 | 7.27 | 99.97 | 100 | 0.473 | 0.473 |
| 15 | 1 | 0.2 | 8.80 | 99.98 | 100 | 0.391 | 0.391 |
| 20 | 1 | 0.2 | 10.08 | 99.98 | 100 | 0.341 | 0.341 |
| 5 | 2 | 0.1 | 6.37 | 99.60 | 100 | 0.538 | 0.540 |
| 10 | 2 | 0.1 | 8.27 | 99.89 | 99.99 | 0.415 | 0.416 |
| 15 | 2 | 0.1 | 9.80 | 99.95 | 99.99 | 0.351 | 0.351 |
| 20 | 2 | 0.1 | 11.08 | 99.97 | 99.99 | 0.310 | 0.310 |
| 5 | 5 | 0.04 | 9.37 | 98.21 | 94.24 | 0.360 | 0.346 |
| 10 | 5 | 0.04 | 11.27 | 99.07 | 99.34 | 0.302 | 0.303 |
| 15 | 5 | 0.04 | 12.80 | 99.35 | 100 | 0.267 | 0.269 |
| 20 | 5 | 0.04 | 14.08 | 99.50 | 100 | 0.243 | 0.244 |
| 5 | 10 | 0.02 | 14.37 | 90.75 | 76.98 | 0.217 | 0.184 |
| 10 | 10 | 0.02 | 16.27 | 92.35 | 81.64 | 0.195 | 0.173 |
| 15 | 10 | 0.02 | 17.80 | 93.11 | 83.76 | 0.180 | 0.162 |
| 20 | 10 | 0.02 | 19.09 | 93.45 | 84.84 | 0.168 | 0.153 |
| 5* | 0.5 | 0.4 | 3.73 | 88.48 | 67.59 | 0.816 | 0.623 |
| 5* | 1 | 0.2 | 4.23 | 82.21 | 62.24 | 0.668 | 0.506 |
| 5* | 2 | 0.1 | 5.23 | 65.23 | 52.29 | 0.429 | 0.346 |
| 5* | 5 | 0.04 | 8.23 | 38.46 | 33.84 | 0.161 | 0.141 |
| 5* | 10 | 0.02 | 13.23 | 22.90 | 20.74 | 0.060 | 0.054 |

$V_i$ = Feed volume, dissolved in water; $c_i$ = concentrations of phenol (P) and *o*-cresol (C) in the feed; $t_c$ = cycle time as the cut-off time of the fraction of *o*-cresol; $R$ = recovery yield; $Pr$ = prodution rate per effective column cross-section, in mmol $min^{-1}$ $cm^{-2}$. Column as in Table 1, flow-rate 1 ml/min. Continuous linear gradients from 0 to 100% methanol in water, except for * linear gradients from 30% to 100% methanol in water.

*o*-cresol and consequently the recovery yield increase. The recovery yield is complete for the sum of the loading factors equal to or lower than 42% of the column saturation capacity (Fig. 1B). The end cut-off time of the fraction of *o*-cresol and the cycle time do not depend on the loading factor, but on the other hand, the production rate decreases with decreasing loading factor, i.e., with decreasing concentrations of the components in the sample feed (Fig. 1A).

The concentrations of the sample components in the isolated pure fractions are higher than their original concentrations in the feed at all loading factors tested. The concentration focusing in overloaded gradient-elution separation of large feed volumes can be explained by simultaneous effects of several factors: high distribution constant of sample compounds in water causes adsorption of sample compounds in a relatively narrow layer of the packing material, starting from the top of the column, like in the solid-phase extraction of water samples. The sample components are later eluted in a lower volume of the mobile phase with a higher elution strength. The concentration focusing of the earlier eluted compound is further assisted by the

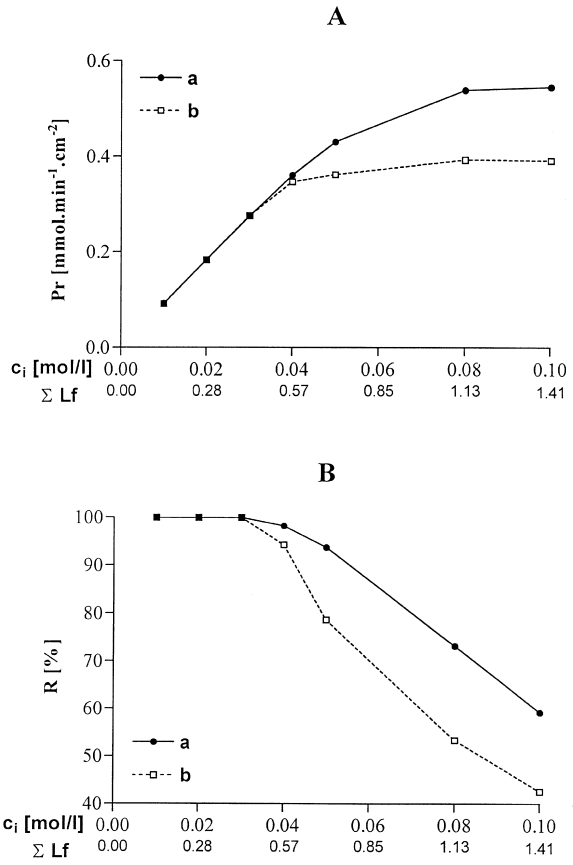


Fig. 1. Dependence of the production rate normalized to the column cross-section area, *Pr*, (A) and of the recovery yield, *R*, (B) of phenol (a) and of *o*-cresol (b) on the sum of loading factors of the two sample components, $L_f$ (in % of the column saturation capacity$\cdot 10^{-2}$), and on the concentrations, $c_i$, of the sample components in the 5 ml feed in overloaded gradient-elution from 0 to 100% methanol in water in 5 min. Column: Separon SGX $C_{18}$, 7 μm, 150×3.3 mm I.D., flow-rate 1 ml/min.

displacement effect of the later eluted compound, which tends to compress the first fraction. Finally, increasing concentration of the stronger solvent during the gradient elution continuously decreases the retention factor and accelerates the sample migration in the rear of the band with respect to the band front – this effect is more significant with cresol eluted in the second fraction.

Combined effects of the sample feed volume and of the gradient steepness were investigated at a constant total loading factor of the two phenolic compounds, equal to 56.5% of the column saturation capacity, which corresponds to 0.2 mmol of each component in the sample feed. Continuous gradients with different steepnesses corresponding to 5, 10, 15 and 20 min gradients from 0 to 100% methanol in water (at 1 ml/min) were compared. The results are given in Table 2. Fig. 2A and 2B show calculated band profiles of the sample components and the experimental chromatograms at the 5 ml feed volumes for the 10 min and the 20 min gradients. The differences in the detector response between the

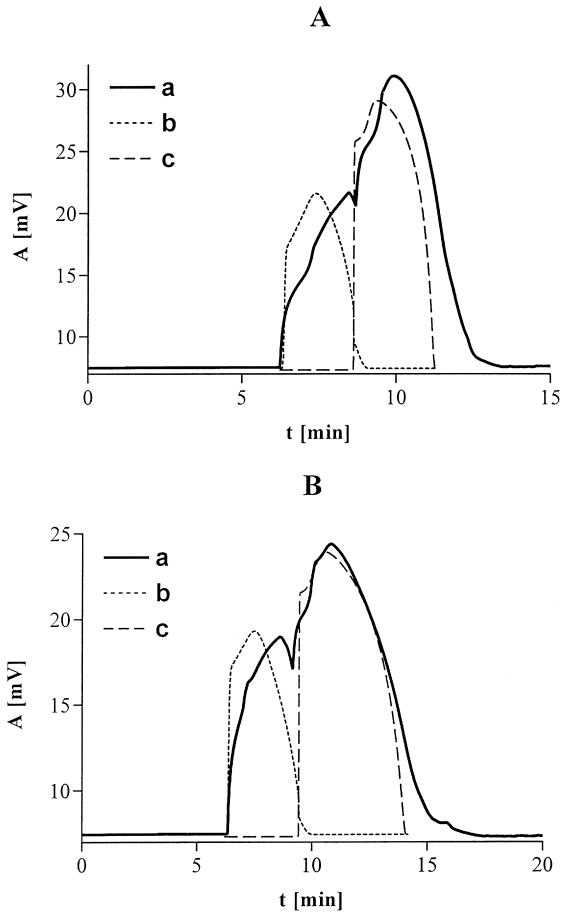


Fig. 2. Calculated (dashed lines, transformed to expected detector response) and experimental [(full lines, a) band profiles of phenol (first peak, b) and *o*-cresol (second peak, c)] in the overloaded gradient-elution separation of a 5 ml sample feed containing 0.04 mol/l (0.2 mmol) of each component dissolved in water. (A): 0–100% methanol in 10 min; (B): 0–100% methanol in 20 min. Column and flow-rate as in Fig. 1. $A$=Response of the UV detector at 289 nm, $t$=time from the start of the feed injection.

calculated and the experimental profiles can be probably partially attributed to less accurate calibration of the detector at high sample concentrations.

Figs. 3 and 4 show the dependencies of the recovery yield and of the production rate of both sample compounds on the volume of the feed at different gradient profiles tested. The separation was almost complete and the recovery yields of phenol and *o*-cresol were close to 100% at the feed volumes up to 5 ml at various gradient steepnesses tested, but they decreased to 77–93% for the feed volume of 10 ml. Decreasing steepness, i.e., increasing time of the gradient did not affect the starting cut-off volume of the first fraction of phenol, which increased proportionally to the feed volume. The end cut-off volume of the fraction of phenol, the start and the end cut-off volumes of the fraction of *o*-cresol and consequently the volume of the middle, impure fraction increased with the time of the gradient. Concentration focusing effect is observed with all gradients tested at feed volumes 5 and 10 ml, but it decreases with decreasing steepness (increasing time) of the gradient. For the 2 ml feed, the concentrations

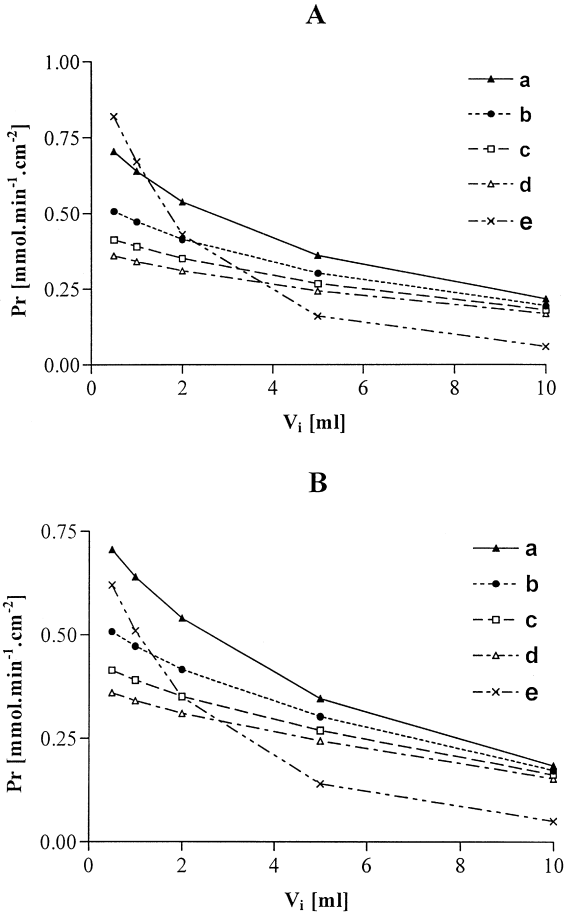


Fig. 3. Dependence of the production rate normalized to the column cross-section area, *Pr*, of phenol (A) and of *o*-cresol (B) on the feed volume, $V_i$, at a constant sum of loading factors (56% of the column saturation capacity) in overloaded gradient-elution from 0 to 100% methanol in water in 5 min (a), 10 min (b), 15 min (c), 20 min (d) and from 30 to 100% methanol in water in 5 min (e). Other conditions as in Fig. 1.

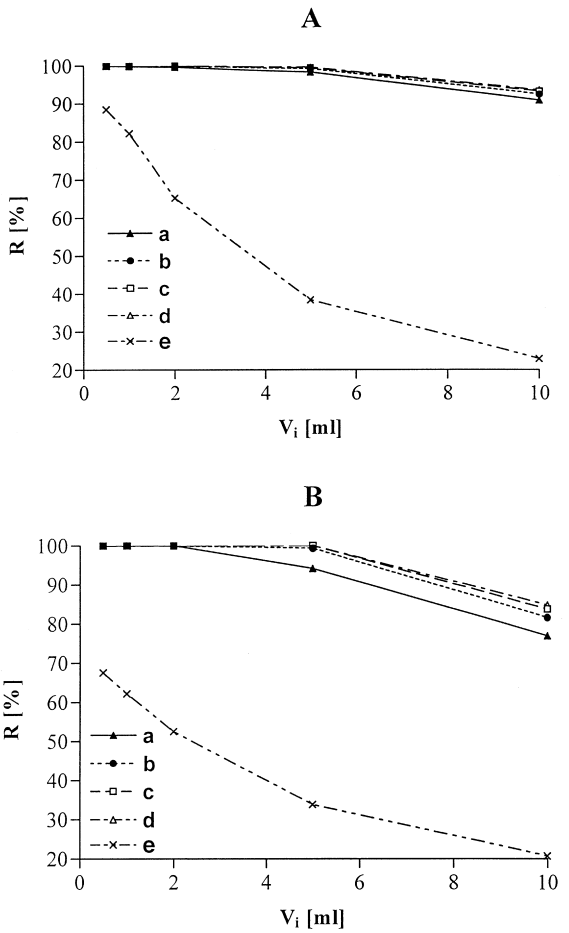


Fig. 4. Dependence of the recovery yield, *R*, of phenol (A) and of *o*-cresol (B) on the feed volume, $V_i$, at a constant sum of loading factors in overloaded gradient-elution from 0 to 100% methanol in water in 5 min (a), 10 min (b), 15 min (c), 20 min (d) and from 30 to 100% methanol in water in 5 min (e). Other conditions as in Fig. 1.

of the sample components in the recovered fractions are higher than in the feed only with the steepest (5 min) gradient.

The time of the separation is proportional to the volume of the feed and the differences between the end cut-off times of the second fractions at the same gradient profile are equal to the differences between the volumes of the sample feed in the individual experiments. The recovery yield of the sample compounds only slightly increase with increasing gradient time (Fig. 4). The production rate decreases as the gradient time and the sample feed volume increase (Fig. 3). As it can be expected intuitively, best recovery yield and production rate are achieved with low feed volumes and with steep gradients. However, under these conditions most of the concentration focusing effect of the overloaded gradient elution is lost. In practice, the concentration of sample components in the feed is often limited by their limited solubility. (For example, it is not possible to prepare the solutions containing more than 0.1 mol/l of phenol and *o*-cresol in water and data for the more concentrated feeds in Table 2 and in Figs. 3 and 4 are only hypothetical).

To illustrate the effect of the initial concentration of methanol at the start of the gradient, the results obtained for various sample feeds with the 5 min gradients from 30 to 100% methanol were included into the data shown in Table 2 and in Figs. 3 and 4 (plots e). Some concentration focusing effect is observed here, too, but the recovery yield and the production rate (except for phenol at low sample volumes) are significantly lower than with gradients starting from pure water.

The characteristics of the overloaded separations under various isocratic conditions are given in Table 3. The effect of the gradient elution on the band profiles is apparent from the comparison of Fig. 2A and 2B with the isocratic band profiles of the 5 ml feeds in Fig. 5A and 5B. In pure water, complete isocratic separation of the sample compounds is achieved for sample feeds up to 5 ml and the recovery for the 10 ml feed is slightly better than with overloaded gradients. However, the time of separation is very long and the production rate is unacceptably low (Fig. 5A). In mobile phases with higher (20% and 50%) concentrations of methanol, both the recovery yields and the production rates are significantly lower than with the same feed in overloaded gradient elution (Fig. 5B).

To investigate if the concentration focusing effect can be utilised also under isocratic conditions, the recovery yields and the production rates with various feed volumes using mobile phase or water as the sample solvent are compared in Table 3 for the mobile phases containing 10–80% methanol. Fig. 6 shows the band profiles obtained for the 5 ml feed containing 0.04 mol/l of each phenol and *o*-cresol in water in gradient elution with a 5 min gradient from 0 to 100% methanol (A) and with 10% methanol as the isocratic mobile phase (B). At similar fraction purities, the isocratic separation is much slower than the gradient run.

The dependencies of the recovery yields and of the production rates on the feed volume with various isocratic mobile phases are shown in Figs. 7 and 8. As with continuous gradients, the production rate increases as the sample feed volume decreases from 10 to 1 ml. The recovery yield increases with decreasing feed volume in the mobile phases containing 30% or less methanol. With mobile phases containing 50% or more methanol, significant concentrations of *o*-cresol are coeluted since the beginning of the elution of phenol, so that pure phenol can not be obtained. Using 30% methanol as the mobile phase, maximum production rate is obtained for a constant feed volume. The cycle time (the end cut-off time of the fraction of *o*-cresol) strongly increases in mobile phases with decreasing concentrations of methanol. Both the recovery yield and the production rate improve with water as the sample solvent for large feed volumes (approximately 1.5-times for the 5 ml feed and twice for the 10 ml feed). With feed volumes of 2 ml or less, using water as the sample solvent has little effect on the recovery yield and on the production rate.

Elution with 30% methanol yields the best compromise between the production rates and the recovery yields under isocratic conditions. Curves a–d in Fig. 9A, 9B and Fig. 10A, 10B show that the gain in the recovery yield and in the production rate of phenol and *o*-cresol for various times of the gradients from 0% to 100% methanol increase with respect to the optimum isocratic separations. The relative increase is more significant as the feed volume increases. The increase in the recovery yield

Table 3
Characteristics of overloaded isocratic separations of phenol and *o*-cresol with various concentrations of methanol in water as the mobile phase

| Methanol concentration (%) | $V_i$ (ml) | $c_i$ (P, C) (mol/l) | $t_c$ (min) | $R$ (P) (%) | $R$ (C) (%) | $Pr$ (P) | $Pr$ (C) |
|---|---|---|---|---|---|---|---|
| 0 | 0.5 | 0.4+ | 34.05 | 99.99 | 100 | 0.101 | 0.101 |
| 0 | 1 | 0.2+ | 34.55 | 99.99 | 100 | 0.100 | 0.100 |
| 0 | 2 | 0.1+ | 35.56 | 99.99 | 100 | 0.097 | 0.097 |
| 0 | 5 | 0.04+ | 38.56 | 99.92 | 99.99 | 0.089 | 0.089 |
| 0 | 10 | 0.02+ | 43.57 | 95.53 | 90.22 | 0.076 | 0.071 |
| 20 | 0.5 | 0.4+ | 11.06 | 96.44 | 87.03 | 0.301 | 0.271 |
| 20 | 1 | 0.2+ | 11.55 | 94.72 | 83.18 | 0.283 | 0.248 |
| 20 | 2 | 0.1+ | 12.55 | 88.98 | 75.32 | 0.244 | 0.207 |
| 20 | 5 | 0.04+ | 15.55 | 62.48 | 56.10 | 0.139 | 0.124 |
| 20 | 10 | 0.02+ | 20.54 | 40.33 | 37.93 | 0.068 | 0.064 |
| 50 | 0.5 | 0.4+ | 2.99 | 57.16 | 40.19 | 0.659 | 0.463 |
| 50 | 1 | 0.2+ | 3.49 | 40.72 | 32.20 | 0.402 | 0.318 |
| 50 | 2 | 0.1+ | 4.48 | 25.31 | 21.82 | 0.195 | 0.168 |
| 50 | 5 | 0.04+ | 6.77 | 11.70 | 3.01 | 0.060 | 0.015 |
| 50 | 10 | 0.02+ | 12.47 | 6.04 | 2.55 | 0.017 | 0.007 |
| 10 | 1 | 0.2* | 20.66 | 99.89 | 100 | 0.167 | 0.167 |
| 20 | 1 | 0.2* | 12.78 | 98.65 | 97.49 | 0.266 | 0.263 |
| 30 | 1 | 0.2* | 8.44 | 91.57 | 81.84 | 0.374 | 0.334 |
| 40 | 1 | 0.2* | 6.06 | 45.01 | 56.11 | 0.256 | 0.319 |
| 50 | 1 | 0.2* | 4.74 | 0 | 31.51 | 0 | 0.229 |
| 10 | 2 | 0.1* | 21.67 | 99.80 | 100 | 0.159 | 0.159 |
| 20 | 2 | 0.1* | 13.79 | 98.34 | 96.20 | 0.246 | 0.241 |
| 30 | 2 | 0.1* | 9.45 | 90.28 | 78.74 | 0.329 | 0.287 |
| 40 | 2 | 0.1* | 7.06 | 38.95 | 55.01 | 0.190 | 0.269 |
| 50 | 2 | 0.1* | 5.72 | 0 | 27.85 | 0 | 0.168 |
| 10 | 5 | 0.04* | 24.66 | 98.81 | 98.37 | 0.138 | 0.138 |
| 20 | 5 | 0.04* | 16.79 | 96.11 | 86.32 | 0.197 | 0.177 |
| 30 | 5 | 0.04* | 12.45 | 86.09 | 69.88 | 0.238 | 0.194 |
| 40 | 5 | 0.04* | 10.06 | 53.49 | 49.30 | 0.183 | 0.169 |
| 50 | 5 | 0.04* | 8.71 | 49.35 | 25.09 | 0.195 | 0.099 |
| 10 | 10 | 0.02* | 29.67 | 91.90 | 82.29 | 0.107 | 0.096 |
| 20 | 10 | 0.02* | 21.80 | 86.75 | 72.54 | 0.137 | 0.115 |
| 30 | 10 | 0.02* | 17.43 | 77.66 | 59.59 | 0.154 | 0.118 |
| 40 | 10 | 0.02* | 15.04 | 64.08 | 40.25 | 0.147 | 0.092 |
| 50 | 10 | 0.02* | 13.73 | 45.72 | 18.99 | 0.115 | 0.048 |

$V_i$ = Feed volume, dissolved in the mobile phase (+) or in water (*); $c_i$ = concentrations of phenol (P) and *o*-cresol (C) in the feed; $t_c$ = cycle time as the cut-off time of the fraction of *o*-cresol; $R$ = recovery yield; $Pr$ = prodution rate per effective column cross-section, in mmol $min^{-1}$ $cm^{-2}$. Column as in Table 1, flow-rate 1 ml/min.

is practically independent of the steepness of the gradient, but the increase in the production rate is more significant for steeper gradients. Fig. 11A and 11B compare the production rate under optimum isocratic conditions with gradient elution where the time necessary to recondition the column with the initial mobile phase is included into the cycle time (six column hold-up volumes). With these cycle times close to the practical separation conditions, the production rates for sample feeds 0.5–2 ml are similar in overloaded gradient elution as in isocratic elution and are 1.3- to 2.3-times higher for larger feeds of 5 and 10 ml. The best improvement in the production rates is obtained with the most steep, 5-min gradients.

However, if such cycle times are considered with steep (5 min) gradients starting at a higher (30%) concentration of methanol, the production rates at the

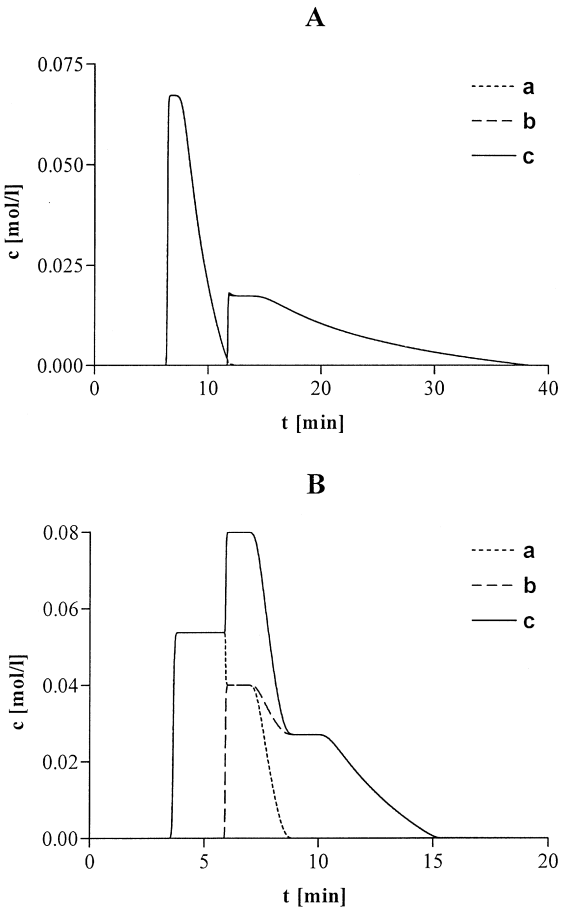


Fig. 5. Calculated concentration band profiles of phenol (first peak, a) and *o*-cresol (second peak, b) and sum of the profiles (c) in the overloaded isocratic separation of a 5-ml sample feed containing 0.04 mol/l (0.2 mmol) of each component dissolved in the mobile phase with water (A) and 20% methanol (B) as the mobile phase. Column and flow-rate as in Fig. 1. $c$ = Concentrations of sample compounds, $t$ = time from the start of the feed injection.

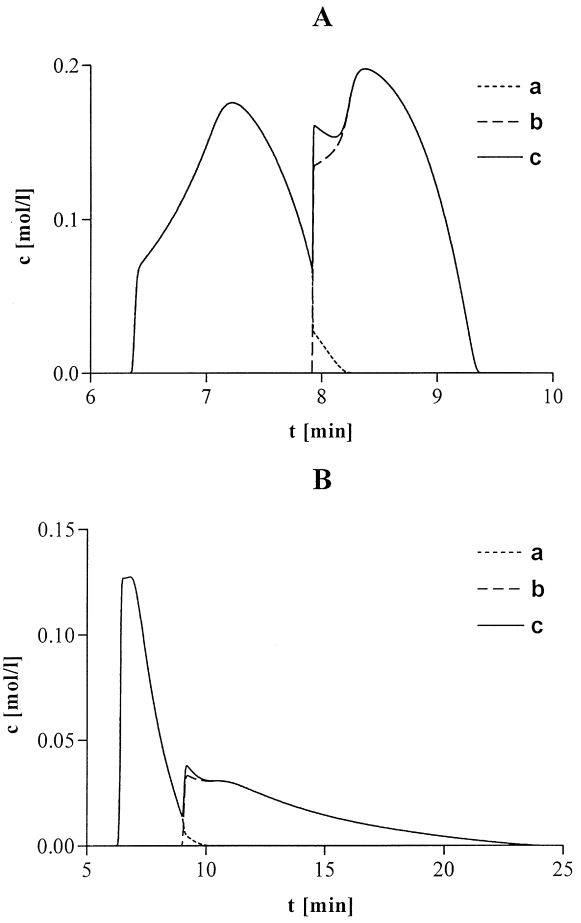


Fig. 6. Calculated concentration band profiles of phenol (first peak, a) and *o*-cresol (second peak, b) and sum of the profiles (c) in the overloaded separation of a 5-ml sample feed containing 0.04 mol/l (0.2 mmol) of each component dissolved in water using continuous gradient-elution from 0 to 100% methanol in water in 5 min (A) and isocratic elution with 10% methanol in water (B). Column and flow-rate as in Fig. 1. $c$ = Concentrations of sample compounds, $t$ = time from the start of the feed injection.

0.5 ml and 1 ml feeds are comparable to the isocratic separation and are lower for larger feed volumes. Moreover, the recovery yields are lower than under optimized isocratic conditions. This behavior is illustrated by curve e in Figs. 9–11.

If isocratic separations in 20% methanol with pure water and with mobile phase as the sample solvent are compared for various feed volumes (curve f in Figs. 9 and 10), the increase in the recovery yield with water as the sample solvent is at 1–10% lower in comparison with overloaded continuous gradient elution and the gain in the production rate is approximately at 10–20% worse. If overloaded continuous gradient elution with steep gradients is compared to the isocratic separation with water as the sample solvent, the recovery yield is better with continuous gradient elution, regardless of the feed volume. The production rate with lower feed volumes (1 and 2 ml) is slightly better for isocratic

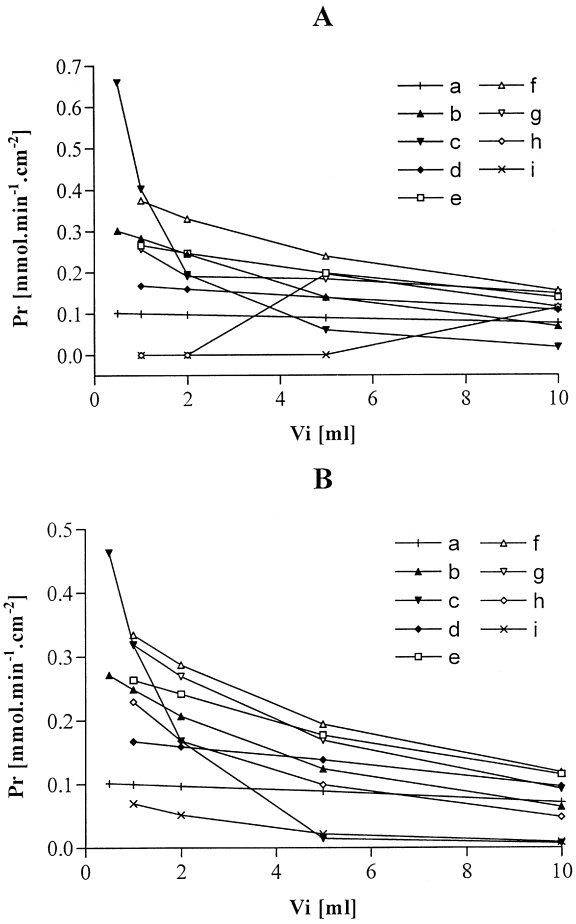


Fig. 7. Dependence of the production rate normalized to the column cross-section area, *Pr*, of phenol (A) and of *o*-cresol (B) on the feed volume, $V_i$, at a constant sum of loading factors (56% of the column saturation capacity) in overloaded isocratic separations of the sample feed dissolved in the mobile phase: water (a), 20% methanol (b), 50% methanol (c) and of the sample feed dissolved in water with 10% (d), 20% (e), 30% (f), 40% (g), 50% (h) and (60%) (i) methanol in water as the mobile phase. Other conditions as in Fig. 1.

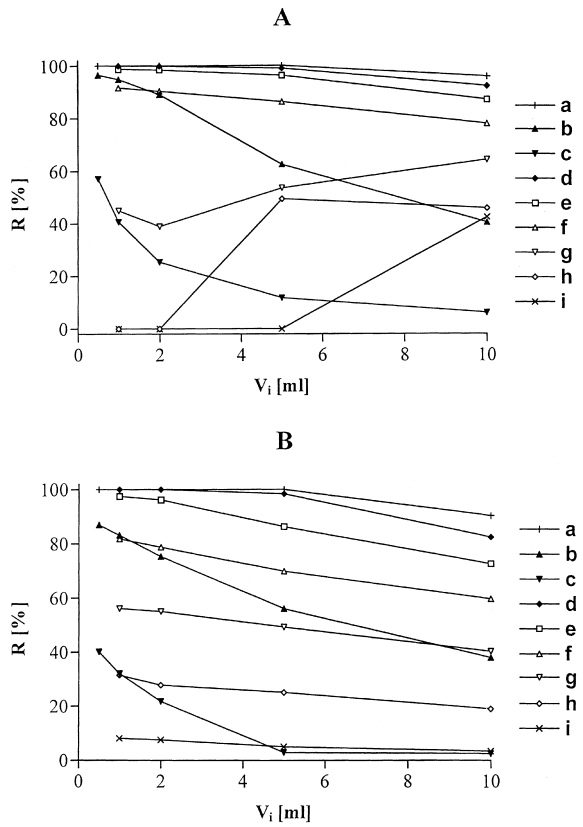


Fig. 8. Dependence of the recovery yield, *R*, of phenol (A) and of *o*-cresol (B) on the feed volume, $V_i$, at a constant sum of loading factors in overloaded isocratic separations of the sample feed dissolved in the mobile phase: water (a), 20% methanol (b), 50% methanol (c) and of the sample feed dissolved in water with 10% (d), 20% (e), 30% (f), 40% (g), 50% (h) and (60%) (i) methanol in water as the mobile phase. Other conditions as in Fig. 1.

separation, contrary to the separation of larger feed volumes (5 and 10 ml).

## 4. Conclusions

If the solubility limits the concentration in the sample feed, large feed volumes are required to increase the production rate. In such a case, overloaded gradient-elution chromatography offers the advantage of increasing the production rate and the recovery yield by the concentration focusing and the displacement effects. Large feed volumes are injected in a solvent with a weak elution strength and then the elution strength is continuously increased to obtain suitable separation factor and to speed-up the elution, which also contributes to increased production rate. The results of the present study with model phenolic compounds show that best improvement of the production rate in preparative separation can be accomplished using steep continuous gra-

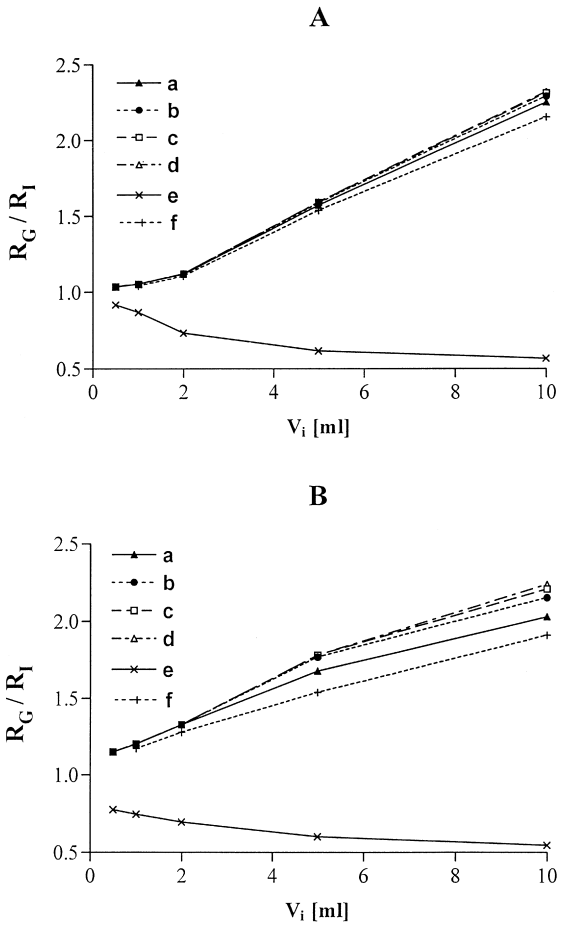


Fig. 9. Dependence of the ratio of the recovery yield under overloaded gradient-elution conditions, $R_G$, to the recovery yield in isocratic elution with 20% methanol in water, $R_I$, for phenol (A) and *o*-cresol (B) on the feed volume, $V_i$, at a constant sum of loading factors in overloaded gradient-elution from 0 to 100% methanol in water in 5 min (a), 10 min (b), 15 min (c), 20 min (d) and from 30 to 100% methanol in water in 5 min (e). Plot (f) applies to the ratio for the sample feed dissolved in water and in the mobile phase (20% methanol), both under isocratic conditions. Other conditions as in Fig. 1.

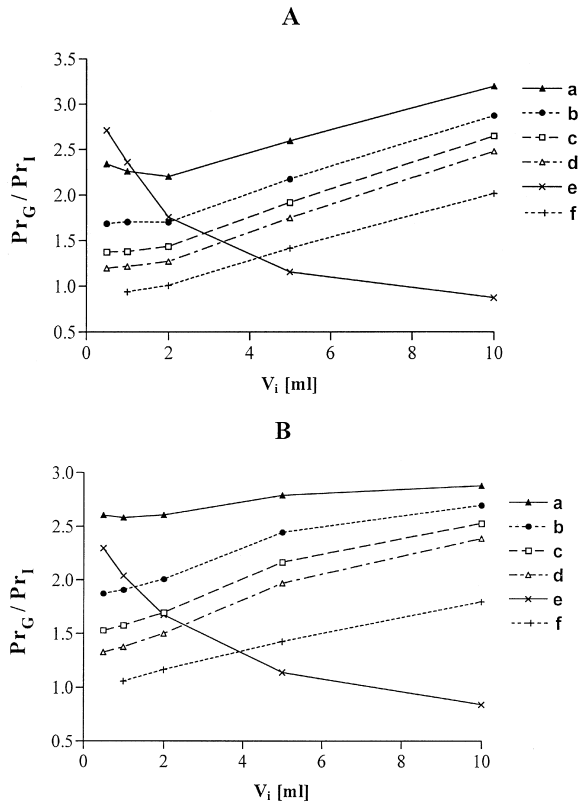


Fig. 10. Dependence of the ratio of the production rate normalized to the column cross-section area under overloaded gradient-elution conditions, $Pr_G$, to the production rate in isocratic elution with 20% methanol in water, $Pr_I$, for phenol (A) and *o*-cresol (B) on the feed volume, $V_i$, at a constant sum of loading factors in overloaded gradient-elution from 0 to 100% methanol in water in 5 min (a), 10 min (b), 15 min (c), 20 min (d) and from 30 to 100% methanol in water in 5 min (e). Plot (f) applies to the ratio for the sample feed dissolved in water and in the mobile phase (20% methanol), both under isocratic conditions. Other conditions as in Fig. 1. End of the cycle at the cut-off time of the second fraction.

dients, which compare favorably with the isocratic separations even if the time necessary for the reequilibration of the column after the end of the gradient is included into the cycle time. The production rate with large feed volumes can be also increased by using isocratic elution with sample feed dissolved in a weak solvent (water) instead in the mobile phase, but continuous gradients generally offer better improvement with respect to this technique. Although the present conclusions are probably applicable to various overloaded reversed-phase separations, more experimental results will be necessary to allow such a generalization.

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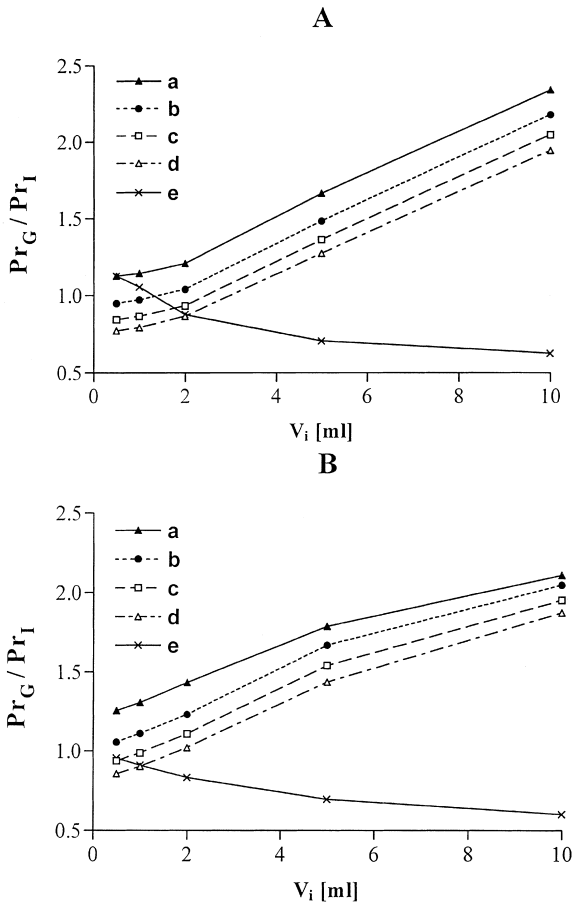


Fig. 11. Dependence as in Fig. 10, but with the end of the cycle after reequilibration of the column with the volume of the initial mobile phase equal to six column hold-up volumes.

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