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Dependence of the production rate on the relative retention of two components in preparative chromatography

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ABSTRACT

The maximum possible production rate which can be obtained with a given chromatographic system, under optimized conditions, increases approximately as the third power of $(\alpha-1)/\alpha$. The optimum conditions themselves depend also on the relative retention. In contrast, the production rate of a given column (constant column length, L, and particle size, d_p) increases more slowly, nearly in proportion to the square of $(\alpha-1)/\alpha$ when the ratio d_p^2/L is lower than its optimum value. These relationships are valid under both touching band and overlapping band conditions.

INTRODUCTION

There has been considerable interest recently in the optimization of the experimental conditions for maximum production rate, and hence in theoretical investigations of the dependence of the production rate on the parameters of a separation [1–6]. As in all chromatographic applications, the most critical factor controlling the difficulty of a separation is $\Delta(\Delta G^0) = \Delta G_2^0 - \Delta G_1^0$, the difference between the molar Gibbs free energies ΔG_1^0 and ΔG_2^0 of transfer from one phase to the other of the chromatographic system used, for the two components of a mixture. In linear chromatography [7]

$$\Delta(\Delta G^0) = -RT\ln\alpha \tag{1}$$

where α is the relative retention of the two components, *i.e.*, the ratio of their column capacity factors at infinite dilution, $\alpha = k'_{0,2}/k'_{0,1}$, or the ratio of the slopes of their equilibrium isotherms at the concentration origin, $\alpha = a_2/a_1$, and R and T are the ideal gas constant and the temperature, respectively. Although preparative chromatography is carried out at high concentrations, theory shows that the production rate depends on α and on the column saturation capacities for the two components [5,8,9].

Admittedly, most mixtures whose separation is of practical interest contain more than two components. A separation procedure may involve several successive chromatographic steps using different modes of chromatography. In addition to the separation of enantiomers for which it is directly relevant, the theory of binary mixture

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separations can be applied to the most important pairs of components of the mixture, including those involving the main compound to be purified and the impurities eluted close by. It gives critical information regarding the need for a multi-step procedure. Therefore, it is important to distinguish two separate problems, the dependence of the production rate on the relative retention of two components on a given column and the dependence of the maximum possible production rate on α. In the former case, the column characteristics are given. In the latter, these characteristics result from the optimization procedure and vary with α.

We briefly review here the relevant results. In both instances two separation objectives are discussed, touching bands and overlapping bands. The former permits a nearly total recovery yield, whereas the latter allows a higher production rate, but at the cost of recycling part of the throughput. In all the following, for the sake of simplicity, we make two assumptions. First, we assume with Knox and Pyper [1] that the reduced plate height equation of the column is $h = Cv$. It has shown previously [5,9,10] that, in order to achieve the maximum production rate with any constraint of recovery yield or fraction purity, preparative columns should always be operated at high reduced velocities (*i.e.*, at velocities between one and two orders of magnitude higher than the velocity providing the maximum column efficiency). Hence this assumption provides a very good and useful approximation. If needed, a simple numerical calculation permits the determination of the optimum conditions with any plate-height equation [5,9,10]. Second, we assume that the equilibrium isotherms of the two components follow the competitive Langmuir isotherm model [5,9,10].

MAXIMUM PRODUCTION RATE

In this section, we summarize previous results relating the maximum production rate to the column saturation capacities and α in the two extreme strategies, touching bands and overlapping bands. The column length, the particle size and the mobile phase velocity are optimized in both instances.

Touching bands

We have shown in a previous paper that the maximum production rate of the second component, Pr_2 (moles per unit time), is given by the following equation (ref. 9, eqn. 38):

$$\frac{Pr_2}{(1-\varepsilon)S} = (\text{constant}_1)\left[\frac{\alpha - 1}{\alpha}\right]^3 \frac{q_{s,2}}{1 + k'_{0,2}} \qquad (2)$$

where $q_{s,2}$ is the ratio a_2/b_2 of the two coefficients of the Langmuir isotherm of the second component. $q_{s,2}$ is the saturation capacity for the second component. The column saturation capacity is the product of $q_{s,2}$ and the amount of packing material in the column. In this equation, ε is the packing porosity and S the column cross-sectional area. The constant is independent of the isotherm parameters, but depends on the mass transfer parameters (molecular diffusion coefficient of solutes, mobile phase viscosity, C coefficient of the plate-height equation) and on the maximum pressure available. Eqn. 2 is correct if we ignore the competitive behavior of the two-component adsorption, as was done by Knox and Pyper [1]. It remains a good approximation if we assume that the equilibrium isotherms of the two components are given by the competitive Langmuir isotherm model, as we have done in our work. The value of the constant is different in the two cases, however. In the case where competitive interactions are neglected, it is

$$(\text{constant}_1) = \frac{1}{24}\sqrt{\frac{\Delta P D_m}{3\phi\eta C}} \tag{2a}$$

where ΔP is the maximum pressure available, D_m the molecular diffusion coefficient of the second component, ϕ the column-specific permeability, η the mobile phase viscosity and C the third coefficient of the plate-height equation. In the case where competitive interactions are taken into account the constant has a different numerical coefficient.

Eqn. 2 assumes that the cycle time, t_c, is equal to $t_{R0,2} - t_0$, where $t_{R0,2}$ and t_0 are the limiting retention time at infinite dilution and the hold-up time, respectively. With the value assumed by Knox and Pyper [1] and by Snyder *et al.* [6], $t_c = t_{R0,2}$, the equation becomes

$$\frac{Pr_2}{(1-\varepsilon)S} = (\text{constant}_1)\left[\frac{\alpha - 1}{\alpha}\right]^3 \frac{q_{s,2}k'_{0,2}}{(1+k'_{0,2})^2} \tag{3}$$

This equation provides for the dependence of the production rate on the isotherm parameters, and more specifically on the relative retention and the limit column capacity factor of the last component.

Overlapping bands

In a previous paper [4], we showed that the production rate is given by

$$\frac{Pr_2}{(1-\varepsilon)S} = \frac{R_2 q_{s,2} L_{f,2} u t_0}{t_c} \tag{4}$$

where u is the mobile phase velocity and $L_{f,2}$ is the loading factor for the second component, *i.e.*, the ratio of the amount of second component in the sample to the column saturation capacity. The recovery yield, R_2, is near 60% under the experimental conditions giving the maximum production rate, regardless of α [3,5]. The optimum loading factor for the second component is given by [4]

$$L_{f,2} = \frac{1}{1+y}\left[\frac{\alpha - 1}{\alpha(1-x)}\right]^2 \tag{5}$$

where

$$x = \sqrt{\frac{1 - Pu_2}{Pu_2 \alpha r_1}} \tag{6a}$$

and

$$y = \frac{q_{s,2}r_1}{\alpha q_{s,1}} \approx \frac{q_{s,2}C_{0,1}}{\alpha q_{s,1}C_{0,2}} \approx \frac{L_{f,1}}{\alpha L_{f,2}} \tag{6b}$$

Pu_2 is the required purity of the more retained component and r_1 is almost equal to the ratio of the concentrations of the two components in the feed, $C_{0,1}/C_{0,2}$. Eqn. 5 has been derived for the ideal model (infinitely efficient column). It has been shown that it remains valid also in the semi-ideal model, for real columns [5]. Although the use of the sample size given by eqn. 5 permits the achievement of the maximum possible production rate for a given purity requirement, the recovery yield will be merely *ca.* 60% [5], which may be considered too low. Higher recovery yields may be achieved by multiplying the sample size by $0.60/R_2$, where R_2 is the required recovery yield [10].

The linear velocity of the mobile phase is related to the column efficiency through the plate-height equation ($h = Cv$, with $H = hd_p$ and $v = ud_p/D_m$). On the other hand, it is related to the pressure drop, ΔP, the mobile phase viscosity, η, and the specific permeability, ϕ, by

$$u = \frac{\Delta P d_p^2}{\phi \eta L} \tag{7}$$

This leads to the following equation:

$$N_0 = \frac{L}{Cvd_p} = \frac{LD_m}{Cud_p^2} = \frac{\phi \eta D_m}{\Delta PC}\left[\frac{L}{d_p^2}\right]^2 \tag{8}$$

where N_0 is the column efficiency under linear conditions. Elimination of L/d_p^2 between eqns. 7 and 8 gives

$$u = \sqrt{\frac{\Delta P D_m}{\phi \eta C N_0}} \tag{9}$$

In analytical chromatography, the resolution between two bands is related to the number of theoretical plates, the relative retention and the column capacity factor by the classical Purnell equation:

$$R_s = \frac{\sqrt{N_0}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_{0,2}}{1 + k'_{0,2}} \tag{10}$$

Combination of eqns. 9 and 10 gives

$$u = (\text{constant}_2) \cdot \frac{\alpha - 1}{R_s \alpha} \cdot \frac{k'_{0,2}}{1 + k'_{0,2}} \tag{11}$$

with (constant$_2$) = $0.25\sqrt{(\Delta P D_m)/(\phi \eta C)}$. Combination of eqns. 4–6 and 11, with a cycle time equal to $t_{R0,2} - t_0$, gives

$$\frac{Pr_2}{(1-\varepsilon)S} = (\text{constant}_2)\left(\frac{\alpha-1}{\alpha}\right)^3 \frac{R_2 q_{s,2}}{R_s(1-x)^2(1+k'_{0,2})(1+y)} \tag{12a}$$

where R_s is the resolution between the bands of an infinitely dilute sample of the feed. The difference with the touching band case, previously discussed, is that R_s, x and y depend on α [5]. The dependence of x is especially strong when the relative concentration of the second component is high (*i.e.*, under conditions where the displacement effect predominates) [4].

If the cycle time is equal to $t_{R0,2}$, we have

$$Pr_2 = (\text{constant}_2)\left(\frac{\alpha-1}{\alpha}\right)^3 \frac{R_2 k'_{0,2} q_{s,2}}{R_s(1-x)^2(1+k'_{0,2})^2(1+y)} \tag{12b}$$

Eqns. 12a and 12b are valid for overlapping bands, with or without a recovery yield constraint. However, the value of the constant and of the necessary resolution depend on the required recovery yield. In the case of a required yield R_2^*, it becomes (constant$_2$ $\cdot$ $0.6/R_2^*$).

PRODUCTION RATE OF A GIVEN COLUMN

In many instances, the optimum column cannot be used, either because it is unavailable or, more generally, because it is impractical to order a new column for each new separation. The relevant equations can be summarized as follows.

Touching bands

In a previous paper on this topic, we have shown that the production rate is given by the following equation (ref. 9, eqn. 21):

$$Pr_2 = (\text{constant}_3)\frac{u q_{s,2}(\alpha-1)^2}{k'_{0,2}}\left\{2 - \frac{1}{\alpha} - \sqrt{4\cdot\frac{\alpha-1}{\alpha} + \frac{16}{N_0}\left[\frac{k'_{0,2}+1}{k'_{0,2}(\alpha-1)}\right]^2}\right\} \tag{13}$$

In this equation, N_0 is given by eqn. 8. Eqn. 13 is valid only for moderate values of the optimum loading factor (exactly when $L_{f,2}$ is negligible compared to $\sqrt{L_{f,2}}$), which usually corresponds to cases when α is close to unity. Eqn. 13 takes the competitive interaction of the two components with the stationary phase into account by using competitive Langmuir isotherms in the derivation. If we neglect this interaction with Knox and Pyper [1], we have (see ref. 9, eqn. 23):

$$\frac{Pr_2}{(1-\varepsilon)S} = \frac{u q_{s,2}}{4k'_{0,2}}\left[\left(\frac{\alpha-1}{\alpha}\right)^2 - \frac{16\left(\frac{k'_{0,2}+1}{k'_{0,2}}\right)^2}{N_0}\right] \tag{14}$$

From the Purnell equation [11], we see that the production rate is positive and different from zero only if the analytical resolution is larger than unity. Given the assumptions of the touching band case, this is an expected result.

We have shown previously, in agreement with Knox and Pyper [1], that there is an optimum value for the ratio d_p^2/L as long as the HETP equation is limited to its third term ($h = Cv$; *cf.*, eqn. 8) [5,9]. If the column characteristics (*i.e.*, its length and the average particle size of the packing) are such that the value of the ratio d_p^2/L is smaller than the optimum value, we cannot operate that column at the optimum mobile phase velocity: the maximum pressure available is not high enough. In this case, the velocity is given by eqn. 7, the plate number, N_0, is given by eqn. 8 and, according to eqns. 13 and 14, the production rate exhibits a primary dependence on $[(\alpha - 1)/\alpha]^2$. Eqns. 13 and 14, however, include corrective terms which may lead to a different dependence in some instances.

If, on the contrary, the column characteristics are such that the ratio d_p^2/L is larger than the optimum value for maximum production rate, there is an optimum mobile phase velocity. This optimum, however, corresponds to an inlet pressure which is lower than the maximum available. The production rate increases nearly in proportion to $[(\alpha - 1)/\alpha]^3$. The decision as to whether to change the column depends in part on the possible gain in production rate. This gain is approximately proportional to the square root of the ratio of the maximum available pressure to the operating pressure of the column considered.

Overlapping bands

Eqn. 4 is also valid in this case if we use the optimum value of the sample size, corresponding to the maximum production rate possible with the given column. Combination with eqn. 5 and a cycle time of $t_{R0,2} - t_0$, gives

$$\frac{Pr_2}{(1 - \varepsilon)S} = \frac{uR_2 q_{s,2}}{(1 + y)k'_{0,2}}\left[\frac{\alpha - 1}{\alpha(1 - x)}\right]^2 \tag{15}$$

As the column is given, L and d_p are now constant. If these values are such that the ratio d_p^2/L is smaller than the optimum value (corresponding to the maximum possible production rate), we cannot operate this column at its optimum velocity: the pressure would exceed the maximum acceptable. The velocity is given by eqn. 7. Then the production rate is approximately proportional to $[(\alpha - 1)/\alpha]^2$, because x, y and R_2 depend also on α.

If the column length and the particle size are such that the ratio d_p^2/L is larger than the optimum value, the column can be operated at its optimum velocity, but this velocity corresponds to a pressure lower than the maximum available. The maximum production rate which can be obtained with this column increases approximately in proportion to $[(\alpha - 1)/\alpha]^3$. As the column is operated at a pressure lower than the maximum possible, the production rate achieved is not the largest possible. The decision regarding whether the column should be changed or not depends on the economics of the choice.

DISCUSSION

Whether a touching band or an overlapping band strategy is adopted, the production rate which can be achieved with a given column is proportional at least to the square of $(\alpha - 1)/\alpha$ and sometimes to the cube of this ratio. This is a strong dependence [5].

In contrast, the maximum production rate which can be achieved with an optimized column, designed to be operated at the maximum pressure available, increases still more rapidly (see Table IV in ref. 5), as the third power of $(\alpha - 1)/\alpha$, a result which happens to be in agreement with previous work [6]. This means that the maximum production rate doubles when α is increased from 1.1 to 1.13 or from 1.2 to

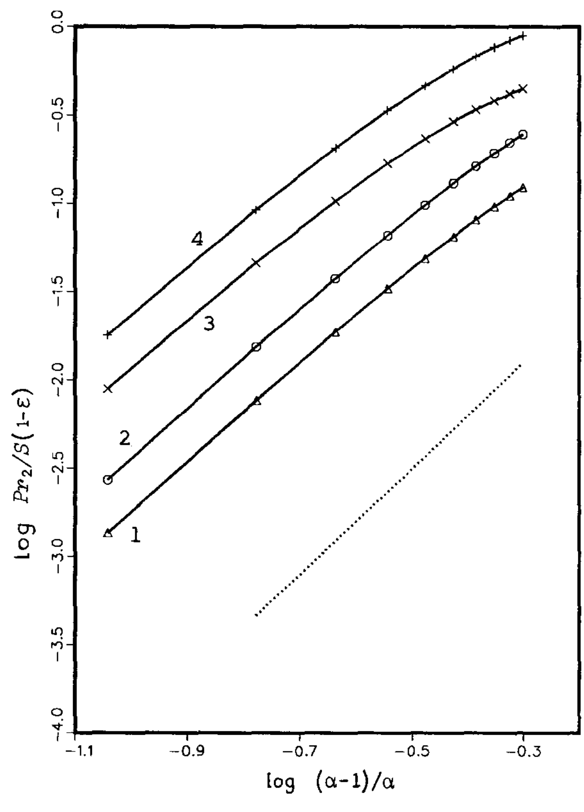

Fig. 1. Plot of the logarithm of the maximum possible production rate per unit column cross-sectional area $[Pr_2/S(1 - \varepsilon)]$ with a given chromatographic phase system *versus* the logarithm of $(\alpha - 1)/\alpha$. The column length and the optimum mobile phase velocity are optimized for each value of α. (The values of α corresponding to the abscissa markers, *i.e.*, $\log[(\alpha - 1)/\alpha]$ equal to -1.1, -0.9, -0.7, -0.5 and -0.3, are 1.086, 1.144, 1.25, 1.46 and 2.0, respectively.) Relative composition of the feed, 1:9; phase ratio, 0.25; column capacity factor, $k'_{0,1} = 6$; column saturation capacity, $q_{s,1} = q_{s,2} = 10$; eluent viscosity, $\eta = 1$ cP; molecular diffusion coefficient, $D_m = 1 \cdot 10^{-5}$ cm^2/s; plate-height equation, $h = 0.1\nu$; cycle time, $t_{R0,2} - t_0$. Curve 1: touching bands, $\Delta P = 50$ atm. Curve 2: touching bands, $\Delta P = 200$ atm. Curve 3: overlapping bands, $\Delta P = 50$ atm. Required degree of purity: 99%. The recovery yield is about 60%. Curve 4: overlapping bands, $\Delta P = 200$ atm. Required degree of purity: 99%. The recovery yield is about 60%. Dotted line: a straight line with a slope equal to 3.

1.27 or from 1.4 to 1.56. The production rate is increased ten-fold if α increases from 1.05 to 1.11 or from 1.1 to 1.24 or from 1.2 to 1.56. If an adjustment of the mobile phase composition permits an increase in α from 1.1 to 1.3, the production rate can be multiplied by 16. These numbers illustrate how the choice of the stationary phase and of the composition of the mobile phase are of paramount importance.

The production rates and their relative retention dependence are different in the four situations discussed here. To illustrate these differences, Figs. 1 and 2 show plots of the production rate versus the ratio $(\alpha - 1)/\alpha$ in double logarithmic coordinates. A production rate dependence on the square or cube of this ratio would result in straight lines with slopes of 2 or 3, respectively, as indicated by the dotted line in Fig. 1 and the two dotted lines in Fig. 2. The actual dependences are as follows.

In Fig. 1, we have plotted the maximum production rate which can be achieved with a given chromatographic system (*i.e.*, for a given value of α) *versus* the ratio

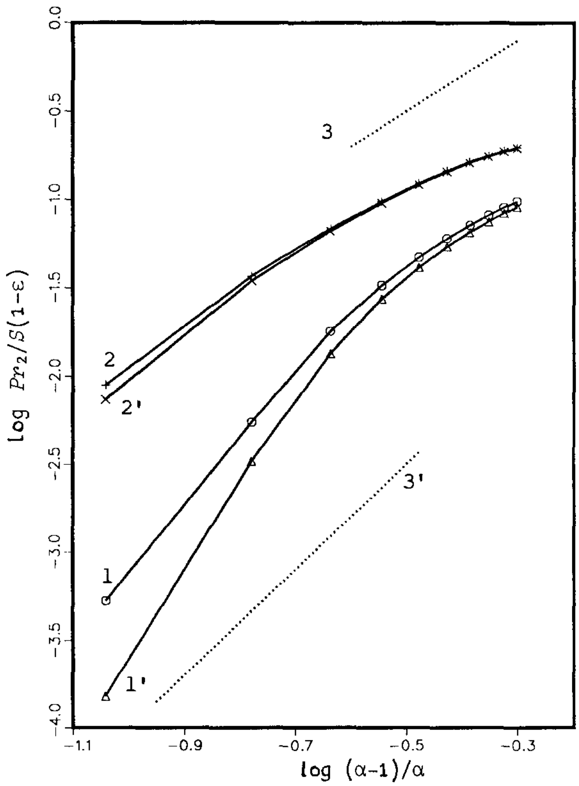

Fig. 2. Plot of the logarithm of the maximum production rate per unit column cross-sectional area $[Pr_2/S(1 - \varepsilon)]$ with a given chromatographic phase system *versus* the logarithm of $(\alpha - 1)/\alpha$ for a given column. Same conditions as in Fig. 1, except column length 30 cm and particle size 20 μm. Curve 1: touching bands, $\Delta P = 50$ atm. Plate-height equation: $h = 0.1\nu$. Curve 1′: touching bands, $\Delta P = 50$ atm. Plate-height equation: $h = 2/\nu + \nu^{0.33} + 0.1\nu$. Curve 2: overlapping bands, $\Delta P = 50$ atm. Required degree of purity: 99%. Plate-height equation: $h = 0.1\nu$. Curve 2′ = overlapping bands, $\Delta P = 50$ atm. Required degree of purity: 99%. Plate-height equation: $h = 2/\nu + \nu^{0.33} + 0.1\nu$. Dotted lines: 3, a straight line with a slope equal to 2; 3′, a straight line with a slope equal to 3.

$(\alpha - 1)/\alpha$. In this case, the column length and the mobile phase velocity are optimized for each value of α. We have considered both the overlapping and the touching band conditions. For each condition, two curves correspond to two different values of the inlet pressure, 50 and 200 atm, respectively. Eqns. 4 and 13 show that for each condition, the maximum production rate is proportional to the velocity used, whereas eqn. 9 shows that this velocity is proportional to the square root of the maximum pressure available. Hence the two curves which correspond to the same condition are shifted by log 2. As can be seen in Fig. 1, the production rate increases nearly as the cube of $(\alpha - 1)/\alpha$ at values of α close to one, and more slowly when α becomes large.

In Fig. 2, we have plotted the maximum production rate which can be achieved with a given column. The choice of the exact conditions (*i.e.*, $L = 30$ cm and $d_p = 20$ μm) is more arbitrary than in the previous instance. In Fig. 2, we show two examples, corresponding to touching band (curves 1 and 1′) and overlapping band (curves 2 and 2′) conditions. In both instances we carried out the calculations using the complete Knox plate-height equation [12] and a simplified plate-height equation. The difference between the results obtained with these two plate-height equations is very small, except at values of the relative retention close to unity, in the touching band case. This is because at values of α close to 1 a high column efficiency is needed and the reduced velocity is low, *e.g.*, 12 for $\alpha = 1.10$.

We observe in Fig. 2 that in both the touching and the overlapping band cases, the production rate at low values of α increases more rapidly with increasing α than what would correspond to the square of $(\alpha - 1)/\alpha$ (see the dotted lines). This is because in this region the column characteristics are such that d_p^2/L is larger than the optimum value of this ratio for the maximum production rate. We must operate the column at a pressure which is below the maximum allowed by the equipment. When α increases, the optimum value of the ratio d_p^2/L increases, the column can be operated at an increasingly high velocity, corresponding to an inlet pressure closer and closer to the maximum available and the production rate increases very rapidly. The dimensions of the column chosen for this discussion give a value of d_p^2/L which is optimum for a relative retention equal to *ca.* 1.4. For this value, curves 1 and 2 in Fig. 2 are tangential to curves 1 and 3 in Fig. 1, respectively. For higher values of α, the ratio d_p^2/L becomes smaller than the optimum, the column must be operated at the maximum available pressure, but the corresponding velocity is below the optimum value and the production rate increases more and more slowly with increasing value of α, approximately as $[(\alpha - 1)/\alpha]^2$.

These results are only valid, however, as far as one critical assumption remains satisfied. We have assumed all along that the column saturation capacity is unchanged by the adjustments made to the composition of the mobile phase. When we compare the production rates for different separations, we tend to emphasize the role of α and to play down the role of the column saturation capacity. Both are important. For example, in the separation of enantiomers on chiral phases, we have observed that the production rate is orders of magnitude smaller than that achieved with less closely related isomers with a comparable relative retention [13]. The column saturation capacity for the chiral recognition sites of immobilized bovine serum albumin is very much smaller than the capacity for the non-specific interaction sites. There are reported cases when the column saturation capacity and the relative retention of proteins vary with the mobile phase composition in opposite directions [14].

Obviously, in such cases the optimum mobile phase composition for preparative chromatography will be different from that for analytical separation.

Finally, we know that most users of preparative chromatography prefer normal-phase to reversed-phase chromatography. The capacity of the mobile phase is higher with the former, because the solubility of most organics is higher in organic solvents than in water-rich solutions. The use of an additive which causes a large increase in the mobile phase solubility of the feed components while decreasing slightly the relative retention may lead to a higher production rate.

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REFERENCES

1 J. H. Knox and H. M. Pyper, *J. Chromatogr.*, 363 (1986) 1.
2 G. Guiochon and H. Colin, *Chromatogr. Forum*, 1, No. 3 (1986) 21.
3 S. Ghodbane and G. Guiochon, *Chromatographia*, 26 (1988) 53.
4 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 61 (1989) 1276.
5 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 61 (1989) 1368.
6 L. R. Snyder, J. W. Dolan and G. B. Cox, *J. Chromatogr.*, 483 (1989) 63.
7 B. L. Karger, *Anal. Chem.*, 39 (1967) 24A.
8 A. W. J. de Jong, H. Poppe and J. C. Kraak, *J. Chromatogr.*, 148 (1978) 127.
9 S. Golshan-Shirazi and G. Guiochon, *J. Chromatogr.*, 517 (1990) 229.
10 S. Golshan-Shirazi and G. Guiochon, *J. Chromatogr.*, in press.
11 J. H. Purnell, *J. Chem. Soc.*, (1960) 1268.
12 J. H. Knox, *J. Chromatogr. Sci.*, 15 (1977) 352.
13 S. Jacobson, S. Golshan-Shirazi and G. Guiochon, *J. Am. Chem. Soc.*, 112 (1990) 6492.
14 J.-X. Huang, J. V. H. Schudel and G. Guiochon, *J. Chromatogr. Sci.*, in press.