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Optimization of experimental conditions in preparative liquid chromatography

Trade-offs between recovery yield and production rate

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ABSTRACT

The optimization of the experimental conditions in preparative chromatography under constraints of recovery yield, product purity and maximum available pressure is discussed. It is shown that there are optimum values of the loading factor and the column limit efficiency which permit the achievement of the maximum production rate under specified constraints of recovery yield and product purity. The optimum loading factor is given by a simple equation. The optimum column efficiency is calculated from numerical solutions of the semi-ideal model. The optimum column length for a given packing material and particle size and the optimum mobile phase velocity are then derived. These optimum values depend on the maximum available pressure, and the production rate increases rapidly with increasing pressure. If a given column is available and it is shorter than the optimum length, it should be operated at the optimum loading factor and at the optimum column efficiency. The mobile phase velocity, and hence the production rate, are less than those for the column of optimum length. If the column is longer than the optimum and cannot be cut, it should be operated at the maximum available pressure, at a mobile phase velocity lower than the optimum and with a loading factor larger than the optimum.

INTRODUCTION

The optimization of experimental conditions in preparative chromatography is a topic of great current interest. This process of separation or purification is expensive. Colin [1] has shown that the main contributions to that cost are, in order of decreasing importance, (i) the mobile-phase components (solvent, additives), including solvent losses and solvent regeneration; (ii) the amortization of the instrument; (iii) the periodic replacement of the stationary phase; (iv) labor; (v) other items, including energy, instrument maintenance and site preparation. Hence, it is important to maximize the production rate of a given instrument–column combination. Obviously, this requires the operation of the process at high concentrations, under non-linear conditions. When the column is "overloaded", bands interfere and overlap. However,

because the displacement effect [2–6] enhances the separation between adjacent bands, high production rates of highly pure fractions may be achieved under these "overlapping band" conditions. The inconvenience of this mode of operation is similar to one of the major difficulties encountered in displacement chromatography, where the recovery yield becomes significantly smaller than unity.

This may be of limited concern for the isolation or purification of low- or medium-molecular-weight chemicals whose conformers exchange rapidly, as opposed to biopolymers. The recovery yield for given injection of the feed of such products may be moderate as long as this permits a significant increase in production rate (or rather a decrease in production costs) over "touching band" conditions. The intermediate fractions can be recycled, and the final recovery yield can be extremely high. The operation is profitable as long as the additional processing costs are offset by the revenue brought by the increase in the production rate. On the other hand, some peculiarities of the specific problem considered may be important and prevent or drastically limit this mode of operation. For proteins, the risk of a change in conformation, and hence in biological activity, is considered so serious that regulatory agencies require batch certification for the fractions which would have to be reprocessed. Given the cost and inconvenience of this procedure, intermediate fractions are better wasted. This results in the formulation of an additional constraint in the optimization development, a recovery yield constraint, which is added to the purity constraints.

Knox and Pyper [7] were the first to discuss the optimization of experimental conditions in preparative liquid chomatography. They addressed the touching-band case for a two-component mixture which they discussed thoroughly, making two critical assumptions, however. First, they ignored the competition between the two components for interaction with the stationary phase and considered the behavior of the two bands to be independent. Second, they assumed that the band profiles are right triangles, which is equivalent to replacing the equilibrium isotherms by their first two-term expansion (parabolic isotherm). This assumption has been rightly criticized by Snyder *et al.* [8], who nevertheless used it implicitly in their reformulation of the Knox and Pyper calculations [9]. Within the framework of their assumptions, Knox and Pyper [7] were able to calculate the sample size needed to achieve touching-band conditions and from there to derive the optimum values of the experimental parameters. They predicted that the optimum column should give a resolution of 1.7 between the peaks of the two components under linear conditions (sample size corresponding to a very small value of the loading factor). They also predicted an optimum value for the ratio d_p^2/L, where d_p is the average particle diameter and L the column length.

Recently, we published a theoretical analysis of the optimization problem for a binary mixture in the overlapping-band case (no yield constraint) [10,11] and in the touching-band case [12]. The practical consequences of this work have been explained in detail in several publications [13–17]. The procedures for the determination of the experimental conditions have been described and illustrated [10–12].

These analyses take into account the competition between the two components by assuming competitive Langmuir isotherms. We know that this isotherm does not account exactly for the competitive interaction between the components of a mixture, but that it predicts properly the trends and gives at least a good semi-quantitative

estimate of the individual band profiles and hence of the production rate[a] and recovery yield[a] [6,18,19]. In the case of touching bands [12], we have shown that the influence of the competitive interaction between the two components changes slightly the optimum column characteristics (optimum length or particle size) or the optimum mobile-phase flow velocity. On the other hand, the optimum loading factor, and hence the maximum production rate, are considerably different from the values predicted by Knox and Pyper [7]. Depending on the feed composition, they may be dramatically increased or decreased. In the case of overlapping bands, an equation was derived between the optimum sample size and the required purity [10]. The optimum operating conditions were related to the characteristics of the separation problem studied [11]. It was shown that allowing the collection of slightly impure (e.g., 99% pure) fractions permits a large increase in the production rate when the second-eluted component is in excess [10,11].

Our previous work, however, considered only two extreme cases, 100% recovery yield (touching-band case) and maximum possible production rate, which results in a low recovery yield (ca. 60%). In this paper, we discuss the influence of a recovery yield constraint on the optimization of these experimental conditions.

THEORY

Summary of previous results

Consider first a two-component mixture, within the framework of the ideal model[b] [10]. The production rate increases in proportion to the sample size until, beyond the touching-band condition, a chromatogram is obtained such that a cut at the retention time of the second component shock (which gives a 100% yield) gives a fraction having exactly the required degree of purity, Pu_2. If we continue to increase the amount of feed injected at each cycle, the production rate remains constant and the recovery yield decreases. The optimum sample size for which this maximum production rate is achieved is such that the corresponding value of the loading factor for the second component, $L_{f,2}^*$, is given by the following equation [10]:

$$L_{f,2}^* = \frac{\left[\dfrac{\alpha-1}{\alpha(1-x)}\right]^2}{(1+b_1 r_1/b_2)} \tag{1}$$

where α is the relative retention of the two compounds ($\alpha = a_2/a_1 = k'_{0,2}/k'_{0,1}$), the coeficient a_i being the first coefficient of the equilibrium isotherm of component i and $k'_{0,i}$ is its retention factor, b_i is the second coefficient of the single-component Langmuir isotherm of component i and x is given by

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[a] The production rate is the amount of the corresponding component in the purified fraction at the required degree of purity which is produced per unit time. The recovery yield is the ratio between the amount of the component of interest that is collected in the product fraction and the amount injected in the column with the feed.

[b] The ideal model assumes that the column efficiency is infinite. In practice, although real columns have a finite efficiency, many results of the ideal model can be applied directly or with only minor corrections.

$$x = \sqrt{\frac{1 - Pu_2}{Pu_2 \alpha r_1}} \tag{2}$$

For touching bands, the purity of the collected fraction is 100%, so $x = 0$ in eqn. 1. Finally, r_1 is the root of the characteristic equation of the problem [20], which has been shown to be practically equal to C_1^0/C_2^0 [3], where C_i^0 is the concentration of component i in the feed.

Thus, eqn. 1 can be rewritten as

$$L_{f,2}^* = \frac{\left[\dfrac{\alpha - 1}{\alpha(1 - x)}\right]^2}{1 + \dfrac{q_{s,2}C_{0,1}}{\alpha q_{s,1}C_{0,2}}} \tag{3}$$

where $q_{s,i} = a_i/b_i$ is the adsorbent saturation capacity for component i. The sample size, n, is given by

$$n = L_{f,2}^*(1 + C_1^0/C_2^0)(1 - \varepsilon)SLq_{s,2} \tag{4}$$

where ε is the packing porosity, S is the column geometrical cross-sectional area and L is the column length. The sample size given by eqns. 3 and 4 gives the maximum production rate and a total recovery yield in the ideal model.

We have shown that for real columns whose efficiency is finite, the optimum sample size for maximum production rate is still given by eqns. 3 and 4 [11]. However, the recovery yield achieved with this optimum sample size, which was total with an ideal column, is now less than 100%. It depends on the column efficiency, increasing with the column limit efficiency (i.e., the efficiency measured at very small loading factors) and tending towards unity when the efficiency becomes infinite. The column efficiency, in turn, depends on the mobile phase velocity, u, which turns out to control both the cycle time (proportional to $1/u$) and the recovery yield. Accordingly, there will be an optimum value of the mobile phase flow velocity at which the production rate is maximum. It turns out that this mobile phase velocity corresponds to a very high value of the reduced velocity (unless the separation is extremely difficult, with α values below 1.05) and that the corresponding recovery yield is around 60% in about all instances [11].

As suggested by Knox and Pyper [7], there is an optimum value for the ratio d_p^2/L the value of which depends on the recovery yield [11]. However, the demonstration of this result [7,11] assumes a simplified column plate height equation ($h = Cv$). For a general plate height equation (e.g., the Knox equation), this result remains valid as a first approximation at high velocities, i.e., for all but the most difficult separations (e.g., with $\alpha \ll 1.1$, and when small particles are used). Thus, if we introduce a recovery yield constraint, as we are going to do here, the optimum column will change with the recovery yield constraint chosen. The fact that there is an optimum value for the ratio d_p^2/L but no separate optimum value of the particle size and the column length means that we can chose arbitrarily one of these parameters and adjust the other. For example, if the optimum value of d_p^2/L is 10 (d_p in μm, L in cm), we can take a 10-cm

long column packed with 10-μm particles or a 40-cm long column packed with 20-μm particles. With the latter column, both the cycle time and the sample size will be four times larger than with the former, so the production rate will remain the same. However, from the point of view of production rate optimization, we could as well chose a 0.9-cm long column packed with 3-μm particles or a 250-cm long column packed with 50-μm particles. Neither of these columns would be very practical.

As in our previous work, we have neglected here the extra-column effects which may modify the band shape and the separation. The interaction between these sources of band broadening and the band profile in non-linear chromatography has been discussed recently [21]. It is easier in preparative than in analytical chromatography to design injection systems, connecting tubes and detectors with a volume that is small compared with that of the column. However, the feed volume injected is always large. The influence of the feed volume on the band profile has also been discussed [22]. It has been found to be small compared with that of the non-linear effects (displacement and tag-along effects).

Statement of the optimization problem

The primary aim of this work was the determination of the optimum experimental conditions for the maximum production rate of the second-eluted component of a binary mixture, at a given degree of purity, with a certain recovery yield. The second aim was an investigation of the trade-offs between production rate and recovery yield.

In this discussion, we assume that the chromatographic system has already been selected. Thus, the retention factors, k'_i, of the two components are constant, in addition to the relative retention, α, and the column saturation capacities, $q_{s,i}$, for the two components, their molecular diffusion coefficients and the coefficients of the Knox plate height equation [23]. Finally, we assume that the chromatograph used cannot be operated above a certain inlet pressure, ΔP.

In previous papers we have already discussed the influence on the production rate of the relative retention [17] and of the maximum available inlet pressure [11,16] and shown that the maximum production rate always increases rapidly with increasing values of these two parameters.

General equations used

For the equilibrium isotherms, we used the classical competitive Langmuir equation [24]:

$$q_i = \frac{a_i C_i}{1 + b_1 C_1 + b_2 C_2} \tag{5}$$

where q_i and C_i are the concentrations of component i in the stationary and the mobile phases (both in mol/l) at equilibrium and a_i and b_i are numerical coefficients. All of the calculations presented here were performed for binary mixtures of variable compositions of two components and a maximum available pressure of 100 bar, except for a study of the influence of the operating pressure. In most instances, the relative retention, α, is 1.20.

For the plate height equation, we used the classical Knox equation [23]:

$$h = \frac{B}{v} + A v^{1/3} + C v \tag{6}$$

where $h = H/d_p$ is the reduced plate height, *i.e.*, the ratio of the actual column plate height to the average particle size, and $v = ud_p/D_m$ is the reduced mobile phase velocity or Peclet number for the particle size. In the calculations the results of which are reported here, the coefficients A, B and C are 1, 2 and 0.1 respectively.

To calculate the relationship between the mobile phase flow velocity, the inlet pressure and the column characteristics, we use the classical Darcy equation and its well known relatives:

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

$$v = \frac{\Delta P d_p^3}{\phi \eta D_m L} \tag{7b}$$

where η is the mobile phase velocity, D_m is the component diffusivity in the mobile phase and ϕ is the column hydrodynamic resistance factor (*ca.* 1000).

Optimization strategies

The recovery yield depends on the degree of band overlap, determined by both the loading factor (which controls the degree of column overloading) and the column efficiency (which controls the thickness of the shock layers and increases the band width beyond the value predicted by the ideal model). The production rate for the second component is [10]

$$\frac{Pr_2}{(1 - \varepsilon)S} = \frac{uL_{f,2}R_2 q_{s,2}}{k'_{0,2}} \tag{8}$$

In this equation, we assume that the cycle time is $t_c = k'_{0,2}t_0$, the time spent by the last-eluted fraction of the second component in the stationary phase. If another value of the cycle time is used, eqn. 8 should be multiplied by $(t_{R,0,2} - t_0)/t_c$. R_2 is the recovery yield for the second component and ε is the packing porosity. Note that the production rate is proportional to the loading factor and independent of the column length, because with a constant column cross-sectional area, the loading factor corresponding to a given sample size is inversely proportional to the column length. The values of the production rate (Pr_2) and the recovery yield (R_2) are related to the experimental conditions.

Many combinations of mobile phase flow velocity and loading factor may result in the same value of the recovery yield, and we need to find out rapidly which combination gives the highest production rate. It is impossible to find out rapidly the exact combination of parameter values giving the maximum production rate for a given fraction purity and a given recovery yield. However, it is possible to derive rapidly approximate values which are close enough to the optimum values for practical purposes. We present here four different methods, which are all simple, compare them and choose the most effective among them. With the first two methods, we start from the experimental conditions giving the maximum possible production rate and a recovery yield of *ca.* 60%, and we vary one of the operational parameters, the sample size or the mobile phase velocity.

Optimization strategy I. We can keep the sample size constant, at the value given by eqns. 3 and 4, and decrease the mobile phase velocity to increase the column efficiency and the recovery yield until the constraint is met. In so doing, we decrease the production rate, because R_2 will increase much more slowly than the cycle time, *i.e.*, $1/u$.

Eqns. 7 and 8 in ref. 11, which give the recovery yield and the production rate, are valid as long as the sample size is given by eqns. 3 and 4. Thus, in this approach, we can use them to calculate Pr_2 and R_2 (Note: in these equations there is an unfortunate typographical error [11,25]; the correct equations are given in ref. 25 and are used here). Accordingly, the constraint on the recovery yield introduces a relationship between the parameters of the separation, and the required limit column efficiency is obtained by solving the combination of eqn. 5 and the correct eqn. 7 in ref. 11 [25]:

$$N_0 = \frac{16(1 + k'_{0,2})^2}{k'^2_{0,2} w^2_{2,\text{th}}} \left(\left\{ \frac{\alpha - 1}{\alpha(1 - x)} (1 - \sqrt{R_2}) \left[2 - \frac{\alpha - 1}{\alpha(1 - x)} (1 + \right. \right. $$

$$ \left. \left. + \sqrt{R_2}) \right] \frac{\gamma}{w_{2,\text{th}}} + 1 \right\}^2 - 1 \right)^{-1} \qquad (9)$$

where R_2 is the required recovery yield and $w_{2,\text{th}}$ and γ are given by the following relationships:

$$w_{2,\text{th}} = \frac{(\alpha - 1) [1 + \alpha + 2q_{s,2}C_{0,1}/(q_{s,1}C_{0,2})]}{\alpha^2 [1 + q_{s,2}C_{0,1}/(\alpha q_{s,1}C_{0,2})]^2} \qquad (10a)$$

and

$$\gamma = \frac{1 + q_{s,2}C_{0,1}/(q_{s,1}C_{0,2})}{1 + q_{s,2}C_{0,1}/(\alpha q_{s,1}C_{0,2})} \qquad (10b)$$

The calculation of N_0 requires only the knowledge of the single-component isotherm coefficients readily obtained by the retention time method[a] [26,27]. We have shown that the production rate always increases with increasing inlet pressure [11,12,16]. Hence the column must be operated at the maximum pressure available, and its efficiency must be N_0. Thus the optimum column length at fixed particle size (or, conversely, the optimum d_p for a given L) is derived from eqns. 6 and 7b. The optimum column length (or particle size) depends on the recovery yield required (see eqn. 9).

Eqn. 9 shows that, for any required value of the recovery yield, there is an optimum limit column efficiency, N_0, which depends also on the required purity of the fractions, the relative composition of the feed and the isotherm parameters (α, $q_{s,i}$ and $k'_{0,2}$). The existence of an optimum value of N_0 is a general result, although eqn. 9 has been derived for a loading factor given by eqn. 3. If another loading factor must be

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[a] The retention time method uses the analytical equation of the single-component band profile in the case of a Langmuir isotherm to determine the parameters of this isotherm.

used, there is still an optimum column efficiency for any required yield. However, this efficiency must be calculated by using the solution of the semi-ideal model.

Optimization strategy II. We can keep the mobile phase flow velocity constant, and hence the rate constant of the mass transfer kinetics and the column limit efficiency, and reduce the sample size until the recovery yield constraint is met. This approach will give the lowest production rate that can be achieved while maintaining a certain value of the recovery yield.

The column efficiency, and hence the column length and reduced flow velocity, are kept constant and equal to those giving the maximum possible production rate. These values are those calculated for the maximum possible production rate (no yield constraint) and are given by eqns. 5, 7 and 8 in ref. 11. The sample size must be reduced from the value given by eqns. 3 and 4 until the required yield is achieved. A series of successive individual profile computations permits the rapid calculation of the loading factor for which the yield is equal to the stated value of the constraint.

Optimization strategy III. Obviously, neither of the above two approaches will give the maximum possible production rate at the requested recovery yield. To achieve this result, both the parameters $L_{f,2}$ and u must be adjusted. We may attempt to keep constant the product $R_2 L_{f,2}$ in eqn. 8. Since the recovery yield at the maximum possible production rate (no yield constraint) is *ca.* 60%, we may reduce the loading factor by the ratio $0.6/R_2$, where R_2 is the requested value of the yield. Thus, the loading factor is given by

$$L_{f,2} = L_{f,2}^* \cdot \frac{0.6}{R_2} \tag{11}$$

where $L_{f,2}^*$ is given by eqn. 3. We then adjust the column efficiency to achieve the desired recovery yield. This procedure provides an adjustment of both parameters. Although not rigorous, the method is reasonable, as it is in agreement with the results of a simplex study which showed a quasi-linear relationship between the recovery yield and both $L_{f,2}$ and u [28]. The required efficiency is obtained by calculating series of chromatograms corresponding to the loading factor given in eqn. 11 and different values of the column efficiency and determining the value of the efficiency for which the recovery yield is equal to the required value. The optimum column length and mobile phase velocity are calculated as described in the first strategy.

Optimization strategy IV. Starting from the optimum values predicted by the third approach, the loading factor is slightly increased, while the mobile phase velocity is decreased (the efficiency is increased) in order to achieve the desired recovery yield. The procedure is repeated until the maximum production rate is observed. The determination of the optimum values of the loading factor, $L_{f,2}$, and the column efficiency is made by calculation of series of chromatograms.

Optima in chromatography are usually rather flat. Hence the third approach will most often give an excellent approximation of the optimum conditions, while the fourth approach will converge rapidly towards the true optimum.

RESULTS AND DISCUSSION

We first compare the results obtained with the different approaches outlined in the previous section. We show that one of these methods performs better than the others. Then, we use the results obtained with this preferred method to investigate the trade-offs between recovery yield and production rate.

Table I compares the production rates obtained with the four different strategies. The calculations were performed for a 1:9 binary mixture with a relative retention, α, of 1.20 and for 10-μm particles. The other experimental conditions (Table I) are typical of current practice in preparative liquid chromatography. For this mixture, the maximum possible production rate, with a 60% recovery yield, is 0.46 mol m^{-2} s^{-1} (see Table II). Note that, in agreement with eqn. 8, the values of the production rate are proportional to the loading factor and the recovery yield.

The different strategies can be ranked in order of the performance allowed. The second strategy is obviously the worst. The first strategy gives twice as large a production rate, but is still less than two thirds of the production rate predicted by the third strategy. The fourth strategy gives only a marginal increase in production rate, about 3% at best, which is hardly significant. On the other hand, strategy IV is more complex and requires more computation time. Accordingly, in the remainder of this paper, we shall use exclusively the third strategy.

The maximum production rate of the second component that can be achieved with 1:9 mixture, while achieving a 95% recovery yield, is two thirds of the maximum possible production rate, which would bring a recovery yield of only 60%. In economical terms, it means that by reducing the throughput by a factor of 2.3, we reduce the production rate by only 1.5. The savings on the throughput and on the amount of feed to be recycled or wasted due to the large increase in yield are certainly significant. Probably the least costly production rate would be achieved for a throughput corresponding to a value of the recovery yield between 60 and 95%.

TABLE I

OPTIMUM EXPERIMENTAL CONDITIONS FOR A SPECIFIC VALUE OF THE RECOVERY YIELD WITH VARIOUS OPTIMIZATION STRATEGIES

Recovery yield constraint: 95%. Experimental conditions: $\alpha = 1.2$, $k'_{0,1} = 3$; column saturation capacity, $q_{s,1} = q_{s,2} = 5$; phase ratio, $F = 0.25$; maximum available pressure, $\Delta P = 100$ bar; solute molecular diffusion coefficients, $D_m = 1 \cdot 10^{-9}$ m^2/s; mobile phase viscosity, $\eta = 1$ cP ($1 \cdot 10^{-3}$ Pa s); packing particle average diameter, $d_p = 10$ μm; degree of purity of the collected fractions, 99%; cycle time, $t_c = t_{R,02} - t_0$; plate height equation, $h = 2/v + v^{0.33} + 0.1v$; composition of the binary mixture, 1:9 (10% of the first component).

Strategy used	N_0	L (m)	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
I	5100	0.32	31.0	4.82	0.198
II	550	0.0884	113.2	0.7	0.104
III	1050	0.127	78.7	3.04	0.316
IVa	1250	0.14	71.2	3.4	0.319
IVb	1400	0.15	66.7	3.7	0.327

Trade-offs between production rate and recovery yield

Using the same binary mixture as for the calculation of the data in Table I, Table II compares the production rates predicted by the third optimization strategy for different values of the required recovery yield, from 60% (in practice the maximum possible production rate without yield constraint) to near 100% (corresponding to the touching-band case). The production rate decreases slowly at first with increasing required recovery yield, the loss of production rate being 10% for an 80% yield and 22% for a required yield of 90%. Beyond 90%, the loss increases rapidly and becomes precipitous above 99%. For the touching-band condition, the production rate is 15% of the maximum production rate possible and still only 22% of the production rate possible with the excellent recovery yield of 95%. Allowing a recovery yield of 99.9%, which is equivalent to total recovery for all practical purposes, still gives a 2.5 times larger production rate than the touching-band case. However, the reason for this remarkable increase in the production rate is that we have accepted to produce 99% pure fractions, not that we have accepted a decrease in yield of 0.1%. Touching bands permit, at least in theory, both a total recovery yield and the production of totally pure products. When the concentration of the less retained component in the feed is much less than the concentration of the more retained component, allowing a small amount of impurity in the product permits a large increase in production rate [10]. This is no longer true at higher concentrations of the less retained component.

The data in the Table II show also that the required efficiency increases rapidly with increasing recovery yield, as well as the column length, while the reduced velocity and the loading factor decreases steadily. Although the throughput decreases, allowing important savings on solvent costs, the column required is longer and the amount of packing material needed per unit amount of feed purified increases.

TABLE II

TRADE-OFFS BETWEEN PRODUCTION RATE AND RECOVERY YIELD; OPTIMUM EX-
PERIMENTAL CONDITIONS FOR MAXIMUM PRODUCTION RATE AT VARIOUS SPECIFIED
VALUES OF THE RECOVERY YIELD

Same experimental conditons as in Table I, except for the recovery yield constraint. R_s is the resolution observed between the two component bands at very low value of the loading factor (linear conditions).

Yield (%)	N_0	R_s	L (m)	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
60[a]	550	0.76	0.084	113	4.82	0.46
80[b]	650	0.83	0.097	103	3.60	0.413
90[b]	825	0.94	0.111	90	3.20	0.361
95[b]	1050	1.05	0.127	78.7	3.04	0.316
99[b]	1800	1.38	0.173	57.7	2.92	0.232
99.9[b]	3000	1.79	0.234	42.9	2.9	0.172
Touching band[c]	3230	1.85	0.244	41	1.24	0.0705

[a] Values calculated using eqns. 5, 7 and 8 in ref. 11 (maximum possible production rate, with no yield constraint).
[b] Values calculated using strategy III, as explained in the text.
[c] Values calculated using the procedure described in ref. 12.

TABLE III

TRADE-OFFS BETWEEN PRODUCTION RATE AND RECOVERY YIELD: OPTIMUM EX-PERIMENTAL CONDITIONS FOR MAXIMUM PRODUCTION RATE AT VARIOUS SPECIFIED VALUES OF THE RECOVERY YIELD AND INFLUENCE OF THE PARTICLE SIZE

Same experimental conditions as in Table II, except average particle size, $d_p = 20\ \mu$m.

Yield (%)	N_0	R_s	L (m)	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
60	500	0.73	0.315	253	4.82	0.505
80	650	0.83	0.363	220	3.6	0.449
90	850	0.95	0.42	190	3.2	0.381
95	1100	1.08	0.483	166	3.04	0.358
99	1800	1.38	0.633	126	2.92	0.254
99.9	3000	1.78	0.841	95	2.90	0.191
Touching band [12]	3380	1.90	0.90	89	1.27	0.0785

Table III shows data similar to those in Table II, but corresponding to packing material with an average particle size of 20 μm. The optimum column length is approximately four times longer than that found with 10-μm particles (see Table II), and the maximum production rates for each stated recovery yield are very nearly the same. The difference between the production rates is *ca.* 10% at both ends (yields of 60 and 99.9%) and 14% for a required yield of 95%. This results confirms the observation by Knox and Pyper [7] and our general demonstration [12] that, when columns are operated at high velocities, there is a near-optimum value of d_p^2/L, but no separate optima of the particle size and the column length.

Table IV shows data corresponding to a binary mixture of the same two components, but with a very different composition, 3:1 instead of 1:9 used for Tables

TABLE IV

TRADE-OFFS BETWEEN PRODUCTION RATE AND RECOVERY YIELD: OPTIMUM EX-PERIMENTAL CONDITIONS FOR MAXIMUM PRODUCTION RATE AT VARIOUS SPECIFIED VALUES OF THE RECOVERY YIELD AND INFLUENCE OF THE MIXTURE COMPOSITION

Same experimental conditions as in Table II, except composition of the feed, 3:1 binary mixture (25% of the second component).

Yield (%)	N_0	R_s	L (m)	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
60[a]	1500	1.26	0.156	64	0.875	0.0473
80[b]	1800	1.38	0.173	57.7	0.656	0.0422
90[b]	2200	1.50	0.195	51.4	0.584	0.0377
95[b]	2700	1.70	0.22	45.6	0.553	0.0333
99[b]	4000	2.06	0.277	36.0	0.53	0.0263

[a] Values calculated from eqns. 5, 7 and 8 in ref. 11.
[b] Values calculated from strategy III, as explained in the text.

I–III. The concentration of the second component decreases from 90 to 25%, *i.e.*, 3.6 times. In the former instance, the displacement effect predominates, and we know how favorable this effect is to the separation chemist. It enhances the production rate for both the second and the first component. In the present instance (Table IV), the tag-along effect predominates and spreads the second-component band over a wide range of retention times. If we compare Tables II and IV, we observe that the production rate for the second component decreases nearly 10-fold, corresponding to a total throughput (amount of feed processed by the column per unit time) decrease by a factor of *ca.* 2.6. When a compound is eluted second, its purification (Table II) is much easier and less costly than its extraction (Table IV). Compared with the optimum conditions for the 1:9 mixture, the column required for the extraction of the second component from the 3:1 mixture is nearly twice as long, the optimum mobile phase velocity is nearly two thirds as large and the loading factor is about six times smaller.

The only possibility of increasing the production rate would be the use of a combination of two chromatographic separations, the mixture collected with the mixed zone during the first stage being reprocessed. This raises new and interesting problems of optimization, which are beyond the scope of this work.

Table V illustrates the influence of the feed composition on the production rate for the second component, with a required recovery yield of 95% (and a product purity of 99%, as in the remainder of this work). The loading factor is calculated from eqn. 11. The production rate increases dramatically with increasing concentration of the second component, from 5 to 95%. At the same time, the optimum column efficiency decreases more than four-fold and the column length about two-fold, while the loading factor increases 43-fold and the optimum mobile-phase velocity more than two-fold. All these effects combined provide for a production rate increase by a factor of 100 and a more than five-fold increase in the total feed throughput. The relative ease with which the second component can be purified from small proportions of the first component, compared with the difficulty in extracting small amounts of this second component from an excess of the first, illustrates the importance of the displacement effect in

TABLE V

TRADE-OFFS BETWEEN PRODUCTION RATE AND RECOVERY YIELD: OPTIMUM EX-PERIMENTAL CONDITIONS FOR MAXIMUM PRODUCTION RATE FOR VARIOUS FEED COMPOSITIONS

Same experimental conditions as in Table II, except variable feed composition. Recovery yield, 95%. Results calculated using strategy III, as explained in the text.

Feed composition	N_0	R_s	L (m)	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
5:95	950	1.0	0.120	83.3	4.62	0.509
10:90	1050	1.05	0.127	78.7	3.04	0.316
25:75	1300	1.18	0.144	69.6	1.93	0.177
50:50	1720	1.35	0.169	59.3	1.15	0.090
75:25	2700	1.70	0.220	45.6	0.553	0.033
90:10	3000	1.78	0.233	43.0	0.217	0.0123
95:5	4100	2.08	0.281	35.5	0.107	0.0050

TABLE VI

TRADE-OFFS BETWEEN PRODUCTION RATE AND RECOVERY YIELD: OPTIMUM EX-PERIMENTAL CONDITIONS FOR MAXIMUM PRODUCTION RATE AT VARIOUS VALUES OF THE SELECTIVITY

Same experimental conditions as in Table II, except various values of selectivity and average particle size d_p = 20 μm. Recovery yield, 95%. Results calculated using strategy III, as explained in the text.

Selectivity, α	N_0	R_s	L (m)	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
1.1	3400	1.04	0.903	88.6	0.93	0.0594
1.2	1100	1.08	0.483	166	3.04	0.358
1.3	620	1.12	0.354	226	5.68	0.783
1.5	330	1.18	0.253	317	11.37	1.91
1.7	225	1.20	0.206	388	16.8	3.05

preparative chromatography [4,5]. It also emphasizes the need to select, as far as possible, the order of elution of the feed components.

Table VI describes the influence of the relative retention of the two components on the maximum production rate of 99% pure fractions of the second component, with a recovery yield of 95%. The optimum loading factor is calculated from eqn. 11. The optimum column efficiency decreases rapidly with increasing relative retention and so does the optimum column length. At the same time, the optimum mobile phase velocity increases rapidly, in addition to the optimum loading factor. The maximum production rate increases nearly as $[(\alpha-1)/\alpha]^3$ [17]. The same dependence has been predicted in both the touching-band case (100% yield) and the overlapping case (maximum possible production rate, no yield constraint) [12,17].

Finally, Table VII illustrates the influence of the operating pressure[a] on the

TABLE VII

TRADE-OFFS BETWEEN PRODUCTION RATE AND RECOVERY YIELD: OPTIMUM EX-PERIMENTAL CONDITIONS FOR MAXIMUM PRODUCTION RATE AT VARIOUS VALUES OF THE OPERATING PRESSURE

Same experimental conditions as in Table II, except various values of inlet pressure. Recovery yield, 95%. Results calculated using strategy III, as explained in the text.

Operating pressure, ΔP (bar)	N_0	R_s	L (m)	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
20	1050	1.05	0.065	30.6	3.04	0.121
50	1050	1.05	0.095	52.6	3.04	0.21
100	1050	1.05	0.127	78.7	3.04	0.316
200	1050	1.05	0.172	116	3.04	0.462

[a] The operating pressure is the highest pressure at which the equipment can be operated safely on a routine basis.

maximum production rate of 99% pure product, with a 95% recovery yield. The optimum loading factor and the optimum column efficiency are both independent of the pressure. Both the optimum velocity and the optimum column length (at a given value of d_p) increase nearly in proportion to the square root of the operating pressure, so the cycle time remains constant. Thus, the production rate increases with increasing sample size; at a constant loading factor, the latter, in turn, increases in proportion to the increasing column length. The production rate is nearly proportional to the square root of the operating pressure [12].

Optimization of a given column

In this instance, the column length and particle size are determined and cannot be adjusted. Only the sample size and the mobile phase flow velocity can be optimized. The value of the ratio d_p^2/L can be either larger or smaller than the optimum value for the separation considered.

If d_p^2/L is larger than the optimum (*i.e.*, the column is too short for the packing material used), it cannot be operated at the maximum available pressure, because the efficiency would be insufficient. We must operate it at the optimum efficiency, with the optimum loading factor calculated above (eqn. 11). Hence we must use a lower flow velocity than with a column of optimum length to achieve the optimum efficiency, and the production rate is lower than that possible with the column of optimum length. The data in Table VIII (last three lines) illustrate this situation. If we compare these with the similar data in Table II, we see that the column length in Table VIII (10 cm) is shorter than the optimum length for production with a recovery yield in excess of about 82%.

TABLE VIII

TRADE-OFFS BETWEEN PRODUCTION RATE AND RECOVERY YIELD FOR A GIVEN COLUMN: OPTIMUM EXPERIMENTAL CONDITIONS FOR MAXIMUM PRODUCTION RATE AT VARIOUS SPECIFIED VALUES OF THE RECOVERY YIELD

Same experimental conditions as in Table II, except fixed column length (0.10 m).

Yield (%)	N_0	R_s	L (m)	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
60[a]	685	0.85	0.10	100	5.4	0.45
80[a]	685	0.85	0.10	100	3.60	0.408
90[b]	825	0.94	0.10	78	3.20	0.313
95[b]	1050	1.05	0.10	57	3.04	0.228
99[b]	1800	1.38	0.10	5.6	2.92	0.103

[a] In this case, the column is longer than the optimum (see Table II) and cannot be operated at the optimum velocity (the inlet pressure would exceed the maximum available pressure). The column efficiency being higher than the optimum (*cf.*, Table II), we must inject a sample larger than that corresponding to the optimum loading factor in order to achieve the required yield. This does not compensate for the decreased flow velocity, and the production rate is lower that would be possible with the column of optimum length.

[b] In this case, the column is shorter than the optimum. We must operate it at the velocity giving the optimum efficiency and use the optimum loading factor given in Table II. Compared with the optimum conditions (Table II), the mobile phase velocity will be lower and the production rate smaller. The production rate decreases rapidly with increasing required yield.

Under such conditions, the column must be operated at the flow velocity that gives the same efficiency as the optimum column (compare the second columns in Table II and VIII), and with the same loading factor as for the optimum column (compare the sixth columns in Tables II and VIII). The production rate is decreased by 15% for a recovery yield of 90% and by more than 50% for a recovery yield of 99%.

For each column, there is a optimum flow velocity for the maximum production rate. This optimum velocity is such that the column has the optimum efficiency (see Table II). Accordingly, there is an optimum value of the inlet pressure [11,16]. If the column is too short, this optimum pressure is lower than the maximum available pressure.

If d_p^2/L is smaller than the optimum (i.e., the column is too long for the packing material used), the optimum pressure is higher than the maximum available pressure, and we cannot operate it at a high enough velocity to achieve the optimum column efficiency (see the first two lines in Table VIII and corresponding data in Table II). The column is operated at the maximum available pressure, at the flow velocity given by eqn. 7b, and it has the efficiency derived from eqn. 6. However, this column efficiency exceeds the optimum efficiency for the maximum production rate. In order to achieve the required recovery yield, we must use this excessive efficiency and overload the column more. The loading factor is increased, but the production rate is lower than is possible with the optimum column, although in the example chosen here the loss is almost negligible (1–2%).

Finally, Table IX compares the performances of various columns packed with the same stationary phase but having different lengths and used to separate the same binary mixture as used for Table I (1:9 mixture), with the same recovery yield of 95%. The loss in production rate that accompanies the use of a column of improper length can be very important. It is especially costly if the column is too long.

TABLE IX

OPTIMUM EXPERIMENTAL CONDITIONS FOR MAXIMUM PRODUCTION RATE WITH A GIVEN COLUMN AT A SPECIFIED YIELD

Same experimental conditions as in Table II, except fixed column length, as indicated. In all instances the required recovery yield is 95% and the required degree of purity of the products is 99%.

L (m)	N_0	v	Loading factor (%)	Production rate, $Pr_2/(1-\varepsilon)S$ (mol m^{-2} s^{-1})
0.10[a]	1050	57	3.04	0.228
0.127[b]	1050	78.7	3.04	0.316
0.20[c]	2300	50	4.4	0.29
0.30[c]	4560	33.3	4.7	0.208
0.40[c]	7300	25.0	4.82	0.162

[a] The column length is shorter than the optimum. In this case, the maximum production rate is obtained at the column efficiency and loading factor of the optimum column (see Table II). However, the linear velocity is smaller than the optimum, and so is the production rate.

[b] Optimum column length (see Tabel II).

[c] The column length is longer than the optimum. The column is operated at the maximum available pressure. Compared with the optimum, the mobile phase velocity is lower, the loading factor higher and the production rate smaller.

CONCLUSION

The theory of optimization presented here is based entirely on the theory of non-linear chromatography and as such is as rigorous as possible. It avoids the pitfalls of uncontrolled empiricism more or less hidden in previous attempts and the use of adjustable parameters [8,9,29]. Nevertheless, the optimum conditions are simple to derive in most cases. However, this approach suffers from two serious limitations.

First, it deals only with binary mixtures. The two-component problem is the simplest separation case; it is relevant for the separation of closely related isomers produced by chemical synthesis (e.g., separation of enantiomers). In practice, a number of impurities may have to be eliminated or the component of interest must be extracted from a complex mixture. In this case, as in the optimization of the experimental conditions for minimum analysis time, the cycle time depends on the retention time of the last-eluted impurity. We may want to operate the column at a higher mobile-phase velocity than the optimum value obtained by considering the binary mixture made of the main compound of interest in the feed and the most closely eluted impurity to be eliminated. This would permit a reduction of the cycle time, although a smaller sample size will have to be used.

Second, the present approach is based on the assumption that the competitive Langmuir isotherm accounts satisfactorily for the interaction behavior of the two main components of the feed. This is not true except, to some extent, for enantiomers [18]. The more rigorous ideal adsorbed solution model [30] gives slightly better results, but it cannot take into account two important effects, the variation of the activity coefficients of solutes with their concentrations and the molecular interactions between the feed components in the stationary phase [31]. Accounting properly for these effects is the last hurdle in the full understanding of the mechanism of preparative chromatography, but a major one.

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