Journal of Chromatography, 537 (1991) 497–506
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 936

Letter to the Editor

Pitfalls in the choice of isotherms for the calculation of band profiles in preparative chromatography

Sir,

The last years have brought significant advances in the theory of non-linear chromatography and, especially, in the prediction of the individual elution band profiles in preparative liquid chromatography by computer simulation [1–9]. With the increasing power of computers, accurate approximations have become possible. Some of the various finite difference algorithms that can be used for the correct simulation of chromatograms have been discussed recently [10]. These algorithms are procedures of propagating the chromatographic signal through a grid of time and space coordinates. Except at very low column efficiencies (number of theoretical plates, $N < 1000$) they all give nearly identical band profiles. The central part of any of these simulation methods is the calculation of the fraction of the sample molecules that has to be propagated, using the proper distribution isotherm (mostly, competitive Langmuir isotherms are chosen for lack of a better equation). Of these methods, the Craig model appears as a particular case that requires that this equilibration be done by iterations at each step along the grid [10]. For this reason, the implementation of a Craig model is bound to consume much longer computing times than other methods that do not require this iterative calculation [11–13].

Attempts have been made at eliminating the time consuming equilibration step of the Craig model [2,14–16]. In a recent publication, an algorithm has been proposed that replaces the two-component competitive Langmuir isotherm with a new numerical approximation [17]. This algorithm is claimed to be "accurate to within $\pm 10\%$ for a wide range of sample concentrations and sample k' values" (k' = capacity factor) [17]. Indeed, many of the peak shapes shown in this work resemble those found in experimental preparative chromatography [18,19] and those obtained in band profile calculations by other groups [3,5–8,10]. Surprisingly, however, at high sample loads, the formation of double peaks is predicted. According to the literature, this should not be possible with a monotonically curved isotherm [20,21], such as the Langmuir isotherm. So far, to the best of our knowledge, it has never been reported to have occurred in any experiment.

Furthermore, the authors report [17,22] that the retention times predicted with their method agree poorly with their experimental data or with the results of calculations we have published [3,6,7,12]. They tried to circumvent this consistent disagreement by using an empirical factor with which they multiply the sample size (or the injection concentration). Agreement is then claimed between experimental results

0021-9673/91/$03.50

and the results of the calculations performed with the "corrected" sample load. The value of the correction factor is given as intermediate between 1.5 and 1.8 [17,22].

In our own work, we have always observed an excellent agreement between experimental band profiles and the results of the simulations when they were carried out using the equilibrium isotherms determined on the same column [23–26]. Thus, the unexpected peak profiles presented in ref. 17 deserved some investigation. We have also noticed that the individual elution band profiles are sensitive to minor changes in the isotherms. As we report here, the exact nature of the isotherms used [17] is the cause for the questionable results. Since the published procedure (Appendix, ref. 17) contains some inconsistencies and typographical errors, we give the version we used in Appendix I, with the list of changes made to the published program [17]. Judging by the excellent agreement between our results and those published, the program in Appendix I is a very close match to what the authors of ref. 17 have used.

PROCEDURE AND EXPERIMENTAL CONFIGURATION

Langmuir isotherm

In Craig simulations, the total amount of a sample component in one column plate must be distributed between the stationary and the mobile phases according to its equilibrium isotherm. Then, the fraction in the mobile phase moves forward to the next plate, whereas the fraction in the stationary phase stays behind and is equilibrated with the mobile phase coming from the preceding plate. Thus, it is necessary to calculate both equilibrium concentrations from the total amount of each component contained in the plate.

For the lack of a better model, the most commonly used isotherm in liquid–solid chromatography is the competitive Langmuir isotherm:

$$q_i = \frac{a_i C_i}{1 + \sum_{j=1}^{n} b_j C_j} \tag{1}$$

where q_i and C_i are the local equilibrium concentrations of the compound i in the stationary and the mobile phases, respectively, and a_i and b_i are coefficients the numerical values of which are characteristic of the compound i and the phase system and determine the saturation capacity of the column.

The Langmuir competitive isotherm is a convenient first order approximation the most serious inconvenient of which is not to satisfy the Gibbs–Duhem equation, unless the column saturation capacities, a_i/b_i, are the same for the two components. There are some possibilities to correct for this drawback [27]. Experimental results show reasonably good agreement with profiles calculated using a competitive Langmuir isotherm model [25].

For the calculation of chromatographic profiles, it is necessary to keep track of the amounts of each compound, rather than of concentrations. Thus, it is convenient to report both amounts in the same units. The amount in the stationary phase is also divided by the volume of mobile phase in one plate. Instead of the a_i, parameter, we use the limiting retention factor at infinite dilution, $k'_{0,i}$:

$$q'_i = \frac{k'_{0,i} C_i}{1 + \sum b_j C_j} \tag{2}$$

This allows us the use of the same numerical values, whether we discuss compound amounts as in ref. 17 (w_s, w_m) or their concentrations (q, C).

When there is only one component, eqn. 1 can be rearranged and solved in closed form for C. For a multi-component mixture, there are no closed form solutions giving the mobile phase concentrations as functions of the total amount of each component. Nevertheless, it is straightforward to calculate the numerical solution by an iterative approximation such as the following one. If the above definition of the stationary phase concentrations is followed, the amounts in either phase are proportional to the quantities C or q', respectively. The total amount of a component in one cell divided by the volume of the mobile phase in this cell then corresponds to the sum of the amounts of the component i in the two phases contained in this cell:

$$T_i = C_i + q'_i \tag{3}$$

For the two components, X and Y, we have:

$$q'_X = T_X - C_X = \frac{k'_{0,X} C_X}{1 + b_X C_X + b_Y C_Y}$$

and

$$q'_Y = T_Y - C_Y - \frac{k'_{0,Y} C_Y}{1 + b_X C_X + b_Y C_Y} \tag{4}$$

The total amounts, T_X and T_Y, of the two components in the cell considered are known. Eqns. 4, then, have to be solved for the two mobile phase concentrations, C_X and C_Y. We can consider the denominator in eqn. 4 as a correction factor and solve for C_i in the numerator (see Appendix II). After each iteration step, the value of the denominator is updated, using the new values of the concentrations C_i. When the difference between two successive values of the two concentrations drops below $1 \cdot 10^{-10}$, the approximation is considered as satisfactory and the resulting concentrations are used in the propagation step. This procedure has proven to be very robust and converges rapidly.

Isotherm parameters

According to the Appendix of ref. 17, "in a Langmuir type system [...] a one-stage equilibrium is assumed, having a mobile phase volume of 1.0 ml and a stationary phase capacity of 0.1 g of sample". For a 400-plate system, the dead volume is 400 ml and the saturation capacity is 40 g. In order to inject an amount of 20% of the column saturation capacity for one of the two components of the mixture, we need a sample solution containing 8 g of each component for 1 ml of mobile phase (the content of one plate). This is quite unrealistic, but the injection had to be carried out during one cycle time in order to duplicate the published results. For a more realistic simulation, the injection should last several cycle times and its profile should mirror the experimental injection profile. In our computations, we have chosen a 10 cm long column and a mobile phase velocity of 0.1 cm/s, which gives a cycle time of $\Delta t = 0.25$ s. The space increment along the column is $\Delta z = 0.025$ cm.

The Langmuir parameters, b_i, can be calculated from the relationship:

$$\frac{k'_{0,i}}{b_i} = 0.1 \tag{5}$$

The values of the various input parameters used are presented in Table I. Note that the values of b_i are not used in the computation method described in ref. 17.

Computation times

The execution times for three simulation runs carried out with different loads are given in Table II. They are reported as the times required for the completion of one band propagation step, including the equilibration in one Craig plate. The equilibration according to the procedure described in the previous section needs an average of five iterations to converge to a concentration difference of $1 \cdot 10^{-10}$. This may take up to 15 iterations in the vicinity of steep concentration gradients, i.e., for the band fronts. The explicit isotherm from ref. 17 avoids these iterations but requires two exponentiations at low sample loads or one square root at high loads. On the machine language level, both these operations require rather complex manipulations. The end result is that there is no clear computational advantage in using the isotherm of ref. 17. Depending on the computer used (VAX or PC with different processors), it may be either somewhat slower or slightly faster than the iteration procedure.

RESULTS AND DISCUSSION

The most striking feature of the isotherm of ref. 17 is the concentration discontinuity observed for a sample amount of 0.175 units (w_{max} for $k_X = 1$). At low concentrations, the isotherm agrees quite closely with the corresponding competitive Langmuir isotherm (eqn. 1). When the cumulative load of both components in one Craig stage exceeds this threshold amount, the algorithm follows a distribution law that is completely different from the one used at lower total loads. The two branches of

TABLE I

PARAMETERS USED IN THE COMPUTER SIMULATIONS

Numerical simulation of band propagation in non-linear chromatography

(1) Input profiles (for a load of 2 × 20% of saturation):
Calculation 1: concentration 8.00 during 0.25 s
Calculation 2: concentration 8.00 during 0.25 s

(2) Column: column length 10 cm
linear velocity 0.10 cm/s
height equivalent to a theoretical plate for $k' = \infty$ 250 μm (400 plates)

(3) Retention at low load, isotherm data:
Calculation 1: 200.0 s $k' = 1.0$ b = 10.00
Calculation 2: 270.0 s $k' = 1.7$ b = 17.00

(4) Craig simulation:
grid spacing [10] $\Delta z = 0.025000$ cm $\Delta t = 0.250000$ s

The sample sizes are given as loading factors, i.e. fractional column saturations.

TABLE II

EXECUTION TIMES ON DEC VAX 6000-440: TIME NEEDED FOR ONE EQUILIBRATION FOLLOWED BY A PROPAGATION STEP. 400 CRAIG STAGES, k' = 1.0 and 1.7.

Load	Ref. 17	Langmuir	Difference
2 × 2.5% of capacity	60 μs	42 μs	+30%
2 × 20% of capacity	60 μs	59 μs	+2%
2 × 30% of capacity	57 μs	62 μs	−8%

the isotherm do not even link up at this point (Fig. 1). As a result, the concentrations of the two components in both phases change abruptly. As the concentration in one phase increases, the concentration in the other phase decreases by the corresponding amount. This property is illustrated in Fig. 1 which shows, for one component, the amount adsorbed in the stationary phase of one stage *versus* the total amount in both phases. This discontinuity is disconcerting and makes no physical sense.

The objective of introducing the upper part of the isotherm seems to have been to force complete saturation of the stationary phase at all sample loads above the threshold. When only one component is present, the concentration in the stationary phase is set equal to the saturation limit. For a binary mixture (Fig. 1), the numerical value of a complicated function (Appendix I) determines the relative amount supplied by each component to complete stationary phase loading. The amounts in the mobile phase are calculated as the leftovers of this process.

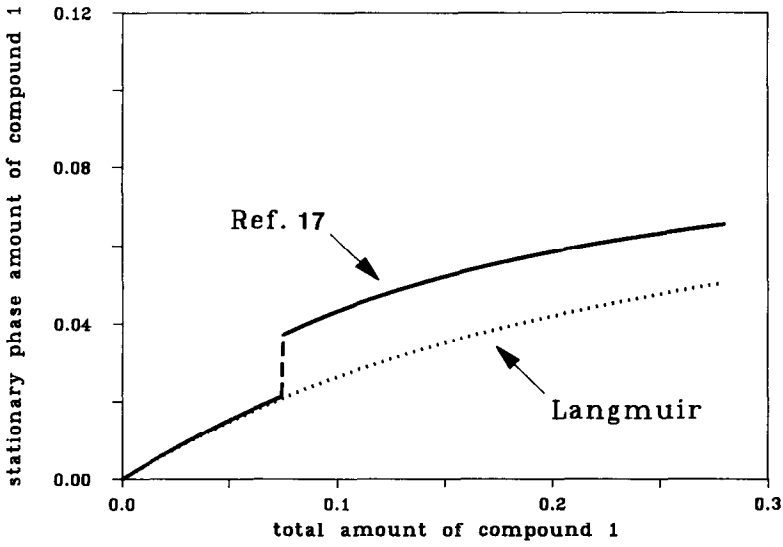

Fig. 1. Distribution isotherm of the first component of a binary system calculated according to the procedure described in ref. 17 and comparison with Langmuir isotherm. Plot of the amount of first component in the stationary phase *versus* the total amount of component 1 in the system (solid line). Langmuir isotherm in dotted lines. Amount of component 2 constant (w_Y = 0.10 g/ml).

This choice of a constant equilibrium concentration at all high mobile phase concentrations is rather unfortunate. According to thermodynamics, when the concentration in one phase is changed, the equilibrium is restored by a proper change in the other phase. This would be impossible if the concentration in one phase were fixed. Therefore a saturation concentration can be only an asymptotic limit.

The effects of the isotherm shape on calculated band profiles are shown by the example in Fig. 2. It compares the chromatograms calculated with isotherms obtained using the algorithms in either Appendix I (solid lines) or Appendix II (true competitive Langmuir isotherm, dotted lines). The "experimental conditions" for this simulation are equivalent to those given in ref. 17 for Fig. 5e, to which the result must be compared.

The chromatogram calculated following the iterative procedure (dotted lines) is in close qualitative agreement with experimental band profiles reported in earlier work [23–26]. It exhibits the typical tag-along effect reported previously [3,6]. Due to the strong blockage of the adsorbent surface by the molecules of the first component, the front of the second component band moves much faster than its tail [28]. This phenomenon leads to the formation of a plateau trailing behind the maximum of the second band and often eroded into a shoulder by the finite kinetics of mass transfer [6,28].

In contrast, the profiles produced by the discontinuous isotherm exhibit a shoulder preceding the band maximum (Fig. 3e in ref. 17) or even a second peak (Fig.

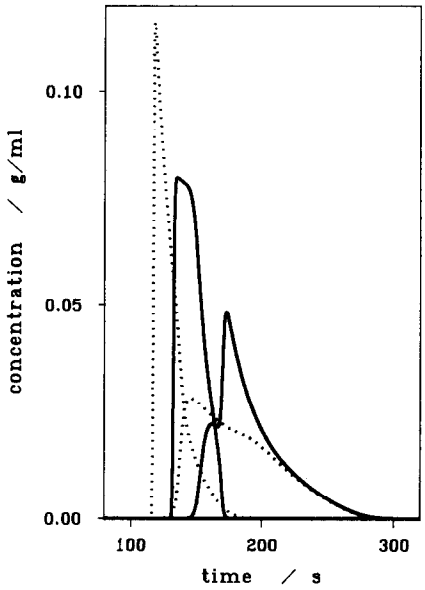

Fig. 2. Chromatograms calculated using the two isotherm calculation procedures described in the present paper. Craig model program. Solid line: individual band profiles obtained with the discontinuous isotherm calculated according to the procedure in ref. 17 (Appendix). Dotted line: individual band profiles obtained with the Langmuir competitive isotherm calculated according to the procedure in Appendix II of this work. Conditions: $k'_1 = 1, \alpha = 1.7$, loading factors: $L_{f,1} = L_{f,2} = 0.20$, 400 plates. Compare to Fig. 5e in ref. 17.

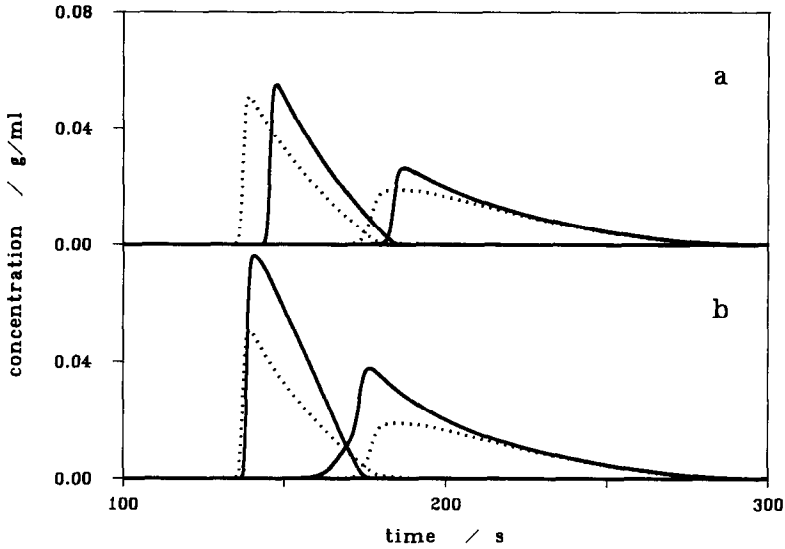

Fig. 3. Chromatograms calculated using the two isotherm calculation procedures described in the present paper. Craig model program. Solid line: individual band profiles obtained with the discontinuous isotherm, calculated according to the procedure in ref. 17 (Appendix I). (a) Calculation made with the true sample amount. (b) Calculation made with an adjusted sample size equal to 1.5 times the true sample amount. Dotted line: individual band profiles obtained with the competitive Langmuir isotherm calculated according to the iterative procedure in Appendix II, for the true sample amount (both in a and b). Conditions: $k'_1 = 1$, $\alpha = 1.7$, $L_{f,1} = L_{f,2} = 0.10$, $n_c = 400$ plates. Compare to Fig. 5d in ref. 17.

2, loading factor 20% for each component, separation factor, $\alpha = 1.7$). The position of the valley between the two peaks of the second band corresponds to the region at the rear of the first band where the cumulative sample concentration drops suddenly below the threshold level. Concurrently, the amount adsorbed falls from complete saturation down to the values determined by the lower part of the isotherm (see Fig. 1). These band shapes are not consistent with a monotonically curved isotherm.

Fig. 3a compares the chromatograms obtained with the two isotherms under the same conditions as in Fig. 2 but with a lower sample size (loading factors = 10%). Both chromatograms have band profiles that look quite reasonable, with almost touching band separation. There is still a serious difference in the retention times however. Both peaks calculated with the discontinuous isotherm (Fig. 3a, solid lines) appear at higher retention times than expected (dotted lines). The explanation lies in the shape of the isotherm. Whereas its lower part is virtually identical to the Langmuir model, the higher part has a much higher fraction of the sample adsorbed. Accordingly, the band fronts move more slowly than with asymptotic saturation behavior and the retention times are too long.

The procedure selected to correct for this discrepancy tries to remedy the effects of an incorrect isotherm by an adjustment of the sample size [17]. If the loading factor for a given sample size is multiplied by an arbitrary factor, the bands elute faster [28]. Values between 1.5 and 1.8 are suggested for the correction factor [17,22]. As seen in Fig. 3b, this approach is only moderately successful. Although the retention times of

the first component band are about the same for the chromatogram calculated using the true Langmuir isotherm (dotted lines same as in Fig. 3a) and the one calculated with a correction factor of 1.5 using the discontinuous isotherm (solid lines), the band profiles are markedly different. Of special importance is the fact that with the Langmuir isotherm we come close to a touching band separation, whereas the chromatogram simulated with the discontinuous isotherm and an adjusted sample size (increased by 50%) exhibits a much poorer resolution. When a correction factor of 1.8 is used, the agreement between the two procedures is still worse. This shows that the use of a fixed correction factor cannot permit the calculation of consistently correct retention times or peak profiles. It cannot be trusted either in the derivation of the correct sample size that would allow touching band separations (Fig. 3b), as is the aim of the CRAIG4 subprogram and software packages built around it [29].

CONCLUSION

In this paper we have shown that it may be both unnecessary and risky to replace an implicit function (the competitive Langmuir isotherm) by an explicit approximation. The approximation proposed in a recent paper [17] does not help to save computation time. Instead, it introduces a discontinuity in the equilibrium concentrations, which renders the isotherm meaningless and leads to incorrect and rather unusual peak shapes. If a Langmuir isotherm has been chosen to represent the experimental results, an iterative solution is to be preferred.

ACKNOWLEDGEMENTS

This work has been supported in part by Grant CHE-8901382 of the National Science Foundation and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory. We acknowledge support of our computational effort by the University of Tennessee Computing Center.

APPENDIX I

Procedure to calculate equilibrium concentrations

Isotherm of ref. 17 (for symbols see ref. 17):
Calculate once, outside loop:

$$\alpha = k_y/k_x \rightarrow \sqrt{\alpha}$$
$$A = -(\alpha - 1)$$
$$D_x = 0.5/k_x + 0.7\, k_x^{0.37}$$
$$D_y = 0.5/k_y + 0.7\, k_y^{0.37}$$
$$C_x = 0.62\, 10^{Dx}\, k_x^{-0.4}$$
$$C_y = 0.62\, 10^{Dy}\, k_y^{-0.4}$$
$$w_{max} = 0.175 - 0.013 \log k_x$$

Calculate inside loop for every plate at every time: (w_x and w_y are the amounts of X and Y in that plate)

$w_{tot} = w_x + w_y$

if $w_{tot} > w_{max}$:

$Q = w_{tot} - 0.1$

$B = w_x(\alpha - 1) + 0.1 + \alpha Q$ (Note 1)

$C = -w_x \alpha Q$

$w_{xm} = [-B + (B^2 - 4AC)^{\frac{1}{2}}]/2A$

$w_{xs} = w_x - w_{xm}$

$w_{ys} = 0.1 - w_{xs}$

$w_{ym} = w_y - w_{ys}$

(if only one compound is present, w_{xs} or $w_{ys} = 0.1$) (Note 2)

if $w_{tot} \leqslant w_{max}$:

$J_x = w_x + w_y\sqrt{\alpha}$ (Note 3)

$J_y = w_y + w_x/\sqrt{\alpha}$ (Note 3)

$R_x = (1/k_x) + C_x J_x^{Dx}$

$R_y = (1/k_y) + C_y J_y^{Dy}$

$w_{ws} = w_x/(1 + R_x)$

$w_{xm} = w_x - w_{xs}$

$w_{ys} = w_y/(1 + R_y)$

$w_{ym} = w_y - w_{ys}$

Changes versus the original version of ref. 17

Note 1: original: $B = [w_x(\alpha - 1)] + 0.1\alpha Q$

Using the product $0.1\alpha Q$ can lead the program to take the square root of a negative value.

Note 2: original: for $w_x = 0$, $w_{ys} = 0.1$, $w_{ym} = 0.1 - w_y$;
for $w_y = 0$, $w_{xs} = 0.1$, $w_{xm} = 0.1 - w_x$.

These instructions do not conserve mass.

Note 3: original: $J_x = w_x + (\alpha^{\frac{1}{2}} w_y)w_y$
$J_y = w_y + (\alpha^{-\frac{1}{2}} w_x)w_x$

The repetition of w_i is obviously an editing error.

APPENDIX II

Procedure to calculate equilibrium concentrations

Langmuir isotherm: for the purpose of this paper, the variables used below can be equated with those from ref. 17 according to:

$T_x = w_x$

$C_x = w_{xm}$

$q'_x = w_{xs}$

The Langmuir parameter b is not used in ref. 17.

Calculate inside loop for every plate at every time:

iterative approximation:

$$\text{denominator} = 1 + b_x C_x + b_y C_y$$
$$C_x = T_x/(1 + k_x/\text{denominator})$$
$$C_y = T_y/(1 + k_y/\text{denominator})$$

repeat until convergence (typically five times)

$$q'_x = T_x - C_x$$
$$q'_y = T_y - C_y$$

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1 J. H. Knox and M. Pyper, *J. Chromatogr.*, 363 (1986) 1.
2 L. R. Snyder, G. B. Cox and P. E. Antle, *Chromatographia*, 24 (1987) 82.
3 G. Guiochon and S. Ghodbane, *J. Phys. Chem.*, 92 (1988) 3682.
4 M. W. Phillips, G. Subramanian and S. M. Cramer, *J. Chromatogr.*, 454 (1988) 1.
5 A. J. Howard, G. Carta and C. H. Byers, *Ind. Eng. Chem. Res.*, 27 (1988) 1873.
6 G. Guiochon, S. Ghodbane, S. Golshan-Shirazi, J.-X. Huang, A. M. Katti, B.-C. Lin and Z. Ma, *Talanta*, 36 (1989) 19.
7 A. M. Katti and G. Guiochon, *Anal. Chem.*, 61 (1989) 982.
8 C. K. Lee, Q. Yu, S. U. Kim and N.-H. L. Wang, *J. Chromatogr.*, 484 (1989) 29.
9 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 61 (1989) 2380.
10 M. Czok and G. Guiochon, *Anal. Chem.*, 62 (1990) 189.
11 P. Rouchon, M. Schonauer, P. Valentin and G. Guiochon, *Sep. Sci. Technol.*, 22 (1987) 1793.
12 G. Guiochon, S. Golshan-Shirazi and A. Jaulmes, *Anal. Chem.*, 60 (1988) 1856.
13 B. C. Lin, Z. Ma and G. Guiochon, *Sep. Sci. Technol.*, 25 (1989) 809.
14 J. E. Eble, R. L. Grob, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 384 (1987) 25.
15 J. E. Eble, R. L. Grob, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 384 (1987) 45.
16 J. E. Eble, R. L. Grob, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 405 (1987) 1.
17 L. R. Snyder, J. W. Dolan and G. B. Cox, *J. Chromatogr.*, 483 (1989) 63.
18 J. Newburger, L. Liebes, H. Colin and G. Guiochon, *Sep. Sci.*, 22 (1987) 1933.
19 J. Newburger and G. Guiochon, *J. Chromatogr.*, 484 (1989) 153.
20 E. Glueckauf, *Proc. Roy. Soc. (London)*, A186 (1946) 35.
21 R. Aris and N. R. Amundson, *Mathematical Methods in Chemical Engineering*, Prentice-Hall, Englewood Cliffs, NY, 1973.
22 G. B. Cox, L. R. Snyder and J. W. Dolan, *J. Chromatogr.*, 484 (1989) 409.
23 S. Golshan-Shirazi, S. Ghodbane and G. Guiochon, *Anal. Chem.*, 60 (1988) 2630.
24 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 60 (1988) 2634.
25 A. M. Katti and G. Guiochon, *J. Chromatogr.*, 499 (1990) 5.
26 S. Jacobson, S. Golshan-Shirazi and G. Guiochon, *J. Am. Chem. Soc.*, 112 (1990) 6492.
27 S. Golshan-Shirazi and G. Guiochon, *J. Chromatogr.*, submitted for publication.
28 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 62 (1990) 217.
29 L. R. Snyder, J. W. Dolan, D. C. Lommen and G. B. Cox, *J. Chromatogr.*, 484 (1989) 425.

(First received February 15th, 1990; revised manuscript received October 17th, 1990)