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Experimental study of the production rate of pure enantiomers from racemic mixtures

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ABSTRACT

Elution band profiles were determined experimentally for the racemic mixture of D- and L-mandelic acid on a bovine serum albumin chemically bonded phase. The results show significantly different profiles for a given amount of either isomer, whether pure or in the racemic mixture. The production rate at 99% purity is more than one order of magnitude higher under overlapping band than under touching band conditions. In the former instance, the recovery yield and production rate are always higher for the less retained enantiomer.

INTRODUCTION

The separation and purification of compounds by preparative liquid chromatography has been an area of increasingly intense interest over the past decade [1]. Important progress has been made in the understanding of the processes that control the migration, broadening and separation of the high-concentration bands injected into chromatographic columns, and also the interactions between the bands of the different components of feedstock mixtures [2–11]. Excellent agreement has been achieved between the experimental band profiles and those which can be calculated by applying the theory of non-linear chromatography [12–16].

The optimization of the experimental conditions for a preparative separation by chromatography has been extensively studied [2,3,17–24]. Several general approaches toward optimization have been undertaken: (1) purely empirical methods [3,17]; (2) semi-empirical methods based on oversimplifying assumptions, such as a non-competitive behavior of the mixture components [2,21,24]; and (3) more complex methods based on the theoretically sound assumption of competitive behavior between the mixture components, but also on the simplifying assumption of a Langmuir isotherm model [18–20, 22,23]. Controversies have arisen regarding the comparative advantages and drawbacks of the touching and the overlapping band approaches [18–24], and even regarding the need to use a competitive isotherm in order to simulate accurately the band profiles [23–25]. On the other hand, the practical usefulness of the displacement effect has been established beyond doubt [3,26].

More practically, debates have also arisen between theorists and experimentalists regarding the selection of the parameters that should be optimized. These parameters should include the efficiency parameters [19] (*e.g.*, the column length, the particle size of the stationary phase [3,20] and the reduced velocity) and the loading factor [19,22]. In some instances, however, the optimization is limited to the sample size [10,27], *i.e.*, to the concentration and/or the volume of the feedstock sample injected. In practice, many chromatographers are limited by the equipment and/or by the available supplies and can only optimize the sample size, especially with wide-bore columns that cannot be operated under high pressure and, for this reason, should be operated at the maximum possible pressure. Another source of argument concerns the acceptable values of the recovery yield and the extent of compromise that is affordable between a high yield and a high

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production rate. This is strictly case dependent. Essentially, two options are available, and all intermediates are hybrids of these two primary choices. If the compound to be purified is expensive compared with the cost of the chromatography, then a touching bands situation [2,23] is desirable in order to ensure the highest yield. If the compound to be purified is inexpensive compared with the cost of the chromatography, then a highly overloaded injection is desirable in order to maximize the production rate [18]. For intermediate cases, a trade-off strategy is available [22].

One of the fundamental reasons why such controversies arise and linger is the lack of relevant experimental data. The acquisition of such data is simple but the choice of separation problems of relevant importance is critical if we really want to clarify some of the current issues. It is necessary to validate the theory of non-linear chromatography as it has been derived [18–20]. If the retention mechanism can be elucidated and the equilibrium isotherms of the main components of the feedstock can be measured in a relatively broad concentration range, then the band profiles can be simulated using the proper algorithm [4–9,11] and the optimum conditions under which to perform the experiment can be determined easily [10,18–20,22,23]. This curtails the number of experiments needed for an empirical determination of these conditions. It was the primary aim of this study to provide such data illustrating the fundamental importance of a sound theoretical approach for a satisfactory optimization of experimental conditions.

Chiral separations [28,29] have become increasingly important and commonplace over the past decade, and the advent of a variety of stationary phases has widened the range of enantiomers that can be analyzed. Bovine serum albumin (BSA) bonded covalently to porous silica [30] or adsorbed on silica [31] has been used for chiral separations, and the two types of columns have recently been compared [32]. Previously, DL-mandelic acid has been separated under linear chromatographic conditions [32], and N-benzoylated amino acid derivatives have been investigated under overloaded conditions [15] and found to have a bi-Langmuir isotherm on this stationary phase.

The production of either pure enantiomer from a racemic mixture is a problem which is simple and can be stated clearly enough to be easily studied and solved from theoretical standpoint. However, it is a relevant problem in the pharmceutical industry. This justifies our choice of system for this study. In this paper, we present experimental results that demonstrate again the displacement and the tag-along effects and permit the measurement of the sample size dependence of the production rates and recovery yields of the two enantiomers for a specified purity on the sample size. A comparison between the performance of touching and overlapping bands is possible.

DEFINITIONS

For the sake of clarity and continuity [18–20,33], we have found it useful to summarize here the definitions of the main optimization parameters as stated previously [18]. These definitions are those used in this paper.

Purity

The purity, Pu, of a component i in the presence of a component j is

$$Pu_i = n_i/(n_i + n_j) \tag{1}$$

where n_i and n_j are the amounts of components i and j in the collected fraction, respectively.

Recovery yield

The recovery yield, R_i, of a component i is

$$R_i = n_i/n_{i,\text{tot}} \tag{2}$$

where n_i is the amount of a component i collected in the purified fraction and $n_{i,\text{tot}}$ is the total amount of this component i injected with the sample. It is assumed that all the solute injected is eluted. Detector calibration proved that this is true within the accuracy of the determination of peak areas. This accuracy, in turn, is limited by the influence of the signal noise on the end-time of the integration of the tail of the second-component peak.

Cycle time

The cycle time, t_c, is the time which separates two consecutive injections. It is usually arbitrarily defined, either as the corrected analytical retention time of the second component, $t_{R,0} - t_0$ [18–20], or as the difference between the time when the first

component concentration exceeds a certain threshold and the time when the second component concentration decreases below the threshold value [10,13,27,33].

Production rate

The production rate, *Pr*, is the amount of a component *i* collected in the fraction at the specified purity per unit time:

$$Pr_i = n_i/t_c \quad (3)$$

Relative production rate

The relative production rate is the production rate of one component relative to its production rate in the case of touching bands.

EXPERIMENTAL

Equipment

A modular chromatograph was assembled, consisting of a Waters (Milford, MA, USA) Model 510 pump, a Waters Gradient Controller, a Scientific Systems (State College, PA, USA), LP-21 pulse damper, a Valco Electric (Houston, TX, USA) injector with a 50-μl injection loop, a Spectroflow Model 757 UV detector (Kratos Analytical, Ramsey, NJ, USA) and a Spectra-Physics (San Jose, CA, USA) integrator with a Labnet Data Acquisition Card hooked up to an IBM (Boca Raton, FL, USA) AT computer. The fractions in the mixed bands region were collected with a Gilson (Middleton, WI, USA) Model 203 fraction collector and analyzed on an HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a diode-array UV–VIS detector and a computer data acquisition system.

Materials

Column. The column dimensions were 150 mm × 4.6 mm I.D. The capacity factors under linear conditions were $k'_L = 3.42$ and $k'_D = 4.75$, giving a selectivity $\alpha = 1.39$. At a flow rate of 1 ml/min, the void time was 1.86 min, and the column efficiency was 1000 plates for both enantiomers.

Stationary phase. A quaternary ammonium anion-exchange stationary phase (301TPB-10; Vydac, Hesperia, CA) with an average particle size of 10 μm and an average pore size of 30 nm was used.

Chemicals. DL-Mandelic acid, D-mandelic acid, L-mandelic acid, and bovine serum albumin (BSA) (No. A-7638) were purchased from Sigma (St. Louis, MO, USA) and used without further purification.

Mobile phase. For the elution profiles, the mobile phase was 50 m*M* aqueous sodium phosphate buffer (pH 6.3) and for the pumping of BSA onto the column the mobile phase was a 10 m*M* aqueous phosphate buffer (pH 6.8).

Procedures

The BSA was affixed in the column by pumping a 1 mg/ml BSA solution in 10 m*M* phosphate buffer (pH 6.8) onto the column until the BSA breakthrough was detected. The amount of BSA loaded in the column was calculated from the breakthrough curve and was 176 mg. Before the enantiomeric separations were undertaken, the column was equilibrated with 50 m*M* phosphate buffer (pH 6.3) for 75 column volumes. Elution of BSA at this pH was negligible.

Two sets of elution profiles were determined. First, samples of increasing amounts of the racemic mixture (sample size 1.6–25.6 μg, increasing in proportions 1, 2, 4, 8 and 16) were injected in order to investigate the effects of the competitive behavior of the enantiomers. The profiles obtained are shown in Figs. 1–3. Second, the pure isomers were injected separately, each in the same amount as before, in order to compare the band profiles obtained under non-competitive with competitive conditions. The elution profiles of the racemic mixtures and the pure enantiomers were converted by direct calibration of the detector response to concentration profiles. At the wavelength used, 250 nm, the calibration graphs were linear and the regressions were carried out using a standard procedure.

For the higher concentration injections (Figs. 1c, 2 and 3), the chromatograms exhibit overlapping bands. In order to determine accurately the individual band profiles, fractions in the mixed bands region were collected and reinjected under linear conditions for quantitative analysis. This procedure permits the exact determination of the concentration of each enantiomer. The interval for collection was one fraction every 6 s, *i.e.*, the limit frequency of the model fraction collector employed. The mixed zone was 7.65–8.15 min for Figure 1c (six fractions),

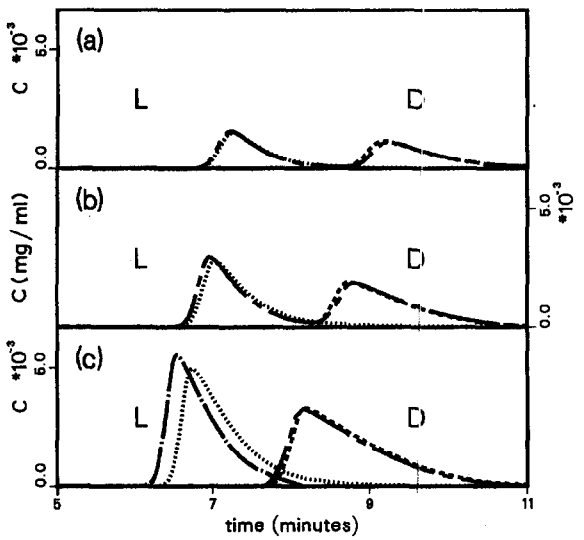

Fig. 1. Comparison between the elution profiles of the enantiomers of DL-mandelic acid with competition, (1) L-isomer (– · – · –) and (2) D-isomer (————); and without competition, (3) L-isomer (.....) and (4) D-isomer (--------). (a)Experimental conditions: stationary phase, BSA ionically immobilized on anion exchanger; mobile phase, 50 m*M* phosphate buffer (pH 6.3); flow-rate, 1 ml/min; sample size, (1) 0.80, (2) 0.80, (3) 0.80 and (4) 0.80 μg. (b) Same conditions as for (a) except twice the sample size: (1) 1.6, (2) 1.6, (3) 1.6 and (4) 1.6 μg. (c) Same conditions as for (a), except four times the sample size: (1) 3.2, (2) 3.2, (3) 3.2 and (4) 3.2 μg.

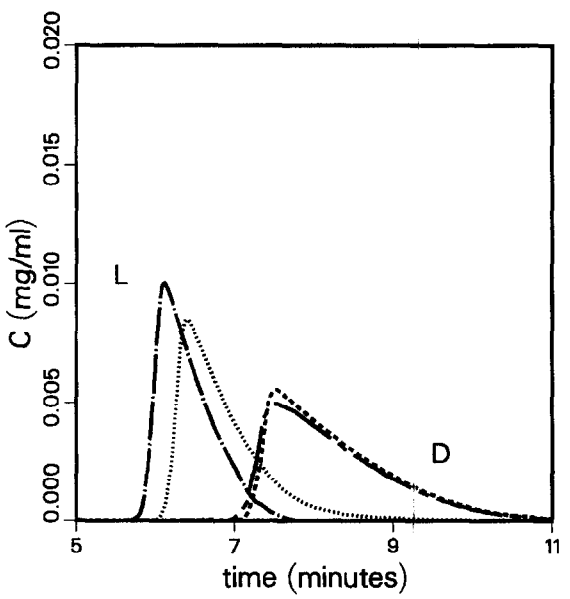

Fig. 2. Comparison between elution profiles of DL-mandelic acid with competition. Same experimental conditions as for Fig. 1a, except eight times the sample size: (1) 6.3, (2) 6.3, (3) 6.4 and (4) 6.4 μg.

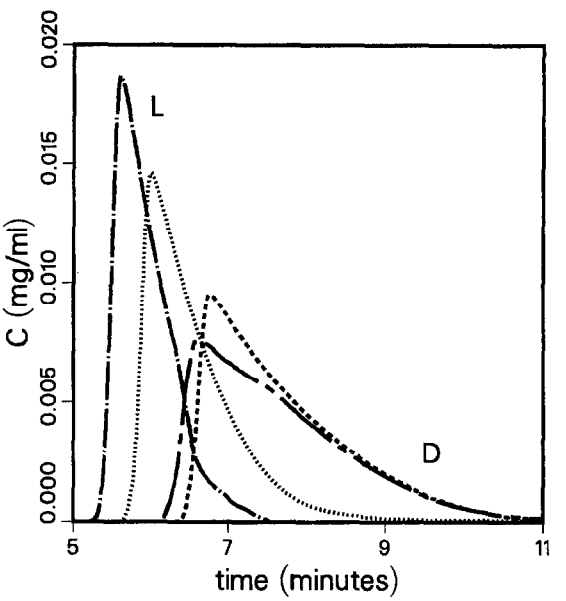

Fig. 3. Comparison between elution profiles of DL-mandelic acid with competition. Same experimental conditions as for Fig. 1a, except sixteen times the sample size: (1) 12.7, (2) 12.7, (3) 12.8 and (4) 12.9 μg.

6.95–7.75 min for Fig. 2 (nine fractions) and 6.15–7.55 min for Fig. 3 (fifteen fractions). In Fig. 1b, the mixed zone was too dilute to collect fractions and analyze them. In Fig. 1a, no mixed zone existed because of the touching bands condition. In order to integrate accurately the area underneath the band profiles to determine the cut points for specified purities, points between collected fractions were linearly interpolated at 1-s intervals.

RESULTS AND DISCUSSION

If an organic synthesis produces a racemate which later must be separated, the ratio of the enantiomers is 1:1. Although stereoselective synthesis may produce mixtures considerably enriched in one of the enantiomers, the racemic mixture remains the most important one to study.

Band profiles

In Figs. 1–3, a series of chromatograms of DL-mandelic acid are illustrated, demonstrating both the displacement and the tag-along effects [7,15,34] by overlaying the elution profiles obtained from the racemic mixture, *i.e.*, under competitive conditions

(solid lines), and the elution profiles obtained from the pure enantiomers (dashed lines), *i.e.*, under non-competitive conditions. The series of chromatograms differ only in the amounts injected (more precisely, in the sample concentrations). In Fig. 1a, the touching bands situation occurs, and the agreement between the chromatogram of the racemate and the chromatograms of the pure enantiomers is very good, *i.e.*, within experimental error, much as in analytical chromatography. From Figs. 1–3, the sample sizes increase in the proportions 1, 2, 4, 8 and 16. As the amount increases, the non-linear effects intensify, in addition to the consequences of the competition for adsorption between molecules of the enantiomers. In such a mixture, however, the displacement effect is more visible than the tag-along effect, although both are present (*e.g.*, Fig. 3).

Given a defined system, the degree to which the tag-along and displacement effects contribute to the distortion of the band profiles of the racemic mixture depends solely on the sample concentration. The higher this concentration the greater is the band overlap observed. For the displacement effect, the sharp front and diffuse rear of the less retained component, the L-isomer, elutes before what is predicted by the elution profile of injection of pure L-mandelic acid. Also, a slight enrichment of the peak maxima is observed. Both effects are seen as early as in Fig. 1b, where the degree of band interference of the elution chromatogram is insignificant. Of course, this observation translates the fact that the bands have severely interfered during part of their migration along the column. Thus, in Fig. 1b and c, although the bands appear to be fairly well resolved with nearly a baseline separation, the memory effects of the displacement are still present. For the tag-along effect, the front of the more retained component, the D-isomer, elutes prior to the time predicted by the injection of the pure D-mandelic acid, and the maximum of the peak is lower and flatter than expected. These consequences of the tag-along effect are seen only in Figs. 2 and 3. As predicted [34], the diffuse rear of the profile of the D-isomer under competition coincides exactly with that of the non-competitive, pure injection of the D-isomer.

The band profiles of the pure enantiomers exhibit quasi-Langmuirian behavior which allows the assumption that the mixed band region follows similar behavior under competitive conditions. Fraction collection has been shown to be a viable means for determining the concentrations of the two enantiomers in the mixed zone of the chromatogram [15]. Following fraction collection and analysis, the mixed zones for Figs. 3–5 are calculated and behave as expected. Consequently, the method of Knox and Pyper [2] and the method of Golshan-Shirazi and Guiochon [18,19,22,23] are implemented as guidelines. The former method employs no competition for adsorption between components and is useful only in the touching bands case (Fig. 1a), whereas the latter includes competition and is useful in all cases, whether the bands are resolved or overlap (Figs. 1b, 1c, 2 and 3).

Production rate and recovery yield

The data associated with Figs. 1–3 concerning the recovery yields (Fig. 4) and the relative production rates (Fig. 5) are listed in Table I. The relative production rate was calculated rather than an absolute quantity as the experimental conditions can fluctuate. The cycle time, usually arbitrarily set, is constant for the five injections and therefore does not have to be established exactly because of the calculation of a relative production rate. The relative production rates are calculated by discarding any intermediate fractions, *i.e.*, the fraction collected between cut times in Figs. 1c, 2 and 3 is not recycled.

The cut times are the starting and stopping points for the collection of fractions of purified components of the L- and D-isomers, respectively. For the first component, the L-isomer, the fraction is collect-

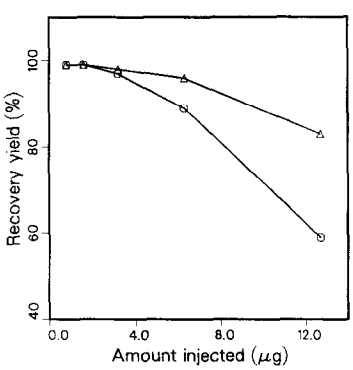

Fig. 4. Variation of the recovery yield with the amount injected for (△) the L-isomer and (○) the D-isomer. See Table I for calculated values.

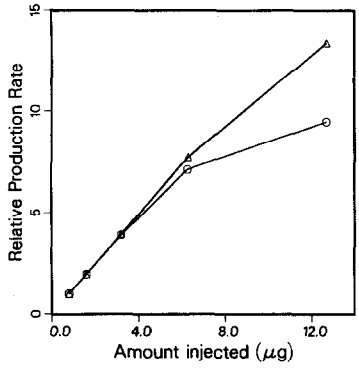

Fig. 5. Variation of the relative production rate with the amount injected for (△) the L-isomer and (○) the D-isomer. See Table I for calculated values.

ed from the start of the elution profile up to the cut time, and for the second component, the D-isomer, the fraction is collected from the second cut time to the end of the elution profile. In Fig. 1a and b, only one cut is necessary to achieve the desired purity and a 100% recovery yield is achieved. Also, although recovery yields and production rates for lower product purities could be calculated easily, only a purity of 99% has been applied to all five instances, for the sake of simplicity in the comparison.

Fig. 4 shows that the recovery yield remains constant and is unity until past the conditions corresponding to touching bands (conditions of Fig. 1c). As long as the purity of each fraction is within specifications, the yield is unity. The recovery yields for the L-isomer (the first eluted) are always better than those for the D-isomer. The yields of the two components remain acceptable up to the conditions corresponding to Fig. 2 (89 and 96%). As long as the recovery yields are high, the production rate increases in proportion to the sample amount (Fig. 5), then it tends to level off. It is important to observe that even under the conditions of Fig. 3, where the two bands are not resolved and an analyst would conclude that there is an excessive degree of overload (rightly so for analytical purposes, wrongly for preparative purposes), the production rate for the L-isomer is far from its maximum.

Comparing Figs. 4 and 5 or the corresponding columns in Table I, one can see that a point of diminishing return is reached when the recovery yield drops below *ca.* 90%. This is clearer with the second-component data, because the phenomenon takes place at lower sample amounts for the second than for the first component. When the recovery yield drops from 99 to 89%, the production rate of the second component increases sevenfold. When the yield decreases further, from 89 to 59%, the production rate increases by only 33%. In practice, the conditions in Fig. 2 are close to the optimum for the production of the second component and those in Fig. 3 for the production of the first component.

These results confirm that the choice of the optimum production rate depends on the recovery yield that is deemed satisfactory. If the starting product (feedstock) is plentiful and can be wasted, then low recovery yields are acceptable, allowing higher production rates (conditions in Fig. 3 or even higher loading). On the other hand, expensive pharmaceuticals may require nearly 100% recovery yields, resulting in lower production rates (conditions in Fig. 1c or 2). The recovery yields (Fig. 4) and relative production rates (Fig. 5) for the L-isomer (first eluted) are better than those for the D-isomer. For the purpose of highest recovery yields and production rates, the displacement effect on the first component is highly desirable while the tag-along effect on the second component is detrimental. The choice of the chiral phase used must be made according to the enantiomer of preference.

One drawback of a stationary phase involving the immobilization of a protein is the poor efficiency exhibited by the column. The reduced plate height of

TABLE I

RELATIVE PRODUCTION RATES AND RECOVERY YIELDS FOR 99% PURE ENANTIOMER FRACTIONS

Fig.	Enantiomer	Cut time (min)	Recovery yield (%)	Relative production rate
1a	L	8.60	99+	1.00
	D	8.60	99+	1.00
1b	L	8.27	99	1.97
	D	8.27	99	1.97
1c	L	7.80	98	3.94
	D	7.90	97	3.89
2	L	7.18	96	7.70
	D	7.47	89	7.12
3	L	6.31	83	13.4
	D	7.23	59	9.47

15 is high and the low column efficiency limits the range of sample amounts that can usefully be investigated. However, the separation factor is large ($\alpha = 1.4$) and the column efficiency (*ca.* 1000 theoretical plates) exceeds the value that would correspond to the optimum for maximum production rate [19,22,33].

CONCLUSIONS

The experimental results reported here are in qualitative agreement with the theoretical conclusions published previously [18,19]. The following conclusions are of practical importance. The recovery yield and the production rate achieved with the less retained enantiomer are higher than with the more retained enantiomer. Depending on which isomer is most strongly needed, a chiral selective stationary phase or its antipode should be selected, whenever possible. In order to perform a preparative separation and purification of a two-component mixture, *e.g.*, a racemic mixture, a decision as to what are acceptable losses in order to achieve the greatest amount of the pure compound(s) desired must be made. If some losses and interfering bands are permitted, the cut points must be established from the competitive, not the non-competitive chromatogram, otherwise, undesirable results are obtained. Overlapping conditions, with apparently total loss of resolution (in the analytical sense) permit a dramatic increase in the production rate (up to tenfold or more, Fig. 5), provided a moderate recovery yield is acceptable.

As this order of magnitude increase in the production rate is achieved by merely increasing the sample amount, all the cost components remain constant. Therefore, the cost of the extraction or purification is divided by the same amount. Major economic losses are thus incurred by those who persist in ignoring the basic results of non-linear chromatography.

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REFERENCES

1 M. Verzele and C. Dewaele, *Preparative High Performance Liquid Chromatography*, TEC, Ghent, 1986.
2 J. H. Knox and H. M. Pyper, *J. Chromatogr.*, 363 (1986) 1.
3 J. Newburger, L. Liebes, H. Colin and G. Guiochon, *Sep. Sci. Technol.*, 22 (1987) 1933.
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. Golshan-Shirazi and A. Jaulmes, *Anal. Chem.*, 60 (1988) 1856.
7 G. Guiochon and S. Ghodbane, *J. Phys. Chem.*, 92 (1988) 3682.
8 S. Golshan-Shirazi, B. C. Lin and G. Guiochon, *J. Phys. Chem.*, 93 (1989) 6871.
9 Q. Yu and N.-H. L. Wang, *Comput. Chem. Eng.*, 13 (1989) 915.
10 A. Katti and G. Guiochon, *Anal. Chem.*, 61 (1989) 982.
11 M. Czok and G. Guiochon, *Anal. Chem.*, 62 (1990) 189.
12 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 60 (1988) 2634.
13 A. M. Katti and G. Guiochon, *J. Chromatogr.*, 499 (1990) 21.
14 A. M. Katti, Z. Ma and G. Guiochon, *AICh J.*, 36 (1990) 1722.
15 S. Jacobson, S. Golshan-Shirazi and G. Guiochon, *J. Am. Chem. Soc.*, 112 (1990) 6492.
16 M. Z. El Fallah and G. Guiochon, *Anal. Chem.*, 63 (1991) 859.
17 L. R. Snyder, G. B. Cox and P. E. Antle, *Chromatographia*, 24 (1987) 82.
18 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 61 (1989) 1276.
19 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 61 (1989) 1368.
20 S. Golshan-Shirazi and G. Guiochon, *Am. Biol. Lab.*, 8, No. 8 (1990) 26.
21 L. R. Snyder, J. Dolan and G. B. Cox, *J. Chromatogr.*, 484 (1989).
22 S. Golshan-Shirazi and G. Guiochon, *J. Chromatogr.*, 536 (1991) 57.
23 S. Golshan-Shirazi and G. Guiochon, *J. Chromatogr.*, 523 (1990) 1.
24 L. R. Snyder, J. Dolan and G. B. Cox, *J. Chromatogr.*, 523 (1990) 11.
25 L. R. Snyder, J. Dolan and G. B. Cox, *J. Chromatogr.*, 484 (1989).
26 J. Newburger and G. Guiochon, *J. Chromatogr.*, 523 (1990) 63.
27 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 444 (1988) 275.
28 D. W. Armstrong and S. M. Han, *CRC Crit. Rev. Anal. Chem.*, 19 (1988) 175.
29 W. H. Pirkle and T. C. Pochapsky, *Chem. Rev.*, 89 (1989) 347.

30 S. Allenmark, B. Bomgren and H. Boren, *J. Chromatogr.*, 237 (1982) 473.
31 P. Erlandsson, L. Hansson and R. Isaksson, *J. Chromatogr.*, 370 (1986) 475.
32 S. Andersson, S. Allenmark, P. Erlandsson and S. Nilsson, *J. Chromatogr.*, 498 (1990) 81.
33 A. M. Katti, E. Dose and G. Guiochon, *J. Chromatogr.*, 540 (1991) 1.
34 S. Golshan-Shirazi and G. Guiochon, *J. Phys. Chem.*, 94 (1989) 4143.