Journal of Chromatography, 464 (1989) 1–25
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 084

IDEAL MIXED CELLS MODEL OF MULTI-COMPONENT PREPARATIVE LIQUID CHROMATOGRAPHY

VRATISLAV SVOBODA
Institute for Research, Production and Application of Radioisotopes, Radiová 1, 102 27 Prague 10 (Czechoslovakia)
(First received July 12th, 1988; revised manuscript received November 2nd, 1988)

SUMMARY

A simple mixed cells model was used for computer simulation of multi-component separation in liquid chromatography. For a description of multi-component adsorption an equation was derived using the concept of adsorption sites blocking by adsorbed molecules in various ratios. With this model the production rate and recovery ratio were followed. At a given load the maximum productivity is attained at the optimum column length. With longer columns with higher loads, higher optimum production rates may be obtained. Optimization of the feed volume increases the productivity only negligibly. If one component in the mixture predominates, the separation is governed by column overloading with this single compound. Higher production rates may be achieved in gradient elution. In displacement chromatography still higher productivities are obtainable, but in this mode not only the overload limitation but also a low limit of injected amount for succesful separation exists. Compounds with crossing isotherms may be separated, contrary to the "golden rule" in displacement chromatography. If the separation of compound pairs with concave and convex hyperbolic isotherms is computed, the two peaks are replaced by a spike at higher loads. This erroneous result is caused by an unlimited increase in the simple hyperbolic (convex) isotherm near the critical solute concentration.

INTRODUCTION

The concept of the theoretical plate model, introduced by Martin and Synge[1], has been complemented by the discontinuous flow model of Mayer and Tompkins[2] and a basis of the theory of column chromatography was developed that has been very fruitful for the understanding of separations of substances. This was preceded by DeVault's[3] attempt to describe the behaviour of solutes on a column by a set of partial differential equations. These two directions in attempts to describe chromatographic separations quantitatively have been followed up to the present.

The first direction in using the description of a continuous model by set of differential equations was further expanded by Glueckauf[4] and by the introduction of the parabolic approximation of the Langmuir isotherm[5], making possible the

0021-9673/89/$03.50

analytical description of peaks in overloaded columns. Numerical methods for the solutions of these equations have been succesfully applied using a digital computer[6]. The Laplace transform of Langmuir's isotherm made it possible to describe multi-component separations in overloaded columns[7]. A more detailed study of the separation of a two-component mixture has been published[8].

The extension of Houghtons' concept of a two-term approximation of Langmuir's isotherm was expanded in recent years[9–11], culminating in a description of the separation of a two-component mixture, where, however, the mutual influence of the components was neglected[12].

The other direction, the discontinuous flow model, was revived by Seshadri and Deming[13], who considered the influence of isotherm curvature on peak shape. Recent application of this concept to a single elution band assuming a Langmuir isotherm demonstrated clearly its applicability to preparative chromatography[14].

Earlier attempts to describe separations by preparative chromatography[15–17] were based on semi-empirical equations and do not enable one to predict the separations of complicated mixtures or to make *ab initio* calculations.

Very interesting from the point of view of preparative chromatography are the gradient and displacements modes of liquid chromatography. The pioneering work of Hagdahl *et al.*[18] has recently been complemented both theoretically and experimentally[19–21]. The elution and displacement modes of chromatography were compared only experimentally; no simple theoretical assessment of these modes was possible until now.

The basic relationship used in all these studies is the Langmuir isotherm. The single-component Langmuir equation was extended to multi-component adsorption by Butler and Ockrent[22]. This isotherm has been criticized because it is inconsistent with the Gibbs adsorption isotherm for ideal adsorbed solution, unless the monolayer capacities are equal. The proposed approximation for binary isotherms is only speculative[23].

Eble *et al.* extended their previous studies[14] to a two-component mixture[24] and were able to extrapolate their results so that gradient chromatography with a moderately overloaded column could be predicted with reasonable accuracy. However, only results from a two-component mixture were presented. In all these previous studies, the purity of the isolated fraction was not defined; only the "separation resolution" was used for the establishment of suitability for preparative separations.

The model based the solution of a two-term Langmuir isotherm expansion was recently extended to a two-component case[25]. The solution of a multi-component case by this method will hardly be possible.

Snyder *et al.*[27] combined the concept of column blockage, formulated by Knox and Pyper[26], with results from the two-component model[24] so that, with empirical equations, even multi-component separations in moderately overloaded column could be formulated.

We start our consideration with the derivation of a multi-component hyperbolic isotherm. Then optimization of production rates and recoveries in multi-component chromatographic separations using isocratic, gradient and displacement modes are examined, using computer simulation.

BASIC RELATIONSHIPS

The equilibrium concentrations of a compound i in the solid and mobile phases can be described by a simple equation:

$$\frac{c_{iF}}{c_i c_F} = K_i \tag{1}$$

where K_i is the equilibrium constant, c_i the concentration of compound i in the mobile phase, c_{iF} the concentration of the adsorbed compound and c_F the concentration of free sorption sites (both in the solid phase). The amount of compound i in one equilibrium cell, G_i, is therefore

$$G_i = c_i V_M + c_{iF} V_s \tag{2}$$

where V_s is the volume of the solid phase and V_M that of the mobile phase. The capacity of the sorbent is the sum of all sorption sites covered by adsorbed compounds plus free sites:

$$G_D = V_s\left(c_F + \sum_{i=1}^{n} c_{iF} R_i\right) \tag{3}$$

where R_i is the blocking factor, defined as the average number of adsorption sites covered by one molecule of compound i bound to one adsorption site. This possibly oversimplified mechanistic approach enables us to describe rationally multi-component equilibria without introducing any artifical empirical coefficients. After simple substitutions we obtain for the concentration of compound j in the mobile phase (in one equilibrium cell)

$$c_j = \frac{G_j}{V_M + G_D K_j \Big/ \left(1 + \sum_{i=1}^{n} R_i K_i C_i\right)} \tag{4}$$

If there is only one compound present, we arrive to the well known Langmuir isotherm:

$$c_{iF} = \frac{c_i K_i G_D / V_s}{1 + K_i R_i c_i} \tag{5}$$

When $R_i = 1$, the classical form of the Langmuir isotherm is obtained. If a linear isotherm describes best the behaviour of an examined compound, then $R_i = 0$. If the isotherm is concave towards the c_{iF} axis, then R_i is negative. In this instance R_i is not related to the number of sites covered by a solute molecule and allows the description of other isotherm systems not identical with the original Langmuir model. Obviously, for positive R_i the maximum adsorbed concentration approaches $G_D/V_s R_i$. If, on the other hand, the blocking factor is negative, then the maximum concentration in the mobile phase is $-1/K_i R_i$. The extension of eqn. 5 to a many-component equilibrium:

$$c_{jF} = \frac{K_j c_j G_D / V_s - \sum_{i \neq j} R_i c_{iF}}{1 + c_j K_j R_j} \quad (6)$$

including a term reflecting the decrease in available adsorption sites caused by other compounds.

This equilibrium model neglects the volume of single components; we assume that molar volumes are zero. The limitation by solubility in mobile or solid phase is also neglected.

The equilibrium cell corresponding to one theoretical plate[1,2] contains equilibrated concentrations of all components. The transport in the chromatographic column is visualized as a discontinuous process, in which the free volume of one theoretical plate (mobile phase volume of the equilibrium cell) is moved step by step, and always equilibrated, with the solid phase contained in one cell. For a low distribution coefficient ($K_i \ll 1$) and a small number of theoretical plates this approximation may lead to severe errors. Another disadvantage of this simulation is that the axial dispersion coefficient is equal and constant for all components and it is impossible to change its value by changing the concentrations of components, as is sometimes the case in real systems.

COMPUTING

For computation the approach of Seshadri and Deming[13] is used, in which the number of mixed cells is equal to the number of theoretical plates[2]. The algorithm for solution of equilibrium eqns. 1–3 by iteration is centred around eqn. 4. The term in parentheses in the denominator is common to all components. Therefore its value is first estimated (from the preceding step), then the concentrations for all components are calculated and finally, the new value of this term and the difference between the new and old values are found. If the relative error is less than $1 \cdot 10^{-5}$, the calculation for this cell is finished. If not, a new value of this term is chosen, according to the rules for iterative calculations, and the whole process is repeated. On average, six steps are needed to solve this set of equations. No approximations are introduced in the equations; the model works with just the arithmetic error in all concentrations and no limit to the concentration or number of components is set. The only limiting factors are technical, *viz.*, the available operating memory and computing time.

With modern personal computers this presents no problem. Eble *et al.*[24], using the same mixed cells model, approximated the two-component Langmuir isotherm by a nine-term polynomial expansion; it was reported that the average error did not exceed 8% for low and moderate surface coverages.

All the computations here were performed with a PDP 11/23 microcomputer (Digital Equipment Co., Maynard, MA, U.S.A.) with a 128K memory under the RT 11 or RSX 11 M system. All programs were written in Fortran F 77. A typical chromatogram [five components, 1000 theoretical plates (T.P.) plus four shorter columns] takes about 15 h of computing time. The values of the concentration of the effluent from all columns in a given computation were stored and subsequently the peak forms, the start and end of all pure fractions, the end of the chromatogram, etc., were evaluated. All fractions were selected so that the purity of separated compound was 99% or higher. In all instances V_M, G_D and V_s were set equal to 1.

RESULTS AND DISCUSSION

To follow the influence of single factors on the separation of a multi-component mixture, a typical example was selected, *viz.*, a sample containing five components with equilibrium constants K_i = 0.5, 1.0, 1.5, 2.0 and 2.5. The volumes of the mobile and stationary phases in one equilibrium cell (theoretical plate) were chosen as 1 and the capacity of the sorbent in this volume was also set equal to 1. The sample amount, the mode of its introduction, values of the blocking factors and column lengths were varied. Three modes of chromatography, isocratic, gradient and displacement, were compared. In some instances, the number of sample components was decreased to three.

From the point of view of preparative chromatography the two most important parameters are the production rate and the amount recovered. The production rate was calculated as the ratio of the amount recovered and the number of steps needed to elute the last component from column, in other words, the amount recovered divided by the time needed to perform the chromatographic separation. The purity of the fractions has to be higher than 99%; in the effluent a step volume containing the greatest amount of component with higher than the required purity was selected and then its volume was increased (forwards and backwards) until the limit of purity was just attained.

Separation of five components with blocking factors of unity

Typical examples of this separation (with increasing sample loads) are displayed in Figs. 1–4. In the first instance, a small amount of sample (1 for all components) was injected in one step. The peak shapes are nearly ideal (Gaussian) with a resolution between adjacent peaks that is so high that the recovery is nearly 100% for all components. If into the same column (1000 T.P.) a 100 times larger sample is injected, only the first and partially the second component can be recovered with the required purity (Fig. 3). All peaks are shifted toward lower volumes, and a mutual influence (*e.g.*, second and third components) on the peak shape is clearly seen. This picture changes drastically if the column length is increased to 2000 T.P. (Fig. 4). Tables I and II give the recoveries and productivities for all five components on two columns that differ only in the number of theoretical plates. With increasing sample load the concentration of the isolated fraction increases and the position of the peak maximum shifts to lower elution volumes (Table I). The productivity initially increases but, after the peaks widen so that the recovery decreases, the productivity starts to decrease.

In Fig. 5 are plotted the production rate and recovery for various lengths of column for the situation when all peaks are Gaussion (and the sample load is constant). Also in this instance the productivity at first increases with increasing resolution, but after the greatest part of the peaks has eluted in high purity, a further increase in the number of theoretical plates only increases the time necessary for a given separation and therefore the production rate declines. When both factors, column length and sample load, are plotted (Fig. 6), the maximum production rates are attained with the longest columns. With decreasing column length the optimum sample load decreases, but the optimum productivity decreases only slightly. The productivity decreases sharply when the column is so short that even at minimum sample loads (Gaussian peak shapes) the resolution between adjacent peaks is less than about 1.5. When a column is this short, its application in the preparative mode is very ineffective.

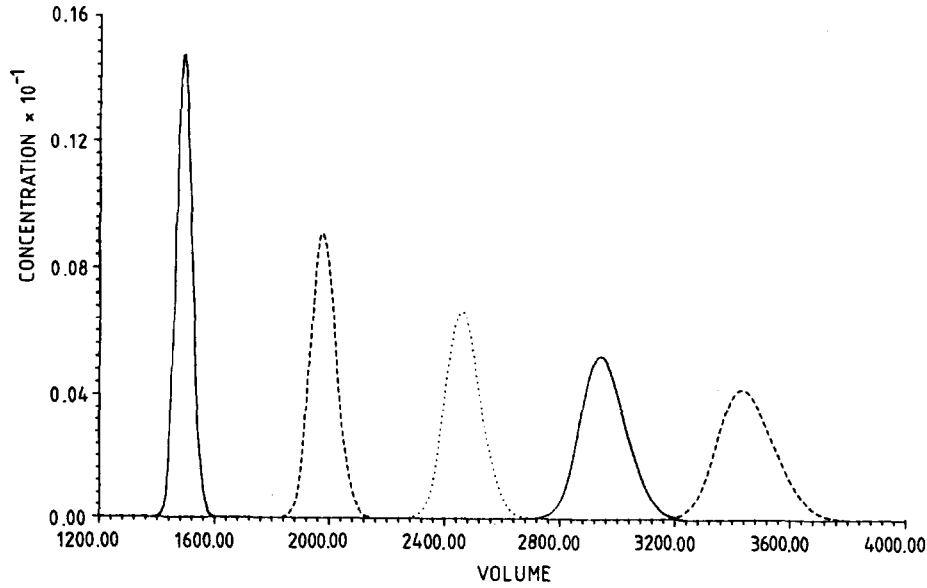

Fig. 1. Separation of a standard five-component mixture. Column, 1000 T.P.; capacity factors, 0.5, 1.0, 1.5, 2.0, 2.5; all blocking factors, 1; volume injected, 1; amount injected (all components), 1.

Knox and Pyper[26] did not directly address the problem of the dependence of production rate (practical throughput in their terminology) on the number of theoretical plates. Their consideration was centred around maximization of the throughput under pressure-limited operation. It should be stressed that in their

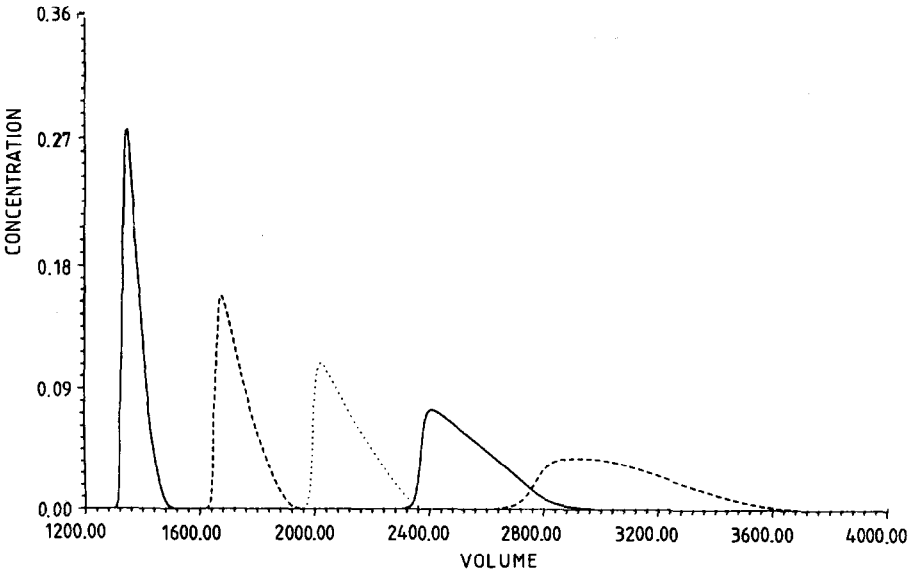

Fig. 2. Separation as in Fig. 1 except amount injected (all components), 20.

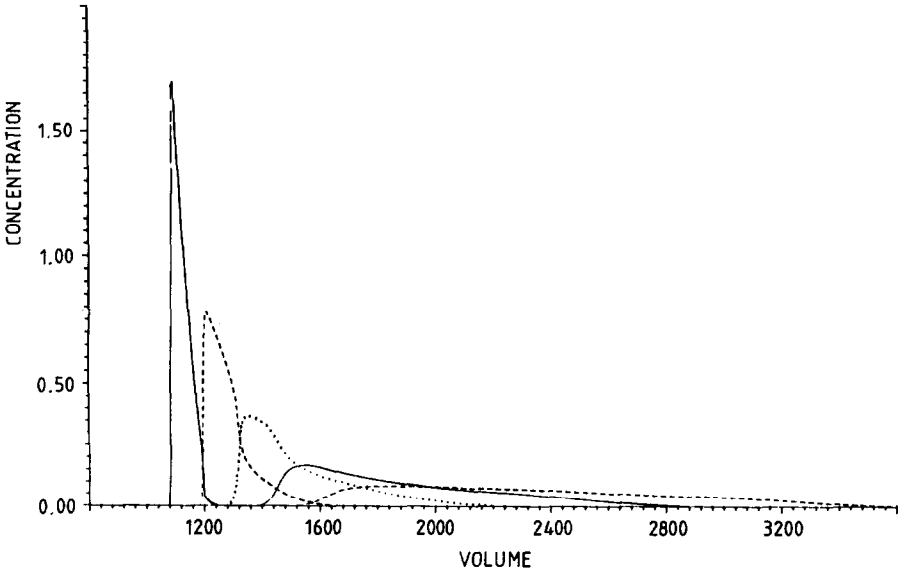

Fig. 3. Separation as in Fig. 1 except amount injected (all components), 100.

discussion it was assumed that there was almost no cross-contamination between adjacent peaks. In our results, optimum throughput is achieved when the peaks strongly interfere and the recovery is around 60%.

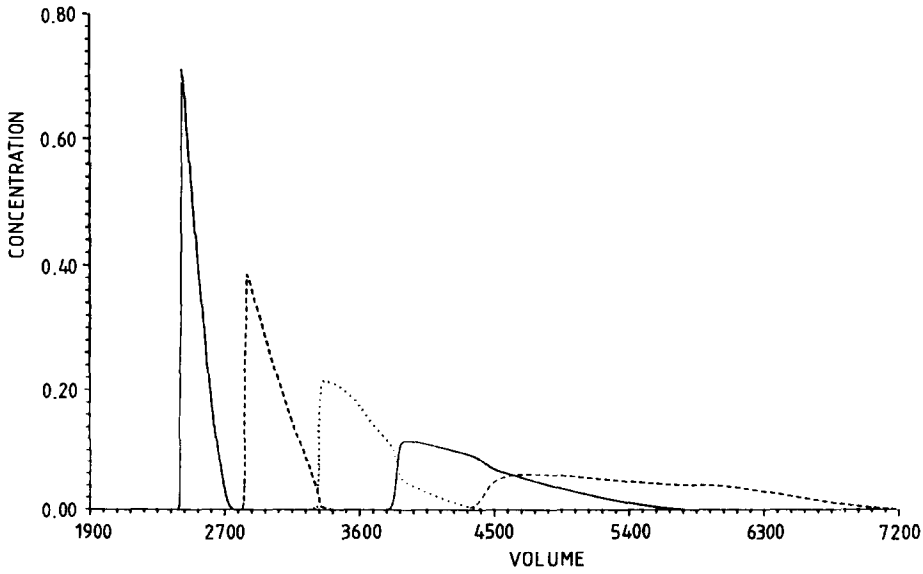

Fig. 4. Separation as in Fig. 3 on a column with 2000 T.P.

TABLE I

INFLUENCE OF SAMPLE LOAD ON RECOVERY AND PRODUCTIVITY

Column, 1000 T.P. Five compounds, capacity factors: 0.5, 1, 1.5, 2.0, 2.5; Blocking factors, 1. REC = Recovery (%); PRD = productivity multiplied by 100; CONC = concentration multiplied by 10^4; P.MAX = peak maximum.

Amount	*Compound 1*				*Compound 2*			
	REC	*PRD*	*CONC*	*P.MAX*	*REC*	*PRD*	*CONC*	*P.MAX*
5 × 1	100.0	0.024	24	1489	100.0	0.024	16	1974
5 × 3	100.0	0.071	73	1469	100.0	0.071	49	1926
5 × 20	100.0	0.472	512	1344	100.0	0.472	432	1674
5 × 25	100.0	0.589	661	1318	100.0	0.589	572	1624
5 × 30	100.0	0.707	852	1294	100.0	0.707	716	1579
5 × 50	100.0	1.179	1866	1215	99.2	1.170	1687	1434
5 × 100	99.0	2.333	7227	1089	51.7	1.218	6152	1202

TABLE II

SEPARATION OF THE SAME COMPOUNDS AS IN TABLE I ON A COLUMN WITH 2000 T.P.

Abbreviations as in Table I.

Amount	*1*		*2*		*3*		*4*		*5*	
	REC	*PRD*	*REC*	*PRD*	*REC*	*PRD*	*REC*	*PRD*	*REC*	*PRD*
5 × 30	100	0.374	100	0.374	100	0.374	99.7	0.373	99.9	0.374
5 × 60	100	0.748	100	0.748	99.2	0.743	65.9	0.493	62.7	0.469
5 × 100	100	1.248	99.7	1.244	78.6	0.981	0	0	38.3	0.478

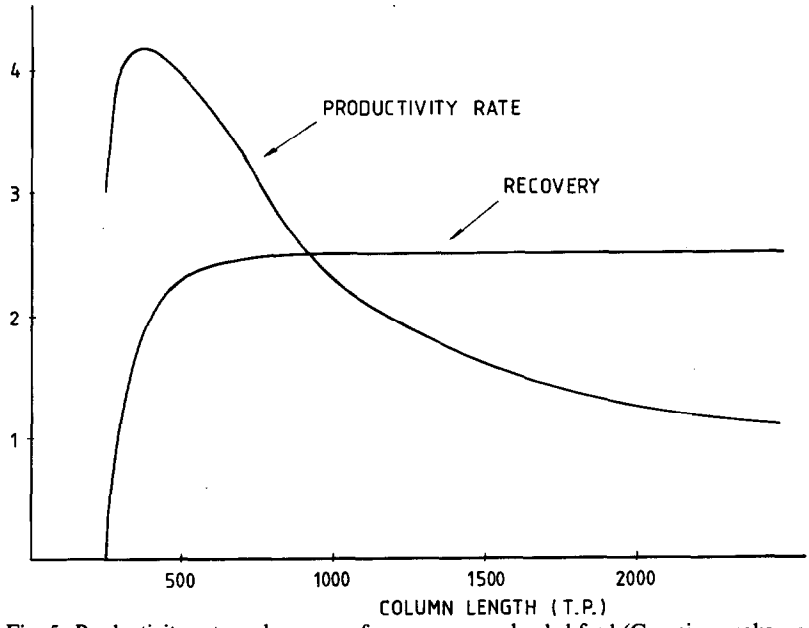

Fig. 5. Productivity rate and recovery from a non-overloaded feed (Gaussian peaks, component 4) with various column lengths. Capacity and blocking factors as in Fig. 1.

Compound 3				*Compound 4*				*Compound 5*			
REC	*PRD*	*CONC*	*P.MAX*	*REC*	*PRD*	*CONC*	*P.MAX*	*REC*	*PRD*	*CONC*	*P.MAX*
100.0	0.024	15	2454	100.0	0.024	17	2942	100.0	0.024	9	3434
100.0	0.071	47	2383	99.9	0.071	53	2847	100.0	0.071	26	3232
98.8	0.471	460	2022	90.4	0.426	506	2412	81.6	0.385	118	2918
99.3	0.585	635	1952	75.7	0.446	651	2333	66.8	0.394	121	2792
97.7	0.691	842	1891	46.0	0.325	738	2259	56.2	0.398	122	2683
66.9	0.789	1672	1691	0	0	0	1985	34.3	0.405	124	2394
0	0	0	1350	0	0	0	1549	17.4	0.410	125	1846

Influence of feed volume

When the feed is injected in one step in a volume equal to the void volume of one theoretical plate and the amount of components is so high that the productivity of separation is slightly higher than the optimum, then the first parts of the column are strongly overloaded. To offset the overloading and to improve the preparative separation, we tried diluting the same amount of feed in various injected volumes. As can be seen from the results (Table III), the productivity and recovery can be improved

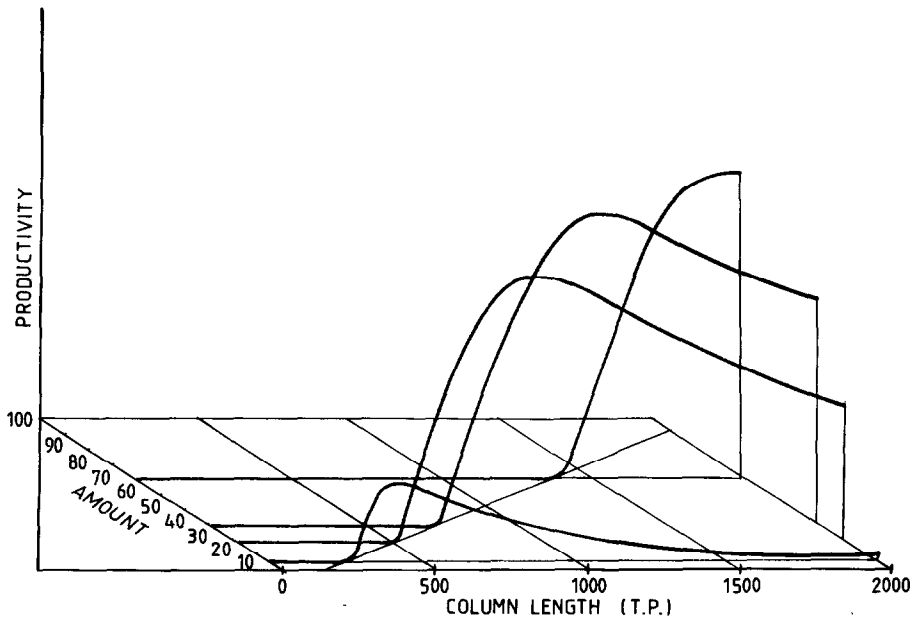

Fig. 6. Production rate of component 4 at various loads (up to 100 for all components) and with various column lengths (up to 2000 T.P.). Capacity and blocking factors as in Fig. 1.

TABLE III

EFFECT OF VOLUME INJECTED

Constant amount: 30 units. Same components as in Table I. Concentrations of all components in feed are identical. Column: 1000 T.P. Abbreviations as in Table I.

Feed volume	*Concentration*	*Component 4*				*Component 3*			
		REC	*PRD*	*CONC*	*P.MAX*	*REC*	*PRD*	*CONC*	*P.MAX*
1	30	46.0	0.325	0.0738	2259	42.0	0.467	0.1701	1023
15	2	54.0	0.381	0.0702	2265	52.4	0.580	0.1621	1035
75	0.4	48.3	0.336	0.0690	2317	38.8	0.421	0.1573	1092
150	0.2	34.2	0.234	0.0670	2377	11.0	0.116	0.1503	1157
30	/0 ... 2/[a]	54.5	0.383	0.0701	2280	53.7	0.592	0.1628	1050

[a] Concentration of feed varies.

only slightly by this approach. If the feed volume is greater than about one tenth of the column void volume, the productivity and recovery decrease. If the concentration of the feed increases linearly during the injection, the separation is about equal to that of uniform injection in half of the volume (see Table III). Chromatograms with two extreme productivities and recoveries of component **4** in Table III are presented in Figs. 7 and 8. It is clear that the separation of other components depends only slightly on the volume injected in the range examined. In both chromatograms the peak shapes are determined predominantly by overloading; the influence of the injection volume is only secondary.

Knox and Pyper[26] predicted that there is a decline in recovery only when the volume injected is increased above about half of the peak volume at the column outlet (without overloading). For component **4**, σ (Gaussian peak) for the peak at the column

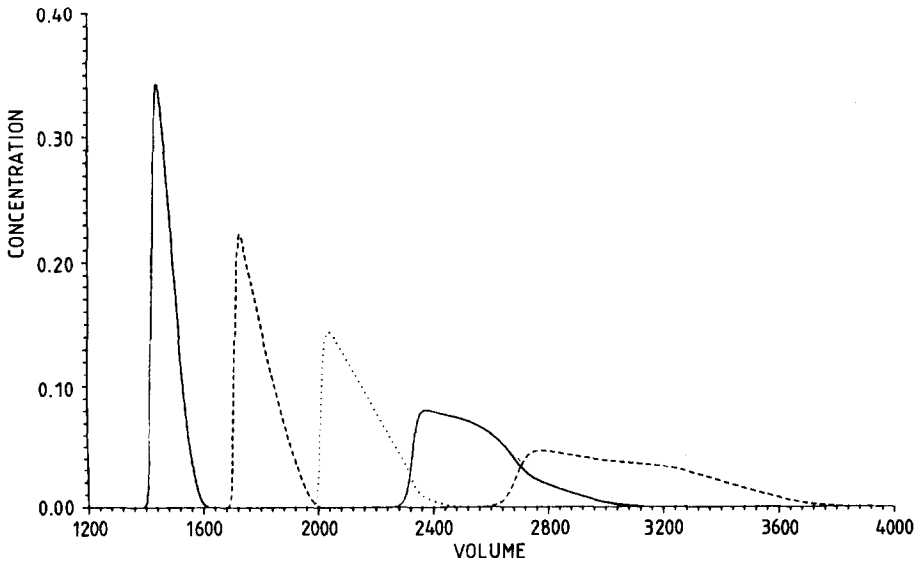

Fig. 7. Separation as in Fig. 1 except amount injected (all components), 30 in volume 150.

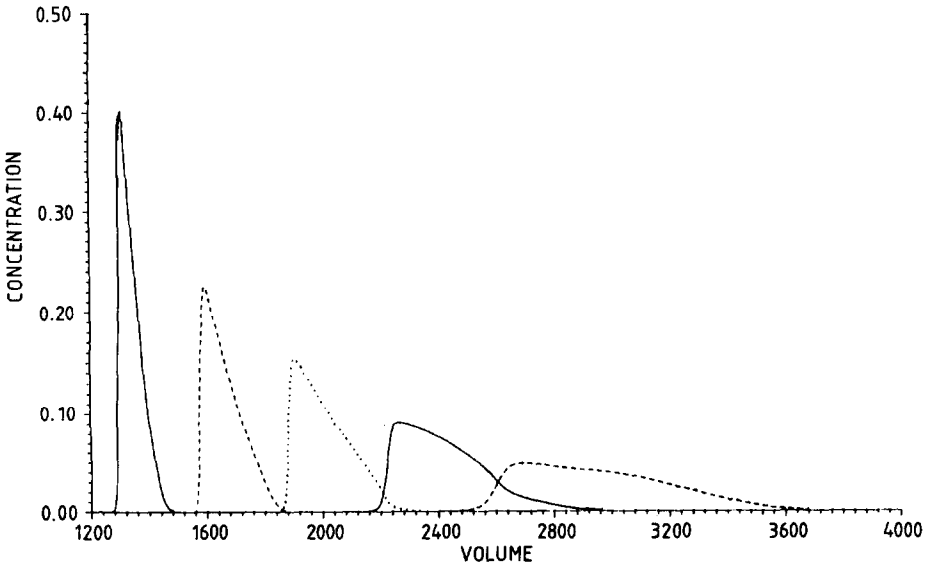

Fig. 8. Separation as in Fig. 1 except amount injected (all components), 30 in volume 15.

outlet (1000 T.P.) is 94.86 and therefore the critical volume should be about 190. Contrary to this prediction, at a feed volume of about 15 an increase of recovery ratio and at a volume of 150 a sharp decline in both recovery and production rate are observed.

Increase in blocking factors

Overloading of the solid phase is determined on the one hand by its capacity and on the other by the sum of the products of the distribution coefficients, concentrations and blocking factors of individual components. Therefore, when the blocking factors of only some of the components are increased, the separation of all components is influenced. In our example, the blocking factors of the first, third and fifth components were increased; the amounts injected and all other parameters remained constant. The recoveries of the second and fourth components decreased on increasing the blocking factors of the surrounding components (see Table IV). In the chromatograms shown in Figs. 9–11 the influence of changing blocking factors is clearly seen. In Fig. 9 the peaks of odd-numbered components would be Gaussian if none of the other components were present. In the example illustrated, however, the third and fifth components are clearly deformed owing to the presence of the fourth component. On the of other hand, the influence of the surrounding components on the peak shape of component **4** is clearly seen. The overloading in the last instance (Fig. 11) is so strong that only the first two components can be isolated; the others are not separated at all. Note also the shift in the peak maxima of all components, even those with constant blocking factors, to lower elution volumes with increase in the blocking factors. This is clear evidence of the mutual influence of sample components in the course of the separation process.

TABLE IV

EFFECT OF INCREASE IN BLOCKING FACTORS

Standard mixture: amount (all compounds), 30. Variable: blocking factors of components **1**, **3** and **5**. All others variables as in Fig. 1.

Blocking factor	*Component 2*		*Component 4*	
	Recovery	*Productivity*	*Recovery*	*Productivity*
0	100.0	0.704	85.2	0.600
1	100.0	0.707	46.0	0.325
2	99.3	0.704	0	0
3	94.5	0.671	0	0
4	86.3	0.613	0	0

Isolation of minor components

If one of the components in the sample predominates, then the recovery is determined not only by its amount but also by its elution volume in relation to other components.

The recoveries listed in Table V vary if the isolated component is eluted before or after the predominant compound. The concentrations of peaks eluted before it are increased, whereas the peaks eluted after the largest peak are smeared and their concentrations are smaller than those which would have been eluted without interference from the predominant component. This is illustrated in Figs. 12 and 13 in comparison with Fig. 1 and confrontation of Tables V and I. too. The effect of

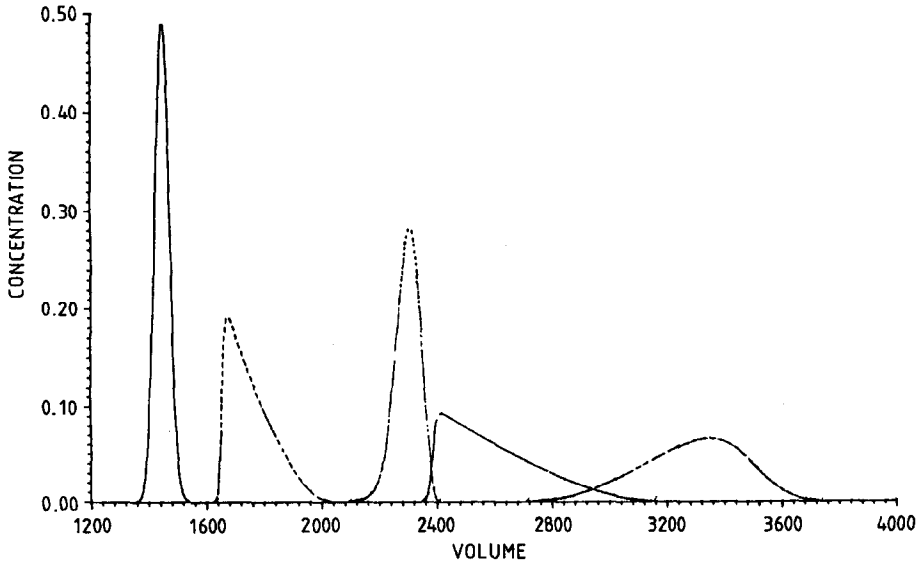

Fig. 9. Separation as in Fig. 1 except amount injected 30; injection volume, 1; blocking factors of components **1**, **3** and **5** = 0.

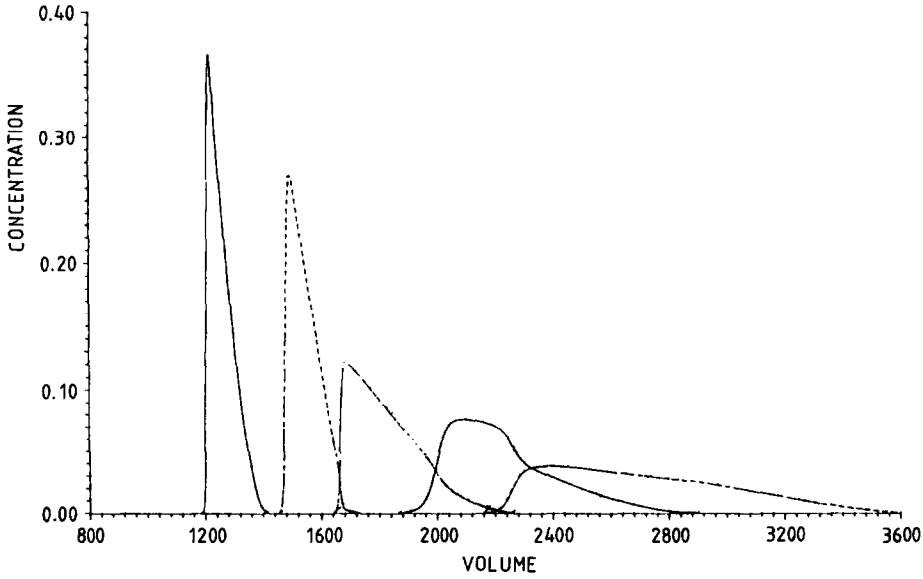

Fig. 10. Separation as in Fig. 9 except blocking factors of components **1**, **3** and **5** = 2.

approximately equal load of first component (475 times 0.5) on recovery is much more adverse than that of the last one (150 times 2.5).

If three components (**1**, **3** and **5**) dominate the sample, then the minor components (**2** and **4**), present in the sample in 100 times smaller amounts, can be

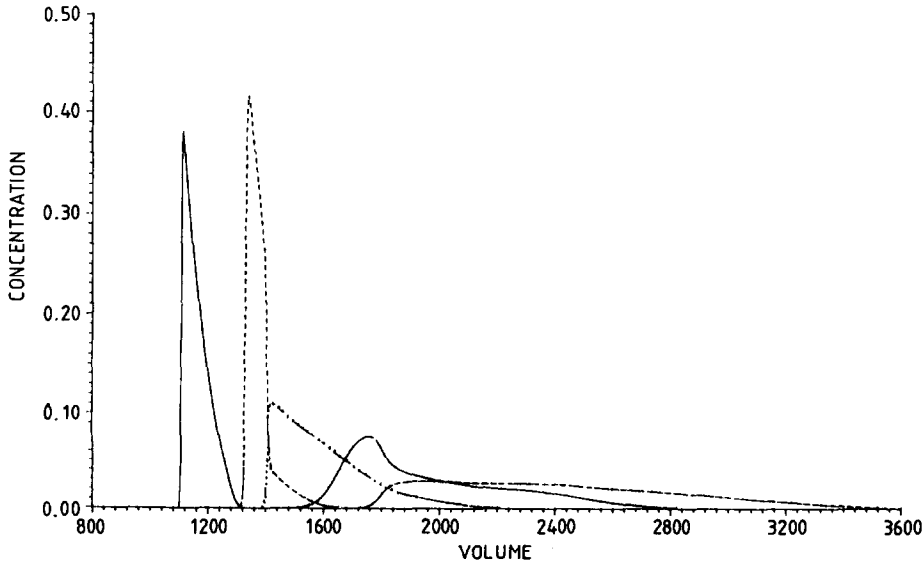

Fig. 11. Separation as in Fig. 9 except blocking factors of components **1**, **3** and **5** = 4.

TABLE V

EFFECT OF A LARGE SURPLUS OF ONE COMPOUND

Column: 1000 T.P.; volume injected, 1; all blocking factors, 1; concentration multiplied by 10^4; k' = capacity factor.

Parameter	***1*** *(k' = 0.5)*	***2*** *(k' = 1)*	***3*** *(k' = 1.5)*	***4*** *(k' = 2.0)*	***5*** *(k' = 2.5)*
Amount	150	1	1	1	1
Peak maximum	1199	1923	2412	2899	3390
Recovery (%)	–	100	99.5	96.0	98.8
Concentration	–	18	18	21	9
Amount	1	1	1	1	150
Peak maximum	1395	1724	1921	1945	1970
Recovery (%)	100	100	44.4	0.0	–
Concentration	28	28	48	–	–
Amount	1	150	1	1	1
Peak maximum	1340	1389	2362	2857	3352
Recovery (%)	98.4	–	14.7	21.9	87.2
Concentration	63	–	16	16	8
Amount	475	1	1	1	1
Peak maximum	1055	1678	2384	2866	3354
Recovery (%)	–	0	0	0	75.2
Concentration	–	–	–	–	7

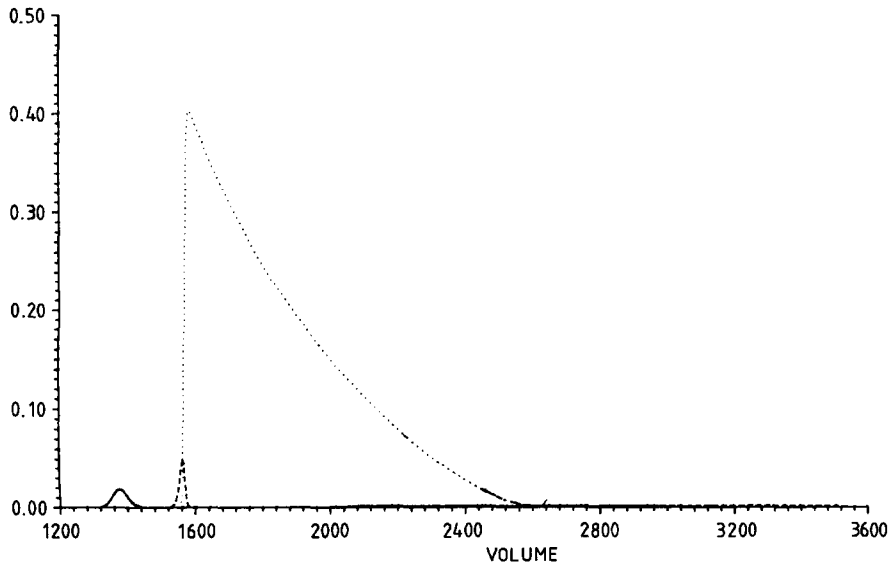

Fig. 12. Separation as in Fig. 1 except amounts injected 1.0, 1.0, 150.0, 1.0, 1.0 (components **1–5**).

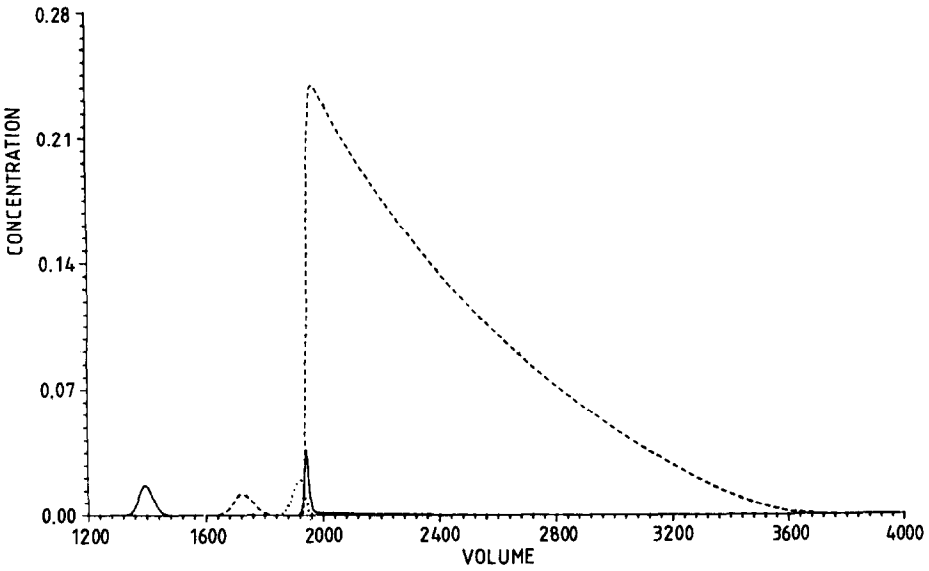

Fig. 13. Separation as in Fig. 1 except amounts injected 1.0, 1.0, 1.0, 1.0, 150.0 (components 1–5).

isolated on the 1000 T.P. column with good recoveries (last entry in Table V). This is true, of course, only when the feed as whole does not overload the column and the separation does not break down.

Gradient elution

To demonstrate the possibilities of gradient elution for preparative separations, three linear gradients were compared. The concentration of the strongest eluent varied from 0 to 1 and the values of its capacity factor were chosen to be 1 (in one instance) and 2 (in another example). The greatest differences are found in the recovery, concentration and productivity of the separation of component **4** when it is isolated from a standard mixture (Table VI). In comparison with isocratic elution, at the same recovery the productivity may be increased by 50% and the concentration of a selected fraction more than doubled (compare the first and fourth separations in Table VI). When the gradient is steeper than the optimum (second line), then the recovery and productivity decrease but the concentration of the selected fraction increases further. It should be stressed that in these computations the eluting agent is not classified as an impurity. It is interesting to follow how its increasing concentration at the column outlet is changed by transport through the column and how the peaks of the separated components are impressed as negative peaks on the trace of eluent concentration (dotted line in Fig. 14).

In an attempt to describe preparative liquid chromatography, Eble *et al.*[24] concluded that gradient elution is equivalent to isocratic elution if "average" capacity factors are equal, but no quantitative treatment of gradient optimization under overload conditions was presented. Therefore, no quantitative comparison with their treatment is possible.

TABLE VI

GRADIENT ELUTION

Column, 1000 T.P.; amount injected, 5 × 20; components, 5. Abbreviations as in Table I.

Capacity factor of eluent	*Length of linear gradient (volume)*	*Component 4*		
		REC	*PRD*	*CONC* ($\times 10^{-5}$)
1	1000	92.2	0.623	1104
2	1000	50.1	0.392	4009
1	2000	92.0	0.567	810
Isocratic	–	90.4	0.426	506

Displacement chromatography

It is generally accepted that displacement chromatography is a more powerful and efficient technique than elution chromatography for the separation of complex mixtures. In an attempt to separate a standard mixture of components, several unsuccessful trials were made to isolate all components from a fully developed train (procession of separated components) in a column with 1000 T.P. or shorter. Only with columns longer than about 1400 T.P. was it possible to isolate all five components (see Table VII). It is characteristic that in all instances when the components could be collected in satisfactory purity, the concentrations of the fractions were higher than in the feed. As in gradient elution, it was assumed that the displacer is not an impurity in

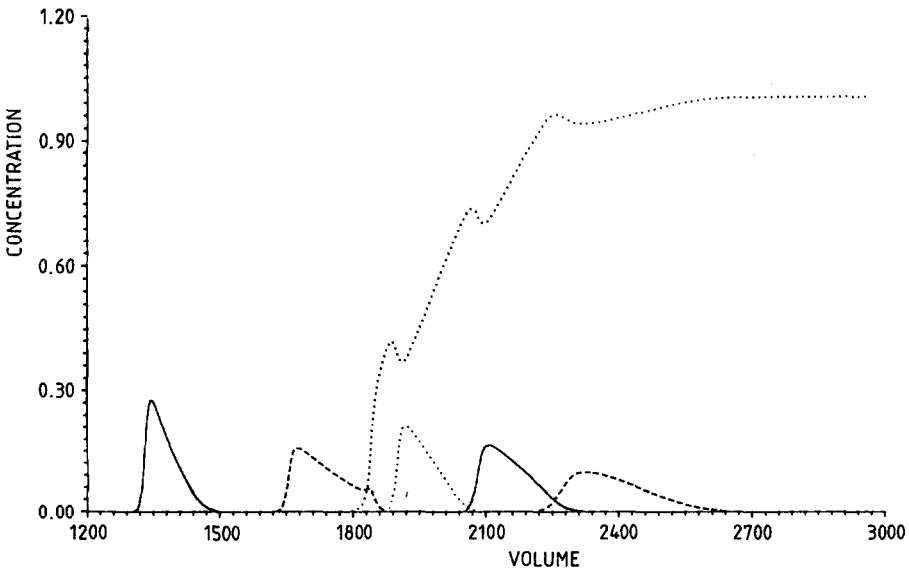

Fig. 14. Gradient elution. Injected amounts: 50 × 20 (volume 1). Capacity and blocking factors of five-component mixture as in Fig. 1. Eluent: capacity factor, 1.0; blocking factor, 1.0. Concentration increasing linearity from 0.0 (volume 0) to 1.0 (volume 1000), later constant (1.0).

TABLE VII

DISPLACEMENT CHROMATOGRAPHY

Feed components: capacity factors, 0.5, 1.0, 1.5, 2.0, 2.5; displacer, 3.0; all blocking factors, 1; injection, 500 × 0.2 (all components); concentration of displacer, 3.0. Productivity is multiplied by 100; recovery is in per cent. Abbreviations as in Table I. CONC = Real values, not multiplied by a quotient.

Column length	*1*			*2*			*3*			*4*			*5*		
(T.P.)	*REC*	*PRD*	*CONC*	*REC*	*PRD*	*CONC*	*REC*	*PRD*	*CONC*	*REC*	*PRD*	*CONC*	*REC*	*PRD*	*CONC*
100	3.4	0.498	0.0710	0	–	–	0	–	–	0	–	–	0	–	–
300	19.9	2.060	0.1592	0	–	–	0	–	–	0	–	–	0.8	0.078	0.0109
450	31.9	2.726	0.1867	0	–	–	0	–	–	0	–	–	3.0	0.257	0.0362
600	43.2	3.147	0.2048	0	–	–	0	–	–	0	–	–	8.1	0.592	0.0865
1000	74.0	3.886	0.2396	18.5	0.969	2.3067	0	–	–	0	–	–	35.1	1.845	0.3004
1300	95.6	4.156	0.3095	87.5	3.804	2.3036	55.5	2.412	2.6432	0	–	–	61.0	2.651	0.4621
1600	95.6	3.550	0.3452	87.6	3.251	2.3051	71.3	2.646	2.6404	39.3	1.458	2.8058	75.5	2.802	0.5393
1800	96.9	3.278	0.3800	87.9	2.972	2.3121	71.3	2.413	2.6415	47.7	1.613	2.8050	75.7	2.560	0.5329
2000	96.9	3.012	0.4177	87.6	2.723	2.3050	71.3	2.217	2.6416	47.7	1.484	2.8077	78.4	2.438	0.5447

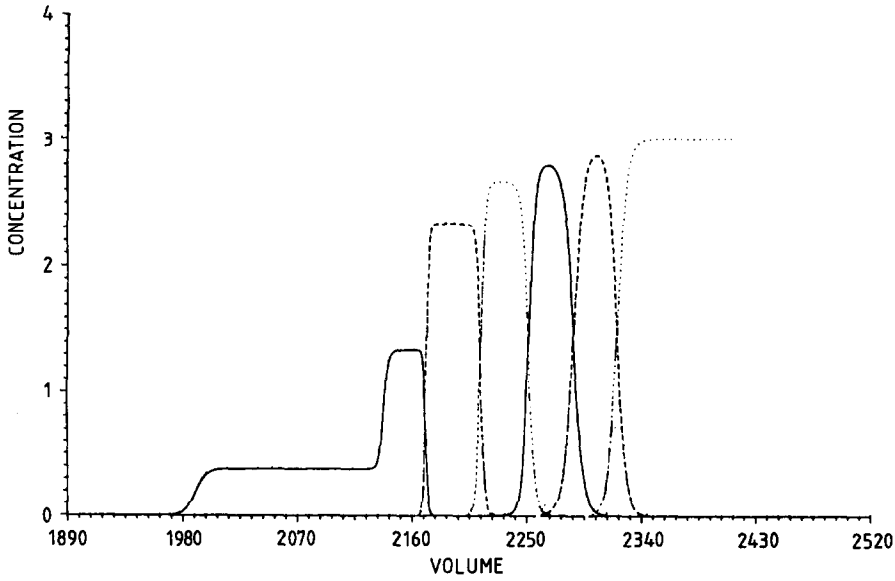

Fig. 15. Displacement chromatography. Column: 1400 T.P. All other parameters as in Table VII.

the collected fractions. This is the reason why the end of last compound fraction reaches far into the volume where predominantly displacer is eluted, and therefore its average concentration is lower than in the preceding fractions. This may be clearly seen from Fig. 15, where a separation on 1400 T.P. column is illustrated. Note that the fourth component cannot be isolated from its separation in satisfactory purity.

The influence of feed volume and amount injected on the recovery of a standard mixture can be seen in Table VIII. The smallest amount injected (30 in volume 1) is not large enough to build up a fully developed train. If a larger amount (100) is injected, then another variable parameter, the volume injected, may influence the recovery ratio of components. From consideration of all the experiments, the optimum volume is 200. In Figs. 16–18 the evolution of separation at three different points in the column (300, 600 and 1000 T.P.) is demonstrated.

TABLE VIII

DISPLACEMENT CHROMATOGRAPHY WITH VARIOUS INJECTED VOLUMES

Standard mixture (see Table VII); column, 1000 T.P. Abbreviations as in Table I.

Feed volume	*Concentration*	*Compound 1*			*Compound 2*		
		REC	*PRD*	*CONC*	*REC*	*PRD*	*CONC*
1	30	86.2	1.663	0.2751	0	0	0
1	100	97.0	6.899	1.2279	39.2	2.786	2.3040
200	0.5	98.0	6.105	0.9337	76.3	4.751	2.3121
400	0.25	81.0	4.488	0.3493	48.4	2.678	2.3034
500	0.2	74.0	3.886	0.2396	18.5	0.969	2.3067

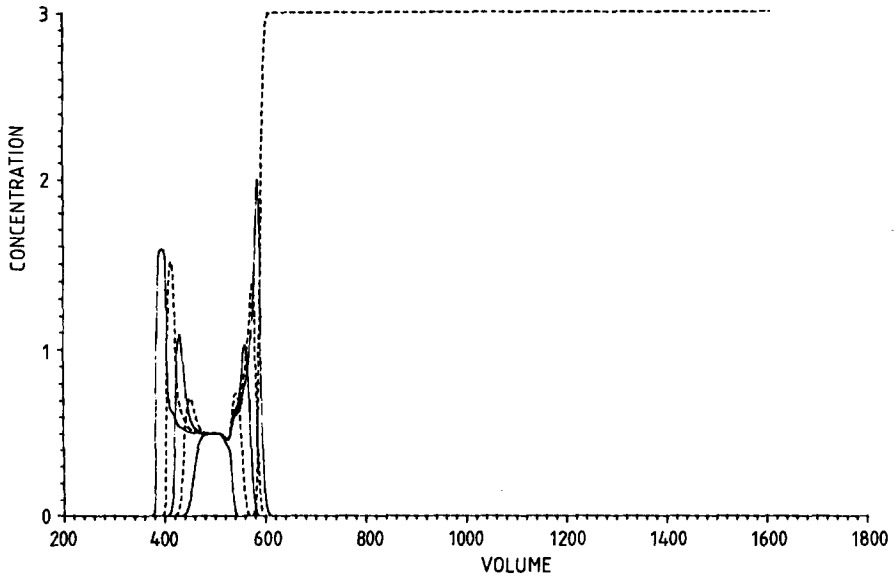

Fig. 16. Displacement chromatography. Components and displacer as in Table VII. Injection: volume 200, concentration 0.5. Displacer concentration, 3.0. Column, 300 T.P.

The recoveries and productivities of separation obtained by elution and displacement chromatography of a three-component mixture are compared in Table IX. From the results we conclude that the productivities using displacement chromatography are about four times higher than those with elution chromatography,

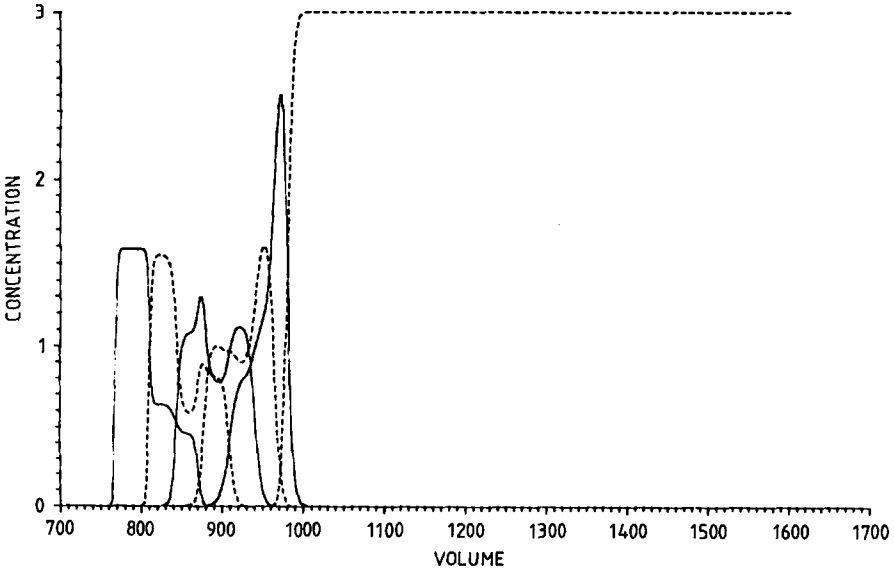

Fig. 17. Separation as in Fig. 16 except column, 600 T.P.

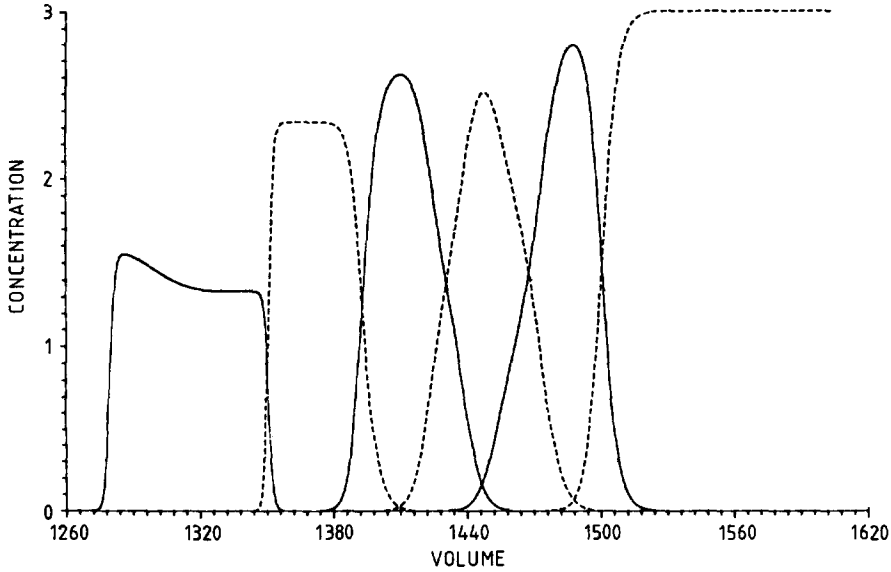

Fig. 18. Separation as in Fig. 16 except column, 1000 T.P.

but the optimization of displacement chromatography is much more difficult. In both elution and displacement chromatography, an upper limit (column overloading) exists. In displacement chromatography, a lower limit exists also; if the amount of sample injected is too small, then the displacement train with a pure component fraction will not be formed and no useful fractions can be isolated. It is interesting that for separation of a standard five-component mixture by displacement chromatography a column longer than 1000 T.P. was needed.

Table X illustrates the separation of a three-component mixture where the isotherms of components **2** and **1** cross each other. This is the case where separation was expected by Frey[28] but was not predicted by the "golden rule"[21].

TABLE IX

COMPARISON OF ELUTION (EL) AND DISPLACEMENT (DI) CHROMATOGRAPHY

Three-component mixture: capacity factors, 0.5, 1.5, 2.5, displacer, 3.0; all blocking factors, 1; column, 1000 T.P.; injection volume, 1; displacer concentration, 3. Abbreviations as in Table I.

Mode	*Amount*	*Compound 1*			*Compound 2*			*Compound 3*		
		REC	*PRD*	*CONC*	*REC*	*PRD*	*CONC*	*REC*	*PRD*	*CONC*
DI	100	99.2	7.047	0.893	89.9	6.387	2.643	93.2	6.623	0.675
DI	200	99.8	14.183	1.559	85.9	12.206	2.6422	93.1	13.235	1.095
DI	300	79.1	16.875	4.964	0	0	0	65.8	14.035	1.135
EL	100	100	2.355	0.2463	99.1	2.334	0.1598	99.4	2.340	0.0462
EL	200	100	4.710	0.9174	66.6	3.139	0.5508	52.1	2.454	0.0480

TABLE X

DISPLACEMENT CHROMATOGRAPHY WITH CROSSED ISOTHERMS

Components: capacity factors, 0.5, 1.5, 2.5; blocking factors, 1, 4, 1; amount injected, 200 (all components); volume, 1. Displacer, capacity factor, 3; blocking factor, 1: concentration, 3. Abbreviations as in Table I.

Compound	*PRD*	*CONC*	*REC*	*P.MAX*
1	0.1420	1.540	99.9	1051.0
2	0.0260	0.665	73.2	1169.5
3	0.138	1.11	97.18	1261.5

The crossing point for both components lies at a concentration of 0.4444. Because injection was made with much higher concentrations and during the whole separation they hardly approached this point, regular behaviour is observed.

Negative blocking factors

The peculiarity of a one-component isotherm with a negative blocking factor lies in the fact that there is not a defined maximum adsorbed concentration, but a maximum attainable mobile phase concentration. If the concentration in the mobile phase were to approach this limit, then the concentration of sample in the solid phase would increase above all limits.

This obviously does not describe any real system; at least the volume of sorbed species would limit the maximum attainable concentration in the solid phase. On the

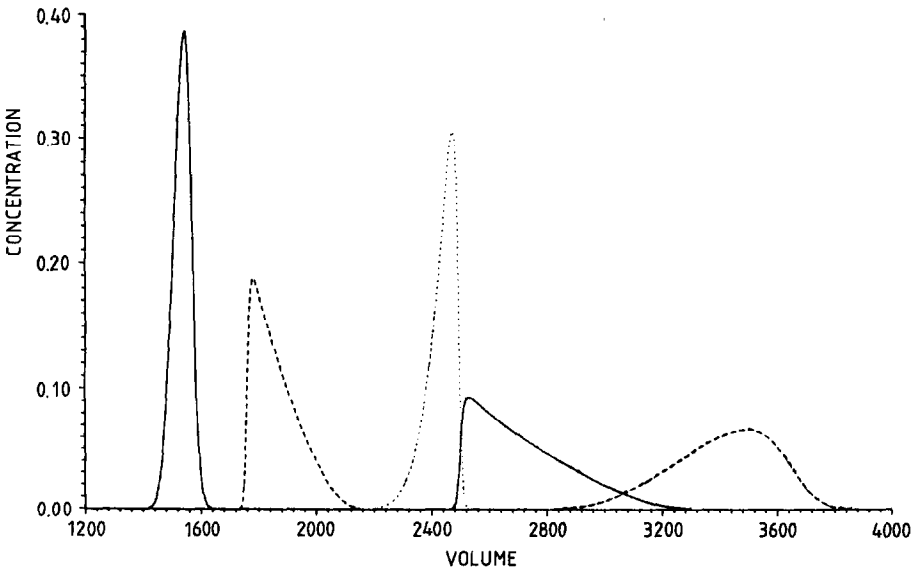

Fig. 19. Isocratic elution chromatography. Column: 1000 T.P. Five components: capacity factors 0.5, 1.0, 1.5, 2.0 and 2.5; blocking factors −0.1, 1.0, −0.1, 1.0, −0.1. Injection (all components): concentration 0.3, volume 1000.

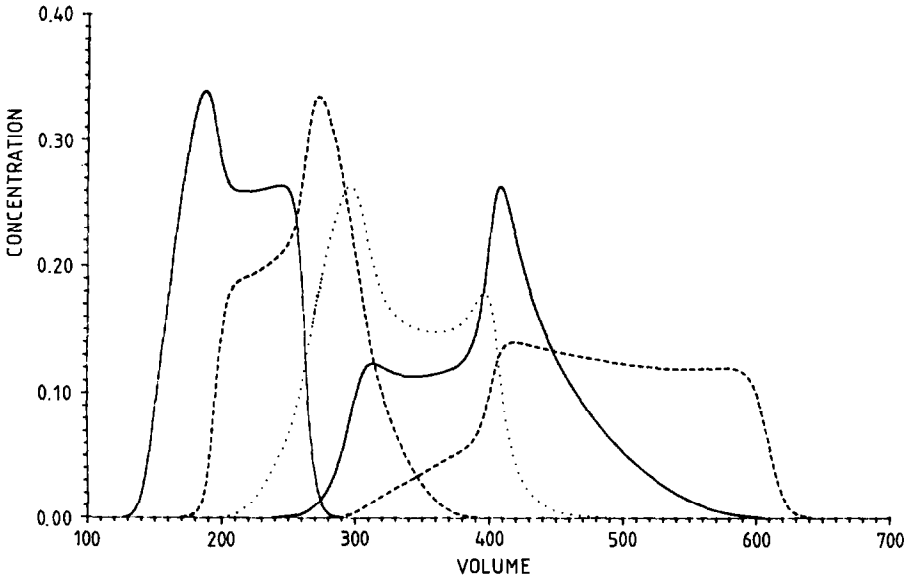

Fig. 20. Separation as in Fig. 19 except blocking factors −1.0, 1.0, −1.0, 1.0, −1.0 and column 100 T.P.

other hand, we may expect that at concentrations much lower than the maximum attainable mobile phase concentration this simple model would describe sorption processes correctly. We have to keep this principal limitation in mind when describing the results of our computations.

In the chromatogram for the five-component mixture with three negative blocking factor components (Fig. 19), the peaks are steeper on the rear side in

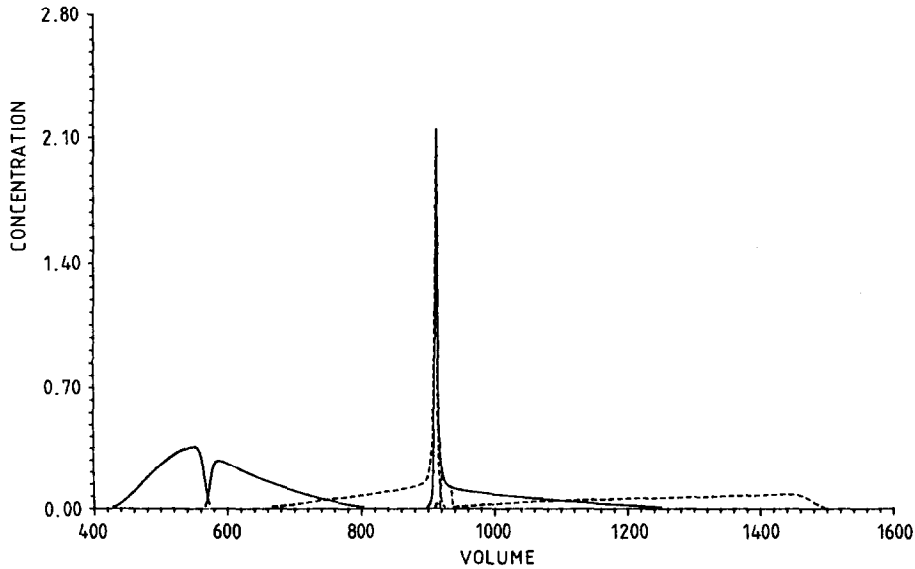

Fig. 21. Separation as in Fig. 20 except column, 300 T.P.

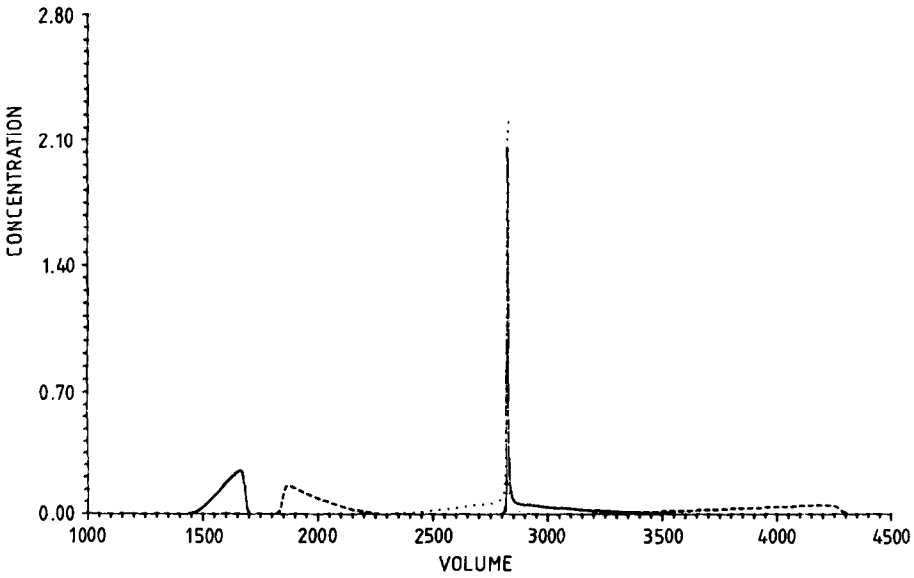

Fig. 22. Separation as in Fig. 20 except column, 1000 T.P.

comparison with a similar chromatogram of components with only positive blocking factors (Fig. 7) and increased peak concentrations of components **1**, **3** and **5** in Fig. 19 are apparent. A shift of the peaks of the second and fourth components toward higher elution volumes is also evident.

When only the chromatogram from a single universal detector is recorded,

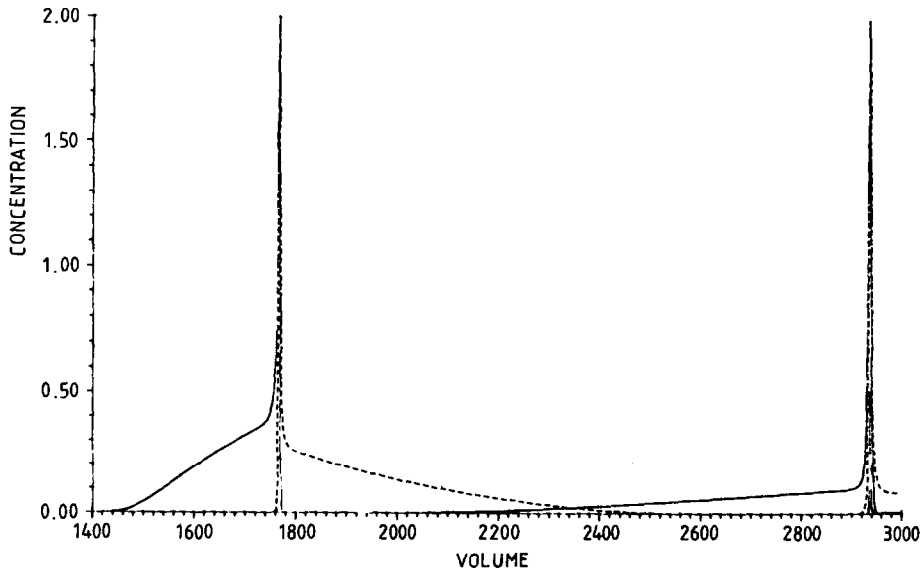

Fig. 23. Separation as in Fig. 22 except amount injected (all components), 100.

components **3** and **4** form a fused peak without any hint of separation. In spite of this, the recovery ratios of these components are fairly high, 87.9 and 81.9%.

When the value of the negative blocking factors is increased, the appearance of the computed chromatogram changes considerably. The third and fourth components form a narrow spike, much thinner than any peak corresponding to a column length of 1000 T.P. The evolution of this peak shape may be traced with three different column lengths. Even with the short 100 T.P. column two areas with higher capacity are formed. These are manifested by secondary peaks at elution volumes of approximately 250 and 400 (Fig. 20). The longer column (Fig. 21) separates components **1** and **2**; the next component pair forms the fused spike; the appearance of the chromatogram does not change substantially with the 1000 T.P. column (Fig. 22). Two virtual peaks are formed when the amount of feed is further increased (Fig. 23).

CONCLUSIONS

It has been demonstrated that a simple ideal mixed cells model is flexible enough to describe all modes of liquid chromatography. Overloading is typical for preparative chromatography. The recoveries and productivities can be easily computed without the introduction of any arbitrary scales as was proposed earlier. Using this model, multi-component systems may be studied if the adsorption of their components can be described by a hyperbolic isotherm. It was shown that under overload conditions the mutual interference of components is always prominent; this is particularly important when trace components are isolated.

The program permits the simultaneous computation of separations with various column lengths. Plotting of effluent concentrations enables the peak forms to be examined closely. From recorded data, production rates and recoveries for various previously defined fraction purities may be computed.

It was demonstrated that isocratic, gradient and displacement modes of preparative chromatography provide the highest throughput when the column is operated in the high overload mode. It is hardly possible to describe the complicated behaviour of these separations with only simple extrapolations from two-component results in a moderately overloaded mode, as was done in recent publications. Our model enables complete chromatograms to be computed with only those data (capacity and blocking factors of every component and capacity and number of theoretical plates of column) which are also necessary for other, oversimplified, models. This work will be supplemented by a comparison of results from the numerical solution of a set of differential equations and will be verified experimentally.

This simple model may lead to erroneous results if components with positive and negative blocking factors are combined in one feed. In a subsequent paper a two-site and two-layer model with a more complicated isotherm shape giving a more accurate picture of components with convex isotherms over the whole concentration range will be described.

REFERENCES

1 A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, 35 (1941) 1358.
2 S. W. Mayer and E. R. Tompkins, *J. Am. Chem. Soc.*, 69 (1947) 2866.
3 D. DeVault, *J. Am. Chem. Soc.*, 65 (1943) 532.

4 E. Glueckauf, *Trans. Faraday Soc.*, 51 (1955) 34.
5 G. Houghton, *J. Phys. Chem.*, 67 (1963) 84.
6 H. Vink, *J. Chromatogr.*, 18 (1965) 25.
7 F. Helfferich and G. Klein, *Multicomponent Chromatography — Theory of Interference*, Marcel Dekker, New York, 1970.
8 J. V. Lankelma and H. C. Smit, *Commun. Math. Chem.*, (1985) 157.
9 H. Poppe and J. C. Kraak, *J. Chromatogr.*, 255 (1983) 395.
10 P. Rouchon, M. Shoenauer, P. Valentin, C. Vidal-Madjar and G. Guichon, *J. Phys. Chem.*, 89 (1985) 2076.
11 A. Jaulmes, C. Vidal-Madjar, H. Colin and G. Guichon, *J. Phys. Chem.*, 90 (1986) 207.
12 M. J. Gonzalez, A. Jaulmes, P. Valentin and C. Vidal-Madjar, *J. Chromatogr.*, 386 (1987) 333.
13 S. Seshadri and S. N. Deming, *Anal. Chem.*, 56 (1984) 1576.
14 J. E. Eble, R. L. Grob, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 384 (1987) 25 and 45.
15 K. P. Hupe and H. H. Lauer, *J. Chromatogr.*, 203 (1981) 41.
16 P. Gareil, C. Durieux and R. Rosset, *Sep. Sci. Technol.*, 18 (1983) 441.
17 G. Gretier and J. L. Rocca, *Chromatographia*, 21 (1986) 143.
18 L. Hagdahl, J. P. Williams and A. Tiselius, *Ark. Kemi*, 4 (1951) 193.
19 P. P. Zolotarev, N. V. Kolosov and N. V. Kurdyumov, *Zh. Fiz. Khim.*, 59 (1985) 1979 and 2837.
20 J. Frenz, C. Horváth, *AIChE. J.*, 31 (1985) 400.
21 Cs. Horváth, A. Nahum and J. H. Frenz, *J. Chromatogr.*, 218 (1981) 365.
22 J. A. V. Butler and C. Ockrent, *J. Phys. Chem.*, 34 (1930) 2841.
23 M. D. LeVan and T. Vermeulen, *J. Phys. Chem.*, 85 (1981) 3247.
24 J. E. Eble, R. L. Grob, P. E. Antle, L. R. Snyder, *J. Chromatogr.*, 405 (1987) 1, 31 and 51.
25 S. Ghodbane, G. Guiochon, *J. Chromatogr.*, 444 (1988) 275.
26 J. H. Knox and H. M. Pyper, *J. Chromatogr.*, 363 (1986) 1.
27 L. R. Snyder, G. B. Cox, P. E. Antle, *Chromatographia*, 24 (1987) 82.
28 D. D. Frey, *J. Chromatogr.*, 409 (1987) 1.