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Multi-component elution overload chromatography of compounds with S-shaped isotherms

A theoretical study

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ABSTRACT

An equation describing two-step adsorption was used for computation in overload elution chromatography of compounds with S-shaped isotherms. An equilibrium mixed cells model permits the study of the separation of multi-component mixtures. The band profiles of compounds with S-shaped isotherms exhibit two local maxima under certain conditions. Minor components may be dragged within the band of a major component.

INTRODUCTION

The preparative chromatography of compounds from biotechnological processes has been gaining in importance recently. Usually the starting point for this separation is analytical chromatography, but with increasing load the mechanism of separation becomes more and more complicated, as the non-linear part of the isotherm and the mutual influence of compounds play a greater role.

The first approach to gain an insight into these complicated mechanisms was the introduction of a simple model based on Langmuir isotherms [1]. No matter what computation method was used, either based on the study of solutions of systems of partial differential equations [2] or the use of a multi-cell equilibrium model [1], this approach may provide results that are in qualitative agreement with those of everyday practical preparative chromatography. On the other hand, there are some results that are impossible to explain by using this simple model, such as peaks with two maxima, the reversal of peak expansion under overload conditions and the dragging of one compound within the band of another.

A theoretical study of band profiles of compounds with S-shaped isotherms was published recently [3], but was limited to isolated bands of compounds without any extension toward the mutual influence of separated compounds. In that paper no

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indication was given of the possible mechanism by which the S-shaped isotherm is formed.

In principle, the deformation of the Langmuir isotherm to an isotherm with an inflection point may be caused by some secondary processes in the mobile or solid phase, *e.g.*, limited solubility of a solute in the mobile phase, two-stage adsorption on homogeneous adsorption sites or two or more kinds of adsorption sites in the solid phase, or by a combination of these influences.

There are only a few published experimental studies of S-shaped isotherms [4–6]. On the other hand, many unusual peak shapes resembling those predicted for S-shaped isotherms can be explained by the interaction of two compounds with Langmuir isotherms [7].

In this paper we postulate two-stage adsorption and extend this approach to solutes with limited solubility. Only elution chromatography will be considered; studies of the displacement chromatography of compounds with S-shaped isotherms and chromatography on solid phases with two or more kinds of adsorption sites will be reported later.

BASIC RELATIONSHIPS

The equilibrium distribution of a compound i between a mobile and a solid phase in a two-step process may be described by a set of equations similar to those used previously for the simple one-step system [1,8]. The first-step equation is identical:

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

where c_i, c_{iF} and c_F are concentrations of compound i in the mobile and solid phase and the concentration of sorption sites, respectively, and K_i is the equilibrium constant.

In the next step, compound i is sorbed on the already covered sorption site:

$$\frac{c_{2iF}}{c_i c_{iF}} = L_i \tag{2}$$

where c_{2iF} is concentration of sites in the solid phase binding two molecules of compound i. The amount of this compound in one cell, G_i, is therefore

$$G_i = c_i V_M + V_S (c_{iF} + 2c_{2iF}) \tag{3}$$

where V_M and V_S are volumes of the mobile and solid phase, respectively, in one equilibrium mixed cell.

The capacity of the sorbent in one cell is the sum of all free sorption sites, sorption sites covered by one molecule and sorption sites covered by two molecules:

$$G_D = V_S \left[c_F + \sum_{i=1}^{n} c_i K_i (R_i + c_i L_i T_i) \right] \tag{4}$$

where R_i is the blocking factor for the first-step sorption and T_i that for the second step. The blocking factor R_i may be understood as the average number of sorption sites covered by one molecule of compound bound to one adsorption site.

For the concentration of a compound j in the mobile phase (in one equilibrium mixed cell) one obtains

$$c_j = \frac{G_j}{V_M + G_D (K_j + 2L_jK_jc_j)/\left[1 + \sum_{i=1}^{n} c_iK_i(R_i + c_iL_iT_i)\right]} \tag{5}$$

The isotherm for one compound is simply

$$c_{iA} = c_{iF} + 2c_{ZiF} = \frac{G_D}{V_S}\left[\frac{K_ic_i(1+2L_ic_i)}{1 + c_iK_i(R_i+c_iL_iT_i)}\right] \tag{6}$$

Recently a similar equation for S-shaped isotherms was published [3]:

$$Q = \frac{25c\ (1+Ac)}{1+2Bc+ABc^2}$$

Obviously, these equations are equivalent with simple relationships between coefficients.

If $L_i = 0$, then eqn. 6 is reduced to the simple Langmuir-type isotherms (see eqn. 5 in ref. 1). For small values of c_i:

$$c_{iA} = \frac{G_DK_ic_i}{V_S} \tag{7}$$

(linear part of isotherms), and for very high values of c_i:

$$c_{iA} \rightarrow \frac{2G_D}{V_ST_i} \tag{8}$$

The shape of the isotherm is governed by the values of the constants R_i, K_i, L_i and T_i. If all are positive, then the isotherm is defined for all positive values of c_i. If R_i is negative, then the isotherm is defined for all positive values of c_i only if

$$T_i > \frac{R_i^2\ K_i}{4L_i} \tag{9}$$

If T_i is lower than this limiting value or if T_i is negative (all other constants being positive), then the isotherm is defined only in range from zero to the critical concentration, which is equal to the lower positive root of

$$c_{i,K} = \left(-R_i + \sqrt{R_i^2 - 4T_iL_i/K_i}\right)/2T_iL_i \tag{10}$$

Near this critical solute concentration the adsorbed concentration increases above all limits. This obviously does not correspond to any real system. Therefore, isotherms that do not obey condition 9 may introduce severe errors in model computation, as has been shown previously [1], and these results have to be examined very carefully.

If Lc is negligibly small in comparison with 1 and LT is negative, we obtain an equation that is similar to that used for the description of the adsorption of liquids with limited solubility [5,6]. The critical concentration then corresponds to the concentration of a saturated solution.

On isotherms with positive R, L and K a maximum of $c_{i,A}$ is observed in range of positive c_i if

$$T_i > \frac{4L_i}{K_i} + 2R_i \tag{11}$$

Isotherms conforming to condition 9 always show a maximum. The maximum occurs at a concentration

$$c_{i,\max} = \frac{1 + \sqrt{1+K_i\ (T_i-2R_i)/4L_i}}{K_i(T_i/2-R_i)} \tag{12}$$

In this instance the isotherm has two inflection points. No simple equation for their localization was found.

It should be pointed out that the binding of two different compounds to one site probably occurs in real systems (*i.e.*, c_{ijF}). This mechanism was not considered here. It is believed that the approach presented is flexible enough to describe most observed cases with satisfactory precision.

Table I and Fig. 1 demonstrate typical shapes of isotherms, all with the distribution coefficient $K_1 = 1.5$. Isotherm 1 is just a simple linear isotherm. The next isotherm (2) is the Langmuir isotherm (single-step adsorption). Isotherms 3, 4 and 5 differ only in the value of T (second-step blocking factor). When $T = 0.3$ (less than the critical value of 0.375 according to eqn. 9), then the isotherm is hyperbolic (curve

TABLE I

INFLUENCE OF CONSTANTS IN EQN. 6 ON ISOTHERM SHAPES

Curve No.[a]	K	R	L	T	Shape of isotherm
1	1.5	0.0	0.0	0.0	Linear
2	1.5	1.0	0.0	0.0	Langmuir
3	1.5	−1.0	1.0	0.3	Hyperbolic (convex)
4	1.5	−1.0	1.0	0.5	S-shaped, with maximum
5	1.5	−1.0	2.0	2.0	S-shaped, with maximum
6	1.5	1.0	1.0	1.0	S-shaped, no maximum
7	1.5	1.0	1.0	5.0	S-shaped, with maximum
8	1.5	1.0	0.01	−10.666	S-shaped, hyperbolic

[a] See Fig. 1.

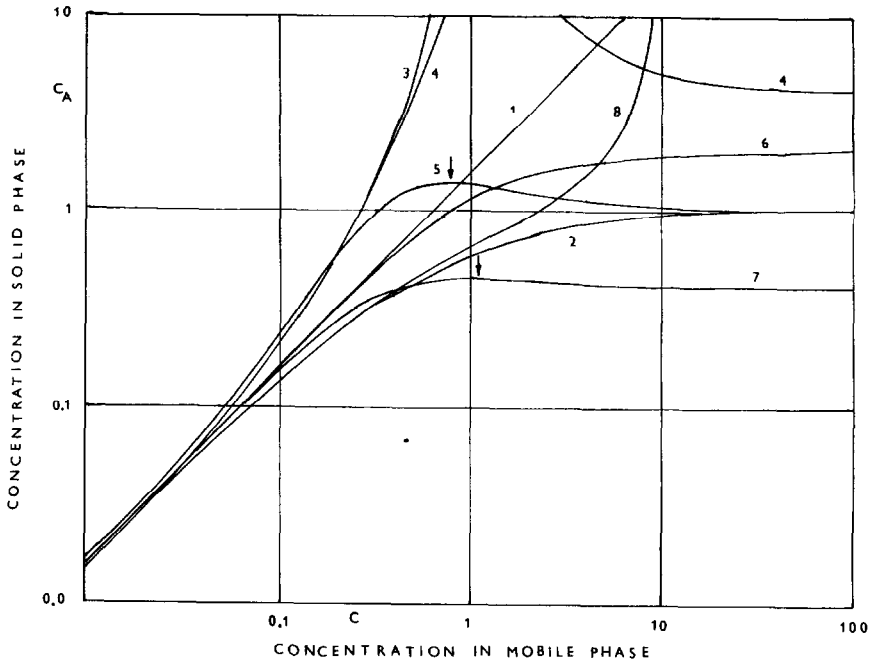

Fig. 1. Various shapes of two-step isotherms. Concentrations in arbitrary units. For values of the constants for the various curves, see Table I. Arrows indicate maxima.

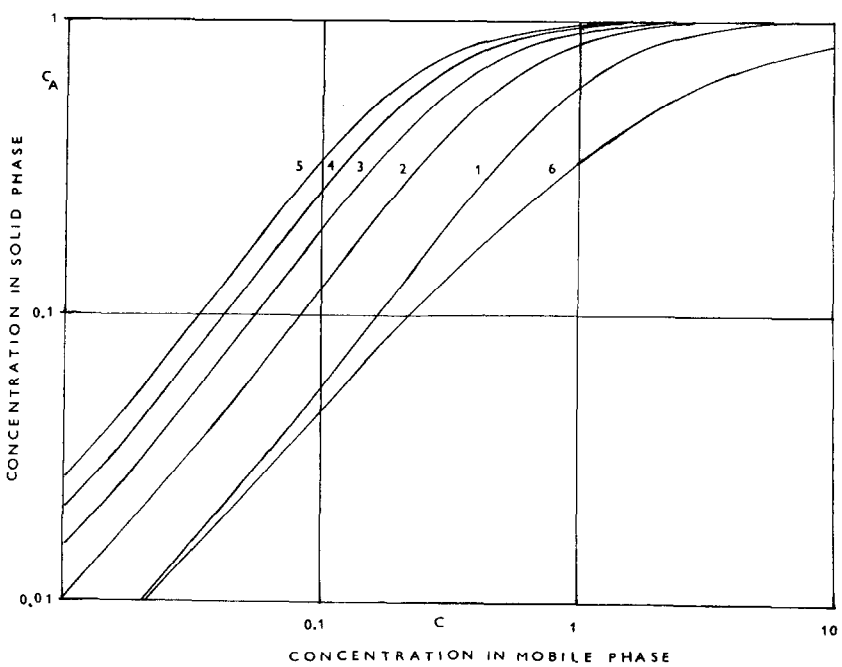

Fig. 2. Family of five parallel isotherms (log–log scale) and a Langmuir isotherm. Curve 1, $K = 0.5$, $L = 1.0$; curve 2, $K = 1.0$, $L = 2.0$; curve 3, $K = 1.5$, $L = 3.0$; curve 4, $K = 2.0$, $L = 4.0$; curve 5, $K = 2.5$, $L = 5.0$. For all these curves $R = 1.0$ and $T = 2.0$. Curve 6: Langmuir isotherm, $K = 0.5$; $R = 1.0$.

3). When T increases (above 0.375), then the isotherms exhibit a maximum (curves 4 and 5). With increasing value of T, the asymptotic value of the concentration adsorbed decreases according to eqn. 8. Isotherm 6 has all four constants positive; it has an inflection point on the ascending part, but no maximum. If its T value is increased above the critical value of 4.666 (eqn. 11), then a maximum appears (curve 7).

The isotherm with $L = 0.01$ and $T = -10.666$ (critical concentration = 10) is plotted as curve 8. This S-shaped isotherm has the first part concave, as in the Langmuir isotherm, and the second hyperbolic part convex toward the mobile phase concentration axis.

A set of parallel S-shaped isothems is depicted in Fig. 2. Compare curves 1 (S-shaped) and 6 (Langmuir).

The multi-component isotherm is an extension of eqn. 6:

$$c_{iA} = \frac{\frac{G_D}{V_S} \cdot K_i c_i \, (1 + 2L_i c_i)}{1 + \sum_{j=1}^{n} c_j K_j (R_j + c_j L_j T_j)} \tag{13}$$

The equilibrium model described by eqns. 1–4 neglects the volumes of solutes; in most instances this omission will not introduce severe errors.

As in the previous paper [1], the equilibrium mixed cells model is considered to

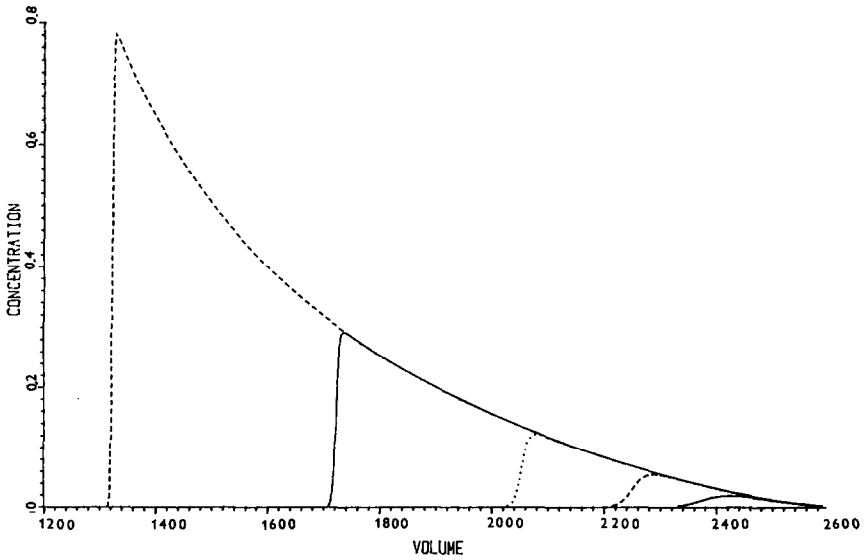

Fig. 3. Peak shapes of a compound with a Langmuir isotherm ($K = 1.5$, $R = 1$, $L = T = 0$); various amounts injected (3, 10, 30, 100, 300). Number of equilibrium cells: 1000. Amounts are measured in sorption capacity of one equilibrium cell. Injected volume (in volumes of sample solution divided by volume of one equilibrium cell): 1. Both sample amount and volume (in examples) are therefore dimensionless.

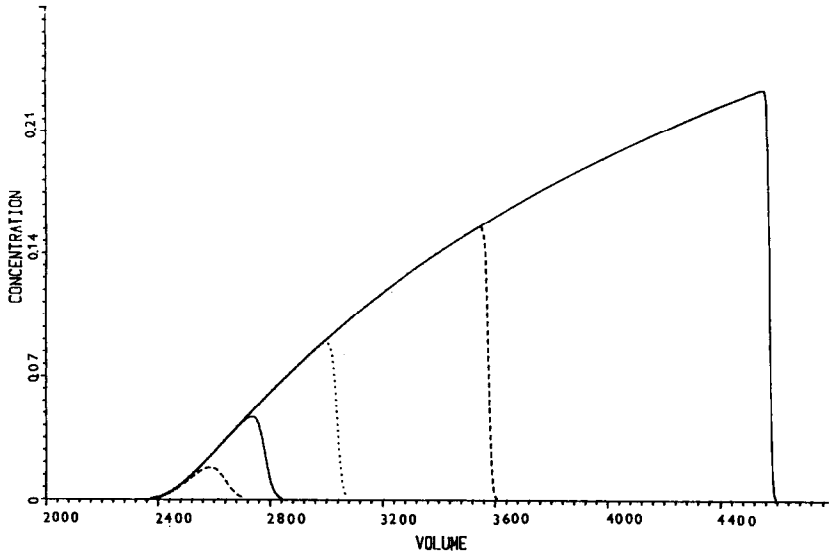

Fig. 4. Peak shapes of a compound with a hyperbolic isotherm ($K = 1.5$, $R = -1$, $L = T = 0$); injected amounts and column length (number of equilibrium cells) as in Fig. 3.

represent the number of theoretical plates in the chromatographic column. As has been pointed out earlier, the relationship between the number of cells and the number of theoretical plates holds exactly only for high enough numbers of these elements and for high values of K. The comparison between the equilibrium mixed cells model [1] and the description of chromatographic separation by a differential equation with a constant axial dispersion coefficient [8] will be published elsewhere.

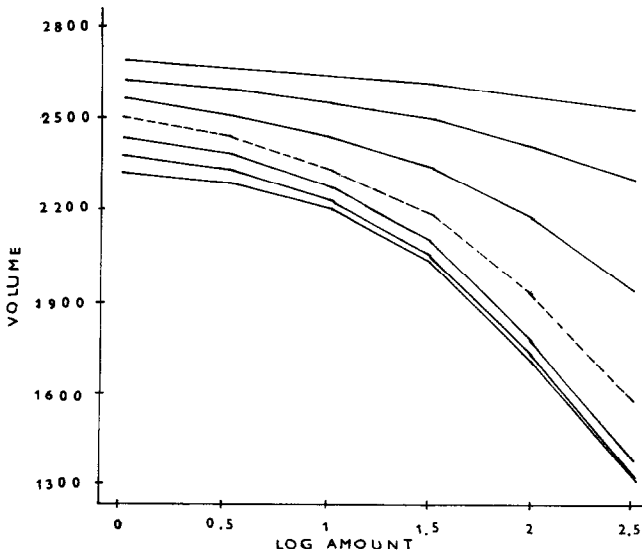

Fig. 5. Peak section positions for compound from Fig. 3 (Langmuir isotherm). Dashed line: median. For further explanation see text.

COMPUTATION

The course of computation follows the procedure described previously [1]. The key equation for computation is eqn. 5. The coverage of binding sites in the solid phase is estimated (from the previous step or set equal to zero) and then the concentration of all components is computed. If the coverage corresponding to these concentrations differs from the previously estimated value by less than $1 \cdot 10^{-5}$, then the computation is finished; if not, the iterative computation proceeds until the desired precision is attained. The amount of injected sample is expressed as sample amount divided by sorption capacity of one equilibrium cell (G_D), sample volume in multiples of V_M. Other details were described previously [1].

RESULTS AND DISCUSSION

Single compounds

Typical peak shapes for simple Langmuir-type isotherms are displayed in Figs. 3 and 4. Similar band profiles have been published for overload column chromatography when a solution based on the difference method was used as the method of computation. For a quantitative description of peak shapes we divided the peak area into seven sections, whose normalized areas (0.00135, 0.02140, 0.13591, 0.34134, 0.34134, 0.13591, ...) were selected so that for an ideal Gaussian peak the distances between section borders are equal to integral multiples of sigma (standard deviation) from the peak centre. When we plot these positions for our two peak families, we obtain for the Langmuir isotherm monotonously descending curves (Fig. 5) and for the hyperbolic (convex) isotherm curves which increase monotonously (Fig. 6).

The band profies of compounds with S-shaped isotherms change their shape

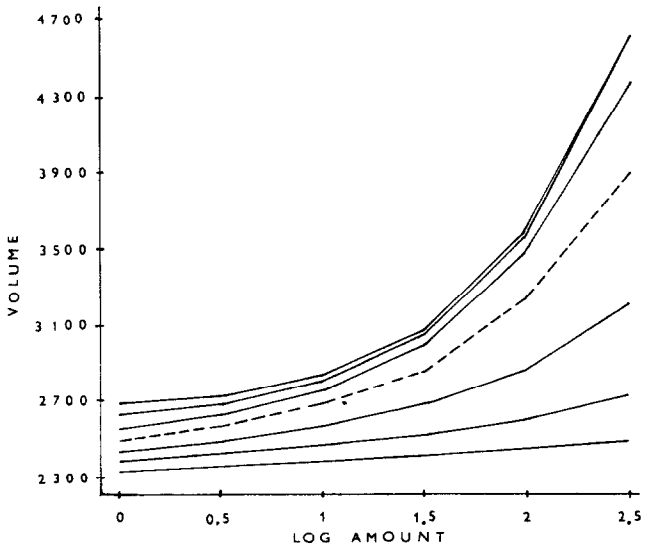

Fig. 6. Peak section positions for compound from Fig. 4 (hyperbolic isotherm). Dashed line: median. For further explanation, see text.

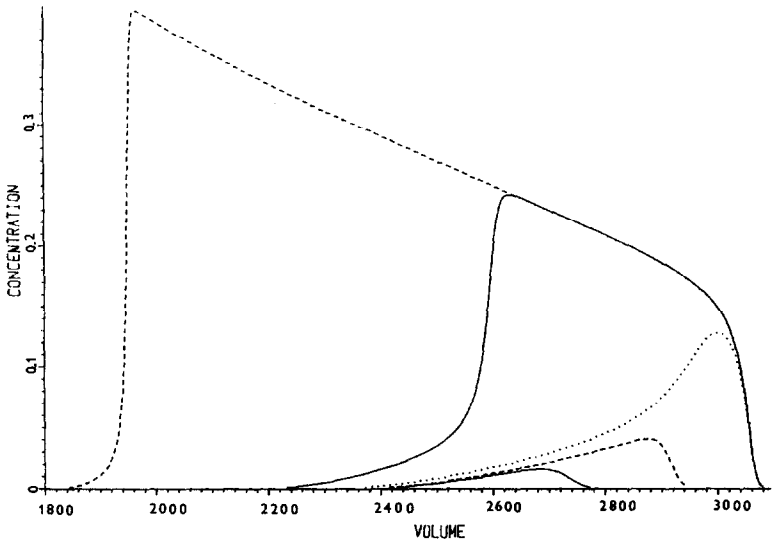

Fig. 7. Peak shapes of a compound with an S-shaped isotherm without a maximum ($K = 1.5$, $R = 1$, $L = 3$, $T = 2$). Isotherm is plotted in Fig. 2 (curve 3). Injected amounts: 3, 10, 30, 100, 300. Number of cells: 1000.

with increasing amount in a more complicated manner. At first, when the convex part of the isotherm predominates the peak extends its rear edge toward higher elution volumes (see Fig. 7) and its front edge remains diffuse. At higher overloadings, when the main role is played by the concave part of isotherm, similarly to the Langmuir isotherm, the front edge of the peak starts to extend to lower elution volumes and

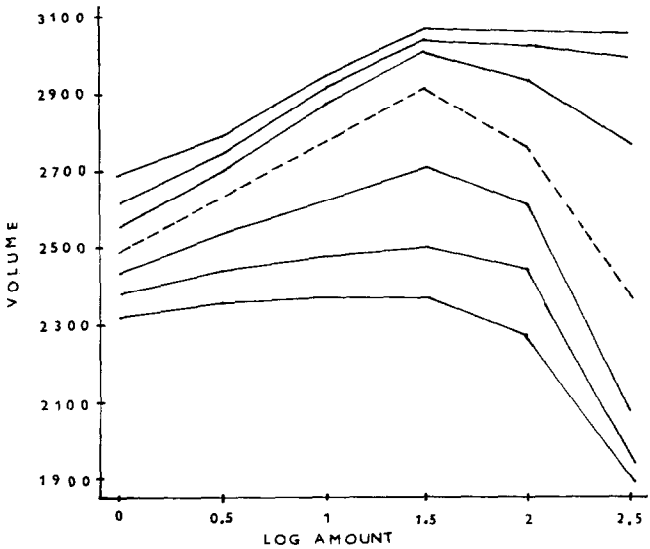

Fig. 8. Peak section positions for a compound with an S-shaped isotherm without a maximum (see Fig. 7). Dashed line: median. For further explanation, see text.

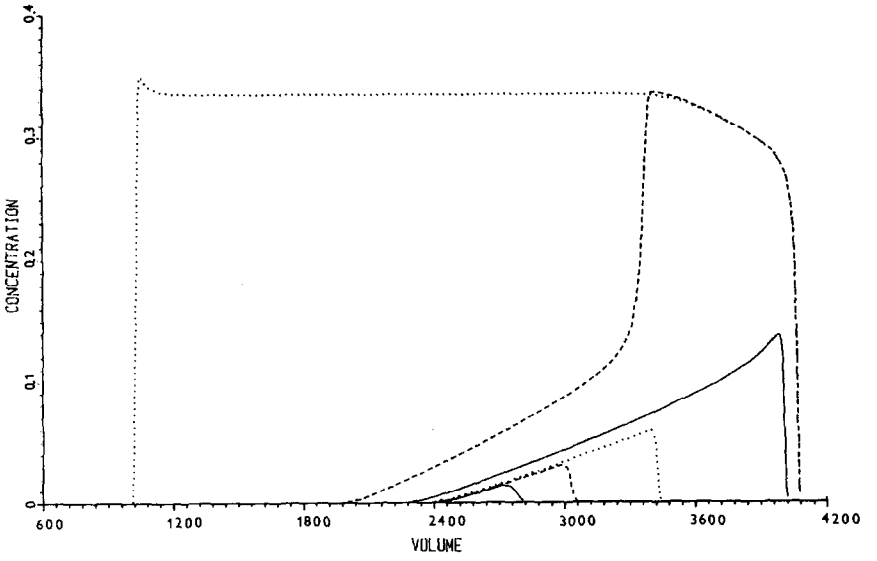

Fig. 9. Peak shapes of a compound with an S-shaped isotherm with a maximum ($K = 1.5$, $R = -1$, $L = 2$, $T = 2$). Isotherm is plotted in Fig. 1, curve 5. Injected amounts: 3, 10, 30, 100, 300, 1000. Number of cells: 1000.

becomes sharper, whereas the rear becomes diffuse and its position remains unchanged. This two-fold character of band profile dynamics is reflected in the plot of peak section positions by the at first ascending and descending shape of the plotted curves (Fig. 8). If the peak shapes of a compound with Langmuir and S-shaped

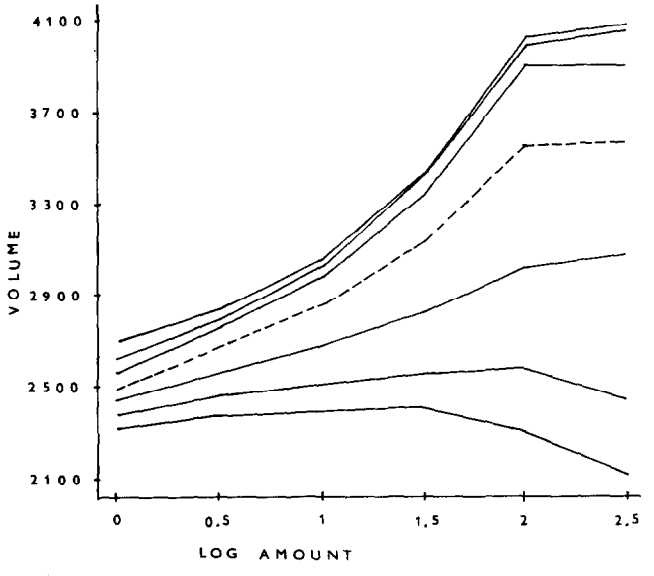

Fig. 10. Peak section positions for a compound with an S-shaped isotherm with a maximum; for parameters, see Fig. 9. Dashed line: median. For further explanation, see text.

isotherms are compared, then the heavily overloaded peak of the former is much broader and its maximum concentration is therefore lower (Figs. 3 and 7).

In the preceding example we examined isolated peaks corresponding to an S-shaped isotherm without a maximum (Fig. 2). The peaks corresponding to the S-shaped isotherm with a maximum (Fig. 1, curve 5) change their forms in a similar manner (Fig. 9). The overloading occurs at higher amounts owing to the higher concentrations at which isotherm maximum occurs; this is also reflected in the plot of peak section positions (Fig. 10). The stabilization of the rear edge position occurs at higher volumes than in the preceding example.

The influence of column efficiency and capacity on the band profile is illustrated in Fig. 11. With increasing column length, and hence with increasing column capacity, the peak form is transformed similarly to when the injected amount is decreased at a constant column length. At the same time, with increasing column efficiency, the relative effect of dispersion decreases.

The band profiles of compounds with limited solubility with isotherms similar to that depicted as curve 8 in Fig. 1 change their shape with increasing overloading in a different manner. At first they behave like bands corresponding to a normal Langmuir isotherm: the front edge is steep and the desorption part of the profile is long. When the load is increased, the profile becomes steeper also at the rear and finally we obtain a peak shape (Fig. 12) that is similar to the peak corresponding to an isotherm with the first part convex (Figs. 7 and 9). The computed shape resembles that published in an experimental study [6].

Comparison of the band profiles of four compounds with moderate (Fig. 13) and heavy overloading (Fig. 14) demonstrates how drastically may be the change in the elution volumes of bands which in linear region all elute as Gaussian profiles at 2500 volume units.

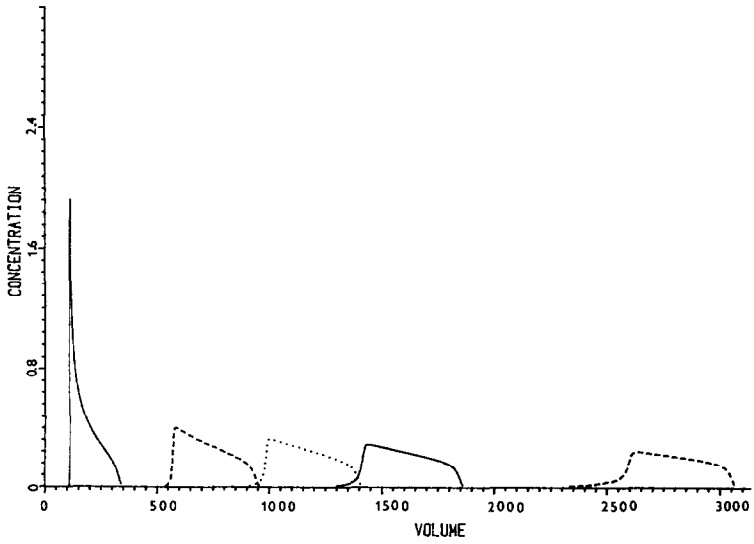

Fig. 11. Peak shapes at various column lengths. Isotherm as in Fig. 9, injected amount: 100 (volume, 10; concentration, 10). Number of cells: 1000, 600, 450, 300, 100.

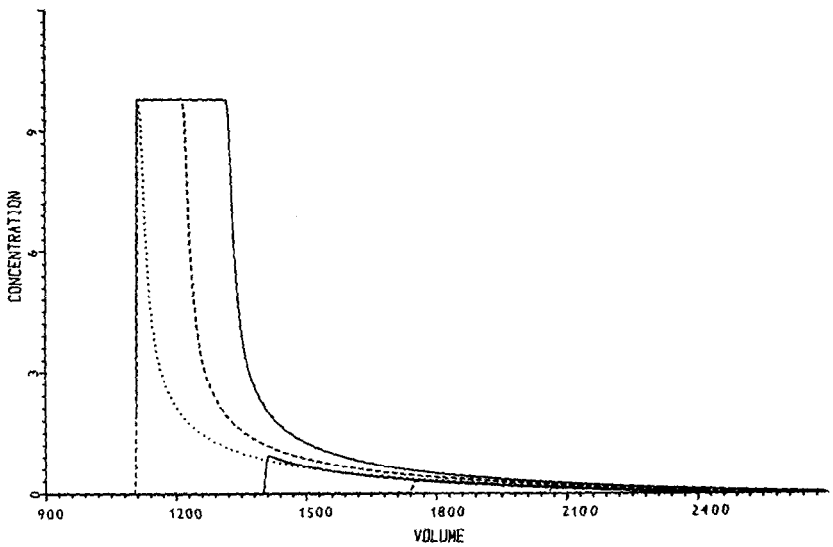

Fig. 12. Peak shapes of a compound with limited solubility (isotherm 8 in Fig. 1). Injected amounts: 3000, 2000, 1000, 300, 100, 30. Number of cells: 1000. Concentration of injected sample (all cases): 9.8.

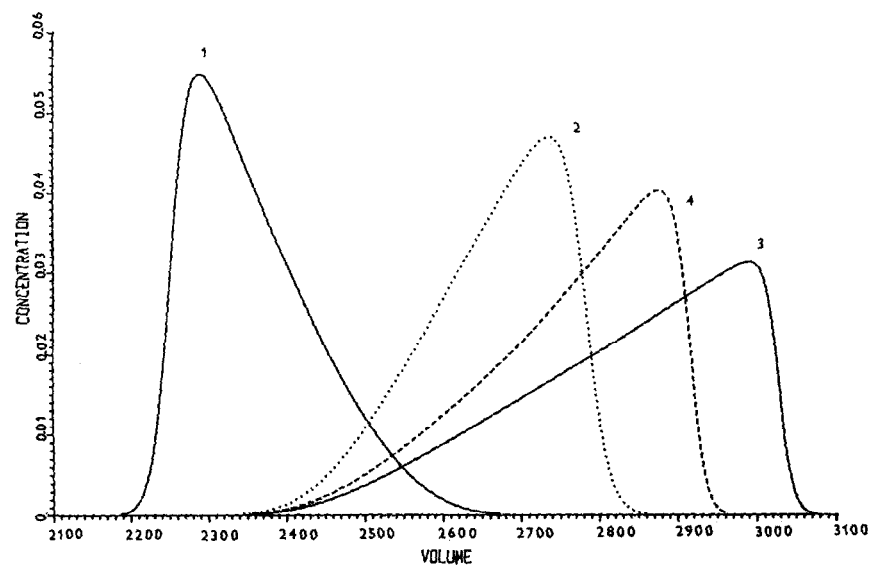

Fig. 13. Four band profiles at moderate overloading. In all instances $K = 1.5$. Injected amount: 10. Number of cells: 1000. Curve 1, Langmuir isotherm, $R = 1$; curve 2, hyperbolic (quasi-Langmuir), $R = -1$; curve 3, S-shaped, $R = -1$, $L = 2$, $T = 2$; curve 4, $R = 1$, $L = 3$, $T = 2$.

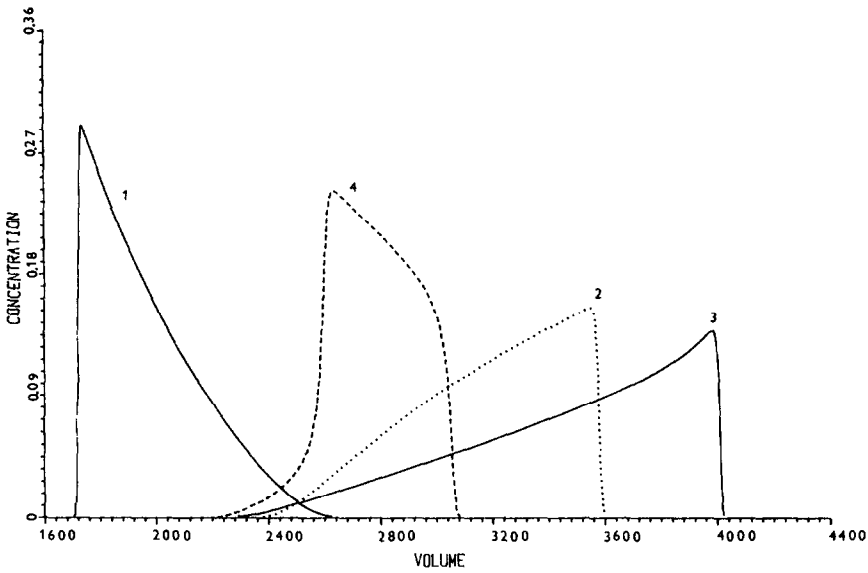

Fig. 14. Same as Fig. 13 but amount = 100.

Interaction of two compounds

Interaction of two bands with normal Langmuir isotherms in the overload region has been described previously [1,7,9] and similar interactions are observed when two compounds with hyperbolic isotherms are eluted (Fig. 15). In the region of

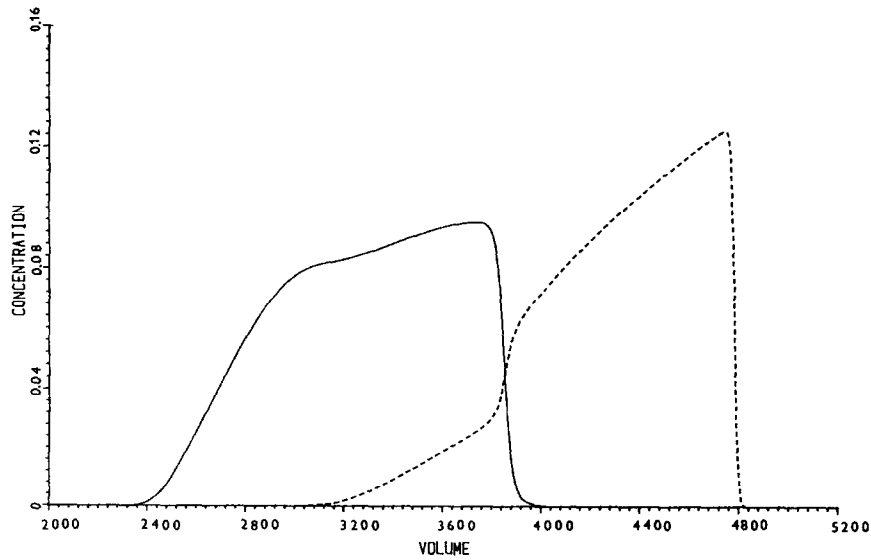

Fig. 15. Band profiles of two compounds with hyperbolic (quasi-Langmuir) isotherms, heavy overloading. Both bands: amount = 100, $R = -1$. Full line, $K = 1.5$; dashed line, $K = 2$. Number of cells: 1000.

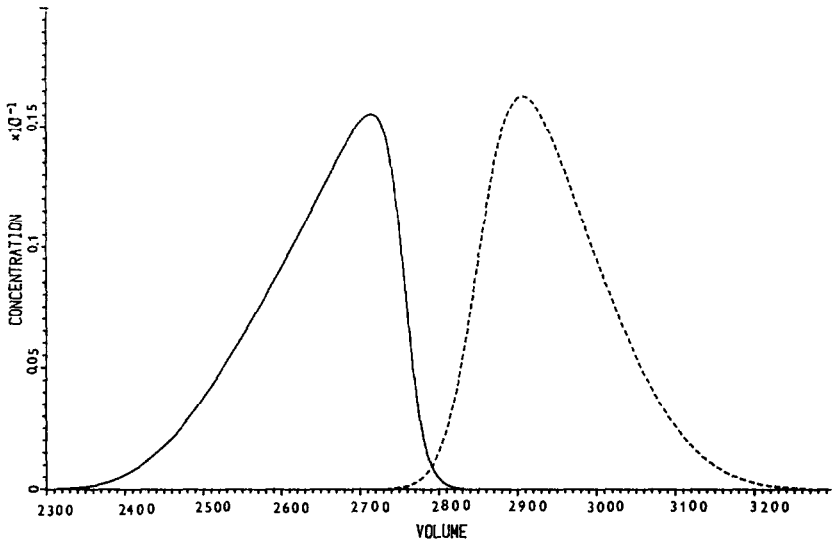

Fig. 16. Two compounds with different types of isotherms. Full line, $K = 1.5$, $R = -1$ (hyperbolic, quasi-Langmuir); dashed line, $K = 2$, $R = 1$ (Langmuir). Injected amount: 3. Injection volume: 10. Number of cells: 1000.

overlap, the compounds seemingly displace each other and the tendency to form bands of pure compounds manifests itself by the formation of an indentation in the front part of the second peak and of a hump in the front part of the first peak. A different situation occurs if one compound has a Langmuir isotherm and the second

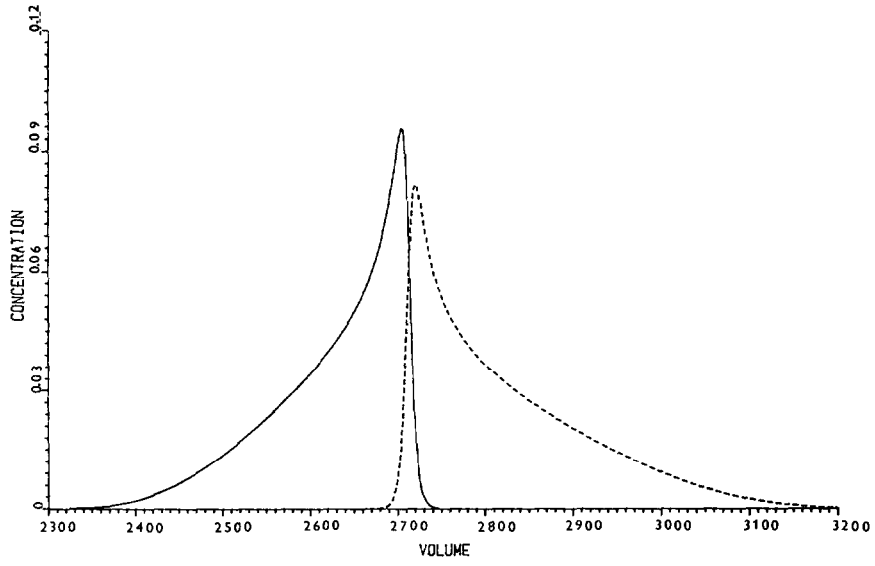

Fig. 17. Same as Fig. 16 but injected amount = 10.

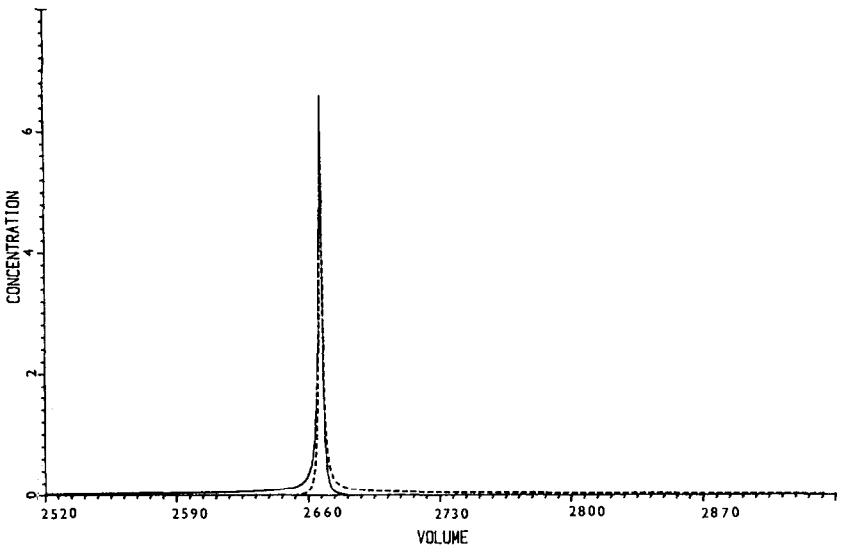

Fig. 18. Same as Fig. 16 but injected amount = 30.

a hyperbolic isotherm. Then at higher overloadings the band profiles do not overlap, but form a region with increased concentration (Figs. 16–18). This tendency for a decreasing width and increasing concentration finally leads to extremely narrow twin band profiles [1].

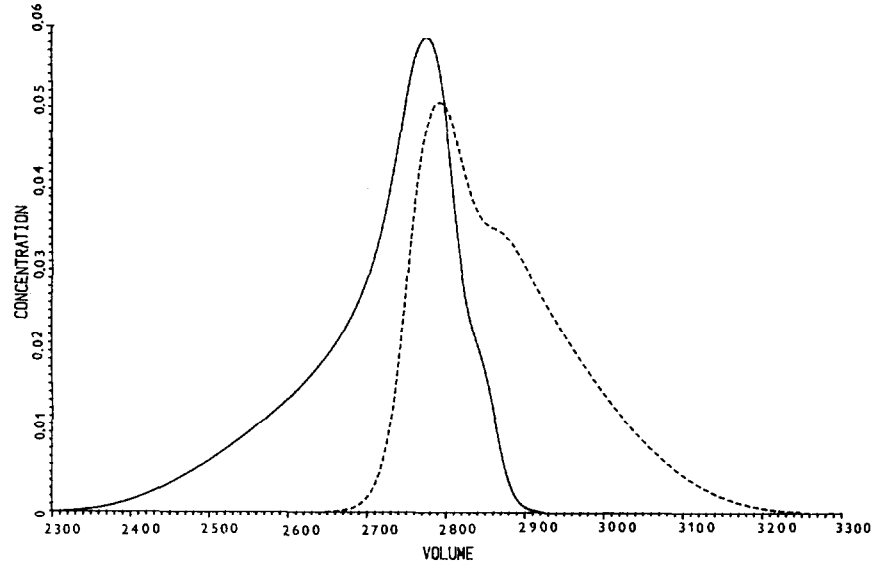

Fig. 19. Two compounds with different types of isotherms. Full line, S-shaped isotherm, $K = 1.5$, $R = -1.0$, $L = 2.0$, $T = 2.0$; dashed line, Langmuir isotherm, $K = 2.0$, $R = 1.0$, $L = T = 0$. Number of cells: 1000, Injected amount: 10.

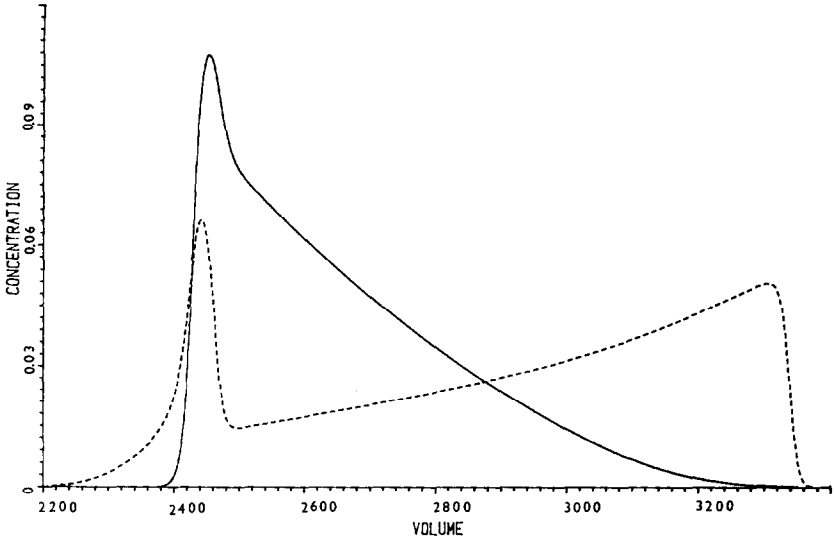

Fig. 20. Same as Fig. 19 but injected amount = 30.

When, instead of a compound with a hyperbolic isotherm together with a compound with a Langmuir isotherm, a compound with an S-shaped isotherm having the first two constants identical is eluted, at low concentrations the band profile is identical with that in Fig. 16. When the injected amount is increased, a narrow twin peak is formed (Fig. 19), similarly to the previous case (Fig. 17) with larger injected amounts, but then the peaks start to overlap (Fig. 20) and at still higher amounts the

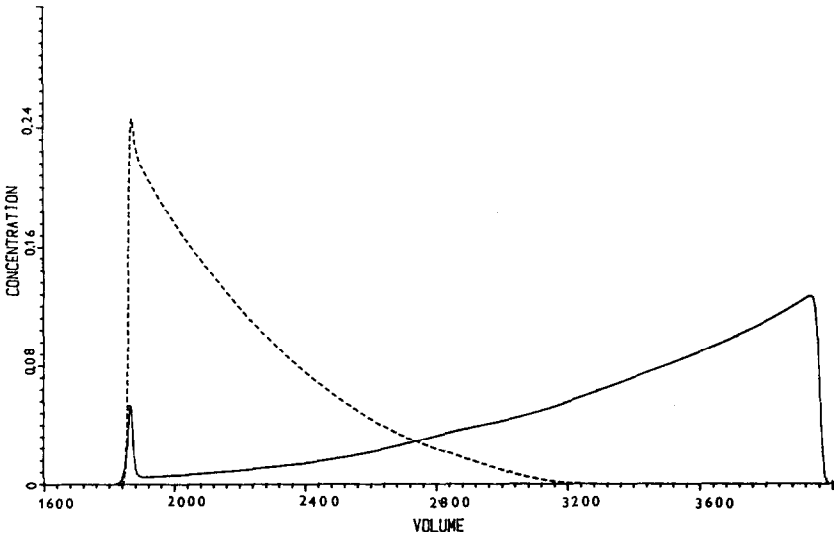

Fig. 21. Same as Fig. 19 but injected amount = 100.

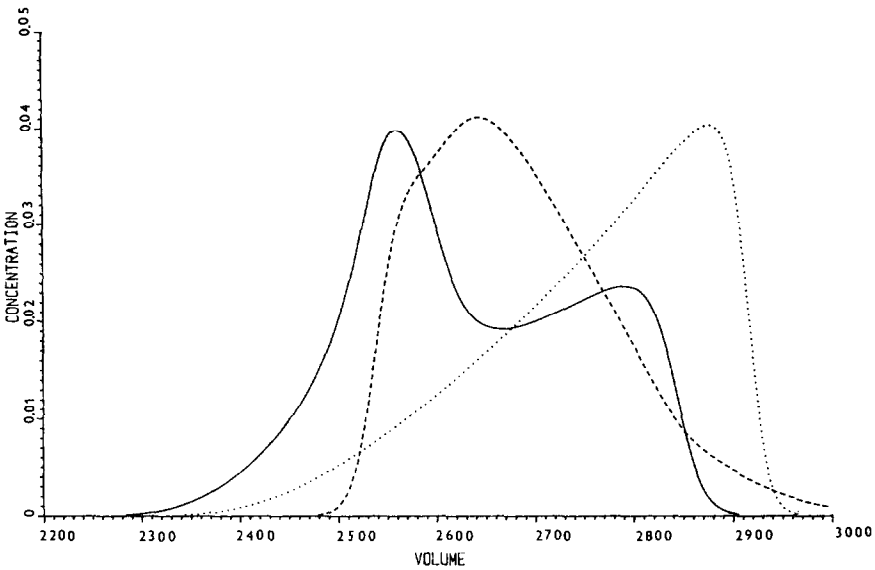

Fig. 22. Band profiles of compounds with S-shaped and Langmuir isotherms. Full line, $K = 1.5$, $R = 1.0$, $L = 3.0$, $T = 2.0$; dashed line, $K = 2.0$, $R = 1.0$, $L = T = 0$; dotted line, same as full line, but compound eluted alone, without interaction. Number of cells: 1000. Injected amount: 10.

band profiles are quite similar to those for pure compounds. The only indication of this inflection point on the isotherm of the compound with a lower distribution coefficient is the formation of a spike on the ascending part of the peak profile. This spike is also reflected on the front edge of the compound with a regular Langmuir isotherm (Fig. 21).

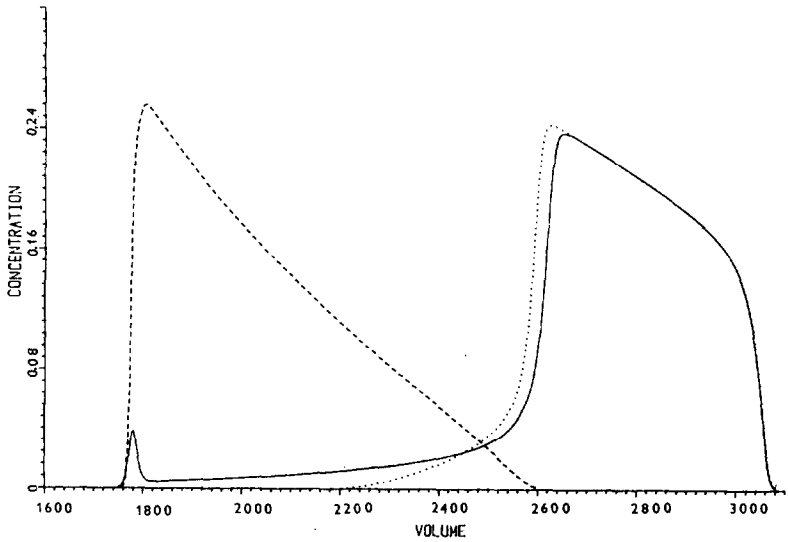

Fig. 23. Same as Fig. 22 but injected amount = 100.

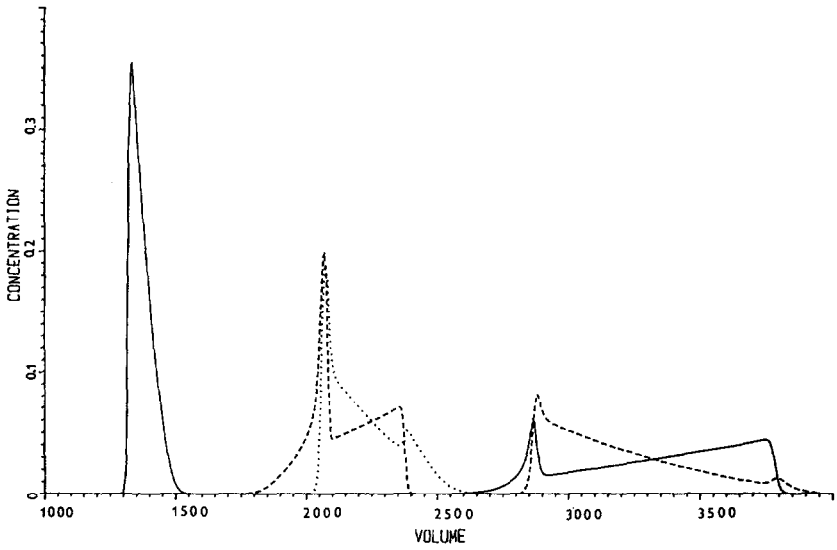

Fig. 24. Separation of five-component mixture. Three components with Langmuir isotherms, $K = 0.5$, 1.5 and 2.5; for all three $R = 1.0$, $L = T = 0$. Two component with S-shaped isotherms: $K = 1.0$, $R = -1.0$, $L = 1.0$ and $T = 0.3$ and $K = 2.0$, $R = -1$, $L = 1.0$ and $T = 1.2$. Injected amount (all components): 30. Number of cells: 1000.

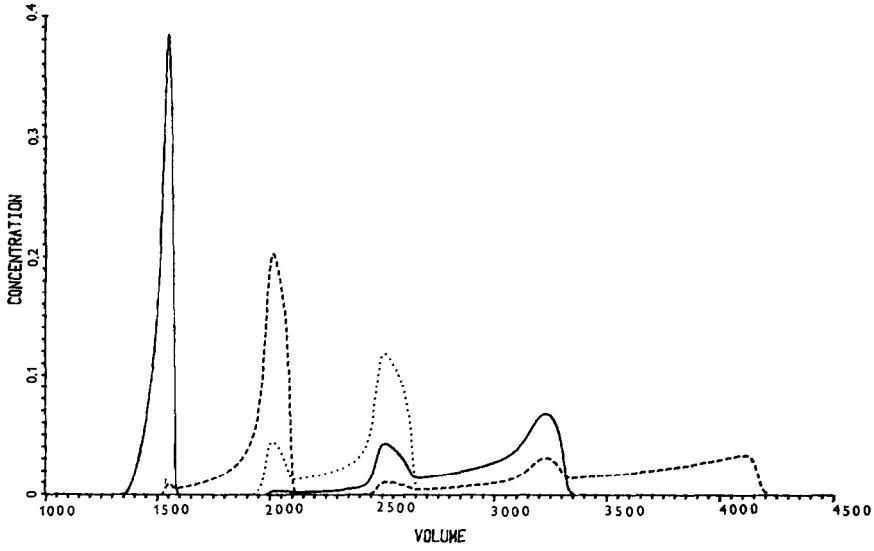

Fig. 25. Separation of five-component mixture. All components: S-shaped isotherms as in Fig. 2. Injected amount (all components): 30. Number of cells: 1000.

TABLE II

RECOVERY OF COMPOUNDS WITH S-SHAPED ISOTHERMS (AS IN FIG. 2) AS PERCENTAGE OF AMOUNT INJECTED

Injected amount	Volume of load	Compound[a]				
		1	2	3	4	5
5 × 10	1	100	80.57	31.92	6.03	40.36
5 × 20	200	100	100.00	37.14	10.16	85.77
5 × 30	1	50.87	16.32	0	0	0

[a] The numbers correspond to the curve numbers in Fig. 2.

The general character of the interaction between compound bands with S-shaped and Langmuir isotherms is preserved even when the isotherm contains only positive constants and does not exhibit a maximum. If small amounts are injected, Gaussian peaks at 2500 and 3000 volume units are eluted. At higher concentrations, the band profile of the first compound is extended towards higher elution volumes and *vice versa*. At the same time, the peak of the first compound is pushed backwards by interaction with the band of the second compound and a secondary peak is formed (Fig. 22). At higher loads the bands change their mutual position, and the band of the compound with an S-shaped isotherm shows a secondary spike at the elution volume of the sharp front of the compound with a Langmuir isotherm (Fig. 23).

Multi-component separations

The five-component mixture of compounds with regular Langmuir and with

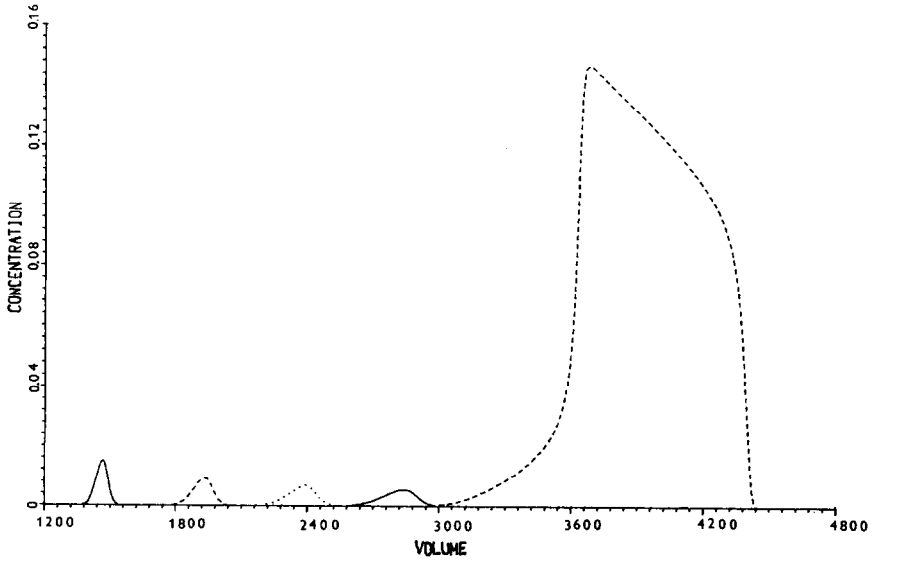

Fig. 26. Separation of five components as in Fig. 25. Injected amounts: component with $K = 2.5$, 100; other four, 1. Number of cells: 1000.

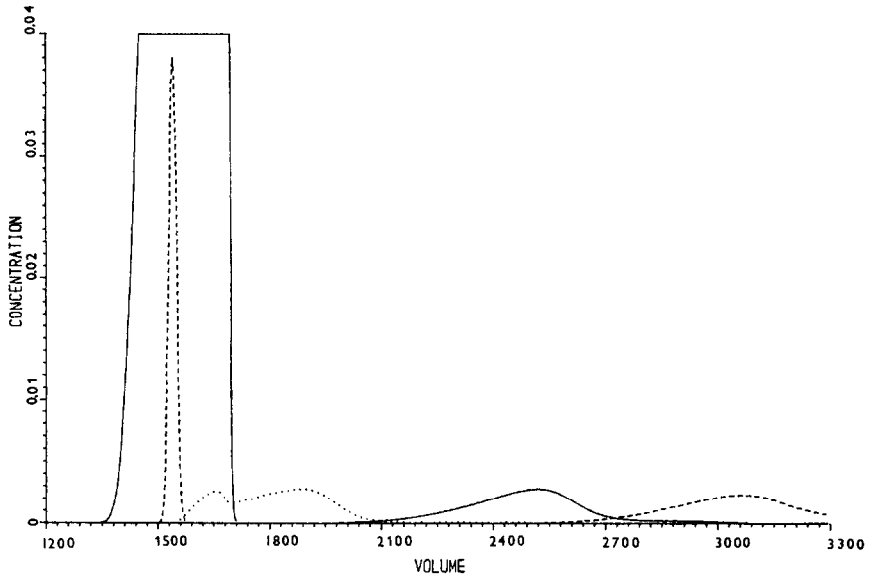

Fig. 27. Separation of five components as in Fig. 26. Injected amounts: component with $K = 0.5$: 100; other four, 1. Number of cells: 1000.

S-shaped isotherms reveals (Fig. 24) similar band profiles to those found for the two-component mixture (Fig. 20). The loading is relatively low in comparison with similar separations of compounds with Langmuir isotherms [1], but the overlap of bands is so severe that the recoveries (with the exception of the first compound) are only a few percent.

Another example is presented by compounds with S-shaped isotherms without a maximum and with all four constants positive (Fig. 2).

Injection of the same amount of compounds as in preceding example leads to severe interferences between all components (Fig. 25). Only after the load has been decreased 3-fold may a small percentage of all components be recovered with the desired purity (99%). As in similar instances, when severe interactions between chromatographic bands exist, dilution of the injected mixture may lead to an improvement in separation. In our case, even when the injected amount is double that in the preceding case the recovery increases (see Table II).

The recovery of trace components from compounds with S-shaped isotherms does not seem to present special problems if the major component is eluted in the last position. All four minor components are then eluted in isolated bands, only their elution volumes are shifted to lower values compared with elution of pure components (Fig. 26). In contrast, when the major compound is eluted first, the first minor component is completely overlapped by the major band and even a substantial part of the second minor component is lost in the first peak (Fig. 27). The shift of elution volumes to lower values is more pronounced than in the previous instance.

CONCLUSION

Two-step adsorption has been postulated as the mechanism for the formation of isotherms with inflection points. The band profiles of isolated peaks can then be computed using the equilibrium mixed cells model. This model permits the computation of multi-component separations and interesting features, not observed when compounds with simple Langmuir isotherms are separated, may be discerned.

However, it must be stressed that similarity of computed and experimental chromatograms may be only circumstantial. Only a thorough study of multi-component isotherms may establish that the hypothetical chromatograms presented in this paper describe real systems. On the other hand, the simplicity of the original postulate increases the validity of the results presented.

SYMBOLS

c_i concentration of ith compound in mobile phase (mol l^{-1})
c_{iA} concentration of ith compound (all adsorbed forms) in solid phase (mol l^{-1})
c_{iF} concentration of sorption sites with one adsorbed molecule of ith compound (mol l^{-1})
c_F concentration of free sorption sites in solid phase (mol l^{-1})
c_{2iF} concentration of sorption sites with two adsorbed molecules of ith compound (mol l^{-1})
G_D sorption capacity of sorbent in one equilibrium cell (mol) (see eqn. 4)
G_i amount of ith compound in one equilibrium cell (mol)
K_i equilibrium constant in single-step adsorption (l mol^{-1}) (see eqn. 1)
L_i equilibrium constant of the second adsorption step (l mol^{-1}) (see eqn. 2)
R_i blocking factor for the first adsorption step (dimensionless)
T_i blocking factor for the second adsorption step (dimensionless)
V_M volume of mobile phase in one equilibrium cell (l)
V_S volume of solid phase in one equilibrium cell (l)

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