Journal of Chromatography, 541 (1991) 195–206
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 937

Effect of the intersection of the individual isotherms in displacement chromatography

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(First received March 20th, 1990; revised manuscript received October 18th, 1990)

ABSTRACT

Neither the existence of an intersection between the two single-component isotherms drawn on the same graph, nor the fact that the column saturation capacity for the more retained component is lower than that of the lesser retained component, have any major consequence on the chromatographic behavior of elution bands or on the formation of the isotachic train, as long as the equilibrium isotherms of the two components are properly described by the competitive Langmuir model. Significant deviation from this model could make impossible the formation of an isotachic train in displacement chromatography, but definitive experimental proof of the existence of this effect is lacking.

INTRODUCTION

The calculation of elution band profiles in preparative chromatography requires the knowledge of the equilibrium isotherms of the components involved [1–3]. It has been shown that the competitive Langmuir isotherm [4] is sometimes an excellent model [5] but that more often it is barely acceptable [6,7]. However, the use of the Langmuir model permits the achievement of realistic results in simulation studies. These results can be used for investigations of the mechanism of band separation and band interactions in overloaded columns. Experimental results agree qualitatively very well with the predictions obtained in these calculations [5,7].

In practice, it is generally observed that the best set of competitive Langmuir isotherms which fit experimental results corresponds to different column saturation capacities for the various components of the mixture. Analysis of experimental data shows that in a number of instances the more retained component is also the one for which the column saturation capacity is the smaller. This situation should be expected especially in reversed-phase chromatography, as the bulkiest component, which has the smallest column saturation capacity, is often also the one which is adsorbed the most strongly. The phenomenon, however, is not peculiar to adsorption chromatography.

When two compounds exhibit this type of behavior, their single-component isotherms plotted on the same graph intersect and, for this reason, the effect has often been referred to as *isotherm intersection*. The isotherm intersection effect has been

0021-9673/91/$03.50

reported recently in overloaded elution [1] and displacement [2,3] chromatography. Cox and Snyder [1] suggested that the consequence of a lower saturation capacity of the more retained compound is that the displacement effect of the first-eluted compound by the second in overloaded elution is minimal. Subramanian and Cramer [3] showed that when the isotherms intersect, an isotachic displacement train cannot be achieved experimentally. They had to change the mobile phase composition to eliminate the isotherm intersection in order to perform displacement separations. They stated that their experimental results cannot be explained by a Langmuir-based model, as such a model predicts that the order of the bands in the isotachic displacement train depends only on the initial slopes of the isotherms and is independent of the displacer concentration, whether the isotherms of the feed components intersect or not.

In a recent paper, we reported a study of the influence of the column saturation capacity on the intensity of the displacement and tag-along effects [8]. We showed that the major contributions to the intensity of these effects are the ratio of the column saturation capacities for the two components and the feed composition which determines the ratio of the loading factors and the sample size. *If we assume that the competitive Langmuir isotherm model remains valid in this case*, the main consequence of a lower column saturation capacity for the more retained compound is that the displacement effect is enhanced and at the same time the separation deteriorates [8]. This is because, for a given sample size, the loading factor increases with decreasing column saturation capacity whereas the degree of column overload increases with increasing loading factor. On the other hand, at constant loading factor (*i.e.*, if the sample size is decreased in proportion to the column saturation capacity), the separation is improved because of the enhanced displacement effect. These results of the ideal model of chromatography are confirmed by those of numerical solutions of the semi-ideal model [9].

The aim of this paper is a detailed discussion of the interactive behavior of the bands of two components in the case of intersecting single-component isotherms, when the competitive isotherms of the two components are assumed to follow the competitive Langmuir isotherm model. This study applies to both overloaded elution and displacement.

THEORY

Properties of the isotherm intersection

The competitive Langmuir isotherm for a component i is given by:

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

where C_i and Q_i are the equilibrium concentrations of the component i in the mobile and stationary phases, respectively, and a_i and b_i are numerical coefficients. The column saturation capacity, $q_{s,i}$, is the product of the specific saturation capacity, $Q_{s,i} = a_i/b_i$, and the volume of stationary phase contained in the column. The column saturation capacity is the natural unit of the sample size used in preparative chromatography. The loading factor for one component is the ratio of the actual amount of this compound in the sample and the column saturation capacity.

As discussed previously [9], the competitive isotherms of the components of a binary mixture are two surfaces which must intersect. This is easily demonstrated. When the relative concentration, C_1/C_2, of the two components in the mobile phase varies from 0 to infinity at constant total concentration ($C_1 + C_2 = C$), the amount, $Q_1(C_1,C_2)$, of the first component adsorbed at equilibrium increases from 0 to $Q_1(C,0)$, whereas the amount of the second component adsorbed, $Q_2(C_1,C_2)$, decreases from $Q_2(0,C)$ to 0. Thus, the intersection of the isotherm surfaces by a vertical cylinder, which are two curves drawn on that cylinder, must intersect somewhere. Hence, the two surfaces intersect along a certain curve.

To help in understanding the situation, a concrete representation of the isotherms is useful. This can be achieved with either false three-dimensional (3-D) plots or contour maps. Fig. 1a shows a view of the 3-D plot of the Langmuir isotherms in a case where the single-component isotherm intersection effect takes place ($RQ_s = Q_{s,2}/Q_{s,1} = 0.5$). This figure does not exhibit any unusual feature resulting from the fact that the two single-component isotherms would intersect if they were drawn in the same plane.

Fig. 1b and c shows contour maps of the two sets of Langmuir isotherms used in the present work [$RQ_s = 1.0$ (Fig. 1b) and $RQ_s = 0.25$ (Fig. 1c)]. It is seen that these contour maps, showing the intersection of the surfaces $Q_i = f(C_1,C_2)$ by horizontal planes of increasing height, are fans of straight lines. For the isotherm of the first component, the general equation of these lines is

$$C_2 = -\frac{1}{b_2} + \frac{1}{b_2}\left(\frac{a_1}{k} - b_1\right)C_1 \tag{2}$$

where k is the height of the horizontal plane. C_2 must be positive; this is possible only if k is smaller than the saturation capacity for the first compound, $Q_{s,1}$ and for values of C_1 larger than $k/(a_1 - b_1k)$. Eqn. 2 defines a family of straight lines which all pass through the point of coordinates $C_1 = 0$, $C_2 = -1/b_2$ (note that only positive concentrations have a physical meaning). The contours of the second-component isotherm are also straight lines whose equation can be obtained by exchanging the indices 1 and 2 in eqn. 2. Their general equation can be rearranged into

$$C_2 = \frac{k}{a_2 - kb_2} + \frac{kb_1}{a_2 - kb_2}C_1 \tag{3}$$

All the straight lines defined by eqn. 3 pass through the point of coordinates $C_1 = -1/b_1, C_2 = 0$. Thus, we obtain two fan-like families of straight lines as contour maps for a binary mixture. This result is seen in Fig. 1b and c. It comes from the properties of eqn. 1, which defines a conoid surface.

The two straight lines of the two families which correspond to the same value of k, *i.e.*, the intersections of the two isotherm surfaces by the same horizontal plane, intersect in a point whose coordinates are such that $C_1 = \alpha C_2$. As a consequence, the intersection of the two isotherm surfaces, $Q_1(C_1,C_2)$ and $Q_2(C_1,C_2)$ is a curve in the vertical plane of trace $C_1 = \alpha C_2$. This result has already been demonstrated otherwise [6,9].

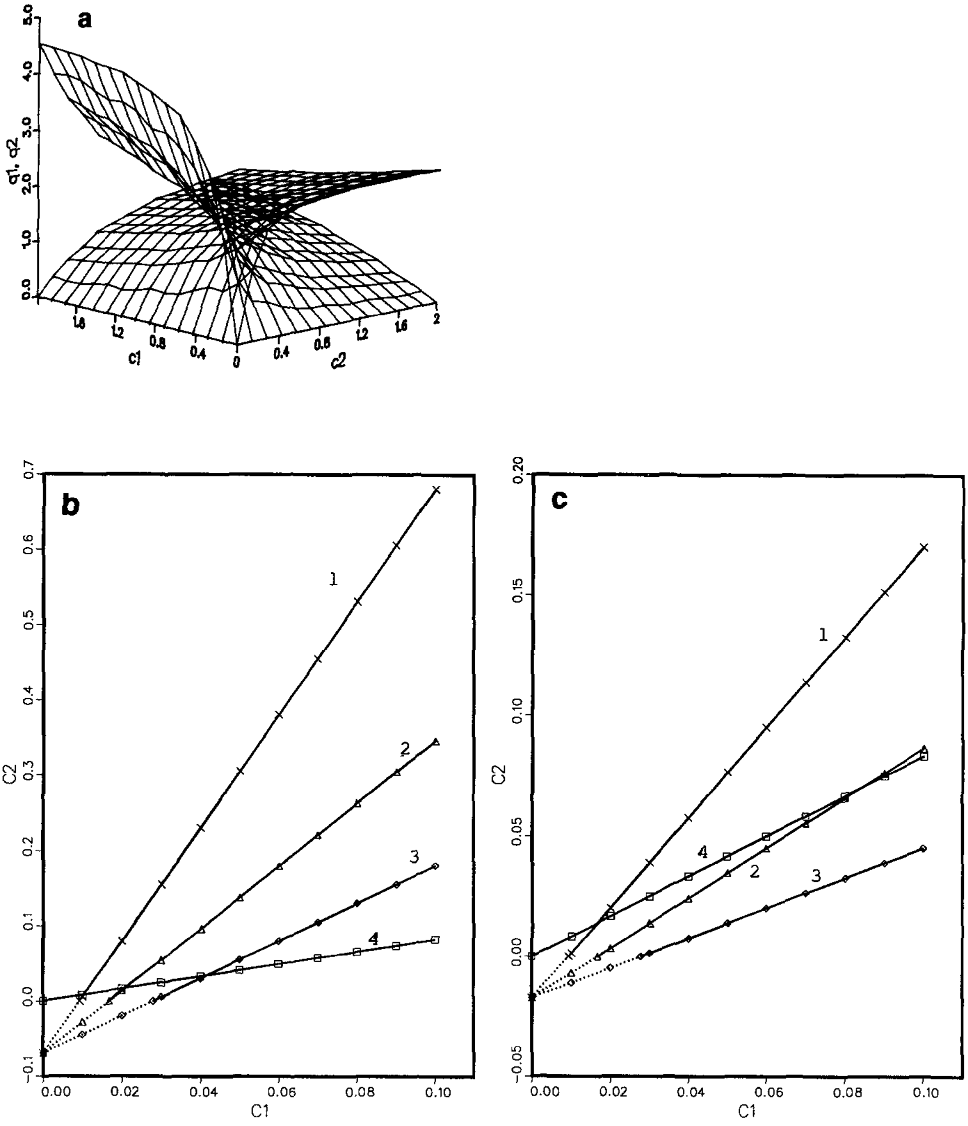


Fig. 1. Three-dimensional representation of the Langmuir competitive isotherm surface for the two components of a binary mixture. (a) False 3-D plot of the isotherm surfaces for the two components. $RQ_s = 0.5$. The specific column saturation capacity is larger for the first component ($Q_{s,1} = 5$) than for the second ($Q_{s,2} = 2.5$). Apparent isotherm intersection takes place ($a_1 = 24$; $a_2 = 28.8$). (b) Contour map of the isotherm surface for the first compound. $RQ_s = 1.0$. Isotherm coefficients: $a_1 = 24$; $a_2 = 28.8$; $b_1 = 12$; $b_2 = 14.4$. The specific saturation capacity is the same for the two components $Q_{s,1} = Q_{s,2} = 2.0$. The pure component isotherms do not intersect. The straight lines are intersections of the isotherm surface with the following planes: 1, $Q_1 = 0.10\,Q_{s,1}$ (×); 2, $Q_1 = 0.167\,Q_{s,1}$ (△); 3, $Q_1 = 0.25\,Q_{s,1}$ (◇); 4, line $C_2 = 1/\alpha C_1$ (□). (c) Same as (b), except $RQ_s = 0.25$ ($Q_{s,1} = 2.0$, $Q_{s,2} = 0.5$, $b_2 = 57.6$). The single-component isotherms intersect (see Fig. 2).

Fig. 1b and c has the same features. For both components, the isotherm surfaces obtained in either instance look similar. The isotherms corresponding to the case when the column saturation capacity is four times larger for the lesser than for the more retained component do not have any special property. In cases where the competitive Langmuir model is valid, isotherm intersection cannot have any direct consequence in chromatography, as the coordinates of the intersection point have no physical significance and the separation factor of the system for the two components $[(Q_2/C_2)/(Q_1/C_1) = a_2/a_1 = \alpha]$ is independent of the concentrations. It is also independent of the column saturation capacities of the two components but depends only on the ratio of the origin slopes of the isotherms.

Fig. 2 shows the overlaid plots of the single-component isotherms of both components of the mixture in the case when the column saturation capacity of the second-eluted component is four times smaller than that of the first. The single-component isotherms which intersect on a $Q_i(C_i)$ *versus* C_i plot are in fact the curves $Q_1(C_1)$ and $Q_2(C_2)$, which have nothing in common, not even the abscissa! The first curve is a plot of Q_1 *versus* C_1, at equilibrium of the first component between the two phases of the system, and the second curve is a plot of Q_2 *versus* C_2 under the same conditions. The intersection point has two different sets of coordinates depending on whether it is

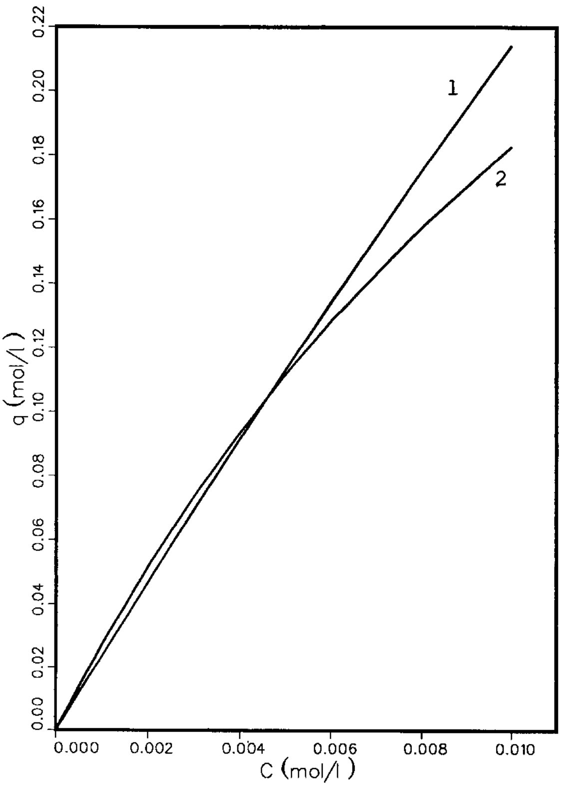


Fig. 2. Single-component isotherm of two solutes. $Q_1 = a_1C_1/(1+b_1C_1)$, $Q_2 = a_2C_2/(1+b_2C_2)$, $a_i = k'_{0,i}/F$, $k'_{0,1} = 6$, $Q_{s,1} = 2$, $Q_{s,2} = 0.5$, $b_i = a_i/Q_{s,i}$. The two isotherms intersect at $C = 0.00463$ *M*.

considered as a point on the first curve or a point on the second curve. It has no physical meaning. It does not exist on Fig. 1c.

The situaton may be different in displacement chromatography; it has been claimed that displacement is impossible in the case when single-component isotherms intersect because the elution order of the plateau concentration of the isocratic train would be different from the elution order at very low concentrations [3]. This requires further investigation.

Effect of single-component isotherm intersection in displacement chromatography

Rhee and Amundson [10] reported a detailed theoretical study of displacement chromatography within the framework of the ideal model, using the competitive Langmuir isotherm. They showed that in this case it is always possible to achieve an isotachic train provided that a sufficiently long column is used and that the displacer concentration exceeds a threshold concentration given by

$$C_\mathrm{d} > \frac{1}{b_\mathrm{d}}\left(\frac{a_\mathrm{d}}{a_\mathrm{f}} - 1\right) \tag{4}$$

where C_d is the displacer concentration, $a_\mathrm{d}/a_\mathrm{f} = k'_{0,\mathrm{d}}/k'_{0,\mathrm{f}}$ is the ratio of the slopes of the displacer isotherm and of the less retained component isotherm at infinite dilution and $b_\mathrm{d} = a_d/q_{\mathrm{s,d}}$ is the second parameter of the competitive Langmuir isotherm of the displacer.

We have recently shown that in the case of an actual column with a finite efficiency, the critical parameters in displacement chromatography are the displacer concentration, the loading factor and the column efficiency. This efficiency is the limiting efficiency obtained with very small size samples and results from the effects of axial dispersion and the resistances to mass transfers [11]. The same condition as stated by eqn. 4 applies in non-ideal and ideal chromatography. However, when the displacer concentration increases, the plateau becomes narrower and narrower. In non-ideal chromatography the plateau is eroded because of the finite rate of the mass-transfer kinetics. It may disappear if the displacer concentration is too large [11].

Thus, the theory of non-linear chromatography predicts that, as long as the competitive Langmuir isotherm model is valid, we must be able to generate isotachic trains in displacement chromatography, even if the single-component isotherm intersect. In this last case, the column saturation capacity for the second component is smaller than that of the first. To illustrate this point, which is important, let us compare the separation of two binary mixtures, A + B and A + B′, and assume that the only difference between these problems is in the column saturation capacity for the second component, which is larger for B than for B′. The isotherm surface for the first component, A, and the coefficient a_2 for the second component, B or B′, is the same in both instances. If we inject the same amount of a feed having the same relative concentration ([A]/[B] = [A]/[B′]) of the two components, the loading factor of the second component is larger in the second case (B′) than in the first (B). The successful formation of an isotachic displacement train in the second case (lower column saturation capacity for B′) requires that we reduce the loading factor for B′ by using either a smaller sample size or a longer column. The proper set of experimental conditions may be more difficult to achieve for B′ than for B.

The calculation of the chromatograms obtained in displacement chromatography under a variety of experimental conditions illustrates these theoretical considerations.

RESULTS AND DISCUSSION

Fig. 2 shows the single-component isotherms corresponding to the competitive isotherms in Fig. 1c. These two isotherms intersect at $C_1 = C_2 = 0.00463$ M. Figs. 3 and 4 compare the profiles of the zones of two compounds in displacement chromatography, for the same sample size. In Fig. 3, the column saturation capacities of the two components are identical ($Q_{s,1} = Q_{s,2} = 2.0$; the isotherms are not shown, since they are classical). In this figure, a successful isotachic displacement train has been formed. In Fig. 4 ($Q_{s,1} = 2$, $Q_{s,2} = 0.5$), in contrast, the isotachic displacement train is not formed although the amounts of the two components injected are the same for both Figs. 3 and 4. The zone profiles observed are typical of those taking place during this isotachic train formation [11,12]. The reason is that, as the sample size is the

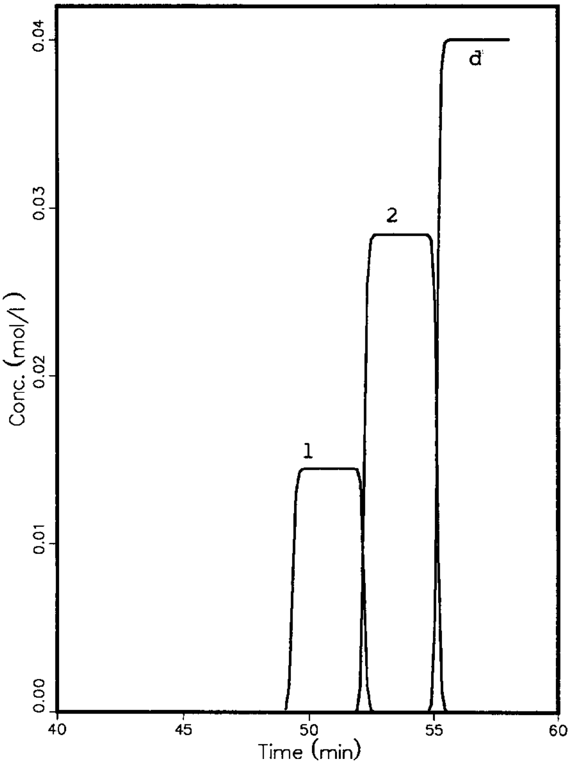


Fig. 3. Calculated zone profiles in displacement chromatography. Experimental conditions: phase ratio, $F = 0.25$; flow velocity, 0.05 cm/s; column length, 25 cm; efficiency, $N = 5000$ theoretical plates. Langmuir competitive isotherm: $Q_i = a_iC_i/(1+b_1C_1+b_2C_2+b_dC_d)$, $a_i = k'_{0,i}/F$, $k'_{0,1} = 6$, $\alpha_{1,2} = k'_{0,2}/k'_{0,1} = 1.2$, $\alpha_{2,d} = k'_{0,d}/k'_{0,2} = 1.2$, $Q_{s,1} = Q_{s,2} = Q_{s,d} = 2$, $b_i = a_i/Q_{s,i}$. Sample size: injection time, $t_p = 250$ s; feed concentration, $C_1^0 = 0.01$ M, $C_2^0 = 0.02$ M, $C_d^0 = 0.04$ M; $L_{f,1} = 0.01$, $L_{f,2} = 0.02$.

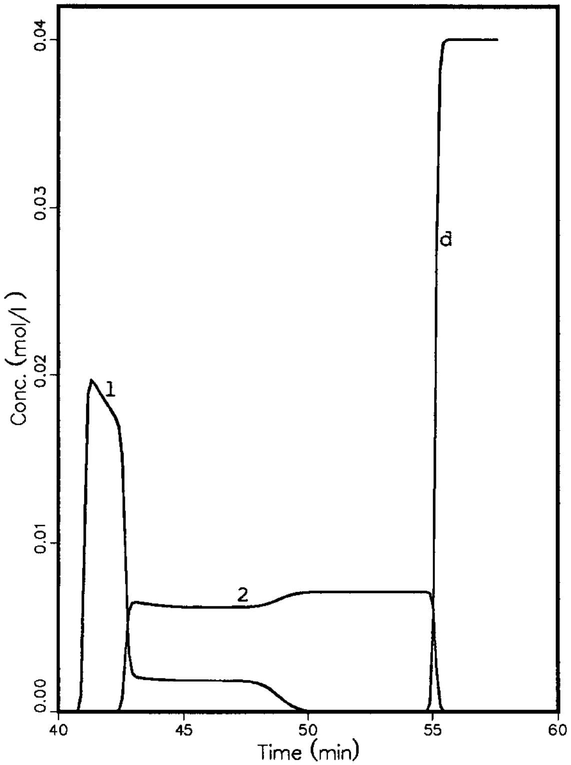
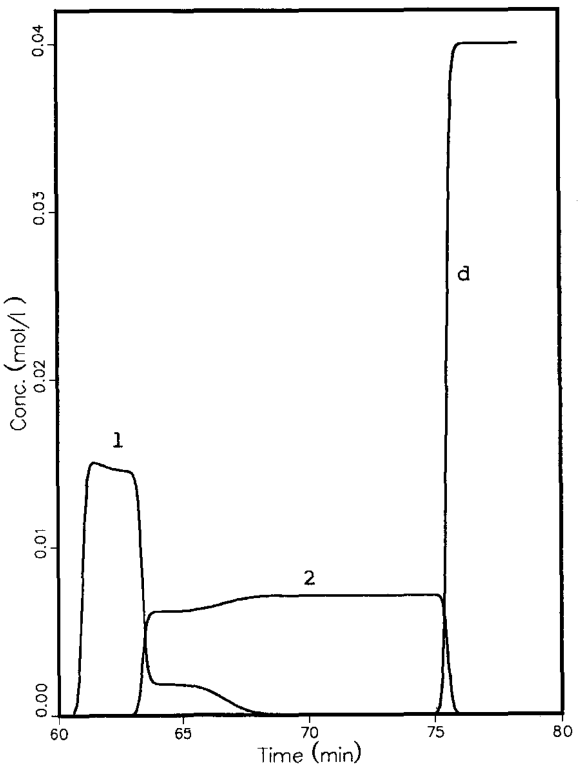


Fig. 4. Calculated zone profiles in displacement chromatography. Experimental conditions as for Fig. 3, except $Q_{s,1} = Q_{s,d} = 2$, $Q_{s,2} = 0.5$, $L_{f,1} = 0.01$, $L_{f,2} = 0.08$.

same for both figures and the column saturation capacity of the second component is four times smaller for Fig. 4 than for Fig. 3, the loading factor for the second component is four times larger in Fig. 4 than in Fig. 3, 8% instead of 2%. The loading factor is inversely proportional to the volume of stationary phase, and hence decreases with increasing column length, in proportion to the reverse of this length.

The zone profiles of the same binary mixture used for Fig. 4 is shown in Figs. 5 and 6, with longer columns, 35 and 50 cm, respectively. Thus, for the same sample size, the loading factors for the second component are 5.7% and 4%, respectively. The formation of the isotachic train is more advanced in Fig. 5 than in Fig. 4 and it is successful in Fig. 6. *We note, however, a remarkable difference between Figs. 3 and 6.* In Fig. 3, the heights of the plateaux of each successive zone increase from the first to the second component and to the displacer. This is the normal behavior, when single-component isotherms do not intersect: the operating lines intersect successively the isotherms of these compounds in this order, corresponding to increasing mobile phase concentrations [10–12]. However, *when the single-component isotherms intersect and if the displacer concentration is high enough, the operating line encounters the second component isotherm before, and at a lower concentration than, the first component isotherm.* Thus, the plateau of the second zone is lower than the plateau of the first.



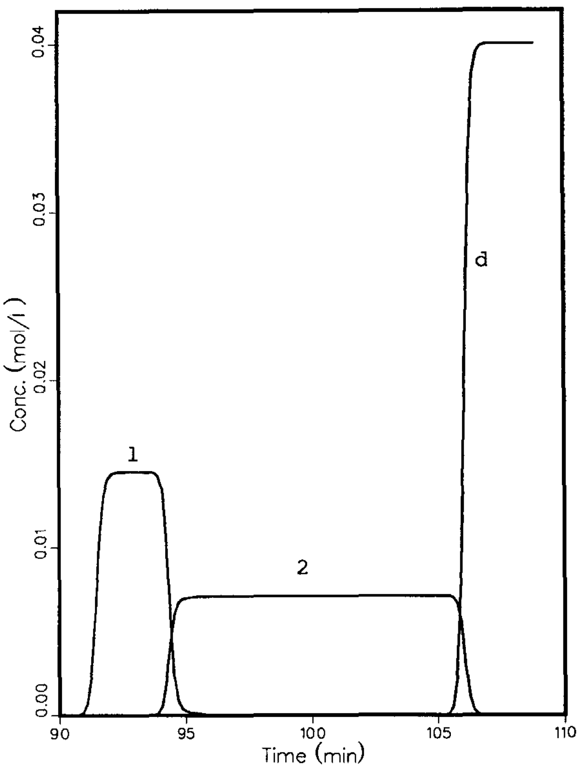
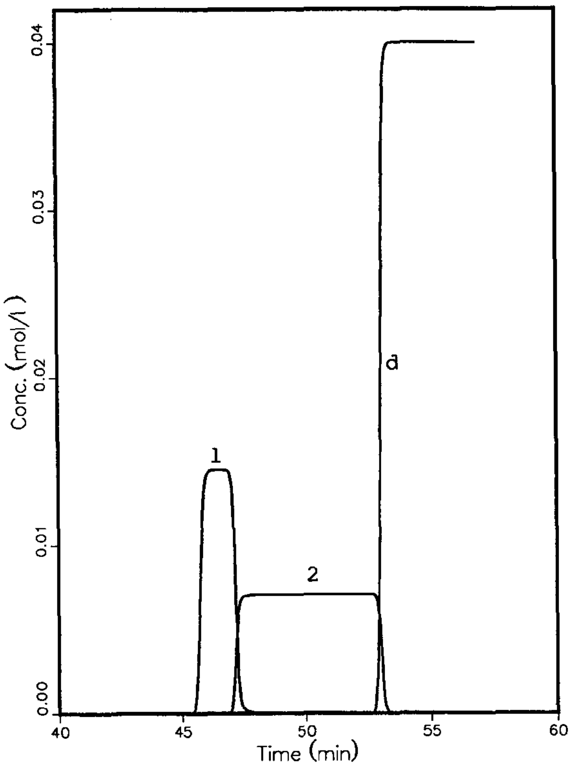


Fig. 5. Calculated zone profiles in displacement chromatography. Experimental conditions as for Fig. 4 (including sample size), except column length, L = 35 cm; hence $L_{f,1}$ = 0.007, $L_{f,2}$ = 0.057.

Fig. 6. Calculated zone profiles in displacement chromatography. Experimental conditions as for Fig. 4, except column length, L = 50 cm; hence $L_{f,1}$ = 0.005, $L_{f,2}$ = 0.04.



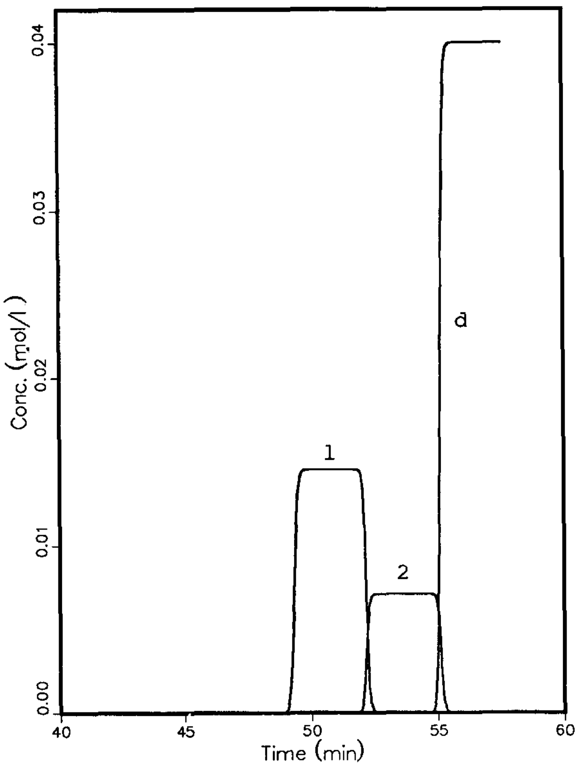


Fig. 7. Calculated zone profiles in displacement chromatography. Experimental conditions as for Fig. 4 (including column length and feed composition), except sample size, t_p = 125 s; hence $L_{f,1}$ = 0.005, $L_{f,2}$ = 0.04.

Fig. 8. Calculated zone profiles in displacement chromatography. Experimental conditions as for Fig. 4 (including injection time), except $C_{0,2}$ = 0.005 *M*; hence $L_{f,1}$ = 0.01, $L_{f,2}$ = 0.02.

This result is also in agreement with the prediction of the equations giving the heights of the zones in displacement chromatography [10,11].

Instead of decreasing the loading factor by using a longer column, we can decrease it by using a smaller sample size. We show in Fig. 7 the chromatogram obtained with a sample size half that in Fig. 4 and a column length of 25 cm; the loading factors for the two components are the same as in Fig. 6. In Fig. 8, the composition of the feed has been changed so that the amount of first component injected is the same as in Fig. 3 or 4, but the amount of second component injected is four times smaller. The corresponding loading factors for the second component are 4% for Fig. 7 and 2% for Fig. 8. The loading factors for the two components are now the same in Figs. 3 and 8. In both instances, the successful formation of an isotachic displacement train is observed, although in Fig. 3 the single-component isotherms do not intersect ($Q_{s,2}/Q_{s,1} = 1$) whereas in Fig. 8 they do ($Q_{s,2}/Q_{s,1} = 0.25$). As expected, the heights of the plateaux of the two components are the same in Figs. 6–8, as the displacer concentration is the same in all instances, but the lengths of the plateaux are two and four times shorter in Figs. 7 and 8 than in Fig. 6, respectively.

These theoretical results and also those derived in overloaded elution chromatography [8,9] are in contradiction with most experimental observations [1–3] whereas they agree well when the column saturation capacities are equal [5]. This suggests that the Langmuir competitive isotherm fails to predict correctly the equilibrium behavior of a binary mixture when the column saturation capacities of the two components are different. A more sophisticated model, such as that provided by the ideal adsorbed solution theory (IAS) [13], must be used. The IAS model predicts that the separation factor of the mixture (Q_2C_1/C_2Q_1) depends on the concentrations of the two components. As a consequence, very different results are obtained with this model which are in qualitative agreement with experimental observations [14,15].

CONCLUSION

The formation of an isotachic displacement train is possible, whether the single-component isotherms intersect or not, under the condition that the equilibrium behavior of the binary mixture studied is correctly accounted for by the competitive Langmuir model. This result completes the demonstration that in this instance single-component isotherm intersection is not a physical phenomenon but an illusion (similarly to optical illusions). The formation of an isotachic displacement train simply requires that the loading factor be lower than a critical threshold. This result can be achieved by adjusting properly either the column length or the sample size.

It should be emphasized that thermodynamic consistency requires that the column saturation capacities for two compounds whose binary competitive adsorption is accounted for by the Langmuir model should be the same. Otherwise, the Gibbs–Duhem equation is not satisfied [16]. Frey [17] has given an interesting demonstration of the thermodynamic impossibility of an isotachic displacement train when the two isotherms intersect and the concentrations exceed those corresponding to the intersection point. He showed that the free energy consumed at the hypothetical front between the two components (with the component more retained at low concentrations placed upstream, as predicted by the Langmuir model) would be negative. Finally, and importantly, the competitive Langmuir isotherm model does not give a very good representation of equilibrium data in most instances and this is especially true when the ratio of the column saturation capacities of the two compounds is significantly different from unity [7]. We consider this to be the main block at present to further progress in the understanding of the separation process in chromatography and in the derivation of accurate predictions of production rates and recovery yields.

Finally, all the conclusions of this work are valid only as long as the competitive Langmuir isotherm model represents correctly the phase equilibria involved. Serious deviations from this model resulting in single-component isotherms which intersect may also lead to situations where displacement would be impossible, even though the isotherms are still convex upwards. If we assume that the single-component isotherms follow the Langmuir behavior, the IAS theory predicts that the separation factor depends on the concentrations of both components when the column saturation capacities of the two components are different. It predicts a reversal of the elution order when the single-component isotherms intersect. Then the formation of an isotachic train may become impossible, at least in some range of experimental conditions.

ACKNOWLEDGEMENTS

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

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