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THEORETICAL STUDY OF MULTI-COMPONENT INTERFERENCES IN NON-LINEAR CHROMATOGRAPHY

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SUMMARY

The semi-ideal model of non-linear chromatography is used to calculate the elution band profiles of each component of various ternary mixtures. It is assumed that these compounds compete for interaction with the stationary phase following the competitive ternary Langmuir isotherm model. Calculated profiles are generated for a series of mixtures of variable relative compositions, using different sample sizes. The patterns obtained are discussed.

The calculated results are easily predicted in most instances from the combination of the displacement effect, the compression of an early eluting band by a later eluting band, and the "tag-along" effect, *i.e.*, the spreading of a band by the band which is eluted just before it. When the concentration of the second component of the ternary mixture is low, and those of the first and third components are high, the second component band is squeezed between the other two and its profile is most unusual.

INTRODUCTION

In previous papers we discussed on a theoretical basis the progressive separation between the two bands of a binary mixture during elution¹⁻⁶ and during displacement⁷ chromatography. These investigations permitted a better understanding of band interference in chromatographic columns under non-linear conditions, *i.e.*, when the concentrations of the sample components are large enough for their equilibrium isotherms between the two phases to be non-linear. The importance of the displacement of the first component band by the second band has been illustrated¹. This effect permits a considerable improvement in the production rate and recovery yield of the first-eluted component of a feed in preparative chromatography⁶. This theoretical prediction has been confirmed by various experimental results⁸⁻¹⁰. The displacement effect depends greatly on the relative concentrations of the two components of the binary mixture. It is especially strong when the first component is at a lower or much lower concentration than the second^{10,11}.

When the reverse is true, the displacement effect is small or negligible, but another effect, the "tag-along" effect, has been found^{1,2}. Because of the competition

for access to the stationary phase, the molecules of the first component crowd out those of the second component. The second component band tags along with the first and is spread over a wide volume of mobile phase. The result is a low production rate and a poor recovery yield at high column loadings^{6,12}.

Experimental results have confirmed the validity of both predictions, and observations of both effects have been reported^{8-10,13,14}. It has been noted in several instances^{9,14} that the displacement effect is stronger than predicted and, conversely, the tag-along effect weaker. In one instance at least, the opposite has been found¹³. This is not surprising, as the theoretical investigations were made using the simplest general equation for competitive isotherms, the Langmuir isotherm. This model assumes that the adsorbent surface is homogeneous, that the column saturation capacities of the two components are the same and that both the mobile phase solution of the sample components and the stationary phase are ideal, *i.e.*, that there are no molecular interactions between the retained components, which is a simplistic assumption. Compared with the assumption of linear chromatography, where there is no interaction between bands, *i.e.*, no competition for retention, the Langmuir competitive isotherm is a considerable improvement. It gives a good first approximation of the competitive isotherm behavior. Molecular interactions must be taken into account, however, to achieve a quantitative prediction of the band profiles of a multi-component mixture. Depending on the comparative strengths of the interactions of the molecules of each compound with those of the same compound and those of the other, deviations from the prediction of the Langmuir isotherm in one or the other direction may be expected.

In summary, the predictions of the ideal and semi-ideal models of chromatography for the elution profiles of the bands of a pure compound pulse^{15,16} or of a binary mixture^{1,17} are sufficiently accurate to predict the phenomena associated with band interference and progressive band separation in chromatography. However, they cannot give accurate elution profiles unless, of course, the exact isotherms are known.

All the previous work referred to here relates to pure compound band profiles or to the separation of binary mixtures. In most practical situations, however, real mixtures are not binary but contain a larger number of components. It is useful at this stage to investigate the separation of multi-component mixtures on a purely theoretical basis, in order to search for possible new effects that could not be accounted for on a straightforward basis by simple combinations of the displacement and tag-along effects taking place between each pair of the components involved.

This paper discusses results obtained using the semi-ideal model^{1,16} applied to a ternary mixture. Ternary Langmuir competitive isotherms have been used, assuming the same column capacity for the three components. The relative retentions of the two successive pairs of components (1-2 and 2-3) have been varied, in addition to the total sample size, expressed as the loading factor, *i.e.*, the fraction of the column saturation capacity. The composition of the mixtures investigated covers a wide range of relative compositions, including all the possible combinations where a component is either major or minor.

THEORY

The model used for the simulation of the elution and separation of the

components of a ternary mixture is the semi-ideal model applied previously to the simulation of the band profiles for a pure compound^{1,6} and for a binary mixture¹⁻⁶ on an overloaded column. It is based on the ideal model of chromatography¹⁸⁻²². The ideal model assumes the column efficiency to be infinite and focuses attention on the phenomena that arise because of the non-linear behavior of the equilibrium isotherms at high concentrations and on the competitive interactions between the components of a mixture. This model stems from the properties of the equation system of chromatography.

The differential mass balance equation for a single compound in a slice of a chromatographic column can be written as

$$\frac{\partial C_m}{\partial t} + F \cdot \frac{\partial C_s}{\partial t} + \frac{\partial(uC_m)}{\partial z} = D \cdot \frac{\partial^2 C_m}{\partial z^2} \quad (1)$$

where C_m and C_s are the concentrations of the compound considered in the mobile and stationary phases, respectively, at time t and abscissa along the column, F is the phase ratio of the column packing, with $F = (1 - \epsilon)/\epsilon$, ϵ being the column packing porosity, u is the mobile phase velocity and D is the coefficient of axial dispersion.

In liquid chromatography, the compressibility of the mobile phase is negligible and the partial molar volumes of the compounds investigated in the mobile and the stationary phases are nearly the same. Accordingly, the mobile phase velocity, u , is constant and can be taken out of the differential operator.

Integration of eqn. 1 requires a relationship between C_m and C_s . Giddings²³ has shown that, provided the column efficiency exceeds a few hundred theoretical plates, which is the general case in modern liquid chromatography, we can take for C_s the value corresponding to thermodynamic equilibrium between the two phases, *i.e.*, the value given by the isotherm:

$$C_s = \partial(C_m) \quad (2)$$

Having made this simplifying assumption, the axial dispersion coefficient in eqn. 1 is replaced by an apparent dispersion coefficient that accounts for the finite column efficiency, *i.e.*, for the deviation from the thermodynamic equilibrium resulting from the finite rate of the radial mass transfer²³. However, it is not possible to find an exact analytical solution following this replacement and it is extremely difficult to write the appropriate computer programs for its calculation.

The task becomes much easier if we assume that the column has an infinite efficiency, making the apparent dispersion coefficient zero. Further, it can then be shown that the computation process itself introduces errors that are equivalent to the addition of a dispersion term (*i.e.*, $k\partial^2 C/\partial z^2$)^{24,25}.

Thus, we obtain the following equation, which constitutes the ideal model for the ternary mixture:

$$\left(1 + F \cdot \frac{dC_{s,i}}{dC_{m,i}}\right) \frac{\partial C_{m,i}}{\partial t} + u \cdot \frac{\partial C_{m,i}}{\partial z} = 0 \quad (3)$$

where dC_s/dC_m is the differential of the equilibrium isotherm and $i = 1, 2$ and 3 represents the three components of the ternary mixture. Numerical solutions of eqn. 3 can easily be calculated if the numerical value of each parameter is previously known¹⁶. As already described²⁶, a finite difference method, using the Godunov algorithm, is especially suitable for this calculation^{27,28}. We take the column efficiency into account by choosing the following values for the space and time integration increments:

$$dz = H \quad (4)$$

and

$$dt = 2H/u_{z,0} \quad (5)$$

where $u_{z,0}$ is the velocity associated with an infinitely small concentration of the compound considered [$u_{z,0} = u_0/(1 + k'_0)$, where k'_0 is the column capacity factor of the compound under analytical, *i.e.*, linear, conditions]. We also need a set of ternary competitive isotherms and a value for $u_{z,0}$, in order to select these integration increments (see the next section).

EXPERIMENTAL

The computations were carried out using ternary competitive Langmuir isotherms:

$$C_{s,i} = \frac{a_i C_{m,i}}{1 + b_1 C_{m,1} + b_2 C_{m,2} + b_3 C_{m,3}} \quad (6)$$

with $i = 1-3$. The numerical values of the six coefficients selected for most of the calculations are given in Table I. The parameters of the three individual isotherms were selected so that the column saturation capacity was the same for the three compounds ($q_i = a_i/b_i = 1.60$ for all values of i). The k' value for the third component at infinite dilution was $a_3 F = 4.0$. In most instances, the two values of the selectivity of the stationary phase (*i.e.*, $\alpha_{1,2} = a_2/a_1$ and $\alpha_{2,3} = a_3/a_2$) were equal to 1.10. In a few cases, a value of 1.4 was used instead.

TABLE I
COEFFICIENTS OF THE TERNARY COMPETITIVE ISOTHERMS

Selectivity	Parameter ($i=1, 2, 3$)	Component 1	Component 2	Component 3
$\alpha_{1,2} = \alpha_{2,3} = 1.10$	k'_i	3.31	3.64	4.00
	aa_i	13.24	14.56	16.00
	b_i (l/mol)	2.07	2.27	2.50
$\alpha_{1,2} = \alpha_{2,3} = 1.40$	k'_i	1.97	2.85	4.00
	a_i	7.88	11.40	16.00
	b_i (l/mol)	1.27	1.78	2.50

The column calculated is 25 cm long, its phase ratio is 0.25 and the flow velocity ($u_0 = 0.125$ cm/s) corresponds to a dead time of 200 s. The column efficiency is 12 500 theoretical plates for the second component (*i.e.*, height equivalent to a theoretical plate = 0.0020 cm), except when the influence of the column efficiency on the separation between the three bands is considered. The values of the integration increments were calculated from the data selected for this component. Accordingly, the column efficiencies for the other two compounds are 11 250 and 13 750 theoretical plates, respectively²⁸. Eqns. 4 and 5 show that, as dt and dz are constant during the integration, the simulated column efficiency is different for the three components^{24,28}.

The sample size is given as the total loading factor, calculated for the whole sample, as the column saturation capacity is the same for all components. In most instances the loading factor used was either 10% or 40%. Values of 5%, 20% and 60% have also been used, and some results are reported. The relative composition is given in fraction of the sample size. An x - y - z mixture means that the concentrations of the three components in that mixture are as x to y to z , *e.g.*, the relative concentration of the second component in the sample is $y/(x + y + z)$.

RESULTS AND DISCUSSION

The results reported were obtained exclusively with the parameters of the ternary isotherms given in Table I. Therefore, only two values of the selectivity are discussed here, 1.10 and 1.40. The value of 1.10 provides strong band interactions for low sample loadings. Compared with the larger selectivity value of 1.40, which requires higher loadings to induce similar band interactions, it permits the investigation of the non-linear effects associated with strong band interactions when using relatively small sample sizes. The use of a low selectivity reduces the relative importance of the displacement effect and increases that of the tag-along effect⁴.

Other calculations, not reported here, were performed using values of the selectivity for either pair of components or for both equal to 1.15, 1.30 and 1.50. No qualitative differences were observed for the chromatograms generated. The degree of column overload and the band interference pattern obtained with a selectivity of 1.15 and a total sample size of 20% of the column saturation capacity were nearly identical with those observed with a selectivity of 1.10 and a loading factor of 10% on the chromatograms presented here. When the selectivities were unsymmetrical (*e.g.*, $a_{1,2} = 1.10$ and $a_{2,3} = 1.30$), the result was the coupling of a strong interaction between one pair of bands, a weak interaction between the other pair and nearly none between the first and the third compounds. In other words, the degree and type of band interaction can be simply predicted from what we know about binary mixtures whether the selectivities are symmetrical or unsymmetrical.

Changes in the sample size provide an easy adjustment of the degree of interference between bands. Compared with a single component band or even a binary mixture, the sample band system of a ternary mixture is spread over a longer section of the column, resulting in a less stationary phase overloading. At a loading of 5% for a mixture of three components, typically used in preparative liquid chromatographic applications, adjacent compounds competed only minimally. Much more interesting results were obtained for loadings of 10%, the most often discussed in this work, or 20%. Under the grossly overloaded conditions corresponding to loading factors of 40% or 60%, even the first component interacted with the third.

The main parameter investigated, that having the strongest influence on the profiles of the three component bands, was the relative composition of the mixture. From this point of view, we can separate our results into three categories: (i) those corresponding to mixtures where the three components are present in equal amounts, (ii) those corresponding to mixtures where one of the three components dominates in the presence of the other two and (iii) those obtained for mixtures where the second component is at low concentration compared with the other two and its band is squeezed between two major component bands. The last series of results are the only ones which were really unexpected.

The displacement effect and the tag-along effect are illustrated in Figs. 1 and 2, respectively. They will be referred to often in the following discussion, as most band systems for ternary mixtures exhibit features which are combinations of displacement (Fig. 1) and tag-along (Fig. 2) effects.

Mixtures with equivalent concentrations of the three components

Chromatograms calculated for samples of increasing size from 5% to 40% are shown in Figs. 3–6. Fig. 7 shows, for comparison, the chromatogram obtained for a 10% loading factor, assuming that there is no competition between the three components. This is the superimposition of the three bands obtained by successively injecting samples of each of the three compounds pure, with a loading factor of 3.33%. The band profiles in Fig. 7 are typical of those associated with a Langmuir isotherm under that degree of overloading.

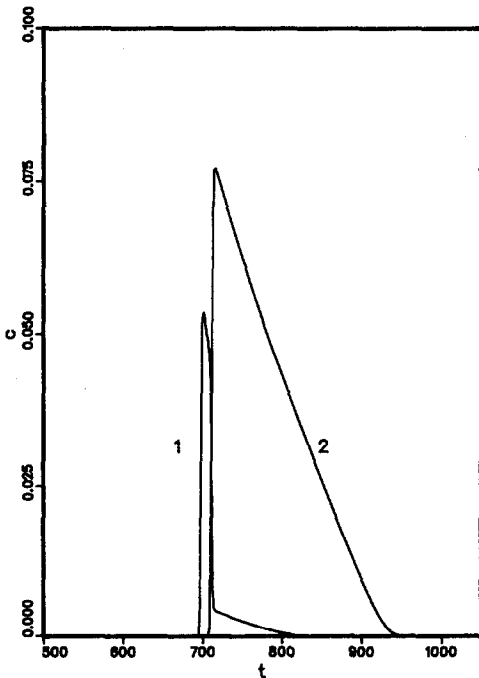


Fig. 1. Chromatogram calculated for a 1:9 binary mixture (concentration, c , in M versus, time, t , in s). Total loading factor: 10%. Isotherms, see eqn. 6. For isotherm coefficients, see Table I with $\alpha = 1.10$. First and second components only.

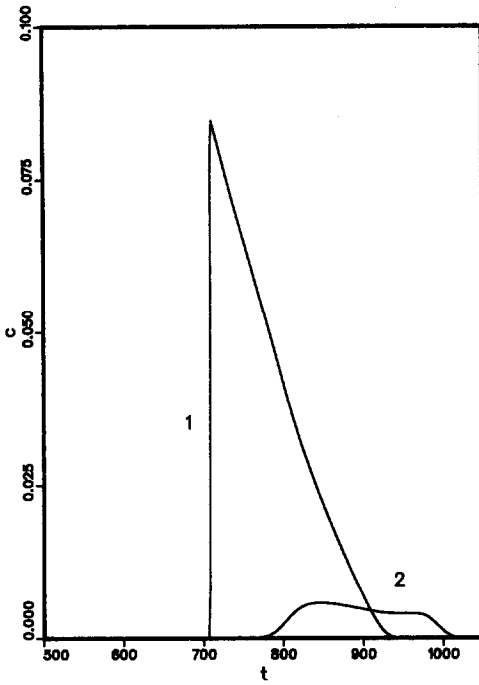


Fig. 2. As in Fig. 1, but for a 9:1 mixture. Total loading factor: 10%. Second and third components only.

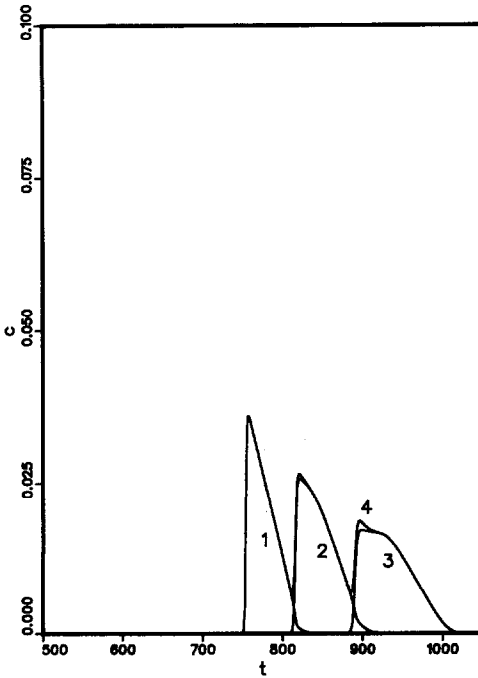


Fig. 3. Chromatogram calculated for a 1:1:1 mixture. Total loading factor: 5%. Isotherms, see eqn. 6. For isotherm coefficients, see Table I with $\alpha = 1.10$. Column characteristics, see Experimental. The number on each profile is the rank of the component; 4 is for the total concentration profile, as recorded by an ideal, non-selective detector.

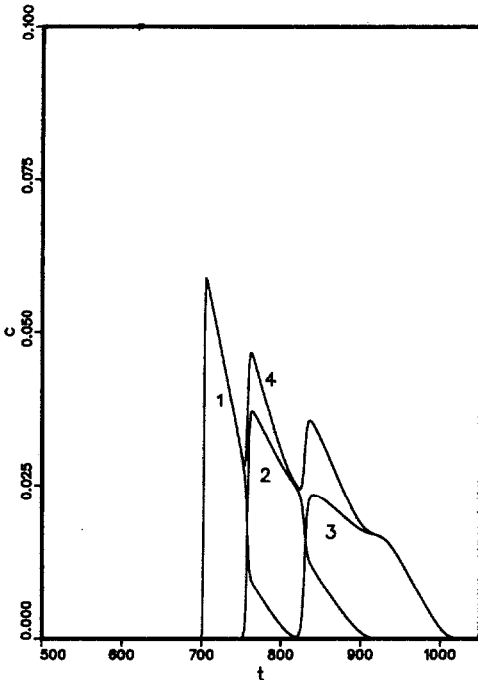


Fig. 4. Chromatogram calculated for a 1:1:1 mixture as in Fig. 3, except total loading factor: 10%.

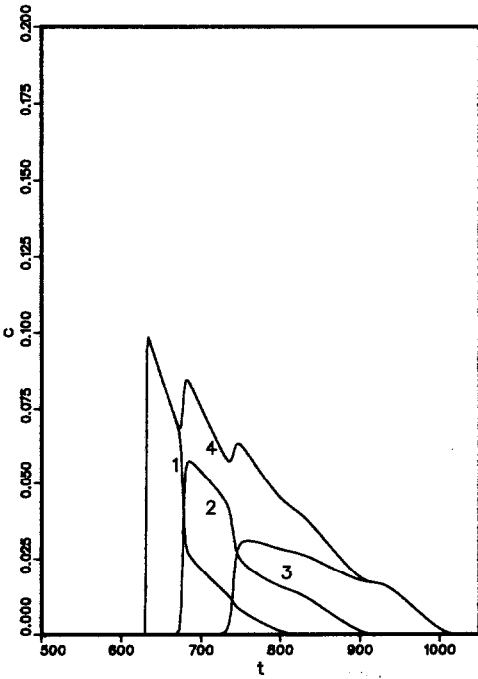


Fig. 5. Chromatogram calculated for a 1:1:1 mixture as in Fig. 3, except total loading factor: 20%.

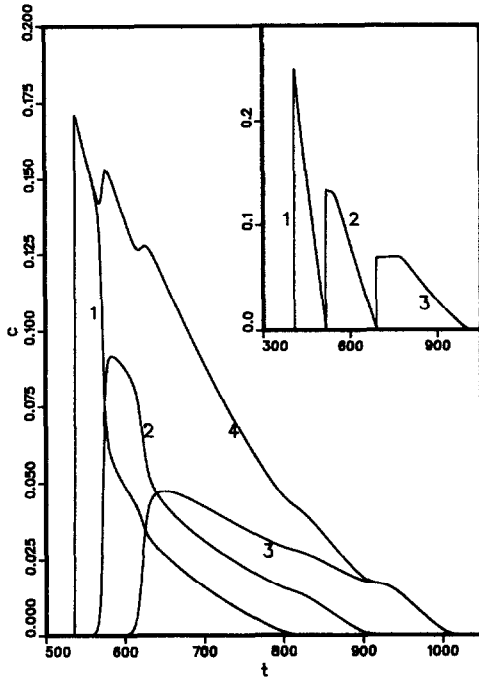


Fig. 6. Chromatogram calculated for a 1:1:1 mixture as in Fig. 3, except total loading factor: 40%. Inset: chromatogram calculated for a 1:1:1 mixture with total loading factor: 40%. Isotherm coefficients as in Table I with $\alpha = 1.40$. Column characteristics, see Experimental.

Compared with Fig. 7, where no interaction takes place between the compounds (because pure compound adsorption isotherms are used), the chromatograms in Figs. 3-6 show moderate to considerable band interaction, depending on the sample size. In Fig. 3, there is a slight displacement effect of the first component band by the second and of the second component band by the third. This is demonstrated essentially by the earlier time at which the elution of these bands is completed and by the inflection points on the back of their profiles, although the rear shock layers are barely discernible. Similarly, a modest tag-along effect is exhibited by the second band and a slightly stronger one by the third band.

In Fig. 4, corresponding to a 10% loading factor, the two effects are stronger. The front shock layers of the three bands are eluted much earlier than in Fig. 7, obtained with the same sample size for each component. The time gain decreases, however, from the first to the second and to the third component. A shock layer is clearly noticed on the rear of the first two bands. These shock layers are followed by slight tails, as the bands are not completely separated. This demonstrates the presence of the displacement effect. The tag-along effect is shown by the wider profiles of the second and third bands, the marked inflection point on the rear of these last two band profiles, with a nearly horizontal tangent for the third band. While the band of the second component simultaneously experiences a displacement by the third band and tags along with the first band, the other two bands are affected only by one effect each, displacement for the first one and tag-along for the third.

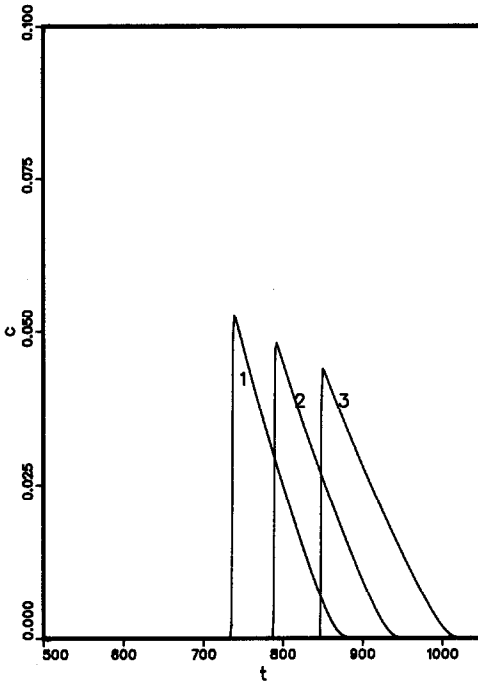


Fig. 7. Chromatogram calculated for a 1:1:1 mixture as in Fig. 4, except the components are not supposed to interact. Alternatively, superimposition of the chromatograms obtained successively for the three pure compounds, with a loading factor of 3.33% each.

In Figs. 5 and 6, which correspond to larger values of the loading factors, the band of the first component experiences a double displacement effect, while the third component band exhibits a double tag-along effect, the column being so overloaded that the first and third component bands interfere. This phenomenon becomes even stronger in Fig. 6, where the width of the third band profile reaches 400 s.

In the inset in Fig. 6, $a_{1,2} = a_{2,3} = 1.40$ (instead of 1.10, Fig. 6) and a column loading of 40% (instead of 10%, Fig. 6) were used. The three bands are resolved, but the second and third bands still show the effects of previous interactions with adjacent components. The third band plateau would shrink and disappear if it were allowed to migrate further. The profile of the second band can be considered as an intermediate stage in the recovery of the third band to its typical triangular shape.

If we compare Figs. 3–6, we see that the first part of the first band, that corresponding to the elution of the pure first component, becomes narrower with increasing sample size, while the recovery yield of the pure product decreases. The width of the third component band increases considerably, but the size corresponding to the last zone of the chromatogram, when the last component is eluted pure, does not change significantly. This confirms earlier results⁶ that, because of the displacement effect, the production rate of the first component increases with increasing sample size until well into the range of severe band interference. In contrast, the production rate for the last component increases with increasing sample size only until its band begins to interfere with the previous band. Above that sample size, the production rate

remains constant, while the recovery yield decreases²⁹. A similar conclusion is reached for the second component¹². The production rate of the second component is certainly lower under the conditions in Fig. 4 than under those in Fig. 3. It seems to be maximum for a sample load of about 5%, corresponding to Fig. 3.

Mixtures in which one component predominates

In this group, we examined five mixtures, three for which one compound is in large excess, corresponding to concentration distributions of 9:1:1, 1:9:1 and 1:1:9, and two mixtures for which the first or third component is minor, 9:9:1 and 1:9:9. The profile of the last possible mixture in this series, 9:1:9, is discussed in the next section.

Fig. 8 shows the chromatogram calculated for a mixture containing a large excess of the first component (9:1:1). The strong tag-along effect of the first component band on the last two is obvious. It is expectedly stronger on the second band than on the third as the degree of interaction is lower and the time during which the two bands interact in the column is shorter. In fact, the third component band is in the process of recovering from the deformation caused by the tag-along effect that it underwent during the first part of its elution. Simultaneously, there is a very weak displacement of the second band by the third, which can be recognized by the slightly shorter elution time of the band end in Fig. 8 compared with Fig. 7 (note that for a pure compound with a Langmuir isotherm, the time at which the band ends does not depend on the sample size).

In the inset in Fig. 8 (calculated under the same conditions as the inset in Fig. 6),

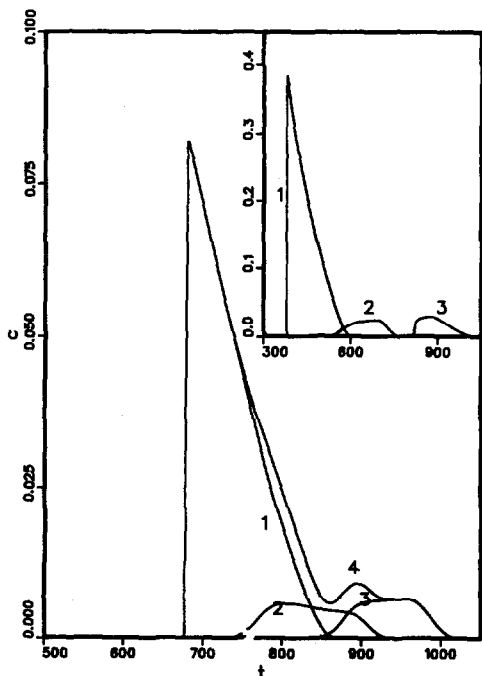


Fig. 8. Chromatogram calculated for a 9:1:1 mixture. Total loading factor: 10%. Conditions as in Fig. 3. Inset: chromatogram calculated for a 9:1:1 mixture with conditions as in Fig. 6, inset.

the second band tags along with the first and the third band, although separated, has not fully recovered from the tag-along effect. The shock front is slowly reforming as the tag-along plateau decays. Eventually, this third band profile will regain its typical triangular shape expected for a pure compound, under overloaded conditions.

Fig. 9 shows the chromatogram corresponding to the mixture having a large excess of the second component (1:9:1). The second component band displaces the first band strongly, while forcing the third to tag along. The first and third component bands do not interact significantly. Nevertheless, it would not be possible to prepare much pure second component under the conditions selected for Fig. 9. The first part of the second band contains about 40% of the amount of the first component injected, while the second part contains nearly 60% of the amount of the third component contained in the sample.

In the inset in Fig. 9 (calculated under the same conditions as for the inset in Fig. 6), no interaction takes place between the first and the third components, which are effectively shielded by the second. We observe merely the sum of two two-component problems. Comparing Fig. 9 and its inset, we see a stronger displacement effect and a weaker tag-along effect in the inset, as expected because of larger values of the selectivity and the loading factor⁴.

Fig. 10a shows the chromatogram calculated with a mixture containing an excess of the third component (1:1:9). In this instance, two strong displacement effects are observed. The third component band displaces the second and in so doing forces it to displace the first component band. Under the conditions selected, with a small loading

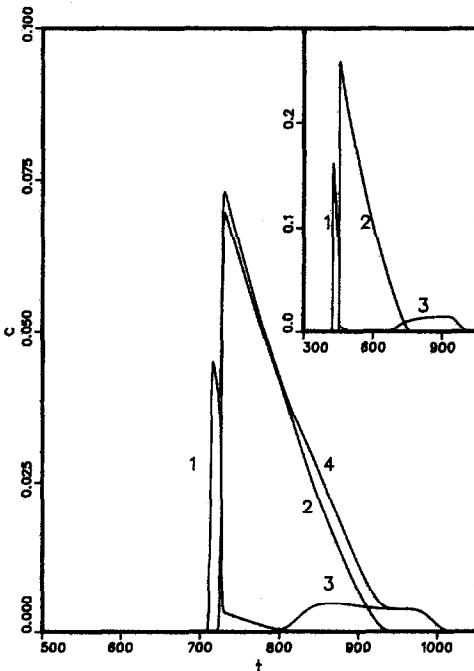


Fig. 9. Chromatogram calculated for a 1:9:1 mixture. Total loading factor: 10%. Conditions as in Fig. 3. Inset: chromatogram calculated for a 1:9:1 mixture with conditions as in Fig. 6, inset.

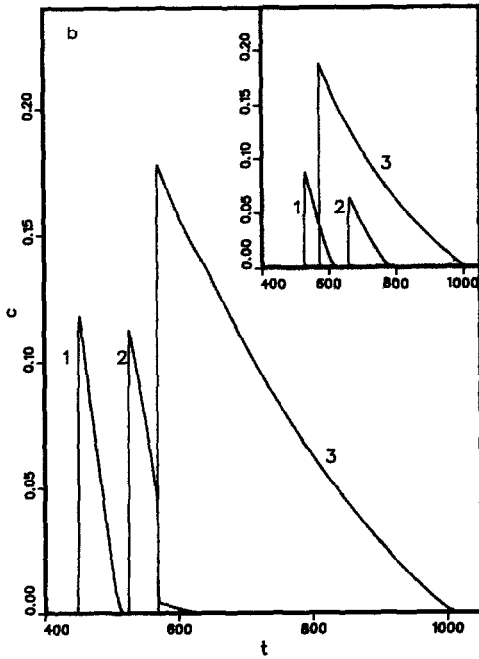
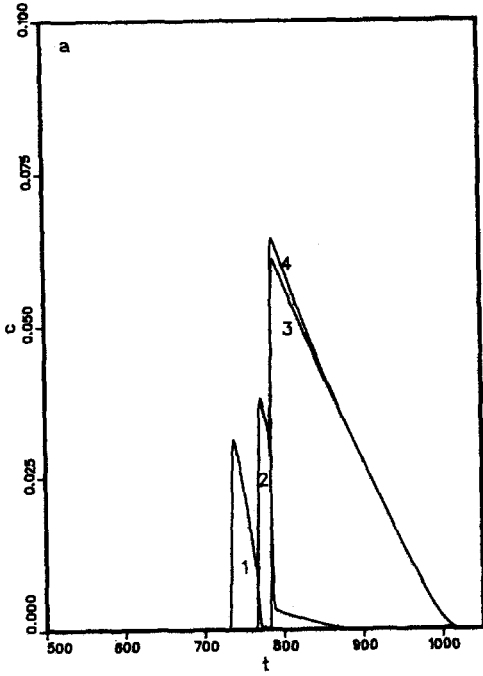


Fig. 10. Chromatograms calculated for a 1:1:9 mixture. (a) Total loading factor: 10%. Conditions as in Fig. 3. (b) Same conditions as in Fig. 6, inset (selectivity: 1.4). Inset: same conditions, except without component-component interaction (similar to Fig. 7).

factor for the first two components (0.9%), there would be no displacement of the first band by the second in the absence of a large excess of the third component (see Fig. 3). The second component has the classical L-shape profile of a band strongly displaced by a more retained compound, when the selectivity of the column is low or moderate. The displacement of the first band, however, is easier to observe by comparing the retention time of that band and its width in Figs. 3 and 10 than by visually comparing its profiles in the two figures.

In Fig. 10b, the selectivity has been increased ($a_{ij} = 1.4$) together with the total loading factor (40%). Comparing this figure with its inset under the same conditions except that the calculation was carried out assuming there are no component-component interactions, the displacement effect of the first two bands by the last is markedly evident. Although the first component appears unaffected in its profile, it is both narrower and eluted earlier after having been displaced strongly by the third component and mildly by the second component. The second component also suffers from the displacement effect, but although present in the same amount as the first component, no tag-along is seen between the first and second components. The displacement effect, therefore, overpowers the tag-along effect under these conditions.

The double displacement effect is illustrated for a very strong column overload in Figs. 11 and 12. Fig. 11 shows the chromatogram calculated for a 40% loading factor with a 1:1:18 mixture and Fig. 12 shows an enlargement of the front parts of the elution profiles of the two minor compounds. These chromatograms are striking. The chromatogram exhibits some similarity to those obtained in displacement chromatography during the formation of the isotachic displacement train⁷. They show that

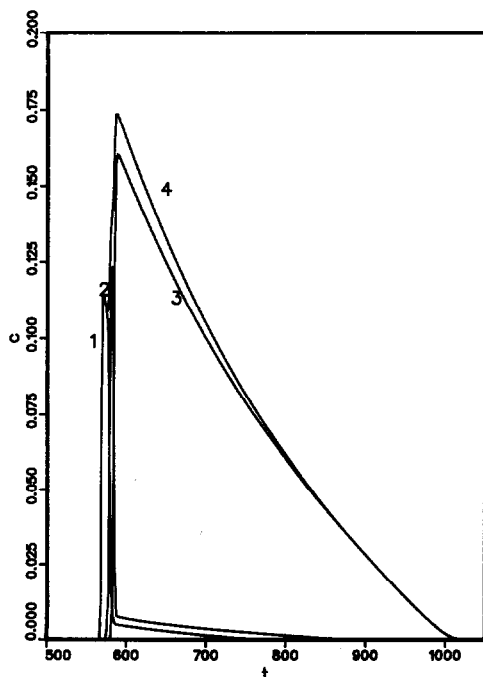


Fig. 11. Chromatogram calculated for a 1:1:18 mixture. Total loading factor: 40%. Conditions as in Fig. 3.

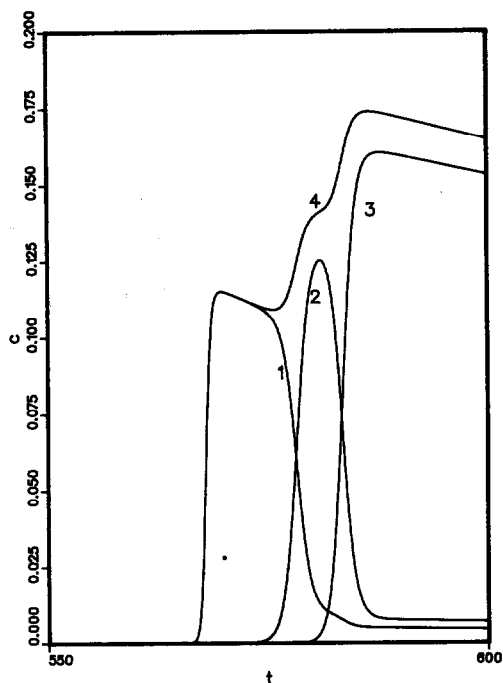


Fig. 12. Chromatogram calculated for a 1:1:18 mixture. Total loading factor: 40%. Enlargement of the front of the chromatogram in Fig. 11.

a large fraction of the impurities of a pure compound can be recovered at the front of the main component band (see Fig. 12). Unfortunately, the recovery yields would not exceed about 70% for the first component and 60% for the second. The maximum concentration of these impurity bands, however, is of the order of that of the main component. This phenomenon can be used in combined liquid chromatography-mass spectrometry for the easier identification of impurities or for the extraction of small amounts of them for further experiments³⁰. It cannot be used for the purification of the main component, as we have already said, or for the total recovery of these compounds.

In Figs. 8-12, as in Figs. 1 and 2, the major component causes a total change in the shape of the elution band of the minor component(s), but its band profile is essentially unaffected. Next, we shall consider the opposite situation where the interference between the bands of the major compounds controls the shape of the chromatogram.

Fig. 13 shows the chromatogram calculated for a mixture where the third component is minor (9:9:1). The chromatogram for the first two components is very much like that corresponding to an overloaded band of a 1:1 binary mixture. The second band displaces the first and at the same time tags along with it. These two effects are easily recognized. The third band tags along with the other two and is spread over a wide retention time range (nearly 200 s). The elution profiles of the first two bands of the 9:9:1 mixture in Fig. 13 are very similar to the elution profiles of the last

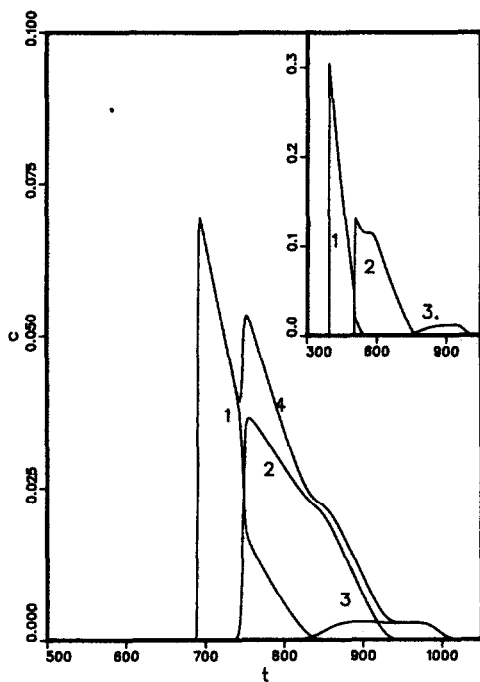


Fig. 13. Chromatogram calculated for a 9:9:1 mixture. Total loading factor: 10%. Conditions as in Fig. 3. Inset: chromatogram calculated for a 9:9:1 mixture with conditions as in Fig. 6, inset.

two bands of the 1:9:9 mixture in Fig. 14. These two bands interact in exactly the same way, the third band displacing the second and tagging along with it at the same time. The difference between the chromatograms in Figs. 13 and 14 is that in the former instance the third band tags along with the other two, whereas in the latter instance the first band is displaced by the system of the other two. However, the displacement effect in Fig. 14 is not as strong as with the 1:9:1 mixture (Fig. 9). In part this is because the actual amount of the second component injected with the sample is markedly smaller in Fig. 14 than in Fig. 9.

Similarly, the same phenomena are observed in the insets in Figs. 13 and 14 as for the respective main figures, except that they take place with larger values of the selectivity and the loading factor in the insets. The interferences between bands are much reduced in the insets, in spite of the higher loading factors. This confirms that both the recovery yield and the production rate of purified individual components are greatly enhanced by an increase in the selectivity^{12,31}.

Mixtures in which the second component is minor

With a ternary mixture in which the intermediate component is a minor one, it simultaneously undergoes a strong displacement effect from the third component band which pushes it forward, and a strong tag-along effect from the first band which pulls it ahead. The combination of these two effects results in very strong squeezing of the band, which acquires an unexpected elution profile (see Figs. 15–18). The squeezing effect results from the superimposition of strong displacement and tag-along effects.

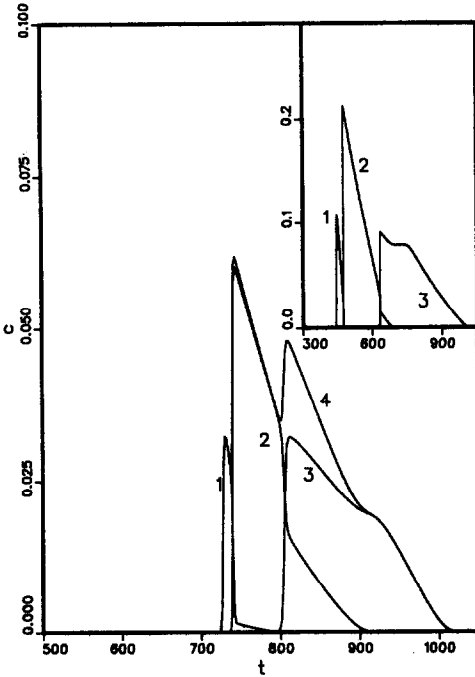


Fig. 14. Chromatogram calculated for a 1:9:9 mixture. Total loading factor: 10%. Conditions as in Fig. 3. Inset: chromatogram calculated for a 1:9:9 mixture with conditions as in Fig. 6, inset.

This causes a much greater change in the band profiles than any other effect or combination of effects observed so far in the investigation of multi-component mixtures.

In Fig. 15a, the column loading factor (10%) is moderate and the first and third bands are well resolved. The second component band is sandwiched between them. If Fig. 15 is compared with Fig. 7, however, it is clear that the first band has been displaced (the retention time of the front is reduced by about 40 s) and the third component band has undergone a slight tag-along effect from which it is beginning to recover. This perturbation is caused by the elution of the second, minor band squeezed between these two major bands.

This squeezing is maximized with increasing efficiency in Fig. 15b (column loading factor 5%). Whereas no pure second component can be recovered with an efficiency of only 1500 theoretical plates as the efficiency improves the second band undergoes a stronger displacement effect from the third component and a weaker tag-along effect from the first component. With an efficiency of 12 500 plates very pure fractions of the individual components can be collected with a high recovery yield. In this instance at least, increasing the column efficiency certainly increases markedly the production rate of fractions of constant purity.

In Fig. 15c, the column loading factor (60%) is high and $a_{1,2} = a_{2,3} = 1.4$. The high loadings were necessary in order to force competitive interactions between adjacent components. The inset in Fig. 15c has a column loading factor (40%) under

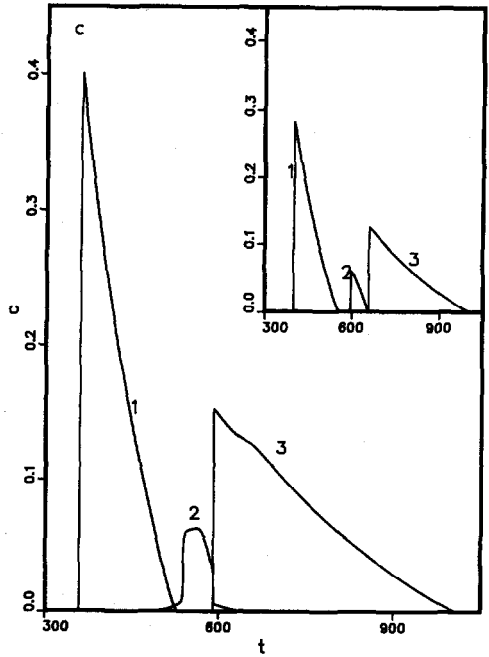
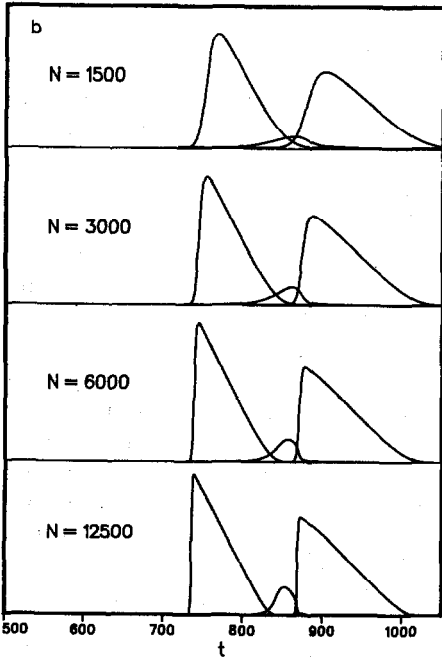
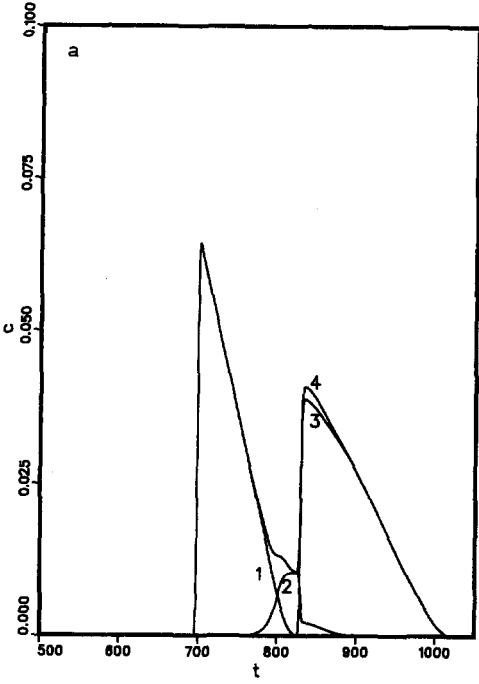


Fig. 15. Chromatograms calculated for a 9:1:9 mixture. (a) Total loading factor: 10%. Conditions as in Fig. 3. (b) Total loading factor: 5%. Conditions as in Fig. 3, except number of theoretical plates, N . (c) Total loading factor: 60%. Conditions as in Fig. 6, inset. Inset: same except total loading factor: 40%.

the same conditions as in Fig. 15c. Only subtle effects are seen in the inset under heavily overloaded conditions. The second profile shows a slight bowing of its rear side. In Fig. 15c under more overloaded conditions, the profiles are very similar to those in Fig. 15a. In fact, the separation is better in Fig. 15c than in Fig. 15a even though in Fig. 15c the column loading factor is six times that in Fig. 15a, 60% vs. 10%. The significant difference comes from the selectivity, 1.4 in Fig. 15c and 1.1 in Fig. 15a.

Clearly, if the sample size is increased from 10% to 20% (with a selectivity of 1.1) (Fig. 16), the first and last bands of the chromatogram will interfere strongly. The first is displaced by the second, which in turn tags along with the first. The second band is squeezed between the other two bands and has a small, but not entirely negligible, effect on the overall profile. The second band causes a very slight displacement of the first band and a weak tag-along effect, seen as a very small hump on the rear of its profile.

Schematically, the second component band looks like a half-Gaussian profile with a rear shock followed by a very long tail. This tail, in turn, looks also like a half-Gaussian profile, but one that is much shorter than the first and much wider. Figs. 17 and 18 show the progressive changes in the profile of the second component band when samples of constant size of an equimolar binary mixture of the first and third components are injected and the mixture contains decreasing concentrations of an impurity eluted between the two main components. The separation of samples whose sizes amount to 10% (Fig. 17) and 20% (Fig. 18) of the column saturation capacity, respectively, and relative compositions of 9:1:9, 25:1:25, 50:1:50 and 100:1:100 were calculated. For the sake of clarity, only the profiles of the second band are shown.

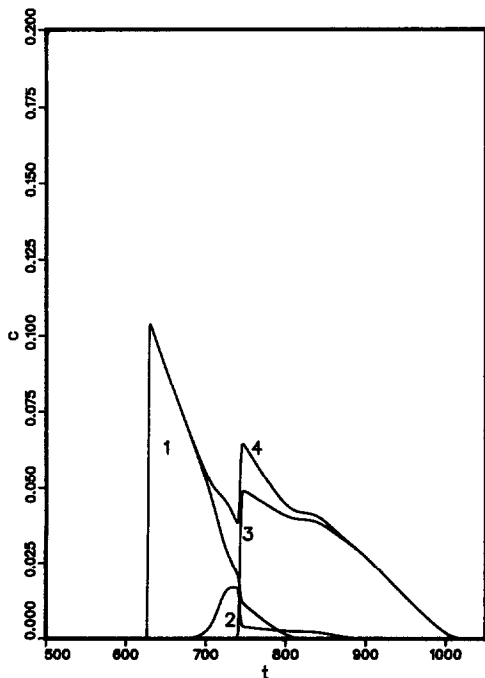


Fig. 16. Chromatogram calculated for a 9:1:9 mixture. Total loading factor: 20%. Conditions as in Fig. 3.

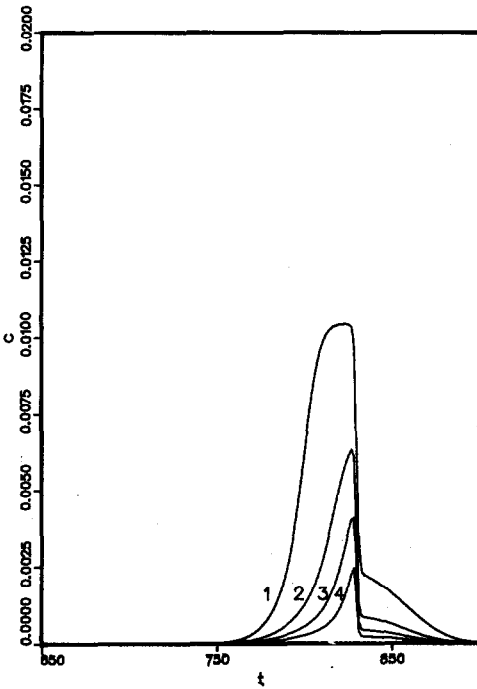


Fig. 17. Chromatogram calculated for the minor second component of a ternary mixture. Conditions as in Fig. 3, except total loading factor: 10%, and mixture composition: (1) 9:1:9; (2) 25:1:25; (3) 50:1:50; (4) 100:1:100.

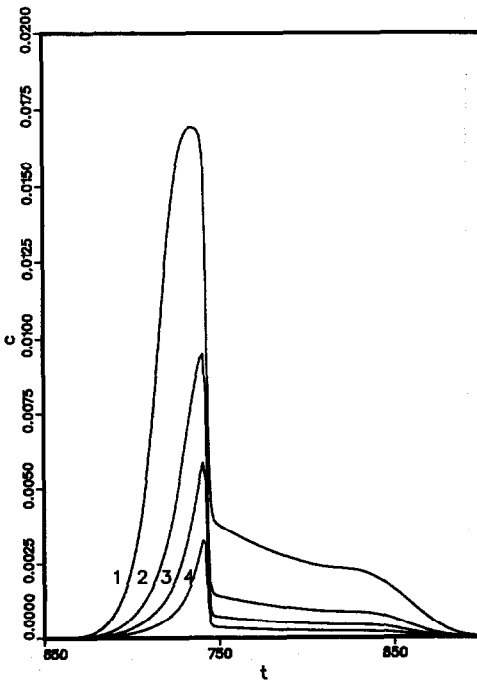


Fig. 18. Same as Fig. 17, except total loading factor: 20%.

The top part of the second component profile (Figs. 17 and 18) becomes sharper with decreasing amount of this component in the sample, but the relative importance of the tail does not change much.

CONCLUSION

This theoretical investigation has shown few unexpected results. On a qualitative basis, almost all the results obtained could have been understood in terms of combinations between the displacement and tag-along effects already discussed. Only with a minor component squeezed between two major components is the band profile intricate enough to be nearly impossible to predict or explain without the help of computer calculations based on the use of the semi-ideal model.

Experimental verifications of computer-predicted profiles for binary mixtures have already shown that both displacement and tag-along effects are observed¹³. Significant deviations from the predictions made on the basis of a competitive Langmuir isotherm model take place, however. Depending on the system studied, these deviations result from one of the effects, displacement or tag-along, being stronger and the other being weaker than predicted^{13,14}. The competitive Langmuir isotherms derived by introducing in eqn. 6 the numerical values of the coefficients measured for the single compound isotherms of the mixture components remains an excellent first-order approximation for the calculation of individual band profiles.

Accordingly, the chromatograms presented give a good description of the type of band interference patterns that can be expected in practical preparative chromatography when columns are overloaded with large samples of multi-component mixtures for the achievement of high production rates.

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REFERENCES

- 1 G. Guiochon and S. Ghodbane, *J. Phys. Chem.*, 92 (1988) 3682.
- 2 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 440 (1988) 9.
- 3 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 444 (1988) 275.
- 4 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 450 (1988) 27.
- 5 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 452 (1988) 209.
- 6 S. Ghodbane and G. Guiochon, *Chromatographia*, 24 (1989) 53.
- 7 A. M. Katti and G. Guiochon, *J. Chromatogr.*, 449 (1988) 25.
- 8 J. Newburger, L. Liebes, H. Colin and G. Guiochon, *Sep. Sci. Technol.*, 22 (1987) 19.
- 9 J. Newburger and G. Guiochon, *J. Chromatogr.*, 484 (1989) 153.
- 10 J. Perry, presented at the 6th Symposium on Preparative Chromatography, Washington, DC, May 8-10, 1989.
- 11 G. Guiochon, S. Ghodbane, S. Golshan-Shirazi, J. X. Huang, A. Katti, B. C. Lin and Z. Ma, *Talanta*, 36 (1989) 19.
- 12 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 61 (1989) 1368.
- 13 A. M. Katti and G. Guiochon, *J. Chromatogr.*, 499 (1990) 21.
- 14 S. Golshan-Shirazi and G. Guiochon, in preparation.

- 15 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 60 (1988) 2364.
- 16 G. Guiochon, S. Golshan-Shirazi and A. Jaulmes, *Anal. Chem.*, 60 (1988) 1856.
- 17 S. Golshan-Shirazi and G. Guiochon, *J. Phys. Chem.*, 93 (1989) 4341.
- 18 J. N. Wilson, *J. Am. Chem. Soc.*, 62 (1940) 1583.
- 19 E. Glueckauf, *Proc. R. Soc. London, Ser. A*, 186 (1946) 35.
- 20 D. DeVault, *J. Am. Chem. Soc.*, 65 (1943) 532.
- 21 H. K. Rhee, R. Aris and N. R. Amundson, *Philos. Trans. R. Soc. London, Ser. A*, 267 (1970) 419.
- 22 G. Guiochon and L. Jacob, *Chromatogr. Rev.*, 14 (1971) 77.
- 23 J. C. Giddings, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965.
- 24 B. C. Lin and G. Guiochon, *Sep. Sci. Technol.*, 24 (1989) 32.
- 25 B. C. Lin, Z. Ma and G. Guiochon, *J. Chromatogr.*, 484 (1989) 83.
- 26 P. Rouchon, M. Schonauer, P. Valentin and G. Guiochon, *Sep. Sci. Technol.*, 22 (1987) 1793.
- 27 S. K. Godunov, *Mat. Sb.*, 47 (1959) 271.
- 28 M. Czok and G. Guiochon, *Anal. Chem.*, submitted for publication.
- 29 A. M. Katti and G. Guiochon, *Anal. Chem.*, 61 (1989) 982.
- 30 A. M. Katti, R. Ramsey and G. Guiochon, *J. Chromatogr.*, 477 (1989) 119.
- 31 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 61 (1989) 1276.