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Multicomponent interferences in overloaded gradient elution chromatography

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Abstract

A theoretical study of multicomponent interferences has been performed by calculating band profiles in nonlinear overloaded gradient elution chromatography. The separation of binary and ternary mixtures has been modeled by means of a finite difference algorithm similar to the Craig mechanism, in the case when the adsorption behavior of the mixture components is accounted for by Langmuir competitive isotherms. The influence of the loading factor, the composition of the sample, and the gradient steepness have been investigated. In most cases, the profiles and the band interactions are qualitatively similar to those obtained under isocratic conditions.Although the profile fronts of the individual bands are very sharp, nearly vertical, the separation of closely eluted bands of 'parallel' or 'divergent' solutes cannot be improved significantly by gradient elution. Obviously, their retention time can be reduced considerably, which may improve the production rate although column regeneration must be carried out after each run, which increases the cycle-time. The elution profiles of impurities and trace components is usually very similar in gradient elution and in isocratic elution. This means that the recovery yield is not changed significantly, regardless of the gradient steepness. The most interesting results and the only one which is not observed under isocratic conditions, are obtained for the separation of 'convergent' solutes. In this case, the elution order of the components may change. This results in a kind of peak splitting, a fraction of the impurity eluting before the major component, the rest forming a flat profile spread along the profile of the major component. This phenomenon can be controlled or eliminated by adjusting the loading factor of the sample and the gradient steepness.

Keywords: Overloaded gradient elution; Adsorption isotherms; Multicomponent interference; Preparative chromatography; Gradient elution; Band profiles

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1. Introduction

Band interferences cause complicated profiles in preparative chromatography. Because a high throughput is required, large samples and high concentrations are used. The band profiles are controlled by the nonlinear, competitive equilibrium isotherms of the mixture components. Although the physicochemical processes involved are simple and rather well understood, there are no analytical solutions for the profiles of components which interfere on a column of finite efficiency. So, the phenomenon of band interferences can be studied only by numerical calculation, even in the simplest case of two components, with Langmuir competitive isotherms, a finite efficiency, and isocratic elution [1,2]. By

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means of systematic numerical calculations, we can acquire a better understanding of the practical consequences of the governing theory. This investigation is also necessary to pave the way for detailed studies of the optimization of the experimental conditions of a given separation by numerical calculation, which is much faster, more economical and less wasteful of expensive samples and large volumes of solvents [3,4].

Gradient elution techniques have long been applied in analytical chromatography whenever the range of retention times of the components of a mixture spans a wide range [5]. They are used for the analysis of macromolecules such as peptides or proteins in reversed phase chromatography because their retention volume depends so strongly on the mobile phase composition that the chromatograms obtained in isocratic analysis would be too irreproducible [6,7]. The strength of the mobile phase is continuously increased by changing the concentration of a suitable modifier. The weakly retained components elute with the less strong mobile phase, while the elution of the components strongly bound to the stationary phase happens at higher elution strength.

The preparative applications of gradient elution has been reviewed recently [8], mostly from a general point of view. Gradient elution is able to produce higher concentrations and shorter cycle-times than isocratic elution, which is favorable for the production rate. On the other hand, the column must be regenerated after each run, which may significantly increase the cycle-time. Only when the increase in production rate is important can the process be economical. The recovery of the mobile phase for recycling requires its intermediate separation, a complex and costly proposition.

2. Theory

The linear solvent strength (LSS) theory predicts that the logarithm of the retention factor, k'_i, of the solute i can be considered, as a first approximation, as a linear function of the solvent strength [5]. The solvent strength is controlled by φ, the volume fraction of the modifier in the mobile phase.

$$\ln k'_i = \ln k'_{0,i} - S_i\varphi \tag{1}$$

where $k'_{0,i}$ is the retention factor of solute i in a modifier-free mobile phase and S_i is the slope of the plot of $\ln k'_i$ versus φ. S_i depends on the nature of both the strong solvent and the solute. To conform to this theory, the parameter a of the Langmuir isotherm, which is proportional to k', changes with the mobile phase composition as

$$a_i(\varphi) = a_{0,i}\exp(-S_i\varphi) \tag{2}$$

El Fallah and Guiochon [9] observed that in many cases, especially with small molecules, the saturation capacity, $q_{s,i} = a_i/b_i$, of the stationary phase for solute i is practically constant. This means that, in this case, the dependence of the coefficients a and b on the mobile phase composition must be the same or very similar:

$$b_i(\varphi) = b_{0,i}\exp(-S_i\varphi) \tag{3}$$

This observation is not valid for proteins in a number of cases, e.g., when the conformation depends on the concentration of the strong solvent. For the sake of simplicity, in this first theoretical discussion of interfering band profiles in gradient elution, we will assume that q_s is constant.

The adsorption isotherms of the strong solvent and of the different components of the mixture were assumed to follow the competitive Langmuir model:

$$q_i = \frac{a_iC_i}{1+\sum_{j=1}^{n} b_jC_j} \tag{4}$$

When we consider the dependence of the isotherm coefficients on the mobile phase composition (Eq. 2 and Eq. 3), the above isotherms become:

$$q_i = \frac{a_{0,i}\exp(-S_i\varphi)C_i}{1+\sum_{j=1}^{n} b_{0,j}\exp(-S_j\varphi)C_j} \tag{5}$$

In the gradient elution of a binary mixture, the values of S_i determine the separation factors, α, for a given mobile phase composition.

$$\ln\alpha = \ln\left(\frac{k'_{0,2}}{k'_{0,1}}\right) - (S_2 - S_1)\varphi \tag{6}$$

Because S_1 and S_2 are different for two compounds, their separation factor may change during gradient elution. If $S_1 = S_2$ (*parallel solutes*), the separation factor is unchanged during elution. If $S_1 < S_2$ (*convergent solutes*), the separation factor decreases with increasing modifier concentration. Finally, if $S_1 > S_2$ (*divergent solutes*), the separation factor increases with increasing modifier concentration. The rate of change of the mobile phase composition during gradient elution can be described by the gradient slope $\Delta\varphi/t_G$, i.e. the ratio of the change in the modifier volume fraction and the gradient time. The gradient steepness G is related to the gradient slope:

$$G = \frac{\Delta\varphi}{t_G} t_0 S \qquad (7)$$

where t_0 is the column hold-up time. In case of convergent or divergent solutes, the values of gradient steepness for the different solutes are not identical. In the following, we always refer to the gradient steepness of the first eluted component.

In practice, linear concentration gradients are used in RPLC and G is constant during elution. The logarithmic mean separation factor has been introduced by Antia and Horváth [10] as a convenient parameter to characterize the average extent of separation in the case of divergent or convergent solutes:

$$\alpha_{\ln} = \frac{\alpha_{\varphi_0} - \alpha_{\varphi^*}}{\ln\left(\dfrac{\alpha_{\varphi_0}}{\alpha_{\varphi^*}}\right)} \qquad (8)$$

where α_{φ_0} is the separation factor at the initial mobile phase composition and α_{φ^*} is the separation factor at the mobile phase composition when the retention factor of the less retained component k_1' is unity.

Numerical calculations of band profiles in gradient elution chromatography cannot be carried out with the usually fast finite difference method based on the forward–backward algorithm [11], since the component isotherms differ at every point of the column [12]. The backward–forward scheme (i.e., the 'Craig' mechanism) or the finite element method offer an alternative in this case [10,13–15].

The total amount of the components in the sample compared to the column saturation capacity is expressed by means of the loading factor:

$$L_f = \frac{V_s \sum_{j=1}^{n} C_j^0}{(1-\varepsilon) s L q_s} \qquad (9)$$

where V_s is the volume of the sample injected, C_j^0 the concentration of component j in the sample injected, ε the porosity of column packing, s the column cross-sectional area, L the column length, and q_s the saturation capacity of the stationary phase.

2.1. Calculation methods

In this study, band profiles were calculated using the backward–forward finite difference scheme [1,7,9,12,16]. The details of the algorithm are provided elsewhere [1,12]. Linear gradient was applied after the sample has been injected into a regenerated column, so the volume fraction of the strong solvent is given by:

$$\varphi = \varphi_0 + G\frac{t - t_{\mathrm{inj}}}{t_0 S_1} \qquad (10)$$

where t_{inj} is the injection time and t_0 is the void time of the column.

Note that the rate by which the retention factor k' changes during the elution does not depend on the absolute value of parameter S, but solely on the gradient steepness G (and in the case of non-parallel solutes on the ratio of S_i/S_1):

$$\ln k_i' = (\ln k_{0,i}' - S_i\varphi_0) + G\frac{S_i}{S_1}\frac{t - t_{\mathrm{inj}}}{t_0} \qquad (11)$$

where the expression in parenthesis on the right-hand side of the equation is constant during the elution. As a consequence, the results of this study are independent of the specific values chosen for S_i.

The components of the mixtures were assumed to follow the competitive Langmuir model with the initial isotherm parameters of the first component being: $a_{0,1} = 400$ and $b_{0,1} = 1.54$ and those of the second component being derived from the value chosen for α. The modifier was assumed not to adsorb in the stationary phase.

3. Results and discussion

Calculations of bands profiles were made for the separation of various binary and ternary mixtures in overloaded gradient elution. Linear gradient profiles of varying gradient steepness were applied. The influence of the relative composition, of the separation factor and the loading factor were investigated. In the case of binary mixtures, all the possible cases, parallel, divergent, and convergent solutes, were considered. The separation of ternary mixtures was modeled by considering only parallel solutes.

3.1. Binary mixtures

In order to study the multicomponent interferences in preparative gradient elution, binary mixtures of different relative compositions were simulated at different separation factors and column efficiencies.

3.1.1. Effect of the elution order and relative concentration

Binary mixtures of relative concentrations 9:1 and 1:9, respectively, were studied. Fig. 1 and Fig. 2 compare the band profiles of these mixtures, at a separation factor $\alpha=1.2$, a loading factor of 30%, and a gradient steepness $G=0.5$. The figures show a strong tag-along effect in the case of the 9:1 mixture (Fig. 1) and a strong displacement effect in the case of the 1:9 mixture (Fig. 2). The same displacement and tag-along effects are observed during the isocratic elution of these mixtures [1], although the shape of the band profiles themselves are very different in gradient and isocratic elution. Note that the intensity of the interaction between the bands, i.e., of the tag-along and displacement effects is nearly the same as under isocratic conditions.

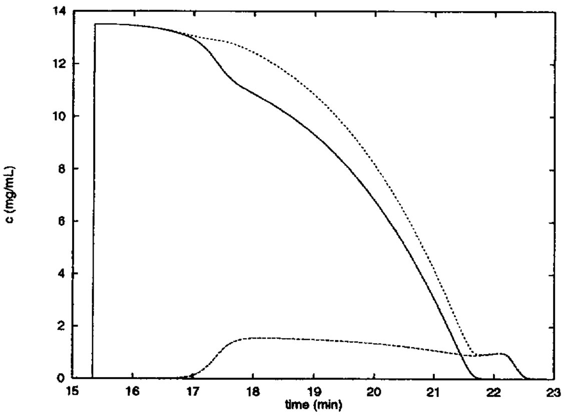

Fig. 1. Separation of a binary mixture with a relative concentration of 9:1; $\alpha=1.2$, $G=0.5$, $N=5000$, $L_f=30\%$.

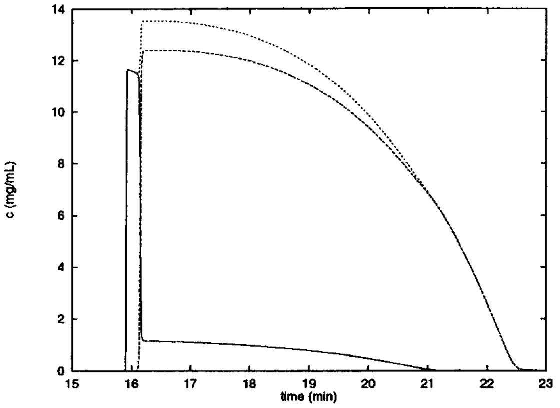

Fig. 2. Separation of a binary mixture with a relative concentration of 1:9; $\alpha=1.2$, $G=0.5$, $N=5000$, $L_f=30\%$.

It has been shown that in isocratic overloaded elution, the peak shapes and the recovery yield depend only on the loading factor and the column efficiency [17], regardless of how the column efficiency was achieved (i.e., of the HETP). In gradient elution, the peak shape depends on the gradient steepness. Nevertheless, it is remarkable that the extent of peak overlap and, consequently, the recovery yield achieved remains practically unchanged when the gradient steepness is altered. For instance, the recovery yield of the first component in Fig. 1 is 48.1% at 99% purity. If a similar separation is carried out in the isocratic mode (i.e. with $\alpha = 1.2$, $L_f = 30\%$, and $N = 5000$), the recovery yield of the first component in the same 9:1 mixture is 46.5%, a change which is nearly negligible.

3.1.2. Effect of the gradient steepness

The effect of the gradient steepness is expected to be two-fold in gradient elution chromatography. First, the retention times decrease with increasing gradient steepness. Second, the band profiles become narrower and taller. In isocratic elution, the Langmuir isotherm causes the rear, diffuse profiles of single component bands or bands of major components in mixtures to be convex downward [1]. In gradient elution, as the mobile phase becomes stronger during the elution of a band, late concentrations migrate in a stronger eluent, where they move faster and their retention time decreases continuously with decreasing concentration. Consequently, the band profiles become convex upward and the peaks are compressed. However, the results of band profile calculations (not shown) demonstrate that the intensity of the tag-along and displacement effects remains approximately constant. As discussed above, the recovery yield is unchanged.

3.1.3. Effect of the loading factor

Fig. 3 illustrates the effect of the loading factor on the interference between bands. The total loading factor was set to 5, 10, and 30%, respectively, for the three chromatograms. The other parameters of the separation were: $\alpha=1.2$, $G=0.5$, and $N=5000$. The figure clearly demonstrates that the rear ends of both bands, those of the main component and the impurity, remain unchanged, irrespective of the value of the loading factor. This means that the amount of an impurity eluting after the main component and whose band is caught in a tag-along effect remains constant, independently of the loading factor. Thus, when increasing amounts of feed are injected, the production rate reaches a constant value achieved when resolution is lost because of the tag-along effect. Beyond that stage, the recovery yield decreases as the rest of the amount of impurity injected is eluted under the main component band.

3.1.4. Effect of the difference between S_i and S_j

The results described in the previous sections were obtained for parallel solutes. For divergent solutes, the calculated chromatograms are similar. They differ only slightly and merely by a lesser degree of band interference caused by the increase in the separation factor with increasing concentration of the strong solvent in the mobile phase. The most peculiar band profiles were obtained for convergent solutes. In this case, the separation worsens with passing time. In some cases, a reversal in the elution order can even be observed.

Fig. 4 shows a series of band profiles of the minor component of a 9:1 mixture of two slightly divergent solutes ($S_1 = 25$, $S_2 = 23$), with three values of the loading factor, as in Fig. 3. The profile of the major component is not shown, it is very similar to the profile shown in Fig. 3. In this case, the gradient steepness has a slight effect on the shape of the band profile of the impurity, but no dramatic differences can be observed with the profiles of parallel solutes shown in Fig. 5, for the sake of comparison. The

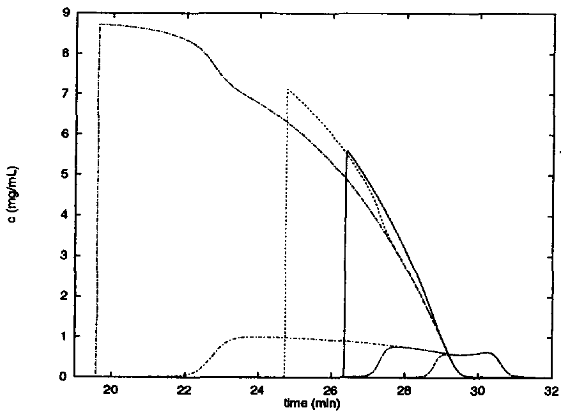

Fig. 3. Separation of a binary mixture with a relative concentration of 9:1; $\alpha=1.2$, $G=0.5$, $N=5000$, loading factor: $L_f=5$, 10, and 30%, respectively.

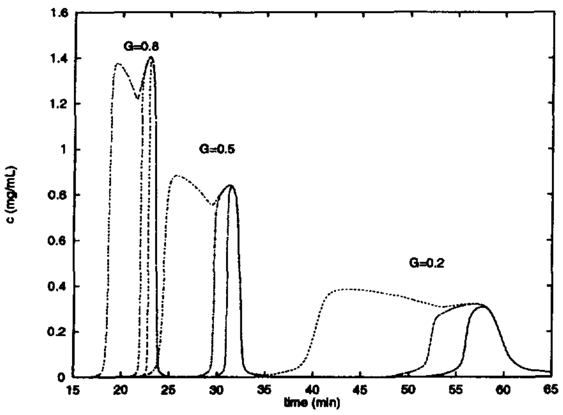

Fig. 4. Band profile of the second component of a 9:1 mixture at loading factors $L_f=5$, 10, and 30%, gradient steepness $G=0.2$, 0.5, and 0.8. Divergent solutes: $S_1=25$, $S_2=23$.

only difference between the calculated chromatograms in Fig. 4 and Fig. 5 is the value of $S_2 = 25$ in Fig. 5 instead of 23 in Fig. 4.

However, if the solutes are convergent and the gradient steep, a fraction of the impurity may elute before the front of the major component. This phenomenon depends on the loading factor, the gradient steepness, and the extent of the convergence. In the case of a slight convergence ($S_1 = 25$, $S_2 = 27$), a small gradient steepness causes a normal behavior of the bands, as shown in Fig. 6. If Fig. 4, Fig. 5, and Fig. 6 are compared, we see a progressive decrease of the retention time of the tail and of the amount of impurity separated from the main component, which corresponds approximately to the part of the profile eluted after its valley.

When the gradient steepness is increased, however, the profiles change dramatically and a spike of the impurity appears when the front of the main component elutes. This can be seen in Fig. 6, for the profiles at high values of G (0.5 and 0.8 but not 0.2) and moderate values of L_f (at 5 and 10% but not 30%). To better illustrate the phenomenon, the convergence of the two solutes is slightly enhanced by taking $S_1 = 25$ and $S_2 = 29$. The calculated chromatograms are shown in Fig. 7. The difference with the chromatograms in Figs. 4–6 is striking. Note that the front shock layer of the band of the 'second' component elutes slightly before that of the 'first' one in this case. Fig. 8 completes the illustration of the peak splitting phenomenon by showing also the profile of the main component in one case. When the gradient steepness is only $G = 0.2$, a very sharp spike of the impurity elutes slightly before the front of the major component at a loading factor of $L_f = 5$ and 10% (Fig. 7). The spike disappears at higher loading factors (30%) and, in this last case, the elution of the second component begins long after that of the first one. When the gradient steepness is increased to

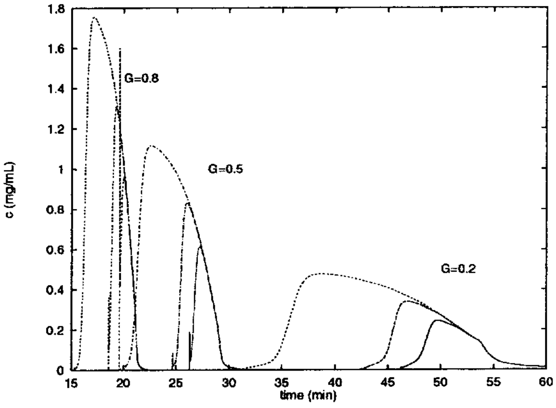

Fig. 6. Band profile of the second component of a 9:1 mixture at loading factors $L_f = 5$, 10, and 30%, gradient steepness $G = 0.2$, 0.5, and 0.8. Convergent solutes: $S_1 = 25$, $S_2 = 27$.

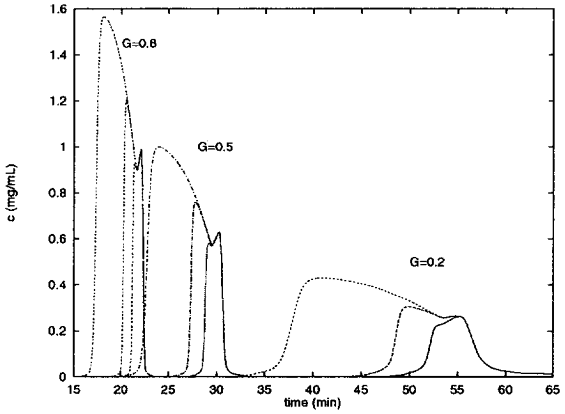

Fig. 5. Band profile of the second component of a 9:1 mixture at loading factors $L_f = 5$, 10, and 30%, gradient steepness $G = 0.2$, 0.5, and 0.8. Parallel solutes: $S_1 = S_2 = 25$.

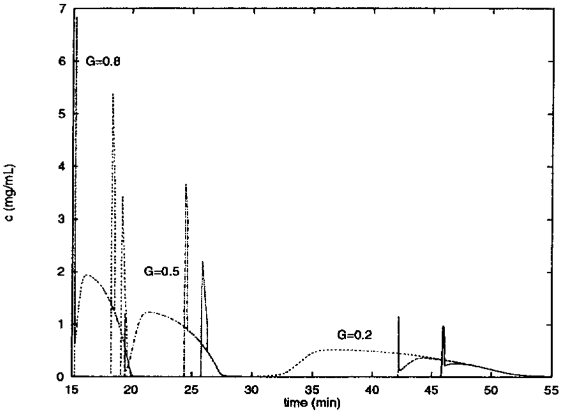

Fig. 7. Band profile of the second component of a 9:1 mixture at loading factors $L_f = 5$, 10, and 30%, gradient steepness $G = 0.2$, 0.5, and 0.8. Convergent solutes: $S_1 = 25$, $S_2 = 29$.

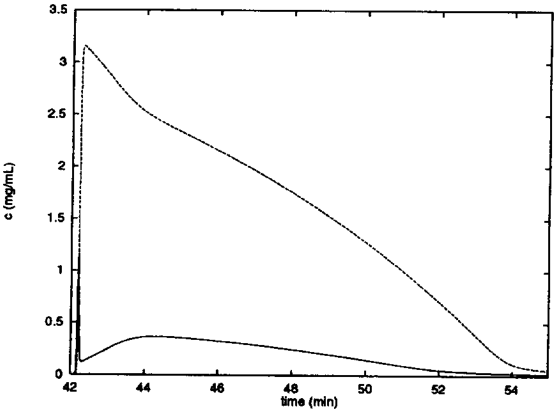

Fig. 8. The effect of peak splitting. $L_f=10\%$, $S_1=25$, $S_2=29$, $G=0.2$.

$G=0.5$, and the loading factor is 5%, a significant amount of impurity elutes before the main component as a high concentration spike at $L_f = 30\%$. At still higher gradient steepness, the spike is very high, even for moderate values of the loading factor.

The steeper the gradient the larger the amount of impurity which elutes before the major component. In many cases, but not all (Fig. 7), a true peak splitting is observed. After a sharp spike, the concentration of the impurity decreases almost to 0 and then rises again toward a second maximum. This kind of elution profiles – i.e. the appearance of a sharp impurity peak – may give the false illusion that the impurity elutes before the main component. Unfortunately, the opposite takes place. It is unusual that more than 10 or 20% of the impurity can be extracted by collecting the spike. The rest of the impurity still contaminates the main component and is spread all along its elution band. Purification becomes impossible under such conditions.

3.2. Ternary mixtures

3.2.1. Effect of the elution order and relative concentration

Ternary mixtures of relative concentrations of 1:1:1, 9:1:1, 1:9:1, 1:1:9, and 9:1:9 were studied. The total loading factor of the three components was $L_f = 30\%$, the separation factor for the two pairs, $\alpha=1.1$, and the column efficiency, $N=5000$ theoretical plates. Only parallel solutes were considered. Fig. 9 shows a typical chromatogram obtained for the separation of the 1:1:1 mixture under these conditions, at a gradient steepness of $G=0.5$. On the total chromatogram, only one band with a few undulations is observed. The profile of the first eluted component has a very sharp front or shock layer, followed by a rapid drop in concentration when the second and then the third component begin to elute; finally, the band flattens out. The front of the second component is less steep, its maximum concentration is smaller than that of the first component, and the rear of the band is very similar to the first one. The last eluted component has a weak front shock layer, and has a rear profile similar to those of the first two components. The second and third components displace the first and second ones, respectively, the displacement effect being more important for the first component band than for the second one. The bands of the second and third components also exhibit a strong tag-along effect, stronger for the second component than for the last one.

Next, the band profiles of two impurities in a major component were investigated. When the two impurities elute before the major component, part of the impurities are compressed as sharp peaks eluted before the front of the major component. However, the second part of these impurities spread out along

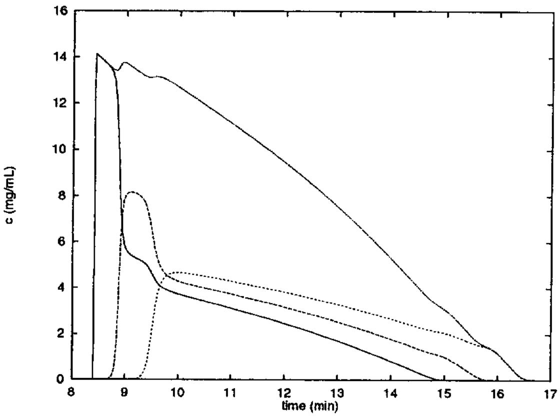

Fig. 9. Separation of a ternary mixture with a relative concentration of 1:1:1; $\alpha=1.1$, $G=0.5$, $N=5000$, $L_f=30\%$.

most of the profile of the main component, contaminating it all the way, nearly until the end of its elution (see Fig. 10). The behavior of the two impurities are very similar. This result is a consequence of the choice, for illustration purposes, of a value of the loading factor which would be excessive in actual practice when the separation factor is so low (1.1).

When both impurities elute after the major component, they undergo important tag-along effects while the front of the major component is a clean, sharp shock layer, as shown in Fig. 11. The impurities begin to elute behind the front of the major component, because the conditions selected for this calculation are not conductive of the peak-splitting phenomenon (parallel solutes). The impurity bands are spread over a wide part of the first component band by an intense tag-along effect, contaminating it in such a way that only a small, initial fraction of the component to be purified can be collected pure. The effects of the two impurities appear to be additive; no real competition can be observed between them. As in the previous case, the considerable intensity of the tag-along effect results from the selection of a value of the loading factor which would be excessively large in practice.

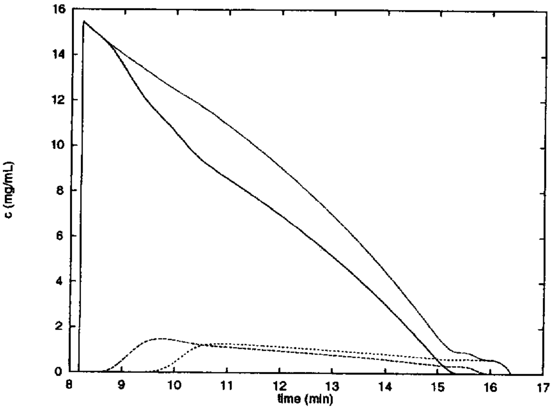

Fig. 11. Separation of a ternary mixture with a relative concentration of 9:1:1; $\alpha=1.1$, $G=0.5$, $N=5000$, $L_f=30\%$.

A combination of the two effects of displacement and tag-along, illustrated separately in the previous figures, is observed when the major component is locked in between two impurities, as shown in Fig. 12, in the case of a 1:9:1 mixture. This case is a schematic of most purification applications, in which one or a few impurities are eluted before and after the main component. As seen in Fig. 10 and Fig. 11, there is no interaction between impurities eluted on the same side of the main component. Figs. 10–12 show that the profile of the major component is never affected by the minor impurities. At the value of the loading factor used to calculate the chromatogram in Fig. 12, only about half of the impurity eluted before the main component can be easily eliminated while the impurity eluted after it cannot

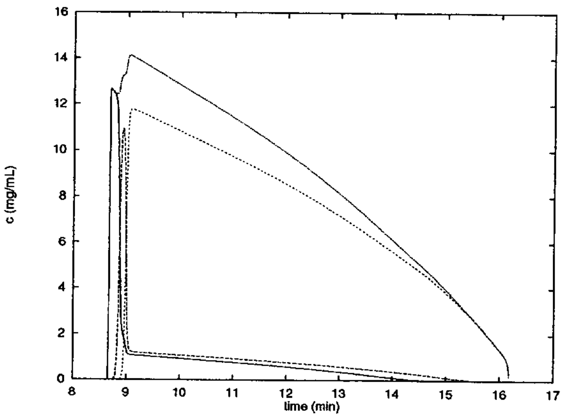

Fig. 10. Separation of a ternary mixture with a relative concentration of 1:1:9; $\alpha=1.1$, $G=0.5$, $N=5000$, $L_f=30\%$.

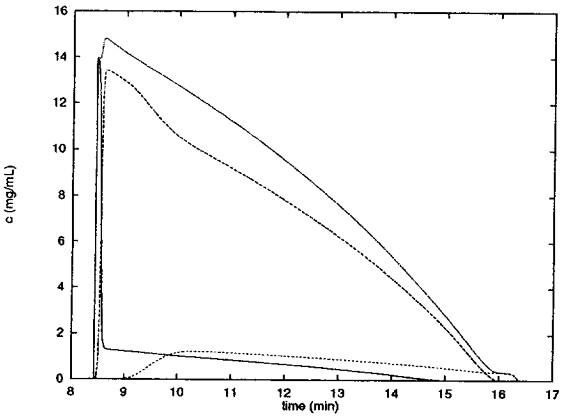

Fig. 12. Separation of a ternary mixture with a relative concentration of 1:9:1; $\alpha=1.1$, $G=0.5$, $N=5000$, $L_f=30\%$.

be extracted without accepting a very low yield. In practice, a much lower value of the loading factor, hence a rather low production rate must be used for an effective purification.

If the loading factor is lowered to $L_f = 5\%$, a value typical for practical separations at this separation factor (i.e. $\alpha = 1.1$), the behavior of the impurities is still similar to what we could observe at $L_f = 30\%$. The band shapes for both impurities, as well as for the major component are hardly changed, although, as seen in Fig. 13, due to the six-fold decrease of the loading factor, the separation is slightly improved. However, even at this much reduced value of the loading factor, no pure fraction of the major component can be collected.

When an impurity is trapped between two high-concentration components (see Fig. 14), its band undergoes a certain concentration due to its displacement by the second component band. However, the same spreading out due to the tag-along effect caused by the first component band can be observed as in Fig. 11. The front of the impurity is not sharp because the impurity concentration is too low to cause local nonlinear effects.

3.2.2. *Effect of the separation factor*

The effect of the separation factor is illustrated by a comparison of Fig. 9 and Fig. 15. In the latter, the separation factor was increased to $\alpha = 1.2$ for the calculation of a chromatogram showing the separation of a 1:1:1 mixture. The other parameters were the same as for Fig. 9. The band fronts are much sharper at higher separation factor, even for the second and the third components. This illustrates the strong influence of the separation factor on the thickness of shock layers between successive bands [1,18]. The intermediate plateaus on the rear of the bands, corresponding to the successive ends of the band profiles of the lesser retained components become clearly visible. So, the elution profiles are not as smooth as in the case of $\alpha = 1.1$, but the general behavior remains similar.

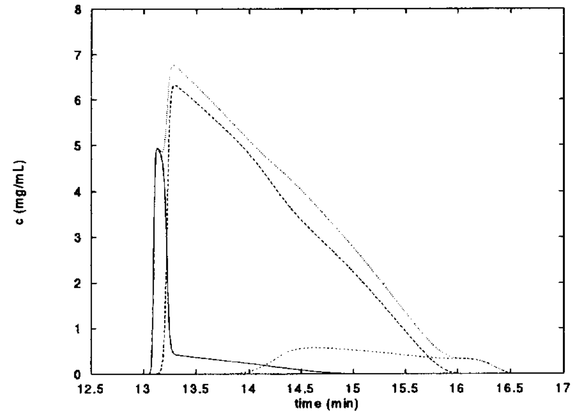

Fig. 13. of a ternary mixture with a relative concentration of 1:9:1; $\alpha = 1.1$, $G = 0.5$, $N = 5000$, $L_f = 5\%$.

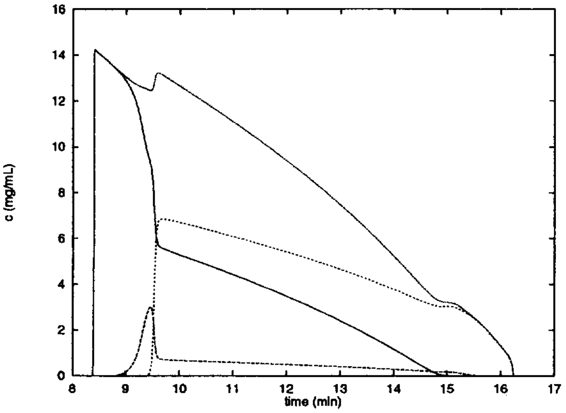

Fig. 14. Separation of a ternary mixture with a relative concentration of 9:1:9; $\alpha = 1.1$, $G = 0.5$, $N = 5000$, $L_f = 30\%$.

3.2.3. *Effect of the column efficiency*

The chromatogram in Fig. 16 has been calculated with the same values of the parameters as used for the one in Fig. 9, except for the column efficiency, 10 000 plates instead of 5000. When comparing Fig. 9, Fig. 15 and Fig. 16, it is clear that the effect of an increase in the column efficiency is comparable to that of an increase in the separation factor. The chromatograms in Fig. 15 and Fig. 16 exhibit comparable improvement on the quality of the chromatogram in Fig. 9, sharper fronts, better resolution between the bands, higher potential recovery yields.

3.2.4. *Effect of the gradient steepness*

The steeper the gradient, the more convex upward the band profiles. However, the gradient steepness

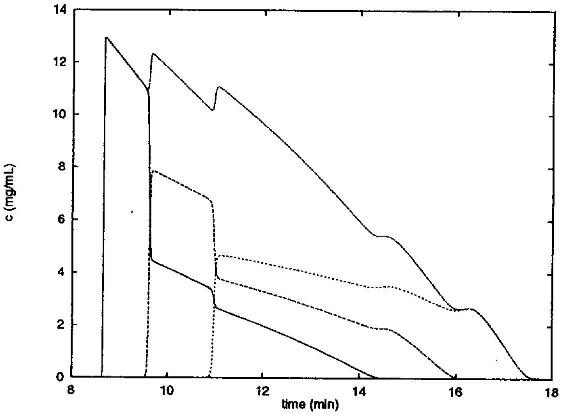

Fig. 15. of a ternary mixture with a relative concentration of 1:1:1; $\alpha=1.2$, $G=0.5$, $N=5000$, $L_f=30\%$.

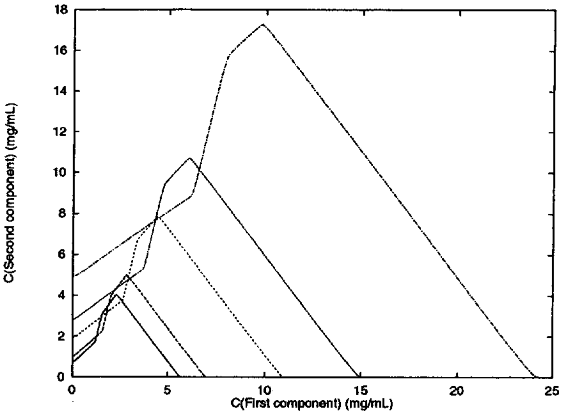

Fig. 17. Plot of the concentration of the second component vs. the concentration of the first component during the gradient separation of a 1:1:1 ternary mixture. $\alpha=1.1$, $N=5000$. $L_f=30\%$. From top to bottom: $G=1.5$, 0.8, 0.5, 0.2, 0.1.

causes the compression of each peak in a uniform manner. This can be illustrated by a plot of the concentration of one component versus the concentration of the other one, or hodograph plot [1]. In Fig. 17, the hodographs of the chromatograms obtained for the 1:1:1 mixture at five different gradient steepness are compared. As the gradient steepness increases, the peaks become more compressed and their concentrations higher. However, the lines are all parallel, whatever the gradient steepness. From the separation point of view, it does not matter how the peak profiles are changing. Since they are all changing together in the same manner, the degree of separation remains unchanged. This is the reason why the recovery yield or the purity of the fraction collected from the individual peaks will not change by increasing the gradient steepness.

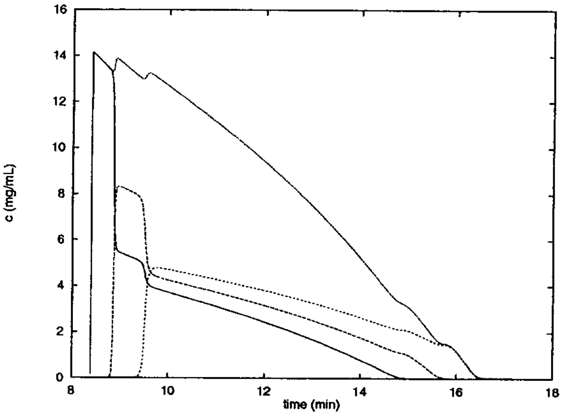

Fig. 16. Separation of a ternary mixture with a relative concentration of 1:1:1; $\alpha=1.1$, $G=0.5$, $N=10\,000$, $L_f=30\%$.

4. Conclusions

By applying a gradient in overloaded elution chromatography, the bands are compressed, the retention times are decreased and the concentrations of the collected fractions are increased. Although the band profiles change with increasing gradient steepness, the relative concentrations of the different components do not change compared to what they are in isocratic separations. As a consequence, although the production rate may be increased, the purity of the fractions collected and the recovery yield remain the same in isocratic and in gradient elution chromatography, whatever the gradient steepness, at least as long as the solutes are parallel. In most cases, the results obtained are similar to those achieved under isocratic conditions [2].

If the solutes are divergent the band spacing improves and a similar behavior is observed. On the other hand, very strange band profiles may appear when the solutes are convergent. In this instance the profile of minor components interfering with the band of the major component may split in two parts, one eluted very slightly before the front of the major component, and the second spreading out and contaminating all the collected fractions of the component to be purified. This splitting effect can be controlled by the gradient steepness and by the loading factor. It can be expected that with an optimization algorithm, the optimum gradient steepness and other experimental conditions can be calculated to exploit fully the complexity of these new types of separations.

The application of these results to the study of the optimization of experimental conditions in preparative gradient elution chromatography will be discussed in a forthcoming publication.

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References

[1] G. Guiochon, S. Golshan-Shirazi, and A.M. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, Boston, MA, 1994.

[2] S. Jacobson, S. Golshan-Shirazi, A.M. Katti, M. Czok, Z. Ma and G. Guiochon, J. Chromatogr., 484 (1989) 103–124.

[3] A. Felinger and G. Guiochon, J. Chromatogr., 591 (1992) 31–45.

[4] A. Felinger and G. Guiochon, J. Chromatogr., 609 (1992) 35–47.

[5] L.R. Snyder, in Cs. Horváth (Editor), High-Performance Liquid Chromatography, Advances and Perspectives, Vol. 1, Academic Press, New York, 1980, pp. 207–316.

[6] S. Yamamoto, M. Nomura and Y. Sano, J. Chromatogr., 512 (1990) 89–100.

[7] M.Z. El Fallah and G. Guiochon, Biotechnol. Bioeng., 39 (1992) 877–885.

[8] G. Crétier and J.L. Rocca, J. Chromatogr. A., 658 (1994) 195–205.

[9] M.Z. El Fallah and G. Guiochon, Anal. Chem., 63 (1991) 859–867.

[10] F.D. Antia and Cs. Horváth, J. Chromatogr., 484 (1989) 1–27.

[11] S. Golshan-Shirazi and G. Guiochon, J. Chromatogr. A., 658 (1994) 149–171.

[12] M. Czok and G. Guiochon, Comput. Chem. Eng., 14 (1990) 1435–1443.

[13] J.E. Eble, R.L. Grob, P.E. Antle and L.R. Snyder, J. Chromatogr., 405 (1987) 51–66.

[14] G. Crétier, M. El Khabchi and J.L. Rocca, J. Chromatogr., 596 (1992) 15–25.

[15] T. Gu, Y.-H. Truei, G.-J. Tsai and G.T. Tsao, Chem. Eng. Sci., 47 (1992) 253–262.

[16] G. Crétier, L. Macherel and J.L. Rocca, J. Chromatogr., 590 (1992) 175–188.

[17] A. Felinger and G. Guiochon, AIChE J., 40 (1994) 594–605.

[18] J. Zhu and G. Guiochon, AIChE J., 41 (1995) 45–57.