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OPTIMIZATION OF CONCENTRATION OVERLOAD IN PREPARATIVE LIQUID CHROMATOGRAPHY

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SUMMARY

    A numerical optimization of concentration overload in preparative liquid chromatography based on a new mathematical model was undertaken. This model is a variant of the ideal model of chromatography and accounts for the finite efficiency of the column. It is shown that in order to maximize production in preparative chromatography, the column should be considerably overloaded. Under optimal conditions, the two bands overlap markedly. This work provides guidelines for predicting the optimum extent of overloading, which depends largely on the required recovery and degree of purity of the final products.

INTRODUCTION

    Whereas quantitative analysis by liquid chromatography requires an excellent resolution between the peaks of the components of interest in the sample mixture, the preparation of pure compounds by the same technique does not. As long as it remains small, the fraction collected between overlapping bands can be either recycled or rejected to waste. To maximize production, we need to increase the column load far beyond conventional analytical practice, which tends to reduce the yield and will eventually increase costs. Hence there is an optimal throughput at which the production costs of a compound of interest at a stated degree of purity is at a minimum.
    This optimal throughput results from a compromise between two components of the cost. When throughput is increased, production rises, and the fixed costs (amortization of the investment) are spread over a larger production. Other costs, such as extracting the pure compound from its dilute solution in the mobile phase, also decrease. On the other hand, the yield decreases and the cost of recycling or wasting the overlapping fraction increases. Accordingly, the optimal throughput is lower than that corresponding to the maximum production. The exact value depends on the specifics of the problem being studied, including the accounting procedures used. Therefore, in this work, we have studied the variation of both production and yield with the throughput and the influence of various parameters, without looking for an optimal cost, which would be arbitrary to define in an academic environment.

In order to achieve a large throughput, we may increase the volume of sample injected, the concentration or both. The former method is called "volume overload" and the latter "concentration overload". In both instances, when the sample size is increased the elution band width remains constant at first, then increases more and more rapidly. In practical terms, a chromatographic column is said to be overloaded when an increase in the sample injected has resulted in the eluted peak being 10–20% wider than that resulting from the injection of a very small dilute sample[1].

In "volume overload", the sample concentration is kept constant and is confined to the linear range of the adsorption isotherm, but the volume injected is very large. For rectangular injection pulses of constant height (concentration) and increasing width (volume), the elution band becomes higher and wider. Ultimately, it becomes flat-topped but remains symmetrical.

In "concentration overload", a small sample volume is injected, but its concentration exceeds the linear range of the adsorption isotherm. Accordingly, the band profile broadens and becomes unsymmetrical, even if there is no volume overload. With convex (e.g., Langmuir) isotherms, the profile becomes close to triangular, with an almost vertical front and a slanted tail[1–4]. The opposite situation, i.e., a slanted front and a vertical tail, occurs when the adsorption isotherm is concave[4].

In discussing the optimization of preparative liquid chromatography (PLC), Knox and Pyper[1] have shown that concentration overload always allows for a higher production than that achieved by means of volume overload. They confirmed earlier predictions made by Gareil et al.[5], who further suggested a ratio of 10:1 between the maximum productions achieved in concentration and volume overload. Some practitioners still maintain that the resolution between closely eluted bands should be at least unity in order to minimize cross-contamination. They recommend that the fraction cuts be made at the bottom of the valley between the resolved bands, which ensures a yield as close as possible to unity. This high yield requirement is arbitrary and unrealistic, as illustrated in the chemical industry where all processes are accompanied by some losses. Some decrease in the recovery yield is acceptable, provided that it is compensated for by a large increase in production. In fact, it is observed experimentally that when the size of the feed sample injected is increased beyond the value shown by Knox and Pyper[1] to mark the limit where total recovery of purified components is possible, the yield decreases only slowly whereas the production continues to increase even after the two bands have merged so much that a valley is hardly discernible[6,7].

A solution to this problem can be derived from the fundamental theory of non-linear chromatography. A general model of the migration of large concentration bands in chromatography is obtained by writing the mass balance equations of each of the chemical species involved in the system (components of the mixture and of the mobile phase), the equations corresponding to the mass-transfer kinetics between mobile and stationary phases and solving the system of partial differential equations obtained. Unfortunately, the mathematical properties of this system are such that a solution of the overall problem is impossible at present[8]. As a result, some additional assumptions have to be made to simplify further the system of partial differential equations. Recently, we reported the successful use of a finite difference method to simulate the separation of a two-component mixture with a known mixed isotherm[3]. In this work, the same method was used to investigate the effect of "concentration

overloading" on the recovery and the production of the components of a binary mixture.

THEORETICAL

This work is derived from the classical ideal model of chromatography[9-12]. An exact model of chromatography can be obtained by combining a mass balance equation and a kinetic equation for each compound involved. As it is based on first principles (the law of conservation of mass), such a model would be perfectly accurate provided that an exact kinetic equation is written. This is not feasible, however, but very good approximations can be used unless the exact influence of the flow velocity and the average size and size distribution of the packing particles are to be predicted. In such a case, the well known difficulties encountered in the prediction of the column height equivalent to a theoretical plate (HETP) in classical high-performance liquid chromatography (HPLC) arise[13,14].

The simplest approximation of the kinetic equations assumes constant equilibrium between the mobile and stationary phases and corresponds to the ideal model of chromatography[9-12]. The kinetics of radial mass transfer are infinitely fast, whereas axial diffusion is negligibly slow, *i.e.*, the column efficiency is infinite. This assumption is unrealistic, although very high efficiencies can be achieved in practice. We have improved it by incorporating a diffusion term in the numerical solution of the system. This term accounts for the effect of a finite column efficiency.

For a two-component mixture in a pure solvent, the system of mass balance equations is[3,9]

$$\frac{\partial}{\partial t}\left(1 + F\frac{\partial f_1}{\partial c_1^m}\right)\frac{\partial c_1^m}{\partial t} + \frac{\partial}{\partial z}(uc_1^m) = 0 \tag{1}$$

$$\frac{\partial}{\partial t}\left(1 + F\frac{\partial f_2}{\partial c_2^m}\right)\frac{\partial c_2^m}{\partial t} + \frac{\partial}{\partial z}(uc_2^m) = 0 \tag{2}$$

where c_1^m and c_2^m are the concentrations of components 1 and 2 in the solution at time t, at the point of abscissa z in the column, u is the local mobile phase velocity, assumed to be constant, F is the ratio $(1 - \varepsilon)/\varepsilon$, where ε is the total porosity of the packing, and f_1 and f_2 are the adsorption isotherms of the two compounds:

$$c_1^s = f_1(c_1^m, c_2^m) \tag{3}$$
$$c_2^r = f_2(c_1^m, c_2^m) \tag{4}$$

There is no mass balance for the mobile phase, which is assumed to be incompressible and to have the same density as the eluites. The adsorption isotherms, f_1 and f_2, are determined with the convention that the solvent is not adsorbed[3].

An exact solution of the system of eqns. 1–4 with the boundary condition corresponding to the injection of a rectangular sample plug (elution mode) would lead to the appearance of concentration shocks on the peak fronts[9]. Serious difficulties have been encountered in previous attempts at calculating numerical solutions[9].

In this work, the results of a numerical simulation based on the Godunov algorithm[15] are presented. The continuous (z,t) plane is replaced by a (z,t) grid, defined by the values of the space (δz) and time (δt) increments. The concentrations are calculated at each point of the grid, starting from the injection profile, until the entire profile is eluted.

To simulate the ideal model exactly, space and time increments equal to zero should be used. This is impossible, as it would require infinite computational time. This drawback of direct numerical methods can be used here to our advantage, as it has been demonstrated[16] that the choice of a finite space increment has the effect on the numerical solution of a dispersion coefficient which is mathematically identical with the simulation of an apparent diffusion coefficient. This use of a finite increment results in numerical smoothing of the sharp concentration discontinuities, in much the same way as finite kinetics of mass transfer relax the large concentration gradient built up by the concentration dependence of the band velocity, so the concentration shocks do not appear. It can be shown that the space increment should be chosen to be equal to the column HETP[16,17]. This permits an exact coincidence of the Gaussian profiles calculated and measured at very low concentrations[18].

The theoretical model described above has been used to structure a computer program which gives the elution profiles of each component of a binary mixture and the overall band profile, provided that the mixed equilibrium isotherms are known[3]. This program allows for the simulation of different experimental conditions. A subroutine is written to determine the recovery and the production rate of each component of the mixture at the outlet of the column.

EXPERIMENTAL

We studied the change in the elution profiles of a binary mixture, the variation of the recovery of each component and their production rate as a function of the relative concentration of solutes $[X_j = C_j/(C_1 + C_2]$ for varying total injected amounts.

We simulated the behaviour of a mixture of two compounds, 1 and 2, having column capacity factors of 5.75 and 6.25, respectively, and a relative retention of 1.09 at infinite dilution. Their competitive isotherms are of the Langmuir type. They are given by the following equations:

$$f_1 = 23 \, c_1^m/(1 + 2.38 \, c_1^m + 2.56 \, c_2^m) \tag{5}$$

$$f_2 = 25 \, c_2^m/(1 + 2.38 \, c_1^m + 2.56 \, c_2^m) \tag{6}$$

The column used was 25 cm long with an efficiency of 5600 theoretical plates at very low sample load, i.e., an HETP of 45 μm. This would be reasonable for a preparative column made with 10-μm particles. This efficiency characterizes the rate constants of mass transfer which are not affected by changes in the eluite concentration[2-4,8]. The band broadening is entirely of thermodynamic origin[1-4,8-12].

We studied binary mixtures ranging in composition from 1:19 to 19:1. The sample size is given in arbitrary units. However, because of the combination of the numerical values selected, the saturation capacity of the column as defined by Eble et al.[19] is equal to 100, so an area of 1 unit corresponds to a 1% column loading.

RESULTS AND DISCUSSION

Numerical solutions of equation systems such as that discussed here (eqns. 1–4) are valid only for the particular set of numerical values of the parameters used in the derivation. It is therefore difficult to generalize from a particular solution and to derive rules and conclusions valid for a whole range of values of the experimental parameters, unless many numerical solutions are calculated and studied. We investigated here the effects of sample size and composition for a given mixture. The numerical results are valid only for the type of mixture studied, *i.e.*, for compounds having the particular pair of competitive isotherms selected (eqns. 5 and 6). The extension of the results presented here to other mixtures, especially those of compounds with relative retentions larger than 1.1, is discussed briefly in the Conclusion.

Figs. 1–5 show the band profiles obtained for different sample sizes and compositions. Fig. 1 shows the elution profiles of a 2-unit sample of a 1:1 mixture (fraction of column saturation capacity = 2%). Although its influence on the elution profiles is moderate, the interaction between the two compounds is conspicuous. Compound 2 tends to displace 1 while tending to tag along with 1. With the combination of these effects, both bands migrate faster than if they were alone. The intensity of each of these effects increases with increasing concentration of the corresponding compound.

These effects are easy to understand. Compound 2 is more strongly adsorbed than 1; its molecules occupy sites on the surface which are not available for molecules of 1, hence the partial displacement effect. When the concentration of 2 decreases
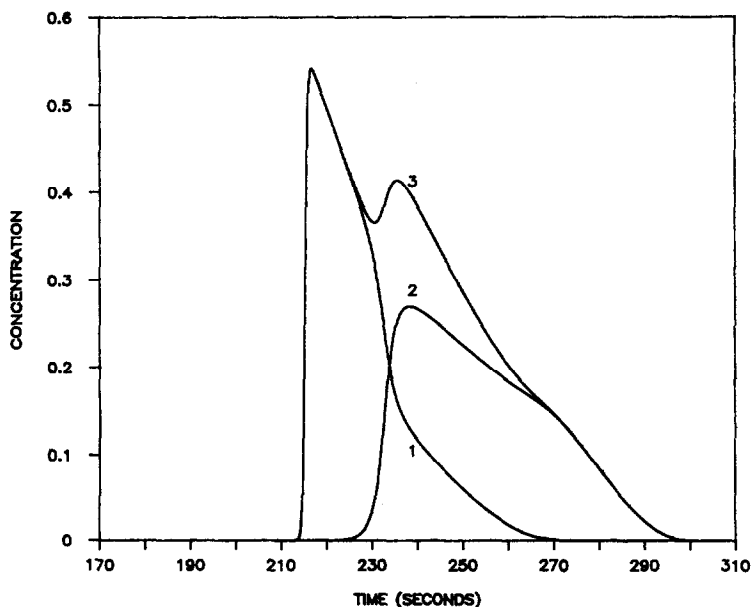

Fig. 1. Chromatogram of a 1:1 mixture (sample size = 2 units; 2% of column saturation limit). (1) Elution profile of component 1; (2) elution profile of component 2; (3) chromatogram obtained with an ideal detector.
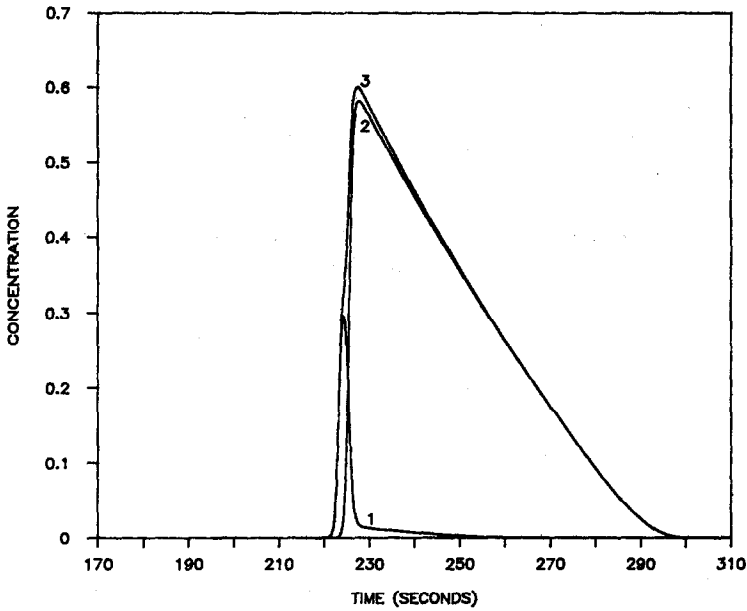
Fig. 2. Chromatogram of a 1:19 mixture (sample size = 2 units). (1), (2) and (3) as in Fig. 1.

after the band maximum of 2 is eluted, the competition becomes less severe and 1 tails. Its molecules occupy sites on the surface, competing with molecules of 2. This reduces the fraction of molecules of 2 which are sorbed and creates a "tag-along"
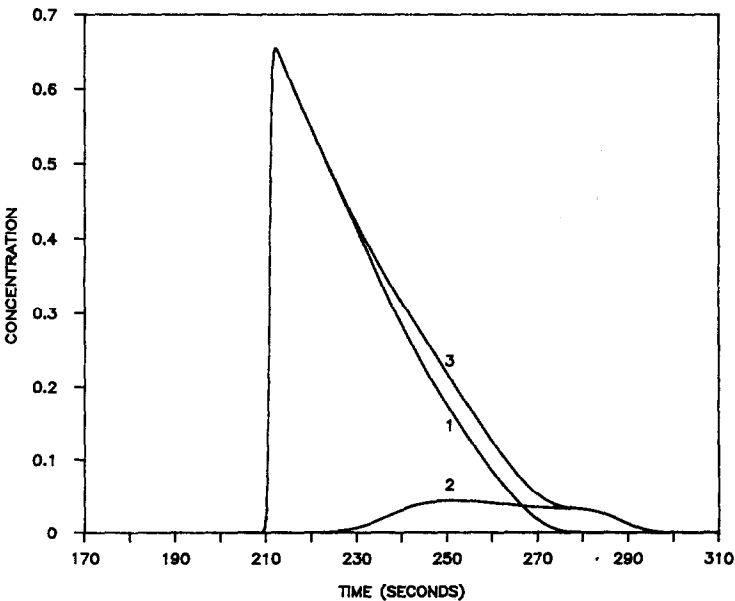

Fig. 3. Chromatogram of a 9:1 mixture (sample size = 2 units). (1), (2) and (3) as in Fig. 1.

effect which is the complement, in the elution mode, of the displacement effect. The balance between these two effects depends on the relative concentration of the mixture.

Fig. 2 shows the separation of the same amount (2 units) of a 1:19 mixture. The displacement of 1 by 2 is now very strong, although the formation of an isotachic train is impossible in elution chromatography. The elution profile of compound 1 exhibits a tail going almost to the retention time of 1 at infinite dilution ($t_R = 270$ s). Because of this tail, it will not be possible to prepare 2 in a high degree of purity and with a very good yield. On the other hand, because the front of 2 is very sharp and pushes the larger fraction of 1 in front of it, it will be possible to recover pure 1 in a good yield. The displacement of compound 1 by 2 is the major effect in this instance, but there remains a very minor "tag-along" effect (see below).

Fig. 3 shows the separation of the same amount (2 units) of a 9:1 mixture and illustrates the "tag-along" effect of compound 1 on 2. Compound 2 is slightly more strongly adsorbed than 1; however the concentration of 1 is much larger than that of 2, so 1 occupies a large fraction of the surface, reducing the apparent retention of 2, which tags along with 1. The broadening of the band of 2 is such that the recovery of very pure 1 and 2 will be low (see below).

Figs. 4 and 5 show the elution profiles of 1- and 5-unit samples of a 1:1 mixture, respectively (fractions of column saturation capacity 1 and 5%, respectively). A slight interaction takes place between the bands of compounds 1 and 2 in the former instance. This interaction becomes important in the latter instance.

Figs. 6–8 permit a comparison of the changes in the elution profiles of the two components when the concentration and sample size are changed. Fig. 6 shows the
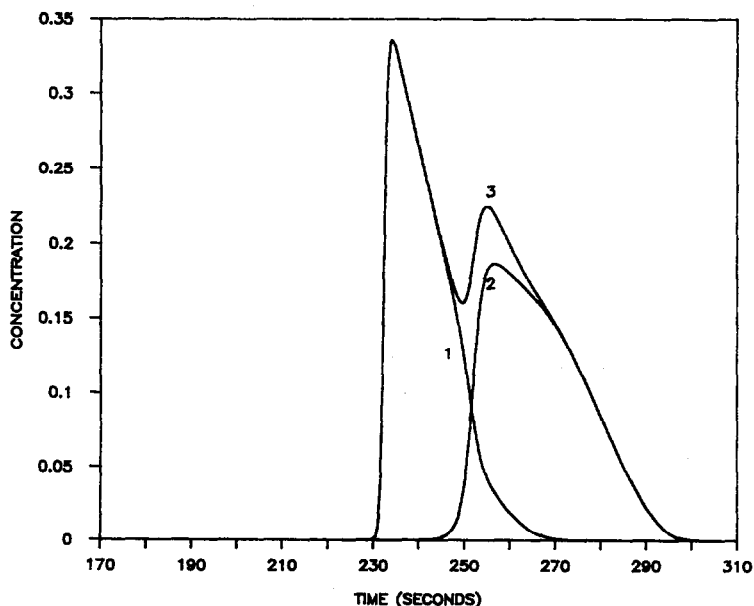

Fig. 4. Chromatogram of a 1:1 mixture (sample size = 1 unit; 1% of column saturation limit). (1), (2) and (3) as in Fig. 1.
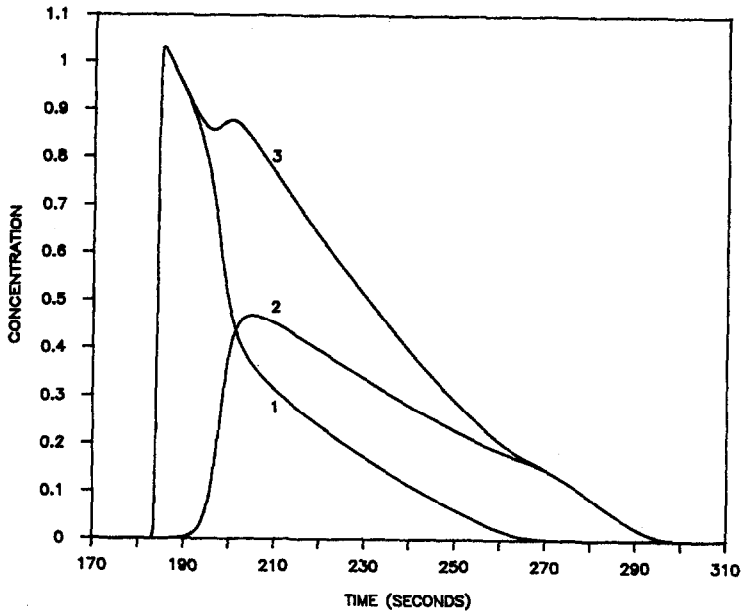
Fig. 5. Chromatogram of a 1:1 mixture (sample size = 5 units; 5% of column saturation limit). (1), (2) and (3) as in Fig. 1.
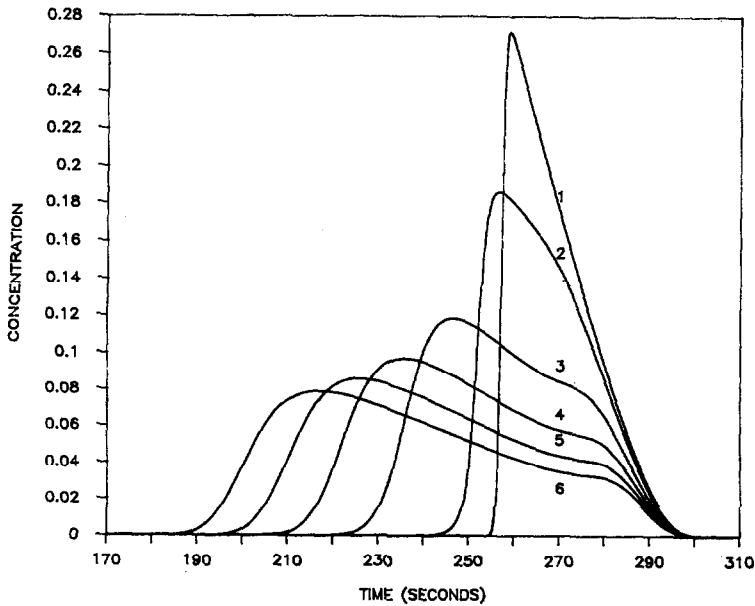

Fig. 6. Superimposed elution profiles of a constant amount (0.5 units; 0.5% of column saturation limit) of component 2 in mixtures when the amount of 1 injected at the same time increases. (1) Area of 1 = 0; (2) area of 1 = 0.5; (3) area of 1 = 1.5; (4) area of 1 = 2.5; (5) area of 1 = 3.5; (6) area of 1 = 4.5.
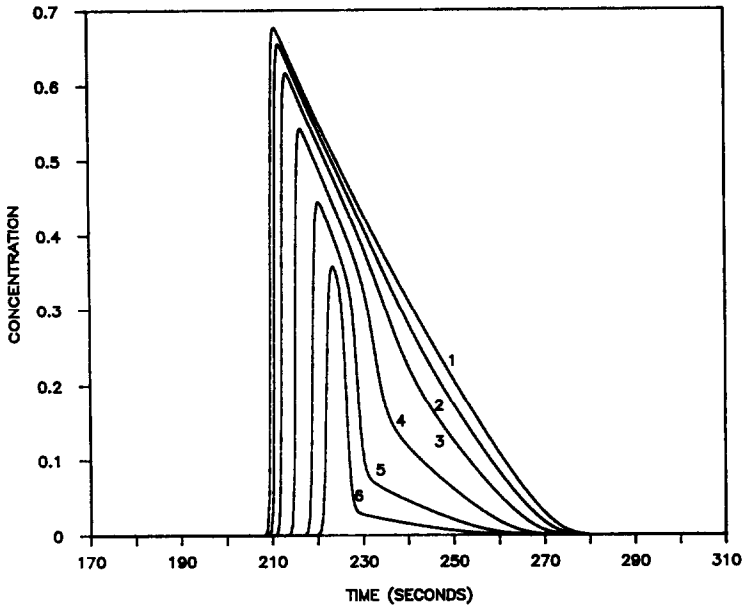
Fig. 7. Superimposed elution profiles of component 1 at a constant sample size (2 units) for mixtures of different compositions of 1 and 2. (1) 2:0; (2) 1.8:0.2; (3) 1.5:0.5; (4) 1:1; (5) 0.5:1.5; (6) 0.2:1.8.
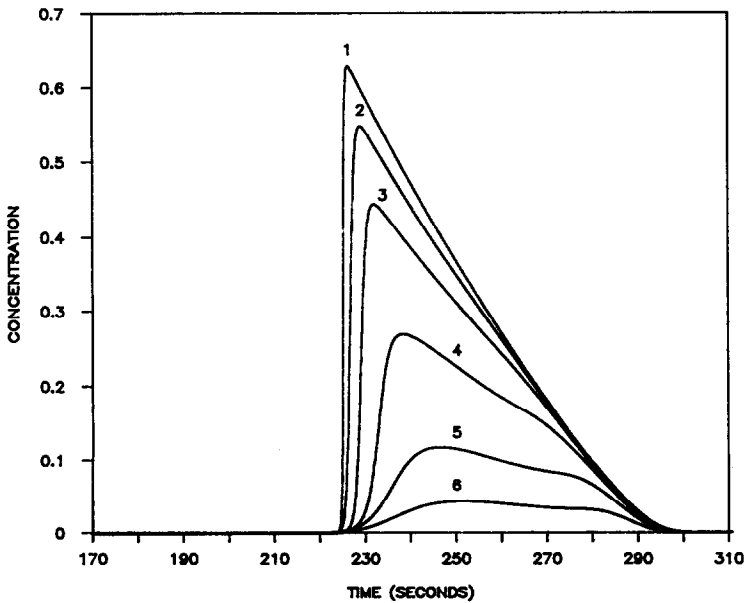

Fig. 8. Same as Fig. 7 for component 2. (1) 0:2; (2) 0.2:1.8; (3) 0.5:1.5; (4) 1:1; (5) 1.5:0.5; (6) 1.8:0.2.

elution profiles of a constant amount of compound 2 equal to 0.5 when the total sample size and composition are varied, so that the amount of 1 injected increases from 0.5 to 4.5 in increments of 1 (the fraction of column saturation increases from 1 to 5%). An elution profile of an amount of pure 2 equal to 0.5 is also shown as a reference. The increasing importance of the "tag-along" effect, due to the increasing amount of 1, is obvious.

Figs. 7 and 8 show the elution profiles of compounds 1 and 2, respectively, when the composition of the injected sample is varied from 1:9 to 9:1 at constant sample size (2 units). The elution profiles of amounts of pure 1 and pure 2 equal to 2 units are also shown. The small range of variation of the band width of 2 is striking. It illustrates the importance of the "tag-along" effect of 1 on 2. On the other hand, the change in profile of 1 from triangular to L-shaped when its concentration is decreased from 100% to 10% illustrates the increasing importance of the displacement of the less retained component of the mixture by the more strongly retained component.

By simple integration of these profiles, it is possible to calculate the production and yield of compound 1 or 2 at any degree of purity. Such calculations have been carried out for various values of the purity between 90 and 99%. The first value would correspond to an extraction problem and the second to the preparation of very pure compounds. Figs. 9–12 show the recoveries of compounds 1 and 2 at 99 and 95% purity as a function of the relative concentration of the corresponding compound in the mixture, for three different sample sizes. As a general rule, the recovery yield decreases with increasing sample size. The recovery of 1 increases very rapidly with its concentration in the feed in the range 0–20% and then levels off until
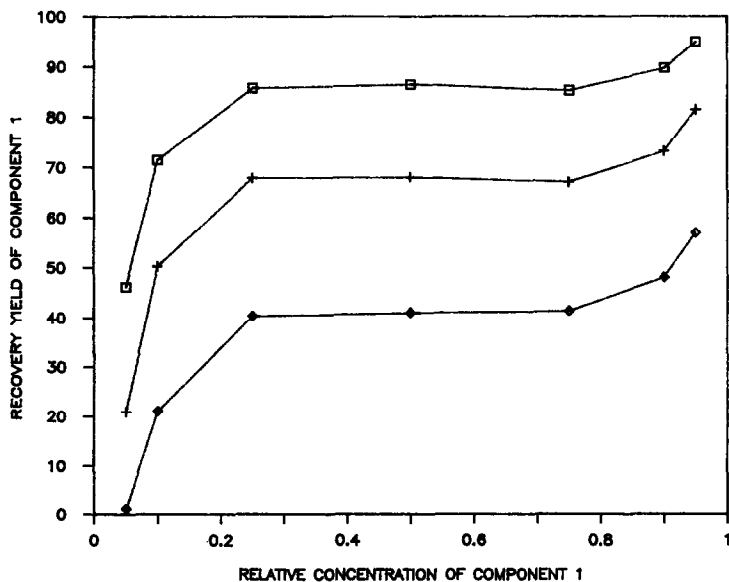

Fig. 9. Recovery of 1 *versus* the relative concentration of 1 in the mixture for three different sample sizes and a required purity of 99%. Sample size: (□) 1 unit, (+) 2 units, (◇) 5 units.
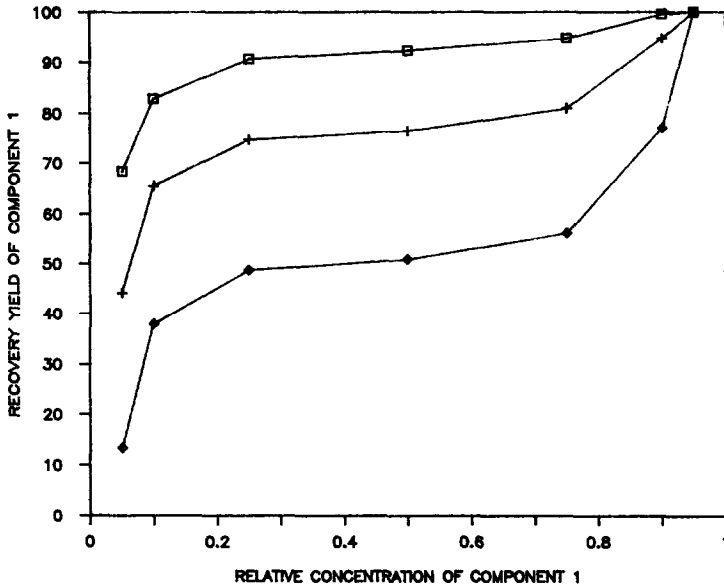
Fig. 10. Same as Fig. 9 for a required purity of 95%.

a feed concentration of about 80% is reached (Figs. 9 and 10). The change in the yield of 2 with concentration is very different. It does not vary rapidly in the 0–20% feed concentration range but increases slowly until a feed concentration of 75–80% is reached, where it begins to rise very rapidly, as it must reach 100% for a feed
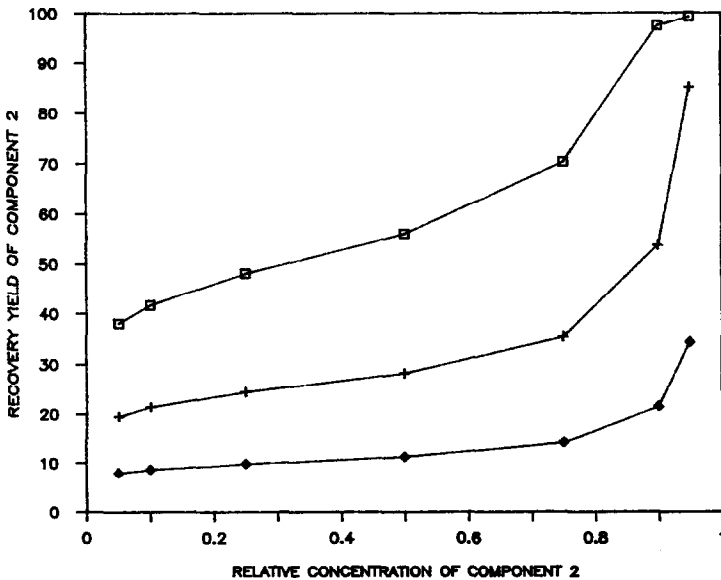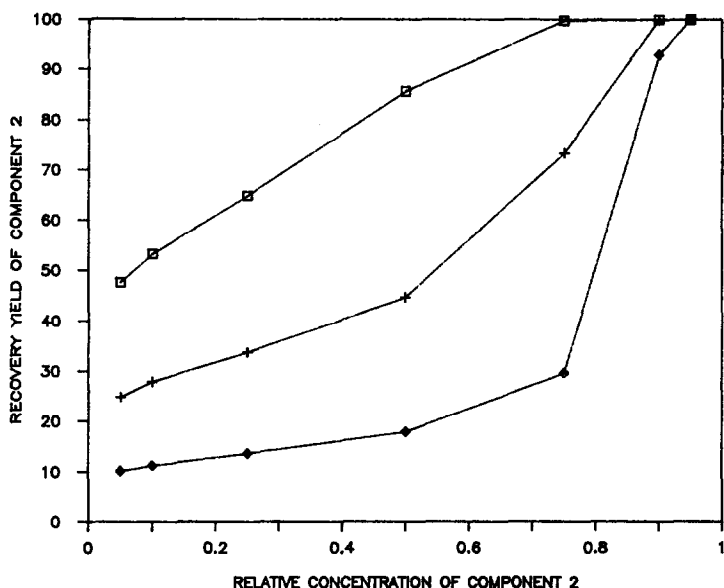

Fig. 11. Same as Fig. 9 for component 2.

Fig. 12. Same as Fig. 10 for component 2.

concentration of 1 unit (exactly 0.95 or 0.99, depending on the required purity). Figs. 9–12 show that the yield of 2 is generally much lower than that of 1. This is due to the influence of the displacement effect of 2 (enhancing the yield of 1) and the long
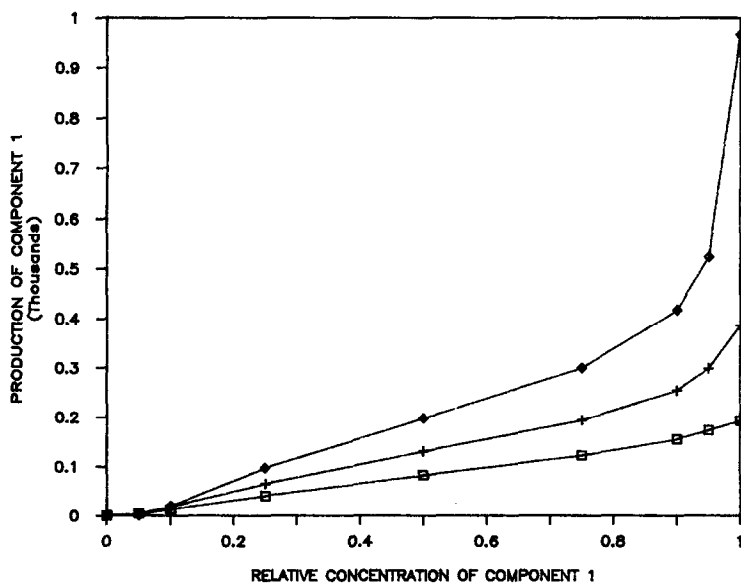

Fig. 13. Production of 1 *versus* the relative concentration of 1 in the mixture for three different sample sizes and a required purity of 99%.
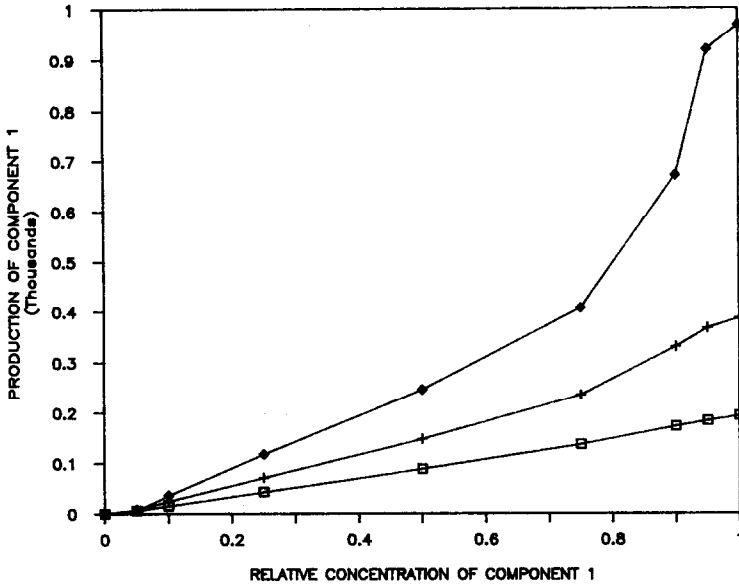
Fig. 14. Same as Fig. 13 for a required purity of 95%.

tailing of 1 (decreasing the yield of 2). At low concentration, 2 will exhibit a poor recovery, because it tags along with 1. As a result, its elution profile is much more spread out than expected.

Figs. 13–16 show the change in the production of compounds 1 and 2 as a function of their relative concentrations in the feed and of the sample size for the
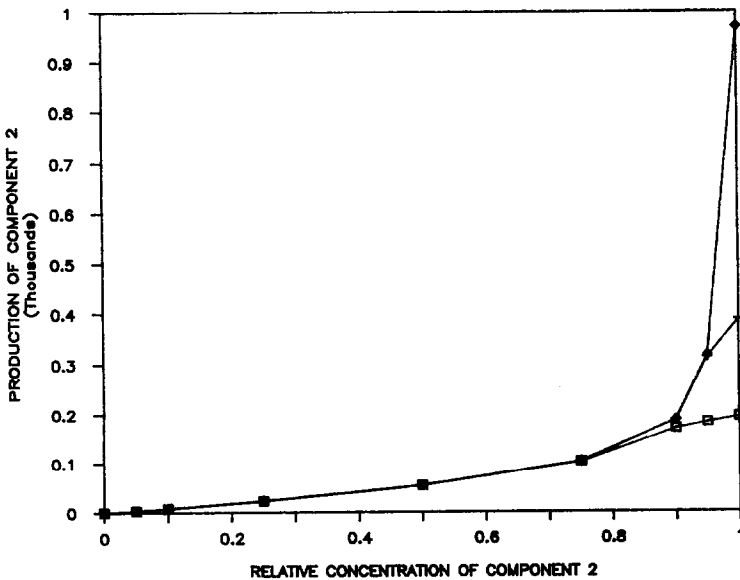

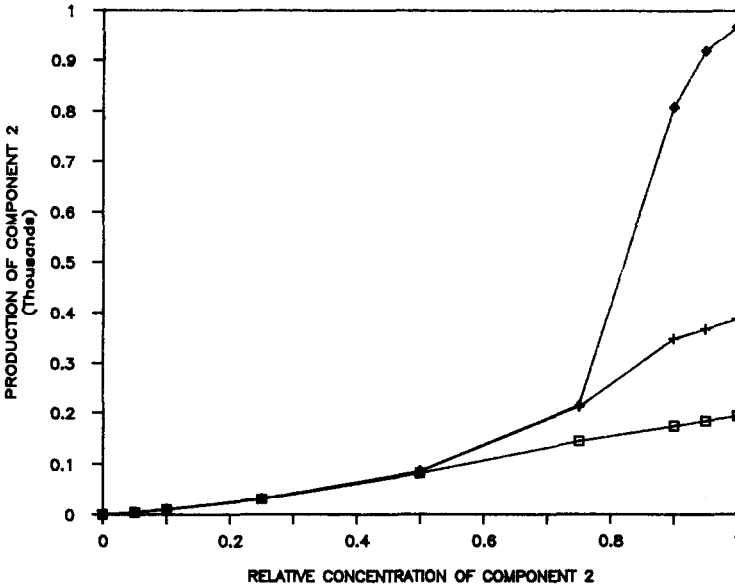Fig. 15. Same as Fig. 13 for component 2.

Fig. 16. Same as Fig. 14 for component 2.

same two degrees of purity, 95 and 99%. The production results from the amount of feed injected (throughput) and the yield. The production of 1 increases rapidly with its concentration in the feed, in the range 0–15% owing to the rapid increase in yield (Figs. 13 and 14). It is almost linear between 20 and 80% (almost constant
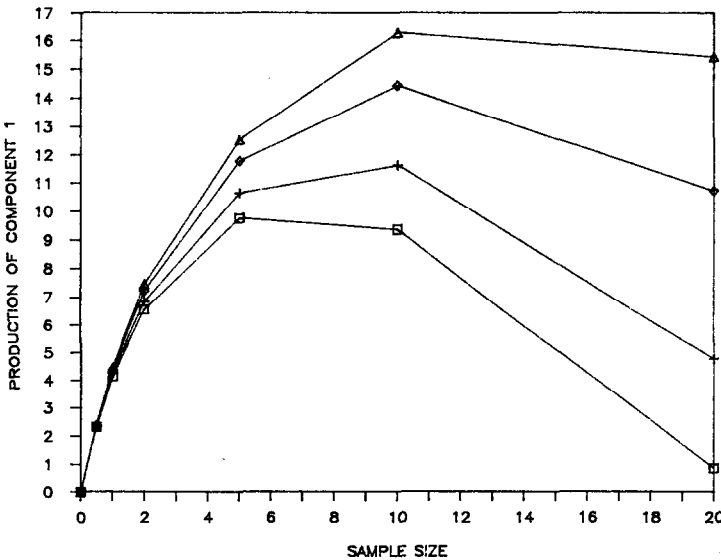

Fig. 17. Production of 1 as a function of sample size for a 1:3 mixture. Purity: (□) 99%; (+) 98%; (◇) 95%; (△) 90%.
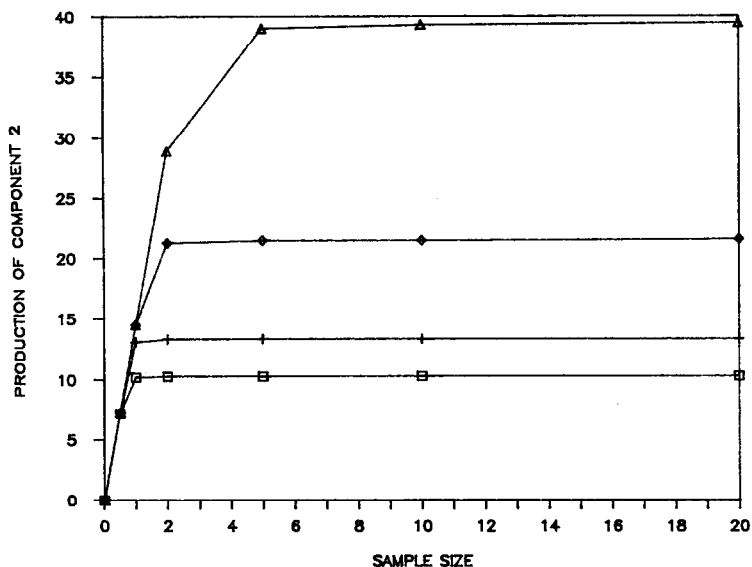
Fig. 18. Same as Fig. 17 for component 2.

yield) and then increases rapidly, as does the yield. The production values for a 100% pure feed are given as a way to relate throughput, production and sample size. It is striking to see in Figs. 15 and 16 that the production of 2 is independent of the sample size until very large values of the feed concentration of 2 are reached. This again is due to the carrying effect of 1. At high feed concentrations, both the production and the yield increase.

The production and yield depend greatly on the sample size. Figs. 17 and 18 show the variation in production of compounds 1 and 2 with sample size for a 1:3 mixture at different purities. It is remarkable that the production of 1 passes through a maximum, corresponding to a throughput that increases with decreasing value of the required degree of purity. On the other hand, the production of 2 increases much more rapidly and levels off for a throughput that is only a fraction (about one fifth) of that corresponding to the maximum production of 1. There is a serious conflict between optimizing the experimental conditions for the production of one component or the other.

CONCLUSION

When the sample size is increased, the recovery decreases, but the production continues to increase well after the two bands have merged. Adsorption is a competitive process, and the two compounds interact. Hence, the presence of large amounts of the more retained solute leads to the onset of a displacement effect. The less retained solute becomes less retained in the presence of the more strongly adsorbed component of the mixture. This is similar in nature to the phenomenon observed in displacement chromatography but with important differences. In elution chromatography, the pseudo-displacer (i.e., the second component) band is too nar-

row and decays too fast for the displacement train to develop fully. Further, the first and second components are injected simultaneously, not one after the other as in true displacement. Nevertheless, displacement takes place to some extent and the yield is always higher for the first-eluting component of the mixture under investigation than for the second.

This observation suggests that it would be interesting to investigate the influence of a third more retained component on the recovery of the first two components. Many mixtures purified by preparative liquid chromatography are complex, and a succession of several overlapping bands is not unusual. It could even prove attractive to inject an auxiliary "pusher", *i.e.*, a band of a compound not contained in the sample and more strongly retained than the last eluted component of this sample, to improve the recovery of the more retained component of a given two-component mixture.

As mentioned above, it is difficult to extrapolate these numerical results to other mixtures, either more complex or merely binary mixtures with larger relative retentions. Qualitatively, it seems, however, that results of the same nature will be obtained. When the relative retention increases, a larger amount of sample will be necessary for the second peak to begin to interfere with the first, but eventually, with large enough loads, this situation will arise. The displacement and the "tag-along" effects will then take place. Their absolute and relative intensities will be different from what has been measured here, however. As the displacement effect is related to enrichment with compound 2 of the stationary phase compared with the mobile phase, we may expect it to become stronger and stronger when the relative retention increases. The front between compounds 1 and 2 will become steeper and steeper and the tails of 1 under 2 less and less important. Similarly, the "tag-along" effect will decrease.

These effects are in agreement with the limited data reported by Eble *et al.*[20]. The profile of their component 1 (HET) shows a very steep front and all the characteristics of a strong displacement effect. This effect appears to be even stronger than that predicted here (Figs. 1, 4 and 5), which is in agreement with the larger α (1.7 instead of 1.09). The tail of HET behind the front of HPT was not reported (ref. 20, Fig. 5) because the individual profiles were not determined, so we cannot make a comparison with the program results, but the difference between the profiles of compound 2 (HPT), pure or in a mixture with HET, shows a slight decrease, similarly to what is observed in our work (Fig. 8, curves 1 and 2), pointing to the onset of a "tag-along" effect. The influence of the relative retention on the band profiles is under investigation and will be reported soon[21].

Finally, it should be emphasized that no validation of the results of the numerical calculations made using a model such as that used here is really necessary, because the model is based on first principles and the assumptions made to solve it are very minor (the mobile phase is not compressible, its density is the same as that ·of the sample, the diffusion coefficients of the eluite in the mobile phase do not depend on concentration in the range used). The only significant discrepancy could come from artifacts introduced by the numerical methods. The fact that thousands of aircraft fly daily, whose wings have been calculated by these methods applied to an equation system (the aerodynamic system) of the same type as the chromatographic system (eqns. 1–4), is witness to the value of these calculations. Nevertheless,

experiments are under way to compare experimental and predicted profiles, with the aim of deriving the isotherms from this comparison. Such a direct derivation would considerably accelerate a long and tedious experimental process, as competitive isotherms are required to run the programme. In the case of a single eluite, the agreement between experimental band profiles and profiles calculated from isotherms measured by an independent method is excellent[18].

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