

Study of the interaction between two overloaded bands injected successively in non-linear chromatography

M. ZOUBAÏR EL FALLAH and GEORGES GUIOCHON*

**Department of Chemistry, University of Tennessee, Knoxville, TN 37996-1600 and Division of Analytical Chemistry, Oak Ridge National Laboratory, Oak Ridge, TN (U.S.A.)*

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ABSTRACT

Band interaction in non-linear chromatography was studied using a delayed injection procedure which permits the easy adjustment of the degree of interference regardless of the real selectivity between the two compounds studied. This work represents an investigation of the mechanism of band interference, independent of the separation process. Special attention was given to the converse displacement effect, which corresponds to the displacement of the more strongly retained compound by the less retained one. Experimental evidence of this converse displacement is shown using two different systems. The intensity of this displacement is discussed in relationship to the concentration of the displacer and the waiting time between the two injections. Calculated band profiles, obtained with the semi-ideal model of chromatography and competitive Langmuir isotherms, are compared with experimental profiles. With one system good agreement between experiments and simulations was found whereas the other system studied showed significant deviations from the competitive Langmuir isotherm model.

INTRODUCTION

Non-linear effects have been observed and reported systematically in preparative liquid chromatography when the column is operated under overloaded conditions [1–10]. So far these effects have been noticed mostly when high concentration bands of binary mixtures are introduced into a column, although gradient elution, systems peaks [11,12] and displacement chromatography [13,14] are other non-linear effects. In overloaded elution, the displacement effect [1–5] and the tag-along effect [7,10] are the most important of these non-linear effects. They are both due to the fact that the velocity associated with a certain concentration of a given component depends on the concentration of the other components locally present [6,7].

These non-linear effects occur whenever two or more different species compete for adsorption on the same sites or, more generally, for interaction with the stationary phase. At low coverages of the adsorbent surface (*i.e.*, at low sample sizes), the effects of this competition cannot be observed. At high surface coverages, however, the competition becomes important and strongly affects the propagation of the band system of a mixture. This influence is especially important for the individual elution profiles of incompletely resolved bands.

As the ability to make accurate predictions of band profiles in overloaded chromatography is critical in any attempt to optimize the operating parameters, a proper understanding of the mechanism of band interactions and especially of the displacement and tag-along effects is very important [15–18]. Theoretical predictions must be compared with experimental results. So far, such comparisons have been performed by injecting high-concentration samples of binary mixtures under conditions where eluted bands are incompletely resolved [4,5]. Then, an accurate determination of the individual band profiles is required. It cannot be made by on-line analysis, which cannot be carried out at a high enough frequency. The best method available involves the collection of *ca.* 100 fractions during the elution of the mixed band and the analysis of these fractions [4,5].

The study of band interactions does not require the simultaneous injection of the two compounds, however. They only need to interact somewhere in the column. This can be achieved very simply by injecting the more strongly adsorbed compound, waiting a certain time and injecting the less retained compound. Depending on the delay between the two successive injections, we have two interesting possibilities: either the faster band of the less retained compound reaches the slower band but does not pass it completely, which leads to the elution of a mixed zone, or the less retained compound passes completely through the more retained one and the resulting chromatogram shows two separated bands. Because of their temporary interaction, however, the profiles of these bands are different from those obtained with the same samples injected in the reverse order. As the bands are resolved, their profiles can be derived simply from the detector signal after proper calibration.

The former situation permits the study of the interaction between a concentration shock layer and a diffuse front, independently of the separation process, under a variety of experimental conditions. The latter situation would be of great potential interest if a large change in the band profiles took place and if either the new band profiles or the change in band profiles due to the band interaction could be related simply to the parameters of the competitive adsorption isotherms of the compounds studied.

Unfortunately, when the faster band has completely passed through the slower one, the band profiles are almost unchanged. Only small shifts in the retention times of their fronts and tails are observed. Of course, these retention-time shifts are related to the band interaction, but it is impossible to extract from these shapes the accurate information needed concerning the nature and the intensity of the interactions, *i.e.* the competitive adsorption isotherms and their parameters.

Obvious changes in the band profiles occur only under conditions where a two-component mixed zone is eluted. This case is discussed in this paper, where the results of theoretical calculations [10,19] are compared with actual profiles. Any pair of compounds can be used, no matter what their actual relative retentions are, to obtain a system of incompletely resolved bands. We may choose these compounds so that one of them can be detected at a wavelength where the other is transparent.

THEORY

The calculations made in this work are based on the use of the semi-ideal model of chromatography and of the calculation programs developed previously in our

laboratory [10,19–21]. All details regarding the theoretical approach and the assumptions made can be found in several recent publications [22,23]. The semi-ideal model assumes that the mass transfers between phases in the column are fast so that the concentrations of any compound in the two phases are never much different from their equilibrium values. The influence of the finite kinetics of mass transfer is accounted for by the use of an apparent coefficient of axial dispersion, which is related to the column efficiency at very low solute concentrations [24–26].

The simulation program used for the calculation of the individual elution band profiles in the case of the delayed injection of two compounds is identical with that used in previous publications to calculate the individual elution bands of the components of a binary mixture [10,20]. In these computations, competitive Langmuir adsorption isotherms were used. The values of the space and time increments were adjusted to account for the finite column efficiency [23,27,28]. Only the injection subprogram had to be changed to provide for the delayed injection of the two solutes. The final program was written in Pascal and run on the VAX 8700 of the Computer Center of the University of Tennessee.

Excellent agreement between the calculated individual band profiles and those determined experimentally has been demonstrated in several previous papers [4,5,29–31]. This agreement has been reported for single- [29,30] and two-component samples [4,5], with a pure mobile phase or a solution of a weak organic solvent (acetonitrile or methanol) in water and also when the mobile phase is a solution of a strong organic modifier in a weak organic solvent [31].

EXPERIMENTAL

Equipment and injection procedure

An HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.) equipped with a diode-array detector was used. The mobile phase was used as solvent A. Solutions of sample components in the mobile phase were used as solvents B and C. Injections were carried out by programming step gradients of solvent B or C followed by a return to solvent A in the time-table monitoring the gradient system. These pump injections were reproducible and led to acceptable rectangular injection profiles provided that they lasted long enough (at least 1 min, *i.e.*, more than 1-ml injections at flow-rates above 1 ml/min).

In order to avoid saturation of the UV detector, we made very large-volume injections (not less than 2 ml) and used dilute solutions. For the compounds selected, this led to values of the loading factor which always exceeded 1% (the loading factor is the ratio of the amount of one component injected to the column saturation capacity for this component).

Column and chemicals

A 25×0.46 cm I.D. column was packed in the laboratory with YMC-gel 120A, 5- μ m particles of octadecyl silica (YMC, Morris Plains, NJ, U.S.A.). The column void volume measured as the retention volume of uracil was 2.66 ml, its porosity, ϵ , was 0.64 and its phase ratio $[(1 - \epsilon)/\epsilon]$ was 0.56. The mobile phase used was acetonitrile (J.T. Baker, Phillipsburg, NJ, U.S.A.)–water (25:75, v/v). All experiments were performed with a mobile phase flow-rate of 1 ml/min. The column was conditioned at 40°C for several hours before use.

In order to simplify the experimental study of overloaded peak interactions, we chose 2,4-dimethylphenol (DMP) (Aldrich, Milwaukee, WI, U.S.A.), which adsorbs at 295 nm, as the slower compound. The faster compound was either 2-phenylethanol (PE) or 2-methylphenethyl alcohol (MPA) (both from Fluka, Ronkonkoma, NY, U.S.A.), which do not show any significant UV absorption at this wavelength.

Isotherm determinations

Single-component isotherms were determined by frontal analysis and retention-time methods. The former method uses the injection of increasing concentration steps and derives isotherm points from the retention times of the breakthrough curves [32]. The latter method, based on the closed-form solution of the ideal model of chromatography [33], derives the two parameters of the best Langmuir isotherm from the retention time of the front of a high-concentration band and the retention time of a very small band, small enough to be eluted under the conditions of linear chromatography [31]. Data were derived from several bands corresponding to different sample sizes.

Data acquisition

The raw data were acquired with the HP 9133 data station. Chromatographic data were then transferred to the VAX 8700 of the University Computer Center for further treatment. Using calibration data acquired from standard solutions, the absorbance profiles were converted into concentration profiles for comparison with calculated profiles.

Separate injections of solutions of each compound were made under the selected experimental conditions. From their retention times, the time between two successive injections or the waiting time needed in order for band interference to occur was obtained. The whole range of waiting times was investigated, from zero (simultaneous injections) to the value for which the front of the first band coelutes with the end of the second band tail. The amount of DMP injected was kept constant throughout the study. A 4-ml pulse of an 11.39 mM solution was pumped into the column. This corresponds to a loading factor of 2.17%.

RESULTS AND DISCUSSION

Results of the calculations

Although we deal in a later section with the comparison between calculated and experimental data, all the input data for the calculations whose results are reported here (*i.e.*, mobile phase flow-rate, phase ratio, column length, isotherm parameters, etc.) were chosen to be as close as possible to the experimental values. Calculations made with our program permit an easy simulation of the different possible degrees of interaction, depending on the value of the injection parameters, *i.e.*, the waiting time, t_w , between the two injections and the concentration of each compound, and a study of the effects of these parameters on the band interference. The waiting time controls the degree of band resolution on elution and the sample sizes the intensity of the non-linear effects.

Bands completely separated

This situation is easily obtained if the two compounds have a large relative retention on the phase system used. It suffices to wait a short period of time between the injection of the slower component and that of the faster one. Fig. 1 compares the band profiles obtained in such a case (solid lines) with those calculated for successive injections of the two components in the reverse order (dashed lines), *i.e.*, when the faster component is injected first. The same profiles in dashed lines would be obtained if we assume no band interaction, *i.e.*, single-component adsorption isotherms, in the simulation whereas the solid line profiles were calculated assuming Langmuir competitive isotherms.

In the calculations for Fig. 1, the concentration of the faster component was ten times higher than that of the slower component and the waiting time was 4 min. The essential effect of the interaction between the two bands seems to be a significant shift

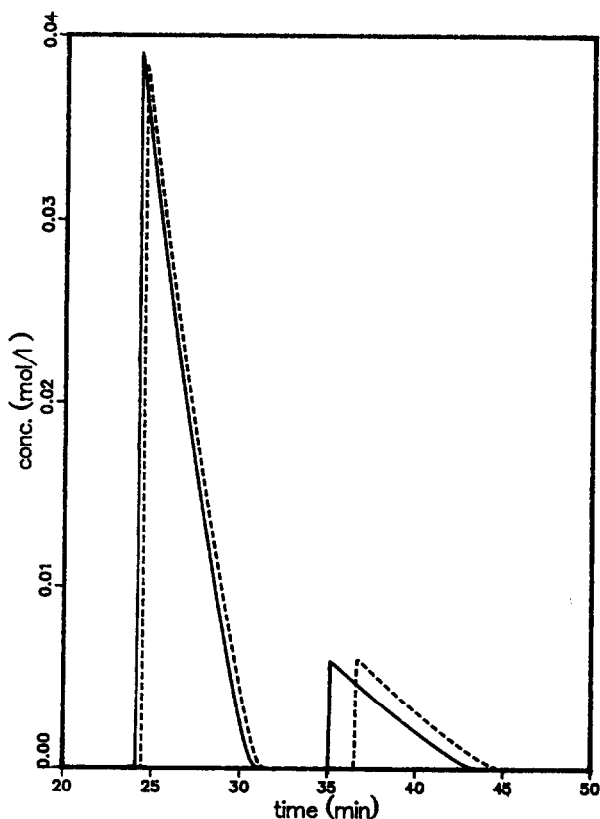


Fig. 1. Interaction between two independent bands. Calculated chromatogram for the successive injection of the slower and the faster moving compounds, with a waiting time $t_w = 4$ min. Injection characteristics: faster compound, $V_1 = 2$ ml, $c_1 = 113.9$ mM; slower compound, $V_2 = 1$ ml, $c_2 = 11.39$ mM. Feed composition: $R = c_1/c_2 = 10$. Solid lines: Langmuir competitive isotherms ($a_1 = 13.82$, $a_2 = 26.13$, $b_1 = 5.26$, $b_2 = 18.56$; a is dimensionless, b in ml/mM). Dashed lines: no competition was assumed (single Langmuir isotherm), or superimposed chromatograms of independent injections. The two bands are completely resolved.

of the whole profile towards shorter retention times. Both bands move faster than when there is no competition. The shift is much larger for the slower than for the faster component.

The waiting time between the two injections was found to have no significant effects on the band profiles over the whole range of waiting times for which band interference has ended before elution of the second band. On the other hand, the amount of one component affects the retention time shift of the band of the other. The profiles obtained for the same amount of the slower component and increasing amounts of the faster component are shown in Fig. 2.

The retention time shift of the band of a constant amount of the slower component increases with increasing amount of the faster component. There is a slight, nearly negligible change in the shape of its tails and the second band becomes slightly shorter (Fig. 2). A similar but much smaller effect is observed for the faster component band profile. Its retention time shift increases at a constant amount of faster component with increasing amount of the slower compound injected.

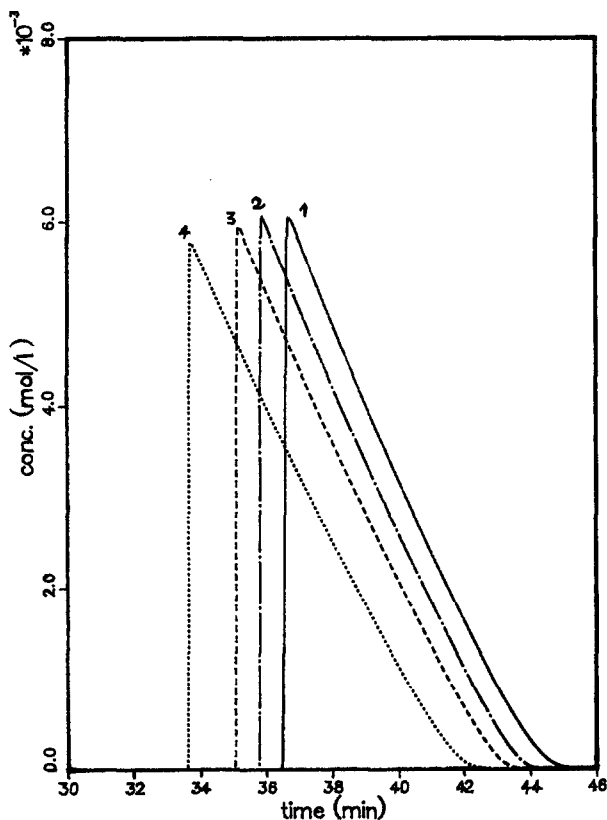


Fig. 2. Influence of the concentration of the faster compound on the elution band profile of the slower one. Same competitive Langmuir isotherm as for Fig. 1. Same injection volumes and waiting time. (1) No faster compound injected; (2) relative concentrations of the two samples = 5; (3) relative concentrations of the two samples = 10; (4) relative concentrations of the two samples = 20.

These phenomena were calculated using the competitive adsorption model. In principle, it would be possible to correlate the retention time shift of one compound band with the loading factor for the other. This could provide a method for the determination of competitive isotherms. The effects calculated are weak, however, and the errors made in the determination of retention data are too large to permit the use of the retention time shift for a study of the possible deviations of the competitive isotherms from the Langmuir model.

Elution of a two-solutes mixed zone

Displacement of the faster solute by the slower one. When the waiting time is chosen so that the front of the faster moving band passes the front of the slower one while the two bands are not fully resolved, the two individual bands profiles are similar to the conventional profiles obtained for the overloaded bands of a binary mixture of closely related compounds, for which the relative retention is not very different from unity [1-5].

Fig. 3 shows the calculated chromatogram obtained in such a case. It is characterized by two concentration shock layers on the faster moving band, one at the front and one at the rear. The latter results from the displacement effect of the slower moving component. As we observed above, the injection procedure provides the possibility of such strong band interaction even if the relative retention is very large. Fig. 3 shows clearly that the displacement effect is due to band interference, to the fact that the velocity associated with a certain concentration of a compound depends (through the competitive isotherm) on the concentration of the other compounds. In other words, a displacement effect of the other components of a system (sample components or mobile phase additives) accompanies a positive concentration shock (or shock layer) of any component. This effect arises because the concentration surge of this component crowds the other ones out of the stationary phase.

As this type of band interaction has already been discussed [8,9], we shall focus on the other case, which has not been reported previously and whose observation would be further proof of the competition between compounds for interaction with the stationary phase.

Displacement of the slower compound by the faster one. When the waiting time is such that the band of the faster compound catches up with the band of the slower one but the front of the slower moving band is still eluted before the front of the faster one, the calculated chromatogram shown in Fig. 4 is typically obtained.

Again, the first-eluted band exhibits two shock layers, the second shock layer marking the beginning of the elution of a mixed zone, but in this instance the chromatogram differs from the previous one (Fig. 3) in two important ways. First, the first eluted band is that of the more strongly retained, slower moving compound (Fig. 4). Hence the second shock layer occurs on the rear of the slower component. Second, this shock layer is positive (Fig. 4), whereas that on the rear of the faster moving band in the previous case was negative (Fig. 3). It produces an increase in the concentration of the slower compound, which physically corresponds to the displacement of the more strongly retained component by the other one.

Such a displacement effect is unlikely to occur in practice in preparative chromatography, but the mutual displacement of solutes is well known in separations and purifications involving adsorption, such as in pressure swing adsorption (PSA)

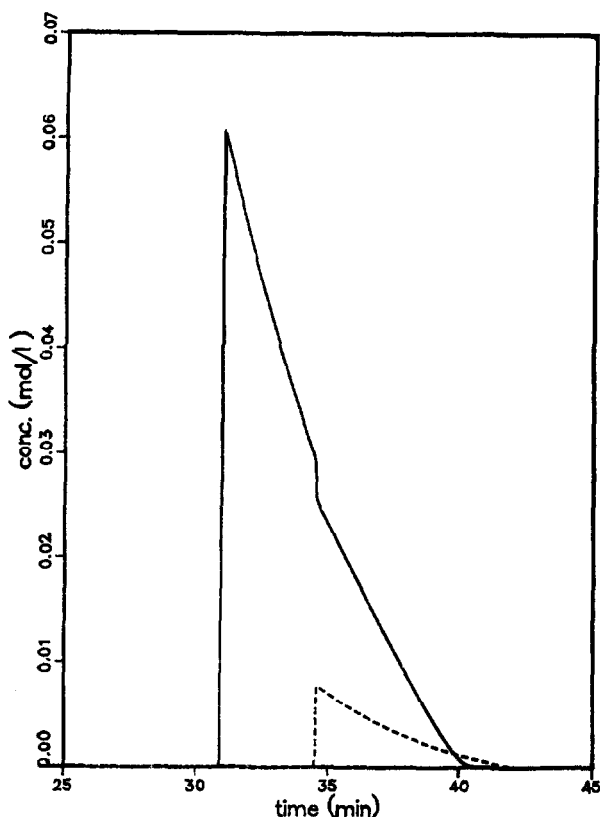


Fig. 3. Influence of the waiting time between the two successive injections on the nature of the displacement effect. Same competitive Langmuir isotherm and concentration as in Fig. 1. Volumes of solution injected: $V_1 = V_2 = 2$ ml. Relative concentrations of the two samples: 10. Waiting time: 12 min. The two bands interact and there is an obvious displacement effect of the faster one by the slower one.

[34,35]. In Fig. 4, all the conditions are identical with those in Fig. 3, except the waiting time. Hence, the displacement of the slower compound by the faster one is part of the interaction pattern involved in the delayed injection experiments as much as the displacement of the faster compound by the slower one. Although in Fig. 3 the only apparent band interaction at the column outlet is the displacement of the faster compound by the slower one, the converse displacement effect has taken place previously, somewhere in the column.

This pattern of interference is general and explains the results shown in Figs. 1 and 2 in the case where the eluted bands are well resolved. The first interaction to take place, the displacement of the slower compound by the faster one, narrows the slower moving band by making its tail move faster than that of a pure compound band. When the faster moving band passes over the slower one, the usual displacement effect takes place and the front of the slower band moves faster. Accordingly, both the front and the rear of the band are accelerated successively, but at different times, by different interaction mechanisms. The result, however, is only a shift of the whole band.

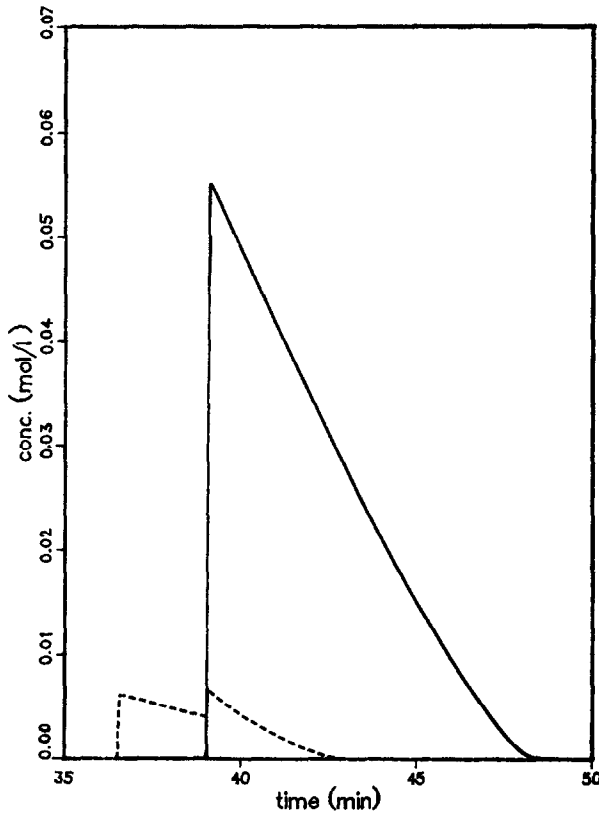


Fig. 4. Same as Fig. 3, except waiting time = 18 min. The two bands interact and there is a converse displacement effect of the slower one by the faster one.

Fig. 5 shows the effect of the waiting time on the intensity of the converse displacement effect when the two sample amounts are kept constant. Fig. 5 shows the profile of the slower band when the concentration of the faster compound is ten times that of the slower one, in order to maximize the effect.

As the waiting time decreases, the faster band reaches the slower one earlier and earlier. Note that the front of the faster band (not shown in Fig. 5) is eluted in the same time as the second shock layer of the slower band (only shown in Fig. 5). When the waiting time, t_w , decreases, the importance of the band interaction increases, the desorption of the slower compound by the faster one occurs earlier and the retention time of its tail decreases, as the interaction lasts longer. We note that when t_w decreases, the ratio of the concentrations of the slower compound just after and before the shock is almost independent of the waiting time. Hence this ratio can be used as a measure of the inverse displacement intensity. A similar ratio has been used to measure the intensity of the normal displacement effect in non-linear chromatography [7,9].

Fig. 6 shows the effect of increasing the amount of faster compound injected at a constant amount of slower compound and constant waiting time.

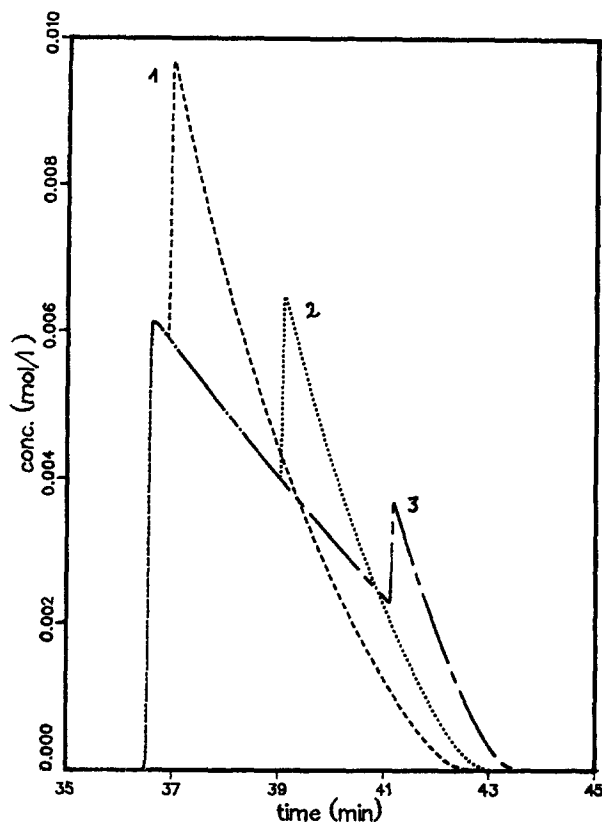


Fig. 5. Influence of the waiting time between the two successive injections on the displacement of the slower compound by the faster one. Same competitive Langmuir isotherm and concentrations as in Fig. 1. $V_1 = V_2 = 2$ ml. Relative concentrations of the two samples: 10. Waiting time: (1) 18 min; (2) 20 min; (3) 22 min.

When the concentration of the faster compound is increased, its band moves faster and, at a constant waiting time, the retention time of its front decreases. At the same time, the relative concentration of the two compounds increases, the ratio of the solute concentrations after and before the shock increases and the intensity of the converse displacement effect increases.

Isotherm determinations

In order to compare quantitatively the results of the calculations with those of experiments, we need to know the adsorption isotherm of each compound used. We selected DMP as the slow-moving compound. Depending on the experiment, either PE or MPA was the fast moving compound. This choice has the advantage that the slower compound can be detected in the presence of the faster one, as DMP absorbs light at 295 nm whereas neither PE nor MPA does.

Two methods were used for the determination of the equilibrium isotherms, frontal analysis (FA) and the retention time method (RTM). FA is a classical method

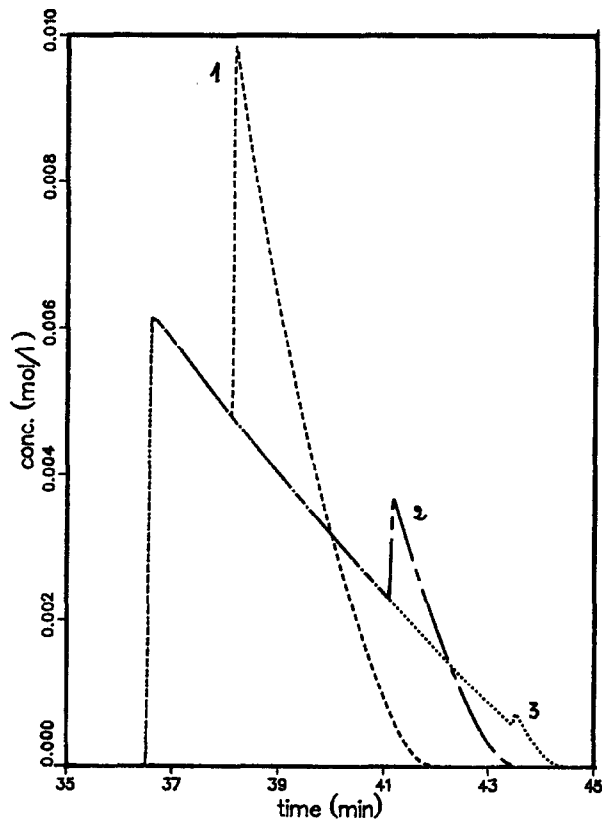


Fig. 6. Influence of the concentration of the faster compound on the elution band profile of the slower one. Conditions as in Fig. 5. Relative concentrations of the two successive samples: (1) 20; (2) 10; (3) 5.

known to be accurate for the measurement of adsorption isotherms in HPLC systems which give convex upwards isotherms [36,37]. RTM [31] is based on the analytical solution of the ideal model of chromatography [33]. It has been shown that when the column efficiency exceeds 5000 plates, the parameters estimated by RTM are in good agreement with those obtained by other methods [31]. The column we used has more than 10 000 theoretical plates, which justifies the use of RTM. This method has the further advantages of being both fast and economical regarding the amount of compound needed to perform a determination.

The FA data were fitted to the Langmuir model [$q = aC/(1 + bC)$ where q and C are concentrations of the compound in the stationary and mobile phase, respectively, and a and b are numerical coefficients]. The values of a obtained by the two methods were found to be in close agreement and slightly different (2%) from the value derived from the capacity factor of small samples ($a = k'/F$, where F is the phase ratio). RTM gave a value of b that was less affected by the concentration range of the data with which the fit was performed than the value given by FA. The value of b given by RTM changed by less than 5% when the amount injected was increased four-fold, whereas decreased by 20% for the FA data. This shows a slight deviation

of the Langmuir model from the exact adsorption behavior of the compounds. The best values of a and b for the three compounds studied in this work are given in Table I.

When using the a and b values obtained from RTM, the simulated individual band profiles for the three compounds were very close to the experimental ones. Fig. 7 compares the band profile recorded for a large amount of DMP with that calculated with the coefficients in Table I. There is excellent agreement. Similar agreement was observed for the other two compounds.

The ratio of the saturation capacities of the two compounds used in this study is markedly different from unity. These ratios are $Q_s(\text{MPA})/Q_s(\text{DMP}) = 1.86$ and $Q_s(\text{PE})/Q_s(\text{DMP}) = 1.30$. The corresponding values of the relative retention are 1.89 and 3.64, respectively.

In order to take into account the competition of the compounds for adsorption, a competitive adsorption model should be adopted. One of the most widely accepted theories of multi-component adsorption is based on the concept of an ideal adsorbed solution (IAS) [38–42]. Its major advantage is that only single-component data are required. Among several IAS competitive adsorption models, the competitive Langmuir isotherm [41] is the simplest and the most popular, although the IAS theory shows that it is thermodynamically consistent only if the two solute saturation capacities are identical [42]. This condition, which is seldom fulfilled in real cases, applies to gas–solid systems. Its extension to liquid–solid adsorption is not straightforward. However, it has been shown that in many instances the competitive adsorption data obtained by frontal analysis of a binary mixture [43] and by the ‘simple wave’ method [44] were fairly well accounted for by a Langmuir competitive isotherm. Conversely, excellent agreement has been reported between the individual band profiles of the components of binary mixtures measured experimentally and those calculated using the competitive Langmuir isotherm model [4,5]. As the competitive Langmuir isotherm model is also convenient, we have used this model as an approximation to actual isotherms in our calculations.

Experimental results

As experimental proof of the converse displacement effect has never been reported before, in this work we mainly investigated the displacement of the slower compound by the faster one.

TABLE I
VALUES OF THE SINGLE-COMPONENT LANGMUIR ISOTHERM COEFFICIENTS DETERMINED BY RTM

See text for RTM procedure.

Compound	a	b
PE	7.18	3.92
MPA	13.8	5.26
DMP	26.1	18.6

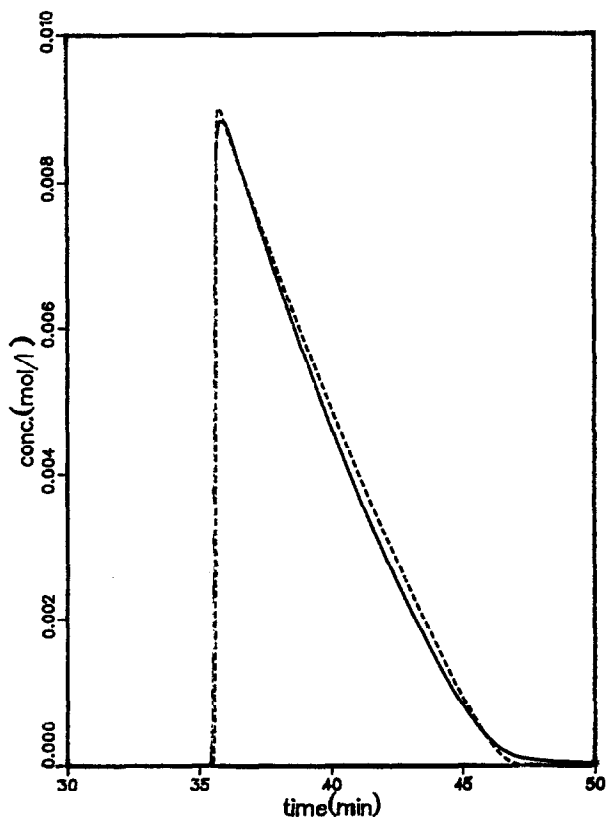


Fig. 7. Comparison between the band profile recorded for a 4-ml sample of an 11.4 mM solution of DMP (solid line) and the profile calculated with the isotherm parameters in Table I (dashed line).

Fig. 8 shows a typical chromatogram obtained with the injection procedure described above (see Experimental). A solution of DMP is first injected and after 28 min a solution of PE (see caption) is injected. The chromatogram shown in Fig. 8 was recorded at 240 nm, where both compounds absorb UV radiation.

Fig. 8 shows that there is some interaction between the two bands. The top of the second band profile (faster moving compound) is different from the top of the profile obtained for an unperturbed injection (see Fig. 7). The change in the band profile of DMP is small, however. The dip in its rear profile is due to an impurity in the PE sample.

Effect of the waiting time on the intensity of the displacement effect

Figs. 9 and 10 show the superimposition of the experimental (curve 1) and simulated (curve 2) concentration profile of the elution band of DMP recorded at 295 nm with waiting times of 23 and 18 min, respectively. In both instance the faster moving component was MPA and its concentration was ten times higher than that of the slower moving compound.

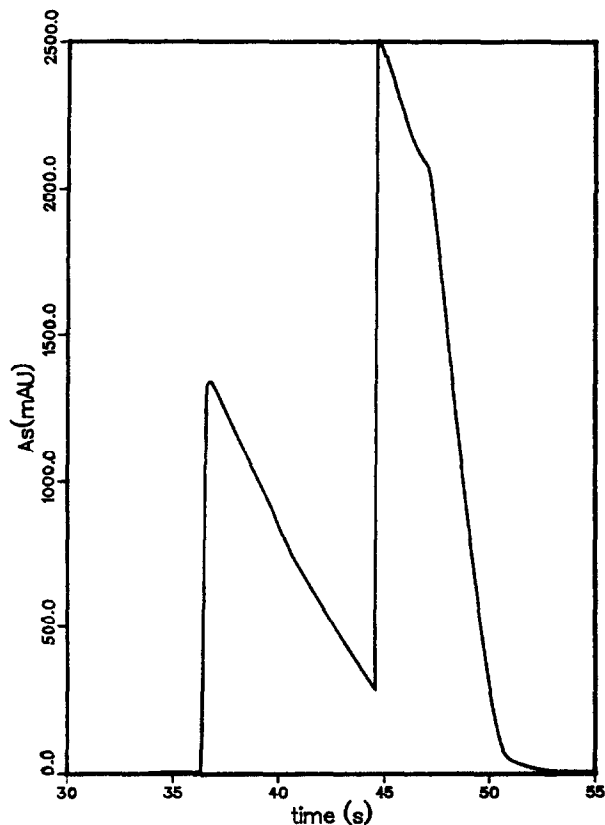


Fig. 8. Experimental chromatogram obtained on injecting first a sample of DMP ($V_2 = 4$ ml of a solution of DMP in the mobile phase, $c_2 = 11.39$ mM), then, after a 28-min waiting time, a sample of PE ($V_1 = 4$ ml of a solution of PE, $c_1 = 6c_2 = 68.34$ mM). UV detection at 240 nm. Flow-rate, 1 ml/min. For other conditions, see text.

In both Figs. 9 and 10 there is excellent general agreement between the behavior of the experimental and calculated band profiles of DMP. The only prominent difference is the small shift in retention time observed. However, this is minor with regard to the total time (less than 1 min in 36 min, *i.e.*, 3% in Fig. 9 and, less than 1% in Fig. 10). Also, the tip of the experimental band is blunter than that of the calculated profile. Except for the top of the two peaks, however, the experimental and calculated profiles are nearly identical. We also note that the only difference in the rear of these profiles is the tailing of the experimental peak, possibly an indication that the adsorbent surface is not completely homogeneous [45].

As we have seen in the numerical study of the displacement effect reported above, when the waiting time decreases, the interaction between the two bands becomes more important. The retention time of the band tail decreases and the concentration at the front shock increases. A quantitative comparison between the profiles in Figs. 9 and 10 shows that the ratios of the concentrations after and before the shock

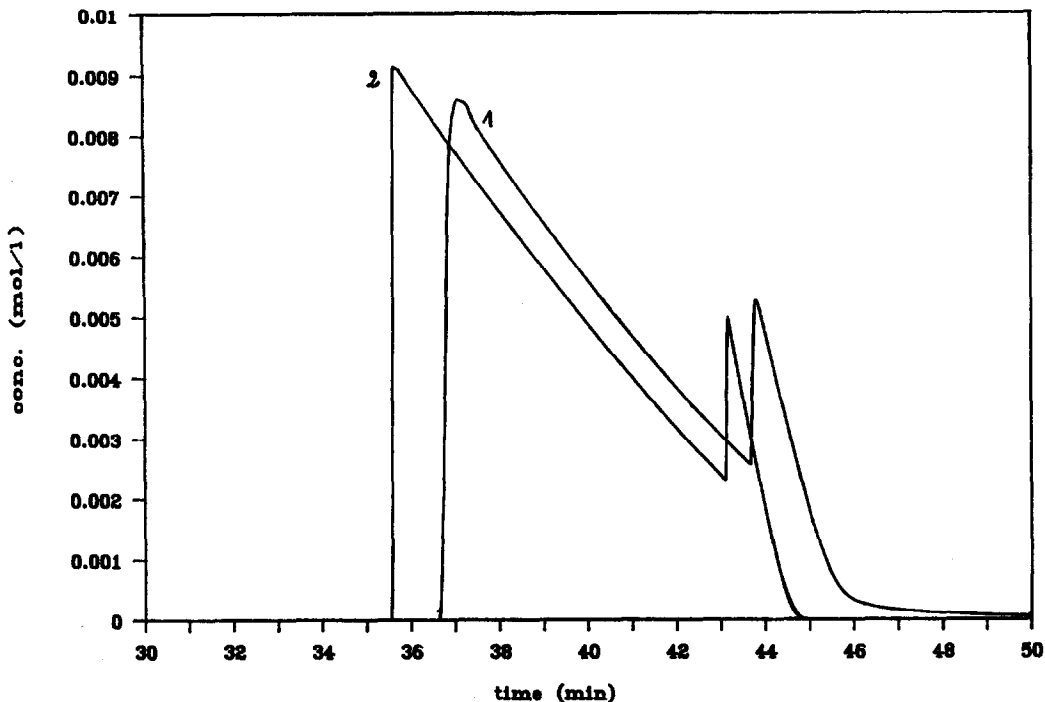


Fig. 9. Comparison between (1) experimental and (2) calculated elution profiles for the slower compound. Experimental conditions: first injection of DMP, $V_2 = 4$ ml, $c_2 = 11.39$ mM; waiting time, 23 min; second injection of MPA, $V_1 = 6$ ml, $c_2 = 113.9$ mM. Calculated profile with the same Langmuir competitive isotherms as in Fig. 1.

on the two experimental or the two theoretical profiles are equal to within less than 2%. In each figure these ratios differ by less than 7%. This difference between experimental and theoretical results is small, given the several possible sources of error (injection profiles, detector calibration, flow-rate and temperature fluctuations).

Influence of the faster compound concentration on the intensity of the displacement effect

This influence was studied by performing successive experiments using higher and higher concentrations of the faster compound with a constant-volume injection, while keeping constant the amount of the slower compound and the waiting time (Fig. 11). The faster compound in this instance was PE. Its concentration in the feed was increased so that the relative amount of PE injected to that of DMP was 3, 6 and 10 for the three chromatograms.

As expected, when the concentration of the faster compound is increased, the front of its band moves faster and the intensity of the displacement effect increases. Accordingly, the retention time of the slower compound band tail decreases and the ratio of its concentrations on the two sides of the shock increases. Hence the trend predicted in the calculations reported above is well reproduced in the experiments.

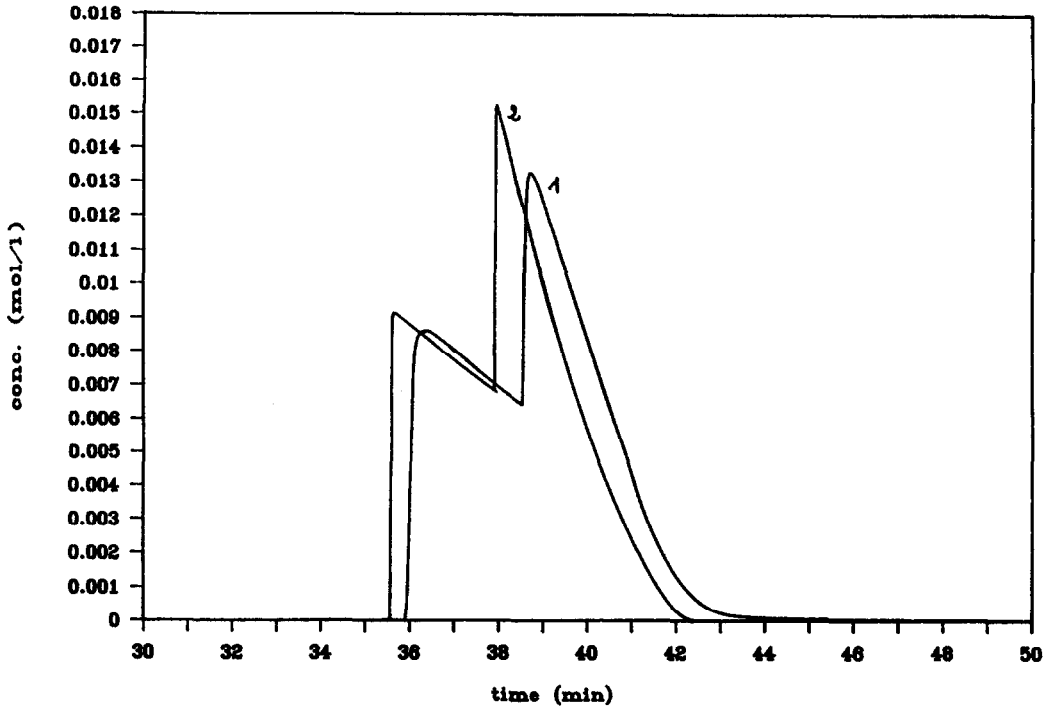


Fig. 10. Same as Fig. 9, except waiting time = 18 min.

For the same concentration, MPA causes a greater displacement effect of DMP than PE. This is demonstrated by the ratio of the slower compound concentrations on the two sides of the shock, *i.e.*, 1.52 and 2.18 for displacement with PE and MPA, respectively. This situation was expected as the relative retention between PE and DMP is almost twice that between MPA and DMP. The intensity of the displacement effect increases with increasing relative retention of the displacer to the displaced compound [8]. This means that the intensity of the converse displacement increases when the two compounds become more closely retained.

The agreement between the experimental and calculated chromatograms is not as good when DMP is displaced by PE than when it is displaced by MPA. As can be seen in Figs. 12 and 13, the calculations predict a much greater displacement effect than that observed experimentally. For example, the calculated ratio of the DMP concentrations on the two sides of the shock differs by more than 10% from the experimental value. The rear profile of the calculated band profile is steeper than that recorded experimentally.

Especially when the band interaction is expected to be important (*e.g.*, in Fig. 13), the faster compound shock appears to move more slowly than predicted by the calculation. On the other hand, there are differences in the degree of disagreement between Fig. 12 and 13. In Fig. 12 we observe that the time shifts of the two shocks are nearly the same, indicating the probably hydrodynamic origin of the band shift.

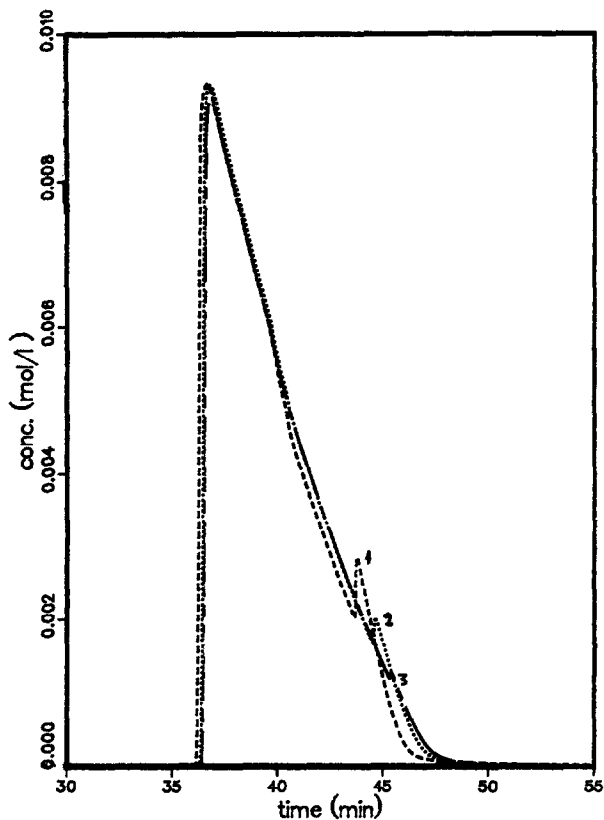


Fig. 11. Influence of the concentration of the faster compound on the intensity of the converse displacement of the slower one. First injection of DMP, $V_2 = 4$ ml, $c_2 = 11.39$ mM; waiting time, 28 min; second injection of PE, $V_1 = 4$ ml. Concentration ratio in the two samples, c_1/c_2 : (1) 10; (2) 6; (3) 3.

In Fig. 13, in contrast, there is almost no hydrodynamic band shift, but the front of the faster moving compound has progressed further than predicted. Note the impurity on the front of the experimental band. This is the origin of the slight dip on the rear part of the slower compound band profile seen in Figs. 8 and 11.

Excellent agreement is observed between the calculated and recorded single-component band profiles in this work, as in previous studies [29,30]. The same agreement was also reported for the individual band profiles of optical isomers [5], and good agreement was observed for the bands of 2-phenylethanol and 3-phenylpropanol [4]. Hence the disagreements between calculated and experimental individual band profiles observed here are due to deviations between the true competitive isotherms and the Langmuir model, a topic of intense investigation.

CONCLUSION

Band interaction in non-linear overloaded chromatography is of critical importance as it determines the exact extent of the separation between components of a

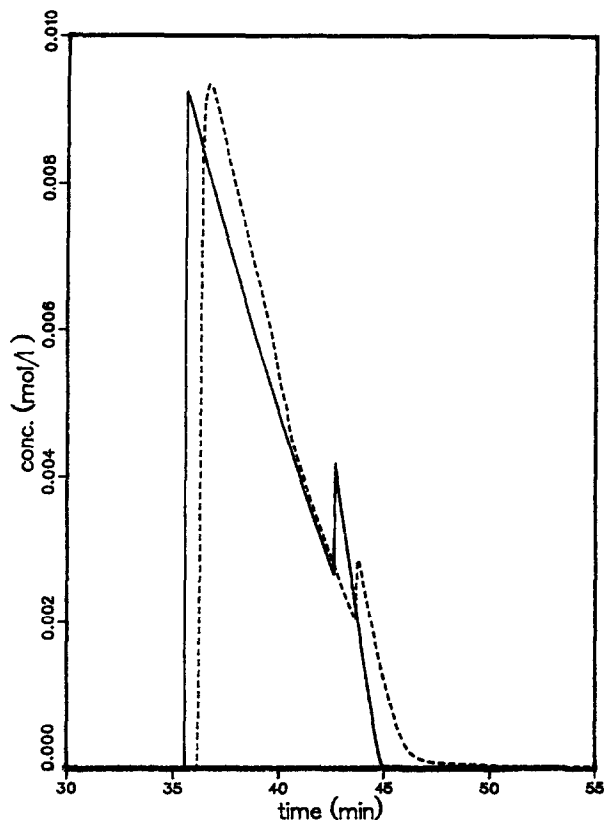


Fig. 12. Comparison between experimental (dashed lines) and calculated (solid lines) elution profiles for the slower compound. First injection of DMP, $V_2 = 4$ ml, $c_2 = 11.39$ mM (loading factor for the second component, $L_{f,2} = 2.17\%$); second injection of PE, $V_1 = 4$ ml, $c_2 = 113.9$ mM (loading factor for the first component, $L_{f,1} = 16.7\%$); waiting time, 28 min.

mixture and controls the exact values of the production rate and the recovery yield. Selectivity, column saturation capacity and, to a lesser extent, column efficiency control the degree of band interaction. The semi-ideal model of chromatography permits an accurate prediction of the propagation of component bands and of their individual elution profiles, taking the finite column efficiency into account. However, the exact knowledge of the competitive isotherm model is crucial for the exact prediction of the individual elution profiles or at least for an accurate description of the separation between components in non-linear chromatography.

The primary aim of this work was an investigation of the phenomena associated with band interference in chromatography. By studying the interaction between two independent bands in a chromatographic column, we were able to isolate band interference from the separation process itself. The conclusion is that band interaction follows a pattern which has not yet been reported, although it derives directly from the theory of non-linear chromatography. Under the proper set of experimental conditions, the less retained compound can displace the more retained compound, a

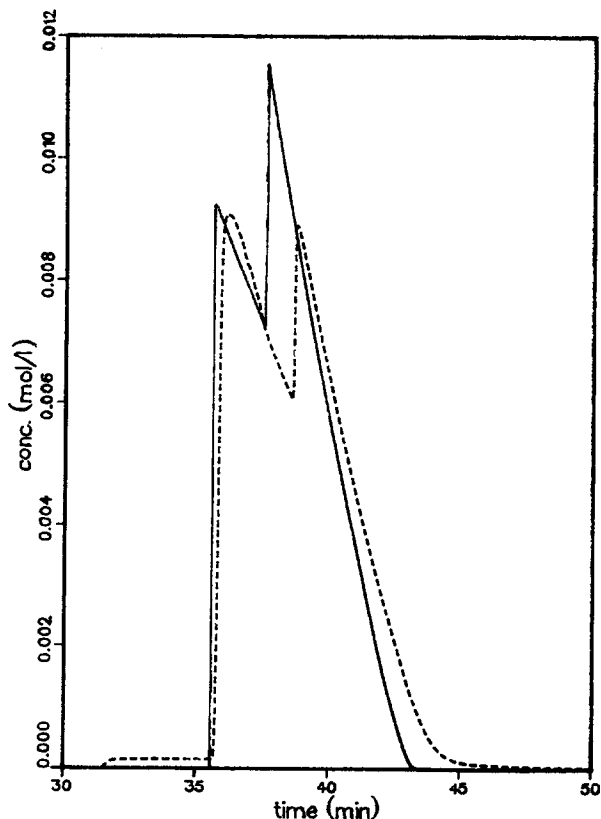


Fig. 13. Same as Fig. 12, except waiting time = 23 min.

phenomenon which cannot take place when the two components are injected together, as usual. This is the paradoxical converse displacement effect. It is due to the decrease in the partition coefficient (ratio of stationary phase and mobile phase concentrations) of the second component which accompanies a surge of the first component concentration at the front of its band. There is a major difference with the classical displacement effect, however. In the classical displacement effect, the concentration of the first component (displaced) behind the front of the second component band (displacer) decreases during the experiment. In contrast, in the converse displacement, the concentration of the second component (now displaced) behind the front of the first component band (now the displacer) increases progressively.

The consequences may be unfavorable in preparative chromatography. A reduction of the cycle time is often attempted by decreasing the time between two successive injections. Then the first eluted component of an injection may interfere with the last eluted component of the previous injection and the phenomenon illustrated in Figs. 8-13 may take place.

A secondary aim of this work was an experimental demonstration of the phenomena associated with band interference in chromatography. The existence of the

converse displacement effect has been demonstrated and its intensity discussed. The injection procedure described in this work makes such investigations easy. Two mixtures were investigated. We found that the experimental data obtained with one of them were well accounted for by a competitive Langmuir model whereas the data obtained with the other mixture were not. In the latter instance, the divergence between the data predicted with the Langmuir competitive model and the experimental data was important. Experimental data of this type must be accumulated until a pattern appears and we understand why the adsorption data for some pairs of compounds are well accounted for by the competitive Langmuir isotherm whereas those for others are not.

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