Journal of Chromatography, 461 (1989) 1–18
Elsevier Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1444

THEORETICAL STUDY OF SYSTEM PEAKS AND ELUTION PROFILES FOR LARGE CONCENTRATION BANDS IN THE CASE OF A BINARY ELUENT CONTAINING A STRONGLY SORBED ADDITIVE

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SUMMARY

The profile of system peaks when using high concentrations and a binary mobile phase was studied using the semi-ideal model of chromatography. The profile of the sample band depends on the concentration of the strong solvent and on the sample size. It is very strongly influenced by the relative adsorption strength of the strong solvent and the sample. This relative strength is measured by the ratio of (a) the origin slope of the equilibrium isotherm of the strong solvent between the stationary phase and the pure weak solvent to (b) the origin slope of the isotherm of the sample (*i.e.*, the ratio between their respective column capacity factors in the pure weak solvent). When this ratio is smaller than 0.2, the sample band profile depends only on the equilibrium isotherm of the sample in the binary mixture. If the ratio becomes larger, the competition between the strong solvent and the sample molecules for interaction with the stationary phase becomes more intense. For Langmuir-competitive isotherms of the strong solvent and the sample, if the concentration of the strong solvent and/or its strength are progressively increased at constant sample size, the retention time of the band decreases (as expected), whereas its typical Langmuirian asymmetry decreases and, past a narrow transition range, reverses. Bands traditionally associated with an anti-Langmuir isotherm are then obtained.

Extremely unusual, broad, characteristic profiles, with a sharp front and a sharp rear, which sometimes exhibit two maxima, are obtained in the transition region. When the origin slope ratio is large, the profile of a high-concentration-sample band in the transition region has two shock layers. The occurrence of such band profiles, under experimental conditions similar to those predicted here, have been reported previously, but their origin has remained unexplained until now.

INTRODUCTION

Since the work of Wilson[1], DeVault[2], Glueckauf[3] and Thomas[4], it has been recognized that the elution profile of high-concentration chromatographic bands is the result of non-linear phenomena. These phenomena can be considered from either a kinetic or a thermodynamic point of view, as we know that thermodynamic equilibria

0021-9673/89/$03.50

are not static but kinetic in nature. The equilibrium constant, for example, is the ratio between the sorption and the desorption rates.

As explained in the following paragraphs, the rigorous solution of a chromatographic problem requires the use of a kinetic approach and, hence, a knowledge of the concentration dependence of the sorption and desorption rates. Equilibrium isotherms are easier to model and measure than mass-transfer rates, which explains the attraction and popularity of equilibrium and semi-equilibrium models that make simplifying assumptions regarding the kinetics of mass transfers. Most retention mechanisms used in chromatography have very fast kinetics. Hence the phase compositions are always, and everywhere in the column, close to equilibrium. Equilibrium isotherms are not linear. At high solute concentrations, they always exhibit some degree of non-linearity, the concentration in the stationary phase at equilibrium increasing either faster or more slowly than the concentration in the mobile phase. Accordingly, solutes at different concentrations tend to move along the column at different speeds, and either the front or rear of the band will tend to become steeper and steeper. However, the finite character of the kinetics of mass transfers between phases tends to relax all concentration gradients, especially those which the non-linear behavior of the chromatographic process at high concentrations tends to build up.

The elution profile of a chromatographic band is obtained as the solution of a classical mass balance equation, relating the time differential in the concentrations of the corresponding compound in the mobile and stationary phases[1,2]. A relationship between these two differentials is required in order to solve the problem, and it can be found by either a thermodynamic or a kinetic approach. The former assumes constant equilibrium between phases. The required relationship between the time differentials of the solute concentrations in both phases is obtained by differentiation of the equilibrium isotherm. The latter attempts to derive a relationship between (a) the rate of sorption/desorption and (b) the composition of the stationary and mobile phases, and is a much more difficult approach.

The first approach assumes, according to Haarhoff and Van der Linde[5], that the mass transfer is fast enough for the two phases to be always very near equilibrium, the effects of axial diffusion and finite rate of radial mass transfer merely combining and resulting in an apparent diffusion, larger than the true axial diffusion but having the same consequence. This is the basis of the semi-ideal model of chromatography, which is valid for columns having more than 1000 theoretical plates. This model is attracting much interest at present, because of the recently increased importance of preparative liquid chromatography in the pharmaceutical and biochemical industries, and because nearly all retention mechanisms used in the various implementations of high-performance liquid chromatography (HPLC) (normal- and reversed-phase chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, ligand-exchange chromatography, size-exclusion chromatography) involve very fast mass transfers between phases. The related ideal model assumes further that the column efficiency is infinite[1–3,6]. An analytical solution of the ideal model can be derived in the case of a Langmuir isotherm[7]. The solutions of the ideal mode are very close to the profiles obtained with real columns of finite efficiency used in the semi-ideal model. It is important to note that the discontinuities or shocks predicted by the ideal model are replaced in the semi-ideal model with shock layers that have the steep fronts or rears recorded with overloaded elution bands, and that propagate at the same velocity as the shocks of the ideal model[8].

The second approach consists in writing the kinetic equation describing the processes of adsorption of the studied compounds by the stationary phase and their desorption as a function of their concentrations in the two phases of the system. In Langmuir kinetics, the corresponding equation has also been solved[4,9]. It gives profiles that are identical with those supplied by the previous approach, in the case of fast kinetics[10]. However, it permits the study of slow equilibria, such as those used in affinity chromatography, in some instances where the sorption/desorption rates are abnormally slow or in various processes of adsorption and absorption.

The choice between the two approaches will depend in many instances on the relative ease with which the thermodynamics or the kinetics of a certain retention mechanism can be studied and described. At present, it seems that thermodynamics leads to an easier route.

In previous papers we described the algorithmic approach to a numerical solution of the single- and two-component problems within the framework of the semi-ideal model[11,12]. We used the programs derived from these analyses to calculate elution profiles of single and two-component pulses and the influence of most experimental parametes on these profiles[11] and on the separation of a binary mixture[13]. However, in all instances, we neglected the adsorption of the mobile phase. This is acceptable provided that the mobile phase is a single solvent, as it can be shown that this is tantamount to making a choice of the reference state in adsorption thermodynamics[14]. However, this assumption falters when the mobile phase is a mixture of solvents[15].

In adsorption chromatography, it is common to use a mobile phase containing a strong solvent, which is adsorbed on the stationary phase, modifies its surface and competes with the eluites for access to the adsorption sites on this surface. Hence the prediction of the band profile of a single-component sample eluted with a binary eluent is really a two-component problem, whereas the prediction of the elution profiles and separation of a two-component sample is a three-component problem. The boundary and initial conditions of the problem are just slightly different from those for the elution of a two- and a three-component sample plug: one of the components (the sample) is injected as a plug, whereas the other one (the strong solvent) is injected continuously, at constant concentration as a component of the mobile phase.

In this paper, we discuss from a theoretical standpoint the phenomena associated with the elution of a single component with a binary eluent. In the accompanying paper[16], we present experimental evidence for the soundness of these theoretical results.

THEORY

As both the strong solvent and the solute can interact with the stationary phase (through whatever retention mechanism is involved), we must consider a mass balance equation for each of them. For the solute we have

$$\frac{\partial C_a}{\partial t} + \frac{V_s}{V_m} \cdot \frac{\partial q_a}{\partial t} + u \cdot \frac{\partial C_a}{\partial z} = D_L \cdot \frac{\partial^2 C_a}{\partial z^2} \tag{1}$$

and for the strong solvent we have similarly

$$\frac{\partial C_s}{\partial t} + \frac{V_s}{V_m} \cdot \frac{\partial q_s}{\partial t} + u \cdot \frac{\partial C_s}{\partial z} = D_L \cdot \frac{\partial^2 C_s}{\partial z^2} \tag{2}$$

where t is the time and z the abscissa along the column, C is the concentration in the mobile phase [the subscripts a and s represent the analyte (sample, eluite or solute) and the strong solvent, respectively], q is the concentration in the stationary phase, V_s and V_m are the fractions of the column volume available for the stationary and the mobile phase, respectively, and D_L is the axial diffusion coefficient, assumed to be the same for both the strong solvent and the sample. In order to solve this system we need a proper set of relationships between the time differentials of the concentrations of the solute and the strong solvent in the stationary phase and their concentrations in the mobile phase (see Introduction).

It is impossible to derive exact solutions of the system of partial differential equations obtained, except when linearity applies (*e.g.*, first-order kinetics)[17]. Such a solution is valid only under analytical conditions. Numerical solutions of this system may be obtained using a finite-difference method[11,12], but only after some simplification to eliminate the right-hand side (RHS) of the equations.

Within the framework of the classical semi-ideal model[18], we make the following three assumptions. First, the axial diffusion term is negligible. Second, the relationship between (a) the concentrations of the solute and the strong solvent in the stationary phase at equilibrium and (b) the mobile-phase concentrations of these two components, at the same time and the same place in the column, are given by the mixed, competitive, ternary isotherms (see below). The combination of these two assumptions results in an infinite column efficiency. Thus, we account for the finite efficiency of the column by setting the length increment in the numerical integration of the system of partial differential equations equal to the column HETP. The time increment is related to the length increment[19]. It can be shown that, with this method, the errors introduced by the calculation in the derivation of the profiles solution of eqns. 1 and 2 with a zero RHS are made exactly equal to the neglected RHS. Accordingly, the procedure gives exact numerical solutions of the system of eqns. 1 and 2.

In the following, we have assumed the simplest possible form for the ternary isotherm, the competitive Langmuir isotherms

$$\frac{q_a}{q_o} = \frac{b_a C_a}{1 + b_a C_a + b_s C_s} \tag{3}$$

and

$$\frac{q_s}{q'_o} = \frac{b_s C_s}{1 + b_a C_a + b_s C_s} \tag{4}$$

where q_o and q'_o are the saturation concentrations of the sample and the strong solvent respectively, in the stationary phase. The column saturation limit is the product of q_o (or q'_o) and the column volume, and b_a and b_s are numerical coefficients.

This sytem of eqns. 1–4 is identical with that used in the study of the separation of the two components of a binary mixture when a sample pulse is injected in elution chromatography[12]. The difference from this previous problem is the set of boundary conditions. A constant stream of mobile phase with a constant concentration of strong solvent is flowing during the whole experiment, and a pulse of solute is injected over a very short period of time. Accordingly, the boundary conditions of the problem are as follows:

$$C_a(x,0) = 0 \tag{5a}$$

$$\begin{aligned} C_a(0,t) &= 0 \text{ if either } t < 0 \text{ or } t > t_p \\ &= C_o \text{ if } 0 < t \leqslant t_p \end{aligned} \tag{5b}$$

where t_p is the width of the sample pulse.

$$C_s(0,t) = C_{s,o} \tag{5c}$$

The calculations are carried out by means of a program similar to that described previously[15]. In all the simulations, the column dimensions are assumed to be 25 cm × 4.5 mm I.D., the flow-rate 1 ml/min (flow-velocity 0.122 cm/s) and the column efficiency 5000 theoretical plates. The isotherm parameters used and the sample size are given in the figure captions.

RESULTS AND DISCUSSION

Chromatography of a sample containing a single compound with a binary mobile phase gives a peak for that compound at a retention time resulting from the combined interaction of both the sample and the strong solvent with the stationary phase and gives two peaks for the strong solvent. The latter are usually called system peaks. They have been investigated extensively by previous workers[20–24] who were essentially preoccupied with the interference of these peaks with those of the sample in analytical chromatography.

In true linear chromatography, these system peaks should not occur. Analytical chromatography with a mixed mobile phase may be linear as far as the sample is concerned, but not as far as the mobile phase is concerned. In fact, the whole purpose of using mixtures as mobile phases is to manipulate the equilibrium constants so as to adjust the selectivity and retention volumes for successful analysis. The concentration of the strong solvent is usually high enough to modify the retention of the sample components, and therefore the column behavior cannot be linear towards this solvent modifier.

The solvent peaks are the result of the modification of the strong solvent–stationary phase equilibrium due to the injection of the sample. Hence they are the result of transitory changes in the composition of the mobile phase. They are detected by a non-selective detector, such as a refractive index detector, and are not detected by a selective detector, such as a UV detector. This is why the chromatograms obtained, even in analytical chromatography, can be very different, depending on the nature of the mobile phase used and whether a selective or a non-selective detector is chosen.

With a non-selective detector, the chromatogram may be nearly impossible to account for if there are numerous resolved components in the sample being analyzed.

If the sample injected is large, the non-linear effects are considerably amplified and a variety of situations may be encountered, depending on the relative strengths of the adsorption of the solute and the strong solvent on the stationary phase. Figs. 1–4 show the results of the simulation of the elution of a high-concentration band of a single-solute sample under different sets of experimental conditions. In all instances we observe three peaks, one for the solute and two for the strong solvent. The first of the latter two peaks is positive (excess of strong solvent, representing the amount replaced by the sample when it is injected). If the strong solvent is less strongly adsorbed than the sample, the first peak is eluted as a non-retained compound. If the strong solvent is equally or more strongly retained than the sample, the first peak is retained and eluted at a time that depends on the nature of the strong solvent (*i.e.*, the characteristics of its adsorption), on its concentration and on the nature and concentration of te sample.

The second solvent peak is negative. Usually, it is eluted at the same time as the solute. Sometimes, the negative peak is more strongly retained than the sample, and

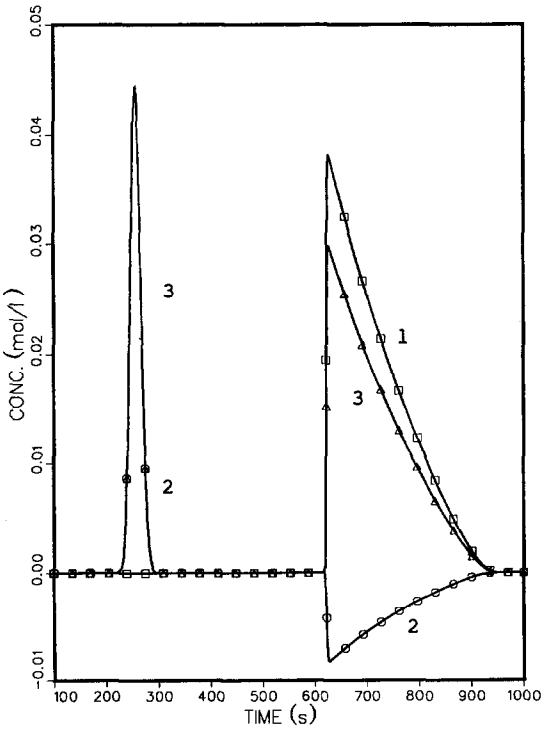

Fig. 1. Chromatogram obtained for a one-component sample on a chromatographic column with a binary mobile phase. Column length, 25 cm; column efficiency, 5000 theoretical plates; flow velocity, 0.122 cm/s ($t_0 = 205$ s); strong solvent concentration, 0.25 M; Langmuir isotherm coefficients, $a_s = 2.0$, $a_a = 20$, $b_s = 1.0$, $b_a = 10$; sample size, 83.3 μmole. 1, Elution profile of the sample (signal from a detector selective for the sample); 2, elution profile of the strong solvent (signal from a detector selective for the solvent); 3, sum of profiles 1 and 2 (signal from a non-selective detector with equal response factors for strong solvent and solute).

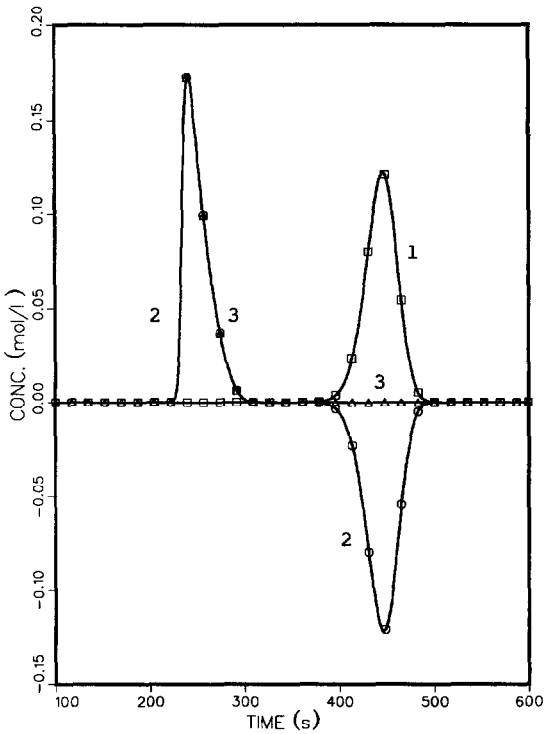

Fig. 2. Chromatogram obtained for a one-component sample on a chromatographic column with a binary mobile phase. Experimental conditions as in Fig. 1, except $a_s = 20$ and $b_s = 10$. 1, Elution profile of the sample; 2, elution profile of the strong solvent; 3, sum of profiles 1 and 2.

then the positive peak is eluted at the same time as this sample. The signal of a truly non-selective detector is a combination of these two concentration profiles. In the figures we have assumed that the total chromatogram was obtained this way.

In practice, however, there is no truly non-selective detector. The response factor of the differential refractive index detector may change by more than one order of magnitude from one compound to another. It may even change in sign[16]. In such an event, the combination of the asymmetric shapes of the two interfering peaks [the solvent (system) peak and the solute peak] and of unusual response factors may lead to unexpected chromatograms, which may be difficult to account for without an excellent understanding of the chromatographic processes involved. The shape of the sample band profile itself may become totally unexpected, very different from what is usually observed for an overloaded column, whether the isotherm is Langmuirian or anti-Langmuirian. Some unusual profiles of that kind have already been reported[25].

Fig. 1 shows the simulation chromatogram obtained for a large sample of a solute that is much more strongly retained than the strong solvent. However, as the concentration of the strong solvent is high its presence changes the retention behavior of the sample and, in particular, reduces its retention time (the column capacity factor is inversely proportional to $1 + b_s c_s$, where b_s and c_s are the second coefficient of the Langmuir isotherm of the strong solvent and its concentration in the mobile phase,

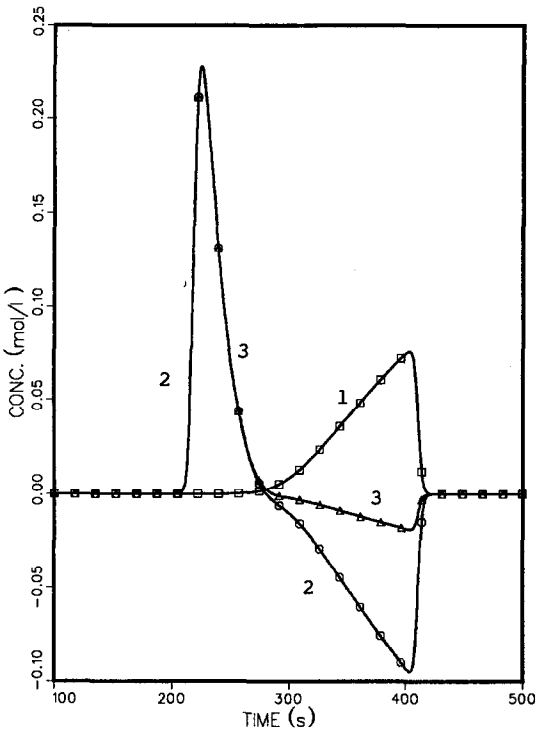

Fig 3. Chromatogram obtained for a one-component sample on a chromatographic column with a binary mobile phase. Experimental conditions as in Fig. 1, except $a_s = 40$ and $b_s = 20$. 1, Elution profile of the sample; 2, Elution profile of the strong solvent; 3, sum of profiles 1 and 2.

respectively[15]). We observe a sample peak with a very sharp front and a tail ending close to the retention time of a very small plug of sample, under the same experimental conditions. This profile is very close to that observed when the solute equilibrium isotherm is Langmuirian. It has been shown that when the ratio of the slopes at the origin of the adsorption isotherms of the sample and the strong solvent in the weak solvent is greater than about 5, the three following profiles are almost identical[15]: (i) the experimental profile, (ii) the profile calculated from the (correct) two-component model and the competitive isotherm and (iii) the profile calculated from the approximate one-component model (using the single-component isotherm, derived from the competitive isotherm by assuming that the strong solvent concentration is constant). Differences appear progressively as the isotherm slope ratio decreases.

As expected, there are two solvent peaks in Fig. 1. One, observed at the dead volume, is Gaussian. The other, appearing at the same time as the sample band, is negative; the concentration of the strong solvent decreases in the eluent during the elution of the sample band. Accordingly, the total concentration of solutes in the weak solvent varies less than would be indicated by the profile of the sample band. The signal of a non-selective detector is smaller than that of a detector selective for the sample only, which would have the same response factor. The strong solvent peak is proportional to the solute peak. This means that a detector that would respond to both

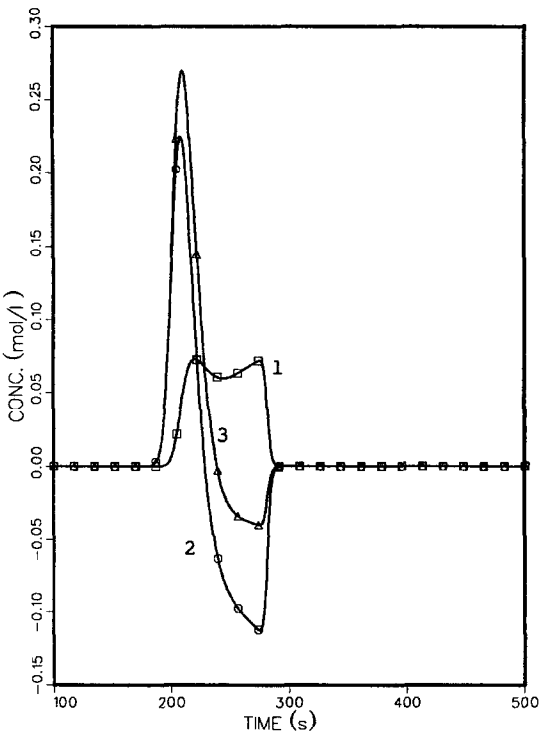

Fig. 4. Chromatogram obtained for a one-component sample on a chromatographic column with a binary mobile phase. Experimental conditions as in Fig. 1, except $a_s = 200$ and $b_s = 100$. 1, Elution profile of the sample; 2, elution profile of the strong solvent; 3, sum of profiles 1 and 2.

the sample and the strong solvent could exhibit a total lack of response during the elution of the composite band, should the two response factors be in the proper ratio. This proportionality is due to the fact that each molecule of sample that leaves the stationary phase is replaced with a certain number of molecules of strong solvent that were displaced at the time of injection of the sample. Obviously, the areas of the two bands of strong solvent are equal, as they correspond to the same amount of material, first displaced from the column, then replenished.

Fig. 2 shows the simulated chromatogram obtained under conditions very similar to those for Fig. 1, except that the slope at the origin of the adsorption isotherm (origin slope), of the strong solvent is steeper. The origin slopes of the sample and the strong solvent are now equal. The sample band has become less retained at infinite dilution and, altough the sample was of the same size for Figs. 1 and 2, the band shown in Fig. 2 is much more symmetrical, even nearly Gaussian. The first solvent peak, on the other hand, is now tailing slightly. Its retention time increases with increasing strength of the organic modifier. The profile of the second, negative, solvent band is equal to that of the sample band, but it is negative. Accordingly, the concentration of the weak solvent in the eluent remains constant during the entire elution of these bands and the only signal recorded by a non-selective detector would be the asymmetric first

solvent band. This is a paradoxical, unexpected result, which could be highly misleading.

Figs. 3 and 4 show simulated chromatograms obtained with samples that are less strongly adsorbed than the strong solvent. The ratios of the origin slopes of the adsorption isotherms for the strong solvent and the sample in the weak solvent are 2 and 10 for Figs. 3 and 4, respectively. Now, the profiles of the three bands, that for the sample and the two for the strong solvent, are all asymmetric. However, the elution profile of the sample has changed direction. Its front is diffuse, whereas its rear is very sharp, as if the adsorption isotherm were anti-Langmuirian. As explained under Theory, the isotherms used here are competitive Langmuir isotherms, wich cannot, in any way, be combined to result in an anti-Langmuir isotherm. The phenomenon is entirely due to the competition between the molecules of sample and strong solvent for access to the adsorbent surface. The increasing strength of the strong solvent is reflected by the decrease in the retention of the sample, at constant composition of the eluent. The mere competition between solute and strong solvent (or one of the mobile phase additives) explains why, in many instances, the profiles of overloaded bands obtained in reversed-phase or hydrophobic interaction chromatography correspond to an anti-Langmuir type of isotherm, not to a Langmuir type. This phenomenon

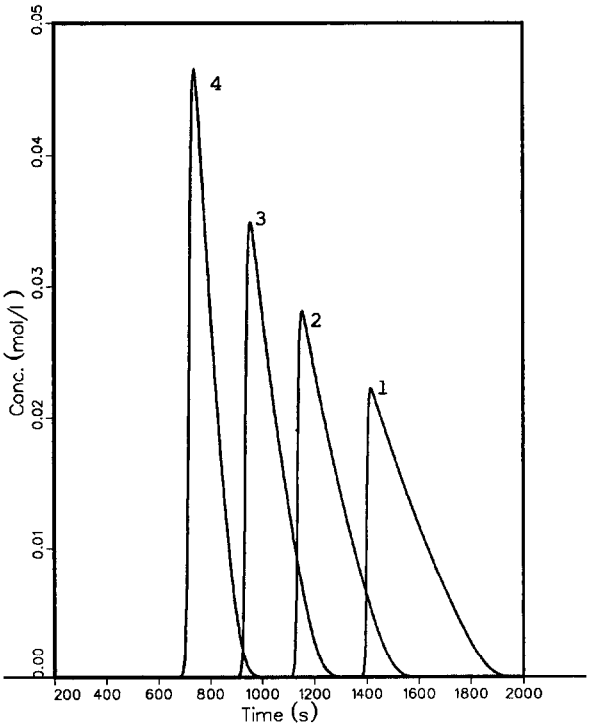

Fig. 5. Influence of the concentration of the strong solvent in the mobile phase on the elution profile of a single compound. Weakly adsorbed strong solvent. Experimental conditions as in Fig. 1, except $a_s = 4$, $a_a = 40$, $b_s = 0.80$; $b_a = 8$, and different strong solvent concentrations. Strong solvent concentration: 1, 0.1; 2, 0.5; 3, 1; 4, 2 *M*.

could explain why a change in the composition of the solvent can reverse the asymmetry direction of the band and replace an apparently anti-Langmuir band profile by a Langmuirian profile.

We also observe in Figs. 3 and 4 that the two solvent bands are now partially merged and that the negative second band is more concentrated than the sample band. As a consequence, the injection of the sample results in a negative signal with a non-selective detector, while a much simpler, positive signal is observed with a selective detector.

These phenomena are similar to those observed in analytical chromatography and reported by previous workers[20–24]. In the case of high-concentration sample bands, there is an additional complexity resulting from the fact that the perturbation due to the injection of the sample cannot be considered to be small and cannot be treated by assuming that the system behaves linearly near the steady equilibrium point.

The retention time of the sample band and the shape of its profile depend not only on the relative strengths of the adsorption of the strong solvent and the sample from their solution in the weak solvent, but also on the column efficiency, the concentration of the strong solvent in the mobile phase, the column saturation capacity and the size of the sample pulse.

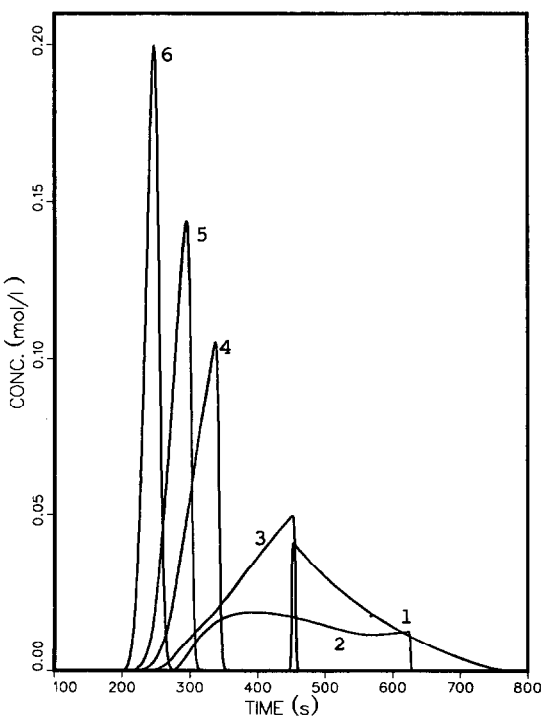

Fig. 6. Influence of the concentration of the strong solvent in the mobile phase on the elution profile of a pure compound. Strongly adsorbed strong solvent. Experimental conditions as in Fig. 5, except $a_s = 28.8$, $a_a = 14.4$, $b_s = 16$ and $b_a = 8$ and different strong solvent concentrations. Strong solvent concentration: 1, 0.017; 2, 0.085; 3, 0.17; 4, 0.34; 5, 0.5; 6, 1 *M*.

Fig. 5 shows a series of simulated chromatograms obtained with the same sample size of a compound that is much more strongly adsorbed than the strong solvent from a dilute solution in the weak solvent. The ratio of the origin slopes of the sample and the strong solvent is 10. The concentration of the strong solvent is increased from 0.1 to 0.5, 1 and 2 *M*. In all instances, the band profile corresponds to that observed in overloading a chromatographic system with a Langmuir isotherm. However, the degree of symmetry of the sample peak increases with increasing concentration of the strong solvent. Its retention time decreases, as does the limiting retention time at zero sample size of the sample. This is expected, as it is the reason why the concentration of strong solvent in the eluent is usually adjusted.

Fig. 6 shows the simulated chromatograms obtained by the same procedure, increasing the concentration of the strong solvent in the mobile phase at constant sample size, but this time the sample is less strongly adsorbed on the stationary phase than the strong solvent. The ratio of the origin slopes of the sample and the strong solvent is now 0.5. At high concentrations of the strong solvent, we observe the same effect as that seen in Fig. 3. The band profile is similar to the profiles associated with an anti-Langmuir isotherm, although the isotherms involved here are not anti-Langmuirian. When the concentration of the strong solvent decreases, the band profile

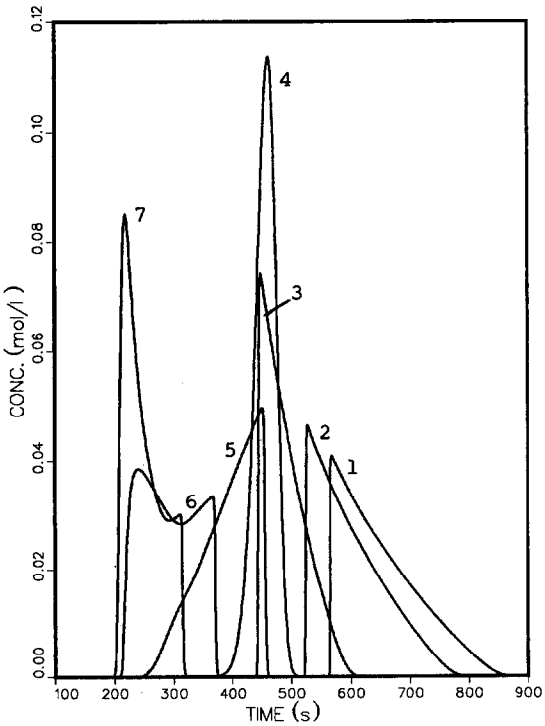

Fig. 7. Influence of the strength of adsorption of the strong solvent in the mobile phase on the elution profile of a single compound. Experimental conditions as in Fig. 6, except strong solvent concentration 0.17 *M* and different Langmuir coefficients a_s and b_s. a_s/a_a: 1, 0; 2, 0.1; 3, 0.5; 4, 1; 5, 2; 6, 5; 7, 10. b_s/b_a: 1, 0; 2, 0.1; 3, 0.5; 4, 1; 5, 2; 6, 5; 7, 10.

changes, a hump appears on its front, then its rear moves backwards rapidly and the profile becomes typical of the band of a compound having a Langmuir isotherm (see Fig. 6). Such a behavior is unexpected. The profiles obtained in the transition region are very unusual. Elution band profiles similar to those described in Fig. 6 have been reported previously by Kirkland[25] and have remained unexplained until now.

These phenomena can be explained by the fact that, even if the strong solvent is strongly adsorbed on the stationary phase, the fraction of the adsorbent surface that is covered by the strong solvent molecules at equilibrium is still small at low concentrations of this solvent (otherwise, with a more weakly adsorbed solute, there would be almost no retention). Hence the competition with the sample molecules has mainly the effect of reducing the retention time, but the band profile is not changed. The modification of the band profile occurs when the competition for adsorption between strong solvent and sample molecules becomes acute, *i.e.*, at strong solvent concentrations, in a range which depends on its strength of adsorption.

Fig. 7 shows a series of simulated chromatograms for the injection of a constant amount of a single-solute sample in a mobile phase of constant composition (*i.e.*, constant concentration of the strong solvent). From one band to the next, the relative adsorption strength of the sample and the strong solvent is changed. The ratio of the origin slopes of the isotherms between the strong solvent and the sample for adsorption

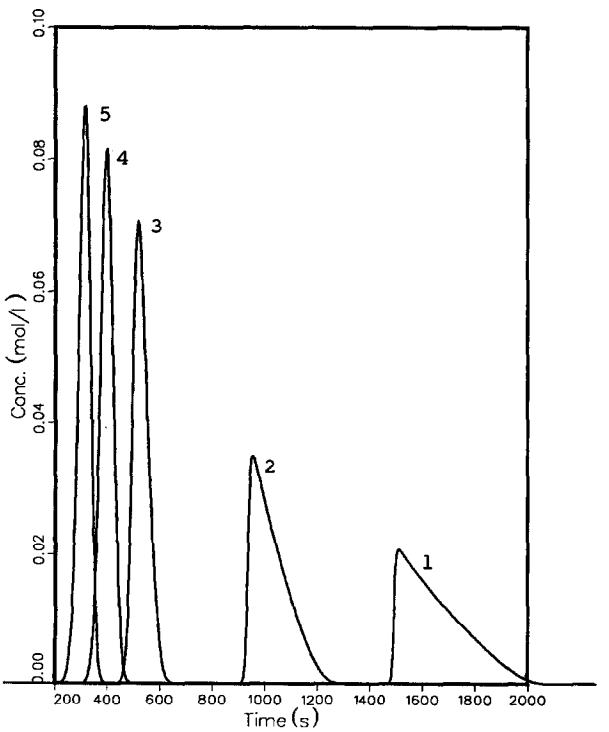

Fig. 8. Influence of the strength of adsorption of the strong solvent in the mobile phase on the elution profile of a single compound. Experimental conditions as in Fig. 7, except strong solvent concentration 1.0 *M* and different Langmuir coefficients a_s and b_s. a_s/a_a: 1, 0; 2, 0.1; 3, 0.5; 4, 1; 5, 2. b_s/b_a: 1, 0; 2, 0.1; 3, 0.5; 4, 1; 5, 2.

from their solutions in the weak solvent is increased from 0 to 0.1, 0.5, 1, 2, 5 and 10. As expected on the basis of our explanations, the sample band profile changes, and a reversal in the direction of the asymmetry is observed. The first three profiles have the shape normally associated with a Langmuir isotherm. The fourth is Gaussian. The fifth is similar to those associated with an anti-Langmuir isotherm. The last two bands have two steep fronts, *i.e.*, two shock layers. They show a progressive transformation of the sample band into a non-retained band (t_0 = 205 s).

Fig. 8 shows a series of simulated chromatograms, similar to those in Fig. 7, the only difference being in the higher concentration of the strong solvent in the mobile phase. The retention time of the sample band decreases rapidly with increasing ratios of the origin slopes of the isotherms between the strong solvent and the sample. The band profile remains similar to the profiles associated with a Langmuir isotherm. By the time the ratio has become large enough to promote a reversal in the direction of band asymmetry, the retention time is too small and the sample band just becomes unretained and symmetrical.

Whereas all Figs. 1–8 showed changes in the band profile associated with variations in the composition of the mobile phase and with the relative strength of adsorption of the sample and the strong solvent, while the sample size was kept constant, Figs. 9–11 show simulated chromatograms obtained with increasing sample

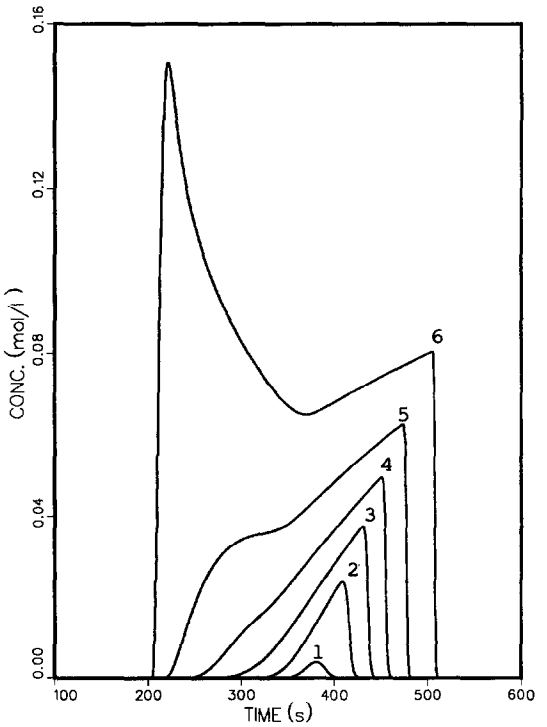

Fig. 9. Influence of the sample size on the elution profile of a single compound by a binary mobile phase. Strongly adsorbed solvent. Experimental conditions as in Fig. 6, except strong solvent concentration 0.17 *M* and different sample sizes. Sample size: 1, 1.66; 2, 16.6; 3, 41.6; 4, 83.3; 5, 166.6; 6, 416.5 μmole.

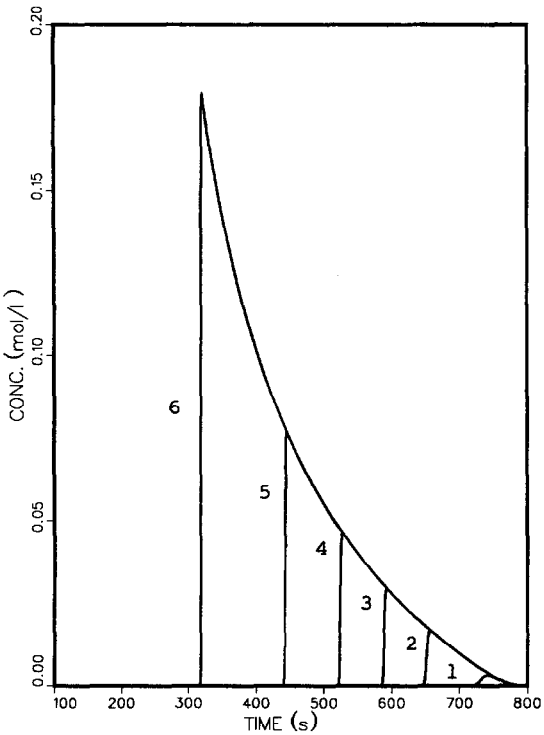

Fig. 10. Influence of the sample size on the eluting profile of a single compound by a binary mobile phase. Weakly adsorbed solvent. Experimental conditions as in Fig. 9, except Langmuir coefficients are $a_s = 1.44$ and $b_s = 0.8$. Same sample sizes.

sizes. Fig. 9 shows a series of such chromatograms corresponding to a strong solvent that is more strongly adsorbed on the stationary phase than the sample (ratio of the origin slopes equal to 2). At small sample sizes, the sample band profile evolves in a way that is typically associated with an anti-Langmuir isotherm, with a shock layer at the rear border of the band. We note, however, that the front does not remain stable, but that the front of the band moves backwards with increasing sample size. This does not occur with a true anti-Langmuir isotherm[11]. However, when the sample size becomes large a hump appears on the slanted front of the band. It grows and progressively gives rise to a second maximum, while a second shock layer[8] appears on the front. Such bands with two maxima for a pure compound are exceptional, but the phenomenon has been reported previously[25].

Fig. 10 shows a series of simulated band profiles obtained for increasing sample sizes of a single compound that is eluted by a binary mobile phase containing a strong solvent that is much less retained than the sample (ratio of the origin slopes of the strong solvent and the sample equal to 0.1). The progressive change in the profile, with a front becoming steeper and steeper and being eluted earlier and earlier, is classical of what is observed when a chromatographic column is overloaded with a compound exhibiting a Langmuir equilibrium isotherm between the stationary and mobile phases[11].

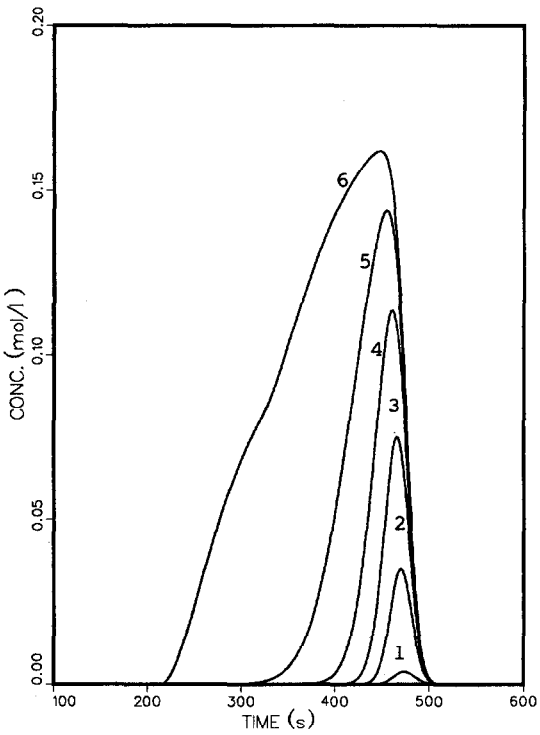

Fig. 11. Influence of sample size on the elution profile of a single compound by a binary mobile phase. Strong solvent and sample equally adsorbed. Experimental conditions as in Fig. 9, except Langmuir coefficients are $a_s = 14.4$ and $b_s = 8$. Same sample sizes.

Fig. 11 shows a series of simulated band profiles obtained for increasing sample sizes of a single compound that is as strongly retained by the stationary phase as the strong solvent (ratio of the origin slopes of the equilibrium isotherms equal to 1). Although the band profile changes progressively, becoming less and less symmetrical, it is remarkable that in this instance the retention time of the band maximum remains nearly constant until very large sample sizes are reached.

Fig. 12 shows a series of simulated band profiles obtained at constant sample size and constant composition of the mobile phase containing a strong solvent such that the ratio of the origin slopes of the strong solvent and the sample isotherms is constant. The values of the coefficients b_a and b_s of the isotherms (see eqns. 3 and 4) change from one band to the next. Accordingly, the column saturation capacity changes and the loading factor changes proportionally at constant sample size. At high values of the column saturation capacity the band profile is of the Langmuir type, but the loading factor is small, and hence the band profile is nearly symmetrical and the profile is close to Gaussian. As the column saturation capacity decreases, the column becomes more and more overloaded, the band asymmetry increases and its retention time decreases. At a certain intermediate value, the band profile shifts, the direction of asymmetry reverses and the profile acquires a rear shock layer, usually characterizing the anti-Langmuir isotherms.

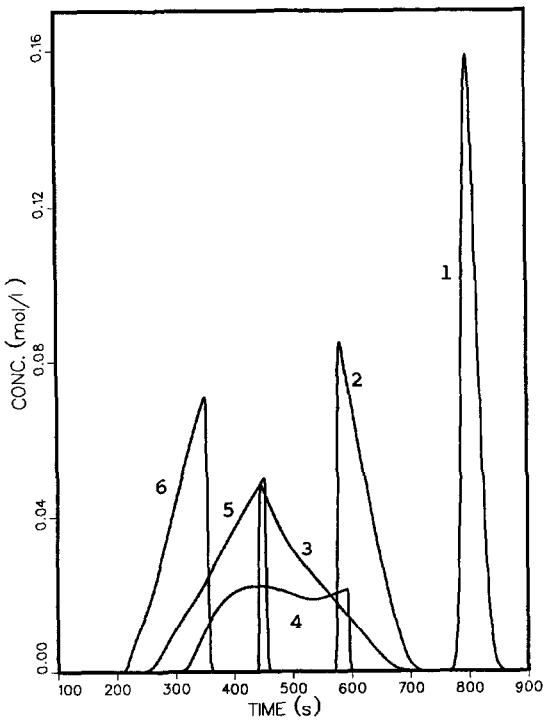

Fig. 12. Influence of the column saturation capacity on the elution profile of a single compound by a binary mobile phase. Experimental conditions as in Fig. 7 (including sample size), except stationary phase saturation capacity, Q. $a_s = 28.8$ and $b_s = 28.8/Q$; $a_a = 14.4$ and $b_a = 14.4/Q$. Stationary phase saturation capacity, Q: 1, 144; 2, 14; 3, 7.2; 4, 3.6; 5, 1.8; 6, 0.9 mol/l.

CONCLUSION

The use of mixed mobile phases containing a strong solvent that is not much less strongly adsorbed than the components of the samples studied generates system peaks that become very complex and may become extremely difficult to account for, especially if these compounds are not very well separated from each other and if a non-selective detector is used to record the chromatograms. The combination of several detectors, including a component-selective and a non-selective detector, may help considerably in accounting for the experimental results.

In the accompanying paper[16], we present a comparison between experimental results obtained in normal-phase adsorption chromatography with mixtures of a weak solvent and alkanols at low concentrations and the present theoretical results[16]. In further work, we shall extend the present observations on a single-component sample to the separation of multi-component mixtures[26].

ACKNOWLEDGEMENTS

This work was supported in part by grant CHE-8715211 from the National

Science Foundation and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory.

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