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Note

Computation of band shape for strong injection solvent and weak mobile phase combinations in liquid chromatography

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Many workers[1–10] have observed the production of distorted and multiple peaks from the injection of a single eluite dissolved in a solvent stronger than the mobile phase in reversed-phase high-performance liquid chromatography (HPLC). Microcomputer simulation of this production has been attempted[11]. Recently, a systematic study of this phenomenon showed some general patterns of peak development [12]. We have computed, using equilibrium distribution theory, band patterns that develop when a strong injection solvent is used. Although a non-equilibrium process is operative in HPLC, we hoped to find general features in band patterns that were similar to those observed in the above systematic study. Our computer approach was based on that used to examine band development when the injection solvent was the mobile phase[13].

METHOD

The chromatography was viewed as having two contiguous mobile phases. (1) The small volume of strong injection solvent which introduced the eluite into the first few column plates followed by (2) the main weaker mobile phase. Plates had equal volumes, and the total injection volume was an integral multiple of the plate volume. The multiples used varied from 1 to 10. The computation was the same as previously used[13] except that initially two capacity factors (k') were used, one of small value representing the injection solvent plug as mobile phase and one of larger value representing the weaker main mobile phase. Later more than two capacity factors were used to represent a partially diluted injection solvent plug.

An AT&T PC 6300 microcomputer with 640 Kbytes RAM was used. A Lotus 1–2–3 spreadsheet program computed the eluite distribution. Cells were reserved for the fraction of eluite in the stationary phase, $k'/(k' + 1)$, for each k' value used in a given equilibrium distribution. Distributions were extended up to 100 plates. Distributions were displayed with a Hewlett-Packard 7475A plotter.

Table I represents the initial part of a spreadsheet distribution where 100 wt. units were injected into a column whose individual plate volume accepted 10 wt. units. The capacity factors were 0.100 for the injection solvent, giving a stationary phase fraction (rounded to one place beyond the decimal for display purposes) shown

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TABLE I

SPREADSHEET GENERATED FOR INJECTION INTO THE FIRST TEN PLATES[a]

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
1	10.0	0.8	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
2	10.9	9.1										
3	11.0	10.7	8.3									
4	11.0	11.0	10.5	7.5								
5	11.0	11.0	10.9	10.2	6.8							
6	11.0	11.0	11.0	10.9	9.9	6.2						
7	11.0	11.0	11.0	11.0	10.8	9.6	5.6					
8	11.0	11.0	11.0	11.0	11.0	10.7	9.2	5.1				
9	11.0	11.0	11.0	11.0	11.0	10.9	10.5	8.9	4.7			
10	11.0	11.0	11.0	11.0	11.0	11.0	10.9	10.4	8.5	4.2		
11	9.2	2.8	11.0	11.0	11.0	11.0	11.0	10.8	10.2	8.1	3.8	
12	7.6	3.9	1.5	11.0	11.0	11.0	11.0	11.0	10.8	10.0	7.7	3.5

[a] $k' = 5$ and 0.1.

in cells C1 to K1 and 5.00 for the mobile phase, giving a stationary phase fraction shown in B1. All other cells were used for distribution. Increasing numerical values of the rows gives increasing extent of distribution.

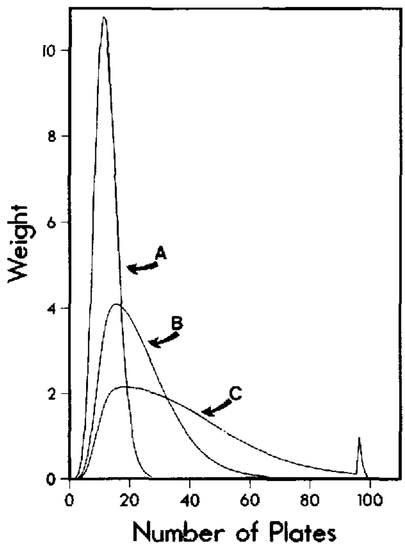
The distributions computed and displayed in this paper are column band distributions. They are not chromatogram simulations because the computation never simulated moving the eluite out of the column. To obtain well developed bands all of the spreadsheet memory was used in computation, and, thus, it was not possible to continue "moving" the plate contents through a "detector".

When simulation of moving a partially developed distribution band through a detector was done the following shape effects occurred and should occur in well developed distributions. The solvent front peak found in Figs. 1–8 retained its height and shape. Other peaks broadened and were somewhat lower. Valleys between peaks were somewhat higher.

RESULTS AND DISCUSSION

Equilibrium distribution predicted that band distortion occurs when the injection solvent strength as measured by the capacity factor was greater than the mobile phase strength. Fig. 1 illustrates the eluite distribution using a weak mobile phase, $k' = 10$. As the strength of the injection solvent increases, k' from 1 to 0.1, the distribution changes from a reasonably symmetric band to a band with a leading edge, and finally to the development of a sharp band near the mobile phase front. Experimentally, chromatographic bands developed a leading edge and a sharp peak at the mobile phase front appeared as the injection solvent strength increased[12].

Fig. 2 shows the distribution of an eluite injected in a strong solvent, $k' = 0.1$. The mobile phase strength is decreased from $k' = 1$ to $k' = 10$. This eluite's main band develops a leading edge that produces more asymmetry as the mobile phase strength decreases. In all three cases a sharp band of similar height and identical location is formed near the mobile phase front. The appearance of this sharp band



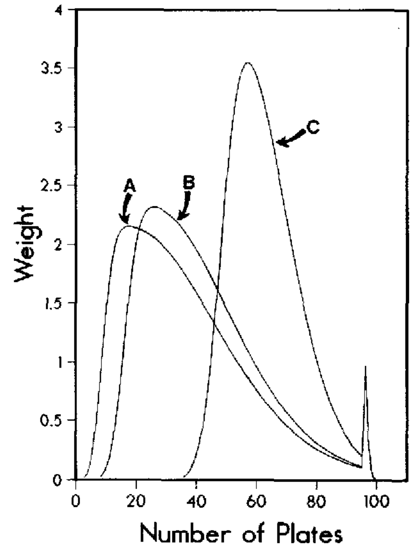


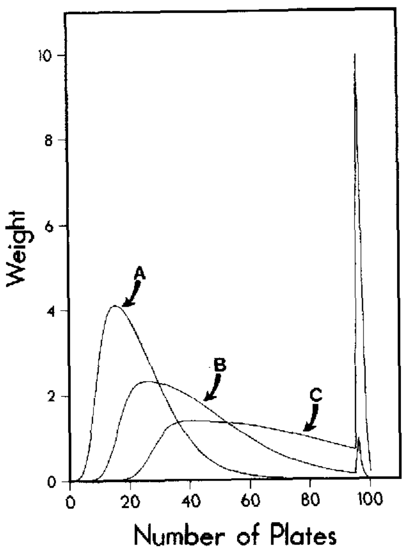
Fig. 1. Distribution of 100 wt. units of eluite after the mobile phase (k' = 10) traverses 100 plates. The eluite injection volume was five plate volumes. Injection solvent strength, expressed as capacity factor, was (A) k' = 1; (B) k' = 0.2; (C) k' = 0.1.

Fig. 2. Distribution of 100 wt. units of eluite after the mobile phase [(A) k' = 10; (B) k' = 5; (C) k' = 1] traverses 100 plates. The eluite was introduced in an injection solvent of k' = 0.1 and a volume of five plate volumes.

indicates that the strong injection solvent will carry the eluite with it whether the mobile phase is moderately weak or very weak, that is, this peak's appearance is largely dependent on the strength of the injection solvent and not that of the mobile phase.

Fig. 3 attempts to simulate the case of the distribution of three eluites when injected into the same weak mobile phase from the same injection solvent. The eluites have different k' values in the mobile phase, and, as a first approximation, the eluites have proportionally different k' values in the injection solvent giving a constant k' ratio for each eluite of 50. The eluite that is retained the most, has the most symmetric distribution although even here a leading edge appears. Furthermore, the peak near the solvent front is highest for the case of the eluite that has the smallest k' for the injection solvent. This effect is in agreement with the constant appearance of such a peak discussed for Fig. 2. The distributions shown in Fig. 3 are in agreement with the chromatographic peaks observed experimentally[12].

Experimental peak distortion was found to increase with increasing injection volume for HPLC with injection solvents stronger than the mobile phase[12]. Fig. 4 shows the distribution of eluite when 100 wt. units were injected three ways. Distribution A resulted from a small injection volume, one plate volume. The peak had a leading edge. When the injection volume was five plate volumes, the 100 wt. units gave distribution B. In this case the leading edge produced more asymmetry, and the sharp peak at the mobile phase front, illustrated previously in Figs. 1, 2 and 3, appeared. If the injection volume was increased further to nine plate volumes, the



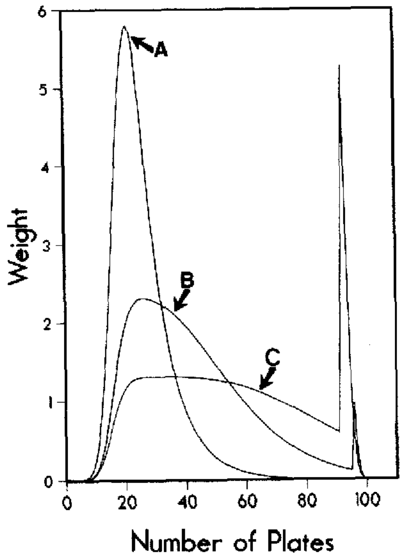
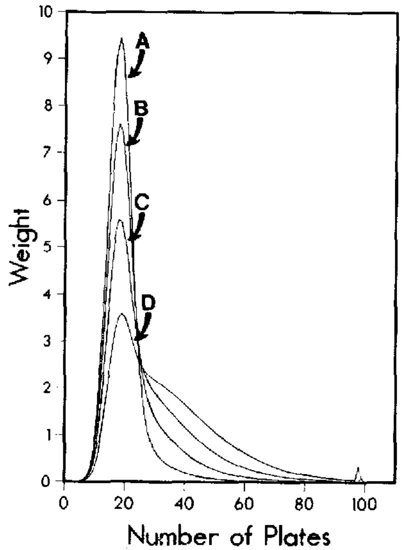


Fig. 3. Distribution of three eluites [(A) $k' = 10$ and 0.2; (B) $k' = 5$ and 0.1; (C) $k' = 2.5$ and 0.05 for mobile phase and injection solvent, respectively] after the mobile phase traverses 100 plates. The eluite injection volume was five plate volumes.

Fig. 4. Distribution of 100 wt. units of eluite ($k' = 5$ and 0.1 for mobile phase and injection solvent respectively) after the mobile phase traverses 100 plates. Injection in a volume of (A) one plate volume, (B) five plate volumes and (C) nine plate volumes.



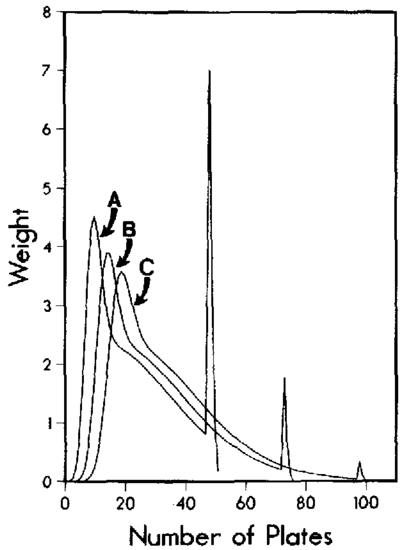


Fig. 5. Distribution of 100 wt. units of eluite ($k' = 5$ and 0.1 for mobile phase and injection solvent respectively) after the mobile phase traverses 100 plates. The volume of the injection solvent plug was five plate volumes. In A the last four fifths of the plug had a $k' = 5$. In B $k' = 5$ was used for the last three fifths, in C the last two fifths and in D the last fifth.

Fig. 6. Distribution of 100 wt. units of eluite ($k' = 10$ and 0.1 for mobile phase and injection solvent respectively) after traversing (A) 50, (B) 75 and (C) 100 plates. The volume of the injection solvent plug was five plate volumes. The last fifth of the plug had a $k' = 10$.

peak was severely distorted and the peak at the mobile phase front was markedly taller.

The general features of these column band patterns were similar to experimentally observed chromatographic peaks. The computed distribution results suggest that, if the volume of strong solvent is large enough, the solvent is capable of carrying part of the eluite with it practically to the end of the column while the remainder of the eluite follows as a peak with a leading edge.

What has been observed experimentally was the development of one or more additional chromatographic peaks between the main peak and the peak near the mobile phase front. It was suggested that one additional peak should develop as the injection solvent volume and strength increased and that more than one additional peak resulted from dilution of the solvent plug[12]. The production of a single additional peak between the two peaks shown in Figs. 1–4 was not supported by distribution theory. However, computation assuming dilution of the plug did give rise to a peak located between the two shown in previous figures.

The consequence of diluting the rear end of the injection solvent plug is illustrated in Fig. 5. When the rear four-fifths of the plug is diluted, the peak develops a slight leading edge. This edge becomes a bulge when only the last fifth of the plug is diluted. A peak near the mobile phase front is present at less dilution.

Fig. 6 shows the effect of traversing an increasing number of plates when the rear fifth of the plug is diluted. The bulge or development of a middle peak is pronounced when only 50 plates have been traversed. The peak near the mobile phase front is very tall early in the distribution development. When 100 plates are traversed, the bulge is smoother and the peak at the mobile phase front is small.

Fig. 7 shows distributions formed when 100 wt. units of eluite are injected into a column whose plate volume is 10% of the injection volume. Both ends of the injection plug were diluted as shown in Table II. These band peaks are very similar to chromatographic peaks observed experimentally by many workers. They illustrate that distri-

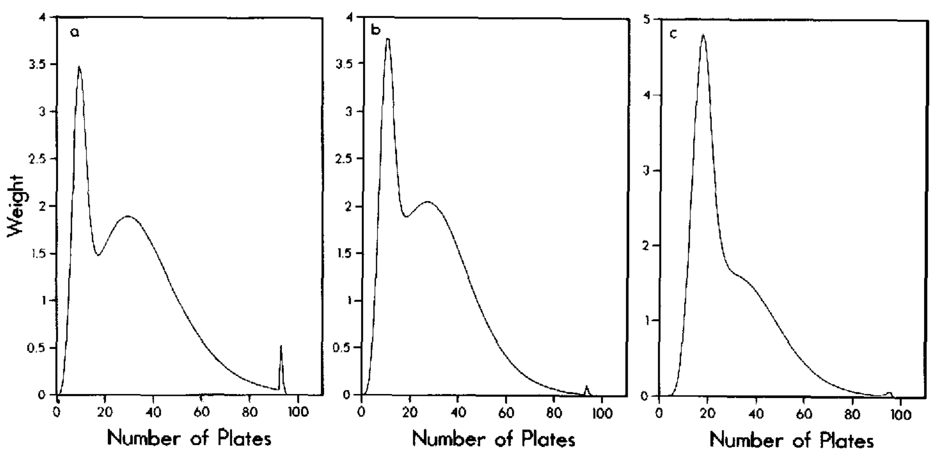


Fig. 7. Distribution of 100 wt. units of eluite after the mobile phase traverses 100 plates. The eluite injection volume was ten plate volumes. Mobile phase $k' = 10$ and injection solvent k' distribution is shown in Table II.

TABLE II

k' DISTRIBUTION IN THE INJECTION PLUG AFTER DILUTION

Plates										Fig.
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	
10.0	10.0	0.15	0.15	0.15	0.15	0.15	10.0	10.0	10.0	7a
5.0	2.0	1.2	0.5	0.2	0.1	0.1	0.2	0.4	0.5	7b
10.0	10.0	0.1	0.1	0.1	0.1	0.5	0.5	0.5	0.5	7c

butions can have three peaks, and their general features can be altered by the dissymmetric dilution of the ends of the strong injection solvent plug.

Finally, Fig. 8 attempts to simulate an experiment in which peak distortion was produced by injecting a strong solvent shortly after the eluite had been injected dissolved in the mobile phase. Experimentally a chromatographic peak with a leading edge was produced[12]. A leading edge in the distribution was produced by computation, and the consequent asymmetry was always present as the volume of strong solvent was varied.

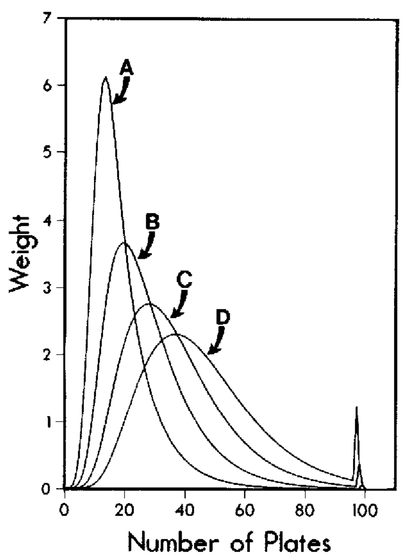


Fig. 8. Distribution of eluite (k' = 10 and 0.1 for mobile phase and injection solvent, respectively). The eluite, 100 wt. units, was introduced into the first plate. For A, B, C and D the injection solvent volumes were one, two, three and four plate volumes, respectively.

CONCLUSION

The general features of the distributions obtained by computation are very similar to the chromatographic peaks obtained experimentally. The similarity suggests the cause of peak distortion and multiplicity is changing partitioning that results from the short lived movement of a strong mobile phase through the column followed by normal movement of a weak mobile phase. More than two mobile phases are needed, however, to produce more than two peaks. Computation results are consistent with the idea that dilution of the solvent plug gives rise to more than two peaks.

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