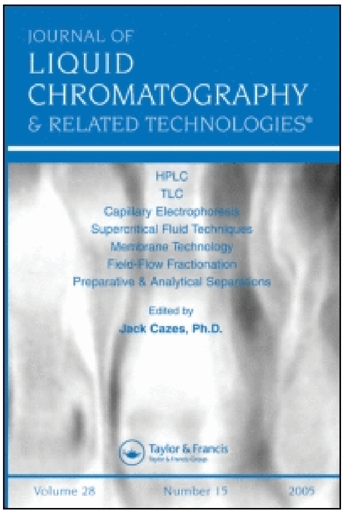

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MICROCOMPUTER SIMULATION OF ELUITE DISTRIBUTION AFTER LC INJECTION

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ABSTRACT

Equilibrium distribution theory was applied to the injection of eluite into several theoretical plates at the head of the column. The use of microcomputer spreadsheet software showed that a symmetric peak quickly develops despite an initial skewed distribution of eluite.

INTRODUCTION

Gas chromatographic sample introduction has been studied theoretically with respect to the effect of volume size on peak characteristics (1). For plug flow, distribution theory predicted a symmetric peak for all injection volumes. For exponential flow, however, theory predicted tailing peaks.

When a sample plug is injected into an HPLC column an eluite band skewness should develop immediately because of the drop in concentration of the eluite in the plug as it moves into the column. The concentration should be highest at the very beginning of the column and drop off proceeding down the column. Yet, as the mobile phase moves into the eluite band the skewness must disappear because, in practice, no skewness is superimposed on the band dispersion resulting from mass transfer. The question arises: from equilibrium distribution theory (2) should a symmetric peak form and, if so, how far down the column must the band move to form a symmetric peak from its skewed initial concentration profile. The degree of skewness should depend on k', and the extent of the initial skewed band should depend on the volume of the plug.

It is the purpose of this paper to report a microcomputer simulation of the injection process. An equilibrium distribution was described using spreadsheet computer software with graphics. The skewed injection profiles were obtained as a function of k' and sample volume. The computer program then simulated movement of the band to show if and when a symmetric or near symmetric band formed.

THEORY

The column was assumed to be divided into theoretical plates whose mobile phase volume was equal to or smaller than the injection volume. Retention was described by:

$$k' = (K/\beta) = (w_s/x-w_s)$$

where K is the partition coefficient, β is the phase ratio, x is the total weight of the eluite in a plate and w_s is the weight of the eluite in the stationary phase in a plate. We chose k' for the distribution equilibrium instead of K, as is often done, because it showed the effect of either the partition coefficient or the phase ratio on the eluite distribution in the plates.

The injection begins with the introduction of the weight x of the eluite into the first plate. Equilibrium is attained and the next volume of injection solvent containing x weight of eluite moves the first plate's mobile layer containing $(x-w_s)$ of the eluite into the next plate. Equilibrium is attained in each of the plates. The process is continued as injection brings in more eluite until finally all of the eluite sample is distributed in the first few plates. The plates have equal volumes and the total injection volume is an integral multiple of the plate volume. The multiples used were 1, 5 and 10.

After injection is completed mobile phase instead of injection solvent moves into the first plate. The mobile eluite in each of the occupied plates moves into the next plate and equilibrium is reattained. The process is repeated as more mobile phase moves into the plates.

PROCEDURE

An AT&T PC 6300 microcomputer with 640K bytes RAM was used. A Lotus 1-2-3 spreadsheet program was used to calculate the eluite distribution. Table 1 shows a typical spreadsheet generated by the computer. Here 100 wt. units, e.g., 100 ng, were introduced

TABLE 1

Spreadsheet Generated for Injection into the First Five Plates[a]

	A	B	C	D	E	F	G	H	I	J
1	20.0	0.5								
2	30.0	10.0								
3	35.0	20.0	5.0							
4	37.5	27.5	12.5	2.5						
5	38.7	32.5	20.0	7.5	1.2					
6	19.4	35.6	26.2	13.7	4.4	0.6				
7	9.7	27.5	30.9	20.0	9.1	2.5	0.3			
8	4.8	18.6	29.2	25.5	14.5	5.8	1.4	0.2		
9	2.4	11.7	23.9	27.3	20.0	10.2	3.6	0.8	0.1	
10	1.2	7.1	17.8	25.6	23.7	15.1	6.9	2.2	0.4	0.0

[a] k' = 1

in five 20 unit portions: in cells A1, A2, A3, A4, A5. Cell B1 did not represent a plate in the separation; it contained k'/1+k', the fraction of x in the stationary phase. The number in each cell (other than B1) represents the total weight of eluite in the plate and not the weight in either phase. The spreadsheet shows distribution of the solute through ten plate volumes. The horizontal row shows the distribution of an eluite along the plates. Fig. 1 shows the cell formulas used to develop the spreadsheet in Table 1. For cases beyond ten plates cell formulas were extended to calculate distributions upto 100 plates. More RAM was required than available to go beyond 100 plates.

Six different k' values (10, 5, 3, 1.5, 1, 0.5) were chosen for calculation. Graphs were generated for each case depicting different stages of distribution in a column depending on the number of plates traversed. A Hewlett Packard 7475A plotter was used to draw the graphs.

```
A1: 20
B1: 1/2
A2: 20+A1*$B$1
B2: +A1-A1*$B$1
A3: 20+A2*$B$1
B3: +A2-$B$1*A2+B2*$B$1
C3: +B2-B2*$B$1
A4: 20+A3*$B$1
B4: +A3-$B$1*A3+B3*$B$1
C4: +B3-$B$1*B3+C3*$B$1
D4: +C3-C3*$B$1
A5: 20+A4*$B$1
B5: +A4-$B$1*A4+B4*$B$1
C5: +B4-$B$1*B4+C4*$B$1
D5: +C4-$B$1*C4+D4*$B$1
E5: +D4-D4*$B$1
A6: +A5*$B$1
B6: +A5-$B$1*A5+B5*$B$1
C6: +B5-$B$1*B5+C5*$B$1
D6: +C5-$B$1*C5+D5*$B$1
E6: +D5-$B$1*D5+E5*$B$1
F6: +E5-E5*$B$1
A7: +A6*$B$1
B7: +A6-$B$1*A6+B6*$B$1
C7: +B6-$B$1*B6+C6*$B$1
D7: +C6-$B$1*C6+D6*$B$1
E7: +D6-$B$1*D6+E6*$B$1
F7: +E6-$B$1*E6+F6*$B$1
G7: +F6-F6*$B$1
A8: +A7*$B$1
B8: +A7-$B$1*A7+B7*$B$1
C8: +B7-$B$1*B7+C7*$B$1
D8: +C7-$B$1*C7+D7*$B$1
E8: +D7-$B$1*D7+E7*$B$1
F8: +E7-$B$1*E7+F7*$B$1
G8: +F7-$B$1*F7+G7*$B$1
H8: +G7-G7*$B$1
A9: +A8*$B$1
B9: +A8-$B$1*A8+B8*$B$1
C9: +B8-$B$1*B8+C8*$B$1
D9: +C8-$B$1*C8+D8*$B$1
E9: +D8-$B$1*D8+E8*$B$1
F9: +E8-$B$1*E8+F8*$B$1
G9: +F8-$B$1*F8+G8*$B$1
H9: +G8-$B$1*G8+H8*$B$1
I9: +H8-H8*$B$1
A10: +A9*$B$1
B10: +A9-$B$1*A9+B9*$B$1
C10: +B9-$B$1*B9+C9*$B$1
D10: +C9-$B$1*C9+D9*$B$1
E10: +D9-$B$1*D9+E9*$B$1
F10: +E9-$B$1*E9+F9*$B$1
G10: +F9-$B$1*F9+G9*$B$1
H10: +G9-$B$1*G9+H9*$B$1
I10: +H9-$B$1*H9+I9*$B$1
J10: +I9-I9*$B$1
```

FIGURE 1: Cell formulas used to generate a spreadsheet.

RESULTS AND DISCUSSION

Fig. 2 shows the weight distribution of an eluite in the first five plates. The eluite weighed 100 weight units and was injected in a plug whose volume was five times the mobile phase plate volume. The five curves represent k' values from 0.5 to 10. The k' values could be the result of eluites with different partition coefficients or could result from columns having different phase ratios. Similar curves, although extended, were obtained when injecting into ten plates.

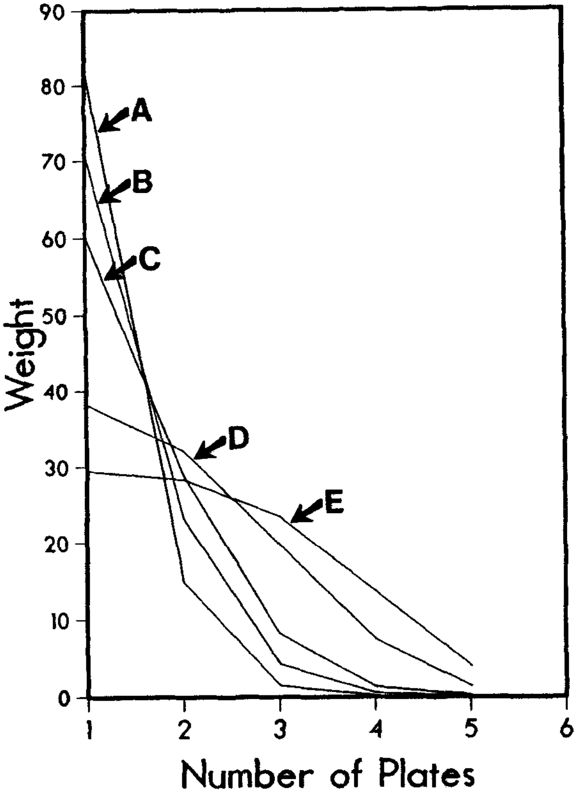

FIGURE 2: Distribution of eluite in the first five plates. For A, B, C, D, E, k'=10, 5, 3, 1, 0.5 respectively.

As the injection plug moves into the first plate the stationary phase removes eluite from the plug so that when the plug moves into the second plate, the amount of eluite has dropped. The stationary phase in the second plate then removes less, and this eluite removal continues on as the rear part of the plug moves into the early plates. Meanwhile the plug is bringing in a higher concentration into the early plates than what was in equilibrium as a result of the front of the plug's entrance. Fig. 2 shows that when k' is large, most of the sample is found in the early plates; e.g., for k'=10 almost all of the sample is in the first two plates. When k' is small the sample is more evenly distributed among the five plates. The distribution would be completely even at k'=0.

Fig. 3 shows, for k'=1, the progression of the eluite band whose injection profile is given in Fig. 2. As the mobile phase enters the first plate following the injection plug, the 5th plate contains about 3% of the eluite content of the first plate. By the time the front edge of the band reaches the 13th plate, a nearly symmetric band has developed with the eluite maximum in plate 7.

By comparison, Fig. 4 shows the changing distribution of eluite after injection in a plug whose volume put all of the eluite in the 1st plate. For k'=1 the band from this kind of injection is always symmetric. When the front of the band reaches the 14th plate (an even numbered plate is required for a single maximum) the band is very similar to the peak with a maximum at the 7th plate shown in Fig. 3. As the mobile phase carries these two eluite bands through more plates the peak symmetries become equal. This equality is shown in Fig. 5 where the bands' maximum weights are located in the 49th and 50th plates.

Another way of comparing the development of peak symmetry when the injection volume is changed is to evaluate the asymmetry when the plate maximum is at a given plate. Table 2 presents peak

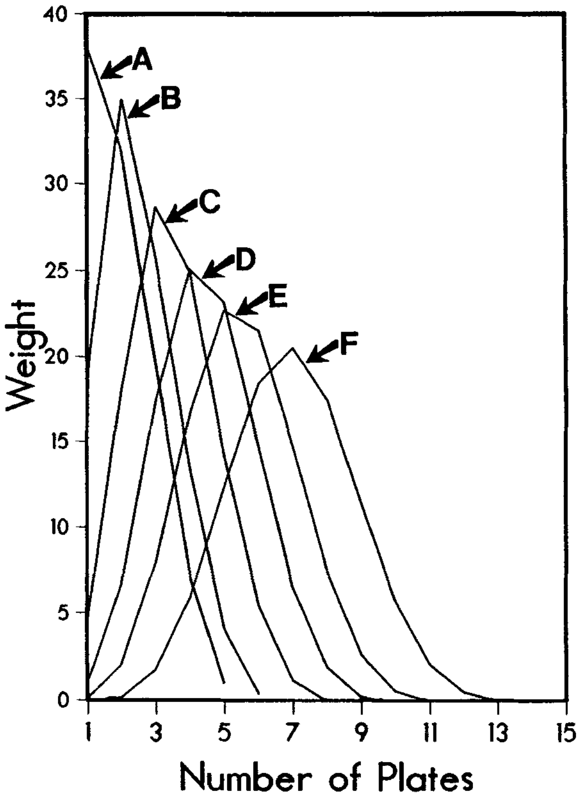

FIGURE 3: Distribution of eluite (k'=1) after traversing several plates. For A, B, C, D, E, F, plates traversed = 5, 6, 8, 10, 12, 15 respectively. Eluite injected into the first 5 plates.

asymmetry early in peak development, namely, when the maximum eluite weight is in plate 8. At a given k' peak leading increases with increasing injection volume. The rate of change of peak leading with change in injection volume decreases with increasing k'

Table 3 shows how peak symmetry has developed when the peak maximum is in plate 17. In most cases the peaks are symmetric.

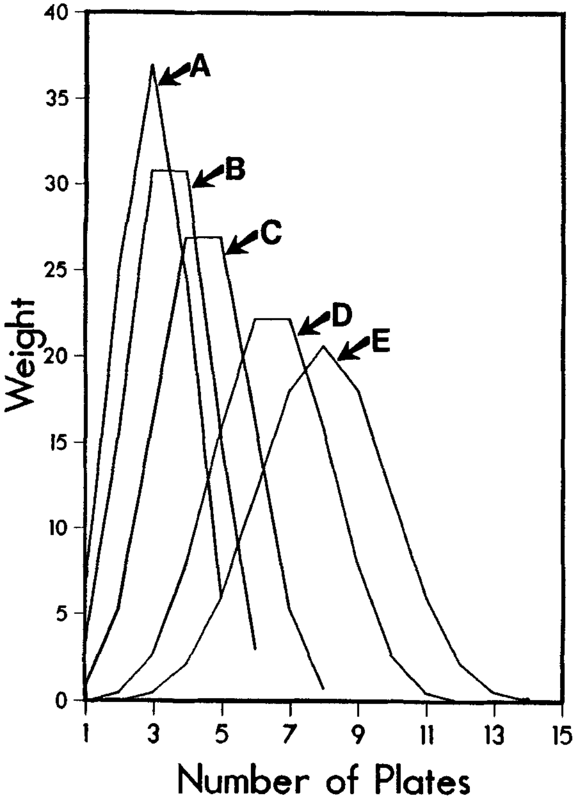

FIGURE 4: Distribution of eluite (k'=1) after traversing several plates. For A, B, C, D, E, plates traversed = 5, 6, 8, 12, 15. Eluite injected into the first plate only.

Within the error of measuring asymmetry, as the injection volume increases, the asymmetry remains constant at 1 or decreases somewhat. In general, the decreasing asymmetry, peak leading, with increasing volume occurs at higher k' values.

In Table 4 the development of peaks following exponential injection (4) is presented. For k'=1, tailing occurs when the peak maximum is in plate 8 but is reduced when the peak maximum

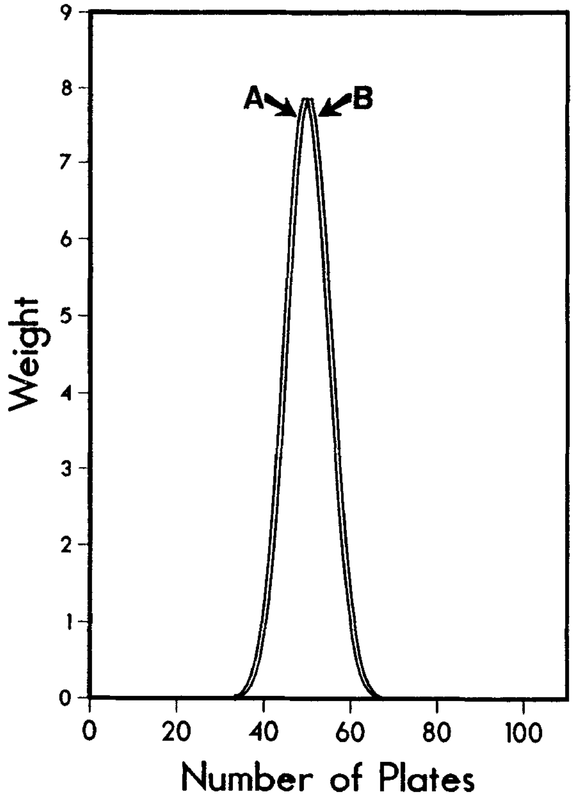

FIGURE 5: Distribution of eluite (k'=1) after traversing 100 plates. A = eluite injected into the first 5 plates; B = eluite injected into the first plate only.

has moved to plate 17. It is absent by the time the peak maximum is in plate 28. When k' is larger than 1 the peaks do not tail but lead. In effect larger k' values keep the eluite in the early plates so that the richest part of the developing band never moves far ahead of the exponential tail.

Overall microcomputer simulation of peak development from equilibrium distribution theory indicates peaks become symmetric

TABLE 2

Peak Asymmetries when the Peak Maximum is in the 8th Plate

Injection Volume[a]	k'	Number of Plates Traversed	Asymmetry[b]
1	0.5	11	1.30
1	1	15	1.00
1	3	29	0.95
1	5	44	0.91
1	10	81	0.84
5	0.5	14	0.83
5	1	17	0.97
5	3	31	0.95
5	5	46	0.86
5	10	84	0.81
10	0.5	17	0.72
10	1	20	0.84
10	3	34	0.87
10	5	49	0.84
10	10	87	0.80

[a] Units: Mobile phase plate volume.

[b] [peak tail]/[peak front] at 10% of peak height (3). Asymmetry measurement from graphics with rsd = 1%.

TABLE 3

Peak Asymmetries when the Peak Maximum is in the 17th Plate

Injection Volume[a]	k'	Number of Plates Traversed	Asymmetry[a]
1	0.5	25	1.04
1	1	33	1.00
1	1.5	41	1.00
1	3	54	0.99
1	5	100	0.88
5	0.5	27	1.01
5	1	35	1.00
5	1.5	43	0.99
5	3	56	0.97
5	5	100	0.90
10	0.5	30	0.89
10	1	37	1.03
10	1.5	45	1.00
10	3	58	0.91
10	5	100	0.78

[a] As in Table 2

TABLE 4

Peak Asymmetries Following Exponential Injection

k'	Plate with Eluite Maximum	Number of Plates Traversed	Asymmetry[a]
1	8	15	1.14
3	8	29	0.99
5	8	43	0.96
1	17	33	1.08
3	17	55	0.96
5	17	100	0.85
1	50	100	0.95

[a] As in Table 2

very rapidly. Whether an eluite is injected in a very small volume or a more moderate sized volume, peaks are reasonably symmetric before their maxima reach the 10th plate. Peaks from eluites with larger k' values require more plates to traverse to become symmetric than those with smaller k' values.

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