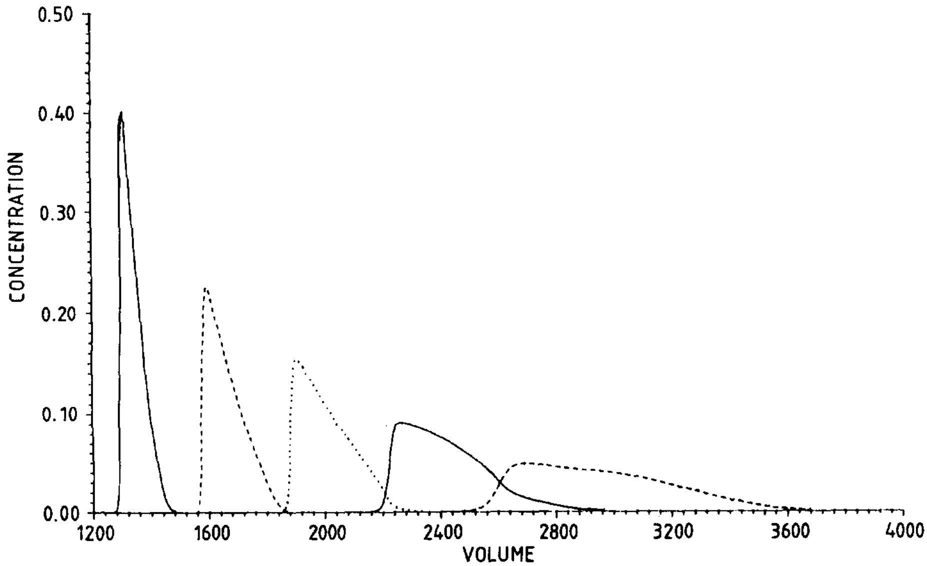

Fig. 8. Separation as in Fig. 1 except amount injected (all components), 30 in volume 15.

outlet (1000 T.P.) is 94.86 and therefore the critical volume should be about 190. Contrary to this prediction, at a feed volume of about 15 an increase of recovery ratio and at a volume of 150 a sharp decline in both recovery and production rate are observed.

Increase in blocking factors

Overloading of the solid phase is determined on the one hand by its capacity and on the other by the sum of the products of the distribution coefficients, concentrations and blocking factors of individual components. Therefore, when the blocking factors of only some of the components are increased, the separation of all components is influenced. In our example, the blocking factors of the first, third and fifth components were increased; the amounts injected and all other parameters remained constant. The recoveries of the second and fourth components decreased on increasing the blocking factors of the surrounding components (see Table IV). In the chromatograms shown in Figs. 9–11 the influence of changing blocking factors is clearly seen. In Fig. 9 the peaks of odd-numbered components would be Gaussian if none of the other components were present. In the example illustrated, however, the third and fifth components are clearly deformed owing to the presence of the fourth component. On the of other hand, the influence of the surrounding components on the peak shape of component **4** is clearly seen. The overloading in the last instance (Fig. 11) is so strong that only the first two components can be isolated; the others are not separated at all. Note also the shift in the peak maxima of all components, even those with constant blocking factors, to lower elution volumes with increase in the blocking factors. This is clear evidence of the mutual influence of sample components in the course of the separation process.

TABLE IV

EFFECT OF INCREASE IN BLOCKING FACTORS

Standard mixture: amount (all compounds), 30. Variable: blocking factors of components **1**, **3** and **5**. All others variables as in Fig. 1.

Blocking factor	*Component 2*		*Component 4*	
	Recovery	*Productivity*	*Recovery*	*Productivity*
0	100.0	0.704	85.2	0.600
1	100.0	0.707	46.0	0.325
2	99.3	0.704	0	0
3	94.5	0.671	0	0
4	86.3	0.613	0	0

Isolation of minor components

If one of the components in the sample predominates, then the recovery is determined not only by its amount but also by its elution volume in relation to other components.

The recoveries listed in Table V vary if the isolated component is eluted before or after the predominant compound. The concentrations of peaks eluted before it are increased, whereas the peaks eluted after the largest peak are smeared and their concentrations are smaller than those which would have been eluted without interference from the predominant component. This is illustrated in Figs. 12 and 13 in comparison with Fig. 1 and confrontation of Tables V and I, too. The effect of

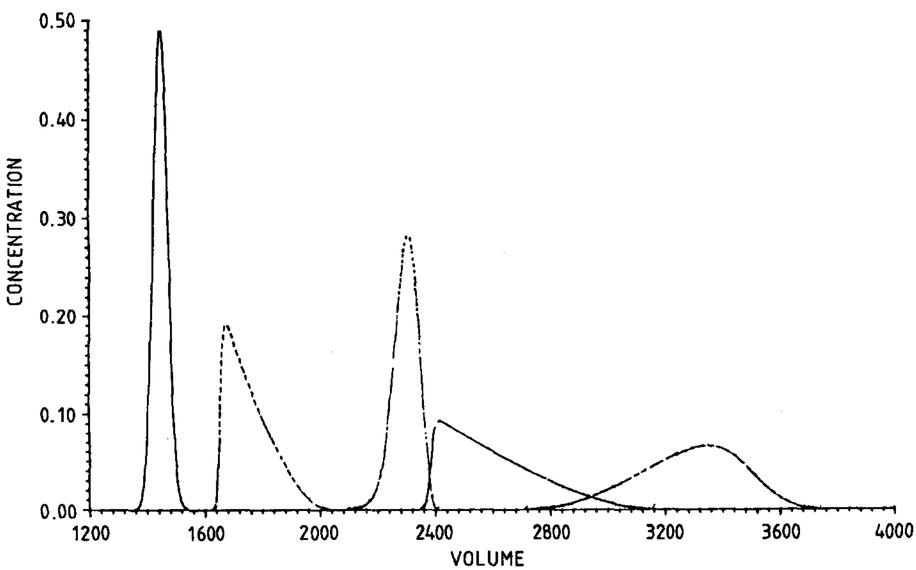

Fig. 9. Separation as in Fig. 1 except amount injected 30; injection volume, 1; blocking factors of components **1**, **3** and **5** = 0.

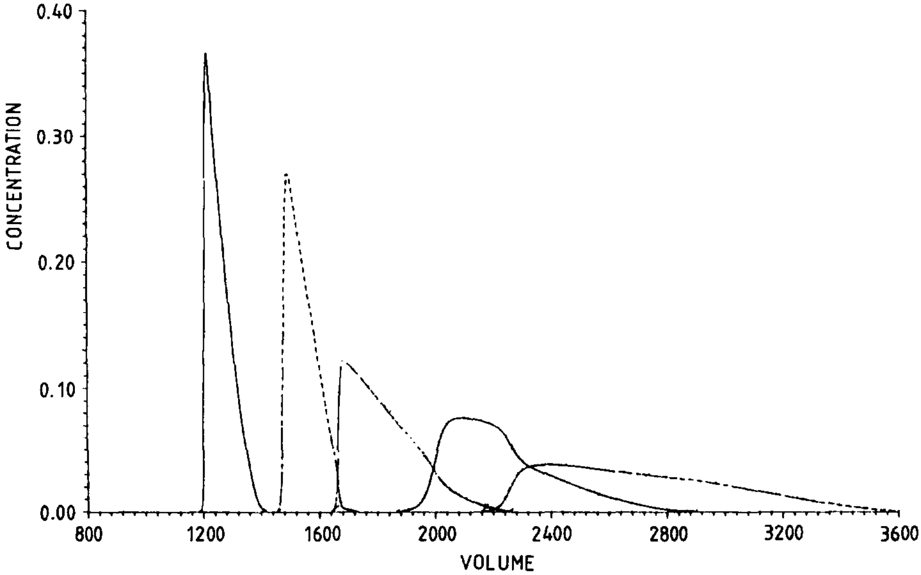

Fig. 10. Separation as in Fig. 9 except blocking factors of components **1**, **3** and **5** = 2.

approximately equal load of first component (475 times 0.5) on recovery is much more adverse than that of the last one (150 times 2.5).

If three components (**1**, **3** and **5**) dominate the sample, then the minor components (**2** and **4**), present in the sample in 100 times smaller amounts, can be

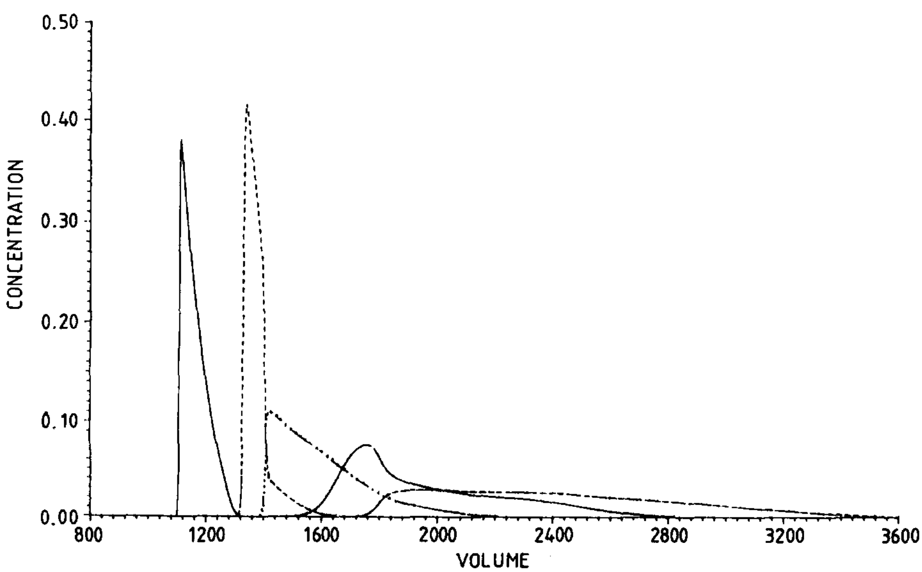

Fig. 11. Separation as in Fig. 9 except blocking factors of components **1**, **3** and **5** = 4.

TABLE V

EFFECT OF A LARGE SURPLUS OF ONE COMPOUND

Column: 1000 T.P.; volume injected, 1; all blocking factors, 1; concentration multiplied by 10^4; k' = capacity factor.

Parameter	*1* (k' = 0.5)	*2* (k' = 1)	*3* (k' = 1.5)	*4* (k' = 2.0)	*5* (k' = 2.5)
Amount	150	1	1	1	1
Peak maximum	1199	1923	2412	2899	3390
Recovery (%)	–	100	99.5	96.0	98.8
Concentration	–	18	18	21	9
Amount	1	1	1	1	150
Peak maximum	1395	1724	1921	1945	1970
Recovery (%)	100	100	44.4	0.0	–
Concentration	28	28	48	–	–
Amount	1	150	1	1	1
Peak maximum	1340	1389	2362	2857	3352
Recovery (%)	98.4	–	14.7	21.9	87.2
Concentration	63	–	16	16	8
Amount	475	1	1	1	1
Peak maximum	1055	1678	2384	2866	3354
Recovery (%)	–	0	0	0	75.2
Concentration	–	–	–	–	7

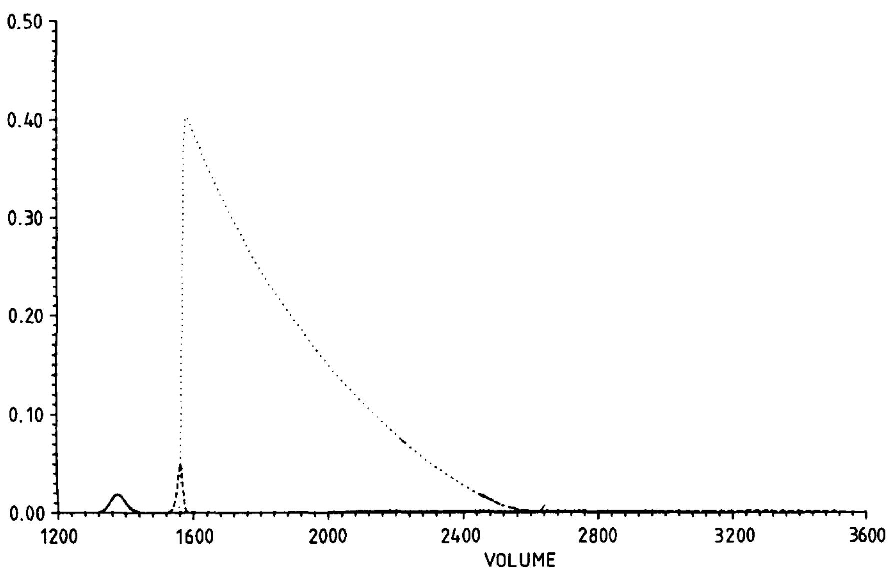

Fig. 12. Separation as in Fig. 1 except amounts injected 1.0, 1.0, 150.0, 1.0, 1.0 (components **1**–**5**).

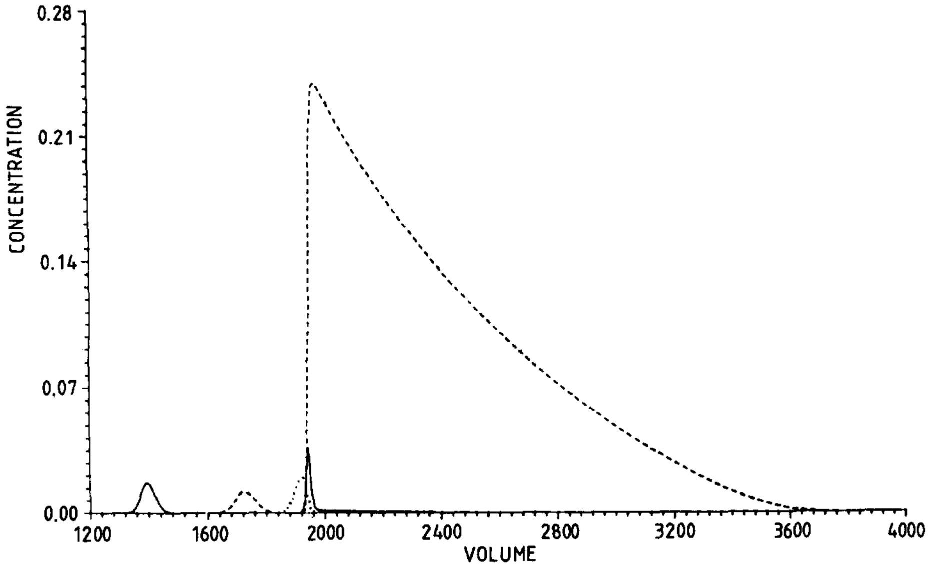

Fig. 13. Separation as in Fig. 1 except amounts injected 1.0, 1.0, 1.0, 1.0, 150.0 (components 1–5).

isolated on the 1000 T.P. column with good recoveries (last entry in Table V). This is true, of course, only when the feed as whole does not overload the column and the separation does not break down.

Gradient elution

To demonstrate the possibilities of gradient elution for preparative separations, three linear gradients were compared. The concentration of the strongest eluent varied from 0 to 1 and the values of its capacity factor were chosen to be 1 (in one instance) and 2 (in another example). The greatest differences are found in the recovery, concentration and productivity of the separation of component **4** when it is isolated from a standard mixture (Table VI). In comparison with isocratic elution, at the same recovery the productivity may be increased by 50% and the concentration of a selected fraction more than doubled (compare the first and fourth separations in Table VI). When the gradient is steeper than the optimum (second line), then the recovery and productivity decrease but the concentration of the selected fraction increases further. It should be stressed that in these computations the eluting agent is not classified as an impurity. It is interesting to follow how its increasing concentration at the column outlet is changed by transport through the column and how the peaks of the separated components are impressed as negative peaks on the trace of eluent concentration (dotted line in Fig. 14).

In an attempt to describe preparative liquid chromatography, Eble *et al.*[24] concluded that gradient elution is equivalent to isocratic elution if "average" capacity factors are equal, but no quantitative treatment of gradient optimization under overload conditions was presented. Therefore, no quantitative comparison with their treatment is possible.