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EVALUATION OF LINEAR GRADIENT LOADED COLUMNS IN TEMPERATURE PROGRAMMED GAS CHROMATOGRAPHY

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SUMMARY

The performance of gradient loaded columns in temperature programmed gas chromatography has been evaluated. It was found that the elution temperatures and elution times are independent of the type of gradient (positive, negative or no gradient). At optimal working conditions (small injection volume and optimal carrier gas flow) resolution is best with evenly loaded columns, intermediate for columns with negative gradient and worst for columns with positive gradient.

When deviating from optimal carrier gas velocity, resolution decreases much more for evenly loaded columns than for gradient columns. On increasing the injected volume, the decrease in resolution is much more pronounced for columns with positive gradient and no gradient than for columns with negative gradient. Thus, columns with negative gradient are superior to evenly loaded columns when large injection volumes are required.

It is suggested that columns with negative liquid load gradient could prove especially useful in preparative temperature programmed gas chromatography.

INTRODUCTION

The isothermal performance of chromatographic columns connected in series has been evaluated theoretically and practically[1,2]. Columns with various amounts of liquid phase along the column (Gradient Loaded Columns) can be considered as a special case of chromatographic columns in series.

The performance of columns with a two[3] and with a multiple[4] stepwise approximation to a continuous linear gradient and to an exponential gradient[14] have been described. Gradient loaded columns with decreasing liquid load (negative gradient) have been claimed to have advantages over uniformly loaded columns with the same amount of liquid phase; for example, a better resolution is obtained for solute-pairs of low or intermediate partition ratio[4,14] and a greater sample size can be injected without loss of resolution[5,14]. The finding that better resolution can be obtained in temperature programmed gas chromatography (TPGC) with a column that has been used for a considerable length of time by changing the direction of the gas flow[6], as

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well as the finding that such a column performs better than a new one[6], prompted us to evaluate the performance of gradient loaded columns in TPGC.

EXPERIMENTAL

5-g portions of Gas-Chrom Q, 80–100 mesh, were loaded with 10 %, 7 %, 5 % and 1 % Polymer EGSS-X (Applied Science Lab.) by the evaporation technique[7]. Three 1.30-m U-shaped columns were deactivated[8] and in order to fill them in a symmetrical manner, a glass wool plug was inserted 8 cm from the end of the column. (The first 8 cm of the column have to remain empty as they come in the flash heater zone.) The gradient column was prepared by sucking consecutively 1 g portions of 10 %, 7 %, 3 % and 1 % loaded stationary phase into the column. The column was further packed under pressure by gently tapping until the packing was exactly 8 cm from both ends of the column. The reference column was packed with a homogeneous mixture of 1 g of each of the packings, and an absolute reference column was packed with the same amount of liquid phase loaded uniformly (5 %). The columns were put in an F & M gas chromatograph, Model 402, purged with nitrogen and conditioned overnight in a nitrogen atmosphere at 220°, after which they were flushed out for a few hours at the same temperature with nitrogen (10 ml/min). The direction of flow for the gradient column was from the region of low liquid loading toward the region of high liquid loading, in order not to reduce the gradient. Temperature programmes were started at 130° (T_i) and the initial isothermal period (t_i) was 4 min in each case. A mixture of fatty acid methyl esters covering a wide range of boiling points (methyl myristate to methyl lignocerate) was used in order to determine the influence of flow rate, programming rate and injection volume upon the elution temperature, the resolution between critical pairs of compounds and other parameters for column performance.

The flow rate was calculated from the time interval between injection and elution of the solvent peak. This time interval was found to be inversely proportional to the flow rate. Elution temperatures were calculated from the elution times, and the resolution[9] and the relative peak dip[10] for the critical pair methyl stearate–methyl oleate were taken as a measure of the separating power of the column.

RESULTS

Influence of flow reversal on the performance of gradient loaded columns

As the columns were packed in a symmetrical way (the columns being symmetrical and the dead spaces at the beginning and at the end of the column being identical) flow reversal could be achieved by turning the column in the oven of the gas chromatograph, *i.e.* by connecting the end that had been connected with the detector with the injector, and *vice versa*.

The effect of the gas flow on the elution temperatures of methyl palmitate ($C_{16:0}$), methyl arachidate ($C_{20:0}$), methyl lignocerate ($C_{24:0}$) and methyl docosahexaenoate ($C_{22:6}$) for both positive and negative liquid phase gradients (programming rate of 4°/min) is presented in Fig. 1.

It is clear that elution temperatures are not dependent on the direction of flow in gradient loaded columns. This has been found to be true for programming rates between 0°/min (isothermal operation) and 10°/min.

The influence of programming rate upon elution temperature (at constant flow rate of 17 ml/min, measured at 1 atm and 25°) was also investigated, and again it was found that elution temperature is independent of the direction of flow. The influence of programming rate on the elution temperature of methyl stearate is shown in Fig. 2.

The influence of gas flow and gas flow reversal on resolution (R), relative peak dip (P_d), peak widths at half height ($w_{1/2}$), and distance between peak maxima (Δv)

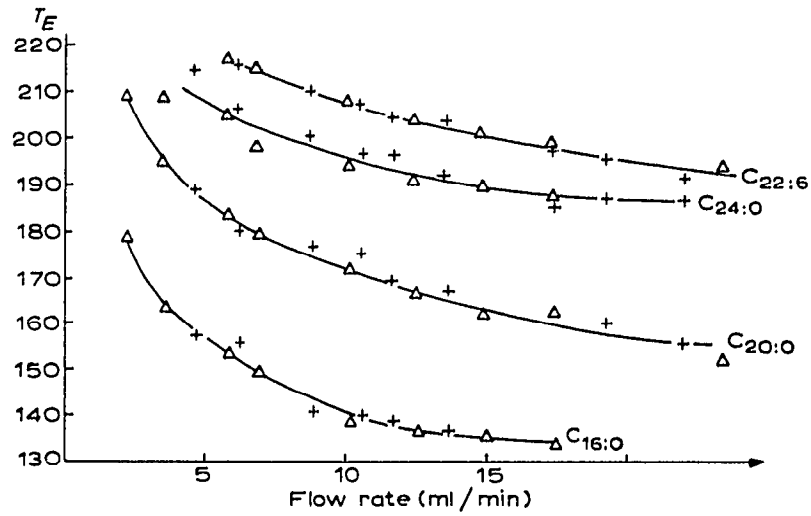

Fig. 1. Elution temperatures (°C) of methyl docosahexaenoate ($C_{22:6}$), methyl lignocerate ($C_{24:0}$), methyl arachidate ($C_{20:0}$) and methyl palmitate ($C_{16:0}$) at various flow rates for columns with positive (+) and negative (△) gradients. Working conditions: T_i = 130°; t_i = 4 min; programming rate = 4°/min.

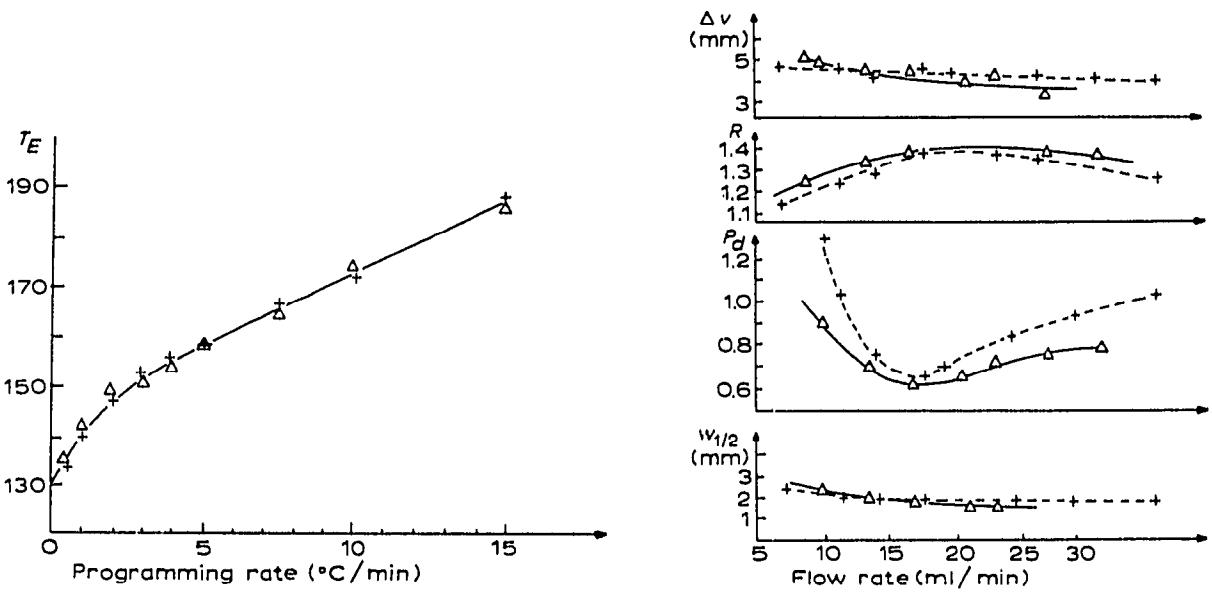

Fig. 2. Elution temperature (°C) of methyl stearate at various programming rates for columns with positive (+) and negative (△) gradients. Working conditions: T_i = 130°; t_i = 4 min; flow rate = 17 ml/min (p = 1 atm; T = 25°).

Fig. 3. Resolution (R), relative peak dip (P_d), peak width at half heigth ($w_{1/2}$) and distance between peak maxima (Δv) as a function of carrier gas flow for columns with positive (+) and negative (△) gradients. Working conditions: T_i = 130°; t_i = 4 min; β = 4°/min.

for the critical pair methyl stearate–methyl oleate was determined. The results are shown in Fig. 3. It can be seen that columns with decreasing gradient exhibit a better performance than columns with increasing gradients, especially when one does not

TABLE I

THE EFFECT OF THE INJECTION VOLUME UPON RESOLUTION BETWEEN $C_{18:0}$ AND $C_{18:1}$

Experimental conditions: $T_i = 130°$; $t_i = 4$ min; $\beta = 4°$/min; F = 17 ml/min.

Injection volume (μl)	*Positive gradient*	*Negative gradient*
0.5	1.26	1.38
1	1.23	1.38
1.5	1.21	1.30
2	1.18	1.28
3	1.07	1.18
4	0.87	1.13
6	—	1.11
8	—	1.09

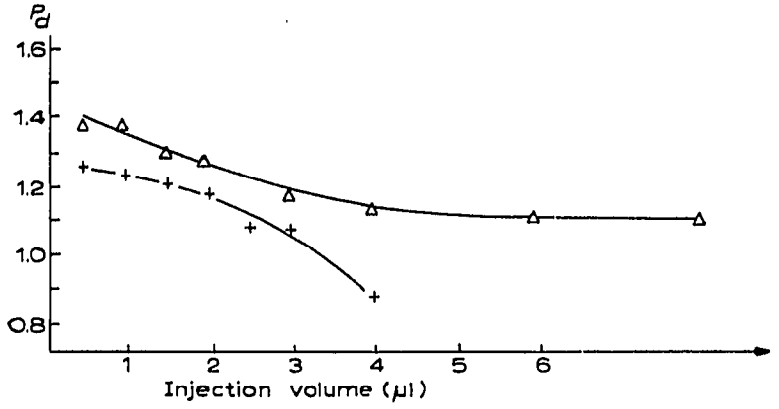

Fig. 4. Resolution as a function of injection volume for columns with positive (+) and negative (△) liquid load gradients. Working conditions: $T_i = 130°$; $t_i = 4$ min; $\beta = 4°$/min; $F = 17$ ml per min.

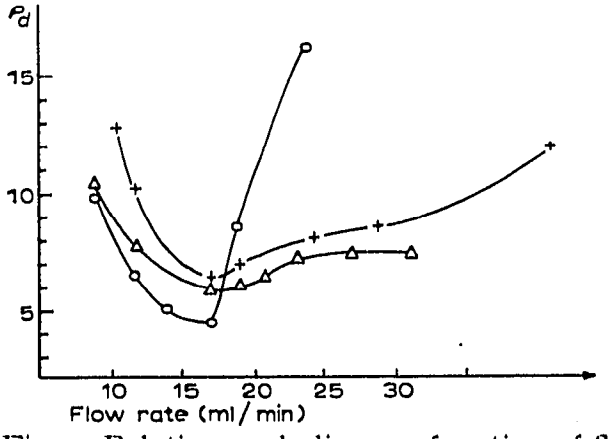

Fig. 5. Relative peak dip as a function of flow rate for columns with positive (+), negative (△) and no (○) gradient. Working conditions: $T_i = 130°$; $t_i = 4$ min; $\beta = 4°$/min; $F = 17$ ml/min.

work at the optimal gas flow velocity (optimal with respect to the resolution of the critical pair under consideration).

The above effect is also found with variation of the injection volume: the more one deviates from the optimal (very small) injection volume, the greater the superiority of the column with a negative liquid load gradient.

The influence of the injection volume upon the resolution between methyl stearate and methyl oleate for columns with positive and negative liquid load gradients in TPGC is shown in Table I and presented graphically in Fig. 4.

As can be seen from Table I, the resolution is dependent in both cases on the injection volume. However, as in isothermal GC[5], columns with negative gradients perform much better than columns with positive gradients.

The comparison of gradient loaded columns to the columns loaded with a homogeneous mixture

The gas flow that gives optimal separation between methyl stearate and methyl palmitate (as judged from the relative peak dip factor) is independent of the nature of the column (positive, negative or no liquid phase gradient; Fig. 5).

Elution temperatures are also independent of the type of column. This is not the case, however, for the resolution between $C_{18:0}/C_{18:1}$. For the mixed column, the optimal resolution ($T_i = 130°$; $t_i = 4$ min; $\beta = 4°$/min) was 1.60, for the absolute reference column (5 %) 1.55 and for the gradient columns 1.39 (positive gradient) and 1.38 (negative gradient).

As in isothermal GC[11], the heterogeneity of the packing does not affect resolution significantly in TPGC. On the other hand, liquid load gradients in the column do decrease the resolution.

As to the deterioration of the resolution with increasing injection volumes, homogeneously packed columns appear to perform better than columns with a positive gradient, but not as well as columns with a negative gradient. Thus, the resolution when a 4-μl sample was injected was only 73 % of that when a 1-μl sample was injected for a positive gradient column, 82 % for a negative gradient column and 78 % for a homogeneously loaded column (with the same amount of liquid phase). Moreover, it was found by plotting the relative peak dip *vs.* the gas flow (Fig. 5) that deviations from the optimal flow affected the resolution much more for homogeneously loaded columns than for columns with either positive or negative gradient.

DISCUSSION

The finding that the elution temperature (or the retention time) is independent of the type of linear gradient in the column (positive, negative or no gradient), (but with the same amount of partitioning liquid) is contrary to the situation in isothermal GC where the retention times do depend on the type of gradient[12,14]. The theory for retention on gradient loaded columns in TPGC which confirms this finding will be published elsewhere.

The fact that the column with negative gradient, which has a larger variation in solute velocity than the column with positive gradient (compared to the latter one, the solute velocity is slower at the inlet (higher liquid loading) and faster at the outlet (lower liquid loading)) performs better than the column with positive gradient

is in accordance with the finding of GIDDINGS[13] that large increases in carrier gas velocity (thus in solute velocity) do not lead to significant loss in resolution.

As columns with negative gradients perform better in TPGC than homogeneously loaded columns when the injection volume is not very small, they will be especially useful when large volumes need to be injected as in temperature programmed preparative gas chromatography.

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