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OPTIMIZATION STRATEGIES FOR THE DEVELOPMENT OF GAS–LIQUID CHROMATOGRAPHIC METHODS

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SUMMARY

The utility of the sequential simplex method in chromatographic methods development is demonstrated by the experimental optimization of separation for several mixtures of isomeric octanes. Column oven temperature and carrier gas flow-rate are varied simultaneously during the optimization process. Factorial experiments and regression analysis are used to understand the factor effects in the regions of the optima.

A chromatographic response function based on the peak separation function of Kaiser is described. Its use as an operational measure of performance in the separation of multicomponent systems is illustrated.

INTRODUCTION

A recurrent objective in the development of chromatographic methods is optimization, the attainment of the best performance from a system by adjusting a set of experimental factors. Achieving this aim in multicomponent separations is difficult because the specification of a general measure of performance is elusive. What objective function of the chromatogram should be optimized?

The goal of chromatographic methods development is to obtain adequate resolution of all components of interest in a reasonable analysis time. For the general case of a multicomponent separation, there is no universally accepted measure of performance. Common practice is to maximize the resolution of the pair of peaks that is most difficult to resolve[1–3]. This procedure is not always successful for multicomponent separations[4–6]. Improving the separation of a given pair of components will not necessarily improve the overall separation: the resolution of other pairs might decrease; further, the amount of time required for the analysis might become unreasonably long.

Existing approaches to the problem of multicomponent separations use measures of performance that relate to the information content of a chromatogram[7]. The essence of chromatographic information is contained in Kaiser's easily-evaluated "peak separation"[8–10] which is applicable to any two adjacent peaks and can therefore be generalized to the multicomponent case. For two components, the peak separation (P) is given by the depth of the valley (f) below a straight line connecting the two ad-

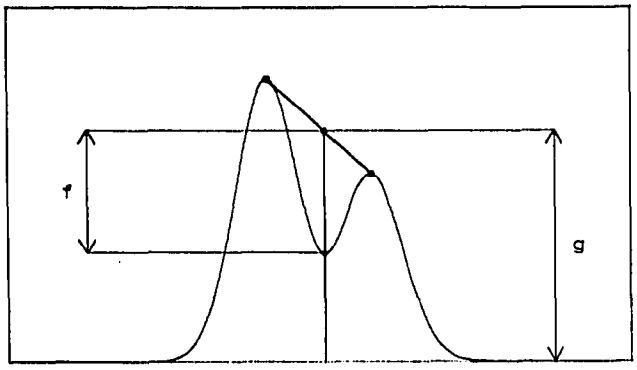

Fig. 1. Peak separation function, $P = f/g$.

jacent peak maxima, divided by the height of the straight line above the baseline at the valley (g) as shown in Fig. 1; that is,

$$P = f/g \tag{1}$$

If some function of the peak separation of adjacent peaks is to be used in a multicomponent measure of performance, it must provide greater sensitivity to highly overlapped peaks and lesser sensitivity to components that are adequately resolved. The logarithm of the peak separation satisfies these requirements: when adjacent peaks are highly overlapped, the peak separation is very small, the logarithm is a large negative number, and sensitivity to change in peak separation is large; when there is little overlap, the peak separation is close to unity, the logarithm is near zero, and sensitivity to change in peak separation is small. The functional relationship between peak separation and its natural logarithm is shown in Fig. 2.

Generalization to multicomponent separation is accomplished by summing the logarithms of the peak separation for all j pairs of adjacent peaks:

$$\mathrm{CRF} = \sum_{i=1}^{j} \log_e (P_i) \tag{2}$$

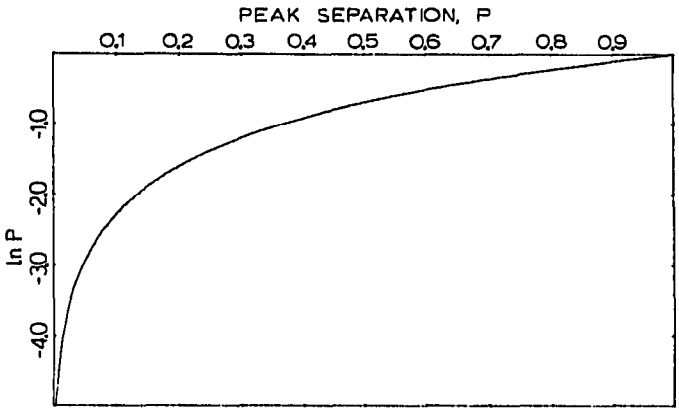

Fig. 2. Relationship between peak separation P and ln P.

where P_i is the peak separation (eqn. 1) of the i-th pair of adjacent peaks. This measure of performance will be referred to as the "chromatographic response function", CRF, or simply "response".

Given a general measure of performance, the attainment of best response from a system by adjusting a set of experimental factors (*e.g.*, column temperature, flow-rate, etc.) can be attempted. For n factors, the response may be visualized as a surface in $(n + 1)$-dimensional space. Optimization may be viewed as finding the factor levels yielding the best response, the maximum in the surface. The question of the best approach to obtain this objective has not yet been resolved. What strategy should be employed to efficiently achieve optimal chromatographic performance?

Chromatographic theory has been successful in developing predictive mathematical models for two-component separations. When good models are available, optimal conditions can be derived by calculus[11–13], by simulation[14], or by numerical optimization[15,16]. Because most theoretical models are based on one- and two-component systems, the predictive accuracy of such models is dependent on the errors involved in both the assumptions behind the model and the extrapolations to the multicomponent case. It is often not recognized that the model is only a tentative approximation to the true response surface and may be valid only within certain ranges of the experimental factors[5]. Theory is helpful in the initial choice of an appropriate chromatographic system. For a given separation, however, the true response surface and its optimum must be determined by experiment.

Experimental techniques for optimization in chromatography have been discussed[2,3,16–18]. The sequential simplex method of optimization[19,20] is an intuitively appealing multifactor search strategy. It is a hill-climbing algorithm that moves a pattern of $(n + 1)$ experimental points away from regions of worse response toward convergence on an optimum in the response surface. It has recently been applied to the optimization of information content for multicomponent separations in ion-exchange chromatography[21]. Various applications of the simplex in methods development have demonstrated its general utility as an experimental approach to efficiently achieve optimal response[22–38].

After locating a suspected optimal region, it is often desirable to further characterize the response surface in that region. What experimental design can be used to verify the optimum and provide an understanding of the factor effects?

A satisfactory experimental design to explore a limited region of factor space should provide as nearly uniform information over the region as possible; it should provide contrasts sufficient for the estimation of all factor effects of interest; it should provide adequate precision in the estimation of those effects as well as an indication of their significance; and lastly, it should accomplish these aims with a minimum of experiments. The effective determination of the nature of a response surface involves the assumption of an empirical model and the estimation of its parameters (the factor effects)[39–42]. In general, there should be more experiments than parameters to be estimated so as to provide degrees of freedom for the estimation of experimental error. Assuming the higher order terms in the Taylor series approximation of the response surface are of lesser importance in the region of the optimum, a polynomial of second degree can be used. For two factors, the form of this model is:

$$\mathrm{CRF} = \beta_0 + \beta_1 x_1 + \beta_{11} x_1^2 + \beta_2 x_2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \qquad (3)$$

where x_1 and x_2 are the two factors, β_0 is the response at the origin, β_1 and β_2 are the linear effects, β_{11} and β_{22} are the quadratic effects, and β_{12} is the interaction effect. Because six parameters are to be estimated, at least six experiments are needed; to estimate the quadratic effects, three levels of each factor must occur in the experimental design.

For a small number of factors ($n = 2$ or 3), a 3^n factorial design, with experiments at all three levels of n factors, is satisfactory for characterization of the response in the region of an optimum. For a larger number of factors ($n > 3$), the number of experiments for a full 3^n factorial is excessive and the efficiency is lowered; varieties of fractional factorials or composite designs[39–41] might then be employed to verify the optimum and provide an understanding of the factor effects in that region.

Two factors of accepted importance in gas–liquid chromatography (GLC) are the column oven temperature and the carrier gas flow-rate. The existence of an optimal region of flow-rate for chromatographic separation is well known[11]. Changes in relative retention with temperature are of frequent occurrence in GLC[43–54], and when the order of elution reverses for several pairs of compounds, optimal regions of isothermal temperature operation exist. The temperature dependence of the retention indices of five isomeric octanes is shown in Fig. 3 (ref. 54). This chemical system is used here to investigate the operational approach of simplex optimization combined with factorial designs in the development of GLC methods.

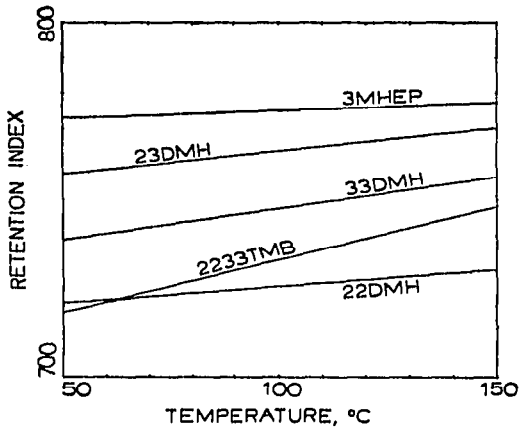

Fig. 3. Variation of retention index with temperature for five isomeric octanes.

EXPERIMENTAL

Chemicals

Five octane isomers were obtained pure (99%) from Chemical Samples (Columbus, Ohio, U.S.A.). Three different samples were prepared: a two-component mixture of 2,3-dimethylhexane (23DMH) and 3-methylheptane (3MHEP) (1:1); a three-component mixture of 2,2-dimethylhexane (22DMH), 2,2,3,3-tetramethylbutane (2233TMB), and 3,3-dimethylhexane (33DMH) (1:2:1); and a five-component 1:1 combination of the first two samples.

Apparatus

A 13-ft. × 1/4-in. O.D., stainless-steel column containing 20% (w/w) GE-SF96 on Chromosorb P (60–80 mesh) (acid washed, dimethyldichlorosilane treated) was used (Chromatographic Technology, Analabs distributor, Houston, Texas, U.S.A.). Sampling was done with a 10-μl syringe (Glenco Scientific, Houston, Texas, U.S.A.). The gas chromatograph employed was a Perkin Elmer 900 equipped with a flame ionization detector (FID) (Perkin Elmer, Norwalk, Conn., U.S.A.) and a strip chart recorder (Leeds & Northrup, Philadelphia, Pa., U.S.A.).

All calculations were performed on a HP-9830A minicomputer (Hewlett–Packard, Palo Alto, Calif., U.S.A.) with a flat-bed plotter (Model 9266A, Hewlett–Packard).

Procedure

All chromatographic runs were made under as nearly identical conditions as possible except for the levels of column oven temperature and carrier gas flow-rate which were dictated by experimental design. Injector and detector temperature controls were set at 150°. The carrier gas, pre-purified nitrogen, was set at constant inlet pressure (26 p.s.i.g.). Gas flow was controlled by a flow controller (Model 8743, Brooks Instrument, Hatfield, Pa., U.S.A.) and read on a flow meter (Model 1355, Tube No. R-2-15-AAA-M, Brooks Instrument) upstream from the column. Compressed air and pre-purified hydrogen for the FID were set at constant pressure levels.

Carrier gas flow calibrations were made by disconnecting the manifold tubing at the detector and measuring volumetric flow-rate at room temperature at the outlet with a soap bubble flow meter and stopwatch. Column oven temperature was monitored by a thermometer in the oven.

Between experiments the column oven was cooled to room temperature. For each indicated observation, the column oven temperature was set on the temperature selector of the instrument and the oven was allowed to equilibrate for 15 min. Temperature settings were selectable at 1° intervals from 50° (the lower limit of adequate temperature control) to 240° (the upper limit of the stationary phase), and settings dictated by experimental design were rounded to the nearest 1°. Unless otherwise specified, the use of °C units refers to instrument settings rather than actual temperature units. Carrier gas flow-rate, measured in uncalibrated units by the flow meter (from 0.00 to 15.00 units), was adjusted by the needle valve on the flow controller.

The sample size for the two- and three-component studies was 0.5 μl; for the five-component study the sample size was 1.0 μl. Three-layer septa of silicone rubber (Hamilton, Reno, Nev., U.S.A.) were changed frequently.

The f and g values for the peak separation (eqn. 1) of all adjacent pairs of peaks were obtained from the chromatograms by drawing with pencil and ruler the lines (indicated in Fig. 1) on the recorder trace and measuring the distances required ($\pm$0.01 cm). The logarithms of these values were summed to form the CRF (eqn. 2). The analysis time, t, defined as the retention time of the last component of the sample to elute from the column, was also recorded ($\pm$0.01 cm with a chart speed of 1/4 in./min).

Experimental design

Certain cautions should be exercised in applying simplex algorithms written

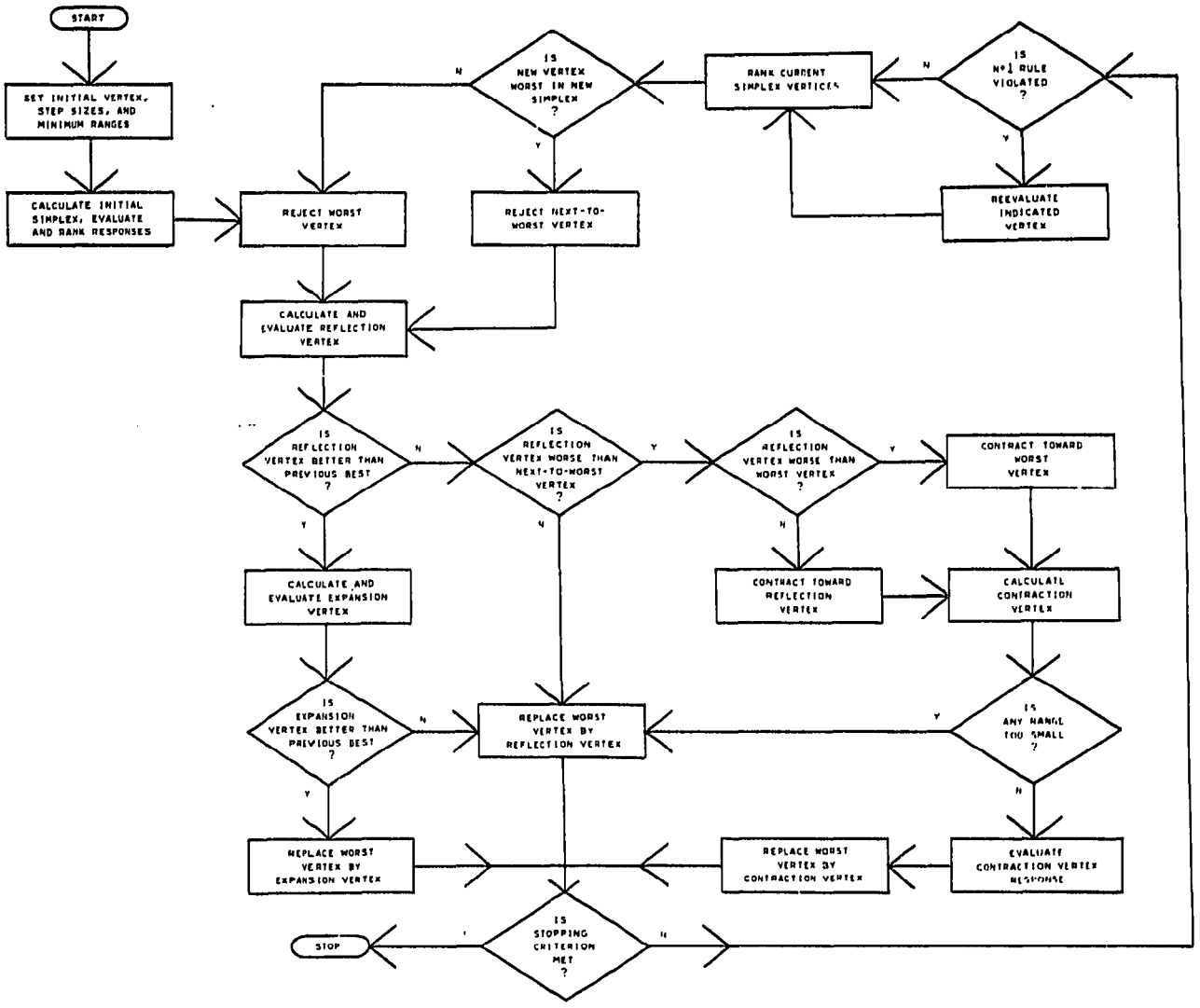

Fig. 4. Flow chart of simplex algorithm for experimental optimization.

for numerical optimization to problems involving experimental optimization. When the size of the simplex is contracted to the extent that differences in response between the vertices are on the same order of magnitude as the experimental error, erratic behavior and false convergence might result[22]. The simplex algorithm employed here is similar to that described previously[29] with two modifications to cope with this problem: (1) In the original Nelder and Mead algorithm[20], when a "failed contraction" results[29], a massive contraction is used to avoid oscillatory collapse. Unfortunately, the massive contraction seriously diminishes the size of the simplex; in the presence of experimental error, massive contractions might cause premature convergence[22]. For the experimental optimizations in this paper, the algorithm was changed: when a

failed contraction occurs, the contracted vertex is retained and the next-to-the-worst vertex is rejected[19,32] when the algorithm is continued. (2) If the range of any factor for the complete new set of simplex vertices after calculation of a contracted vertex is less than a predefined minimum permissible level, the contraction is disallowed; when the algorithm is continued the next-to-the-worst vertex is rejected. Minimum ranges were set at 5.0° for temperature and 0.5 units for flow-rate. These two uses of a "next-worst-reflection" rule might permit the simplex to reorient itself without shrinking its size unnecessarily. A flow chart of the logic for the simplex algorithm used in this study is given by Fig. 4.

The initial simplex was identical for each of the optimization studies in this paper. The first vertex was located at 100° and 6.0 units flow-rate, the step size[55] was 15° for temperature and 3.0 units for flow-rate.

Each 3^2 factorial design was centered on the vertex of best response. Spacings of the levels were 5° in temperature and 1.0 units in flow-rate. The center point of the design was replicated and the experiments were run in randomized time order to minimize trends. Carrier gas flow-rate and column oven temperature were calibrated under the experimental conditions at the center point of the factorial.

RESULTS AND DISCUSSION

Two-component system

The simplex algorithm is illustrated in Fig. 5, which shows the optimization of the separation of the two-component sample (23DMH and 3MHEP). The objective function is the CRF for the two-component case (eqn. 2). Vertex numbers, indicating the time order of the experiments, are given beside each vertex. The eight chromatograms from this simplex search are shown in Fig. 6; to the right of each chromatogram are given the vertex number, CRF, and analysis time in minutes.

The initial simplex vertices (1, 2, and 3) gave the responses −0.577, −1.407, and −1.778. Vertex 4 was generated by rejecting vertex 3 (which had the worst response) and reflecting through the center of the face remaining between vertices 1 and 2. Because the reflection vertex had neither a better response than the previous best

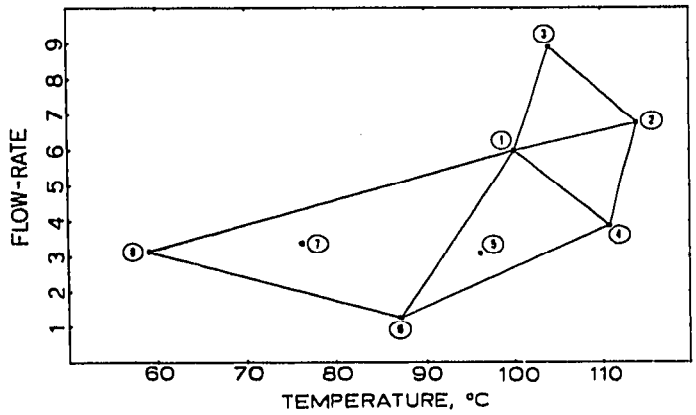

Fig. 5. Simplex progress for two-component system, no time constraint.

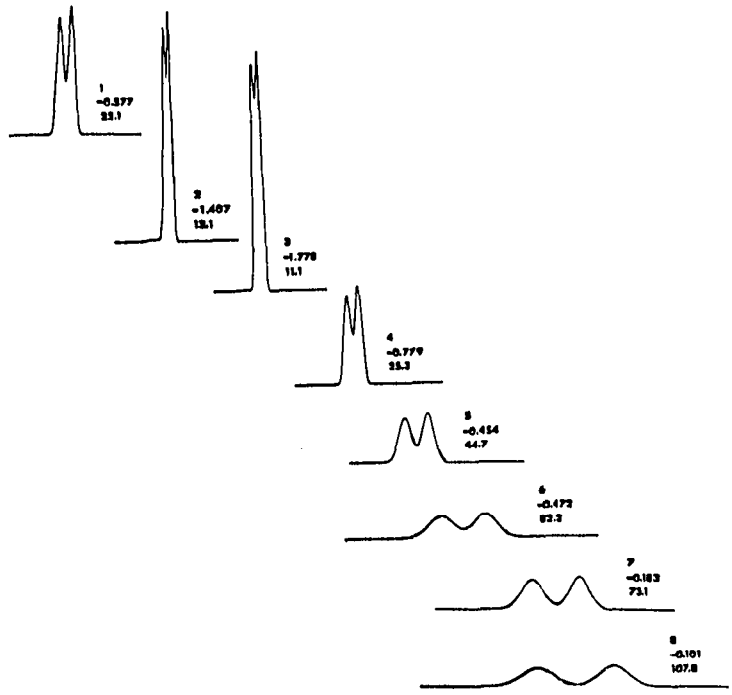

Fig. 6. Chromatograms from optimization of two-component system, no time constraint. Vertex number, CRF, and analysis time are given at the right of each chromatogram.

(vertex 1) nor a worse response than the next-to-the-worst (vertex 2), it was retained and the simplex size remained the same. Vertex 5 was generated by reflecting the vertex of worst response (vertex 2) through the center of the face between vertices 1 and 4; because the new vertex had a response (CRF = −0.454) better than the previous best (−0.577) the simplex was expanded. The expansion at vertex 6 (CRF = −0.472)

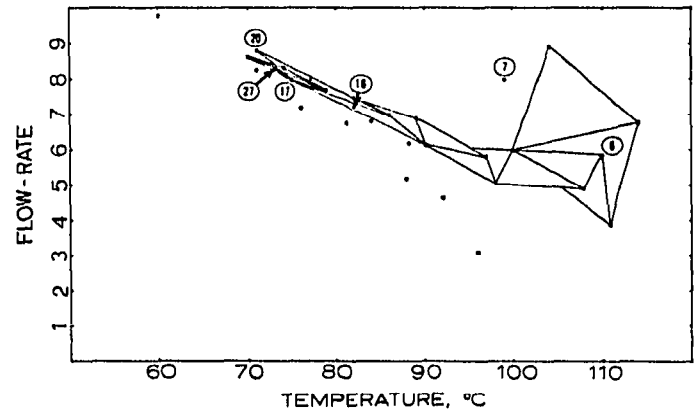

Fig. 7. Simplex progress for two-component system, 30-min time constraint.

was successful. The rejection of vertex 4 from the simplex containing vertices 1, 4, and 6, generated vertex 7, which led to another successful expansion at vertex 8. Vertices 5 and 7 are non-retained reflections and are represented as lone dots rather than vertices of completed simplexes.

While there is a dramatic increase in the resolution of these two components during the optimization, the increased analysis time and decreased sensitivity when operating at reduced flow and temperature is often impractical. Practical separation requires only that adequate resolution be achieved for all components of interest in a reasonable analysis time. For the following three simplex optimizations, the CRF was optimized with the added constraint that the analysis must be completed within 30 min. Any vertex with an analysis time longer than 30 min was treated as a boundary violation[28]; an undesirable response value (−100) was assigned to that vertex to contract the simplex to within the time limit.

The progress of a second simplex optimization on the two-component sample is shown in Fig. 7. Six representative chromatograms are given in Fig. 8. The coordinates of the first five vertices were the same as those of the previous study. Because the analysis time at vertex 5 was 46.1 min, vertex 6 was generated by a contraction. Seven of the eleven non-retained vertices were time boundary violations. This resulted in a flattening of the simplex against the 30-min boundary. After 14 vertices, a next-worst reflection was performed and the simplex began to orient itself to move along

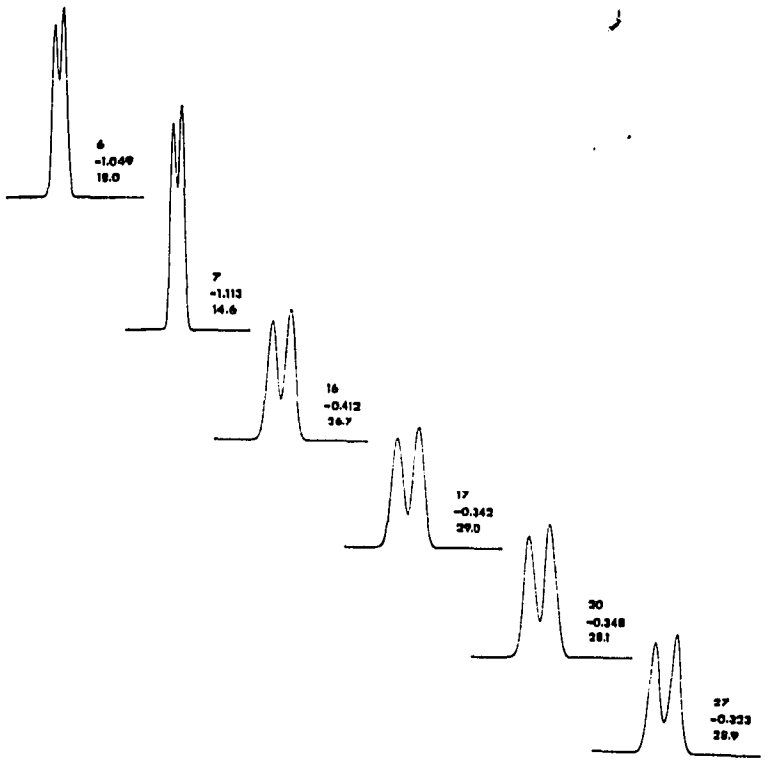

Fig. 8. Representative chromatograms from the optimization of two-component system, 30-min time constraint. Vertex number, CRF, and analysis time are given at the right of each chromatogram.

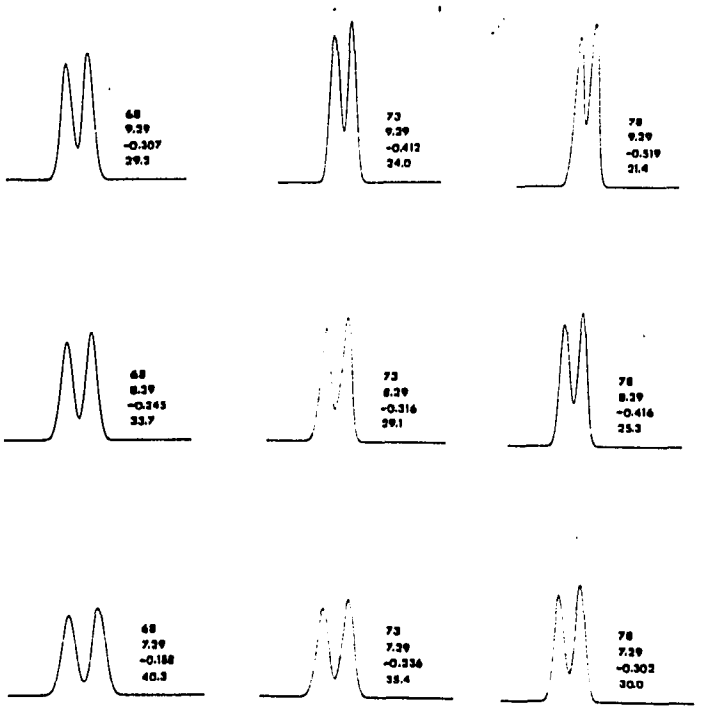

Fig. 9. Chromatograms from factorial design on two-component system. Temperature, flow-rate, CRF, and analysis time are given at the right of each chromatogram.

the boundary in the direction of increasing response while keeping the analysis time under the desired 30 min. A successful expansion at vertex 17 doubled the size of the simplex. After 29 vertices the search was terminated.

Fig. 9 shows the results of a 3^2 factorial design centered on the vertex of best response (vertex 27). The temperature and flow levels are given to the right of each chromatogram along with the resulting CRF and analysis time. Calibration of column oven temperature at the center point was 89°; the outlet flow-rate was 51.7 ml/min. Regression analyses[42,56] for second-order empirical models (eqn. 3) gave:

$$\mathrm{CRF} = -3.244 + 0.07216x_1 - 0.0003295x_1^2 + 0.3012x_2 - 0.001737x_2^2 - 0.004900x_1x_2 \quad (4)$$

$$t = 370.7 - 4.911x_1 + 0.02046x_1^2 - 26.26x_2 + 0.7161x_2^2 + 0.1260x_1x_2 \quad (5)$$

where x_1 is the temperature factor and x_2 is the flow factor.

CRF information from the factorial design is presented in Fig. 10 in a pseudo-three-dimensional plot[57] of eqn. 4; only that portion of the response surface for which the analysis time is $\leqslant$30 min is shown. The predominance of linear effects for temperature and flow in this limited region of the entire response surface is readily apparent. Canonical analysis[39,40,58] confirms that both the CRF surface and the analysis

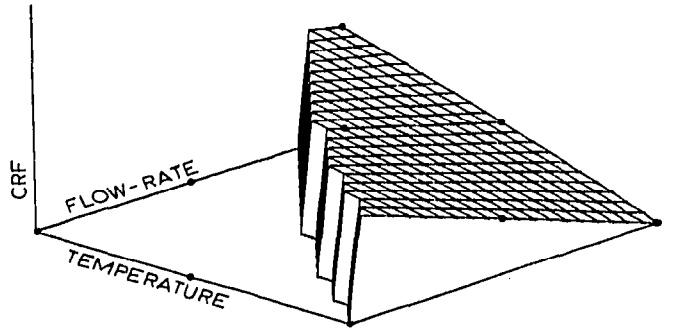

Fig. 10. Time-constrained CRF regression surface for two-component system. Dots correspond to factorial experiments in Fig. 9.

time surface are approximately planar in the region of the factorial and predicts that the expected stationary point (maximum or minimum) is removed from the region of the factorial.

For many separation problems, the pursuit of increased resolution greater than some adequate level is wasteful[11]; in such cases, the optimization can be terminated whenever the desired goal has been met. Results from the factorial design indicate that an improvement in CRF might be possible by moving along the time boundary

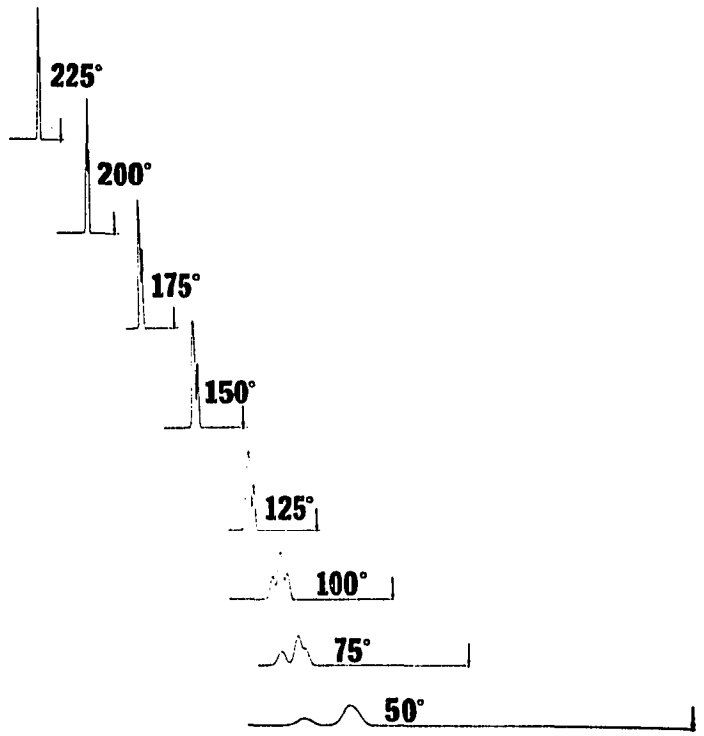

Fig. 11. Univariate temperature mapping at constant flow-rate (6.0 flow units) for the three-component system.

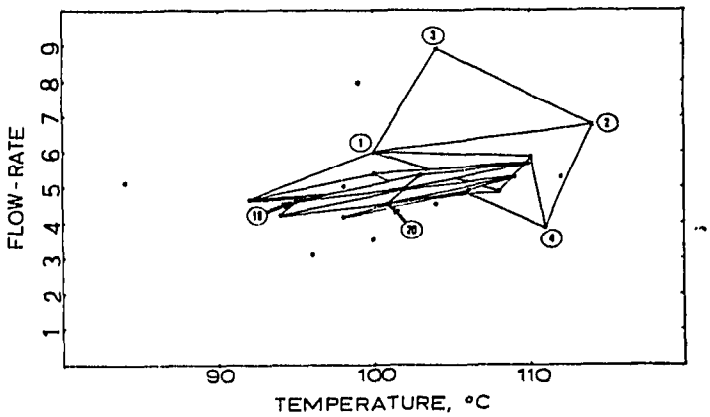

Fig. 12. Simplex progress for three-component system, 30-min time constraint.

toward lower temperature and higher flow. It is expected that the improvement in CRF would be small: the fundamental sensitivity of the peak separation P to changes in resolution becomes less as the peaks become better resolved; also, the logarithm of P (the CRF) is less sensitive to changes in P as P increases.

For a two-component sample that exhibits a reversal in elution order with temperature, there are two possible regions of operation, one above and one below

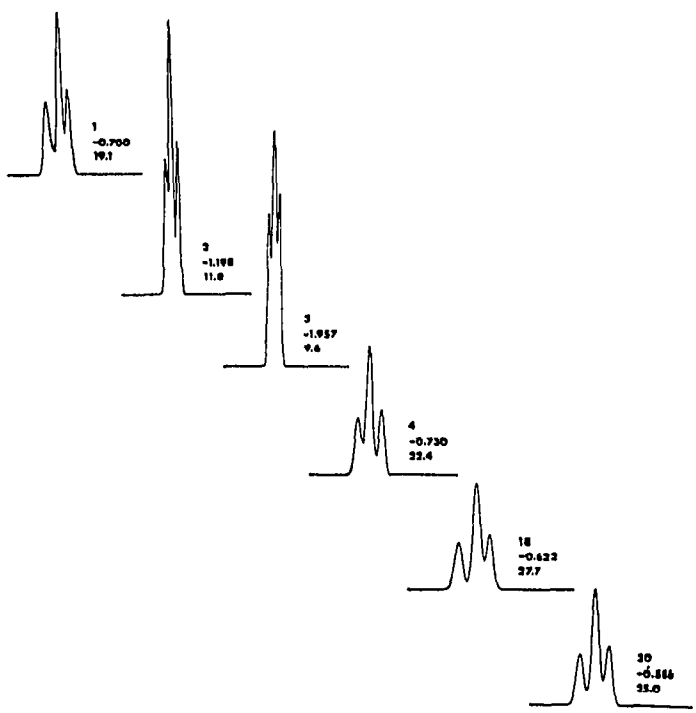

Fig. 13. Representative chromatograms from optimization of three-component system, 30-min time constraint. Vertex number, CRF, and analysis time are given at the right of each chromatogram.

the inversion temperature. These optimizations of separation for the 23DMH–3MHEP sample (inversion at 234°) were performed in the lower temperature region.

Three-component system

For a multicomponent sample that exhibits more than one reversal in elution order with temperature, several distinct temperature regions might offer improved resolution of the components of interest. Separate optimizations within each region might be desirable, especially if the global (overall) optimum is required. A preliminary univariate temperature mapping at constant flow will often reveal the existence of such local optima. In some cases, there might not exist a set of experimental conditions that allows adequate separation of every component of interest from its neighbors and compromises must be made[1,59].

Fig. 11 shows a temperature mapping for the three-component sample consisting of 22DMH, 2233TMB, and 33DMH. The shifting in position of the larger 2233TMB peak can be seen as the temperature is changed. There is a region of good resolution between 50 and 125°. Fig. 3 predicts this local optimum and suggests two other regions for potential investigation, one below the inversion temperature of

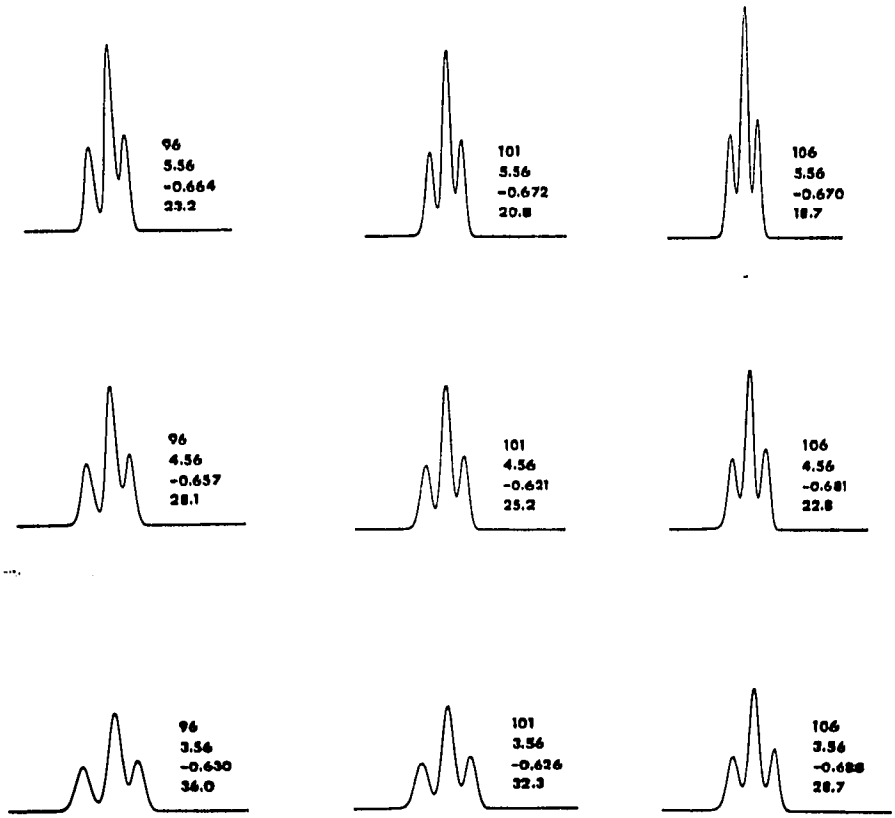

Fig. 14. Chromatograms from factorial design on three-component system. Temperature, flow-rate, CRF, and analysis time are given at the right of each chromatogram.

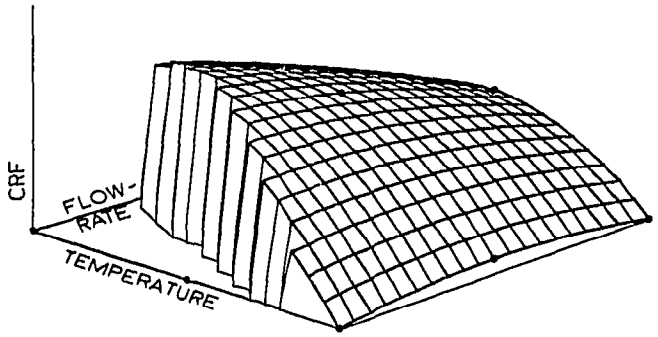

Fig. 15. Time-constrained CRF regression surface for three-component system. Dots correspond to factorial experiments in Fig. 14 (two of the experiments are not visible in this view).

2233TMB with 22DMH, and one above the inversion temperature of 2233TMB with 33DMH. The potential region at high temperature was impractical because it is close to the upper limit of the stationary phase used in this study. The potential region at low temperature was impractical because of inadequate temperature control below 50° and because of the probable long retention times involved.

A simplex optimization on this three-component sample is shown in Fig. 12. The objective function is the three-component case of eqn. 2. The simplex quickly contracted about the optimal region, and the search was terminated after 25 vertices. Some representative chromatograms are shown in Fig. 13.

Fig. 14 shows the chromatograms from the 3^2 factorial design in the region of

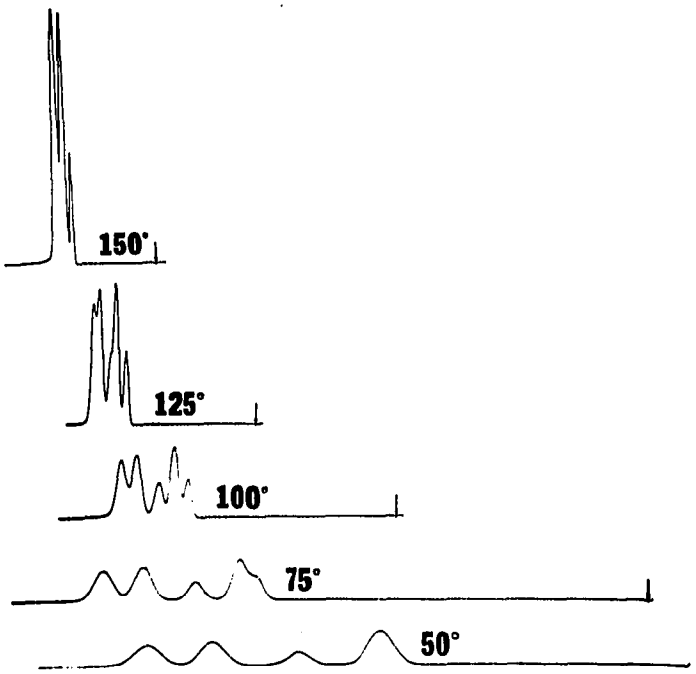

Fig. 16. Univariate temperature mapping at constant flow-rate (6.0 flow units) for five-component system.

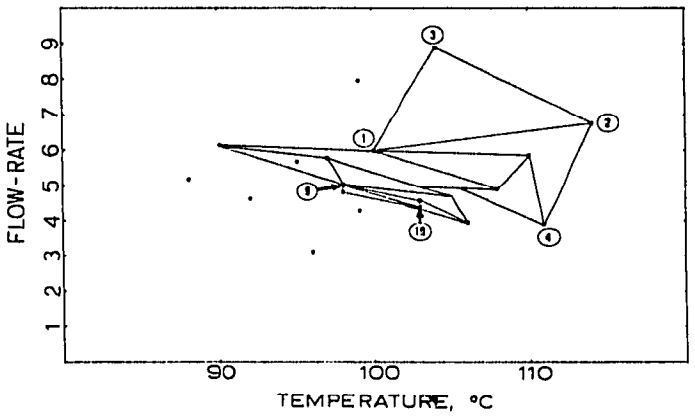

Fig. 17. Simplex progress for five-component system, 30-min time constraint.

termination. The column oven temperature, calibrated at the center point (vertex 20), was 118°; the flow-rate was 21.1 ml/min. Regression analysis on the factorial data gave the prediction equations:

$$\mathrm{CRF} = -9.462 + 0.1884x_1 - 0.001006x_1^2 - 0.2260x_2 - 0.005143x_2^2 + 0.002600x_1x_2 \quad (6)$$

$$t = 308.3 - 3.320x_1 + 0.01044x_1^2 - 32.30x_2 + 1.364x_2^2 + 0.1402x_1x_2 \quad (7)$$

Curvature in the CRF surface is visible in the view given by Fig. 15. Canonical analysis indicates an optimum CRF region with elliptical contours centered at 97° and 2.52 flow units. The analysis time surface of eqn. 7 is slightly different from that of eqn. 5: this is expected because 33DMH elutes sooner than 3MHEP (see Fig. 3).

Five-component system

A univariate temperature mapping for the separation of the five-component sample (a mixture of the previous two samples) is illustrated in Fig. 16. A simplex optimization on this sample, using the five-component case of eqn. 2 as the objective function, is shown in Fig. 17. The search was terminated after 21 vertices. Vertex 9 was the best vertex found. The lower four non-retained vertices had analysis times greater than 30 min. Several of the chromatograms from this study are given in Fig. 18. The second and third vertices were arbitrarily ranked by assigning the responses indicated. The loss in resolution in comparision with the earlier studies was due to changes in column characteristics during the intervening time.

Chromatograms from the factorial design centered in the region of termination are shown in Fig. 19. The column oven temperature at the center point was 112°; the flow-rate was calibrated at 25.0 ml/min. Regression analysis on this data yielded the equations:

$$\mathrm{CRF} = -16.30 + 0.3461x_1 - 0.002000x_1^2 + 0.01752x_2 - 0.04250x_2^2 + 0.001550x_1x_2 \quad (8)$$

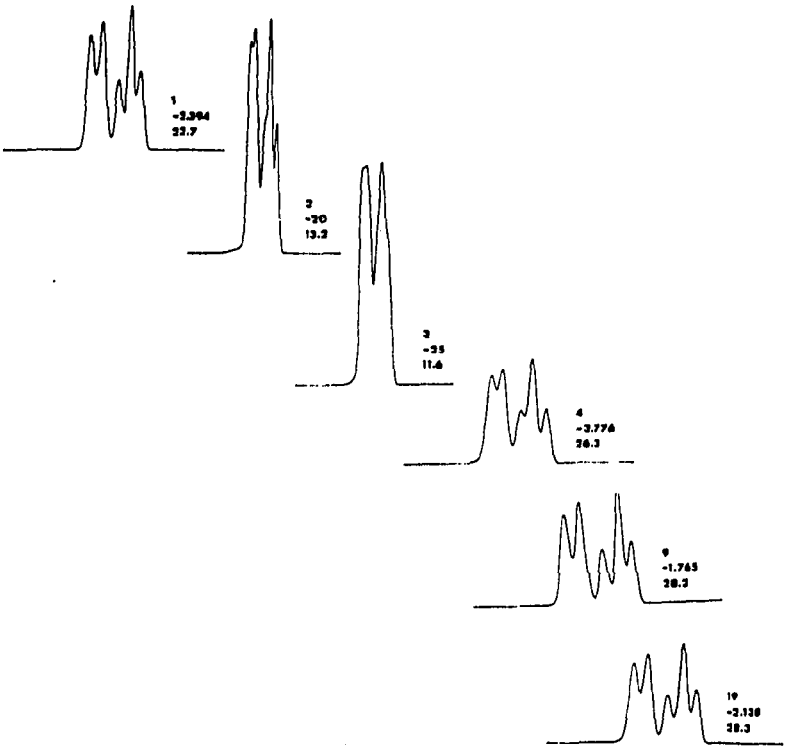

Fig. 18. Representative chromatograms from optimization of five-component system, 30-min time constraint. Vertex number, CRF, and analysis time are given at the right of each chromatogram.

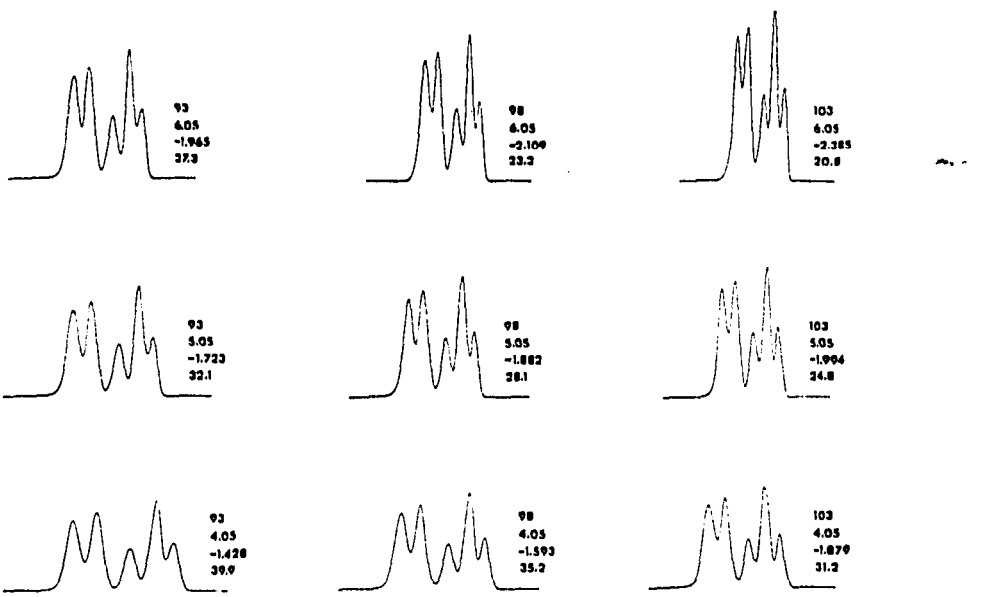

Fig. 19. Chromatograms from factorial design on five-component system. Temperature, flow-rate, CRF, and analysis time are given at the right of each chromatogram.

$$t = 357.7 - 4.235x_1 + 0.01496x_1^2 - 27.99x_2 + 1.122x_2^2 + 0.1102x_1x_2 \quad (9)$$

The effect of adding 23DMH and 3MHEP to the three-component sample to make up this five-component sample is revealed in the CRF surface shown in Fig. 20: eqn. 8 is roughly the sum of the two- and three-component surfaces (eqns. 4 and 6, or Figs. 10 and 15). Canonical analysis of eqn. 8 indicates that the factorial lies to one side of an optimal region with elliptical contours centered at 87° and 1.80 flow units. Canonical analysis of eqn. 9 indicates that, as with the corresponding two- and three-component results, the region of the factorial lies on a sloping surface with analysis time increasing toward low temperature and low flow. Eqn. 9 is almost identical to eqn. 5; this is to be expected because it is 3MHEP that is determining the analysis time for both equations.

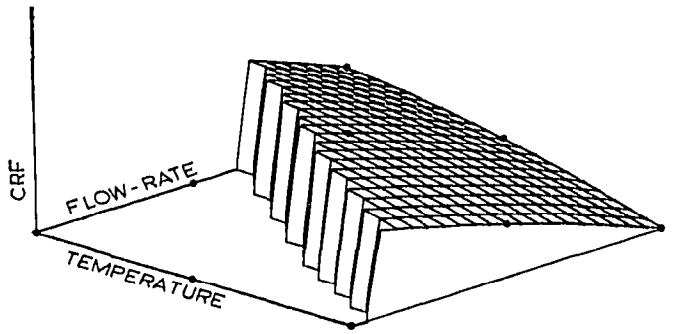

Fig. 20. Time-constrained CRF regression surface for five-component system. Dots correspond to factorial experiments in Fig. 19.

CONCLUSIONS

The chromatographic response function (CRF), defined in eqn. 2, has been shown to be a viable objective function for the experimental optimization of multi-component separations. The function is useful both for studies in which the best possible separation is desired as well as for studies aimed only at improving separation to some adequate level.

Many quantitative measures of performance can be formulated for use in various other aspects of chromatographic methods development (*e.g.*, sensitivity, preparative sample throughput, etc.). The form of any particular objective function will depend on the particular requirements of the optimization, and must be chosen to provide an effective measure of what is chromatographically desired.

With many multicomponent samples, the separation of only a few of the components might be of interest. In such cases, the CRF can be calculated on the basis of the components of interest. Within the set of peaks of interest, it might further be required that some of these be separated to a greater degree than others; weighting coefficients (w_i) can be applied to appropriate pairs of ln P_i terms (one for each side of the peak of interest) to reflect the relative importance of the various peaks. The

CRF might also be normalized for the number of peaks and for the relative weights involved, as shown in eqn. 10:

$$\mathrm{CRF}' = \left[\sum_{i=1}^{J} w_i \log_e(P_i)\right] / \left[\sum_{i=1}^{J} w_i\right] \quad (10)$$

In three of the four studies in this paper, CRF was maximized by varying flow-rate and temperature under the constraint that the analysis must be completed within 30 min. This approach is, in fact, a special case of time normalization chromatography (TNC)[60–65], a technique in which two operating conditions are changed simultaneously to increase the resolution while keeping the analysis time constant. The requirement in TNC that the analysis time be constant constrains out one degree of freedom: in Fig. 7, a one-dimensional search for optimum CRF could be done along the analysis time boundary if the location of that boundary were known. The experimental response surface approach to optimization used here provides a powerful means of locating both the analysis time boundary and the optimum region of response within that boundary.

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