Separation & Purification Reviews
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597294

Experimental Optimization of Chromatographic Systems

Stephen L. Morgan[a]; Stanley N. Deming[a]
[a] Department of Chemistry, University of Houston, Houston, TX

To cite this Article Morgan, Stephen L. and Deming, Stanley N.(1976) 'Experimental Optimization of Chromatographic Systems', Separation & Purification Reviews, 5: 2, 333 — 360
To link to this Article: DOI: 10.1080/03602547608069359
URL: http://dx.doi.org/10.1080/03602547608069359

SEPARATION AND PURIFICATION METHODS, 5(2), 333-360 (1976)

EXPERIMENTAL OPTIMIZATION OF CHROMATOGRAPHIC SYSTEMS

Stephen L. Morgan* and Stanley N. Deming
Department of Chemistry
University of Houston
Houston, TX 77004

INTRODUCTION

Figure 1, a block diagram of a generalized chromatographic system, suggests that the improvement of systems performance is a major goal in the development of chromatographic methods. One measure of systems performance is the separation of the components of a given sample, evaluated at a given set of experimental conditions.

The experimental optimization of chromatographic systems involves varying the experimental conditions in some directed way so as to find new conditions that will produce improved results.

MEASURES OF CHROMATOGRAPHIC PERFORMANCE

The development of systematic optimization procedures for chromatographic systems has been limited

*Present address: Department of Chemistry
University of South Carolina
Columbia, SC 29208

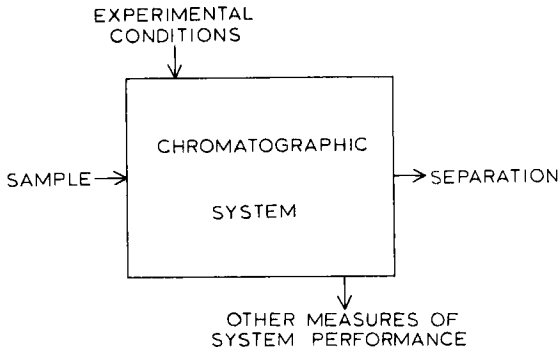

FIGURE 1
Block diagram of a generalized chromatographic system.

somewhat by the lack of universally accepted measures for the extent of separation[1,2]. Although there are numerous characteristics of the chromatographic process that are useful for evaluating a separation[3], most of these characteristics are based upon one- or two-component samples and are not always useful for the more general case of a multicomponent mixture. Realizing that the end result of an optimization study depends upon the choice of a measure of chromatographic performance, the chemist must first answer the specific question, "What is it that I want to optimize?"

Single-Component Measures

One of the most common measures of chromatographic performance is the number of theoretical plates[4], which may be calculated from the chromatogram in several different ways[3,5]. An expression for the number of theoretical plates, n, is:

$$n = 16(t/W)^2 \qquad (1)$$

where t is the time for elution of a peak maximum, and W is the baseline width of the peak in units of time.

The number of theoretical plates is an extensive property of a chromatographic system and depends on the length of the separation medium. For the comparison of systems of different lengths, the height equivalent to a theoretical plate (HETP)[3], an intensive property, is more useful:

$$HETP = L/n \qquad (2)$$

where L is the length of the separation medium.

The statistical moments[3,5-10] of a peak provide similar information. The numerical value of the first moment is the retention time, the numerical value of the second moment is the variance of the peak distribution, and the ratio of the first moment to the second moment is an alternate way of calculating the number of theoretical plates. Higher moments provide information on peak asymmetry and flattening.

These three measures of chromatographic performance describe only the efficiency in terms of peak broadening with which the sample is transmitted through the chromatographic system[11]. As such, they can be calculated on the basis of each sample component and might vary numerically from component to component within the same chromatogram. Further, and more importantly, they are not, by themselves, direct measures of the separation for two or more peaks.

Two-Component Measures

The separation between two adjacent peaks may be measured by the difference in retention times of the peak maxima. Because this measure is an extensive

property, the ratio of the two adjusted retention times, the separation factor, α, is more frequently employed. Although the separation factor does describe the time interval between two peak maxima, it does not take into account the possible overlap between the two peaks[11,12].

Resolution, R, is an operational measure of separation that includes both the time between the two peak maxima and the widths of the two peaks. One way of calculating resolution is:

$$R = 2(t_j - t_i)/(W_j + W_i) \quad (3)$$

where t and W are as defined previously, and i and j are the two adjacent peaks.

A more readily calculable measure of the separation between two peaks is the valley-to-peak ratio, V, defined as the ratio of the height above baseline of the minimum between the two peaks to the height of the smaller of the two peaks[13].

A closely related measure of performance, the peak separation, P, is defined as the depth of the valley (f) below a straight line connecting the two adjacent peak maxima, divided by the height of the straight line above the baseline at the valley (g)[3,14,15]:

$$P = f/g \quad (4)$$

Unlike measures of separation that are based upon Gaussian peak shape (e.g., resolution), the valley-to-peak ratio and the peak separation are measures of chromatographic performance that are not biased by peak asymmetry.

Multicomponent Measures

In seeking to improve multicomponent separations, two-component measures are often applied to the pair of adjacent peaks that are the least separated[16-18]. This approach is based on the assumption that when the two most overlapped peaks become separated, the separation will also have increased between all other pairs of peaks. This assumption, however, is not always justifiable or successful: improving the separation of a given pair of peaks in a typical mixture of chemically different components will often cause two or more other peaks in the chromatogram to become seriously overlapped[2,19-21]. What is clearly needed, then, is a measure of chromatographic performance that includes the separation of all pairs of peaks in the chromatogram.

The lack of complete separation of all peaks in a chromatogram is directly related to the total overlap, ϕ, of the peaks, which might be approximated by a function of the form:

$$\phi = \sum \exp(-2R_{ij}) \qquad (5)$$

where R_{ij} is the resolution between the two peaks i and j, and the summation is for all possible pairs of peaks[2]. The rationale for using this function is that if the total overlap is minimized, the overall separation will be optimized. In addition, this function is more sensitive to peaks that are highly overlapped and less sensitive to peaks that are well separated; that is, this function emphasizes the separation of highly overlapped peaks.

Other measures of multicomponent separation that are closely related to the total overlap of a chromato-

gram can also be formulated. For example, the peak separation function (Equation 4) can be incorporated into a function expressing overall chromatographic separation[21]. To provide greater sensitivity to highly overlapped peaks and lesser sensitivity to components that are adequately resolved, the logarithm of the peak separation can be used. 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. Generalization to multicomponent separation is accomplished by summing the logarithm of the peak separation for all k pairs of adjacent peaks of interest:

$$CRF = \sum_{i=1}^{k} \log_e(P_i) \qquad (6)$$

where P_i is the peak separation (Equation 4) of the ith pair of peaks, and the CRF is a chromatographic response function[21]. The evaluation of the CRF is relatively easy, involving only the direct measurement of the P_i values on the chromatogram.

A third multicomponent measure of overall chromatographic separation involves the application of information theory[22] to the evaluation of separation[23-25]. The informing power, P_{inf}, of a chromatogram is given by:

$$P_{inf} = \sum_{i=1}^{k} \log_2(S_i) \qquad (7)$$

where S_i = 1/(2 x fractional overlap) for the ith pair of peaks.

Other Measures of System Performance

Although in many separation problems the primary response of interest is the overall separation, there might be other measures of system performance that should also be taken into account, either individually or combined in a mathematical function. Examples of other system outputs that might be important are analysis time[16,18-20,26-30], throughput[31], sensitivity of detection[32], sample size[33-36], and cost[37].

PERFORMANCE CRITERIA

Considerations such as these lead naturally to the concept of performance criteria[38,39]: thresholds of system performance above or below which the system should perform to achieve acceptable results.

The shaded region in Figure 2 represents those experimental conditions of column temperature and carrier gas flow rate that produce an overall chromatographic separation better than some specified threshold level. The more stringent the criterion, the smaller the region of acceptable response; the more relaxed the criterion, the larger the region.

In Figure 3, the shaded region represents those conditions of column temperature and carrier gas flow rate for which the analysis time is less than some given value. Again, the area of acceptable performance is dependent on the level at which the threshold is set.

Those conditions of temperature and flow rate for which both of the above criteria are met are shown as the shaded region in Figure 4. In general, if one or more of the performance criteria is set at too strict a level, it is possible that all performance criteria cannot be satisfied simultaneously; in that case, one

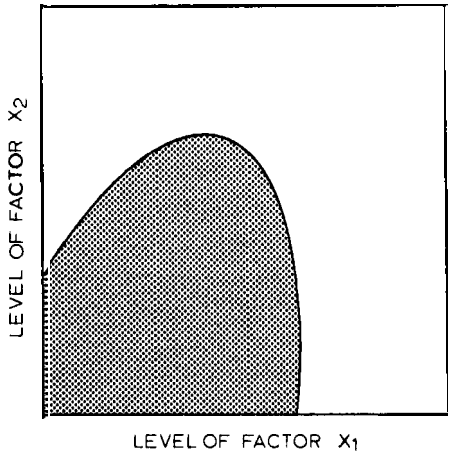

FIGURE 2
Experimental conditions of column temperature (x_1) and carrier gas flow rate (x_2) for which the overall chromatographic separation is better than some specified threshold level (shaded region).

or more of the performance criteria must be relaxed until a satisfactory compromise is achieved.

Mathematical functions that combine criteria of separation and time into a single response include resolution divided by time (R/t)[40] and the informing power divided by time (P_{inf}/t)[25]. As pointed out by Smits et al.[25], certain cautions must be exercised in the use of this type of function. For example, although the P_{inf}/t might increase significantly, close inspection could show that it does so, not because the P_{inf} is being increased to any great extent, but rather because the analysis time is being decreased rapidly.

Time normalization chromatography (TNC) is an approach that fixes the analysis time at a predetermined level and adjusts experimental variables to maximize chromatographic resolution given that constraint[41-48].

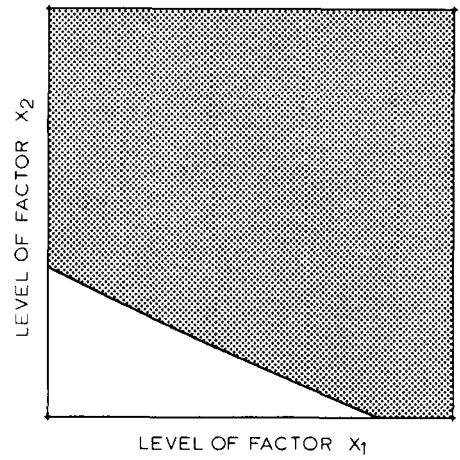

FIGURE 3
Experimental conditions of column temperature (x_1) and carrier gas flow rate (x_2) for which the analysis time is less than some specified threshold level (shaded region).

Time normalization by adjusting column temperature and flow rate is equivalent to searching for maximum resolution along the lower left edge of the feasible region shown in Figure 3.

OPTIMIZATION OF CHROMATOGRAPHIC PERFORMANCE

Efficient multifactor optimization strategies based upon the systematic perturbation of experimental variables have been used for some time by Russian analytical chemists[49], but have been almost completely ignored by other chromatographers. Instead, theoretical models based largely upon the results of single-factor experiments over a limited region of the factor space have resulted in descriptions of the chromatographic process that often ignore possible interactions

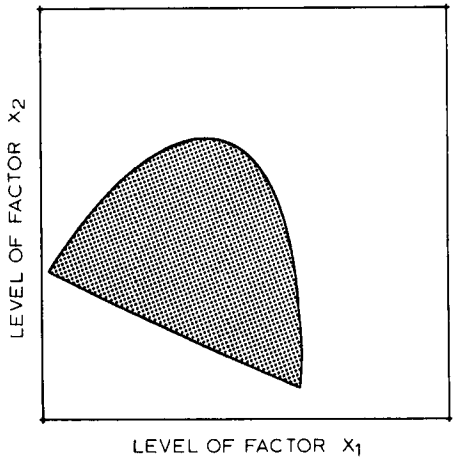

FIGURE 4
Experimental conditions of column temperature (x_1) and carrier gas flow rate (x_2) for which both the overall chromatographic separation criterion and the analysis time criterion are met (shaded region).

among variables. In those rare instances that interactions among variables are negligible, highly accurate predictive models can be formulated; see, for example, the papers of Purnell et al.[50-52] and previous workers[53,54] on the use of mixed solvents as stationary phases in gas-liquid chromatography for the optimization of overall separation of a multicomponent sample. When good models are available, optimal conditions can be derived by calculus[20,39], by simulation[55], or by numerical optimization[19,26,29,56].

It is not often recognized, however, that the theoretical model is usually a tentative approximation to the true behavior of the system and may be valid only within certain ranges of the experimental variables[19]. In 1960 the prediction was made "that chromatograms can eventually be worked out completely by

theory, thus eliminating the considerable time normally spent in the empirical approach to analytical problems"[57] Theory is helpful in the initial choice of an appropriate chromatographic system, but at present the detailed thermodynamic information that is required for calculation of optimum conditions from first principles is usually not available for routine analyses: for a given separation the true behavior of the system and its optimum must be determined by experiment.

Although the most common procedure for experimentally investigating chromatographic systems is to examine the effect of each variable while holding all other variables constant, the inefficiency and possible failure of this single-factor-at-a-time approach has been well documented[58,59] and will not be discussed here. In the remainder of this paper, we present a review of selected multifactor approaches to the experimental optimization of chromatographic systems.

Factorial Designs

To estimate an optimum level for a single factor, at least three experiments must be carried out, one experiment at each of three different levels as shown in Figure 5. (If only two levels are investigated, it is not possible to obtain an estimate of curvature.)

If two factors are to be optimized simultaneously, the five experiments shown in Figure 6 might be carried out. The interpretation of the results from this experimental design assumes that the behavior of each factor is the same at all levels of the other factor. If, in fact, the behavior of one factor is dependent on the level of the other factor (that is, factor dependence exists and the variables are said to interact

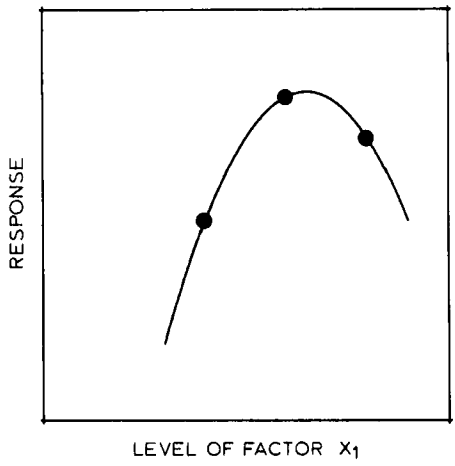

FIGURE 5
An experimental design for the estimation of optimal conditions when only one factor is to be investigated.

with one another[58]), the design shown in Figure 6 will be unable to detect it.

A more informative design is shown in Figure 7 where all combinations of all three levels of each factor are included. This type of design is known as a factorial design; the design shown in Figure 7 would be referred to specifically as a three-level, two-factor full factorial design (a 3^2 full factorial)[58,60].

An example of the use of a factorial design in the optimization of gas-liquid chromatographic performance is found in a paper by Scott[18]. In this work, the objective was to obtain an adequate level of resolution in a minimum analysis time by specifying optimal levels of stationary phase loading, column oven temperature, and carrier gas velocity. The factorial design involved experiments at five levels of stationary phase loading,

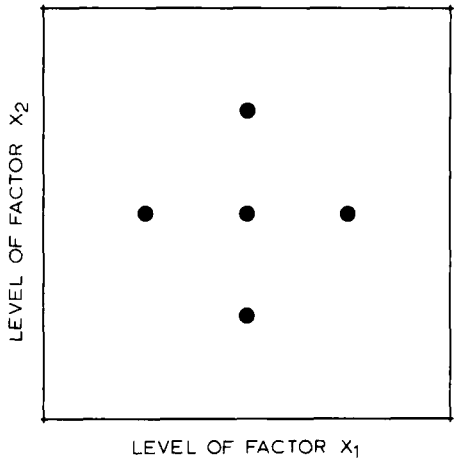

FIGURE 6
An experimental design for the estimation of optimal conditions when two factors are to be investigated and there is no factor dependence.

six levels of temperature, and five levels of carrier gas velocity for a total of 5 x 6 x 5 = 150 different experimental conditions.

Each experiment investigated the separation of methyl, ethyl, and propyl acetates on diethyleneglycol adipate. The two components in the sample that were most difficult to separate were the methyl and ethyl acetates; the resolution of this pair of peaks was calculated as the ratio of the distance between the peaks to the sum of the peak widths measured at 0.607 of the peak height. The elution time of propyl acetate (the last eluting peak) was also recorded.

A flow chart for the graphical analysis of the data is presented in Table 2 of reference 18. From this analysis, the optimum stationary phase loading, the optimum column temperature, and the optimum carrier

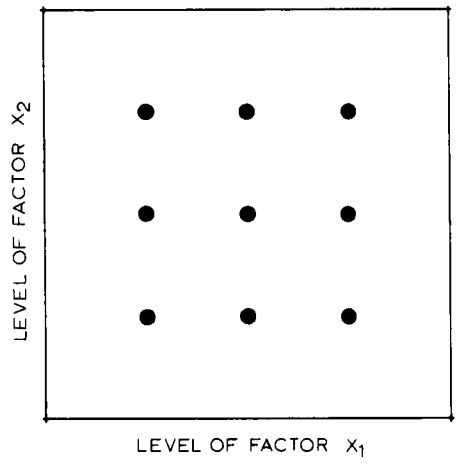

FIGURE 7
An experimental design for the estimation of optimal conditions when two factors are to be investigated and there is factor dependence.

gas velocity were obtained to give a resolution of 1.45 between the methyl and ethyl acetates and to give a minimum elution time for propyl acetate.

The number of experiments in this factorial design might appear to be excessive. Scott has considered this and states[18]:

> The experimental work required to determine the optimum conditions to effect a given resolution would take a skilled technician about 2 wk, and about 4 days of a graduate's time would be necessary for supervision and calculation of results. However, the cost involved for routine repetitive analysis would be recovered after two or three months of operation.

Swingle and Rogers[61] have used an on-line computer system for data acquisition and data processing in a similar factorial study investigating the effects of column length, stationary phase loading, temperature,

and flow rate on the resolution of pairs of n-alkane homologs (C_6 - C_{11}). It was concluded that the use of a computer could reduce the time necessary to complete an optimization such as this to "about four 24-hour days."

In addition to the factorial design's capability of revealing information on interactions between variables, information is gained about the degree of control over the variables that is necessary to maintain a desired level of performance[38,39]. Factorial experiments have been used extensively for this latter purpose by Grant and Clarke[62-64] and others[65,66] in studies of analytical precision in gas chromatography.

Fitting Empirical Models

The determination of optimal solvent composition for the resolution of two components by thin-layer chromatography is the subject of a paper by Turina et al.[67].

Their first study optimized the resolution of a lipid mixture in a two-component solvent system (chloroform-methanol) for which the relative amount of only one solvent component can be varied independently. Thus, this system involves only one factor and was investigated with three experiments at 2, 5, and 8 ml of methanol per 100 ml chloroform (see Figure 5). Measured resolution ranged from 0.56 to 0.92. The behavior of the system was approximated by the model:

$$R = a + bx + cx^2 \qquad (8)$$

where a, b, and c are the parameters of the parabola drawn through the points in Figure 5. The optimal solvent composition was predicted to be 5.68 ml methanol

for 100 ml chloroform by setting the first derivative of Equation 8 to zero. An experiment at this presumed optimum composition gave a resolution of 0.98. This predictive use of an empirical model is reasonable because the data to which the model is fit are obtained directly from the system under consideration and the predicted optimum lies within the region of experimentation.

In a second study, Turina et al.[67] optimized the resolution of dimethylol-4,5-dihydroxyethyleneurea and dimethylolpropyleneurea in a three-component system (benzene-pyridine-water) for which the relative amounts of two solvent components can be varied independently. This system involves only two factors (pyridine and water were chosen) and was investigated with five experiments at 10, 12, and 14 ml of pyridine and 0.5, 1.0, and 1.5 ml of water per 10 ml of benzene. Measured resolution ranged from -0.1 to 0.77. Because it was assumed there would be no significant interactions, the "star" design shown in Figure 6 was used. The behavior of the system was approximated by the model:

$$R = a + bx_1 + cx_2 + dx_1^2 + ex_2^2 \qquad (9)$$

The optimal solvent composition was predicted to be 18.2 ml of pyridine and 10.1 ml of water for 10 ml benzene. An experiment at this presumed optimum gave a resolution of 0.88. Although the predicted optimum did provide improved resolution, it does lie considerably outside the region of initial experimentation; for that reason, there is little confidence that the location of the predicted optimum is the true location of the optimum.

The third study by Turina et al.[67] optimized the resolution of Mg^{2+} and Al^{3+} in a five-component solvent

system (n-propanol, methanol, conc. HCl, water, and 8-hydroxyquinoline) for which the relative amounts of only two components were varied independently. In this study, the interaction between the two components which were varied (methanol and 8-hydroxyquinoline) could not be neglected and a full 3^2 factorial design was used (see Figure 7). Methanol was set at 15, 20, and 25 ml and 8-hydroxyquinoline was set at 0, 10, and 20 mg, while n-propanol, conc. HCl, and water were held constant at 10, 5, and 5 ml respectively. Measured resolution ranged from 0.6 to 0.89. The behavior of the system was approximated by the model:

$$R = a + bx_1 + cx_2 + dx_1^2 + ex_2^2 + fx_1x_2 \qquad (10)$$

The optimal solvent composition was predicted to contain 7.3 mg 8-hydroxyquinoline and 29.75 ml of methanol. An experiment was carried out at this predicted optimum and found to be better than previously obtained.

Factorial designs and the fitting of empirical models have been applied to the understanding of the effects of column oven temperature and carrier gas flow rate in the region of a previously determined optimum for the separation of octane isomers by gas-liquid chromatography[21]. Figure 8 shows the nine chromatograms from a 3^2 factorial design on a three-component sample consisting of 2,2-dimethylhexane, 2,2,3,3-tetramethylbutane, and 3,3-dimethylhexane. The nominal temperature in degrees centigrade and scaled flow rates are given to the right of each chromatogram along with the value of the CRF (Equation 6) and the analysis time in minutes (the retention time of the last component to elute, 3,3-dimethylhexane). Regression analysis[68],

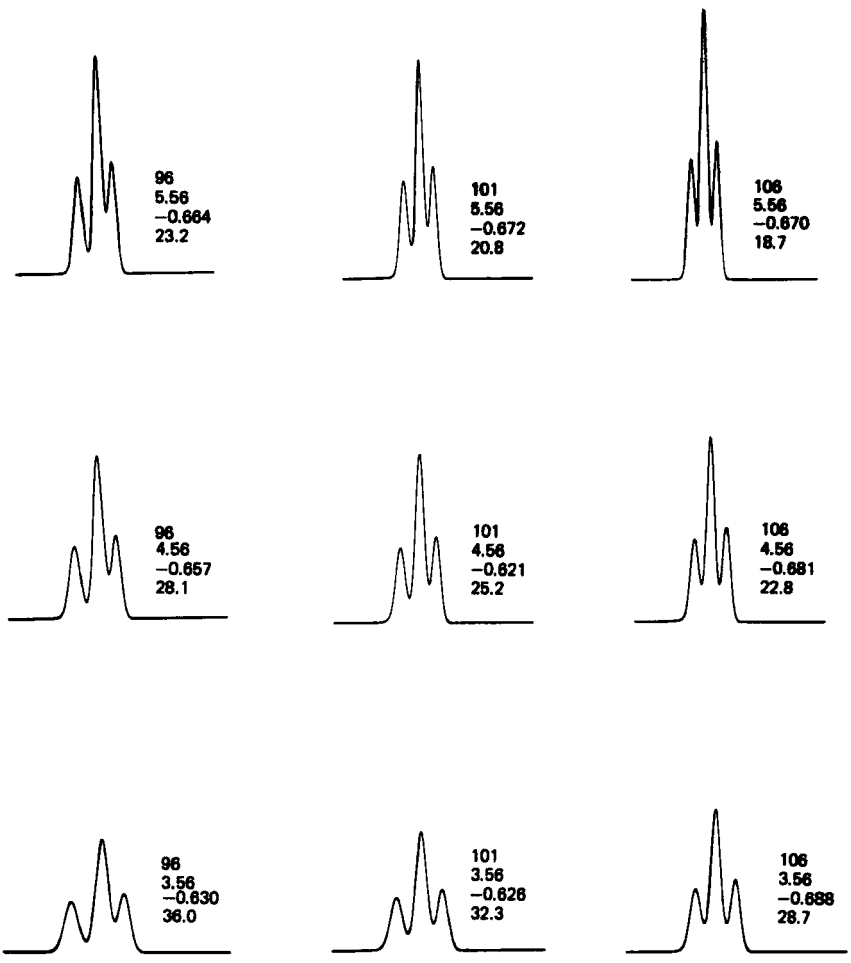

FIGURE 8
Chromatograms from a factorial design on a three-component sample. Temperature, flow-rate, CRF, and analysis time are given at the right of each chromatogram. (Reprinted from J. Chromatogr., 112, 267 (1975) with permission of Elsevier Scientific Publishing Company.)

fitting Equation 10 first to the CRF values and then to the analysis time values, gave:

$$CRF = -9.462 + 0.1884x_1 - 0.001006x_1^2 - 0.2260x_2 - 0.005143x_2^2 + 0.002600x_1x_2 \quad (11)$$

$$t = 308.3 - 3.320x_1 + 0.01044x_1^2 - 32.30x_2 + 1.364x_2^2 + 0.1402x_1x_2 \quad (12)$$

where x_1 is the column oven temperature and x_2 is the carrier gas flow rate.

Figures 9 and 10 show the calculated contours of constant chromatographic performance for Equations 11 and 12 in the region of this factorial design. The optimal CRF region has elliptical contours centered just outside the factorial at 97°C and 2.52 flow units. The analysis time surface is approximately planar in the region of the factorial and, not unexpectedly, predicts a stationary point far removed from the region. It is clear from these contours that both maximum overall separation and truly minimum analysis time cannot be achieved simultaneously; however, specifying a maximum allowable analysis time and superimposing that contour line on Figure 9 defines a feasible region similar to that of Figure 4.

Evolutionary Operation (EVOP)

It is evident that the sequential application of factorial designs might be used as a strategy for optimizing chromatographic systems when the predicted opti-

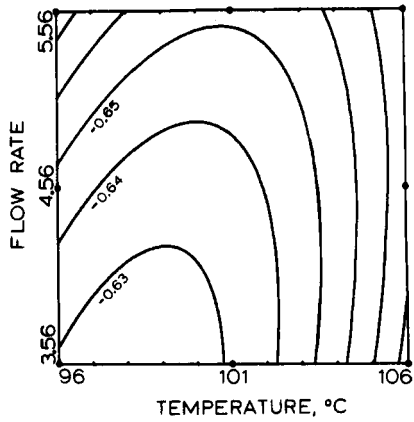

FIGURE 9
Calculated contours of constant CRF, calculated from Equation 11.

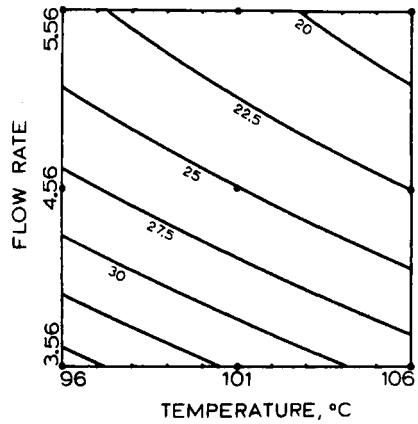

FIGURE 10
Calculated contours of constant analysis time, calculated from Equation 12.

mum lies outside the region of the initial factorial: if a second factorial design is carried out in the region (or in the direction) of the predicted optimum,

the results will either confirm the estimated location, or suggest another region for further study. The sequential use of factorial designs is known as evolutionary operation[58,69] in analogy to the adaptation of natural species to their environment[70].

Examples of the use of EVOP strategies applied to chromatographic systems may be found in the review by Alimarin[49].

Sequential Simplex Optimization

The simplex design is a more efficient experimental design for estimating the direction in which experimental variables might be changed to improve system performance. The sequential application of simplex designs[71,72] is thus an alternate evolutionary operation strategy.

A simplex is a geometric figure that defines a number of different experimental conditions equal to one more than the number of factors being optimized. (Compare this number to the number of experiments required for a factorial design.) If two factors, say temperature and flow rate, are to be optimized, the appropriate simplex design would be defined by only three different combinations of temperature and flow rate.

An attractive feature of the simplex design is that a new simplex adjacent to the current simplex can be generated by the addition of a single experiment. This new experiment is positioned opposite the location of the experiment that gave the worst response in the previous simplex; in this manner, the sequential experiments are forced to move away from regions of low performance toward the optimum. Details of the simplex algorithm may be found in the literature[59,71-75].

Figure 11 shows the pattern of experiments in the simplex optimization by gas-liquid chromatography of the CRF for a five-component mixture of 2,2-dimethylhexane, 2,2,3,3-tetramethylbutane, 3,3-dimethylhexane, 2,3-dimethylhexane, and 3-methylheptane[21]. Column temperature and carrier gas flow rate were optimized with the constraint that the analysis time (elution time of the last peak) not exceed 30 min. The progress of the sequential experiments is seen to move toward lower flow rate in a region of temperature around 100°C and to flatten against the 30-min time constraint. Representative chromatograms from this study are shown in Figure 12; the second and third vertices were arbitrarily ranked by assigning the responses indicated. The operation of the simplex algorithm may be seen by comparing the location of the first four vertices

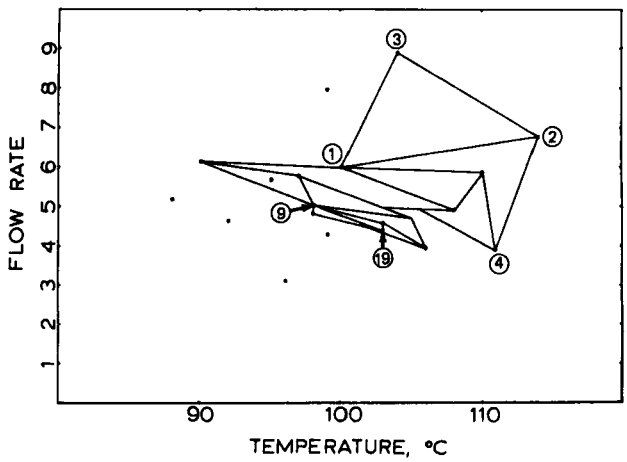

FIGURE 11
Simplex progress for a five-component sample, 30-min time constraint. (Reprinted from J. Chromatogr., 112, 267 (1975) with permission of Elsevier Scientific Publishing Company.)

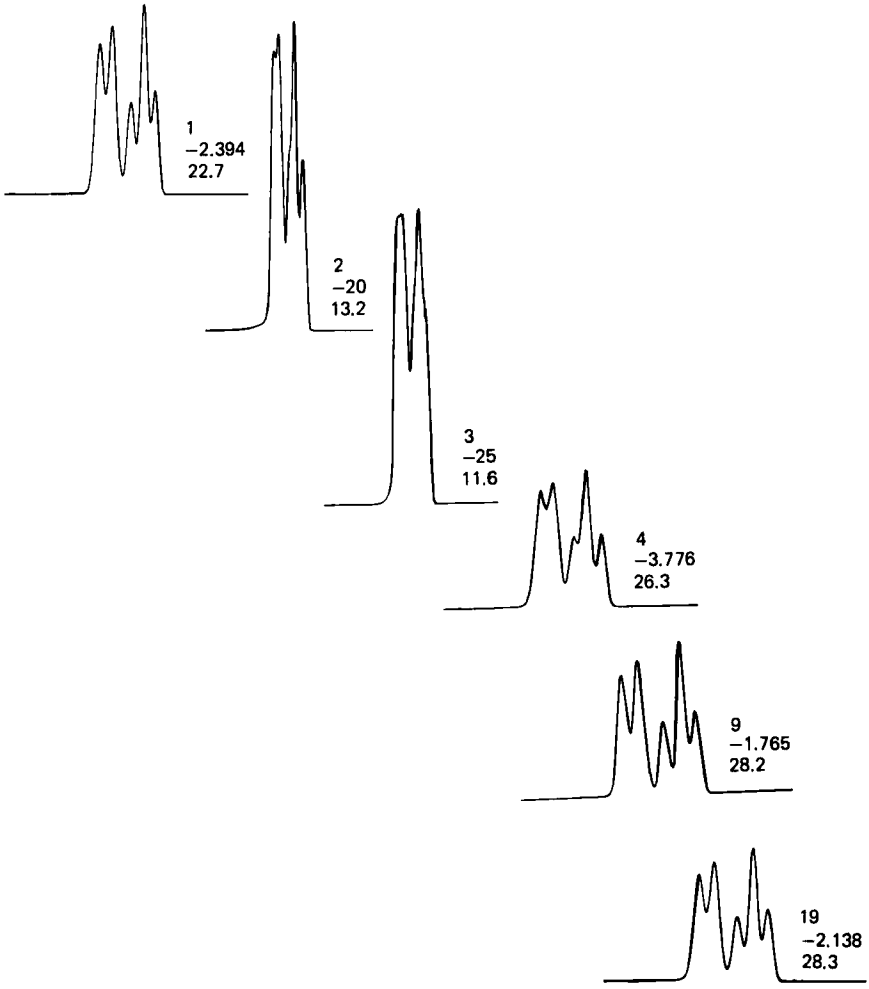

FIGURE 12
Representative chromatograms from the optimization of a five-component sample, 30-min time constraint (see Figure 11). Experiment number, CRF, and analysis time are given at the right of each chromatogram. (Reprinted from J. Chromatogr., 112, 267 (1975) with permission of Elsevier Scientific Publishing Company.)

(Figure 11) with their respective responses (Figure 12). The third experiment (CRF = -25) was eliminated from the first simplex and the next experiment (number four) gave a better response (CRF = -3.776). The best conditions for overall separation were found at experiment number nine; the remaining experiments did not show improved response but served to add confidence that the conditions of the ninth experiment were close to optimal within the 30-min constraint. Additional factorial experiments were also conducted in the region of this suspected optimum and confirmed the conclusion that the region was truly optimal.

Other examples of the use of sequential simplex designs for experimental optimization of chromatographic performance can be found in the work of Smits et al.[25] and Holderith et al.[76].

REFERENCES

1. P. R. Rony, Separ. Sci., 3, 239 (1968).

2. J. C. Giddings, Anal. Chem., 32, 1707 (1960).

3. L. S. Ettre, J. Gas Chromatogr., 1, 36 (1963).

4. J. J. van Deemter, F. S. Zuiderweg, and A. Klinkenberg, Chem. Eng. Sci., 5, 271 (1956).

5. B. L. Karger, C. Horvath, and L. R. Snyder, "An Introduction to Separation Science," Wiley, New York, 1973.

6. E. Grushka, M. N. Meyers, P. D. Shettler, and J. C. Giddings, Anal. Chem., 41, 889 (1969).

7. E. Grushka, Anal. Chem., 44, 1733 (1972).

8. H. M. McNair and W. M. Cooke, J. Chrom. Sci., 10, 27 (1972).

9. H. M. McNair and W. M. Cooke, American Laboratory, 5, 12 (1973).

10. S. P. Cram and J. L. Leitner, Chromatographia, 7, 567 (1974).

11. S. S. Ober, in "Gas Chromatography," V. J. Coates, H. J. Noebels, and I. S. Fagerson, Eds., Academic Press, New York, 1958, p. 41.

12. E. B. Sandell, Anal. Chem., 40, 834 (1968).

13. A. B. Christophe, Chromatographia, 4, 455 (1971).

14. R. Kaiser, "Gas Chromatographie," Geest and Portig, Leipzig, 1960, p. 33.

15. O. E. Schupp, III, "Gas Chromatography," Wiley, New York, 1968, p. 22.

16. B. O. Ayers, R. J. Loyd, and D. D. DeFord, Anal. Chem., 33, 986 (1961).

17. I. Halasz and E. Heine, in "Progress in Gas Chromatography," J. H. Purnell, Ed., Wiley, New York, 1968, p. 153.

18. R. P. W. Scott, in "Advances in Chromatography," Vol. 9, J. C. Giddings and R. A. Keller, Eds., Marcel Dekker, New York, 1970, p. 193.

19. T. B. Rooney and W. Aznavourian, Anal. Chem., 36, 2112 (1964).

20. T. W. Smuts and V. Pretorius, Separ. Sci., 6, 583 (1971).

21. S. L. Morgan and S. N. Deming, J. Chromatogr., 112, 267 (1975).

22. H. Kaiser, Anal. Chem., 42, 24A (1970).

23. D. L. Massart and R. Smits, Anal. Chem., 46, 283 (1974).

24. D. L. Massart, J. Chromatogr., 79, 157 (1973).

25. R. Smits, C. Vanroelen, and D. L. Massart, Z. Anal. Chem., 273, 1 (1975).

26. S. J. Hawkes, J. Chrom. Sci., 7, 526 (1969).

27. J. H. Knox and M. Saleem, J. Chrom. Sci., 7, 614 (1969).

28. R. P. W. Scott, J. Inst. Petrol., 47, 284 (1961).

29. T. W. Smuts and V. Pretorius, Anal. Chem., 44, 121 (1971).

30. J. C. Giddings, Anal. Chem., 34, 314 (1962).

31. J. J. Stefano and J. J. Kirkland, Anal. Chem., 47, 1103A (1975).

32. H. W. Johnson, Jr. and F. H. Stross, Anal. Chem., 31, 1206 (1959).

33. R. M. Bethea and M. Smutz, Anal. Chem., 31, 1211 (1959).

34. R. M. Bethea and T. D. Wheelock, in "Gas Chromatography," Proceedings of the 2nd International Symposium, June 1959, H. J. Noebels, R. F. Wall, and N. Brenner, Eds., Academic Press, New York, 1961.

35. R. A. Bernhard, Nature, 185, 311 (1960).

36. J. Albrecht and M. Verzele, Chromatographia, 4, 419 (1971).

37. M. Martin, C. Eon, and G. Guiochon, Research/Development, 26, 24 (1975).

38. A. L. Wilson, Talanta, 17, 21 (1970).

39. J. C. Giddings, "Dynamics of Chromatography," Part I, Marcel Dekker, New York, 1965.

40. R. J. Loyd, B. O. Ayers, and F. W. Karasek, Anal. Chem., 32, 698 (1960).

41. B. L. Karger and W. D. Cooke, Anal. Chem., 36, 985 (1964).

42. B. L. Karger and W. D. Cooke, Anal. Chem., 36, 991 (1964).

43. G. Guiochon, Anal. Chem., 38, 1020 (1966).

44. R. W. McCoy and S. P. Cram, Pittsburgh Conf. Anal. Chem. Appl. Spectrosc., Cleveland, Ohio, 1970, Paper No. 147.

45. E. Grushka and W. D. Cooke, J. Chrom. Sci., 9, 310 (1971).

46. E. Grushka, Anal. Chem., 43, 766 (1971).

47. E. Grushka and M. Yepes-Baraya, J. Chrom. Sci., 9, 653 (1971).

48. E. Grushka and F. B. Lo, J. Chromatogr., 91, 39 (1974).

49. I. P. Alimarin, O. M. Petrukhin, and G. I. Malofeeva, Z. Anal. Chem., 26, 2019 (1971).

50. R. J. Laub and J. H. Purnell, J. Chromatogr., 112, 71 (1975).

51. J. H. Purnell and J. M. Vargas de Andrade, J. Amer. Chem. Soc., 97, 3585 (1975).

52. J. H. Purnell and J. M. Vargas de Andrade, J. Amer. Chem. Soc., 97, 3590 (1975).

53. G. P. Hildebrand and C. N. Reilley, Anal. Chem., 36, 47 (1964).

54. R. A. Keller and G. H. Stewart, Anal. Chem., 36, 1186 (1964).

55. E. M. Sibley, C. Eon, and B. L. Karger, J. Chrom. Sci., 11, 309 (1973).

56. G. Guiochon, L. Jacob, and P. Valentin, Chromatographia, 4, 6 (1971).

57. J. H. Purnell, J. Chem. Soc., 1960, 1268.

58. G. E. P. Box, Biometrics, 10, 16 (1954).

59. S. L. Morgan and S. N. Deming, Anal. Chem., 46, 1170 (1974).

60. G. E. P. Box, Analyst, 77, 879 (1952).

61. R. S. Swingle and L. B. Rogers, Anal. Chem., 44, 1415 (1972).

62. D. W. Grant and A. Clarke, Anal. Chem., 43, 1951 (1971).

63. D. W. Grant and A. Clarke, J. Chromatogr., 92, 257 (1974).

64. D. W. Grant and A. Clarke, J. Chromatogr., 97, 115 (1974).

65. C. Van Eenaeme, J. M. Bienfait, O. Lambot, and A. Pondant, J. Chrom. Sci., 12, 398 (1974).

66. C. Van Eenaeme, J. M. Bienfait, O. Lambot, and A. Pondant, J. Chrom. Sci., 12, 404 (1974).

67. S. Turina, M. Trbojevic, and M. Kastelan-Macan, Anal. Chem., 46, 988 (1974).

68. N. R. Draper and H. Smith, "Applied Regression Analysis," Wiley, New York, 1966.

69. G. E. P. Box and N. R. Draper, "Evolutionary Operation," Wiley, New York, 1969.

70. G. E. P. Box, Appl. Statist., 6, 81 (1957).

71. W. Spendley, G. R. Hext, and F. R. Himsworth, Technometrics, 4, 441 (1962).

72. J. A. Nelder and R. Mead, Computer J., 7, 308 (1965).

73. S. N. Deming and S. L. Morgan, Anal. Chem., 45, 278A (1973).

74. S. N. Deming and P. G. King, Research/Development, 25, 22 (1974).

75. L. A. Yarbro and S. N. Deming, Anal. Chim. Acta, 73, 391 (1974).

76. J. Holderith, T. Toth, and A. Varadi, J. Chromatogr., 119, 215 (1976).