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# QUANTITATIVE OPTIMIZATION OF SYSTEM VARIABLES FOR CHROMATOGRAPHIC SEPARATIONS

## THE COLUMN TEMPERATURE

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SUMMARY

Our previously-reported window diagram optimization technique is here extended to the optimization of analysis temperature. Examples drawn from gas–liquid chromatography, gas–liquid chromatography and high-performance liquid chromatography are presented. The generality of the technique is demonstrated and its potential in analytical areas other than chromatography is pointed out.

## INTRODUCTION

We have, in recent years, described a technique which allows the quantitative optimization of chromatographic column compositions to allow complete resolution of all sample components[1]. Briefly, we have pointed out that if multi-component substrates are used either as series columns, as striated packings, or as mechanically mixed packings, the partition coefficient of any sample component, $K_R$, must be defined by the equation:

$$K_R = \Sigma \Phi K_R^0 \tag{1}$$

where $\Phi$ defines a sorbent volume fraction and $K_R^0$ the corresponding partition coefficient for the pure sorbent. Thus, for a binary (A + S) liquid phase:

$$K_R = \Phi_A K_{R(A)}^0 + \Phi_S K_{R(S)}^0 \tag{2}$$

Alternatively,

$$V_g^T = W_A V_{g(A)}^{T,0} + W_S V_{g(S)}^{T,0} \tag{3}$$

where $V_g^T$ is the specific retention volume (at column temperature), $W_i$ is the weight fraction of the $i$th component of the stationary-phase mixture, and $V_{g(i)}^{T,0}$ is the value of $V_g^T$ for pure component $i$.

For any pair of sample components (1 and 2), eqn. 2 can be written:

$$\alpha_{2/1} = \frac{K_{R_2}}{K_{R_1}} = \frac{\Phi_A K^0_{R(A)_2} + \Phi_S K^0_{R(S)_2}}{\Phi_A K^0_{R(A)_1} + \Phi_S K^0_{R(S)_1}} \tag{4}$$

from which $\alpha$ values of all solutes with respect to all other solutes may be calculated as a function of $\Phi_A$. These values of $\alpha$ may then be plotted *versus* $\Phi_A$, lines for individual pairs, wherein retention inversion occurs, appearing as approximate inverted triangles since we arrange that $\alpha$ always equals or exceeds unity. The overall picture obtained is defined by the overlapped triangles which, where no overlaps occur, provide separation "windows". Each window side comprises data from a pair of components and so the maximum corresponds to data for two pairs having the same $\alpha$, which is the minimum value for all pairs in the mixture at the corresponding value of $\Phi_A$.

The envelope of the windows defines the minimum value of $\alpha$ for the entire sample as a function of $\Phi$ and, hence, in terms of theoretical plate requirement for complete separation of all components, ($N_{req}$), the optimum value of $\Phi$ corresponds to that at the peak of the highest window.

The window diagram procedure has been applied with complete success to the separation of solute mixtures ranging from hydrocarbons[1] to underivatized sterols[2] with an equally-wide range of stationary phases. It has also been fully computerized for binary[2,3] and for multiple stationary phases[4]. More, recently[5], we have shown its applicability to mixed-bed gas–solid chromatography (GSC) and gas–liquid–solid chromatography (GLSC), and to permutations of these with gas–liquid chromatography (GLC). The most powerful aspect of the procedure is illustrated by our demonstration that it can be readily applied to the analysis of mixtures of unknown composition and complexity[7].

Having ascertained the minimum $\alpha$ value which must be dealt with at the optimum stationary-phase composition, the number of plates required to effect baseline resolution of the particular pairs in question, in addition to all other solutes in the mixture, can be readily calculated via the equation[8]

$$N_{req} = 36\left(\frac{\alpha}{\alpha - 1}\right)^2 \left(\frac{k' + 1}{k'}\right)^2 \tag{5}$$

where $k'$ is the capacity factor of the second component of the pair of sample components offering the most difficulty in separation. Since the values of $N$ per unit length of column attainable can be ascertained from the columns of A and of S used to generate $K_{R(i)}$ data, the required column length is than readily calculable.

It is occasionally found that a window diagram yields more than one optimum stationary-phase composition of the same $\alpha$. In such a situation, the secondary criterion of fastest analysis, *i.e.*, the total time required to elute the last component, decides the choice. This is readily ascertained by reading up a $K_R/\Phi_A$ plot at appropriate values of $\Phi_A$. In practice, of course, this secondary consideration may attain importance when windows offer disparate minimum $\alpha$. Two situations arise. First, if the minimum $\alpha$ in any window is so large that $N_{req}$ is essentially trivial, time of

analysis then clearly assumes primary importance. Secondly, even if $N_{req}$ is not trivial, much improved analysis times may be traded off against the need for longer columns if this does not impose unacceptable practical difficulties.

We recognise, of course, that if $k'$ is not very large, the most difficult separation may not be that of lowest $\alpha$ but one of low $k'$ (*cf.* eqn. 5). This can readily be ascertained by calculation and can be overcome in practice either by adjusting the solvent/support ratio to eliminate the problem or increasing the column length to achieve the needed higher $N_{req}$. We will not take this matter into further account here but have chosen our examples to illustrate the analysis time/column length choice in defining what is optimized.

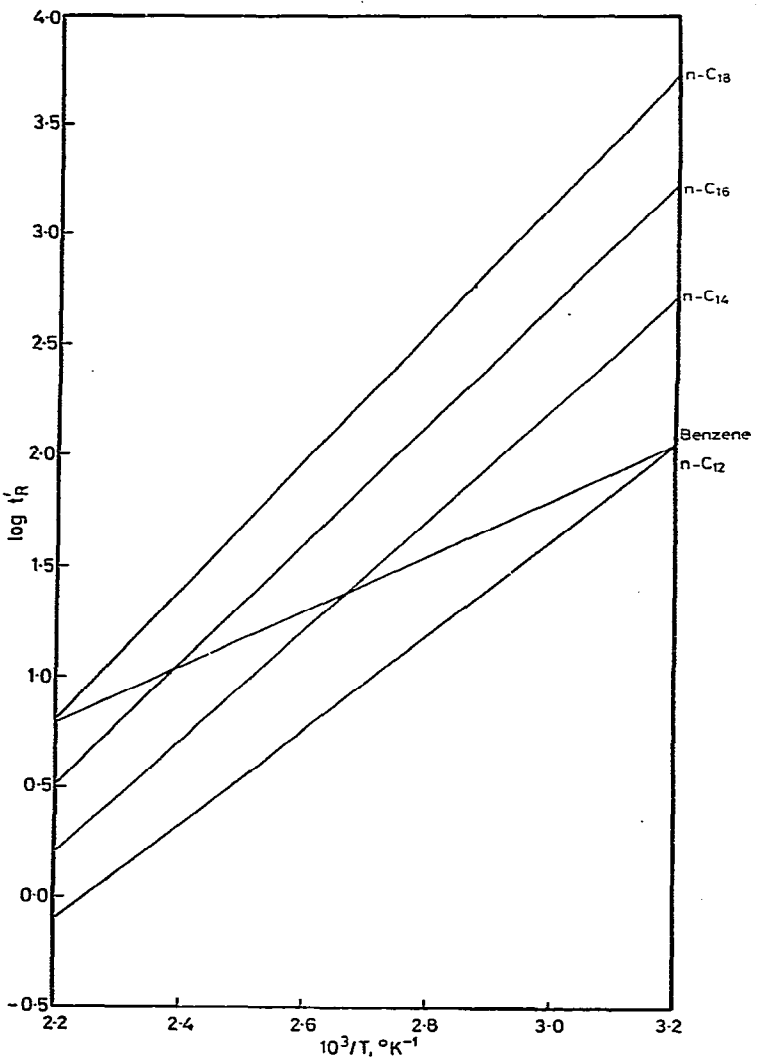


Fig. 1. GLC. Plots of log $t'_R$ *vs.* $10^3\ T^{-1}$ for the solutes: *n*-dodecane, *n*-tetradecane, *n*-hexadecane, *n*-octadecane and benzene with the stationary phase, N,N-bis(2-cyanoethyl)formamide. Data of Rogozinski and Kaufman[9].

In the foregoing we have described the general principles of our approach in terms of already published examples. It is self-evident that the method is directly applicable to optimization of analysis temperature and, equally, since log $K_R$ is virtually a linear function of $T^{-1}$ ($K_R$ also depends on the small temperature dependence of liquid density in GLC), that log $\alpha$ against $T^{-1}$ plots will provide the most efficient approach to construction of a window diagram since, in principle, data for only two temperatures are required to allow evaluation over a wide range.

*Example A: GLC*

We take for this example the data of Rogozinski and Kaufman[9] for elution of $C_{12}$, $C_{14}$, $C_{16}$ and $C_{18}$ *n*-alkanes, in a mixture with benzene, from a column of 40% (w/w) N,N-bis(2-cyanoethyl)formamide. This system provides an example where the authors[9] believe that both bulk and liquid surface solution (adsorption) effects occur. Fig. 1 illustrates plots of their data for log (retention time) against $T^{-1}$ for the temperature range, 40–180°C. At the extremes of this range, benzene cannot be separated from either $C_{12}$ or $C_{18}$ while, at two intermediate temperatures, it cannot be separated from either $C_{14}$ or $C_{16}$. Fig. 2 shows the window diagram in the form of a plot of log $\alpha$ against $T^{-1}$. Three windows appear, their heights and temperatures corresponding, respectively, to $\alpha = 1.406$ ($T = 165$ °C), $\alpha = 1.552$ ($T = 124$ °C) and $\alpha = 1.900$ ($T = 73$ °C). The largest $\alpha$ corresponds to the lowest temperature but, in fact, even the lowest $\alpha$ above offers an extremely easy separation requiring no more than about 400 theoretical plates. We thus have an example where total analysis time is more relevant than column length. Referring back to Fig. 1 where the three windows are indicated, we

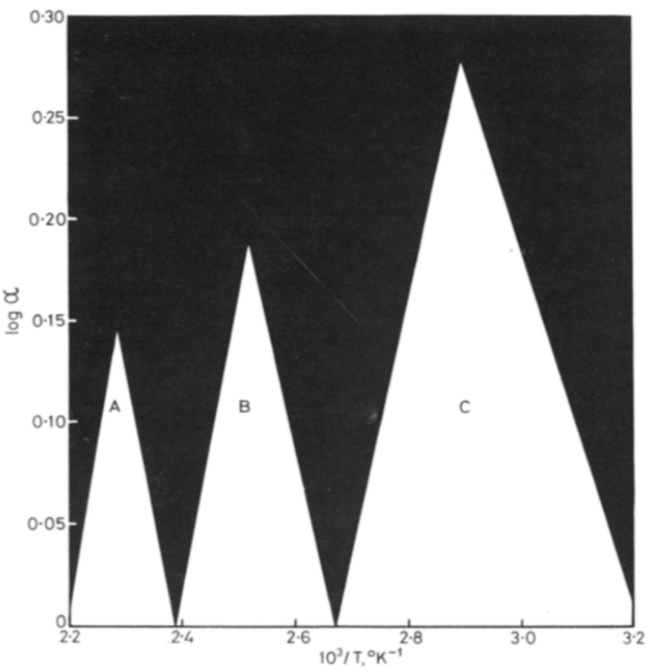


Fig. 2. Window diagram for data of Fig. 1.

see that the analyses (at constant solvent volume) will require total elution times in the approximate ratios A:B:C = 1:5:60. Clearly, window A, although of lowest $\alpha$, is overwhelmingly favourable since little more than 50 cm of column would be needed.

The foregoing relatively simple example well illustrates the secondary advantage of the technique in permitting quantitative decision where analysis time is important.

*Example B: GSC*

We consider now analysis of a somewhat more complex mixture of alkenes, dienes and benzene eluted from F-1 alumina coated with 10% (w/w) of $Na_2SO_4$ for which the necessary data have been provided by Sawyer and Brookman[10]. Their data span the temperature range, 165–225 °C. In Fig. 3, however, we show plots of log (capacity factor) against $T^{-1}$ for temperatures outside this range as well. The use of capacity factor is, of course, entirely legitimate since it is linearly related to $K_R$.

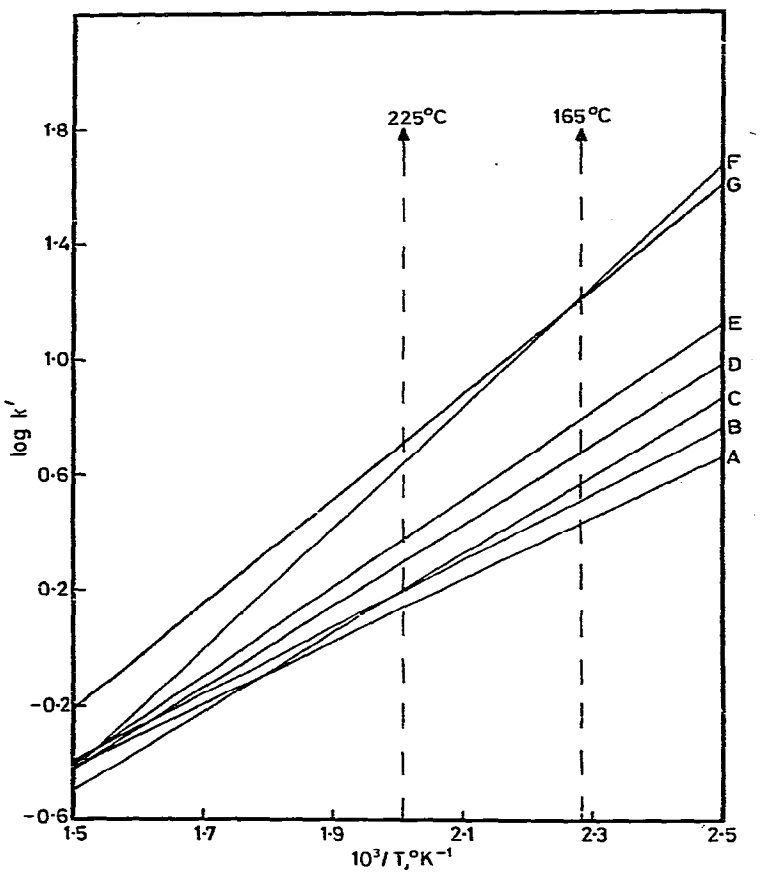


Fig. 3. GSC. Plots of log $k'$ *vs.* $10^3\ T^{-1}$ for the solutes: (A) *trans*-2-hexene, (B) 1-hexene, (C) *cis*-2-hexene, (D) *trans*-1,4-hexadiene, (E) *cis*-1,4-hexadiene, (F) *trans,trans*-2,4-hexadiene and (G) benzene with the stationary adsorbent phase, 10% (w/w) sodium sulfate on acid-washed F-1 alumina. Data of Sawyer and Brookman[10].

Further, in GSC, as in high-performance liquid chromatography (HPLC), it is an easier quantity to ascertain and define.

The window diagram derived is shown in Fig. 4 and we see that, within the experimental range reported, there is a single window (window C) at 190 °C with $\alpha = 1.075$. Outside this range, however, other windows appear with a particularly good one at 127° C, where $\alpha = 1.154$. Complete separation at these $\alpha$ values will require, respectively, about 8000 and 2000 theoretical plates at high $k'$. The reported experiments indicate column efficiencies of around 250 theoretical plates per foot, hence, column lengths of about 32 and 8 ft., respectively, are indicated. In contrast to example A, therefore, the decision is clear-cut in terms of column length requirement. However, 32 ft. is by no means an excessive length of column to construct and operate, hence, total elution time may well again be decisive. The ratio of total time for the two windows, ascertained from Fig. 3, is almost exactly four, the longer column window providing the faster analysis. Since the volumes of sorbent are proportional to column length, the factors effectively cancel (the longer column will take somewhat longer on account of carrier gas compressibility effects resulting from the higher inlet pressure needed). Thus the 8-ft. column operated at 127 °C provides, without question, the better practical solution.

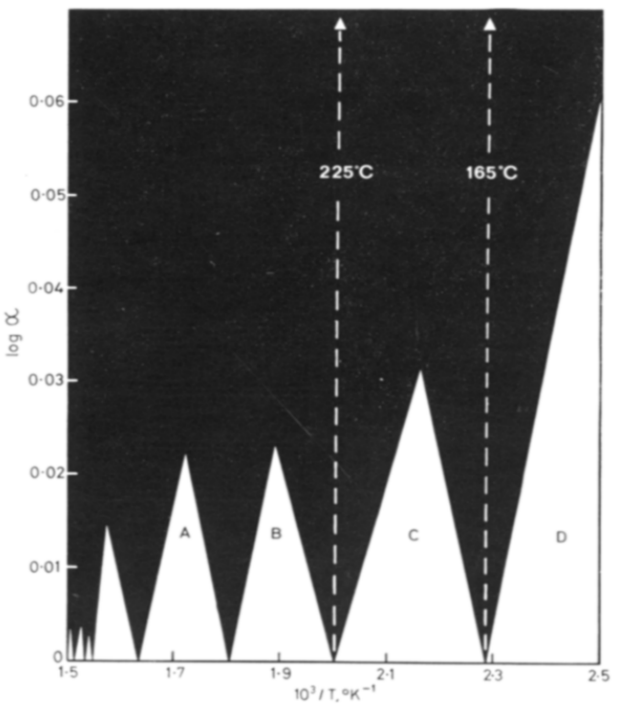


Fig. 4. Window diagram for data of Fig. 3.

*Example C: HPLC*

There are few reports of detailed study of temperature effects on retention in HPLC. A notable acquisition to the literature is a recent paper by Kraak *et al.*[11],

where such data for four systems are provided. We here use those for a system of nine underivatized amino acids, plus $NH_4^+$, eluted from a column of $C_8$-bonded silica with an eluent comprising 0.01 *M* sodium citrate (pH 2.60) + *n*-propanol (49:1, v/v) + sodium dodecyl sulphonate (0.3%, w/w). Fig. 5 shows plots of log (capacity factor) against $T^{-1}$ (the authors presented these as plots against $T$ for the range 20–50 °C). We have, again, extended the data outside the reported temperature range, over what very roughly comprises the practically useful range of the mobile phase.

Complete separation in this system within the range 20–50 °C is self-evidently difficult. This is brought out in the window diagram of Fig. 6 where four windows, all of $\alpha$ less than 1.03, are seen. In contrast, at 54.6 °C, a window at $\alpha = 1.058$ occurs while at 14.9 °C another, more or less equally good window of $\alpha = 1.054$ is seen. The two best windows within the 20–50 °C span require 100,000 ± 10,000 theoretical plates for complete separation of all ten components at high $k'$. The two windows outside this temperature range demand, respectively, 17,000 and 21,000 theoretical plates and thus, superficially, offer five times faster analysis. Reference to Fig. 5 shows that the last emerging components have retentions that are only slightly temperature dependent. Thus, the choice lies between the two windows of low $N_{req}$. Fig. 5 again then establishes

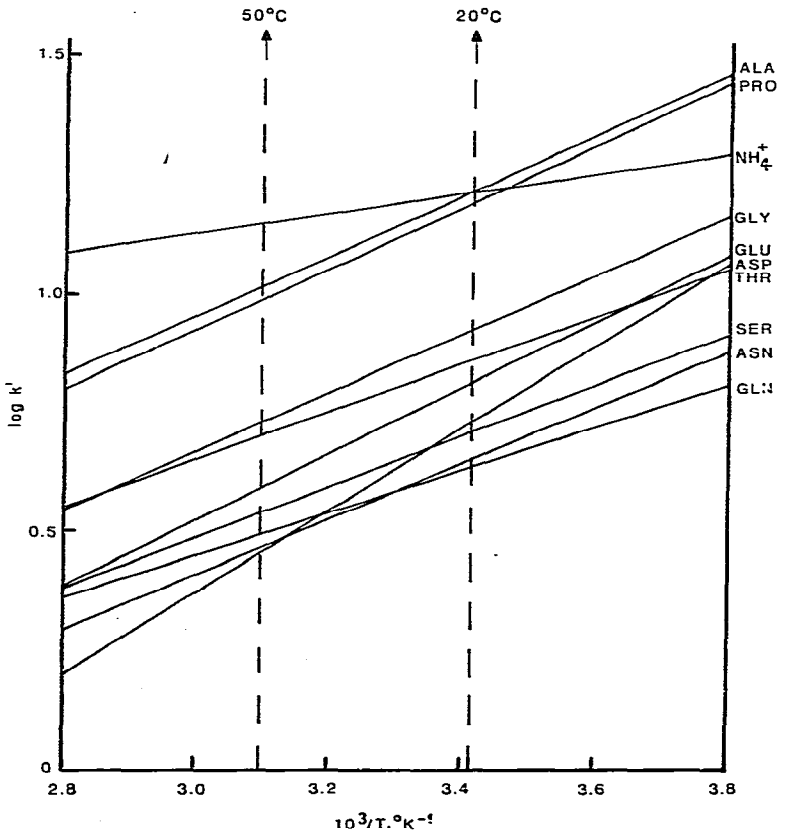


Fig. 5. HPLC. Plots of log $k'$ *vs.* $10^3\ T^{-1}$ for the indicated amino acids and $NH_4^+$ with a buffered mobile phase (pH 2.60) and $C_8$-bonded silica support. Data of Kraak *et al.*[11].

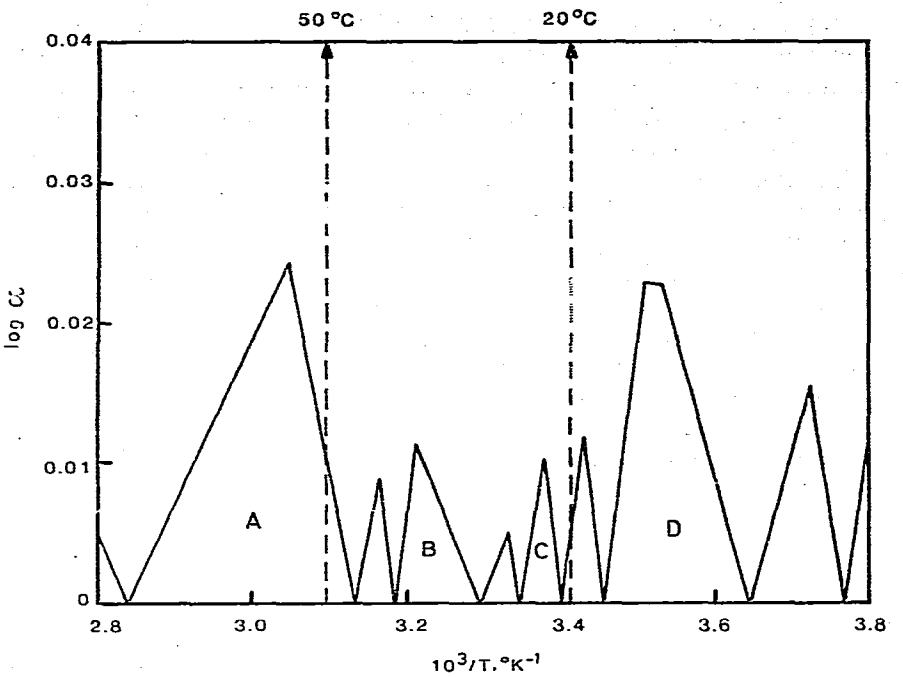


Fig. 6. Window diagram for data of Fig. 5.

a near compensation of length and $k'$ so that total analysis time will be essentially the same with either column. We thus have an example where neither the primary nor secondary criteria differentiate. We must thus choose on the grounds of practical convenience, which probably specifies the 15 °C window.

## DISCUSSION

The examples illustrated show the predictive power of the window diagram technique and the way in which difficult, subjective judgements may be put on an almost automatic and objective basis. Although we have not tested our predictions experimentally, they cannot be seriously questioned since they are based on reliable data from reputable laboratories.

We hope also to have illustrated the generality of our method. It seems self-evident that, with adequate data, optimization with respect to any system variable influencing chromatographic separation can be achieved. Indeed, Deming and Turoff[6] have recently shown an excellent example of the use of the technique to optimise mobile phase composition in the HPLC analysis of aromatic acids. We recognise that an extension of our method will be required when we attempt simultaneously to optimise in terms of more than one parameter, *e.g.*, the optimal condition of pH in an HPLC experiment may itself be a function of temperature. However, our method is admirably suited to computation and, as more information becomes available, it should be possible to handle the situation via modification of programs already described by us[2,3].

In conclusion, it seems to us that our basic approach should find application and utility in many areas of analytical chemistry other than the chromatographic techniques.

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