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## SYSTEMATIC APPROACH TO OPTIMIZING RESOLUTION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY, WITH EMPHASIS ON THE ROLE OF TEMPERATURE

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### SUMMARY

A general model for the easy optimization of separation in reversed-phase liquid chromatographic systems has been developed, for use on hand-held or desk-top programmable calculators. A small number of experimental data (minimum of four different conditions) are used to generate semi-empirical estimates of  $k'$  and  $\alpha$  for various values of temperature and mobile-phase composition (methanol-water, in present study). These latter  $k'$  and  $\alpha$  data are then combined with theoretical values of  $N$  from the Knox equation to allow the calculation of resolution as a function of all experimental parameters. Resolution can easily be mapped for time-normalized separation, fixed or variable column length, fixed operating pressure, etc. This allows the easy optimization of the final separation in terms of any set of initial constraints.

It is seen that temperature plays an important role in such separations, when mobile-phase composition and temperature are adjusted simultaneously to maintain fixed (*i.e.*, optimum) values of  $k'$ . Resolution is generally improved at higher values of separation temperature (and accompanying lower concentrations of organic in the mobile phase). Exceptions to this rule are noted and discussed.

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### INTRODUCTION

Reversed-phase (RP) separation on alkyl-bonded-phase packings is now the most versatile and popular of the various liquid chromatographic (LC) procedures. Judging from recently reported applications of modern LC, it seems probable that 75% of these are now carried out in the RPLC mode. This situation seems likely to continue for the next several years. It is therefore important to develop an in-depth understanding of these RPLC separations, and to translate this knowledge into a systematic approach that can be used by practical workers during method development.

The optimization of separation in chromatography (including RPLC) is normally carried out by trial-and-error procedures, guided by certain fundamental considerations and by empirical intuition. This approach is generally successful, because of the inherent power of modern chromatographic separation. That is, true optimiza-

tion is rarely required, and procedures that are in fact far from "optimum" are usually adequate for the separation goal at hand. Occasionally, however, one is faced with a more difficult separation problem. For example, the achievement of adequate resolution for several sample components of interest may elude the first few attempts at "optimization". Or it may be required that the separation time not exceed a few minutes for a reasonably complex sample (as in process control, clinical analysis, etc.). Alternatively, it may be important to reduce the time spent on each "optimization" to a minimum, consistent with adequate resolution of the sample within an acceptable time of analysis. This would be particularly true in a laboratory with a heavy demand for method development (e.g., an applications lab in an LC equipment company), but it is an important consideration wherever LC method development is carried out.

In each of the above cases, a systematic scheme is required so that a closer approach to true optimization is achieved with minimum expenditure of time and effort. One widely used scheme (e.g., discussion of ref. 1) is afforded by the resolution equation

$$R_s = (1/4) \underbrace{(\alpha-1)}_{(i)} \underbrace{\sqrt{N}}_{(ii)} \underbrace{[k'/(1+k')]}_{(iii)} \quad (1)$$

Here, resolution  $R_s$  is related to the separation factor  $\alpha$ , the column plate number  $N$ , and the average capacity factor  $k'$  of the band-pair of interest. It is assumed that terms i-iii of eqn. 1 are approximately independent, which allows their separate optimization. Thus the capacity factor  $k'$  is first adjusted into an "optimum" range of 1-5 for the band-pair of interest. In RPLC systems this is easily achieved by varying the volume fraction  $\Phi$  of organic solvent in the mobile phase; e.g., a change from 40% methanol-water to 60% (v/v) methanol. If further improvement in separation is required (increased  $R_s$  or decreased separation time), then either term i or ii can be further varied. The separation factor can be optimized in various ways: by changing the organic solvent (e.g., from methanol to tetrahydrofuran), by invoking chemical equilibria (pH effects, ion-pair formation, etc.), or by varying the temperature  $T$  of separation. While initially it was believed that the selectivity of RPLC mobile phases is dominated by the water present, more recently Karger and co-workers<sup>2,3</sup> have shown that significant changes in  $\alpha$  can be achieved by changing the organic solvent. Variation in mobile phase pH (e.g., ref. 4) or the use of ion pairing (e.g., ref. 5) allow major changes in separation selectivity, but these effects are limited to ionized or ionizable solutes. Temperature effects in RPLC are less well understood at present, although several studies<sup>6-9</sup> suggest that temperature can significantly affect  $\alpha$  values.

Column plate number (term ii of eqn. 1) is now well understood as a function of experimental conditions due in large part to the important work of Knox and co-workers (see ref. 1, Chap. 5 for a general discussion). A rapid scheme based on Knox's work has been reported for maximizing  $N$  in small-particle RPLC columns<sup>12</sup>. Several workers have noted the general improvement in column efficiency with increasing temperature (e.g., refs. 10, 11).

One limitation of the foregoing scheme based on eqn. 1 is the assumption that terms i-iii are independent and hence can be separately optimized. While this is a

useful first approximation, it is known not to be rigorously correct. Thus, a change in the percentage of organic solvent in the mobile phase mainly affects  $k'$  but some effect on  $\alpha$  can be expected, and in some cases  $N$  is changed slightly. Similarly, a change in  $T$  affects all three terms significantly, although separation temperature has not been much used for controlling resolution in RPLC separations.

When a mixture of several components is to be separated, eqn. 1 must be applied to each of the adjacent band-pairs of interest. This further complicates the overall optimization process. Since separation selectivity is of greater concern in this situation, the separate optimization of  $\alpha$  values is a useful first step. Laub and Purnell<sup>8</sup> have discussed a scheme for plotting experimental  $\alpha$  values for a complex mixture *versus* a single separation variable (temperature in their examples). It is readily apparent from such plots whether separation of all sample components is possible, and what temperature(s) is optimum. Since mobile phase composition (e.g., the percentage of organic solvent) is of equal importance in affecting  $\alpha$  in RPLC, the generalization of such plots for both composition and temperature is suggested. Once regions of optimum  $\alpha$  are defined, the further optimization of  $N$  and  $k'$  in eqn. 1 could be pursued as needed.

Another approach to optimization of separation in RPLC is suggested by the work of Deming and Turoff<sup>13</sup>. They use the mathematical SIMPLEX scheme to search for optimum experimental conditions. The concept is as follows. An initial experimental separation is carried out, the results are fed back into the SIMPLEX program, the program chooses a logical second set of conditions, the separation for these conditions is carried out experimentally, the results are fed back into the program, etc. In this way the SIMPLEX program will search for an experimental optimum in a "brute force" but systematic fashion. Two objections to the SIMPLEX approach are: (1) the "brute force" approach is limited unless chromatographic intuition and guidance are provided by the operator (e.g., see ref. 14), and (2) with a large number of experimental variables plus a complex sample, many experiments will be required during the course of the optimization; unless there is an on-line interface between the experimental system and the SIMPLEX program, the overall process will in turn be time consuming.

In the present paper we wish to explore another approach to optimizing separation in RPLC systems. This scheme is both syncretistic and complementary with respect to the approaches described above. It is based on the following considerations that reflect recent developments in certain areas.

(1) The availability of simple but rigorous theory for plate number  $N$  as a function of separation conditions;

(2) the observation that semi-empirical relationships can be used to define  $k'$  and  $\alpha$  values in RPLC systems as a function of  $T$  and  $\phi$ , on the basis of a limited number of experimental measurements for a given combination of column, organic solvent (e.g., methanol) and sample;

(3) the wide availability of powerful desk-top or hand-held programmable calculators, such as the Texas Instruments Model 59 used in the present study;

(4) the desirability of involving the chromatographer and his experience during the optimization process;

(5) the need to include all important parameters in the optimization process (e.g., temperature).

The above considerations have led us to develop a general computer approach to separation optimization which is adaptable to small programmable calculators. Experimental retention data are first obtained for the sample of interest in a particular RP system (given column, given organic modifier; e.g. methanol), for four different sets of experimental conditions (different percentages of organic modifier  $\Phi$ , temperature  $T$ ). Appropriate reduction of the latter data generates a set of semi-empirical parameters which describe solute retention as a function of  $\Phi$  and  $T$ , and these are inserted into the program. Further optimization of the separation is now carried out (using the calculator) by mapping resolution (and other pertinent measures of separation, e.g., peak height for sensitivity) as a function of  $\Phi$ ,  $T$  and other separation variables.

We also wish to report data on the combined effects of temperature and mobile phase composition on resolution. While it has been known that  $k'$  values generally decrease with either increase in  $T$  or %-organic in RPLC systems, the effect of simultaneous change in these latter two variables on  $\alpha$  has so far not been reported. As we will see, this dependence of  $\alpha$  on  $T$  and the percentage of organic solvent is important in optimizing  $R_s$ .

Finally, the present limited study suggests certain generalizations for the dependence of  $k'$  in RPLC on the various separation variables, generalizations which transcend sample type and the separation system. While the consequences of these observations will be explored in greater detail elsewhere, they allow some preliminary guidelines to the optimization of RPLC separations in the absence of theoretical calculations for a particular case.

The present discussion is restricted to isocratic RPLC systems. However, recent work from this laboratory<sup>15-17</sup> has established that gradient elution separations can be related to isocratic separation in simple fashion. The further discussion of the impact of the present study on RPLC gradient elution, and *vice versa*, will be deferred until later.

## THEORETICAL

### General resolution equation

The present approach to optimizing separation is based on an alternative form of eqn. 1:

$$R_s = 2(t_2 - t_1)/(w_1 + w_2) \quad (2)$$

Here,  $t_1$  and  $t_2$  are the retention times of bands 1 and 2, and  $w_1$  and  $w_2$  are their base-line bandwidths (in time units).  $R_s$  can be related directly to values of  $k'$  for the two bands ( $k_1$  and  $k_2$ ) and to the column plate number  $N$ , since

$$t_1 = t_0(1+k_1) \text{ and } t_2 = t_0(1+k_2) \quad (2a)$$

and

$$N = 16(t_1/w_1)^2 = 16(t_2/w_2)^2 \quad (2b)$$

The assumption that  $N$  is generally the same for different bands in an RPLC chromatogram is usually valid, but values of  $N$  for each band can be determined as below.

If we are to calculate  $R_s$  as a function of experimental conditions from eqns. 2-2b, we require  $k_1$ ,  $k_2$  and  $N$  as a function of experimental conditions.

*N as a function of experimental conditions*

Knox and coworkers (e.g., refs. 1, 18) have shown that the reduced plate height  $h$  for any LC column is given by

$$h = 2/\nu + A\nu^{0.33} + C\nu \quad (3)$$

Here,  $A$  and  $C$  are constants characteristic of a given column, and  $h$  is related to plate height  $H$  and particle diameter  $d_p$  as

$$h = H/d_p \quad (3a)$$

The reduced velocity  $\nu$  is given as

$$\nu = u d_p/D_m \quad (3b)$$

The quantity  $u$  is the velocity of the mobile phase, equal to  $L/t_0$ ;  $L$  is column length and  $t_0$  is the dead-time for the column (see ref. 1 for further comment). The solute diffusion co-efficient  $D_m$  is commonly given by the Wilke-Chang equation<sup>19</sup>:

$$D_m = 7.4 \cdot 10^{-8} (\psi_B M_B) T/\eta V_A^{0.6} \quad (4)$$

$M_B$  is the molecular weight of the mobile phase and  $\psi_B$  is an association factor; values of  $\psi_B M_B$  for RPLC systems are estimated in ref. 12.  $T$  is the absolute temperature ( $^{\circ}K$ ),  $\eta$  is the mobile phase viscosity (cP), and  $V_A$  is the molar volume of the solute (ml). Eqn. 4 is inapplicable to macromolecular solutes such as proteins and polymers.

The mobile phase velocity  $u$  can be calculated for a particular LC system as (e.g., ref. 1):

$$u = P d_p^2/\Phi^* \eta L \quad (5)$$

Here,  $P$  is the pressure drop across the column (cgs units), and  $\Phi^*$  is an empirical column permeability. For columns of porous, spherical particles,  $\Phi^*$  is generally close to 500 for well-packed columns.

For a given column and RPLC system, the various parameters above will be defined, so that  $N$  can be calculated as a function of experimental conditions. In the case of "well-packed" columns of "good" packing material, eqn. 3 for porous particles becomes

$$h = 2/\nu + \nu^{0.33} + 0.05\nu \quad (6)$$

For such columns, the parameters  $A$  and  $C$  of eqn. 3 need not be determined. For values of  $\eta$  in RPLC systems, see ref. 6. Using methanol as organic solvent, we found that the latter data obey the empirical fitting function below (eqn. 6a), within  $\pm 2\%$  over the range  $0 \leq \Phi \leq 1$  and  $15 \leq T \leq 65^{\circ}C$ .

$$\log \eta = \log \eta_{40} + 10^3 (0.65 + 0.2 \eta_{40}) \left( \frac{1}{T} - \frac{1}{313} \right) \quad (6a)$$

where

$$\eta_{40} = 0.64 + 2.1 \Phi - 2.32 \Phi^2 \quad (6b)$$

and  $\eta_{40}$  is the viscosity at 40 °C.

Values of  $\eta$  as a function of  $\Phi$  and  $T$  for organic solvents other than methanol will require other empirical fitting functions. Note that  $\eta$  need not be estimated to better than  $\pm 10\%$ .

*k'* as a function of experimental conditions

Because retention in chromatographic systems is determined by the equilibrium characteristics of the system, *k'* values for a given solute at two temperatures  $T_a$  ( $k_a$ ) and  $T_b$  ( $k_b$ ) are generally related (other conditions constant) as

$$\log k_a = \log k_b - a(1/T_b - 1/T_a) \quad (7)$$

Eqn. 7 has been verified in numerous RPLC systems (e.g. ref. 6 and present study).  $T$  in eqn. 7 refers to absolute temperature and  $a$  is a constant which is proportional to the standard molar enthalpy for the transfer of solute from one chromatographic phase to the other. It will prove convenient to replace  $T_b$  by a reference temperature  $T_R$  (and  $k_R$ ), to give

$$\log k_a = \log k_R - a(1/T_R - 1/T_a) \quad (7a)$$

The parameter  $a$  will vary with the solute and with mobile phase composition.

For a given solute and separation temperature  $T$ , it is generally observed that  $k'$  varies with the volume fraction  $\Phi$  of organic solvent in the RPLC mobile phase as

$$\log k' = b - c \Phi. \quad (8)$$

Numerous studies summarized in refs. 15–17, as well as the present studies, show that eqn. 8 is generally obeyed within experimental error. Schoenmakers *et al.*<sup>20</sup> report curvature of  $\log k' - \Phi$  plots in representative RPLC systems, but this effect seems to be slight and is probably not general (particularly over the narrow range in  $k'$  values that is of interest in chromatographic optimization). Where deviations from eqn. 8 are significant as far as optimization of resolution is concerned, it is possible to correct for these (see below).

If eqns. 7a and 8 are obeyed by a solute in a RPLC system, then it follows that the temperature coefficient  $a$  must be of the form

$$a = d - e \Phi \quad (9)$$

where  $d$  and  $e$  are constants for a given solute and system. This can be seen by solving for  $a$  in eqn. 7a:

$$a = (\log k_R - \log k_a)/(1/T_R - 1/T_a) \quad (9a)$$

$k_a$  is given by an equation of the form of eqn. 8, as is  $k_R$ :

$$\begin{aligned} \log k_a &= b_a - c_a \Phi \\ \log k_R &= b_r - c_r \Phi \end{aligned}$$

Here,  $b_a$ ,  $b_r$ ,  $c_a$  and  $c_r$  are constants for a given solute and RPLC system. Substituting the above equations for  $\log k_a$  and  $\log k_r$  into eqn. 9a gives

$$a = [(b_r - b_a)/(1/T_R - 1/T_a)] - [(c_r - c_a)/(1/T_R - T_a)] \Phi \quad (9b)$$

Since the various terms of eqn. 9b are constant (for fixed temperatures  $T_R$  and  $T_a$ ), except for  $a$  and  $\Phi$ , eqn. 9b is seen to be of the same form as eqn. 9.

It is convenient now to define a "standard state" for the RPLC system (specified by solute, column and nature of the organic solvent; e.g., methanol). The standard state is defined by a temperature  $T_s$  and mobile phase composition  $\Phi_s$ . In the present study,  $T_s = 51^\circ\text{C}$  ( $324.3^\circ\text{K}$ ) and  $\Phi_s = 0.60$  (unless otherwise noted). The standard state value of  $k'$  for the solute of interest is  $k_s$ . From eqn. 8 we can write:

$$\log k_{\phi,s} = \log k_s - c(\Phi - \Phi_s) \quad (10)$$

Here,  $k_{\phi,s}$  refers to a value of  $k'$  for some value of  $\Phi$  and the temperature  $T_s$ . From eqns. 9a and 10, for a value of  $k'$  at any value of  $T$  and  $\Phi$ , we then have

$$\log k' = \log k_{\phi,s} - a(1/T_s - 1/T) \quad (10a)$$

Eqns. 10 and 10a allow the calculation of  $k'$  for any value of  $T$  and  $\Phi$ , as follows:

- (1) determine the value  $k_s$  for  $T_s$  and  $\Phi_s$ ;
- (2) determine the value of  $c$  from eqn. 10 for experimental values of  $k_s$  and  $k'$  at another value of  $\Phi$  ( $\Phi \neq \Phi_s$ ) and  $T = T_s$ ;
- (3) determine experimental values of  $a$  from eqn. 10a as applied to values of  $k'$  at the same  $\Phi$  and different values of  $T$ ; obtain  $a$  values for at least two different values of  $\Phi$ ;
- (4) determine the coefficients  $d$  and  $e$  of eqn. 9 by plotting experimental  $a$  values vs.  $\Phi$ ;
- (5) to calculate  $k'$  for any value of  $T$  and  $\Phi$ , first calculate  $k_{\phi,s}$  from eqn. 10; then calculate  $k'$  from eqn. 10a (after determining  $a$  from eqn. 9).

The foregoing scheme allows calculation of  $k'$  as a function of  $T$  and  $\Phi$ , given a minimum of four well-chosen values of  $k'$  at different values of  $\Phi$  and  $T$ . Thus, assume the initial measurement of  $k'$  for  $\Phi = 0.5$  and  $T = 25^\circ\text{C}$  gives a value  $k' = 3.5$ . A second measurement might then give  $k' = 2.0$  for  $\Phi = 0.5$  and  $T = 60^\circ\text{C}$ . To maintain accurate values of  $k'$ , the second two measurements should then be carried out at the same values of  $T$  (25 and  $60^\circ\text{C}$ ), and a lower value of  $\Phi$  (e.g.,  $\Phi = 0.2$ ). This might in turn yield  $k'$  values of 16 ( $25^\circ\text{C}$ ) and 8.2 ( $60^\circ\text{C}$ ). The standard temperature could then be selected arbitrarily (e.g.,  $T = 25^\circ\text{C}$ ), or as discussed below. If a standard temperature other than either 25 or  $60^\circ\text{C}$  is desired, values of  $k'$  for that temperature can be determined by interpolation according to eqn. 7.

A comparison of calculated  $k'$  values as a function of  $T$  and  $\Phi$  (as described above) is made with experimental values in a later section. Generally good agreement is noted.

When might a particular standard state be chosen in preference to some arbi-

trary  $T$ ,  $\Phi$  combination? The standard state value of  $k'$  ( $k_s$ ) can be determined either directly (by experiment) or interpolated from direct experimental observations. Thus,  $k_s$  will be known fairly precisely. Extrapolation to other values of  $k'$  can lead to experimental error or imprecision, depending on minor deviations of experimental data from the relationships predicted by eqns. 7a and 8. Thus maximum accuracy in predicted values of  $k'$  occurs when conditions for  $k'$  are similar to those for  $k_s$ .

When might it be important to obtain maximum precision in estimates of  $k'$ ? Resolution  $R_s$  is quite sensitive to values of  $k'$  when values of  $\alpha$  are close to 1.0. Therefore, optimization of  $R_s$  requires accurate values of  $k'$  in this region of  $T$  and  $\Phi$ . For example, it might be found that  $\alpha = 1.00$  for two compounds of interest over the range of conditions  $T = 60^\circ\text{C}$ ,  $\Phi = 0.30$  to  $T = 25^\circ\text{C}$ ,  $\Phi = 0.45$ . A good choice for the standard state would then be  $T = 45^\circ\text{C}$  and  $\Phi = 0.37$ .

## EXPERIMENTAL

### *Chromatography*

The liquid chromatograph was assembled from commercially available modules consisting of an LDC Constametric II pump and Model 1203 254-nm UV monitor (Laboratory Data Control, Riviera Beach, Fla., U.S.A.), a six-port sampling valve (Model CV6-UHPa-N60), Valco, Houston, Texas, U.S.A.), a constant-temperature water-bath (Technicon, Tarrytown, N.Y., U.S.A.), and a Varian Model A-25 recorder (Varian, Palo Alto, Calif., U.S.A.). The separations were performed on  $C_8$  reversed-phase columns ( $150 \times 4.6$  mm I.D.,  $d_p = 5 \mu\text{m}$ , Fast-LC-8<sup>TM</sup> Columns; Technicon).

Duplicate  $k'$  values were measured after 1 h of system equilibration. The column dead time  $t_0$  was measured by injecting mixtures of methanol-water differing in composition from the mobile phase.

All solutes were reagent grade, used as received and diluted in methanol-water (50:50). Mobile phases were prepared on a volume basis using Photrex grade methanol (J. T. Baker, Phillipsburg, N.J., U.S.A.) and Milli-Q water (Millipore, Bedford, Mass., U.S.A.).

### *Calculations*

All calculator-generated data were obtained with a TI-59 programmable calculator (Texas Instruments, Dallas, Texas, U.S.A.). The use of appropriate equations developed in preceding sections provided a calculator program capable of completely describing the separation of any two adjacent solutes, as a function of pertinent experimental conditions. The actual sequence of calculations is summarized in Table I and detailed in the Appendix.

The experimental variables and parameters required in the program are listed in Table I. Final calculated quantities ( $R_s$ ,  $P$ , etc.) are also shown in Table I. The computational steps listed in Table I were carried out as follows.

(1) Values of  $k'$  for the two bands ( $k_1$  and  $k_2$ ) are calculated using eqns. 9, 10 and 10a. The necessary experimental parameters ( $\log k_s$ ,  $c$ ,  $d$ ,  $e$ ) can be derived by application of these same equations to pertinent data. Values of these parameters for



TABLE I

## CALCULATION SEQUENCE

Calculated parameters:  $R_s$ ,  $N$ ,  $P$ ,  $k'_1$ ,  $k'_2$ ,  $u$ ,  $v$ ,  $\eta$ ; required inputs:  $\Phi$ ,  $T$ ,  $t_s$  (primary variables);  $\Phi^*$ ,  $L$ ,  $d_p$  (held constant in these experiments); solute coefficients ( $b$ ,  $c$ ,  $d$ ,  $e$ ), molar volume ( $V_A$ ).

## Calculations

1. Calculate  $k'_1$ ,  $k'_2 = f(\Phi, T)$
2. Determine  $k'_{\max}$ . ( $k'_1$  or  $k'_2$ ?)

3.  $t_0 \approx t_s / (1 + k'_{\max})$   
 $u = L/t_0$

4. Calculate  $\eta = f(\Phi, T)$

5. 
$$\bar{D}_M = \frac{7.4 \cdot 10^{-5} (\psi_B M_B)^{1/2} T}{\eta V_A^{0.6}}$$

$$V_A = \frac{V_1 + V_2}{2}$$

6.  $v = u d_p / \bar{D}_M$

7.  $h = 2/v + v^{0.33} + 0.05v$

8.  $H = h d_p$

9.  $N = L/H$

10.  $t_1 = t_0 (1 + k'_1)$

11.  $t_2 = t_s$

12.  $W_1 = 4t_1/N^2$

12.  $W_2 = 4t_2/N^2$

13.  $R_s = \frac{2(t_2 - t_1)}{(w_1 + w_2)}$

14.  $P = \frac{u \Phi^* \eta L}{d_p^2}$

$$k' = f(\Phi, T):$$

$$\log k' = (b - c\Phi) - (d - e\Phi) \left( \frac{1}{T_R} - \frac{1}{T} \right)$$

where  $b$ ,  $c$ ,  $d$ ,  $e$  are determined experimentally.

$$\eta = f(\Phi, T):$$

$$\log \eta = \log \eta_{10} + 10^3 (0.65 + 0.2 \eta_{10}) \left( \frac{1}{T} - \frac{1}{313} \right)$$

where  $\eta_{10} = 0.64 + 2.1\Phi - 2.32\Phi^2$

TABLE II

 $k'$  DATA FOR  $R_s$  OPTIMIZATION

Solutes	$k'$ (60%, 51 °C)	$b^*$	$c = S^*$	$d$	$e$	$\bar{a}$
<i>p</i> -Nitrophenol	0.34	0.896	2.345	2540	2360	1125
Phenol	0.38	0.892	2.215	1474	1590	523
Acetophenone	0.63	1.310	2.505	1414	1554	480
Anisole	1.09	1.595	2.595	854	485	563
Methyl benzoate	1.10	1.755	2.835	1340	1180	632
Benzene	1.20	1.587	2.535	1264	1184	555
Phenetole	1.75	2.056	3.028	1266	975	681
Toluene	2.03	2.080	2.965	1341	1016	732
Ethylbenzene	3.28	2.582	3.450	1770	1595	813

\* At 51 °C.

TABLE III  
EXPERIMENTAL VERSUS CALCULATED  $k'$  VALUES  
 $\Phi = 0.7$ .

Solute	Temperature ( $^{\circ}\text{C}$ )									
	31		41		51		59.5		69.5	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
<i>p</i> -Nitrophenol	0.26	0.27	0.20	0.22	0.18	0.18	0.14	0.15	0.08	0.13
Phenol	0.26	0.26	0.24	0.24	0.22	0.22	0.21	0.21	0.18	0.19
Acetophenone	0.40	0.42	0.38	0.39	0.36	0.36	0.34	0.34	0.30	0.32
Methyl benzoate	0.70	0.75	0.63	0.66	0.59	0.59	0.52	0.54	0.47	0.48
Anisole	0.71	0.76	0.65	0.67	0.60	0.60	0.54	0.55	0.47	0.49
Benzene	0.77	0.80	0.72	0.72	0.65	0.65	0.61	0.60	0.54	0.55
Phenetole	1.07	1.13	0.96	0.99	0.87	0.86	0.77	0.78	0.68	0.69
Toluene	1.24	1.36	1.13	1.17	1.01	1.01	0.87	0.90	0.76	0.79
Ethylbenzene	1.92	1.99	1.68	1.70	1.47	1.47	1.30	1.30	1.13	1.14

the solutes studied by us are listed in Table II (and were derived from the experimental data of Tables III-V).

(2) Since it is not always known in advance which band will elute first, the larger of the two  $k'$  values calculated in step No. 1 is determined ( $k_{\text{max}}$ ).

(3) The quantity  $t_0$  is calculated from the specified separation time  $t_x$  and the latter value of  $k_{\text{max}}$ .

(4) The mobile phase viscosity  $\eta$  is calculated from eqns. 6a, b.

(5) The average solute diffusion coefficient  $D_m$  for the two solutes is estimated from eqn. 4 (using an average value of  $V'$ ).

(6-9) Eqns. 3b and 6, plus definitions of  $h$  and  $N$ .

(10-13) Eqns. 2, 2a, 2b and 5.

TABLE IV  
EXPERIMENTAL VERSUS CALCULATED  $k'$  VALUES  
 $\Phi = 0.6$ .

Solute	Temperature ( $^{\circ}\text{C}$ )									
	29		41.5		50.5		61		70.5	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
<i>p</i> -Nitrophenol	0.57	0.55	0.42	0.45	0.34	0.31	0.27	0.24	0.22	0.22
Phenol	0.49	0.48	0.42	0.41	0.38	0.37	0.34	0.33	0.30	0.30
Acetophenone	0.79	0.82	0.69	0.71	0.63	0.64	0.57	0.58	0.50	0.53
Methyl benzoate	1.51	1.57	1.24	1.30	1.10	1.14	0.96	0.99	0.83	0.88
Anisole	—	—	1.23	1.23	1.09	1.10	0.95	0.94	0.82	0.87
Benzene	1.56	1.55	1.33	1.31	1.20	1.17	1.05	1.03	0.92	0.93
Phenetole	2.44	2.47	2.00	2.01	1.75	1.75	1.50	1.50	1.28	1.32
Toluene	2.82	2.92	2.31	2.34	2.03	2.02	1.74	1.71	1.48	1.49
Ethylbenzene	4.82	4.95	3.84	3.87	3.28	3.28	2.74	2.74	2.31	2.34

TABLE V  
EXPERIMENTAL VERSUS CALCULATED  $k'$  VALUES  
 $\Phi = 0.5$ .

Solutes	Temperature ( $^{\circ}\text{C}$ )									
	30		41		52		59		71	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
<i>p</i> -Nitrophenol	0.93	1.03	0.71	0.72	0.51	0.51	0.40	0.42	—	—
Phenol	0.85	0.85	0.70	0.71	0.60	0.60	0.55	0.54	—	—
Acetophenone	1.54	1.56	1.29	1.31	1.12	1.12	1.01	1.02	0.88	0.87
Methyl benzoate	3.19	3.15	2.58	2.58	2.14	2.14	1.92	1.91	—	—
Anisole	2.80	2.68	2.29	2.28	1.95	1.96	1.75	1.78	—	—
Benzene	2.91	2.90	2.41	2.43	2.06	2.06	1.87	1.86	1.57	1.58
Phenetole	5.14	5.11	4.14	4.15	3.44	3.42	3.04	3.05	—	—
Toluene	5.84	5.96	4.69	4.78	3.89	3.89	3.45	3.43	2.82	2.81
Ethylbenzene	11.24	11.60	8.78	8.96	7.03	7.04	5.99	6.09	—	—

## RESULTS AND DISCUSSIONS

The first consideration is how well the present approach predicts resolution and other separation parameters. The main question in this regard is the ability of eqns. 9, 10 and 10a to estimate values of  $k'$  as a function of  $\Phi$  and  $T$ . Table II provides derived values of  $\log k_s$ ,  $c$ ,  $d$  and  $e$  for 9 solutes, obtained from the experimental data of Tables III–V. Tables III–V compare experimental and calculated values of  $k'$  for 9 solutes over the range in conditions:  $29^{\circ}\text{C} \leq T \leq 71^{\circ}\text{C}$  and  $0.5 \leq \Phi \leq 0.7$ . For these substituted benzene derivatives, the 127 experimental  $k'$  values are predicted with an average variation of  $\pm 2\%$  (1 S.D.). In this case the reliability of eqns. 9–10a approaches the precision of the raw experimental data. We believe that similar accuracy of these relationships will be found for most other solutes in RP systems.

Another test of the present approach is provided in Table VI. Here we have selected two of the solutes of Tables III–V (benzene, methyl benzoate) and calculated  $R_s$ ,  $N$ , etc. by means of the present program. Comparison of the resulting values with experimentally determined data (Table VI) shows close agreement with all parameters. The actual chromatogram is shown in Fig. 1.

TABLE VI  
PREDICTED VERSUS EXPERIMENTAL SEPARATION OF METHYL BENZOATE-BENZENE;  $\Phi = 0.5$ ,  $T = 28^{\circ}\text{C}$

$$R_s = 2 \left( \frac{t_2 - t_1}{w_1 + w_2} \right)$$

	$t_s$	$R_s$	$k'_B$	$k'_{MB}$	$u$	$N$
Predicted	140	1.24	3.00	3.27	0.46	6140
Experimental	144	1.14	3.04	3.30	0.45	6314

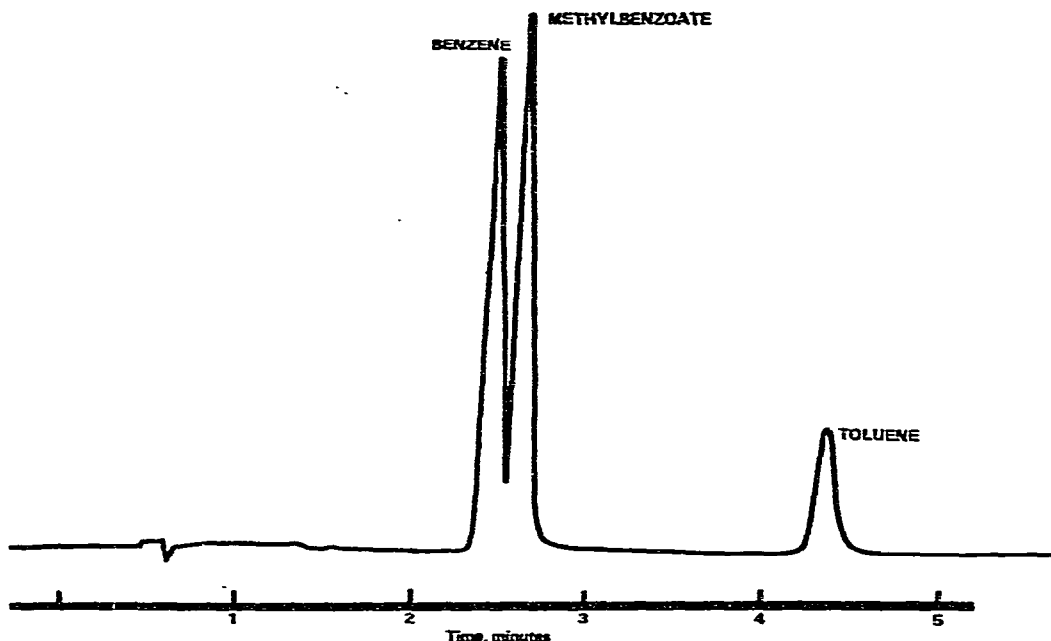


Fig. 1. Separation of methyl benzoate-benzene.  $C_8$  column,  $4.6 \times 150$  mm,  $d_p = 5 \mu\text{m}$ , methanol-water,  $\Phi = 0.5$ ,  $T = 28^\circ\text{C}$ ,  $u = 0.45$  cm/sec.

*Use of the present program (two adjacent bands)*

The application of the present approach is illustrated using data from Tables II-V, for the simplest problem of the separation of two compounds. This is pertinent for a sample containing only two difficultly separable solutes, but a similar approach can be adopted for more complex samples (see below). In essence, the present program allows the operator to adopt any optimization strategy he wants, but it eliminates the need for intermediate experimental work; *i.e.*, the calculator replaces the LC unit during optimization. Tables VII-IX provides an example, for several different solute

TABLE VII

SEPARATION AS A FUNCTION OF  $\Phi$  AND  $T$  AT NORMALIZED  $k'$  FOR THE SOLUTE PAIR BENZENE-TOLUENE

$\Phi$	$T$	$R_s$	$P$	$k'_1$	$\alpha$	$\nu$	$\eta$	$N$
$t_s = 100$ sec, $k'_2 = 3.01$ , $u = 0.60$ cm/sec								
0.64	11.5	7.61	5092	1.52	1.98	57.9	1.94	4446
0.60	27.4	8.05	3644	1.59	1.89	39.45	1.39	5570
0.55	47.0	8.62	2499	1.65	1.83	25.56	0.95	7025
0.50	67.0	9.23	1746	1.67	1.80	16.92	0.67	8554
$t_s = 300$ sec, $k'_2 = 3.01$ , $u = 0.20$ cm/sec								
0.64	11.5	10.24	1697	1.52	1.98	19.30	1.94	8053
0.60	27.4	10.53	1215	1.59	1.89	13.14	1.39	9525
0.55	47.0	10.86	833	1.65	1.83	8.52	0.95	11158
0.50	67.0	11.14	582	1.67	1.80	5.64	0.67	12467

TABLE VIII

SEPARATION AS A FUNCTION OF  $\Phi$  AND  $T$  AT NORMALIZED  $k'$  FOR THE SOLUTE PAIR METHYL BENZOATE-BENZENE $t_s = 100$  sec;  $k'_1 = 3.00$ ;  $u = 0.64$  cm/sec. Note:  $k'_1$  is normalized, not  $k'_2$ .

$\Phi$	$T$	$R_s$	$P$	$k'_1$	$k'$	$\alpha$	$\nu$	$\eta$	$N$
0.5	28	1.13	4002	3.00	3.27	1.09	46.28	1.43	5083
0.45	46.6	1.27	2726	3.00	3.26	1.09	29.88	0.98	6481
0.4	64.3	1.49	1957	3.00	3.28	1.09	20.46	0.70	7836
0.38	71	1.60	1743	3.00	3.30	1.10	17.9	0.62	8336

pairs. In Tables VII-IX the column was held fixed ( $L$ ,  $d_p$  and  $d_c$  do not vary), along with separation time  $t$  and  $k_{max}$ . That is, the separation was time-normalized and an "optimum" value of  $k' = 3$  was assumed. Since the present program does not input a value of  $k_{max}$  (although it could be rewritten to do so),  $k_{max}$  was maintained equal to 3.0 by varying  $T$  at each value of  $\Phi$  studied. For  $\Phi = 0.64$  in the initial "experiment" of Table VII, trial-and-error variation in  $T$  gave a value of  $T = 11.5^\circ\text{C}$  for  $k_{max} = 3.0$ . The value of  $\Phi$  was then lowered to 0.60 in Table VII, and a new value of  $T$  (for  $k_{max} = 3.0$ ) determined. The value of  $R_s$  was observed in each case for final values of  $\Phi$  and  $T$  ( $R_s = 7.61$  and  $8.05$ ). Since  $R_s$  was increased by decrease in  $\Phi$  (and increase in  $T$ ), the optimization process was continued to give  $R_s = 9.23$  for  $\Phi = 0.5$  and  $T = 67^\circ\text{C}$ . Higher column temperatures were considered impractical, but would have given larger values of  $R_s$ .

The next step in the optimization is to note that column pressure is only 1746 p.s.i. (for  $\Phi = 0.5$ ,  $T = 67^\circ\text{C}$ ), and it is assumed that the system allows 5000 p.s.i. Further optimization in  $R_s$  can therefore proceed by allowing  $k_{max}$  to increase. This is accomplished by decreasing  $\Phi$  and holding  $T$  constant. When this was pursued it was found that  $R_s$  improved to 11.7 at  $\Phi = 0.3$  ( $P = 5027$  p.s.i.,  $k'_{max} = 10.97$ ).

Alternatively, optimization could have been carried out by varying  $T$  at each value of  $\Phi$  to give some fixed (maximum) value of  $P$  (rather than fixed  $k_{max}$ ). This would have essentially yielded a similar set of final conditions for maximum  $R_s$ . During the optimization, other separation parameters can be checked. In some applications, maximum detection sensitivity for some minimum value of  $R_s$  may be desired. This can lead to a different set of final conditions, chosen as "optimum" for  $W_1$  and  $W_2$ , rather than for  $R_s$ .

TABLE IX

SEPARATION AS A FUNCTION OF  $\Phi$  AND  $T$  AT NORMALIZED  $k'$  FOR THE SOLUTE PAIR *p*-NITROPHENOL-PHENOL $t_s = 100$  sec;  $k'_2 = 3.01$ ;  $u = 0.60$  cm/sec.

$\Phi$	$T$	$R_s$	$P$	$k'_1$	$\alpha$	$\nu$	$\eta$	$N$
0.40	20	5.53	4506	1.90	1.58	51.72	1.72	4761
0.35	28	4.42	3694	2.15	1.40	41.56	1.41	5408
0.30	35.4	3.11	3059	2.42	1.24	33.82	1.17	6065
0.20	48.5	0.00	2148	3.00	1.00	23.10	0.82	7389
0.15	58	2.98	1702	2.52	1.19	17.91	0.65	8337
0.10	67	6.03	1351	2.12	1.42	13.94	0.51	9299

Where separation time is to be optimized (minimum  $t$ ), the input value of  $t$  can be successively reduced while  $R_s$  is maximized as a function of  $\Phi$  and  $T$  for each value of  $t$ . Or after optimum values of  $\Phi$  and  $T$  (for maximum  $R_s$ ) are obtained at one value of  $t$ ,  $t$  can be reduced until  $R_s$  reaches the minimum acceptable value. Column length  $L$  can also be varied in this process, for absolute minimization of  $t$ . Other examples of the use of this program will be obvious to the experienced worker.

*Use of the present program (more than two bands)*

Where three (or more) overlapping bands of interest are present in the sample, optimization can be obtained by mapping  $R_s$  values for the various band-pairs which have marginal resolution. While this is more cumbersome than for the case of a single band-pair (as above), in most cases, optimization by calculator proceeds more rapidly than experimental optimization. The present conceptual approach could also be extended to a larger computer program capable of simultaneously handling more than two sample bands. For more complex samples, however, a more practical approach may be the initial mapping of  $\alpha$  values for all solutes in the sample, as carried out by Laub and Purnell<sup>3</sup>. This allows the chromatographer to focus on regions of  $\Phi$  and  $T$  of major interest (maximum  $\alpha$  value for the most difficulty separable band-pair), following which further optimization can be pursued using the present program.

*Resolution as a function of  $\Phi$ ,  $T$  and normalized  $k_{max}$ .*

The present program allows additional insight into the various factors that determine resolution in a particular case (given pair of solutes, LC system, etc.). Thus, a particular parameter can be varied, and all the consequences of that variation are available from the computer program. We will explore this aspect further, using the data of Tables VII-IX.

In Tables VII-IX, values of  $R_s$ ,  $P$ , etc. are mapped for three representative solute pairs as a function of  $\Phi$  and  $T$ . In each case, column length was held fixed, along with separation time and  $k_{max}$ .

Tables VII-IX show data for the solute pairs benzene-toluene, methyl benzoate-benzene and *p*-nitrophenol-phenol. In each example, as  $\Phi$  is decreased and  $T$  is simultaneously increased to maintain normalized  $k'$ ,  $N$  increases and its contribution to  $R_s$  increases. Closer examination of the data reveals that this is simply a result of  $\nu$  approaching its optimum value, because  $\eta$  is decreasing. Note that this occurs at constant  $u$ . Furthermore, since  $\eta$  is decreasing, it follows that  $P$  must also decrease. For the solute pairs benzene-toluene and methyl benzoate-benzene,  $N$  has a dominant influence on  $R_s$ ; *i.e.*,  $R_s$  also increases with decreased  $\Phi$  and increased  $T$ . However, this is not the case with *p*-nitrophenol-phenol where the influence of  $\alpha$  dominates (see detailed discussion below).

At this point it is appropriate to subdivide the various contributions to  $R_s$  (terms i-iii of eqn. 1) to provide additional insight into the influence of  $\Phi$  and  $T$  on separation. The data of Tables VII-IX provide some pertinent examples. Fig. 2 further illustrates the effects shown in Tables VII-IX, in terms of percentage changes in  $R_s$  with change in  $\Phi$  and  $T$ . Corresponding changes in  $R_s$  arising from terms i-iii of eqn. 1 are also plotted in Fig. 2. The reference point for these plots (0% change) corresponds to the highest  $\Phi$  value in each case. The influence of the  $k'$  term (iii)

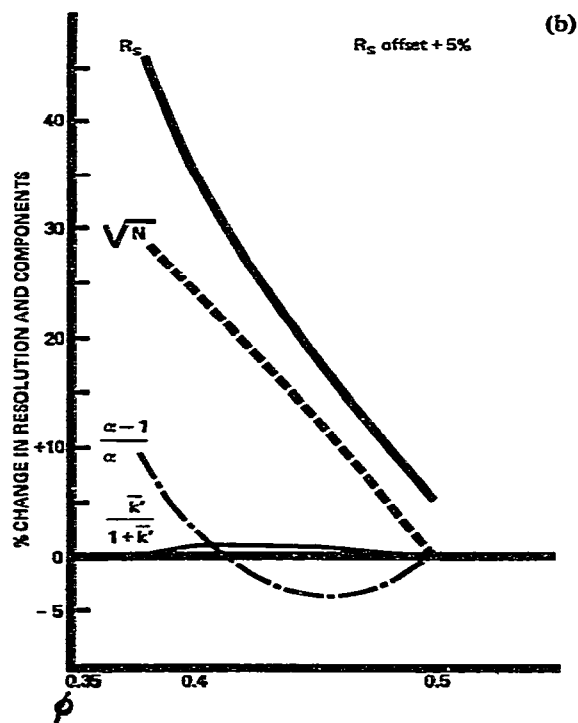
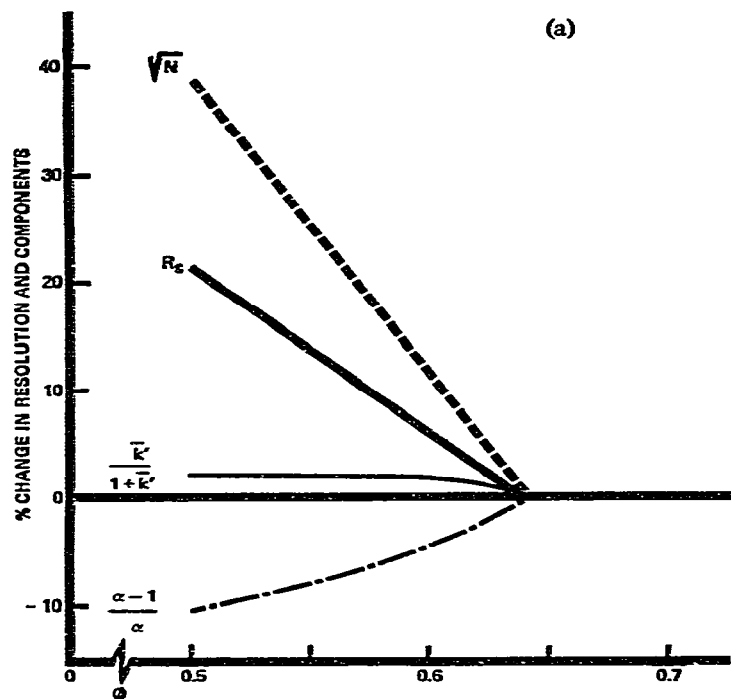


Fig. 2.

(Continued on p. 168).

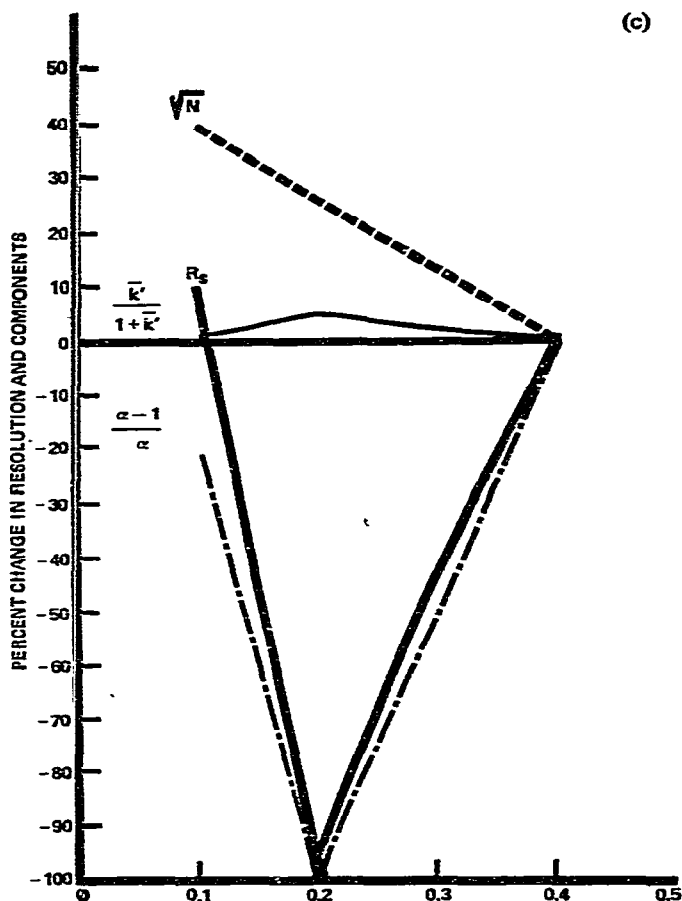


Fig. 2. Percent change in resolution with decreased  $\Phi$ . Temperature adjusted to normalize  $k'$ . Solute pair: (a) benzene-toluene. (b) methyl benzoate-benzene ( $R_s$ , offset by  $\pm 5\%$ ). (c) *p*-nitrophenol-phenol.

is roughly constant, because  $k_{\max}$  is held constant. The contribution of  $N$  is to increase  $R_s$  for larger values of  $T$ , as discussed below. In the case of the separation of benzene-toluene in Fig. 2a, the latter effect is sufficient to overcome a small decrease in  $\alpha$  as  $\Phi$  decreases. For the solute pair methyl benzoate-benzene (Fig. 2b), the  $\alpha$  contribution decreases slightly (approx. 3.5%) and then begins to increase as  $T$  increases. Again, the influence of  $N$  is sufficient to produce a net increase in  $R_s$  for the conditions studied. Notice however that the  $R_s$  line begins to diverge from the  $\sqrt{N}$  line when the  $\alpha$  contribution begins to increase.

The most dramatic example of the influence of  $\alpha$  on resolution is provided by the solute pair *p*-nitrophenol-phenol (Fig. 2c).

The  $k'$  and  $N$  terms contribute to  $R_s$  as in the two previous examples. However, the  $\alpha$  contribution is seen to rapidly decrease ( $\alpha \rightarrow 1.0$ ) as  $\Phi$  decreases ( $T$  increasing), and then increases with further decrease of  $\Phi$ . Resolution effectively follows  $\alpha$  in this



case, and the desired resolution can be achieved at two combinations of  $\Phi$ ,  $T$ . The combination lower  $\Phi$ , higher  $T$  is preferable since it results in a lower operating pressure ( $\eta$  is smaller).

From these examples, it is a simple matter to describe several general categories capable of defining  $R_s$  of solute pairs under conditions of  $k_{\max}$ . normalized (via adjustment of  $\Phi$ ,  $T$ ) and constant  $t_s$ .

In every case, the  $k'$  contribution is approximately constant as long as  $k'$  is not small, and the  $N$  contribution increases as long as  $v_{opt}$  is being approached.

*N dominates.* If changes in  $\alpha$  are not great, the  $R_s$  will obviously improve as long as  $v_{opt}$  is being approached. For this type of solute pair it is generally beneficial to operate at higher  $T$ , lower  $\Phi$ . Figs. 2a and b illustrate this case for both slight increase and slight decrease in  $\alpha$ .

*$\alpha$  dominates.* There are several possibilities when  $\alpha$  dominates  $R_s$  since we have seen that it can decrease, increase, or do both with increase in  $T$  under normalized conditions. If  $\alpha$  decreases (as  $\Phi$  decreases and  $T$  increases), then it is best to operate at the highest reasonable  $\Phi$  and lowest  $T$  to achieve separation. If  $\alpha$  increases, the opposite extreme is desirable. In both cases it is wise to check that the complete usable temperature range is explored to be sure that a crossover does not occur. In cases where crossover does occur (*i.e.*,  $\alpha = 1.0$ ), it may be possible to achieve the desired  $R_s$  at two sets of  $\Phi$ ,  $T$ . It seems generally advisable to operate at the lower  $\Phi$  (hence, higher  $T$ ), since operating pressure will be lower (as long as  $v$  is approaching its optimum value). Fig. 2c illustrates this situation.

#### *Generalizations regarding the dependence of $k'$ on temperature and mobile phase composition*

The variation of solute  $k'$  values with temperature and mobile phase composition  $\Phi$  is given by the coefficients  $c$ ,  $d$ , and  $e$  of eqns. 9 and 10. However, these coefficients are not totally independent. This can be seen in the tendency of temperature coefficients  $a$  (or transfer-enthalpies  $\Delta H$ ) to increase regularly with  $k_s$  for different solutes (and  $\Phi$  constant); *e.g.*, see discussion in ref. 6. Similarly, the coefficient  $c$  of eqn. 8 now appears to increase regularly with  $k_s$  in many RPLC systems, although this effect has not been obvious in some studies (*e.g.*, refs. 16, 20 and others). For example, the data of Tanaka and Thornton<sup>21</sup> are instructive in this regard. These workers obtained extensive data on  $k'$  vs.  $\Phi$  for 31 compounds of widely varied structure and  $k_s$  value. Derived values of  $c$  vs.  $k_s$  ( $T = 30^\circ\text{C}$ ,  $\Phi_s = 0.6$ ) are plotted in Fig. 3. A regular increase in  $c$  with increasing  $k_s$  is apparent, and is noted also in the present (and other) studies.

In fact, to a first approximation it seems that  $c$ ,  $d$  and  $e$  are all roughly predictable from values of  $k_s$  in a given RPLC system. However, this dependency or functional relationship may vary from one column type to another (the extent of surface coverage by bonding alkyl groups may be a factor), and for different classes of solutes. In the present  $\text{C}_8$  RPLC system we found that extrapolated values of  $a$  vs.  $\Phi$  converge to a value of 158 for  $\Phi = 1.0$ . That is, temperature coefficients were similar for all solutes studied in pure methanol as mobile phase. For a more water-rich mobile phase, more strongly retained solutes had larger  $a$  values for a given value of  $\Phi$  (as expected from ref. 6 and other studies). To summarize, it was possible to predict values of  $c$ ,  $d$  and  $e$  (present study) with reasonable precision, given

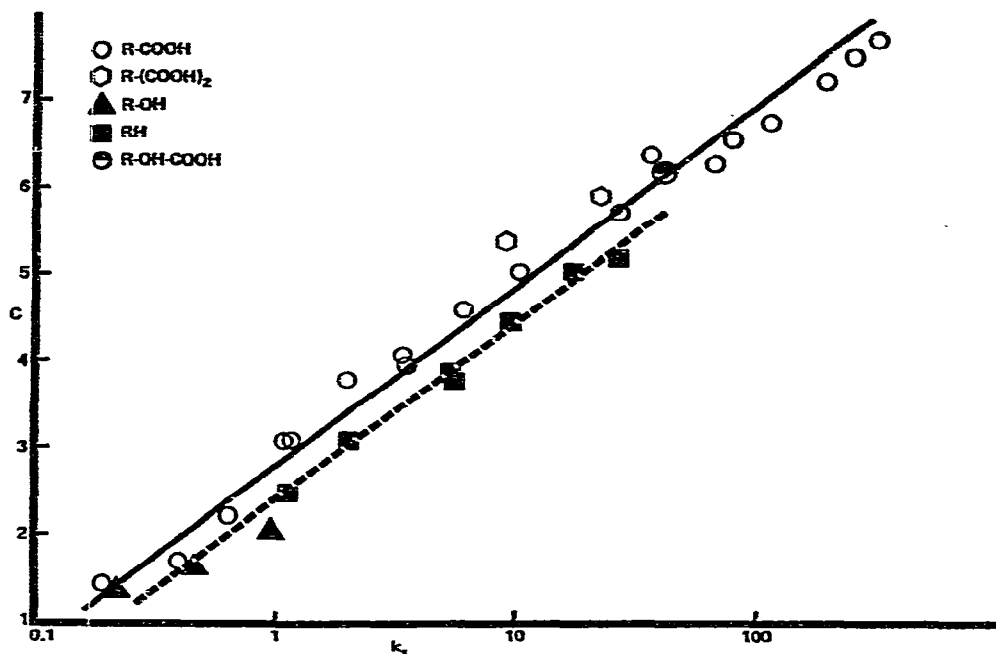


Fig. 3. Correlation of values of  $c$  (eqn. 8) with  $k_s$ . Methanol-water,  $C_{18}$  column, 30 °C. Values of  $k_s$  calculated for  $\Phi_s = 0.6$ ,  $T = 30$  °C. Data of ref. 21.

only a value of  $k_s$  for the compound. The derived equations for the present RPLC system were:

$$c = 2.3 + 1.3 \log k_s \quad (11)$$

$$e = 1450 + 1100 \log k_s \quad (11a)$$

$$d = 158 + e \quad (11b)$$

The ability of eqns. 11–11b to predict values of  $k'$  for the compounds of Table II is summarized in Tables X–XII. As expected, agreement between experimental and calculated values is poorer than using empirically derived values of  $c$ ,  $d$  and  $e$  (Tables III–V), but is still good ( $\pm 9.4\%$ ).

One practical consequence of the above correlations (eqns. 11–11b) is that  $\alpha$  values remain essentially constant for variation in  $T$  and  $\Phi$ , if  $k'$  is held constant during such change in separation conditions. This means (in the general case) that an increase in  $T$  (with decrease in  $\Phi$ ) will usually provide larger  $R_s$  values, due to the accompanying increase in  $N$ . Thus we can generally expect better resolution at higher  $T$  values in RPLC separation, although the preceding examples show that there are exceptions to this rule.

TABLE X

EXPERIMENTAL VERSUS CALCULATED  $k'$  VALUES; AVERAGE COEFFICIENTS (Eqns. 11-11b) $\Phi = 0.7$ .

Solutes	Temperature ( $^{\circ}\text{C}$ )									
	31		41		51		59.5		69.5	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
<i>p</i> -Nitrophenol	0.26	0.28	0.20	0.26	0.18	0.23	0.14	0.21	0.08	0.20
Phenol	0.26	0.31	0.24	0.28	0.22	0.25	0.21	0.23	0.18	0.21
Acetophenone	0.40	0.50	0.38	0.44	0.36	0.39	0.34	0.36	0.30	0.32
Methylbenzoate	0.70	0.85	0.63	0.73	0.59	0.64	0.52	0.57	0.47	0.51
Anisole	0.71	0.84	0.65	0.73	0.60	0.63	0.54	0.57	0.47	0.50
Benzene	0.77	0.92	0.72	0.79	0.65	0.69	0.61	0.62	0.54	0.54
Phenetole	1.07	1.31	0.96	1.12	0.87	0.96	0.77	0.85	0.68	0.74
Toluene	1.24	1.51	1.13	1.28	1.01	1.09	0.87	0.96	0.76	0.84
Ethylbenzene	1.92	2.36	1.68	1.97	1.47	1.66	1.30	1.44	1.13	1.24

Standard state,  $\Phi = 0.6$ ,  $T = 50.5^{\circ}\text{C}$ .

TABLE XI

EXPERIMENTAL VERSUS CALCULATED  $k'$  VALUES; AVERAGE COEFFICIENTS (Eqns. 11-11b) $\Phi = 0.6$ .

Solutes	Temperature ( $^{\circ}\text{C}$ )									
	29		41.5		50.5		61		70.5	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
<i>p</i> -Nitrophenol	0.57	0.45	0.42	0.38	0.34	0.34	0.27	0.30	0.22	0.27
Phenol	0.49	0.51	0.42	0.43	0.38	0.38	0.34	0.34	0.30	0.30
Acetophenone	0.79	0.88	0.69	0.72	0.63	0.63	0.57	0.56	0.50	0.48
Methylbenzoate	1.51	1.61	1.24	1.29	1.10	1.11	0.96	0.94	0.83	0.81
Anisole	—	—	1.23	1.28	1.09	1.10	0.95	0.93	0.82	0.86
Benzene	1.56	1.78	1.33	1.42	1.20	1.21	1.05	1.02	0.92	0.88
Phenetole	2.44	2.71	2.00	2.10	1.75	1.77	1.50	1.46	1.28	1.25
Toluene	2.82	3.19	2.31	2.45	2.03	2.05	1.74	1.69	1.48	1.43
Ethylbenzene	4.82	5.40	3.84	4.03	3.28	3.32	2.74	2.67	2.31	2.22

TABLE XII

EXPERIMENTAL VERSUS CALCULATED  $k'$  VALUES; AVERAGE COEFFICIENTS (Eqns. 11-11b) $\Phi = 0.5$ .

Solutes	Temperature ( $^{\circ}\text{C}$ )									
	30		41		52		59		71	
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.
<i>p</i> -Nitrophenol	0.93	0.68	0.71	0.58	0.51	0.49	0.40	0.45	—	—
Phenol	0.85	0.78	0.70	0.66	0.60	0.56	0.55	0.51	—	—
Acetophenone	1.54	1.47	1.29	1.20	1.12	0.99	1.01	0.88	0.88	0.73
Methylbenzoate	3.19	2.95	2.58	2.32	2.14	1.85	1.92	1.62	—	—
Anisole	2.80	2.91	2.29	2.30	1.95	1.84	1.75	1.60	—	—
Benzene	2.91	3.29	2.41	2.57	2.06	2.05	1.87	1.78	1.57	1.42
Phenetole	5.14	5.27	4.14	4.02	3.44	3.13	3.04	2.69	—	—
Toluene	5.84	6.34	4.69	4.79	3.89	3.69	3.45	3.16	2.82	2.45
Ethylbenzene	11.24	11.54	8.78	8.46	7.03	6.34	5.99	5.32	—	—

## CONCLUSIONS

The present study suggests a general approach to method development and optimization of separation in RPLC systems. A small number of initial experimental data are collected on the sample of interest, and solute parameters are derived to predict  $k'$  as a function of  $T$  and  $\Phi$ . These are then entered into a programmable calculator (present program) to allow the calculation of  $R_s$  (and other separation parameters) for any two solutes of interest. The calculator then replaces the LC unit in further optimization experiments (mapping of  $R_s$ , etc. as a function of experimental variables). This approach can greatly facilitate method development for certain demanding cases.

The present approach also provides insight into the role played by different separation variables on the overall separation. Particular separation parameters can be followed during the variation in separation conditions, to provide information that would not be directly assessable in an actual chromatogram (values of  $N$ ,  $\alpha$ , etc.). An examination of temperature effects in RP separations suggests that in most cases higher temperatures provide better separation. However, there are occasional exceptions to this rule. In any case, many of the generalizations previously offered on temperature effects in RP separations must either be qualified, or in some cases are actually misleading.

## SYMBOLS

$A, C$	coefficients in eqn. 3 (see ref. 12)
$a$	constant which is proportional to the enthalpy for transfer of solute from one chromatographic phase to the other (see eqn. 7)
$b, c$	coefficients in eqn. 8 (for temperature $T_s$ in later discussion)
$d, e$	coefficients in eqn. 9
$D_m$	solute diffusion coefficient as given by the Wilke-Chang equation (Eqn. 4) (see ref. 19)
$\overline{D_m}$	average diffusion coefficient for two solutes using eqn. 4 and an average value of $V_A$
$d_c$	column internal diameter (cm)
$d_p$	particle diameter (cm)
$H$	height equivalent to a theoretical plate
$h$	reduced plate height, eqn. 3a
$k'$	capacity factor (see ref. 1)
$\overline{k'}$	average of $k_1$ and $k_2$
$k_1, k_2$	$k'$ values for solutes 1 and 2
$k_a, k_b$	$k'$ values for a given solute at two temperatures, $T_a$ and $T_b$
$k_R$	$k'$ value at a reference temperature, $T_R$
$k_s$	$k'$ value at standard state defined in present study as $T_s = 51^\circ\text{C}$ and $\Phi_s = 0.00$
$k_{\Phi, s}$	value of $k'$ for some value of $\Phi$ (other than $\Phi_s$ ) and $T_s$
$k_{\max.}$	the larger of the two $k'$ values calculated for the solute pair of interest
$L$	column length (cm)
$M_B$	molecular weight of the mobile phase
$N$	number of theoretical plates (see ref. 1)
$P$	pressure drop across the column (eqn. 5)

$R_s$	resolution (see eqns. 1 and 2)
$T$	absolute temperature in °K (eqn. 4)
$T_a, T_b$	subscripts <i>a</i> and <i>b</i> used to distinguish between different temperatures
$T_R$	reference temperature
$T_s$	standard state temperature ( $T_s = 51^\circ\text{C}$ in present study)
$t_1, t_2$	retention times of bands 1 and 2 (eqn. 2)
$t_s$	separation time where $t_s = t_2$
$t_o$	column void time
$u$	linear velocity (cm/sec)
$V_A$	molar volume of solute A
$V_1, V_2$	$V_A$ values for solutes 1 and 2
$w_1, w_2$	baseline width of bands 1 and 2
$\alpha$	selectivity factor (see ref. 1)
$\Phi$	volume fraction organic solvent in water-organic mixture (methanol in present study)
$\Phi_s$	standard-state volume-fraction organic, $\Phi_s = 0.60$ in present study
$\Phi^*$	empirical column permeability. $\Phi^* = 500$ for well-packed columns
$\psi_B$	association factor in eqn. 4
$\eta$	mobile phase viscosity (cP)
$\eta_{40}$	mobile phase viscosity at $40^\circ\text{C}$
$\nu$	reduced velocity (eqn. 36)
$\nu_{opt}$	optimum reduced velocity (see ref. 1)

## APPENDIX

The program used in this study is listed in Table A-I. Having loaded this program into the TI 59 calculator, parameters describing the chromatographic system of interest are entered into the appropriate storage registers (see "Inputs", Table A-II). Systematic optimization can now be accomplished by varying the appropriate parameters as detailed above. Outputs of interest are found by simply recalling the appropriate storage register (Table A-II, "Outputs").

TABLE A-I  
PROGRAM

The program should be read in the order A, B, C, etc.

A	B	C
LRN	STO 16	( $\log k'_{51}$ ) +
0 X $\leftarrow$ t	RCL 07	273.2
RCL 05	-	=
-	RCL 08	STO 18 (T °K)
RCL 00	X	1/X
X	RCL 00	+/-
RCL 06	=	+
=	STO 17	( $a_1$ ) 1/X
	RCL 01	

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TABLE A-I (continued)

D	E	F
0.0030845	$10^x$	2.32
=	STO 20	$(k'_2)$
X	-	+/-
RCL 17	RCL 19	+
+/-	=	2.1
+	$x \geq t$ 127	X
RCL 16	RCL 20	
=	STO 21	
$10^x$	RCL 19	RCL 00
STO 19	$(k'_1)$ STO 20	
RCL 09	RCL 21	X
-	STO 19	=
RCL 00	=	STR 23 $(v_{40})$
X	=	log X
RCL 10	=	STO 24 $(\log v_{40})$
=	RCL 00	RCL 23
STO 16	+	X
RCL 11	0.64	200
-	+	+
RCL 12	1	650
X	=	=
RCL 00	1/X	STO 25
= 0	X	RCL 18
STO 17	$(a_2)$ RCL 03	1/X
RCL 18	=	-
RCL 20	STO 21	$(t_0)$ 0.003193
+/-	RCL 02	=
-	+	X
0.0030845	RCL 21	RCL 25
X	=	+
RCL 17	STO 22	$(u)$ RCL 24
+/-	RCL 00	=
+	$x^2$	$10^x$
RCL 16	X	

TABLE A-I (continued)

<i>G</i>		<i>H</i>		<i>I</i>
STO 27	(η)	RCL 22		1
RCL 28		X		=
X		RCL 04		X
0.000006074		÷		RCL 21
=		RCL 29		=
STO 29		=		STO 33
14		STO 30	(o)	RCL 20
+		RCL 30		+
46.8		$y^x$		1
=		0.33		=
$\sqrt{x}$		=		X
STO 28		+		RCL 21
RCL 13		2		=
+		÷		STO 34
RCL 14		RCL 30		RCL 33
=		+		=
÷		0.05		X
2		X		2
=		RCL 30		÷
$y^x$		=		RCL 37
9.6		RCL 32		=
=		$\sqrt{x}$		=
1/x		1/x		STO 35
÷		X		RCL 32
RCL 27		RCL 33		$\sqrt{x}$
X		X		1/x
RCL 18		4		X
X		X		RCL 34
STO 31	(h)	RCL 02		X
X		=		4
RCL 04		STO 32	(N)	=
=		RCL 19		STO 36
1/x		+		RCL 35

(Continued on p. 176)

TABLE A-I (continued)

J		K	L
+		X	$1.455 \times 10^{-7}$
RCL 36		RCL 27	=
=		X	STO 39 (P)
STO 37		RCL 02	Pause
RCL 34		+	RCL 38
-		RCL 04	R/S
STO 38	(R <sub>S</sub> )	+	LRN
RCL 22		RCL 04	
X		X	
RCL 15			

TABLE A-II

## LOCATION OF CHROMATOGRAPHIC PARAMETERS IN RESOLUTION PROGRAM

	Parameter	Storage register
Inputs	$\Phi$	00
	T	01
	L	02
	$t_2$	03
	$d_p$	04
	$b_1$	05
	$c_1$	06
	$d_1$	07
	$e_1$	08
	$b_2$	09
	$c_2$	10
	$d_2$	11
	$e_2$	12
	$V_1$	13
	$V_2$	14
$\Phi^*$	15	
Outputs	$K'_1$	19
	$K'_2$	20
	$u$	22
	$\eta$	27
	$v$	30
	N	32
	P	39

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