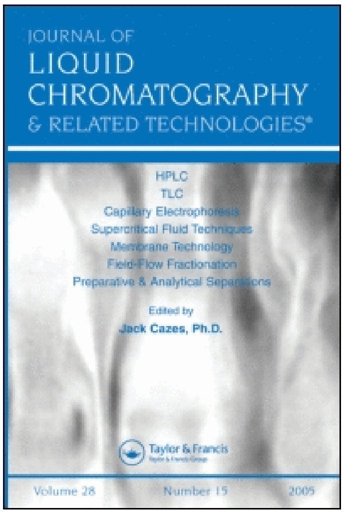

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SOLUTE RETENTION IN COLUMN LIQUID CHROMATOGRAPHY. II. OPTIMIZATION OF MOBILE-PHASE COMPOSITIONS

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ABSTRACT

Retentions are reported for nine phenol solutes with a reverse-phase column packing and acidified aqueous mobile phases containing tetrahydrofuran, acetonitrile, and methanol additives. Plots of $\log(k'_{(M)})$ against additive volume fraction ϕ exhibit both positive and negative deviations from linearity. In contrast, plots of inverse capacity factor against ϕ are in every instance convex to the abscissa. The latter, as well as several systems comprising ionic solutes and/or solvents, are fitted exactly by a semi-empirical relation; however, only one of the fitted parameters appears to exhibit identifiable trends and then only very approximately so. It is deduced by simple inspection of the plots and without resorting to higher-component carriers, then verified experimentally, that the test mixture is completely separable with 30% methanol. Window diagrams of α, S_f, and R_s ordinates are used to identify alternative mobile phases and compositions in the instance that one of the solutes is present in large excess. The three methods of data reduction prove to be entirely equivalent since the solute capacity factors approach 10. When this is not the case, window diagrams constructed with the separation-factor parameter are said to be favored.

INTRODUCTION

The analytical utility of mixed phases is firmly established at the present time both in gas chromatography (gc) and in variants of liquid

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chromatography (lc). Moreover, much of the sucess of resultant systems optimization strategies (1) is due directly to the formulation of functions which describe quantitatively, that is, within reasonable limits of experiment, the variation of solute retentions with composition of the respective phase. For example, it has been argued on various occasions (see preceding paper) that inverse retentions vary in a well-defined fashion with concentration (in whatever units) of one or another of the mobile-phase components in lc, while many have shown that with few exceptions, solute specific retention volumes, capacity factors, and partitition coefficients are additive in stationary-phase weight- or volume-fraction compositional units in gc. Indeed, novel interpretations inter alia of solutions thermodynamics notwithstanding, the preponderance of reputable experimental evidence derived to date points strongly in the direction of relations appropriate for lc which are in fact intimately related to (if not directly based upon) those pertinent to gc (2).

Upon selection of the appropriate description of partitioning, the only valid criterion for which self-evidently being faithful representation of the experimental data, optimization of the relevant system parameter(s) in terms of maximal separation in minimal time of the solutes at hand is straight-forward. Since the initial report of the data-reduction procedure in 1975 by Laub and Purnell (christened by them the window-diagram strategy) (3), a number of variants of the optimization technique has been detailed. For example, Deming and Turoff (4) utilized the methodology for optimization of mobile-phase pH for the lc separation of benzoic acids, while Sachok, Kong, and Deming (5) later extended its use to include multiple and interdependent system variables. The strategy has also been employed in mobile-phase gradient elution (6,7), the most promising approach being that taken by Edwards and Wellington (8).

Recently, Glajch, Lehrer, and colleagues (9) and Issaq and coworkers (10) have shown that substitution of a triangular coordinate system for one of three-dimensional Cartesian coordinates also provides the means of optimization (window diagrams) of ternary- (or higher-) component lc mobile phases. However, since the results from one coordinate system self-evidently must be identical to those derived from the other, there appears to be little from which to choose with regard to each other than on the basis of convenience of presentation.

The separation factor S_f derived by Jones and Wellington (11) appears to offer considerable advantage when used as the (dependent) ordinate parameter for representation of separations in lc, where $S_f = 2R_s/N^{1/2} = (t_{R2} - t_{R1})/(t_{R2} + t_{R1})$. Thus, measurement is no longer required of column void volumes, which frequently proves to be troublesome (12), nor of peak baseline-widths, as required for utilization of R_s.

Nevertheless, there remains some controversy with regard to each of these aspects of liquid chromatography. We have therefore examined in this work, first, appropriate relations for graphical representation of retentions in terms of mobile-phase compositions and, secondly, the accuracy of the resultant window diagrams constructed with values of relative retentions α, separation factors S_f, and resolution R_s.

The analysis of phenols with reverse-phase packings was chosen as the test system, in part because of the report by Lehrer (9) where it was claimed that a quaternary-component solvent was required for resolution of a few simple phenolic compounds, and in part because the separation of these species continues to be of considerable interest in a variety of fields. For example, phenols are known to be present in hardwood smoke used in curing meat and fish products which incorporate the compounds directly. Thus, heavily-smoked bacon has been shown to contain up to 250 ppm of phenols (13). It has also been reported that they can inhibit (14,15) or accelerate (16-18) the formation of carcinogenic nitrosamines depending upon the reaction conditions.

Chlorinated phenols are of course also of intense interest at the present time, these having been used extensively over the years as insecticides, fungicides, antiseptics, and disinfectants. Moreover, effluents from pulp and paper industries are known (19) to contain derivatives of guaiacol, catechol, and trihydroxybenzes (20-22). However, difficulties are often encountered in the separation of these and related materials due to the variety of positional isomers. We therefore intend in this work to provide also at least a basis for choice of systems and conditions pertinent to their analysis.

THEORY

Retention and selectivity with "reverse-phase" systems are taken generally to be more complex than with "normal" (i.e., underivatized) sorbents. In point of fact in either case, the actual retention mechanism(s) remain largely undefined. Nevertheless, there is today agreement, broadly speaking, that retention and selectivity are related in the former mode of lc to carbon chain length (23-27), surface coverage (carbon content) (25-31), eluent composition and type (23,26,27,31-38), solute "polarity" (31,39), and column temperature (32-34,40). In particular, equations of the general form:

$$\log k'_{(M)} = \log k'_{(S)} - S\,\phi_A \qquad (1)$$

have been observed by a number of workers (31,36,39-51) to describe retentions with specifically-defined reverse-phase systems comprising aqueous mobile phases with organic additives, where $k'_{(S)}$ is the solute capacity factor with pure water eluent, ϕ_A is the volume fraction of organic modifier, and S is an empirically-derived constant for the system at hand. For a given column and modifier, S appears to be approximately constant for solutes of varying molecular size and structure. However, it is found to vary somewhat from one additive to the next as well as with the type of reverse-phase sorbent (e.g., different chain length of surface-attached organic species). Schoenmaker and his coworkers (41) have also noted that eqn. 1 is not strictly obeyed by the systems studied by them, where their plots of log $k'_{(M)}$ against ϕ_A were slightly concave to the abscissa. Further work by others has since verified the approximate nature of the relation (52).

A model purporting to account for interactions between the analyte and each phase has alternatively been proposed wherein the solute inverse capacity factor is said to be related to mobile-phase composition in the manner (30,53-56; see also preceding paper):

$$\frac{1}{k'_{(M)}} = \frac{\phi_A}{k'_{(A)}} + \frac{\phi_S}{k'_{(S)}} \qquad (2)$$

where the subscripts A and S pertain to a pure mobile phase. Thus, eqn. 2 predicts that $1/k'_{(M)}$ will vary linearly with ϕ_A (= 1 - ϕ_S), which is frequently borne out in practice. However, the relation is by no means universally applicable. As a result of study of very many systems that in fact give plots of $1/k'_{(M)}$ which are curved, Madden, McCann, Purnell, and Wellington (57,58) have recently proposed a modification of eqn. 2 that applies also to the "normal" mode of lc:

$$\frac{1}{k'_{(M)}} = \phi_A \left\{ \frac{1}{k'_{(A)}} + \frac{b\ \phi_S}{1 + b'\ \phi_S} \right\} + \frac{\phi_S}{k'_{(S)}} \qquad (3)$$

The equation has in all cases studied thus far been shown to account for the (at-times sharp) curvature found with many systems at either end of the mobile-phase volume-fraction range as well as those which show curvature over the entire compositional span. However, the values of b and b' must at the present time be deduced from experimental data. Moreover, there has yet to be provided a rationalization of these in terms of properties either of the solutes or solvents. Thus, this work is intended as well not only to explore the applicability of eqns. 2 and 3 but in addition, the derivation and interpretation in particular of values of b and b' in terms of compound structure, elution order, and composition and type of mobile-phase additive.

EXPERIMENTAL

Solvents. Spectrophotometric-grade methanol (MeOH) and acetonitrile (ACN) mobile-phase components (A) were employed as received, while reagent-grade tetrahydrofuran (THF) (A) was distilled prior to use. Demineralized and doubly-distilled water + reagent-grade acetic acid comprised the solvent component (S).

Mobile-Phase Preparation. Acetic acid at the level of 1% v/v was added to the water component of the mobile phase in order to diminish any interactions between residual unreacted or accessible silanol groups of the reverse-phase packing as well of course to supress dissociation of the phenols, and did indeed sharply reduce peak asymmetry. Solutions of the aqueous blend with organic modifier were then prepared by volume over the additive concentration range of 25-45% (MeOH) or 20-35% (ACN and THF) at intervals in each case of 5%.

TABLE 1

Test-Solute Properties[a]

No.	Name	M/daltons	m.p./°C	b.p./°C	pK_a (25°C)
1	Phenol	94.11	41.8	182.	9.99
2	p-Nitrophenol	139.11	114.	-	7.16
3	2,4-Dinitrophenol	184.11	114.	-	4.09
4	o-Chlorophenol	128.56	7.	175.6	8.48
5	o-Nitrophenol	139.11	45.	216.	7.21
6	2,4-Dimethylphenol	122.17	27.8	211.5	10.45
7	4,6-Dinitro-o-cresol	198.06	86.5	-	-
8	4-Chloro-m-cresol	142.52	66.	235.	-
9	2,4-Dichlorophenol	163.00	45.	210.	7.51

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[a] **CRC Handbook of Tables for Organic Compound Identification,** Third Ed., Rappoport, Z., Ed., CRC Press, Boca Raton, Florida, 1979.

Solutes. Solutions of phenols in methanol comprised the test samples, these being phenol (1), p-nitrophenol (2), 2,4-dinitrophenol (3), o-chlorophenol (4), o-nitrophenol (5), 2,4-dimethylphenol (6), 2,4-dinitro-o-cresol (7), 4-chloro-m-cresol (8), and 2,4-dichlorophenol (9). Their properties are provided below in Table 1.

Instrumentation. The liquid chromatograph employed in this work was a Varian Model 5000 with an LDC UV Monitor or Vari-Chrom variable-wavelength UV detector, each being utilized at 254 nm. The injector was a Valco valve fitted with a 10-ul external loop, while the column was 25 cm by 4.5-mm id of 8 um Zorbax C_8. Both the injector and column were immersed in a water-bath, the temperature of which was maintained constant at 50°C. The mobile phase was also brought to this temperature prior to entering the column.

Procedures. The column was allowed to equilibrate with each new mobile phase under constant flow until the solute retention times remained constant. Dead volumes, which were found to vary with mobile-phase composition (see below), were then calculated from the flow rate and the retention time of the methanol solvent peak.

RESULTS AND DISCUSSION

Column Dead Volume. The values of V_A found in the present work are provided in Table 2, the smallest being that with 40:60 (v/v) THF and the largest with 25:75 MeOH. In general, depending upon the presumed-unretained marker compound employed, the apparent void volume of the column is expected to vary as a function of surface coverage of the mobile-phase additive, as pointed out inter alia by Boehm and Martire (2) and by Billiet, Van Dalen, Schoenmaker, and De Galan (59). That is, a void volume calculated from chromatographic measurements may well be larger than the true value, the excess comprising the sum of contributions arising from interactions with sorbed mobile-phase additive as well as those with underivatized surface silanol groups and the chemically-bound alkyl groups. Nor, clearly, would isotope marking, as in the work of McCormick and Karger (60), speciate the individual contributions (61).

Presumably, retentions for methanol would be largest in those instances, first, where the surface is least covered by additive, i.e., available for sorption and, secondly, when interactions with sorbed additive are most favored. In general, surface coverage of hydrocarbon reverse-phase packings would be expected to be smallest with MeOH, increased with ACN, and highest with THF. The trends followed in Table 2 appear to bear out this notion; thus, at 25% mobile-phase composition, V_A is largest for MeOH (smallest surface coverage; most-favorable interaction with injected MeOH), decreases with ACN, and is lowest for THF (highest surface coverage; least-favored interaction with injected MeOH). In addition, and without exception, V_A decreases upon increasing concentration (surface coverage) of additive irrespective of its identity.

From the standpoint of analysis, the spread on V_A in Table 2 (ca. 8% of the average of the extremes, 2.82 cm^3) emphasizes the utility of separation factors rather than values of alpha in calculating window diagrams, since the dead volume need not be determined with the former.

TABLE 2

Apparent[a] Column Dead Volumes V_A/cm^3

Composition, % v/v	Organic Additive MeOH	 ACN	 THF
20		2.94	2.91
25	3.03	2.88	2.76
30	2.94	2.84	2.68
35	2.91	2.78	2.64
40	2.91	2.72	2.61
45	2.91		

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[a] MeOH solvent peak; flow rate 3 cm^3 min^{-1}

The same holds true also in calculating the resolution, where $R_s = (t_{R2} - t_{R1})/w_m$ and where w_m is the peak mean baseline width. That is, the apparent dead volume (or time) does not enter into the calculations. Nor is the matter a trivial one since the apparent capacity factors of the phenol solutes (see later) are in many instances less than 2.

Graphical Representation of Retention Data. Plots of $1/k'_{(M)}$ against % v/v mobile-phase additive are shown for all systems studied in Figures 1-3. All are clearly convex to the abscissa to varying degrees, and eqn. 2 must therefore be said not to hold in these instances. We also show plots of $\log(k'_{(M)})$ against ϕ_A for the phenol solutes with THF additive in Figure 4. Both convex and concave curves as well as straight lines were found and, in addition, solute no. 5 appeared to pass through a point of inflection. The log plots for all solutes with MeOH and ACN gave, respectively, concave and convex curves. Hence, eqn. 1, too, is not obeyed for the systems studied here. Nor can the curves be ascribed to inaccuracies in the dead-volume determinations, since the apparent capacity factors e.g. for solutes 7-9 were often in excess of 20. In order to force conformity of the data either to eqns. 1 or 2, we would therefore have to assume that the dead volume pertinent to these solutes varied with mobile-phase composition from 0 to in excess of 10 cm^3. This seems unlikely, since the total volume of the empty column tube was only 3.9 cm^3 (61).

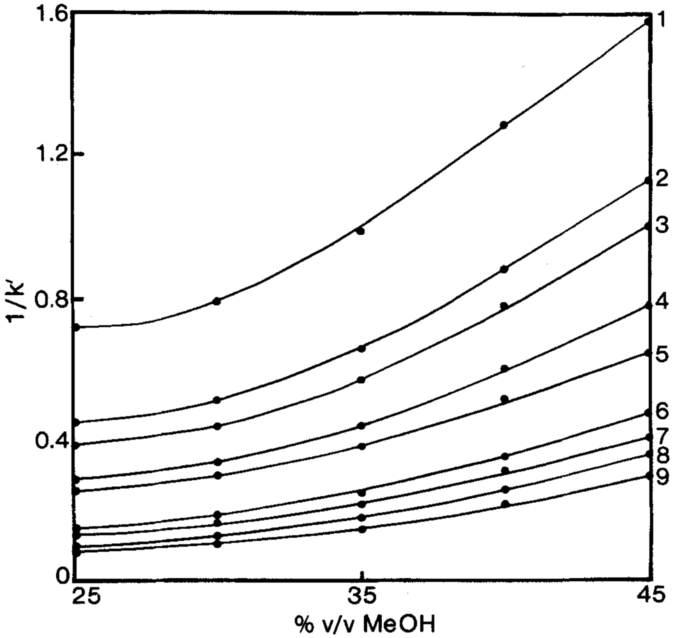

FIGURE 1

Plots of inverse capacity factors of solutes of Table 1 against volume percent MeOH additive. Curves constructed with fitted parameters of eqn. 3 (cf. Table 3).

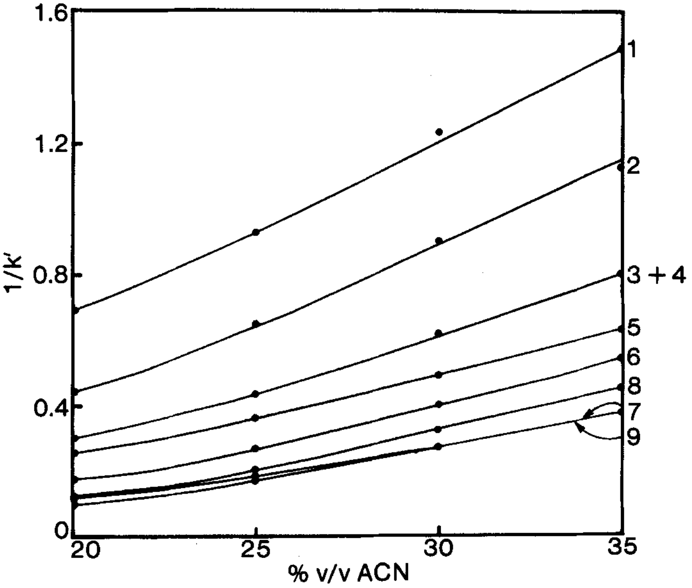

FIGURE 2. As in Figure 1; acetonitrile additive.

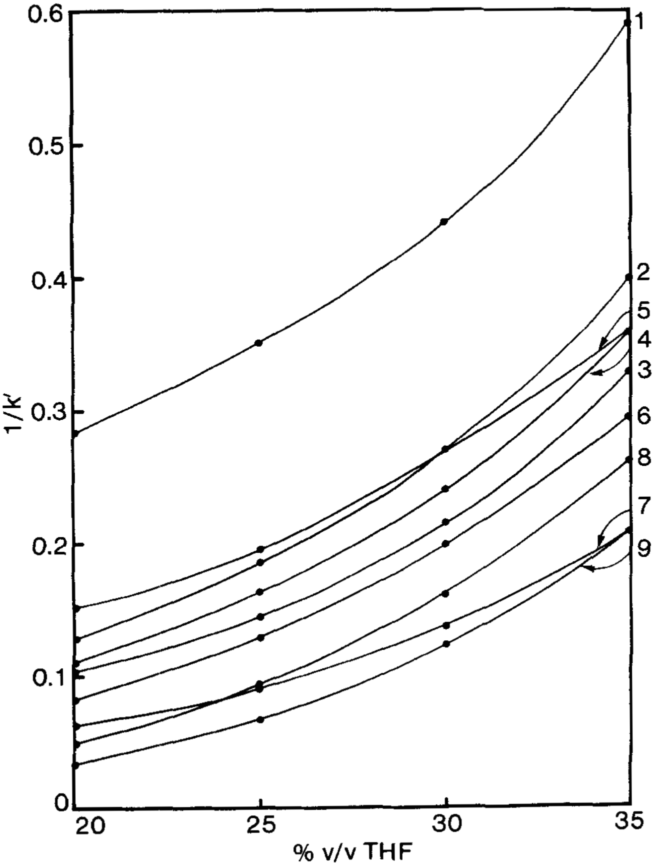

FIGURE 3. As in Figure 1; tetrahydrofuran additive.

The data of Figures 1-3 (solid curves in each) were found to be fitted exactly by the semi-empirical relation eqn. 3, the values of b and b' being presented in Table 3. However, there is no immediately-apparent correlation of these either with solute molecular weight or with pK_a (cf. Table 1). Nevertheless, and while b' appears to vary randomly, the values of b, excluding phenol solute and in contrast to those presented in the preceding paper are, very roughly, constant from one solvent system to the next. In addition, the **averaged** values are nearly identical with MeOH and ACN (ca. -0.3), but are diminished by a factor of 3 with THF (ca. -0.1). This seems

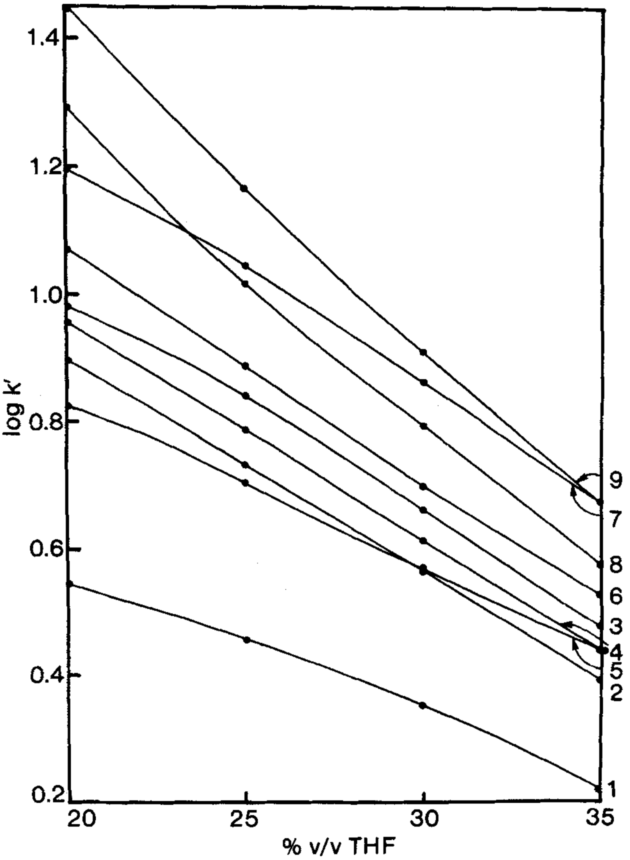

FIGURE 4

Plots of $\log(k'_{(M)})$ against volume percent THF additive for solutes of Table 1.

to suggest that b reflects, at least to some extent, the degree to which solutes interact with additive sorbed on the surface of the stationary phase (62). For example, the values of pK_a for p- and o-nitrophenol are virtually identical yet the latter is eluted much more quickly than the former with mobile phases containing either MeOH or ACN. On the other hand, the solutes nearly coelute with THF additive. Presumably, intramolecular hydrogen bonding with the latter solute diminishes its interactions with sorbed MeOH and ACN but, since hydrogen bonding with THF is small to

TABLE 3

Fitted Parameters of Eqn. 3

Solute No.	Organic Additive					
	MeOH		ACN		THF	
	-b	b'	-b	b'	-b	b'
1	1.23	2.06	0.325	6.27	0.135	-0.644
2	0.650	0.410	0.338	4.33	0.124	-0.522
3	0.554	0.395	0.229	1.89	0.142	-0.338
4	0.410	0.123	0.229	1.89	0.117	-0.426
5	0.298	0.215	0.376	11.6	0.122	-0.379
6	0.275	0.268	0.395	6.18	0.098	-0.239
7	0.219	0.115	0.109	1.50	0.092	-0.241
8	0.197	-0.156	0.275	3.22	0.113	-0.235
9	0.184	-0.146	0.345	8.19	0.099	-0.354

begin with, the compounds are eluted together with this carrier modifier. However, we can infer little else from the data at this time, and the situation thus remains as one which invites further and comprehensive study.

Separation of the Test Mixture. The retention orders of the phenols are altered in several instances on passing from one organic modifier to another. For example, solutes 3 and 4 are well separated with MeOH additive (Figure 1), overlap completely with ACN (Figure 2), and elute with order reversal with THF (Figure 3). However, **by inspection,** and after having examined only three solvent systems (utilizing only four or five compositions of each), we see immediately that the **binary** MeOH system offers the best separation in the fastest time. With the flow rate fixed at 3 cm^3 min^{-1} (in order to compare the results with those of ref. 9), and utilizing Purnell's relation (63) for the number of plates N_{req} requisite for a particular separation at a given a and capacity factor, we deduce further that a methanol concentration of 30% should provide baseline resolution of all solutes with the order of elution being compounds 1 through 9 in numerical sequence. The **first-time** chromatogram is shown in Figure 5 and corresponds very nearly exactly to that predicted (see also ref. 10).

Alternative Mobile-Phase Combinations. Selection of a mobile phase for separation of simple mixtures of solutes, such as the test sample

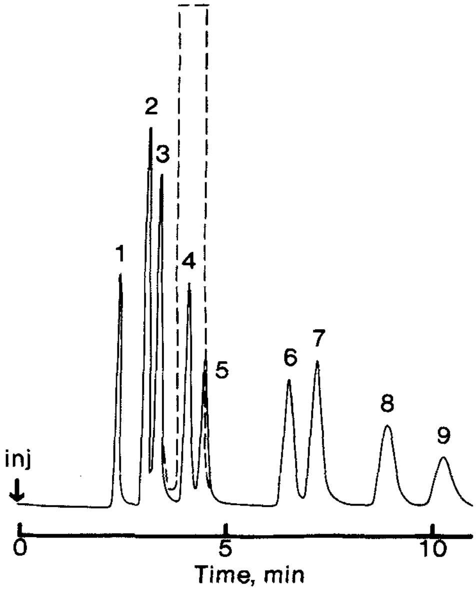

FIGURE 5

First-time chromatogram of the solute test mixture with mobile phase containing 30% MeOH additive. Dashed curve corresponds to hypothetical large amount of solute no. 4.

utilized above, generally presents no real difficulties. More often than not an analyst has simply to plot the retentions in accordance with some or other preferred method of data reduction (e.g., eqns. 1-3) and deduce the optimal mobile-phase composition by inspection. However, only rarely are such mixture types encountered in practice. Those confronted most frequently generally comprise some solutes (often of unknown identity) that are present in overwhelming quantity (hence masking the compounds of interest); a natural-products matrix (or matrices) superimposed on the chromatogram; peak distortion due to this or that column/mobile-phase/stationary-phase effect(s) or artifact(s); and so on. Thus, for purposes of quantitation, preparative collection, spectrometric identification, etc., it is often found in practice that a particular separation must be adjusted such that the compound(s) of interest is(are) eluted well away from others. For these reasons, it may well be that the optimum **practical** mobile-phase composition is much different from that with which all solutes could be baseline-resolved when present in equal amounts.

For example, suppose that solute no. 4 (dashed curve) were in great excess over no. 5 in Figure 5, and that quantitative data were required for the latter. Because of the overlap, quantitation would be rendered less accurate than if the two compounds were present, as in the solid curves, in nearly identical amount. Other mobile phases with which the solute elution order can be changed must therefore be considered.

Reference back to Figure 2 shows, as mentioned earlier, that solutes 3 and 4 coelute at all compositions of ACN considered and so, this mobile-phase additive is not useful in terms of the analysis desired. In contrast, Figure 3 shows that, with small amounts of THF additive (left-hand ordinate), solute no. 5 will elute second (after phenol), followed by 2 and then by 4. Moreover, solutes 4 and 3 approach coelution at 20% THF; 5 and 2 overlap at ca. 30%; while 2 and 4 are not resolved at 35%. Thus, there are substantial shifts in elution order with this mobile-phase modifier, and it is therefore ideally suited for adjustment of the separation in the desired manner.

The resultant window diagrams formulated in terms of α, S_f, and R_s were superimposable in shape and are shown in Figure 6 composited with the relevant ordinate values. The most favorable window is that occurring at 22% THF, with the solute pair 4/3 being the most difficult to resolve at this mobile-phase composition. The most-difficult alpha is 1.095 which, since the capacity factors are ca. 10, requires 4800 theoretical plates for complete separation. Further, the order of solute elution is predicted from Figure 3 to be 1, 5, 2, 4, 3, 6, 7, 8, and 9.

The **first-time** chromatogram is shown in Figure 7, where resolution has been achieved precisely as predicted. In addition, separation of the major component, solute 4 (dashed curve), is more than adequate for quantitation of it as well as its nearest neighbor, no. 3. Solute 5 (that of main interest from the standpoint of this example) elutes well away from 1 and is also baseline-resolved from 2. Thus, the overall optimum **practical** mobile-phase additive and concentration turn out to be much different from those utilized to produce Figure 5.

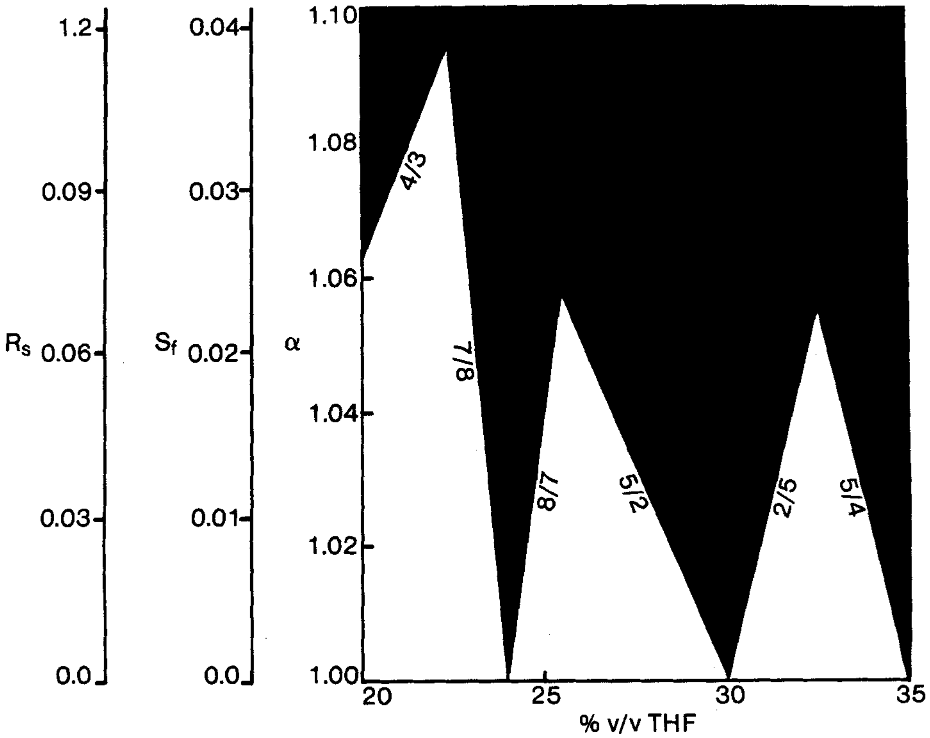

FIGURE 6

Window diagram (1) for test solutes with mobile phase containing THF additive. Ordinates: resolution R_s; separation factor S_f; and relative retention α. Optimum concentration of THF is predicted to be 22% v/v.

Window-Diagram Ordinate Parameters. The equivalence of the three versions of window diagram appears to confirm that the values taken for the system dead volume were not seriously in error since, if that were the case, the alpha plot would have been substantially different from that utilizing S_f. In fact, the various forms of window diagrams must approach coincidence when the solute capacity factors are on the order of 10 or more. However, when that is not the case, there may arise substantial differences in the optimum mobile-phase compositions deduced from each. The separation-factor parameter S_f should then be employed. Plots utilizing an ordinate of R_s would also be correct in such instances, although

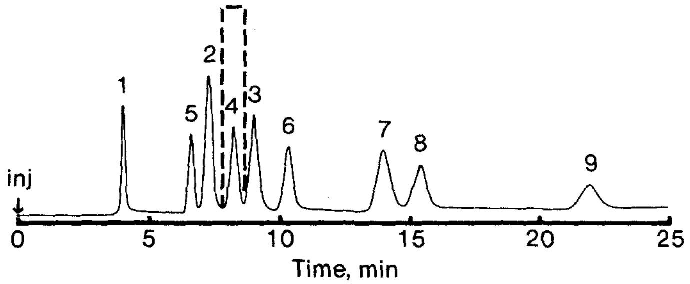

FIGURE 7

First-time chromatogram of the the solute test mixture with 22% THF additive, the optimum mobile-phase composition deduced from Figure 6.

there is some inconvenience in having to measure peak baseline widths in addition to retention times with this method. (There may also be introduced some uncertainty if the pure components or some other suitable reference materials are not available for separate evaluation of w_m.) In any event in this case, where the capacity factors of the most-difficult solutes approach 10, the three versions of window diagrams prove to be entirely equivalent.

Generality of Eqn. 3. It has been established at this point that eqn. 3 applies to very many solvent systems in liquid chromatography (57,58; this work; preceding paper). In addition, and now that a generalized function is in place with which retentions can be represented quantitatively (hence predicted after measurement of only a few datum points), the window-diagram optimization strategy can be employed directly and on a routine basis even for the analysis of mixtures of initially-unknown content and complexity (64,65). Moreover, it would seem that the methodology detailed here could easily be automated: to do so would require only a simple algorithm with which to acquire, thence fit, retention data to the relation, followed by application of a second program to provide plots (window diagrams) of S_f against the system parameter(s) to be optimized (66-68). We take this matter up in the following paper; however, before doing so, we note that all systems shown to date to be described by eqn. 3 have comprised non-ionic solutes and solvents. We therefore enquire at this point whether the relation does in fact apply to ionic systems.

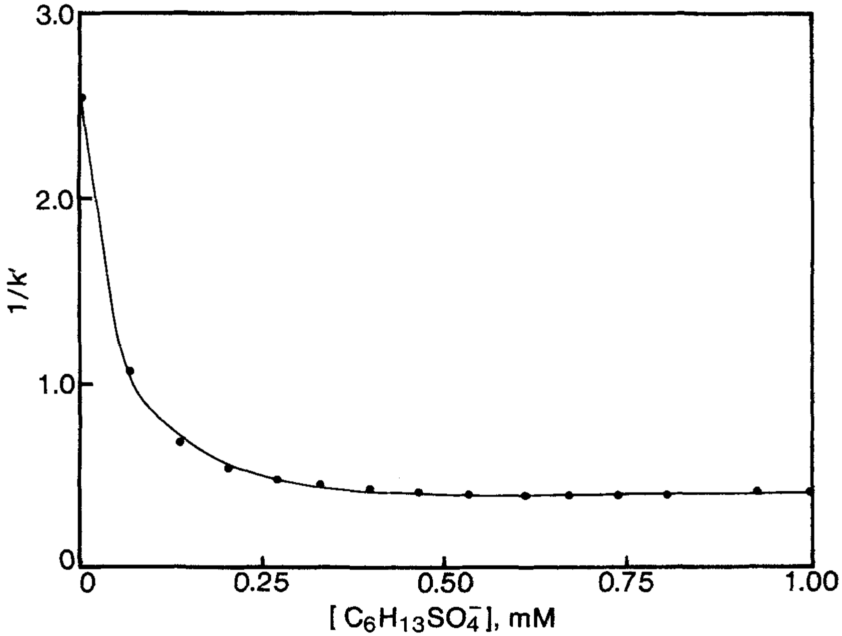

FIGURE 8

Plot of inverse capacity factor for epinephrine solute against mobile-phase surfactant concentration. Data from ref. 68; solid curve constructed with eqn. 3.

Figure 8 presents as an example a plot of the inverse capacity factor data exhibited by epinephrine solute with millimolar concentrations (0 to 1 mM) of hexyl sulfate additive in water:MeOH (80:20; 2.55 pH) mobile phase (69). The circles represent the experimental data, while the solid curve was constructed from eqn. 3 with the fitted values: b = -56.88 and b' = 24.15. The inverse retentions fall off, as shown, very rapidly upon the introduction of some surfactant, and pass through a very shallow minimum at ca. 0.65 mM. Even so, eqn. 3 clearly yields an exact fit over the entire range of additive concentration considered in the work.

The capacity factors $k'_{(S)}$ and $k'_{(A)}$ were taken, for purposes of data reduction, to be those at zero and at unit millimolar amounts of additive; i.e., as if these concentrations corresponded to hypothetical

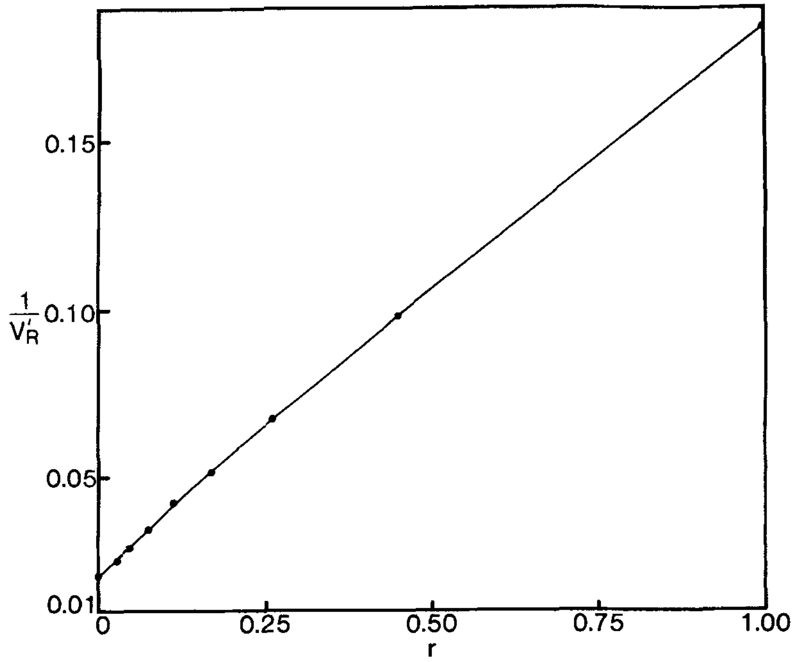

FIGURE 9

Plot of inverse adjusted retention volume for 4-hydroxybenzoic acid against anion/acid ratio for mobile phase containing acetic acid, sodium hydroxide, and sodium chloride. Data from ref. 4; solid curve constructed with eqn. 3.

values of "ϕ" of 0 and 1. Thus, the **actual** units on the abscissa were disregarded; self-evidently, this can be done with data of any form (e.g., pH, ionic strength, concentration of additive, etc.). For example, we show in Figure 9 a plot of the inverse corrected retention volumes of 4-hydroxybenzoic acid solute against the anion/acid concentration ratio, where the mobile phase consisted of various amounts of acetic acid, sodium hydroxide, and sodium chloride so as to maintain a constant ionic strength (4). The experimental data (slightly concave to the abscissa) are again represented by circles, while the solid curve was constructed with b = 0.0361 and b' = 2.504. Figure 10 offers further illustration of the matter, where the inverse capacity factors of tyrosinamide solute are plotted against

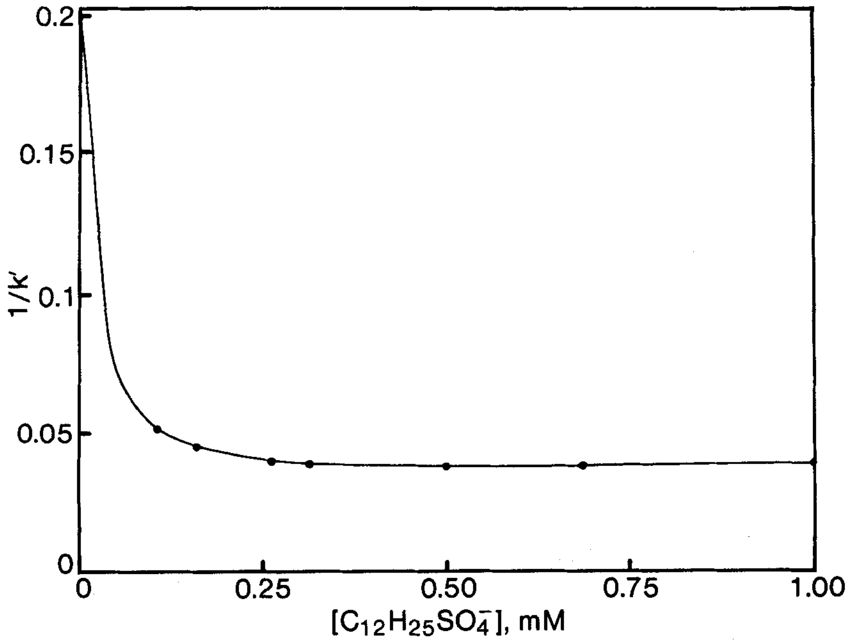

FIGURE 10

Plot of inverse capacity factor for tyrosinamide solute against mobile-phase surfactant concentration. Data from ref. 69; solid curve constructed with eqn. 3.

millimolar concentration of dodecyl sulfate anion in water:MeOH mobile phase (80:20; buffered at pH 6 with 0.018 M KH_2PO_4 and 0.002 M $NaHPO_4$) (70). The values of b and b' were in this instance -10.45 and 61.9.

It therefore appears that eqn. 3 can be fitted also to systems wherein the solutes and/or solvents are ionic. In fact, we have thus far been unable to identify a single case to which it does not apply. Nevertheless, this does not preclude the generality of its form. Indeed, given its complete success up to this time, the relation invites at the very least consideration of models of partitioning from which it may be derived, as well as compel reinterpretation of those already extant.

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61. Even in the absence of interactions of marker species with the stationary phase there remains open the question of the relevance of such data in terms of solutes (here phenols) of differing molecular volume, for which the method (12) of determining an **effective** V_A with an homologous series of compounds closely related to the analytes of interest appears to have merit.

62. The injected amounts of each (effectively at infinite dilution) are completely soluble in the mobile-phase combinations employed here and in fact, are soluble even in pure water. For example, the weight-based aqueous solubility of phenol at 50°C is 33%.

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67. Our experience has been that commercially-available systems heretofore purported capable of automated data acquisition and reduction fail when there occur reversals in solute elution order. Yet such reversals are precisely what is desired in optimizing separations.

68. An nth-order polynomial (or some other function) could obviously be substituted for the relation in order to achieve the same end. However, eqn. 3 is overwhelmingly appealing as a result of the close proximity of its form to what would be derived from fundamental considerations of the partitioning process; see preceding paper.

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