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GRADIENT SELECTION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The rational selection of an optimal gradient in reversed-phase liquid chromatography is discussed from two viewpoints. Firstly, a general expression is derived for the numerical calculation of retention data for the case when the relationship between the capacity factor, k , and the mobile phase composition, φ , is known. For a linear gradient a graphical solution is demonstrated and analytical solutions are presented for simple but widely applicable k - φ relationships. Retention data thus calculated agree well with experimental results.

Secondly, the multi-component solubility parameter theory is used to predict the k - φ relationship. Modification of the stationary chemically bonded phase under the influence of the organic modifier is introduced to improve the accuracy of the predictions. Empirical correlations using solubility parameters are presented as an alternative to the theoretical expressions.

INTRODUCTION

Gradient elution in reversed-phase systems using chemically bonded stationary phases has become a very important technique in high-performance liquid chromatography. Estimates from different workers^{1,2} indicate that 60-80% of the separation problems described in the literature have been solved in the reversed-phase mode. Gradient elution can be used both to extend this number and to improve the separations involved.

The problems encountered in adapting the gradient system to a particular separation problem can be broadly divided into two groups. Firstly, there is the selection of a suitable organic modifier to be mixed with the polar component of the eluent, nearly always water. Secondly, there is the choice of the appropriate gradient (initial and final conditions and the transition curve between them). Solution of the latter problem is facilitated by a simple expression for the retention time as a function of the applied gradient. In this respect, the treatments of Jandera and Churáček³ and Liteanu and Gocan⁴ are less suitable, because their expressions are too complicated.

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Abbott *et al.*⁵ gave a correct mathematical treatment which was not fully expanded. Therefore, an alternative approach is developed in the first part of this paper, resulting in fairly simple mathematical relationships, especially for linear gradients.

Use of these equations presupposes a knowledge of the capacity factor as a function of the eluent composition. Theoretical prediction of this relationship would permit a rational selection of the organic modifier, but is a much greater problem. The indicated wide applicability of reversed-phase chromatography has stimulated attempts to clarify the retention mechanism of chemically bonded phases. So far, these attempts have been only partially successful. The thermodynamic approach used by Locke⁶ incorporates too many simplifying assumptions to be of general interest. Telepchak⁷ offered arguments for a mixed adsorption-partition mechanism. Karger *et al.*⁸ tried to predict hydrophobic selectivity by using a so-called topological index. Horváth *et al.*¹ derived an expression for the capacity factor from the solvophobic theory of Sinanoğlu (cited in ref. 1). The latter was tested, however, on liquid properties such as surface area and surface tension rather than on chromatographic data.

In a previous paper⁹, we discussed the applicability of the extended solubility parameter theory for the description of retention behaviour in various partition chromatographic systems. In the second part of this paper, we extend this approach to reversed-phase systems using chemically bonded stationary phases.

THEORY OF GRADIENT ELUTION CHROMATOGRAPHY

In this section expressions are derived for the retention time of a solute subjected to a chromatographic separation with a continuously changing eluent and hence a continuously changing capacity factor. For this purpose, we assume that the capacity factor is a known function $k(\varphi)$ of the eluent composition, denoted by the volume fraction, φ , of the less polar component.

The solvent delivery system imposes a gradient which, as a function of time (t), can be formulated as

$$\varphi = f(t) \quad (1)$$

If this gradient is transported unchanged through the chromatographic system, it reaches the sample introduction point after a delay time τ and the position z in the chromatographic column after a further period of z/u , where u is the velocity of the mobile phase. Hence at time t the solvent composition at z is given as

$$\varphi(z,t) = f\left(t - \frac{z}{u} - \tau\right) \quad (2)$$

Introduction of the inverse function $f^{-1}(\varphi)$ then yields

$$t = \tau + \frac{z}{u} + f^{-1}(\varphi) \quad (3)$$

or

$$dt = \frac{dz}{u} + d[f^{-1}(\varphi)] \quad (4)$$

The solute migration velocity at position z and time t is given by

$$v(z,t) = \frac{u}{1 + k(\varphi)} = \frac{dz}{dt} \quad (5)$$

Substitution of dt from eqn. 4 into eqn. 5 gives

$$\frac{d[f^{-1}(\varphi)]}{k(\varphi)} = \frac{dz}{u} \quad (6)$$

Now we introduce the usual chromatographic practice that at time $t = 0$ the sample is introduced at $z = 0$, after which the gradient programme is started immediately. Consequently, until the solvent change overtakes the solute, *i.e.*, at $t = \tau + z/u$, or in terms of eqn. 3 for negative values of the inverse function $f^{-1}(\varphi)$, the solvent in the column retains its initial composition so that $k(\varphi) = k(a)$. Further, with τ running from 0 to the column length L , the time varies between 0 and the retention time t_R , so $f^{-1}(\varphi)$ varies between $-\tau$ and $t_R - t_m - \tau = t'_R - \tau$. Imposing these boundaries, eqn. 6 can be integrated to give

$$-\int_{-\tau}^0 \frac{d[f^{-1}(\varphi)]}{k(a)} + \int_0^{t'_R - \tau} \frac{d[f^{-1}(\varphi)]}{k(\varphi)} = \int_0^L \frac{dz}{u} = t_m \quad (7)$$

or

$$\int_0^{t'_R - \tau} \frac{d[f^{-1}(\varphi)]}{k(\varphi)} = t_m - \frac{\tau}{k(a)} \quad (8)$$

This is a basic integral equation for gradient elution, from which the retention time can be calculated numerically for any gradient, provided that $k(\varphi)$ is known. Almost any practical gradient can be described to a good approximation by

$$\varphi = f(t) = a + bt^n \quad (9)$$

so that

$$t = f^{-1}(\varphi) = \left(\frac{\varphi - a}{b} \right)^{1/n} \quad (10)$$

and

$$d[f^{-1}(\varphi)] = \frac{1}{nb} \left(\frac{\varphi - a}{b} \right)^{\frac{1-n}{n}} d\varphi \quad (11)$$

Substituting into eqn. 8 and considering that φ varies between a and $a + b(t'_R - \tau)^n$, we find

$$\frac{1}{nb} \int_a^{a+b(t'_R - \tau)^n} \left(\frac{\varphi - a}{b} \right)^{\frac{1-n}{n}} \frac{d\varphi}{k(\varphi)} = t_m - \frac{\tau}{k(a)} \quad (12)$$

TABLE I

ANALYTICAL SOLUTIONS FOR THE RETENTION EQUATION IN THE CASE OF A LINEAR GRADIENT ($\varphi = a + bt$ UP TO $\varphi = y$)

Relationship between k and φ	Net retention time
<i>Elution before completion of the gradient:</i>	
$k(\varphi) = k_0 + m\varphi$	$t'_R \leq \frac{y-a}{b} + \tau$
$\ln k(\varphi) = \ln k_0 + m\varphi$	$t'_R = \frac{k(a)}{mb} \left(\exp \left\{ mb \left[t_m - \frac{\tau}{k(a)} \right] - 1 \right\} - 1 \right) + \tau$
$\ln k(\varphi) = \ln k_0 + m\varphi$	$t'_R = \frac{-1}{mb} \ln \left\{ 1 - mb \left[t_m - \frac{\tau}{k(a)} \right] \cdot k(a) \right\} + \tau$
$\ln k(\varphi) = A\varphi^2 + B\varphi + C$	$t'_R = \frac{1}{b\sqrt{A}} \operatorname{erf}^{-1} \left\{ \frac{\sqrt{A}}{\pi} \cdot b \left[t_m - \frac{\tau}{k(a)} \right] \cdot \exp \left(\frac{AC - B^2/4}{A} \right) + \operatorname{erf} \left(a\sqrt{A} + \frac{B}{2\sqrt{A}} \right) \right\} - \frac{Aa + B/2}{Ab} + \tau$
<i>Elution after completion of the gradient:</i>	
	$t'_R > \frac{y-a}{b} + \tau$
$k(\varphi) = k_0 + m\varphi$	$t'_R = k(y) \left[t_m - \frac{\tau}{k(a)} \right] - \frac{k(y)}{mb} \ln \left[\frac{k(y)}{k(a)} \right] - \frac{a-y}{b} + \tau$
$\ln k(\varphi) = \ln k_0 + m\varphi$	$t'_R = k(y) \left[t_m - \frac{\tau}{k(a)} \right] + \frac{k(y)}{mb} [k(y) - k(a)] - \frac{a-y}{b} + \tau$
$\ln k(\varphi) = A\varphi^2 + B\varphi + C$	$t'_R = k(y) \left[t_m - \frac{\tau}{k(a)} \right] - \frac{k(y)}{2b} \sqrt{\frac{\pi}{A}} \exp \left(\frac{B^2/4 - AC}{A} \right) \cdot \left[\operatorname{erf} \left(y\sqrt{A} + \frac{B}{2\sqrt{A}} \right) - \operatorname{erf} \left(a\sqrt{A} + \frac{B}{2\sqrt{A}} \right) \right] - \frac{a-y}{b} + \tau$

The special case of a linear gradient results when we substitute $n = 1$:

$$\frac{1}{b} \int_a^{a+b(t'_R-\tau)} \frac{d\varphi}{k(\varphi)} = t_m - \frac{\tau}{k(a)} \quad (13)$$

Clearly, both eqns. 12 and 13 can be solved by numerical integration for any $k(\varphi)$. In the important case of a linear gradient, analytical solutions of eqn. 13 can be formulated for a few simple relationships between the capacity factor (k) and the mobile phase composition (φ). Table I presents analytical solutions for three situations:

(i) a linear relationship between k and φ , which will hardly ever be observed in practice;

(ii) a linear relationship between $\ln k$ and φ , which has been frequently assumed in the literature and may be valid over a restricted range of mobile phase composition;

(iii) a quadratic relationship between $\ln k$ and φ , which will be shown below to provide an accurate description of many experimental data.

In deriving eqns. 12 and 13, it has been assumed that all solutes are eluted from the column before the gradient programme reaches its final composition (at the column outlet). In practice, this is not always observed, so that eqn. 12 must be modified to allow for this. The integral must be split into two parts: one running from an initial composition $\varphi = a$ to a final composition $\varphi = y$ with variable $k(\varphi)$ and the other running from $\varphi = y$ to $\varphi = a + b(t'_R - \tau)^n$ with constant $k(y)$. Consequently, eqn. 12 becomes

$$\frac{1}{nb} \int_a^y \left(\frac{\varphi - a}{b}\right)^{\frac{1-n}{n}} \frac{d\varphi}{k(\varphi)} + \frac{1}{nb} \int_y^{a+b(t'_R-\tau)^n} \left(\frac{\varphi - a}{b}\right)^{\frac{1-n}{n}} \frac{d(\varphi)}{k(y)} = t_m - \frac{\tau}{k(a)} \quad (14)$$

from which the equivalent expression for a linear gradient can again easily be found and solved analytically for the $k(\varphi)$ relationships considered above. The results are included in Table I. In order to avoid unnecessary lengthy calculations, it is recommended that the retention time should first be calculated from the expressions in the top section of Table I. If it then appears that $t'_R > \tau + (y - a)/b$, this means that the solute elutes from the column after the gradient programme has been run to completion. If so, the correct result should be calculated from the appropriate expression in the bottom part of Table I.

In addition to the numerical calculation of retention times from eqn. 13 for arbitrary $k(\varphi)$, Fig. 1 presents a simple graphical solution of this problem based on eqn. 13. In a plot of $1/k(\varphi)$ versus the solvent composition (φ), the initial composition (a) is easily identified. According to eqn. 13, we must select an area under the curve equal to $b[t_m - \tau/k(a)]$, and this area is reached at position $\varphi = a + b(t'_R - \tau)$, from which the desired retention time, t'_R , can then be derived. Elution after completion of the gradient programme is easily allowed for by keeping $1/k$ constant for $\varphi > y$. This graphical solution is less accurate but also much easier than numerical calcula-

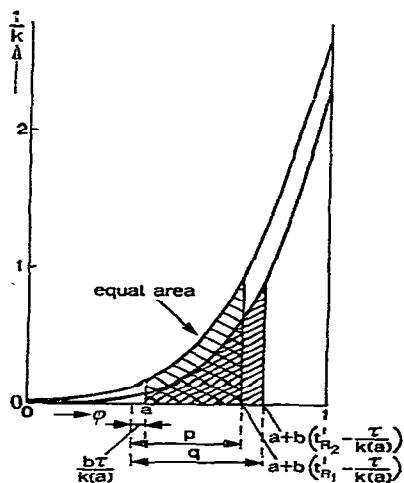


Fig. 1. Solution of the retention equation for a linear gradient (eqn. 13) by graphical integration for two solutes.

tions, especially if several solutes are involved. Moreover, the ratio of the projections p and q on the φ -axis, including a small correction for $b\tau/k(a)$, is equal to the relative retention $\alpha = k_2/k_1$ (see Fig. 1).

Predictions of $k(\varphi)$ by the solubility parameter theory

The expressions for gradient elution in Table I will be even more useful if the relationship between k and φ can be predicted theoretically. In a previous paper⁹ we discussed the possibilities of the extended solubility parameter treatment for the description of retention behaviour in partition chromatographic systems. The appropriate expression is

$$\ln k = \frac{v_i}{RT} \left[(\delta_{T,m}^2 - \delta_{T,s}^2) + 2(\delta_{d,i} + \delta_{ind,i})(\delta_{d,s} - \delta_{d,m}) + \right. \\ \left. 2\delta_{o,i}(\delta_{o,s} - \delta_{o,m}) + 2\delta_{a,i}(\delta_{ind,s} - \delta_{ind,m}) + 2\delta_{a,i}(\delta_{b,s} - \delta_{b,m}) + \right. \\ \left. 2\delta_{b,i}(\delta_{a,s} - \delta_{a,m}) \right] + \ln(V_s/V_m) + v_i(1/v_s - 1/v_m) \quad (15)$$

In this expression, the subscripts m , s and i refer to the mobile phase, the stationary phase and the solute, respectively. δ_d , δ_o , δ_{ind} , δ_a and δ_b are the partial solubility parameters for dispersion, orientation, induction, acid and base interactions, respectively, which together determine the total solubility parameter, δ_T . Finally, v is the molar volume and V is the phase volume.

Eqn. 15 differs slightly from the expression presented previously⁹ because it includes the induction parameter δ_{ind} as defined by Keller *et al.*¹⁰. A major practical, although not principal, objection to the solubility parameter theory is the need for very accurate values of all partial parameters. The determination of dispersion and

acid–base parameters was discussed previously⁹. The subdivision into induction and orientation parameters can be achieved by the use of the internal pressure according to Scigliano¹¹.

The application of eqn. 15 to the prediction of elution on chemically bonded phases is confronted by three problems. Firstly, in realistic reversed-phase systems water is used as the polar component. Unfortunately, water behaves so uniquely that it is difficult to describe in terms of solubility parameters. Nonetheless, Kirchnerova and Cave¹² and Arro and Melder¹³ have recently successfully applied the solubility parameter theory to aqueous systems.

A second problem is the fact that chemically bonded phases may not have the properties of bulk phases for which the solubility parameter theory has been derived. It is more likely that they constitute an interfacial layer between the mobile phase and the silica support material. As will be shown below, this problem can be reduced by assuming that the effective properties of the stationary phase change with the composition of the mobile phase.

A third important problem is the description of the solubility parameters for mixed solvents. After Hildebrand and Scott¹⁴, for a mixture of two liquids, p and q , we use

$$\delta_{T,m} = (1 - \varphi) \delta_{T,p} + \varphi \delta_{T,q} \quad (16)$$

where as before φ is the volume fraction of the less polar component (q). Although experimental measurements¹⁵ indicate a slightly non-linear relationship between the total solubility parameter and the solvent composition, eqn. 16 has been used successfully by Chao and co-workers^{16,17} for the prediction of the vapour pressures of mixtures of weakly polar compounds. Therefore, it seems a valid approximation for non-polar compounds where only dispersion forces are active. Hence,

$$\delta_{d,m} = (1 - \varphi) \delta_{d,p} + \varphi \delta_{d,q} \quad (17)$$

For simplicity, we assume that expressions similar to eqn. 17 are valid for all partial polarities. This assumption will be more questionable for increasingly polar interactions. Especially for a mixture of an acid and a base it is hardly to be expected that acid and base parameters can be described adequately by eqn. 17, because some compensation by “neutralization” must be expected. For the molar volume of the mobile phase, we used

$$v_m = (1 - x) v_p + x v_q \quad (18)$$

where x is the molar fraction of q .

Substitution of expressions similar to eqn. 16 and 17 into eqn. 15 now yields an expression for the capacity factor as a function of the mobile phase composition (φ) of the general form

$$\ln k = A' \varphi^2 + B' \varphi + C' + D'(\varphi) \quad (19)$$

where $D'(\varphi)$ is the size correction term based on the Flory–Huggins expression^{9,18–20}.

TABLE II
EXPRESSIONS FOR A' , B' , C' AND $D'(\varphi)$, AND A'' , B'' , C'' AND $D''(\varphi)$, IN TERMS OF SOLUBILITY PARAMETERS AND MOLAR VOLUMES

Stationary phase	Expression
Independent of mobile phase	$A' = \frac{v_1}{RT} (\delta_{T,q} - \delta_{T,p})^2$ $B' = \frac{2v_1}{RT} [\delta_{T,p}(\delta_{T,q} - \delta_{T,p}) - (\delta_{h,t} + \delta_{lmd,t})(\delta_{u,q} - \delta_{u,p}) - \delta_{o,t}(\delta_{o,q} - \delta_{o,p}) - \delta_{d,t}(\delta_{lnd,q} - \delta_{lnd,p}) - \delta_{a,t}(\delta_{h,q} - \delta_{h,p}) - \delta_{h,t}(\delta_{u,q} - \delta_{u,p})]$ $C' = \frac{v_1}{RT} [(\delta_{T,p}^2 - \delta_{T,s}^2) + 2(\delta_{u,t} + \delta_{lmd,t})(\delta_{u,s} - \delta_{u,p}) + 2\delta_{o,t}(\delta_{o,s} - \delta_{o,p}) + 2\delta_{d,t}(\delta_{lnd,s} - \delta_{lnd,p}) + 2\delta_{a,t}(\delta_{h,s} - \delta_{h,p}) + 2\delta_{h,t}(\delta_{u,s} - \delta_{u,p})] + \ln(V_s/V_m)$ $D'(\varphi) = \frac{v_1[\varphi v_s(v_q - v_p) + v_q(v_p - v_s)]}{v_p v_q v_s}$
Modified by the organic modifier according to eqn. 21	$A'' = \frac{v_1}{RT} (\delta_{T,p} + \delta_{T,s} - 2\delta_{T,p}) (\delta_{T,p} - \delta_{T,s})$ $B'' = \frac{2v_1}{RT} [(\delta_{T,p} + \delta_{T,s} - \delta_{T,p})(\delta_{T,s} - \delta_{T,p}) + (\delta_{u,t} + \delta_{lmd,t})(\delta_{u,p} - \delta_{u,s}) + \delta_{o,t}(\delta_{o,p} - \delta_{o,s}) + \delta_{d,t}(\delta_{lnd,p} - \delta_{lnd,s}) + \delta_{a,t}(\delta_{h,p} - \delta_{h,s}) + \delta_{h,t}(\delta_{u,p} - \delta_{u,s})]$ $C'' = \frac{v_1}{RT} [(\delta_{T,p}^2 - \delta_{T,s}^2) + 2(\delta_{u,t} + \delta_{lmd,t})(\delta_{u,s} - \delta_{u,p}) + 2\delta_{o,t}(\delta_{o,s} - \delta_{o,p}) + 2\delta_{d,t}(\delta_{lnd,s} - \delta_{lnd,p}) + 2\delta_{a,t}(\delta_{h,s} - \delta_{h,p}) + 2\delta_{h,t}(\delta_{u,s} - \delta_{u,p})] + \ln(V_s/V_m)$ $D''(\varphi) = \frac{v_1(v_p - v_s) [-\varphi^2(v_p - v_q) + \varphi(v_p - 2v_q) + v_q]}{v_p v_q} \frac{[\varphi(v_p - v_s) + v_s]}{[\varphi(v_p - v_s) + v_s]}$

Resulting expressions for $D'(\varphi)$ as well as for A' , B' and C' are presented in Table II. As will be discussed later, these equations do not give a good description of the experimental data. Evidence can be found for at least a certain amount of stationary phase modification.

The effective properties of the stationary phase vary with the mobile phase composition, probably owing to preferential adsorption of the organic modifier at the non-polar surface. If such preferential adsorption takes place to a large extent, then we might expect a distribution over two similar phases when the mobile phase consists of 100% modifier. This means a partition coefficient of about 1 for all solutes and consequently no selectivity. Qualitatively, this conclusion agrees reasonably well with practical experience (*e.g.*, see Fig. 3). To investigate this phenomenon, we assume a linear variation of the effective stationary phase properties with the volume fraction of the organic modifier:

$$\delta_{T,s}^{\text{mod}} = (1 - \varphi) \delta_{T,s} + \varphi \delta_{T,a} \quad (20)$$

and similar expressions for v_s (using x instead of φ) and all partial polarities. The expressions resulting from substitution in eqn. 15 are given in Table II and are denoted by A'' , B'' , C'' and $D''(\varphi)$ according to

$$\ln k = A''\varphi^2 + B''\varphi + C'' + D''(\varphi) \quad (21)$$

Before discussing the applicability of this approach, it is important to note that eqn. 19 predicts that the expression for the capacity factor is the sum of a quadratic and a hyperbolic function D' or D'' of φ . In general, the hyperbolic term is not negligible in comparison with the quadratic term, but over the complete range of $0 \leq \varphi \leq 1$ it can be described to a good approximation by a quadratic function of φ . Moreover, if there is no stationary phase modification the hyperbolic function simplifies to a linear function. Therefore, we can write

$$D'(\varphi) = \beta'\varphi + \gamma' \quad (22)$$

and

$$D''(\varphi) = \alpha''\varphi^2 + \beta''\varphi + \gamma'' \quad (23)$$

So with eqns. 19 and 21,

$$\ln k = A'\varphi^2 + (B' + \beta')\varphi + (C' + \gamma') \quad (24)$$

when no stationary phase modification is assumed or

$$\ln k = (A'' + \alpha'')\varphi^2 + (B'' + \beta'')\varphi + (C'' + \gamma'') \quad (25)$$

when there is modification of the stationary phase. Both eqns. 24 and 25 lead to a quadratic form for $k(\varphi)$:

$$\ln k = A\varphi^2 + B\varphi + C \quad (26)$$

This expression is borne out in practice, as shown in Table III and by the examples below in Figs. 2 and 3. This supports our earlier remark that $\ln k$ is a quadratic rather than a linear function of the eluent composition, ϕ .

EXPERIMENTAL

The chromatographic equipment consisted of two M 6000 A pumps coupled via a Model 660 solvent programmer and a U6K sample injector and a UV detector (all from Waters Assoc., Milford, Mass., U.S.A.). Columns of I.D. 4 mm and length 10 cm (30 cm for methanol-water) were slurry packed with LiChrosorb RP-18 (Merck, Darmstadt, G.F.R.). Reagent-grade methanol, ethanol and *n*-propanol from J. T. Baker (Phillipsburgh, N.J., U.S.A.) and specially distilled water were used as solvents. High-purity solutes were dissolved in pure organic modifier and 5- μ l samples were injected on to the column. The detector output was coupled on-line to a PDP 11/45 laboratory computer with sampling frequencies up to 5 Hz. The peak positions of the eluted solutes were determined from the first statistical moment of the digitized chromatogram.

RESULTS AND DISCUSSION

Prediction of retention and separation

The $\ln k$ versus ϕ curves were measured for sixteen solutes using either methanol or ethanol as the organic modifier. Six solutes were also measured in mixtures of *n*-propanol and water at four different temperatures. A solution of potassium dichromate in water was used to determine the mobile phase time, t_m . Representative examples are shown in Figs. 2 and 3. The solid lines drawn through the data points

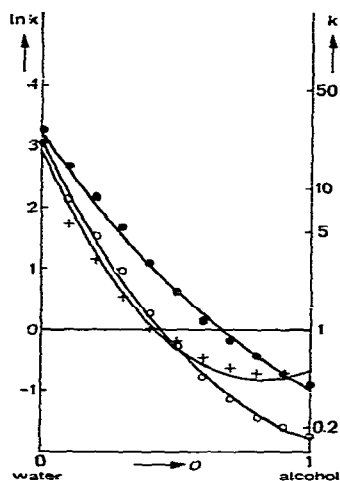


Fig. 2. Relationship between the capacity factor and the mobile phase composition for phenol using different modifiers. ●, Methanol; ○, ethanol; +, *n*-propanol.

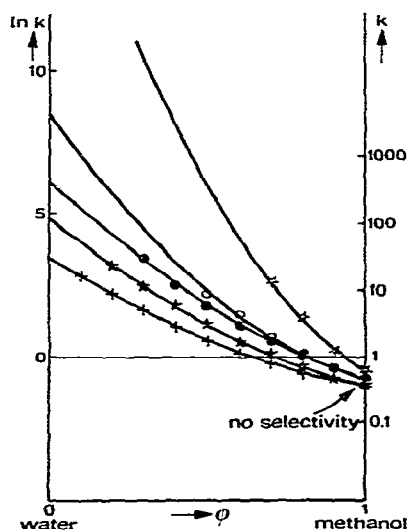


Fig. 3. Relationship between the capacity factor and the mobile phase composition for several solutes in the methanol-water system. ★, *m*-Cresol; ●, 2,4-dimethylphenol; +, aniline; ○, diethyl phthalate; ☆, dibutyl phthalate.

represent best fitting quadratic curves, from which the coefficients A , B and C in eqn. 26 were derived. The complete results are collected in Table III. The standard deviations given indicate the error that might be expected in capacity factors calculated from the quadratic expression. Since the standard deviations are usually very low, the fitted curves match the experimental data very well, as can also be seen from Figs. 2 and 3. However, no experimental data were obtained for very large values of k , so that the standard deviations given do not represent the possible error in calculated $\ln k$ -values of 100 or more ($\ln k \geq 5$). This can most easily be illustrated from the C -values given in Table III.

The C -coefficient in eqn. 26 equals the retention for $\varphi = 0$, *i.e.* for pure water. Therefore C -values for the same solute from different series of experiments using different organic modifiers should be the same. This proves to be correct in most cases, certainly when one considers the differences in chromatographic conditions and columns for the different modifier systems. High C -values are inevitably found by extrapolation, which becomes more uncertain for higher values. This is indicated by the C -values for benzophenone and dibutylphthalate from the methanol-water and ethanol-water systems, showing differences of factors 10 and 10^3 respectively. All coefficients will tend to be more accurate when more experimental data points are available, in other words when the C -value is lower. In all cases however, k -values up to 100 can be calculated with good accuracy and higher values are of very little significance for chromatographic elution. Re-calculated data from the literature^{8,21,22} also result in quadratic dependences, although this was not recognized by the authors concerned. The commonly suggested linear relationship between $\ln k$ and φ appears to be a useful approximation for only a limited number of systems or

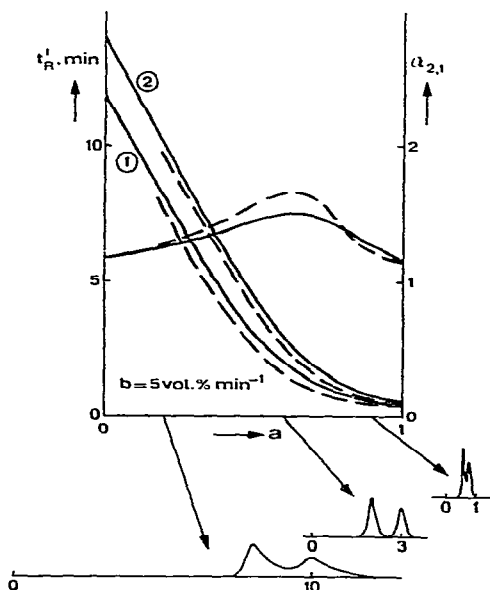


Fig. 4. Comparison of experimental retention data (broken lines) with those calculated from Table I (solid lines), using the equations for the quadratic form. Compounds: 1, *m*-cresol; 2, 2,4-dimethylphenol.

TABLE III
COEFFICIENTS FOR THE RELATIONSHIP BETWEEN $\ln k$ AND φ EQN. 26

Solute	A			B			C			Standard deviation*		
	Methanol	Ethanol	Propanol	Methanol	Ethanol	Propanol	Methanol	Ethanol	Propanol	Methanol	Ethanol	Propanol
Benzene	1.57	1.38	4.68	-7.42	-7.22	-9.72	5.31	4.58	4.57	0.04	0.14	0.13
Toluene	2.20	4.77		-9.72	-12.83		7.12	7.10		0.03	0.04	
Ethylbenzene	1.54	5.19		-10.10	-14.45		8.13	8.31		0.04	0.05	
Diethyl <i>o</i> -phthalate	6.50	10.03	7.64	-15.81	-19.14	-12.77	8.55	7.70	4.53	0.03	0.07	0.21
Dibutyl <i>o</i> -phthalate	12.75	9.82		-32.02	-22.80		18.79	11.65		0.08	0.07	
Acetophenone	4.12	5.87	5.85	-10.34	-11.90	-9.77	5.46	4.68	3.43	0.04	0.05	0.13
Benzophenone	8.23	9.66		-19.86	-19.72		11.11	8.89		0.05	0.09	
Aniline	2.74	2.99	3.82	-7.19	-7.63	-6.53	3.48	3.02	2.08	0.05	0.12	0.06
N-Methylaniline	4.29	6.02		-11.02	-13.62		5.99	6.26		0.02	0.03	
N,N-Dimethylaniline	4.07	6.35		-12.47	-15.02		7.99	7.75		0.03	0.02	
Quinoline	7.69	8.92		-16.73	-16.69		8.60	6.94		0.07	0.04	
Benzyl alcohol	2.52	3.92		-6.96	-8.53		3.48	2.95		0.06	0.06	
Phenol	1.92	3.65	5.64	-6.10	-8.49	-9.19	3.20	3.06	2.59	0.08	0.07	0.20
2,4-Dimethylphenol	3.29	5.68		-10.28	-13.13		6.16	5.86		0.05	0.08	
<i>o</i> -Cresol	3.03	4.19		-8.81	-10.25		4.88	4.34		0.07	0.08	
<i>m</i> -Cresol	3.00	4.41	5.86	-8.85	-10.48	-10.12	4.88	4.31	3.54	0.04	0.08	0.13

* Standard deviation = $\sqrt{\frac{\sum [\ln k(\text{exp}) - \ln k(\text{calc})]^2}{n}}$

over a restricted range of solvent compositions. This means that for the general situation, the retention time in linear gradient elution chromatography could be calculated from the quadratic expressions in Table I.

The fairly complicated expressions may be avoided by using the graphical or numerical alternatives discussed above. Obviously, for non-linear gradients, numerical integration of eqn. 8 is the only possibility. The accuracy of the calculated retention times can be judged from Fig. 4, where the calculated data for two solutes are compared with practically measured net retention times. The systematic deviation is due to an uncertain delay time, τ , rather than to the calculation procedure. Obviously, the retention time of both solutes decreases strongly with increasing initial amount of organic modifier, a , but the separation factor, α , is seen to reach a maximum value at $a = 0.65$.

More extensive calculations using the coefficients for the quadratic form from Table III are presented in Figs. 5 and 6. Again the retention times decrease rapidly with increasing initial amount of organic modifier, a , and with increasing gradient, b (Fig. 5). More important, however, is the optimum in the separation factor that shifts towards higher values of a with increasing slope, b , of the gradient (Fig. 6). This means that for a given analysis time the separation becomes better with decreasing gradient. Also, the initial amount of organic modifier should not be taken too low. Indeed, Fig. 4 shows that for small values of a the separation of two solutes takes a long time and is incomplete. At too high a value of a the separation is very rapid, but also incomplete. Good separation within reasonable time is obtained at $a = 0.6$ for the two solutes in the present example.

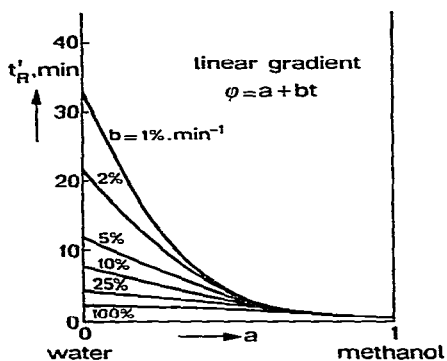


Fig. 5. Calculated net retention times for *m*-cresol, applying a linear gradient.

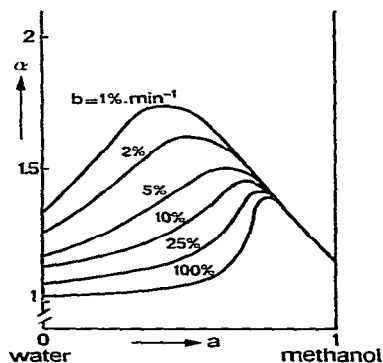


Fig. 6. Calculated relative retentions applying a linear gradient.

Prediction of the relationship between $\ln k$ and φ

Now that the quadratic relationship between $\ln k$ and φ predicted from the solubility parameter approach has been borne out in practice, we turn our attention to the calculation of the coefficients A , B and C in eqn. 26 from the solubility parameter expressions. Some representative results are given in Table IV, where the calculated coefficients are compared with the experimental values taken from Table III.

The first entries in the tables refer to coefficients calculated from the expressions at the top of Table II. Here the solubility parameters of the mobile phase are continuously changed according to the mixing rules (eqns. 16 and 17), but the stationary phase is kept invariable. The size correction term, $D'(\varphi)$, for the unmodified stationary phase, based on the Flory-Huggins theory, is a linear expression and the respective coefficients β' and γ' were added to the corresponding coefficients B' and C' to yield estimates for B and C (eqns. 22 and 24). For the case of a modified stationary phase, the size correction term, $D''(\varphi)$, is approximated by the best quadratic fit (eqn. 23) and A'' , B'' and C'' are corrected according to eqn. 25. Although in all instances the signs of the coefficients are predicted correctly, there is a large difference with experimental data. To gain insight into the retention process, we determined the enthalpy and the entropy contributions to $\ln k$ separately from plots of experimentally measured values of $\ln k$ versus the reciprocal temperature, $1/T$. A representative example is shown in Fig. 7, which shows that the entropy reaches a minimum at $\varphi = 0.5$, where the enthalpy term goes through a maximum at $\varphi = 0.2$. For other solutes the latter maximum was found to be at variable φ values between 0.2 and 0.6. Both the enthalpy and the entropy terms can be well fitted with a quadratic function of φ , so that also the total $\ln k$ versus φ curve follows this behaviour. The expressions at the top of Table II are based on the solubility parameter theory, which makes it tempting to equate the enthalpy term with $A'\varphi^2 + B'\varphi + C'$ and to equate the entropy term with $D'(\varphi)$. However, closer inspection of Table II indicates that this cannot be a viable proposition. Because A' in Table II is always positive, the enthalpy term thus calculated must pass through a minimum rather than show a maximum as observed in Fig. 7. In general, this minimum is situated outside the physically meaningful range of φ (usually at $\varphi > 1$). Alternatively, the Flory-Huggins size correction term, $D'(\varphi)$, in Table II, will never show a minimum as observed experimentally for the entropy term. In view of the small molar volume of water, $D'(\varphi)$ and $D''(\varphi)$ generally increase steadily over the entire range from $\varphi = 0$ to 1.

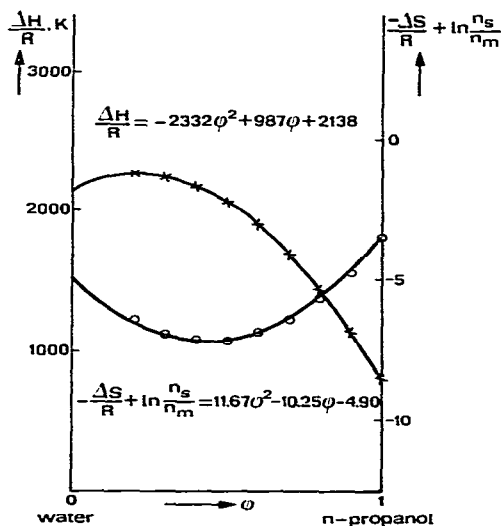


Fig. 7. Experimental enthalpy and entropy functions for phenol in the *n*-propanol-water system.

In principle, the observed variations of the enthalpy and the entropy might be explained if in addition to a variation of the mobile phase, we assume that the effective parameters of the stationary phase also change with varying content of organic modifier. Arguments for such a modification have been advanced in the theoretical section. In that case, the enthalpy term is calculated as the difference of two concave quadratic forms: one for the mobile phase interaction and one for the stationary phase interaction with the solute. Such a difference might well show a maximum. In a similar way, the entropy term, which is a difference between two size correction terms, might show a minimum. This approach leads to the expressions at the bottom of Table II and the coefficients calculated from these expressions are collected in Table IV.

Although it is clear that in comparison with the unmodified stationary phase the values of the coefficients A , B and C change in the correct direction, the change is generally too small to provide a reasonable description of the experimental data. Therefore, we are forced to conclude that the best estimates of the partial solubility parameters presently available do not provide a quantitatively correct prediction of the retention behaviour in gradient elution chromatography using chemically bonded phases. The calculations also show that for the systems presently considered it is probably not permissible to use the regular solution concept to write down separate theoretical expressions for enthalpy and entropy contributions. A similar conclusion has already been drawn by Hildebrand and Scott¹⁴.

A major reason for the limited success of the solubility parameter approach in reversed-phase systems is the need for accurate data on the highly polar mobile phase component, water. The solubility parameter theory has always been more successful with non-polar or only slightly polar components. However, the multi-parameter regression analysis described in a previous paper⁹ enables us to derive effective values for the solubility parameters of water. To this end, retention data for sixteen components in methanol-water and ethanol-water mixtures were used to find empirical relationships for the coefficients A^* , B^* and C^* . This approach is based on the expressions at the bottom of Table II using the data for water as an unknown parameter to be fitted to the experimental data. In this way, the following empirical relationships were obtained:

$$A^* = 0.037 v_i \text{ for methanol;}$$

$$A^* = 0.046 v_i \text{ for ethanol;}$$

$$B^* = 3.38 \cdot 10^{-3} v_i (7.18\delta_{T,q} + 0.99\delta_{d,i} + 1.00\delta_{o,i} - 0.12\delta_{a,i} - 1.36\delta_{b,i} + 0.91v_q - 178.35)$$

where $\delta_{T,q} = 15.85$ and $v_q = 40.41$ for methanol and $\delta_{T,q} = 13.65$ and $v_q = 58.37$ for ethanol;

$$C^* = 1.69 \cdot 10^{-3} v_i (17.88\delta_{d,i} - 4.82\delta_{o,i} - 3.26\delta_{a,i} - 0.44\delta_{b,i} - 105.66)$$

where the asterisks denote the empirical nature of the expressions. The accuracy of the predictions afforded by these equations is illustrated in Fig. 8, where for the sixteen solutes considered the empirically predicted values of A^* , B^* and C^* are compared with the experimental values of A , B and C . Of course, it should be pointed out that the same substances were used to formulate the empirical relationships. It is clear,

however, that within the set of sixteen substances considered there is a general consistency. This is also illustrated by the last entries in Table IV, where the empirical predictions of A^* , B^* and C^* are seen to agree much more closely with the experimental data than either of the theoretical predictions. This means that using the empirical correlations, the retention behaviour of the sixteen solutes can be predicted reasonably well. Absolute retention data on other compounds can be predicted within a factor of 3, but the calculated relative retentions on a few preliminary experiments and literature data demonstrate that for these other solutes (not included in the regression analysis) the calculated relative retentions are usually more accurate.

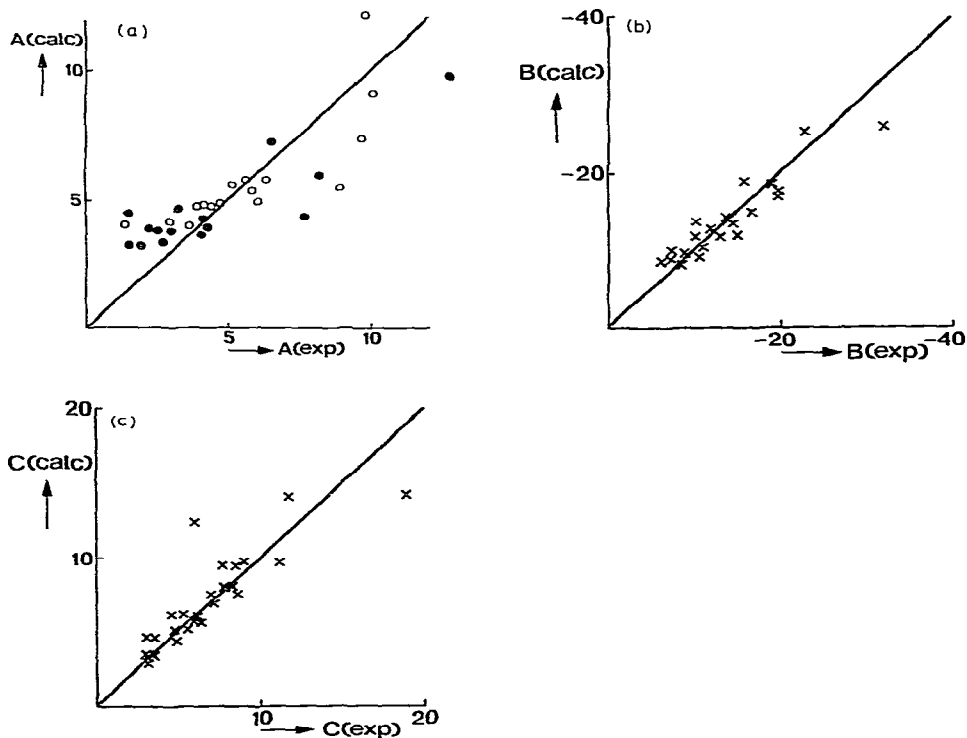


Fig. 8. Calculated *versus* experimental values for the coefficients (a) A^* , (b) B^* and (c) C^* from the empirical correlations. (a) ●, Methanol; ○, ethanol.

CONCLUSION

The mathematical model described enables us to make a good estimate of chromatographic retention data, provided that the relationship between $\ln k$ and φ is known. The solubility parameter theory suggests a quadratic form for this relationship, and this appears to be borne out in practice. *A priori* prediction of the coefficients in the quadratic expression have yielded insufficient results so far. Experimental data show that a separation into an enthalpic contribution described by solubility parameters and an entropic contribution described by the Flory-Huggins theory is not justified. These entropy and enthalpy data suggest modification of the

stationary phase by the organic modifier in reversed-phase systems. As long as no accurate theoretical predictions of the $k(\varphi)$ relationship are possible, empirical correlations might be used with some success.

SYMBOLS

A, B, C	coefficients in the quadratic expression for $k(\varphi)$
a	initial mobile phase composition (volume fraction units)
b	gradient slope (min^{-1} in case of a linear gradient)
$D(\varphi)$	size correction term
$f(t)$	gradient curve function
k	capacity factor
L	column length (cm)
n	exponent in gradient curve function (eqn. 9)
R	gas constant ($R = 1.9865 \text{ cal} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$)
T	absolute temperature ($^{\circ}\text{K}$)
t	time (min)
t_m	mobile phase time (min)
t_R	retention time (min)
t'_R	net retention time (min)
u	mobile phase velocity (cm/min)
V	phase volume (cm^3)
v	solute migration velocity (cm/min)
v	molar volume (cm^3/mol)
y	final mobile phase composition (volume fraction units)
z	distance along column axis (cm)
α	relative retention ($\alpha = t'_{R,2}/t'_{R,1}$)
α, β, γ	coefficients describing the size correction term $D(\varphi)$
δ	solubility parameter (cal/cm^3) [‡]
τ	gradient delay time (min)
φ	volume fraction of modifier

Subscripts

a	acid
b	base
d	dispersion
i	solute
ind	induction
m	mobile phase
o	orientation
p	weakest eluting mobile phase component
q	modifier (strongest eluting mobile phase component)
s	stationary phase
T	total

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