Journal of Chromatography A, 656 (1993) 3–18
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 269

Review

Current strategies for prediction of retention in high-performance liquid chromatography

C.H. Lochmüller*, Charles Reese and Allison J. Aschman

Department of Chemistry, Duke University, Durham, NC 27708 (USA)

Steven J. Breiner

Department of Chemistry, Appalachian State University, Boone, NC 28608 (USA)

ABSTRACT

The desire to model chromatographic retention derives from both theoretical and practical needs. There are fundamental questions concerning the interactions of molecules in separation systems and the modeling of chromatographic retentions is critical for methods development. This paper reviews the current strategies for predicting retention in HPLC with emphasis on RP-HPLC. Three main categories are discussed: methods utilizing a physical model, methods which assume no model, and methods with an abstract or hidden model. The theoretical background, practical application, and relative merits of each approach are discussed.

CONTENTS

* Corresponding author.

0021-9673/93/$24.00

1. INTRODUCTION

The history of the development of chromatography is one of combined empirical efforts and more quantitative chemical/physical modeling of the partition process. Michael Tswett is credited with the discovery of chromatography although there was prior work which would be recognized, today, as chromatography. The distinction is that Tswett's work was the first to define a physical model for the distribution process involving equilibrium conditions in which the solutes were adsorbed on the stationary phase or dissolved in the mobile phase in quantities described by an equilibrium constant characteristic of the solute of interest in the chromatographic system. It was almost half a century later that the work of A.J.P. Martin and his colleagues on liquid–liquid and gas–liquid chromatography was performed. The search for rational and useful models describing the chromatographic process continues today.

Gas and liquid chromatography are, in many ways, mature areas of separation science when related to small molecules. The major growth areas of liquid chromatography applications development in recent years appear to be in the area of biology, especially modern molecular biology, and in the separation of complex mixtures such as those found in environmental systems. The biological area has, for many years, made substantial use of size exclusion separations as well as conventional ion-exchange chromatography for protein and polypeptide purification. A rapid increase in the use of HPLC in the pharmaceutical industry can also be seen. All fields which rely on chromatographic systems require techniques which aid in the rapid development of a useful separation method, however, many researchers are not aware of the many tools that are available to the state-of-the-art chromatographer. The current need is for these methods development tools to be made known to both experienced and new users.

It would be impossible to cite all of the work done in developing models and methods for the prediction of retention and resolution in chromatography in the space of an article such as this. What follows is an attempt to give representative examples of successful approaches and to have those examples span the range of physical to abstract models for retention. The examples are limited to modern HPLC and focus on RP-HPLC as a most common application of HPLC.

Clearly, a universal, rugged physical model for retention in reversed-phase liquid chromatography would be ideal in both a fundamental and practical context. The development of physical models in RPLC is complicated by indications that there is no simple measure of system dead volume. Indeed, it appears that there may be a characteristic dead volume for every different molecule implying that not all molecules undergo even the same distribution equilibria in terms of the identity of the stationary phase. For this reason, some methods development techniques utilize no theoretical model and rely solely on experimental data for retention prediction.

For the purposes of our discussion of current state-of-the-art, it is convenient to make the following classifications for modeling of retention and relative retention HPLC: True physical models from which a method utilizing an assumed functional relationship may be derived. Methods which assume no model. Methods with an abstract or hidden model.

2. TRUE PHYSICAL MODELS

A physical model in the sense used in this paper is one which combines physical properties into an equation or assembly of equations in contrast to abstract (or hidden) models in which the terms *may* have a relation to physical properties but that relation is neither drawn nor necessary for their use. Physical models for retention behavior are many and varied. Some models are purely thermodynamic. Pure thermo-

dynamics does not "know" molecules and directed forces such as dipole–dipole, induction–dipole or hydrogen bonding. However, since chromatography is, in the limit, an equilibrium separation method, thermodynamics can be applied to model behavior and general trends may be predictable based on knowledge of the endo- or exoergic nature of a phase transfer process.

Chemistry *is* molecules and chemists prefer models which permit the use of the developed sense of "chemical insight" gained by study and experience. Physical models with chemical inference can involve microscopic and macroscopic chemical properties. The molecular level models are the most interesting as they can provide insight into the stereochemistry of the interactions which result in differential migration behavior for closely related molecules. Differences in solubility in the mobile phase are the basis for one chemical model describing differential migration rates or retention behavior. Another route to a model is to adapt existing solution theory to the chromatographic experiment and to add terms to the equations which are attributed to specific molecular interactions. It is not possible to do justice here to a great deal of the pioneering work in this area nor can particular models be described in detail. What is presented are representative examples and, where ever possible, an outline of experimental testing of a given prediction or relationship.

2.1. Linear solvent strength theory

Linear solvent strength (LSS) theory [1] predicts that RP-HPLC retention, for a binary solvent pair, varies linearly with mobile phase composition as is shown in eqn. 1:

$$\log k' = mc + b \tag{1}$$

Where k' is the capacity factor of the solute, c is the concentration of the strong eluate and m and b are constants. This model can be applied to binary mobile phase systems with moderate accuracy [2]. The plots of log k' *vs.* concentration which are predicted to be linear are actually somewhat curved but the fit of the model is not bad over a reasonably limited mobile phase composition. The model has increasing difficulty with ternary and higher order mobile phase systems. These mobile phase systems require that the c "independent variable" be adjusted with solvent strength parameters for each of the individual strong solvents. One advantage of this theory is that it does not require many retention measurements before it can be used. The main weakness is its limited accuracy which is generally insufficient to predict retentions well enough to separate difficult-to resolve compounds. The accuracy of the prediction does not improve much with additional data as it is the model itself which is inaccurate.

Two or more gradient runs can be used to predict isocratic retention in the same HPLC system providing an efficient approach to mapping retention as a function of separation conditions and derivation of optimum isocratic parameters [1,3–5]. Comprehensive theory now exists to describe gradient separations in terms of equivalent isocratic parameters (and *vice versa*). Snyder and others have further developed this theory for the special case of linear solvent strength gradient elution [1,6–12]. A short discussion of this approach, extracted from ref. 2, is presented below.

Retention data in isocratic and gradient elution for the same HPLC system are connected by the relationships:

$$\int_0^{V_g} \mathrm{d}V/V_a = 1 \tag{2}$$

and

$$t_g = (V_g/F) + t_0 + t_D \tag{3}$$

Where V_g is the corrected gradient retention volume, V is the volume of the mobile phase after sample injection, V_a is the corrected isocratic retention volume, t_0 is the column dead time, and t_D is the system dwell time, F = flow-rate.

The concentration, C_B, of the strong solvent in the mobile phase varies with time during the linear gradient:

$$C_B = (C_B)_0 + [(C_B)_f - (C_B)_0]t/t_G \tag{4}$$

Where $(C_B)_0$ and $(C_B)_f$ are the initial and final

concentrations of B, t is the time after sample injection, and t_G is the gradient time. If the corrected retention volume ($V_a = V_g - Ft_0$) for the corresponding isocratic system is known as a function of C_B:

$$V_a = f(C_B) \quad (5)$$

then eqn. 2 can be solved as a function of experimental conditions (eqn. 3).

Eqn. 5 may be obtained by an alternative approach based on the LSS approximation to actual gradient systems [6,8], so that two gradient runs can be used to develop the generally accepted reversed-phase approximation:

$$\log k' = \log k_w - S\varphi \quad (6)$$

Where φ is the volume fraction of organic in the water–organic mobile phase; S represents the change in log k' for unit change in φ in isocratic elution; and k_w is the capacity factor in pure water. The use of linear gradients allows eqn. 6 to be expressed as:

$$\log k_i = \log k_0 - b(t/t_0) \quad (7)$$

since the condition for LSS is satisfied. Here k_i represents the value of k' at the column inlet during gradient elution ($k_0 = k_i$ when $t = 0$) and b is proportional to the steepness parameter which has been defined in terms of experimental conditions of mobile phase volume, dead-time, composition change and flow-rate as:

$$b = \Delta\varphi S V_m / t_0 F \quad (8)$$

$$b = 1/(1.15k) \quad (8a)$$

When k is the value of k' when it has migrated halfway down the column in a gradient separation.

The gradient retention time, t_g, is now given as:

$$t_g = t_0/b \log [2.3k_0 b(t_S/t_0) + 1] + t_S + t_D \quad (9)$$

in which k_0 is the k' value at the beginning of the gradient; t_D represents the delay time between the pump and the column; and t_S is the retention time of the solute under non-retaining conditions. For small molecules, it may be assumed that $t_S = t_0$: Also, under gradient conditions, $k_0 \gg 1$.

By carrying out two gradient runs with different gradient times (t_{G1}, t_{G2}), eqn. 9 allows for the explicit solution of the steepness parameter, b, by:

$$b_1 = t_0 \log \beta / [t_1 - (t_2/\beta) - (t_0 + t_D(\beta - 1/\beta)] \quad (10)$$

where $\beta = b_1/b_2 = t_{G2}/t_{G1}$.

By rearranging eqn. 8, the S value (representing the change in log k' with changing solvent composition as produced by the gradient) may be determined.

Values of k for each gradient run (k_1 and k_2) can also be calculated (eqn. 8a) and corresponding values of φ_1 and φ_2 (the average or effective values of φ, values at band center when band is at midpoint of the column, for gradient elution) can be obtained via eqn. 4. When the actual dependence of log k' *vs.* φ is non-linear, the gradient derived values of k *vs.* φ represent an approximation to the true dependence. This approximation becomes poorer for values of φ that do not lie within the range of φ_1 and φ_2. In this case, additional gradient runs can be used to map the exact dependence of k on φ. For these non-LSS cases, it is useful to calculate φ directly for each gradient run:

$$\varphi = \varphi_0 = [t_g - t_0 - t_D - 0.3(t_0/b)]\Delta\varphi/t_G \quad (11)$$

This then yields values of k *vs.* φ (or k' *vs.* φ) under isocratic conditions, for the case of $k_0 \gg 1$. For small values of k_0.:

$$k = 1/[1.15b + (1/k_0)] \quad (12)$$

In this case, values of φ can be obtained from k and eqn. 6.

Generally, LSS behavior for solute retention may be approximated for reversed-phase separations and "non-LSS" errors [7,8] are usually small and can be ignored in practical applications. The same is true for ion-exchange separations, when the effective charge, z, on the solute molecule is >3. For the case of $z \leq 3$, a simple correction is available that minimizes errors due to non-LSS behavior [2]. Other "non-ideal" effects can limit the accuracy of gradient-derived isocratic retention data. These effects may be minimized by utilizing "optimum gradient con-

ditions" described by Quarry *et al.* [2]. Therefore, this procedure for obtaining gradient-derived isocratic retention parameters seems broadly applicable.

Dylab programs produced by LC Resources use a computer to simulate LC runs. After one or more initial separations of a sample in the laboratory, Drylab simulates the effect of additional experimental conditions. Drylab I may be used to develop optimum isocratic LC methods based on initial gradient runs. This example of an "expert system" applies the theory for LSS described above. Drylab G extends this approach to methods optimization for gradient elution.

2.2. Retention in RP-HPLC using ternary mobile phases

The evaluation of phase systems in liquid chromatography can be evaluated in terms of three parameters: retention, selectivity, and specificity. Ternary mobile phases greatly increase flexibility in the search for optimum specificity (relative to binary mobile phases). Practical examples of the advantageous use of ternary mobile phases and the combination of three organic modifiers with water in RP-HPLC have been published [13–17].

Schoenmakers *et al.* [18] report a systematic study of the retention behavior of two ternary mobile phase systems and use the information to define ternary compositions of equal polarity and to analyse specific separation effects.

The solubility parameter concept has been successfully used to express the relationship between the capacity factor, k', and a binary mobile phase composition yielding good estimates of the compositions of different binary solvents that lead to equal retentions [19]. It is, therefore, reasonable to use it in a similar way for ternary systems. Schoenmakers *et al.* [18] derived eqn. 2 from solubility parameter and solute activity considerations.,

$$\ln k'_i = A_1\varphi_1^2 + A_2\varphi_2^2 + B_1\varphi_1 + B_2\varphi_2 + C + D\varphi_1\varphi_2 \quad (13)$$

Where φ_1 and φ_2 are the concentrations of the organic components in a ternary mobile phase and the constants are terms containing solvent parameters and the activities of the solutes. Such relations can be derived also from regular-solution theory or other equivalent lattice models. A brief synopsis of the derivation presented by Schoenmakers in ref. 18 is given.

In LC the capacity factor may be expressed in terms of activity coefficients [20]:

$$k'_i = (\gamma_{i,m}/\gamma_{i,s})(n_s/n_m) \quad (14)$$

Where $\gamma_{i,m}$ and $\gamma_{i,s}$ are the activity coefficients of the solute i in the mobile and stationary phase, respectively, and n_s and n_m are the number of moles of the two phases present in the column. (Eq. 14 is based on the pure liquid solute as the standard state for both phases.)

Activity coefficients can be expressed in terms of total solubility parameters [18]. If entropy effects are neglected:

$$RT \ln(\gamma_{i,f}) = v_i(\partial_i - \partial_f)^2 \quad (15)$$

Where R is the gas constant (1.9865 cal K^{-1} mol^{-1}), T is the absolute temperature (K), $\gamma_{i,f}$ is the activity coefficient of solute i in phase f, v_i is the molar volume of the solute (cm^3 mol^{-1}) and ∂ is the solubility parameter ($cal^{1/2}$ $cm^{-2/3}$). Combining eqns. 14 and 15 produces:

$$\ln k'_i = (v_i/RT)[(\partial_i - \partial_m)^2 - (\partial_i - \partial_s)^2] + \ln(n_s/n_m) \quad (16)$$

Assuming that the solubility parameter of a mixture may be determined from its constituents and knowing that the sum of volume fractions must be equal to 1, the solubility parameter for a ternary mixture may be written as:

$$\partial_{mix} = \phi_1\partial_1 + \varphi_2\partial_2 + (1 - \varphi_1 - \varphi_2)\partial_3 \quad (17)$$

The subscript 1 may be assigned to methanol and 3 to water with 2 referring to the second organic modifier (THF or acetonitrile). The substitution of eqn. 17 into eqn. 16 with rearrangement leads to eqn. 13 for the dependence of capacity factor on the composition of the mobile phase with:

$$A_1 = (v_i/RT)(\partial_1 - \partial_3)^2 \quad (18)$$

$$A_2 = (v_i/RT)(\partial_2 - \partial_3)^2 \quad (19)$$

$$B_1 = (2v_i/RT)(\partial_i - \partial_3)(\partial_3 - \partial_1) \quad (20)$$

$$B_2 = (2v_i/RT)(\partial_i - \partial_3)(\partial_3 - \partial_2) \quad (21)$$

$$C = (v_i/RT)[(\partial_3 - \partial_i)^2 - (\partial_s - \partial_i)^2] + \ln(n_s/n_m) \quad (22)$$

$$D = (2v_i/RT)(\partial_3 - \partial_2)(\partial_3 - \partial_1) = 2(A_1A_2)^{1/2} \quad (23)$$

Eqn. 13 expresses the non-linear dependence of ln k' on the two volume fractions of organic modifier. In binary mobile phase systems, the generally non-linear relationship between ln k' and composition can be approximated by a straight line over a limited range of k' values ($1 < k' < 10$) [21]. For ternary compositions, however, it does not appear to the authors to be feasible to approximate the surface described by eqn. 13 by a plane over a wide range of ternary compositions. Predictions as to the values of A_1, A_2, B_1, B_2, C, and D can be made using the values of the solubility parameters of the different mobile phase constituents [20].

Schoenmaker's model has the advantage over Snyder's more limited LSS theory in that it gives a better fit to experimental data. It has the disadvantage that it requires multiple retention measurements to make a first prediction. This is because, in practice, the constants must be determined by a regression technique. The accuracy of this model's predictions does improve as more data is included. It is difficult to evaluate the validity of this equation with conventional techniques because it contains multiple terms with functions of the same variable.

2.3. Iso-eluotropic diagrams

For certain solute pairs in ternary systems, the *relative* retention can be increased considerably, while retention itself remains roughly constant. Theoretically, as is shown in eqn. 16, different mobile phases will lead to the same retention times if their polarities (solubility parameters) are equal. All ternary mixtures of water, methanol, and a second organic modifier that possess a given polarity follow a straight line between two limiting binary compositions (the binary methanol–water system and the binary "organic modifier"–water system). The straight line connecting the two binary compositions is called a (theoretical) iso-eluotropic line. Empirical iso-eluotropic lines can be constructed from experimental data. Good agreement with the theoretical lines has been observed by Schoenmakers *et al.* [18], except for the very strong solvents, which are of a limited practical value. Theoretical iso-eluotropic lines offer a guideline to the elution of a given sample with different mobile phases, but with roughly constant retention. The authors found that the specificity of ternary systems along one isoeluotropic line appears to vary quite regularly, and, therefore, can be expected to lie between those of the limiting binary mixtures, at the end of the corresponding iso-eluotropic line. Also, variations from the theoretical for certain solutes, referred to as "specific effects", may be classified relative to binary mixtures.

2.4. Mobile phase complexation

Katz *et al.* [22] have presented an analysis of binary mixtures of associating solvents, such as methanol and water. They have suggested that the partial molar volume change on mixing for methanol–water arises from the formation of a methanol–water adduct and that chromatographic models for reversed-phase separations should take this into account. The Scott model considers binary mixtures of water and methanol to be mixtures of: water, methanol is free form, and associated methanol–water. In the simplest form, the association complex would be a 1:1 complex. Based on studies of the association of water with organic solvents such as methanol, it seems likely that more than just a 1:1 complex is present. Nevertheless, Katz *et al.* used volume change on mixing and a 1:1 association model to determine the fraction of free water, free methanol, and associated methanol–water at various volume percents of water–methanol. Analyzing these results, Katz *et al.* demonstrated that free methanol exists over the range where ln k' *vs.* volume percent of methanol gives an apparent linear relationship. The form of this solvent–solvent association equilibrium is represented by:

$$W + [OW] + O$$

where W represents water, O is any organic modifier which can associate with water, and [OW] represents the association complex. The equilibrium constant K is determined by fitting the experimental volume change on mixing data for a series of 0% to 100% O solutions as shown by Katz *et al.* Lochmüller *et al.* [23] have discovered an anomaly in ternary RP-HPLC data. They found that many compounds showed an increase in retention when methanol was replaced volume-for-volume with acetonitrile in methanol–water mixtures, Lochmüller *et al.* applied the simple association model of Katz *et al.* but taking into account the weaker association of water–acetonitrile as a competing equilibrium. They were able to show that adding acetonitrile to methanol–water "frees" water in the sense of unassociated water as defined by Katz. The model's prediction for the increase in free water upon addition of small amounts of acetonitrile (replacing methanol) closely correlated with the volume percent over which retention increased in the observed experimental results.

The assumptions in this model are that adding water generally increases retention in RP-HPLC; that the first-order approximation that the equilibrium constant for water–methanol (1:1) association is dominant; and that "free" organic modifier reduces retention. There are no assumptions about the texture or dynamics of the bonded phase and retention is considered to be a mobile phase dominated phenomenon.

2.5. Retention models for polymers

2.5.1. Critical composition theory (CCT)

Boehm and co-workers [24–26] developed a model for the equilibrium distribution of infinitely dilute, and therefore isolated, flexible polymer molecules between a binary solvent mobile phase and a planar stationary phase based on the Flory–Huggins lattice model. Presumably the phase preference of the solute depends on the degree of polymerization, the solvent composition, and a variety of solvent, solute, and surface interactions including inter- and intra-polymer segment interactions which are not accounted for by traditional chromatographic theories. The retention of a polymer in gradient LC is defined as:

$$k' = \exp[\{A\}M(Xc - X)] \quad (24)$$

in which k' is the capacity factor of the solute, M is the degree of polymerization of the solute, X is the mobile phase composition (volume fraction) of the good solvent, Xc is the critical mobile phase composition for the specific polymer, and A is a constant for polymers with a molecular weight of greater than 10^4 daltons. The significance of this equation is that the critical mobile phase composition (Xc) of a polymer (the mobile phase composition at which k' is unity) is defined as dependent on its molecular mass. This accounts for polymer flexibility affecting inter- and intra-molecular interactions. A polymer is either infinitely retained or tends to elute rapidly depending on whether the mobile phase is above or below the critical composition. This model applies for polystyrene of molecular masses of 10^4 daltons or higher.

2.5.2. The precipitation–redissolution model (PRM)

Glockler and van den Berg [27] have proposed a multi-stage process for precipitation chromatography in a porous medium. In this model, size exclusion of the solute from the pores is used to continuously precipitate the sample in a gradient mobile phase. Solvent molecules are treated as small spheres which can penetrate the pores of the stationary phase while solute molecules are excluded. If the volume of the mobile phase outside of the pores of the stationary phase is Ve and the volume inside is Vi, the polymer molecules in the mobile phase will move through the column at a velocity of μp defined as:

$$\mu p = (LF)/Ve \quad (25)$$

in which L is the length of the column and F is the flow-rate. The velocity of the mobile phase molecules through the column, however, is represented by μs defined as:

$$\mu s = (LF)/(V_e + V_i) \quad (26)$$

which reflects that these solvent molecules have access to the interior of the stationary phase

pores. Therefore, the polymer molecules in the mobile phase will move along the column at a faster rate than the solvent molecules. In this way, the polymer tends to move from a better solvent to a poorer solvent in the mobile phase gradient. As the polymer experiences the poorer solvent it precipitates out of, and is excluded from, the mobile phase until a better solvent is experienced, at which point the polymer redissolves and again migrates along the column. This precipitation–redissolution occurs repeatedly during column migration resulting in fractionation of a sample based on its solubility and size. This model also predicts that the reversed-phase chromatography of a polymer cannot be performed isocratically as it is entropically forbidden [27].

2.5.3. Linear solvent strength theory (LSST)

According to Snyder and co-workers [28–31], traditional chromatographic theories which describe small molecules are applicable to macromolecules. Anomalous behavior reported in conjunction with macromolecular separations (decreased plate number, pore size effects, and steep plots of log k' *vs.* φ) can be explained by existing theory.

2.5.4. Classification of solvents

Classification of solvents to facilitate the selection of an optimum mobile phase in liquid chromatography has been a major goal in methods development. Approaches have been developed for separating the contributions of dipolarity, hydrogen bond acidity (HBA) and hydrogen bond basicity (HBB) to the overall solvent strength. The Snyder solvent triangle approach assumes that any two solvents that are similar in all three of the above properties should behave similarly in terms of their elution properties [32,33].

In studies of steroid retention in RP-HPLC by West [34], several discrepancies in the solvent selectivity triangle concept proposed by Snyder were observed. Examination of the slopes describing the change in solute retention indices as a function of φ, showed that they varied considerably among solvents from the same selectivity group. In some instances, slopes were actually more similar for solvents in different groups than for those in the same group. West suggests that this discrepancy is possibly due to the underlying assumptions of the solvent triangle theory which discounts dispersion interactions between solute and solvent and does not consider the role of the stationary phase and the nature of the solutes themselves. Also, only three solutes may not suffice to encompass all of the important characteristics that contribute to the experimentally observed selectivity for more complex molecules.

The use of the phenomenon of solvatochromism, in conjunction with linear solvation energy effects (LSEE) is also currently being used to elucidate the role of specific chemical processes in gas–liquid and liquid–liquid partitioning [35–37].

Rohrscheider's gas–liquid partition data for six prototypical solutes in 81 common liquids [38] is used as the basis for the Snyder model and by other researchers to test their models. In Snyder's approach, the P' polarity scale is an overall measure of solvent strength that is a composite of all types of solute-solvent interactions, except for dispersive interactions. Rohrshneider's data has been redetermined by a new methodology which circumvents most of the shortcomings and assumptions inherent in his measurements [39]. Also, the gas–liquid partition coefficients of a new set of alkanes in the sample solvents have been measured. In light of this new data, Rutan *et al.* [40] have reexamined two of the approaches used to classify and quantify solvent properties: the solvent triangle-P' scale [32,33] and the Kamlet and Taft approach [35] in which gas–liquid partition coefficients are correlated with solvatochromic scales describing solvent dipolarity–polarizability (π^*), hydrogen bond acidity (HBA) (α), and basicity (HBB) (β). In this study an additional term accounting for solvent reorganization effects in self-associating solvents has been employed. A summary of the examination by Rutan *et al.* in ref. 40 follows:

2.6. P' polarity scale

Snyder's approach assumes that the dispersive interactions and cavity formation contributions can be eliminated from the partition coefficient

by first multiplying the partition coefficient by the solvent molar volume, V_s, as is shown in eqn. 27, and then referencing this quantity to that which would result for a hypothetical alkane with the same molar volume as the solute.

$$\log K'_{i,s} = \log (K_{i,s}V_s) \quad (27)$$

$$\log K''_{i,s} = \log K'_{i,s} - (V_i/163) \log (K_{0,s}V_s) \quad (28)$$

Where $K_{i,s}$ is the gas–liquid partition coefficients for solute i in solvent s, V_s is the solvent molar volume, $K_{0,s}$ is the gas–liquid partition coefficient for octane in solvent s, and V_i is the molar volume of the probe solute. The term 163 is the molar volume of octane.

The above correction is utilized due to the absence of measured values for the partition coefficients for a series of *n*-alkanes. This approach assumes that the intercept of a plot of the log of the partition coefficient for the *n*-alkanes *vs.* molar volume (V_{alkane}) is insignificant.

The log $K''_{i,s}$ value from eqn. 28 is modified by subtracting the average of the log $K''_{i,s}$ values for the ith solute in the solvents hexane, cyclohexane, and isoctane (log $K''_{i,hci}$) as is shown in eqn. 29:

$$P'x_i = \log K''_{i,s} - \log K''_{i,hci} \quad (29)$$

Eqns. 27–29 were used to calculate P' values for the solutes: ethanol ($P'x_e$), *p*-dioxane ($P'x_d$), and nitromethane ($P'x_n$) with the following condition:

$$1 = x_e + x_d + x_n \quad (30)$$

Snyder suggests that x_e, x_d, and x_n should be measures of solvent HBB, HBA, and dipolarity, respectively.

Poppe and Slaats [41] suggested two additions to the approach described above. First, that the historically applied Flory–Huggins correction factor be included in eqn. 27 to account for the entropic contribution to the partition coefficient due to differences in molecular size. The net effect is the arithmetic elimination of the dependence on the solvent molar volume in eqn. 27 producing the following equation:

$$P'x_i = \log K''_{i,s} - (V_i/163) \log K_{0,s} - \log K_{i,hci} + (V_0/163) \log K_{0,hci} \quad (31)$$

In general, the magnitude of the Flory–Huggins factor is relatively small for molecules with similar sizes.

The second correction involves an improvement in the estimate of the contribution from a hypothetical *n*-alkane with the same molar volume as the solute, so that the expression for $P'x_i$ becomes:

$$P'x_i = \log K''_{i,s} - (V_i/163) \log K_{0,s} - \log K_{i,hci} + (V_0/163) \log K_{0,hci} + (\beta_{hci} - \beta_s)[1 - (V_i/163)] \quad (32)$$

Where β_s is a term which accounts for a plot of log $K_{alkane,s}$ *vs.* V_{alkane} having a non-zero intercept. The Flory–Huggins correction was found by the authors to produce $P'x_i$ values for the non-polar alkane solvents that were virtually independent of the solvent. Also, changes in the P' values and the x_e, x_d, x_n factors were negligible in terms of the solvent classification scheme. The correction shown in eqn. 32 was not evaluated due to the difficulty in estimating the β_s values.

Recent gas–liquid partition coefficient data for a series of alkanes in the Rohrscheider solvents has permitted the estimation of the contribution from a non-zero intercept. Precise linear relationships of the form:

$$\log K_{alkanes,s} = m_s V_{alkane} + b \quad (33)$$

were found for the four alkanes studied. Here, m_s and b ae the solvent-dependent slope and intercept. Using this linear extrapolation and omitting the V_s correction of eqn. 27 produces the equation:

$$P'x_i = \log K_{i,s} - m_s V_i - b_s - \log K_{i,hci} + m_{hci} V_i + b_{hci} \quad (34)$$

This approach is somewhat different from that of Poppe and Slaats (eqn. 32).

2.7. Correlation with solvatochromic parameters

A solvent characterization scheme reported by Kamlet *et al.* [35], similar to the one above, is based on a dissection of the partition coefficient into contributions from solvent dipolarity (π^*),

HBB (β), and HBA (α). Partition coefficient data are corrected for dispersion and cavity formation by referencing to an alkane of similar size to the solute as described by Rutan *et al.* [40]. These values are then correlated to solvatochromic scales. The solvent parameters, π^*, α, and β and ∂ (the polarizability correction factor) are used as linear energy parameters in an LSEE. The correlation take the following forms for aliphatic and aromatic solvent respectively:

$$\log K_{i,s} - \log K_{\text{alkane},s} = SP0 + s\pi^* + a\alpha + b\beta \quad (35)$$

and

$$\log K_{i,s} - \log K_{\text{alkane},s} = SP0 + s\pi^* + d\partial + a\alpha + b\beta \quad (36)$$

where ∂ is 0 for non-chlorinated, aliphatic solvents, 0.5 for polychlorinated, aliphatic solvents, and 1.0 for aromatic solvents, $SP0$ is the solute-dependent intercept, and s, a, b, and d are the solute dependent coefficients for dipolarity–polarizability, HBA, HBB and polarizability correction factor contribution, respectively.

Using gas–liquid coefficients which are referenced to a hypothetical alkane with a molar volume equal to the solute molar volume, the equation for the estimation of the selectivity parameters in terms of solvatochromic parameters can be given as:

$$P'x_i = SP0 + s\pi^* + d\partial + a\alpha + b\beta \quad (37)$$

Since K_{hci} and m_{hci} used in eqn. 34 are constants, eqn. 37 differs from eqn. 36 by a constant.

Recently, it has been demonstrated that an additional term, $\alpha\beta$ (the product of solvent HBA and HBB) must be added to eqn. 37 to model adequately the gas–liquid partition behavior for a wide range solvents (used in this work by Rutan *et al.*). This term accounts for the additional reorganization of self-associating solvents which occurs when a solute is capable of hydrogen bonding. The final equation used to examine the solvent selectivity parameters is:

$$P'x_i = SP0 + s\pi^* + d\partial + a\alpha + b\beta + h\alpha\beta \quad (38)$$

Rutan *et al.* have suggested that knowledge of the solvatochromic parameters should permit the selection of more appropriate probe solutes for the development of a solvent triangle with better ability to allow discrimination between solvents. Snyder's solvent triangle classification is limited by the lack of an explicit selectivity parameter describing dispersive interactions which have been shown by Meyer and co-workers [42–45] to predominate over all other interactions in organic solvents. An approach based directly on solvatochromic parameters may be used to develop a more accurate classification scheme using cluster analysis methods [46].

2.8. Bonded-phase selectivity

Many attempts to model retention behavior involve assumptions that mobile phase change is the controlling factor in both retention and selectivity changes. In the case of RP-HPLC, "solvophobic" models derived from "hydrophobic effect" models has met with some success. As with other such models, it assumes that the stationary phase is a passive member in the partition process in the sense of being non-changing. Some would have it that the hydrocarbon bonded phases of RP-HPLC serve the role of converting silica to a graphite-like surface or even something akin to the "basal-plane of graphite" [47,48]. As evidence for a dynamic behavior of the stationary phase in response to the change in mobile phase composition and number of components in the mobile phase, efforts have been made to examine theoretically the effects that changes in bonded phase texture may have on retention and selectivity [49–53].

In describing the role of the bonded phase, the central phenomenon is considered to be the dynamic reorganization of the bonded-phase chains in response to mobile phase changes [49,52,53]. Solvents that are quite compatible with the bonded phase are expected to solvate the phase and promote extension and dissociation of the immobilized molecules which compose the phase. If the extended phase behaves as theorized, there should be penetration of the mobile phase into the bonded-phase mass. Also, it is likely that solute molecules are afforded

deeper penetration/intercalation in the phase mass.

If one considers the importance of solvophobic effects on solute molecules, one must examine the influence solvophobic effects would have on a hydrocarbon-chain-based phase, such as those commonly used in RP-HPLC. Using the same logic as the invocation of the hydrophobic effect for solute–stationary phase association, "hostile" solvents should cause association of bonded-phase chains. A collapse of the bonded chains upon themselves and their neighbors would reduce extension and reduce the intercalation of solute molecules [49]. From this view, a model for a "breathing" stationary phase has evolved in which the surface and its texture adjusts to new mobile phase conditions such that the stationary phase maintains its essential non-polar properties. If the breathing stationary phase model is correct and intercalation of solute is important to retention and selectivity, models for retention behavior cannot ignore the stationary phase changes and the dynamics of that process.

Martire and Boehm [54] have developed a "unified molecular theory" for the solute distribution process in RP-HPLC based on a lattice model for the bonded phase. Their formalism incorporates stationary phase variables such as bonded-chain length, stiffness, surface coverage (relates to density and promimity of neighboring chains), and the nature of the mobile phase (as a "good" or "hostile" solvent). This model predicts the effect of variable change on the composition and the microscopic texture of the bonded phase as well as on the solute distribution process. Solute distribution effects are predicted in detail for the two limits of full extension and collapse of the bonded phase chains. The collapsed limit is of practical concern as the common mobile phases which are used in RP-HPLC are mixtures of water and organic modifier. The resulting polar mixtures are likely to promote varying degrees of chain collapse. Although the bonded phase may be locally wetted by the organic modifier, water is certainly also present. The presence of water in a hydrocarbon phase is a difficult concept for some who think of octadecane as insoluble in water. However, the solubility of water in hydrocarbons is well understood by those confronted with water content in aircraft and vehicle fuels.

"Chemically bonded phases exhibit shape selectivity which increases as the chains become more fully extended. The predicted order of solute retention is as follows: rigid-rod solutes > "plate solutes" > flexible chain solutes" [54]. (A rigid-rod solute could be anthracene and a plate solute could be pyrene. Rods and plates are related, in that rods are plates of unit cross-section.) It is important to realize that RP-HPLC experiments identical in all respects including mobile phase but differing in stationary phase must take the stationary phase into account. After all, if the mobile phase is identical, its contribution to the free energy or to the differential free energy change in the case of a pair of solutes is the same. Since both net retention and selectivity are critical components of a successful chromatographic method, an experimental verification of the predictions of the Martire–Boehm formalism would show its importance as one part of the development of a quantitative and physical model for chromatography.

To produce this experimental verification, the challenge is to synthesize a bonded phase which is inherently more rigid than a corresponding octadecyl moiety. One may consider a conjugated polyene, but such a molecule would not be readily converted to a reactive silane by known hydrosilylation chemistry. Lochmüller *et al.* [55] synthesized and used a 4,4′-dipentylbiphenyl as an inherently more rigid molecule of extended length almost identical to octadecyl. Bonded phases were prepared which were brush-type using the mono-dimethylchlorosilane-terminated analogue of this substituted biphenyl. The new phase is essentially a C_{18} length molecule with a rigid rod inserted midway "up the chain".

Various probe solutes chosen to minimize any complications from stronger force interactions and to serve as rod, plate, and flexible chain probes were used. Both C_{18} and dipentylbiphenyl phase columns were used with identical mobile phases. The results agreed with the predictions of the Martire–Boehm theory as can be seen in Table I from ref. 55. The chromatography was performed using methanol water mixtures with different volume percents of metha-

TABLE I

PERCENT CHANGE IN CHROMATOGRAPHIC SELECTIVITY (α) BETWEEN OCTADECYL AND BIPHENYL PHASES FROM EQN. 39

Compound	$\varphi = 0.7^a$	$\varphi = 0.8$	$\varphi = 0.9$
Butylbenzene	−2	−5	−7
Amylbenzene	−2	−7	−2
Phenylhexane	−9	−8	−10
Phenylheptane	−11	−12	−12
Chrysene	155	147	151
Benzanthracene	134	127	127
Pyrene	68	67	64
Anthracene	107	92	80
Phenanthrene	77	65	61
Napthalene	43	33	31
p-Terphenyl	210	192	159
Biphenyl	58	46	41

[a] φ is volume % methanol in water in mobile phase.

nol. Table I shows the percent change in chromatographic selectivity (α) between octadecyl and biphenyl phases as is shown in eqn. 39. Mesitylene was utilized as a reference solute

$$\%(\Delta\alpha) = (\alpha_{\text{biphenyl}} - \alpha_{\text{ODS}})/(\alpha_{\text{ODS}}) \quad (39)$$

Wise *et al.* [56] have been successful in relating the selectivity in RP-HPLC for polyaromatic hydrocarbons to a "length to breadth ratio" and retention increase to increasing length/breadth. Radecki *et al.* [57] investigated this with respect to a shape parameter defined as the ratio of the longer side to the shorter side of a rectangle which envelops the molecule and has a minimum area. The work of Lochmüller, of Wise *et al.*, and of Radecki *et al.* all point to a confirmation of the Martire–Boehm predictions. The conclusion is that models which invoke only mobile phase effects will be less accurate because of the dynamic nature of the texture of the bonded phase and the effect that this has on selectivity as well as retention.

3. METHODS WHICH ASSUME NO MODEL

The most common of the "no-model methods" is the undirected "cut-and-try" approach in which measurements are made on a single column based on an "educated guess" by the operator as to the next best combination of eluent components. A more rational approach is one in which retention is assumed to be a smooth, continuous variable with respect to mobile phase composition changes. The resolution of critical pairs is then tested at various compositions and a contour map of the resolution surface is produced.

A commercially available example of the latter approach may be found by the generation of "critical resolution maps" in the CHROM3 package of the Perkin-Elmer Corporation. Glajch *et al.* [58] first suggested the "overlapping resolution surface". In the Glajch approach, the resolution of pairs of solutes is studied over a range of solvent/mobile phase compositions and the results are then folded into a map of resolution for all the solutes of concern. It is then possible to estimate conditions where a minimum resolution for all components will be observed.

The CHROM3 approach extends this idea utilizing automated chromatography. In this approach, the "critical resolution" is determined as the spacing between the two most-closely eluting pairs *in the mixture*. There can be problems with reversal of elution order over the range of mobile phases used and the number of observed components can change because of co-elution as well. In this empirical approach, co-elution becomes a truly low value of critical resolution ($\approx$0) and the method remains quite rugged.

A chromatographic column and three mobile phase components are chosen, *e.g.* a 5-cm C_{18} column using 5 μm particles and water, acetonitrile and methanol. Chromatograms are obtained for a variety of mobile phase component variations. For a ternary mobile phase the diagram is that of a triangle and the information plotted is the distance in time or volume of elution between the two most closely eluting pairs. This is illustrated in Fig. 1.

In this figure, the darkest squares are areas of highest resolution between the closest eluting pairs. There are three such regions and the method developer chooses the solvent combination which best meets the need for resolution, quantitation and speed of separation. The development of a critical resolution map such as is

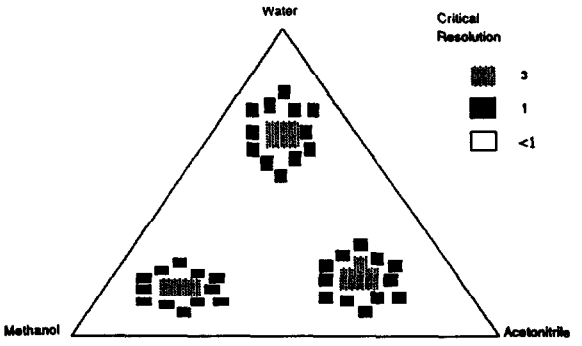

Fig. 1. Critical resolution map where each square is a separate chromatographic trial. Better resolution is indicated by a larger value of critical resolution.

shown may require 150 separate chromatograms. An approximation of the results shown in the example can be made by fewer measurements made over a wider range of solvent combinations. Such a survey can indicate *regions of likely success*. The retention model used here assumes that retention changes as a monotonic function of mobile phase strength. There is no ability to predict the entire surface form the first measurement or any combination of measurements but clear indications of good and poor regions are possible. This method cannot answer the question: "Will this C_{18} column and these solvents ever give me a usable method?" until many measurements are made.

3.1. Retention index systems

Various retention index systems, analogous to the Kovat's retention index system in gas chromatography, have been developed for HPLC in an attempt to accurately predict retention behavior and resolution [59–63]. The 2-keto alkane retention index system developed by Baker and Ma [60] has been successfully utilized by West and Mowrey [64] to characterize the HPLC selectivities of 12 reversed-phase solvents. The characterization was performed using three probe molecules (nitrobenzene, benzaldehyde, and anisole) with retention indices that were adjusted to compensate for the relative slopes of the 2-keto alkane retention index standard calibration line. Regression analysis was then used to predict adjusted retention indices (I') with all 12 reversed-phase solvents for 13 aromatic compounds containing a wide variety of functional groups. The solute-specific retention coefficients were used in combination with the solvent-specific I' values of the three probe molecules to accurately predict the adjusted retention indices of the thirteen aromatic compounds. These adjusted retention indices were used for the accurate prediction of compound resolution for all solvents based on the experimental resolution obtained with only one of the solvents.

The prediction of resolution using this technique requires the highly precise prediction of the adjusted retention indices as very small errors in retention can result in relatively high percentage errors in predicted resolution. The authors have shown, however, that the 2-keto alkane retention index system can be used successfully on a small scale to predict accurately solvent selectivity.

4. METHODS WITH AN ABSTRACT OR HIDDEN MODEL

It would be very advantageous to chromatographic methods development if a technique could be devised which allowed the strengths of the current methods development systems to be combined while avoiding all of the weaknesses. Equations (LSS) and (Schoenmakers) both predict that retention data should conform to the requirements of principal components factor analysis. These requirements are that the data should be a linear sum of product terms. This requirement is illustrated in eqn. 40.

$$\log k' = f_1^s f_1^c + f_2^s f_2^c + f_3^s f_3^c \quad (40)$$

Where f^s is a function of solvent composition and f^c is a function of the solute structure. The φ terms in eqn. 2 would be the f^s functions and the constants would be the f^c functions. The subscripts 1, 2 and 3 indicate that three factors are required to span this chromatographic data space. Eqn. 1 predicts a two factor solution and eqn. 13 predicts a six-factor solution.

A chemometric approach permits the use of a model, but this model can be an *abstract* one; that is, the *values* for the terms in eqn. 40 can be

determined without knowing the *form* of the functions. This abstract model permits the estimation of new data by several techniques. One technique that can be used is simply to interpolate between the known measurements using the abstract solvent co-factors. A second technique is target testing which allows the prediction of retentions for new compounds based on measurements made on a set of standards. These techniques and others can be combined to give a very powerful methods developments system.

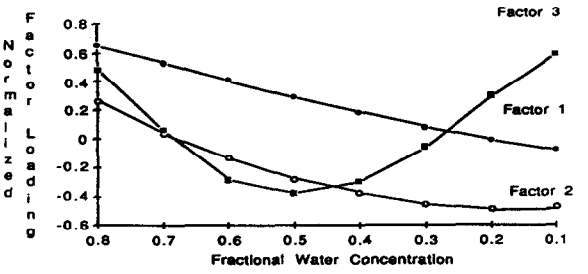

Fig. 2. Plot of solvent co-factor loadings as function of volume fraction water.

4.1. Principal components analysis

Principal components analysis is a technique which can be used to solve data sets where the individual data points can be represented by a multi-term sum of products equation such as eqn. 40. It allows the number of independent terms needed to describe the data to be determined and it gives a set of co-factors which are the (normalized) values of the terms of eqn. 40 [65].

The number of factors required to span a data set is known as its rank; it is the minimum dimensionality of the space needed to represent the relationships within the data. It is the minimum number of independent terms that eqn. 3 must have. To give a concrete example of an abstract (PCA) factor analysis of simple retention data, retention data for eight methanol–water mobile phases on 31 compounds were analyzed. The results of the factor analysis are summarized in Table II.

This table shows that 99.95% of the data variance can be accounted for using only two factors. Note however that the *F*-ratio test (*F*) gives a probability (*P*) of only 0.1% that the third factor is random, we must therefore conclude that this data spans a three-dimensional space. IE and RE are the imbedded error and real error respectively; they measure the amount of error which cannot be extracted from the data and the total error in the reproduced data respectively. The solvent co-factors, which correspond to the normalized values of the f_n^s functions are plotted with the identification of the fractional water composition in Fig. 2. Interpolation of the solvent co-factors allows prediction of retention at new compositions of the mobile phase.

TABLE II

RESULTS OF FACTOR ANALYSIS OF 8 METHANOL–WATER MOBILE PHASES ON 31 COMPOUNDS

EV = Eigenvalue; Var = variance; IE = imbedded error; RE = reduced eigenvalue; *F* = Malinowski *F*-test value; *P* = Probability from *F*-test.

Factor	EV	Var	IE[a]	RE[a]	IND	REV	*F*	*P*
1	$1.18 \cdot 10^{3}$	98.33	5.19	28.92	0.03	4.75	$1.86 \cdot 10^{2}$	0.000
2	$1.95 \cdot 10^{1}$	1.62	1.30	5.10	0.01	$9.27 \cdot 10^{-2}$	$8.82 \cdot 10^{1}$	0.000
3	$5.71 \cdot 10^{-1}$	0.05	0.38	1.21	0.00	$3.28 \cdot 10^{-3}$	$4.01 \cdot 10^{1}$	0.001
4	$2.03 \cdot 10^{-2}$	0.00	0.27	0.76	0.00	$1.45 \cdot 10^{-4}$	3.06	0.155
5	$6.57 \cdot 10^{-3}$	0.00	0.21	0.53	0.00	$6.09 \cdot 10^{-5}$	1.60	0.295
6	$2.95 \cdot 10^{-3}$	0.00	0.17	0.38	0.00	$3.78 \cdot 10^{-5}$	$9.88 \cdot 10^{-1}$	0.425
7	$2.31 \cdot 10^{-3}$	0.00	0.08	0.16	0.00	$4.62 \cdot 10^{-5}$	2.14	0.382
8	$5.19 \cdot 10^{-4}$	0.00	0.00	0.00	0.00	$2.16 \cdot 10^{-5}$	0.00	1.000

[a] IE and RE are multiplied by 100.

4.2. Target testing

To attach chemical significance to the abstract factors, one may presume that a column (or row) of real, measurable or calculable parameters (called a target vector) can be cast as a real factor and then test whether the hypothesis holds. This technique, known as target transformation factor analysis, uses a least-squares criterion for finding a transformation vector that best constructs the real target vector as a linear combination of the abstract co-factors [65,66]. When the transformation is able to reproduce closely target vector from the abstract co-factors, the target is presumed to be real factor. If the components of the target vector and the vector constructed via transformation closely agree, the parameter in question can be reasonably treated as an underlying variable in explaining the behavior encoded in a data matrix.

Target Testing Factor Analysis (TTFA) may be used to predict retention in RP-HPLC with the following advantages: The method gives reasonable predictions over a wide range of solutes and solvent conditions; a minimal number of experiments are required; and extension to new solvent compositions and solutes is relatively simple within a given chromatographic system. Further investigations may lead to the transportability of this method to other chromatographic systems.

A prediction strategy utilized by Lochmüller *et al.* [67] starts with the ln k' values for four "training solutes" (rows) chromatographed in each of three "training solvents" providing a core matrix of retention data. A column vector is constructed for each of the remaining solvents which consists of the ln k' values for each of the core solutes acquired in that solvent. These vectors are concatenated to the core matrix, resulting in an augmented matrix with four solute rows and with $3+n$ solvent columns (3 core and n remaining solvents).

The prediction of the retention behavior involves using the three core vectors (one for each key solvent, containing ln k' values for four core solutes) as a basis set in terms of which ln k' values for training solutes in other mobile phases are expressed. The augmented matrix is subjected to TTFA, using as targets the three core solvent vectors. A three-component loading vector for each solvent is produced in which each component describes the fractional contribution from one of the core solvent vectors toward the value in the new solvent. The loadings for each solvent as, modeled by TTFA, are characteristic of the solvent itself, independent of solute effects, so the predicted ln k' of the solutes in a given (modeled) solvent is simply the sum of three products:

$$\text{predicted ln } k' \text{ in solvent } x = \sum_{i=1}^{3} (\text{loading of solvent } x \text{ on core solvent } i) \cdot (\ln k' \text{ of solute in core solvent } i) \quad (41)$$

Once the core matrix is available, only three capacity factors are needed to model either a new solvent or solute. The size of the predication matrix expands as follows: given a set of m solvent loading vectors and a set of n solutes for which capacity factors in core solvents are known, the prediction matrix will be of the dimension $m \cdot n$; thus, if $m = 100$ and $n = 100$ then, from 600 measurements ($3m + 3n$), 10 000 predictions can be made.

5. CONCLUSIONS

Methods utilizing a physical model, methods which assume no model, and methods with an abstract or hidden model have been discussed. Although it is impossible to cite all of the work performed in developing models and methods for the prediction of retention and resolution in chromatography, representative examples of successful approaches have been given.

REFERENCES

1 L.R. Snyder, in Cs. Horváth (Editor), *High-performance Liquid Chromatography*, Academic Press, New York, Vol. 1, 1980, p. 207.
2 M.A. Quarry, R.L. Grob and L.R. Snyder, *Anal. Chem.*, 58 (1986) 907–917.
3 P.J. Schoenmakers, H.A.H. Billiet and L. de Galan, *J. Chromatogr.*, 205 (1983) 13.
4 I. Molnar, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 276.

5 R. Lehrer, *LC Mag.*, 1 (1983) 84.
6 J.P. Larmann, J.J. DeStefano, A.P. Goldberg, R.W. Stout, L.R. Snyder and M.A. Stadalius, *J. Chromatogr.*, 255 (1983) 163.
7 M.A. Quarry, R.L. Grob and L.R. Snyder, *J. Chromatogr.*, 285 (1984) 1.
8 M.A. Quarry, R.L. Grob and L.R. Snyder, *J. Chromatogr.*, 285 (1984) 19.
9 M.A. Stadalius, H.S. Gold and L.R. Snyder, *J. Chromatogr.*, 296 (1984) 31.
10 M.A. Stadalius, H.S. Gold and L.R. Snyder, *J. Chromatogr.*, 327 (1985) 27.
11 K.A. Cohen, J.W. Dolan and S.A. Grillo, *J. Chromatogr.*, 316 (1984) 359.
12 M.I. Aguilar, A.N. Hodder and M.T.W. Hearn, *J. Chromatogr.*, 327 (1985) 47.
13 S.R. Bakalyar, R. Mellwrick and E. Roggendorf, *J. Chromatogr.*, 142 (1977) 353.
14 N. Tanaka, H. Goodell and B.L. Karger, *J. Chromatogr.*, 158 (1978) 233.
15 R. Spatz and E. Roggendorf, *Z. Anal. Chem.*, 299 (1979) 267.
16 E. Roggendorf and R. Spatz, *J. Chromatogr.*, 204 (1981) 263.
17 J.L. Glajch, J.J. Kirkland, K.M. Squire and J.M. Minor, *J. Chromatogr.*, 199 (1980) 57.
18 P.J. Schoenmakers, H.A. Billiet and L. de Galan, *J. Chromatogr.*, 218 (1981) 261–284.
19 P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
20 R. Tijssen, H.A.H. Billiet and P.J. Schoenmakers, *J. Chromatogr*, 122 (1976) 185.
21 P.J. Schoenmakers, H.A.H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.
22 E. Katz, K. Ogan and R.P.W. Scott, *J. Chromatogr.*, 352 (1986) 67.
23 C.H. Lochmüller, M. Hamzhavi-Abedi and C. Ou, *J. Chromatogr.*, 387 (1987) 105–102.
24 R.E. Boehm and D.E. Martire, *Anal. Chem.*, 61 (1989) 471.
25 R.E. Boehm, D.E. Martire, D.W. Armstrong and K.H. Bui, *Macromolecules*, 16 (1983) 466.
26 R.E. Boehm, D.E. Martire, D.W. Armstrong and K.H. Bui, *Macromolecules*, 17 (1984) 400.
27 G. Glockner and J.H.M. van den Berg, *Chromatographia*, 19 (1984) 55.
28 M.A. Stadalius, M.A. Quarry, T.H. Moury and L.R. Snyder, *J. Chromatogr.*, 358 (1986) 17.
29 M.A. Stadalius, M.A. Quarry, T.H. Moury and L.R. Snyder, *J. Chromatogr.*, 358 (1986) 1.
30 L.R. Snyder, M.A. Stadalius and M.A. Quarry, *Anal. Chem.*, 55 (1983) 1412A.
31 M.A. Quarry, R.L. Grob and L.R. Snyder, *J. Chromatogr.*, 285 (1984) 19.
32 L.R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.
33 L.R. Snyder, *J. Chromatogr.*, 92 (1974) 223.
34 S.D. West, *J. Chromatogr. Sci.*, 25 (1987) 122.
35 M.J. Kamlet, R.W. Taft, P.W. Carr and M.H. Abraham, *J. Chem. Soc., Faraday Trans. I*, 78 (1982) 1689.
36 P.W. Carr, *J. Chromatogr.*, 194 (1980) 105.
37 C.R. Yonker and R.D. Smith, *J. Phys. Chem.*, 92 (1988) 2374.
38 L. Rohrschneider, *Anal. Chem.* 45 (1973) 1241.
39 J.H. Park, A. Hussam, P. Couasnon, D. Fritz and P.W. Carr, *Anal. Chem.*, 59 (1987) 1970.
40 S.C. Rutan, P.W. Carr, W.J. Cheong, J.H. Park and L.R. Snyder, *J. Chromatogr.*, 463 (1989) 21–37.
41 H. Poppe and E.H. Slaats, *Chromatographia*, 14 (1981) 89.
42 E.F. Meyer and R.E. Wagner, *J. Phys. Chem.*, 70 (1966) 3162.
43 E.F. Meyer, T.A. Renner and K.S. Stec, *J. Phys. Chem.*, 75 (1971) 642.
44 E.F. Meyer and C.A. Holtz, *J. Chem. Eng. Data*, 21 (1976) 274.
45 E.F. Meyer, M.J. Awe and R.E. Wagner, *J. Chem. Eng. Data*, 25 (1980) 371.
46 M. Chastrette, M. Rajzmann, M. Chanon and K.F. Purcell, *J. Am. Chem. Soc.*, 107 (1985) 1.
47 C.J. Tanford, *J. Am. Chem. Soc.*, 84 (1962) 4240.
48 Cs. Horváth, W. Melander and I. Molnar, *J. Chromatogr.*, 125 (1979) 129.
49 C.H. Lochmüller and D.R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
50 C.H. Lochmüller, H.H. Hangak and D.R. Wilder, *J. Chromatogr. Sci.*, 19 (1981) 130.
51 N. Tanaka, K. Sakagami and M. Araki, *J. Chromatogr.*, 199 (1981) 327.
52 R.K. Gilpin, *Am. Lab.*, 14 (1982) 104.
53 C.R. Yonker, T.A. Zwier and M.F. Burke, *J. Chromatogr.*, 241 (1982) 269.
54 D.E. Martire and R.E. Boehm, *J. Phys. Chem.*, 87 (1983) 1045.
55 C.H. Lochmüller, M. Hunnicut and J. Mullaney, *J. Phys. Chem.*, 89 (1985) 5770.
56 S.A. Wise, W.J. Bonnett, F.R. Guenther and W.E. May, *J. Chromatogr. Sci.*, 19 (1981) 457.
57 A. Rodecki, H. Lamparcyk and R. Kaliszan, *Chromatographia*, 12 (1979) 595.
58 J.L. Glajch, J.J. Kirkland, K.M. Squire and J. Minor, *J. Chromatogr.*, 199 (1980) 57–79.
59 M. Popyl, V. Dolansky and J. Coupek, *J. Chromatogr.*, 130 (1977) 195.
60 J.K. Baker and C. Ma, *J. Chromatogr.*, 169 (1979) 107.
61 R.M. Smith, *J. Chromatogr.*, 236 (1982) 313.
62 M.N. Hasan and P.C. Jurs, *Anal. Chem.*, 55 (1983) 263.
63 H.J. Hockel, G. Welter and H. Melzer, *J. Chromatogr.*, 388 (1987) 255.
64 S.D. West and D.H. Mowrey, *J. Chromatogr. Sci.*, 29 (1991) 497.
65 E.R. Malinowski and D.G. Howery, *Factor Analysis in Chemistry*, Wiley, New York, 1980.
66 P.H. Weiner, E.R. Malinowski and A. Levinstone, *J. Phys. Chem.*, 74 (1970) 4537.
67 C.H. Lochmüller, S.J. Breiner, C.E. Reese and M.N. Koel, *Anal. Chem.*, 61 (1989) 367.