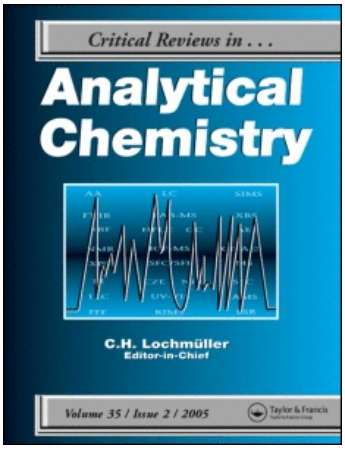

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QUANTITATIVE RELATIONSHIPS BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC RETENTION. IMPLICATIONS IN PHYSICAL, ANALYTICAL, AND MEDICINAL CHEMISTRY

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I. INTRODUCTION

The beginning studies of quantitative relationships between the structure of solutes and their chromatographic retention may be dated back as early as 1949. At that time, Martin,[1] in his fundamental paper, suggested that a substituent changes the partition coefficient of a solute by a factor that depends on the nature of the substituent and both the mobile and stationary phases employed, but not on the remaining part of the molecule. The phenomenon is now recognized as an example of a linear free-energy relationship (LFER).

Generally, LFERs may be regarded as linear relationships between the logarithms of the rate or equilibria constants for one reaction series and those for a second reaction series subjected to the same variation in reactant structure or reaction conditions.[2] The chromatographic retention parameters used in correlation studies are assumed to be proportional to the free energy change associated with the chromatographic distribution process.

Following Green et al.,[3] who found that substituent increments to the $R_M = \log (1/R_f - 1)$ are additive for a number of benzenoid compounds, Iwasa et al.[4] suggested that chromatographic data for studies of Quantitative Structure-Biological Activity Relationships (QSAR) may be useful. At the same time, Boyce and Milborrow[5] published a paper in which they correlated biological activity of a homologous series of amines with R_M data.

Successful applications of chromatography in QSAR studies, together with the availability of computers and the advanced statistical methods which have been used in QSAR calculations, produced the still-increasing interest in analogous studies of chromatographic retention. By analogy, the term Quantitative Structure-Retention Relationships (QSRR) has been proposed.[6]

Papers concerning the relationship between solute structure and chromatographic retention have as yet to be reviewed from the point of view of medicinal chemistry.[7-9] The meaning and limitations of QSRR as a tool for quantitation of physicochemical properties of solutes and for prediction of analytical parameters determining solute separation have only been briefly mentioned.

As the QSRR are derived statistically, there is some confusion in applying particular structural descriptors and in interpreting the regression equations obtained. The generalization of the results derived for an individual group of solutes and/or a specific chromatographic system over a wider range of variables is often unsubstantiated. Also, the predictive power of QSRR equations may be overestimated as a result of chance correlations.

A majority of QSRR studies are concerned with one fixed separation condition. Thus the eluate structure is the single variable considered. Obviously, to describe the molecular

structure of a solute quantitatively one should usually use several numerical structural descriptors. Recently, attempts have been reported where the changes in a liquid chromatographic mobile-phase and/or stationary-phase composition were taken into consideration. In such instances, linearity is assumed between the retention data and mobile-phase composition according to Soczewiński and Wachtmeister.[10]

The criterion of reliability of QSRR equations is in the consistency of the calculated retention data with what is actually observed. The fitness of an equation to the experimental data is evaluated by means of common statistical procedures described in detail in every statistics textbook. The most popular statistical descriptors are: correlation coefficient, r, standard deviation from regression equation, σ, and the value of the F-test, F. The square correlation coefficient, r^2, determines the fraction of the variances of retention data considered, which are "explained" by a given equation. When the number of experimental data analyzed is limited, high correlations can be obtained by including large numbers of independent variables when deriving regression equations. The correlations thus obtained can be statistically insignificant unless the F-test value for a given number of degrees of freedom is lower than the value calculated for a respective significance level. The sequential F-test allows one to decide whether an introduction of an individual independent variable into the regression equation is statistically justified. Chance correlations may also be obtained if one puts a great number of independent variables to the test, especially if the amount of dependent variables, i.e., retention data, is limited. If one is patient enough, it may happen that one or another of the many structural descriptors tested yields a QSRR equation that satisfies the formal statistical requirements. Such a fortuitous correlation is of no value either for retention prediction or for any mechanistic interpretation. As the number of structural descriptors is practically unlimited, especially if one uses various mathematical transformations of individual parameters, the final QSRR equations involving squares, cubics, reciprocals, square roots, etc. of structural descriptors must be carefully analyzed. In order to avoid chance correlations, the number of independent variables (usually molecular structural descriptors) considered together in deriving an individual QSRR equation should be at least five times less the number of dependent variables (usually individual solute retention data). The situation must also be avoided when one of the independent variables assumes similar numerical values for all the sets of solutes studied, except one or two of them. It is especially important that the independent variables be as orthogonal as possible. The occasionally observed colinearity of the structural parameters used in the same equation eliminates its informative value.

Although QSRR equations are derived and verified statistically, different strategies or approaches can be distinguished. The fundamental problem in QSRR studies is to find the proper molecular or submolecular features of solutes that determines their retention behavior. Next, it is indispensable that the molecular properties possibly important for retention be quantified. The quality of the relationships obtained when using these quantities is the criterion of the correctness of the starting assumptions.

The existing theories of chromatographic separations[11-13] provide information concerning the nature of the intermolecular interactions between solute molecules and the molecules of both the stationary and mobile phases. As it will be subsequently shown, the results of QSRR studies, especially of reversed-phase liquid chromatography (RPLC) systems and of gas chromatography (GC) on nonpolar stationary phases, are generally in accordance with the theory. Thus the QSRR obtained may illustrate the role of individual molecular interactions in a given separation process. Conversely, the ability of a solute to participate in particular molecular interactions can be exploited for retention prediction in certain given separation conditions.

The other approach to the structure-retention correlation is based on a linear dependence of the overall free energy changes on the corresponding enthalpy change for intrinsically

similar physicochemical phenomena. The theoretical treatment for chromatographic purposes has been described by Melander et al.[15]

As the RPLC has been long since extensively exploited for characterization of hydrophobic properties of drug candidates, many reports have been published dealing with the relation between retention parameters and the partition coefficients as determined by the classical equilibration procedures. For such QSRR studies, the solute structure has been characterized either by an overall molecular partition coefficient or by the substituent (fragmental) hydrophobic constants.

Regression analysis is most frequently applied in deriving QSRR. Occasionally, other statistical models are used. There are a number of papers in which abstract factors[16] have been determined as influencing solute retention. Usually a physicochemical meaning can be given to the solute factors, at least to a part of them.

As QSRR are extensively exploited in medicinal chemistry, a separate section is devoted here to the applications of chromatographically derived structural information in drug research.

II. CORRELATIONS BETWEEN RETENTION DATA AND SOLUTE MOLECULAR DESCRIPTORS

A. Molecular Interaction Approach

The distribution of a solute between a mobile phase and a stationary phase during the chromatographic separation process results from the forces that operate between solute molecules and the molecules of each phase. Thus, if the nature of the interactions between the solute molecule and the two phases can be determined, the behavior of a particular solute in a given chromatographic system could be predicted.

In his theoretical work, Scott[11] assumed that these interactions could be separated into three groups. One group would be the forces that are polar in nature, arising from permanent or induced electrically associated fields. The second group of forces would form the London's dispersion nonpolar forces. Specifically, in ion-exchange chromatography, the separation-determining forces are ionic in nature, but may include the previously indicated forces as well. Thus the distribution coefficient, K, of a solute between the two phases in a chromatographic system can be defined in the following way:[11]

$$K = \frac{[\Phi_i F_i P_i f_i(T) + \Phi_p F_p P_p f_p(T) + \Phi_d F_d P_d f_d(T)]_s}{[\Phi_i F_i P_i f_i(T) + \Phi_p F_p P_p f_p(T) + \Phi_d F_d P_d f_d(T)]_m} \quad (1)$$

where Φ is a constant and incorporates the probability of position of contact of a solute molecule with another molecule of a specific type (Φ is decided by the size and geometry of the molecules concerned); F is the magnitude of the respective force between the solute molecule and the phase molecule: P is the probability of molecular interaction; and f(T) is the thermal energy of the molecule at the time of contact and is constant at constant temperature, T. The subscripts i, p, and d denote ionic, polar, and dispersive interactions, respectively, whereas the subscripts s and m denote the stationary and mobile phases, respectively. The probability of interaction of a solute with one of the phases, P, will be proportional to the concentration of the interacting moieties in each of the respective phases. At any given stationary-mobile phase system, P_i, P_p, and P_d will be constant and can be incorporated in Φ. Furthermore, if the separations are carried out at a constant temperature, f(T) will be constant and can also be incorporated in Φ. Therefore,

$$K = \frac{(\Phi_i' F_i + \Phi_p' F_p + \Phi_d' F_d)_s}{(\Phi_i' F_i + \Phi_p' F_p + \Phi_d' F_d)_m} \quad (2)$$

If we consider GC, there are no ionic interactions, and the interactions in the gas phase are insignificant. Thus the value of K will be directly related to interactions only in the stationary phase:

$$K = m(\Phi'_p F_p + \Phi'_d F_d)_s \quad (3)$$

where m is constant. If one assumes that the differences in ϕ'_p and ϕ'_d values for different solutes are of little importance for their distribution in GC in comparison to the differences in F_p and F_d, one can write for a given j-th solute:

$$K_j = a\, F_{p_j} + b\, F_{d_j} \quad (4)$$

where a and b are constants.

The distribution coefficient is proportional to the retention parameters. If one is able to quantify the ability of a solute to participate in polar and dispersive interactions, then, taking into account all the approximations involved, one may expect to describe the GC retention index of a j-th solute, I_j, by the following equation:

$$I_j = \alpha \times \text{Polarity descriptor} + \beta \times \text{Dispersive descriptor} + \gamma \quad (5)$$

where α, β, and γ are constants.

In their expanded solubility parameter treatment, Karger et al.[12] have detailed how the abilities of a substance to participate in intermolecular interactions can be calculated from various nonchromatographic data. These authors subdivided interactions determining solubility parameters into dispersive interactions, dipole orientation interactions, and interactions consisting of induction of a dipole moment in surrounding molecules and those determining the species functioning as proton donors or acceptors. Solubility parameters, δ, can be determined from the energy of vaporization and the molar volume of a given compound. Considering orientation, induction, and hydrogen bonding interactions together as polar interactions, the solubility parameter may be described as a sum of polar, δ_p, and dispersion, δ_d, solubility parameter contributions:

$$\delta^2 = \delta_p^2 + \delta_d^2 \quad (6)$$

Karger et al.[12] assumed that δ_d does not change significantly from compound to compound in a homologous series. Thus, having δ experimentally calculated, they could evaluate δ_p from Equation 6. For a homologous series of a given functional group, the plots of log δ_p vs. log V (V is molar volume of the solute) are linear. The lines are parallel for different monofunctional homologous series (Figure 1).

The relationship, as illustrated in Figure 1, is as expected from the equation derived by Karger et al.[12] for the polar solubility parameter, δ_p:

$$\delta_p^2 = C \cdot \mu^2 f(V) \quad (7)$$

where C is a constant. The permanent dipole moment or bond dipole, μ, may also be assumed to be approximately constant for a homologous series of a given functional group. If there are no hydrogen bonding interactions involved, the δ_p for the solutes from different series, but having the same molar volume, should be directly proportional to μ, as it is illustrated in Figure 2.

It is evident from Figure 2 that the ester, RCO_2CH_3, is more polar than predicted from the dipole moment. As pointed out by Karger et al.,[12] this results from the fact that the

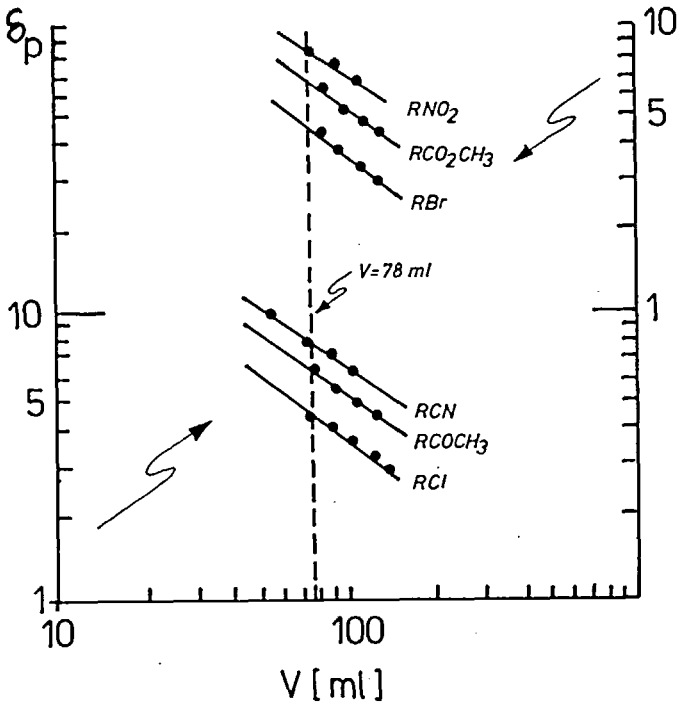


FIGURE 1. Logarithmic plot of polar solubility parameter, δ_p, against molar volume, V, for homologous series of monofunctional group compounds. (After Karger, B. L., Snyder, L. R., and Eon, C., *J. Chromatogr.*, 125, 71, 1976. With permission.)

overall dipole moment of the ester is the sum of two dipole vectors that are partially in opposite directions, causing some cancellation. Yet in solution, single (bond) dipoles from two molecules interact at close range with each other, and therefore these are the individual bond dipoles that interact effectively. It became clear then that for QSRR purposes the overall gas-phase dipole moment should not be considered, but some effective polarity measure should.

Although dispersive forces are common to the interaction of all molecules whether or not they possess a permanent dipole, for nonpolar substances these forces are the sole interacting forces. For quantitation of a dispersive solubility parameter, Karger et al.[12] applied the classical Lorentz-Lorenz expression.

It may be summarized then that the expanded solubility treatment by Karger et al.[12] leads to similar conclusions concerning QSRR as previously expressed here by Equation 5. Even if all the approximations are valid, the quantitation of a solute "effective" polarity can be extremely difficult.

The majority of successful QSRR studies concerns the data obtained from LC with nonpolar stationary phases. The separation of solutes in RP chromatography is explained theoretically[13,17] in terms of the so-called hydrophobic (solvophobic) effect.

A hydrophobic "bond" (the result of a hydrophobic effect) is formed when two or more nonpolar groups in an aqueous environment come into contact, thus decreasing the extent of interaction with the surrounding water molecules. The hydrophobic effect originates from a net repulsion between the water and the unpolar ligand of the stationary phase as well as the unpolar fragment of the solute.

As may be concluded from the detailed studies of Horváth et al.,[13] the high-performance liquid chromatography (HPLC) capacity factor, k′, of different solutes in hydrophobic chro-

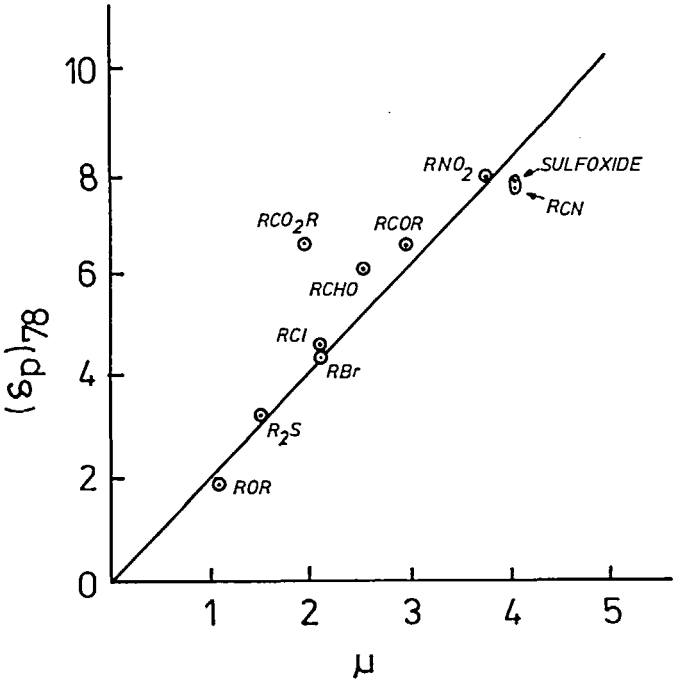


FIGURE 2. Plot of polar solubility parameter normalized to molar volume 78 mℓ, $(\delta_P)_{78}$, vs. gas-phase dipole moment, μ, for monofunctional group compounds. (After Karger, B. L., Snyder, L. R., and Eon, C., *J. Chromatogr.*, 125, 71, 1976. With permission.)

matography (when the same eluent and column are used) may be related to polar (the second term of Equation 8) and dispersive (the third term of Equation 8) properties of the solutes:

$$\ln k' = A + B\frac{1-\lambda}{2\lambda}\frac{\mu_s^2}{v_s}\frac{1}{1-(\alpha_s/v_s)} + C's \qquad (8)$$

where A, B, and C are constants; μ_s, v_s, and α_s are static dipole moment, molecular volume, and polarizability of the solute, respectively; λ is the proportionality factor reflecting the volume change occurring upon the binding of the solute to the ligand of a stationary phase; and s is the contact surface area of the associated solute and ligand. According to Horváth et al.,[13] the term A of Equation 8 can be considered constant for solutes having commensurable molecular dimensions. Thus the observed variation of the capacity factor with solute properties depends on the last two terms. The second term entails the dipole moment, the molecular volume, and the polarizability of the solute as well as the proportionality factor, λ. The third term of Equation 8 is essentially the product of the contact area in the complex and the C constant that reflects the surface tension of the eluent. In other words, the second term of Equation 8 may be related to the solute polarity. The contact area, s, is directly proportional to the hydrocarbonaceous surface area of the solutes. This in turn may be related to the increment of dispersive interactions to a solute capacity factor. The experimental k′ values obtained with a neat aqueous phosphate buffer at the same conditions for three groups of closely related aromatic solutes, i.e., carboxylic acids, amino acids, and amines, prove the general validity of Equation 8 despite all the approximations applied at its deriving (Figure 3).

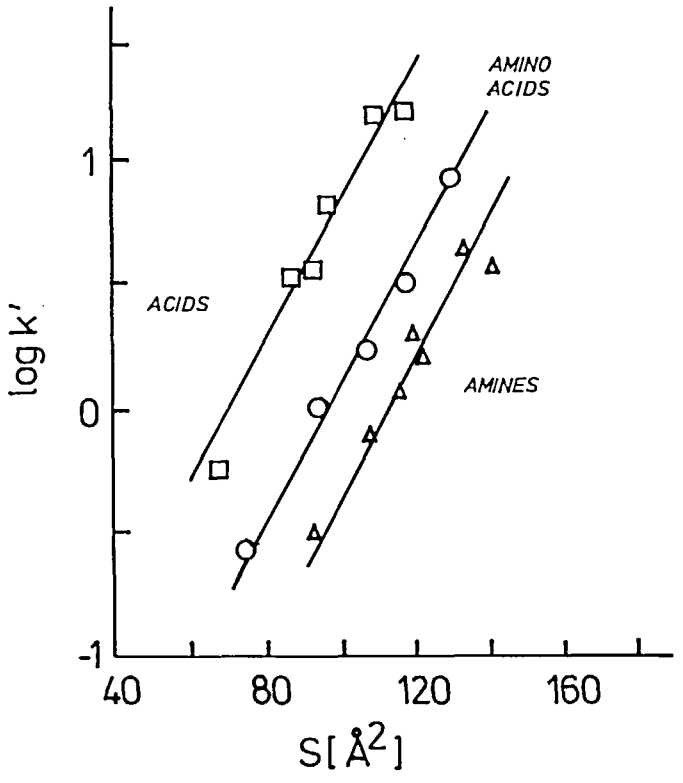


FIGURE 3. Relationship between the logarithm of the capacity factor, log k', and the hydrocarbonaceous surface are of different classes of solutes, S. (After Horváth, C. S., Melander, W., and Molnar, I., *J. Chromatogr.*, 125, 129, 1976. With permission.)

The straight lines of a similar slope obtained when capacity factors were plotted against the hydrocarbonaceous surfaces area suggest that the second term in Equation 8 is about the same for all members of a group. For closely related substances, the second term of Equation 8 may be assumed to be constant at the first approximation, and then Equation 8 can be simplified:

$$\ln k' = A' + C s \tag{9}$$

where A' is a new constant describing the first and second terms of Equation 8. The relative displacement of the lines representing the three different families of compounds in Figure 3 can be accounted for by the differences mainly in the second term in Equation 8 (the sign of that term is negative so that its absolute value would be smaller for the acids and greater for the bases). The second term in Equation 8 is rather complex. If it is related to solute polarity, it is evident that a dipole moment gives only an approximate description of this polar term.

For practical QSRR purposes, i.e., for detection of the solute structural properties quantitatively determining its chromatographic behavior, all three theoretical approaches discussed lead to similar conclusions. The specific molecular interaction treatment,[11] the expanded solubility parameter approach,[12] and the solvophobic theory[13] all lead to the assumptions expressed here in Equation 5, that chromatographic data can be related at the first approximation to both polar and dispersive properties of solutes.

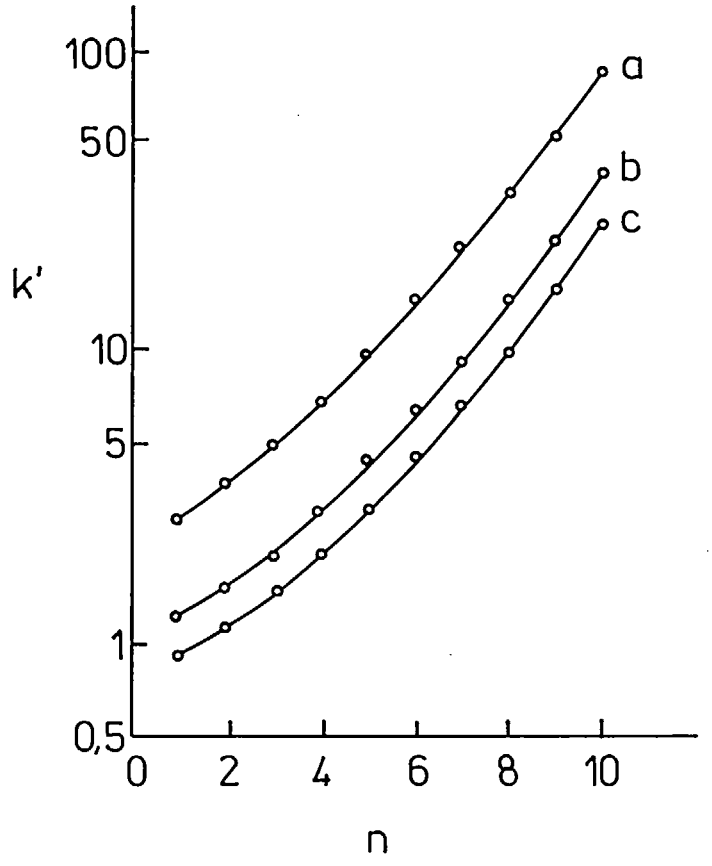


FIGURE 4. Relationships between capacity factor, k', and carbon number of alkyl chain, n, for homologous series of (a) 3-Cl, (b) 2-OCH_3, and (c) 2-NO_2-substituted alkylbenzoates. (After Tomlinson, E., Poppe, H., and Kraak, J. C., *Int. J. Pharm.*, 7, 225, 1981. With permission.)

The ability of a solute to undergo dispersive interactions seems to be unequivocally determinable. The polar interactions are much more complex, and to characterize them by means of a single quantity is difficult, but possible, for diverse sets of solutes.

1. Application of Additive and Additive-Constitutive Structural Parameters

a. Carbon Number, Molecular Mass, Parachor, Molar Refractivity, Polarizability, Molecular Volume, and Specific Surface Area

The linear relationships between the retention data and carbon number for different homologous series have long since been observed in various types and modes of chromatographic separation. It can be noted here that the deviations from linearity occasionally observed may result from differences in shielding that a given functional group present in the series by alkyl chains of various length. That effect may be encountered often in RPLC systems. For example, such deviations were reported by Clifford et al.[18] for R_M values of a series of alkyl-substituted dinitrophenols and by Tomlinson et al.[14] for log k' from HPLC of alkylbenzoates (Figure 4).

The relationship between retention data and carbon number holds only for a homologous series. For the compounds belonging to closely related but different groups, the relation is no longer valid, e.g., the incompatibility of log k' measured by RP-HPLC for alkylbenzenes and polyaromatics has been reported.[19]

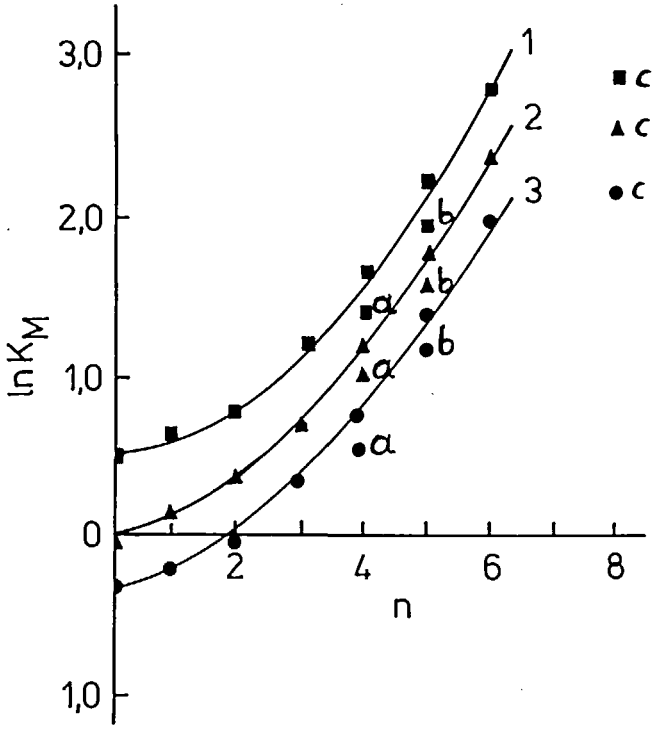


FIGURE 5. Correlation between carbon number, n, of alkylamines and logarithm of the selectivity coefficient, ln K_M, in cation-exchange chromatography. Column: 1 = K^+ form; 2 = Na^+ form; 3 = Li^+ form. (a) Isobutylamine; (b) isoamylamine; (c) β-phenylethylamine. (After Murakami, F., *J. Chromatogr.*, 198, 241, 1980. With permission.)

In GC, linearity between the isothermal relative retention data and carbon number is generally valid among the classes of substances. Basing their work on this observation, Ladon and Sandler[20] were able to derive regression equations relating the solute carbon number to its retention time on each of the three selected stationary phases. This relation holds for classes of compounds containing the same kind and number of functional groups such as alkanes, cycloalkanes, alkenes, cycloalkenes, and alkadienes. On the other hand, the position of the hydroxyl group in primary and secondary straight-chain alcohols affects the retention of that class of solutes.[21]

In cation-exchange chromatography of aliphatic amines, β-phenylethylamine, and aminoalcohols,[22] the plots of the selectivity coefficients, K_M, of the amines for the counter ion, M, against carbon number for individual subgroups of solutes are curvilinear (Figure 5).

Comparing aliphatic amines and aminoalcohols, Murakami[22] observed that the introduction of a hydroxyl group into the aliphatic amine molecule caused a decrease in adsorption, corresponding to a decrease in carbon number of about two.

In cases where specific interactions within a particular group of compounds contribute to retention to the same extent, a linear correlation between retention parameters and molecular mass is observed. Such relationships involving logarithm of GC retention volume and molecular mass of the solute were reported for various series of organometallic compounds[23,24] or nitrogen containing heterocycles.[25]

A linear correlation between logarithms of GC retention volumes and solute molar volumes was demonstrated by Wurst and Churacek[26] for organosilicon compounds. Several authors[27-31] found high correlations between Kováts retention indexes and either molar volume

or van der Waals volume for closely related sets of solutes. In the case of nonpolar solutes, e.g., alkylbenzenes,[30] the correlations between retention indexes and molar volume, van der Waals volume, molar refractivity, and index of refraction are significant. For 61 alkylbenzenes, correlation coefficient, r, of indexes obtained on a nonpolar stationary phase (squalane) against van der Waals volume was 0.94; in the case of molar volume, molar refractivity, and refraction index, the r values were 0.88, 0.92, and 0.23, respectively. The corresponding correlations obtained in the case of Kováts indexes determined on a polar phase (Carbowax® 20M) were significantly lower (except the refraction index) and amounted to 0.88, 0.77, 0.84, and 0.40, respectively. The authors[30] derived a two-parameter regression equation for a subgroup of 26 derivatives, which in their opinion could be used for the identification of components of industrial mixtures. Unfortunately, the subgroup selected is not defined precisely and the possible intercorrelation between the two parameters used in the equation, i.e., van der Waals volume and refraction index, is not determined for the data set considered.

In their studies on aliphatic esters, Bermejo and Guillen[31] observed high correlations between GC retention indexes determined on five phases of varying polarity and solute boiling points (bps). The correlation coefficients ranged from 0.9987 on SE-30 to 0.9724 on Carbowax® 1000. Having the predictive power of their relations in mind, these authors introduced additional parameters into their regression equations. In effect, they had sets of two- and three-parameter equations relating the retention index to bp, reciprocal of molecular volume, and dipole moment or refraction index. The standard deviations from the enlarged regression equations diminished, but from a statistical point of view the introduction of additional terms to the retention-bp relationship may be questioned.

The significance of improvement of correlation after introduction of successive parameters may be determined by the F-test:

$$F = \frac{(r_1^2 - r_2^2)/p}{(1 - r_1^2)/[N - (m + p) - 1]} \tag{10}$$

If one says that r_1 is the multiple correlation obtained when $(m + p)$ predictors are used and that r_2 is the multiple correlation found when only the m of them is retained, then Equation 10 provides a test of whether r_1 is significantly greater than r_2. This F-test has p and $[N - (m + p) - 1]$ degrees of freedom, and the value calculated is compared to the number tabulated for a given significance level, usually 95%. Another problem is the orthogonality of the data used in multiple correlation equations. The bps and the reciprocals of molar volumes used in the Bermejo and Guillen[31] equations are apparently intercorrelated and should not be used together.

Jinno and Kawasaki[32] studied the correlations between the logarithm of RP-HPLC capacity factors and the van der Waals volumes of 19 isomeric alkylbenzenes. Three RP-stationary materials (C-2, C-8, and C-18) of increasing alkyl chain lengths were used for generating retention data. Significant correlations between log k′ and van der Waals volume were found, especially in the case of C-8 and C-18 phases (r equals 0.93 and 0.92, respectively). The authors joined together the van der Waals volumes, connectivity index,[33] and a hydrophobic parameter (log P)[34] as the supposedly independent variables into one regression equation describing log k′ values. The following physical meaning has been assigned to individual parameters: van der Waals volume describes solute size, connectivity index characterizes its shape, and log P determines its hydrophobic properties. The correlation coefficient of the resulting three-parameter equations increases with respect to that obtained with a single parameter. However, the strong intercorrelations among the molecular descriptors used make the physical significance of the three-parameter equations reported questionable. The information concerning molecular size, shape, and hydrophobicity are contained in each of the individual descriptors used for the set of compounds studied. In other words, individual

descriptors used in the three-parameter equations may be represented by a linear function of one of them. Thus the physical meaning of the three-parameter equations reduces to that expressed by a respective one-parameter relation.

Among the other additive molecular descriptors, parachor has occasionally been used. For example, Wurst[35] demonstrated a linear correlation between parachor and logarithms of GC retention indexes for a series of related solutes.

In GC, linearity between retention parameters and solute molar refractivity was often observed, especially when congeneric solutes were chromatographed on nonpolar stationary phases. Basing their work on such relationships, Ellrén et al.[36] proposed the "refraction number", which is analogous to the Kováts retention index.

The relationships observed between the molecular polarizabilities of solutes and their chromatographic data were applied in assessing the electron polarizability.[37,38] In their studies on GC retention indexes of ten polycyclic aromatic hydrocarbons (PAH) on five stationary phases, Lamparczyk et al.[39] found linear relationships with average molecular polarizabilities. The discussion of the results in the original paper[39] is somewhat obscured by the incorrect assumption of the identity of the dimensionless Kováts index and the term in the classical Debye equation related to inductive solute-stationary phase interactions. In any case, linear relationships between retention indexes and experimental polarizability data were found for that closely related group of solutes. The percentage error of polarizability calculated from the regression equations ranged from 2 to 4% when compared with experimental values applied for deriving the relationships. In a recent paper, Lamparczyk and Radecki[40] have attempted to substantiate the polarizability-retention relationship previously observed for PAH. They started from the equation for the potential energy of van der Waals interactions, E:

$$E = -\frac{1}{r^6}\left[\frac{2}{3kT}\mu_a^2\mu_b^2 + \alpha_a\mu_b^2 + \alpha_b\mu_a^2 + \frac{3I_aI_b}{2(I_a + I_b)}\alpha_a\alpha_b\right] \quad (11)$$

where subscripts a and b denote the interacting molecules, μ is the dipole moment, T is the absolute temperature, r is the distance of interaction, α is the molecular polarizability, I is the first ionization potential, and k is Boltzmann constant. Equation 11 has previously been analyzed by Gassiot-Matas and Firpo-Pamies,[41] but these authors omitted the second and third terms in the equation, assuming that the induction effect is never important in interactions between neutral molecules.

In considering closely related compounds, Lamparczyk and Radecki[40] assumed an ionization potential constant among the series. At any given phase and temperature conditions, the terms r, T, μ_{phase} and α_{phase} can also be considered constant. Thus, if the assumption of a constant ionization potential in the series of solutes considered holds, after simple calculations one obtains the relation:

$$I = a \cdot \mu_{solute}^2 + b \cdot \alpha_{solute} - c \quad (12)$$

where I is the Kováts index and a, b, and c are constants. Thus the relation that can be practically verified in QSRR studies is of the same form as suggested in the late 1950s by Kováts and applied practically in QSRR by Gassiot-Matas and Firpo-Pamies[41] and by Kaliszan and Höltje.[42]

Lamparczyk and Radecki[40] assumed for PAH the dipole moment $\mu_{solute} = 0$ and thus they got a description of Kováts indexes determined for these compounds on various phases as a linear function of their polarizability. They also discussed the slope and intercept of their relations in terms of the constants a, b, and c of Equation 12, which were related to a stationary-phase polarizability and dipole moment.

Summarizing, one may note that there are a number of simple, easily determined or calculable molecular descriptors that may be related to the ability of a solute to participate in nonspecific intermolecular interactions. These parameters may be used in prediction of retention behavior of closely related solutes, i.e., in situations when the more structurally specific interactions for a given set of compounds are either negligible or very similar among the members of the series.

b. Topological Indexes

From the point of view of QSRR, especially interesting are the efforts to translate molecular structures into unique characteristic structural descriptors, expressed as numerical indexes. This can be attempted by means of the chemical graph theory, where a chemical structural formula is expressed as a mathematical graph. The formula shows how bonds connect different atoms in a molecule. The mathematical graph describes abstract vertices joined by edges. Each molecular graph may be represented either by a matrix, a polynomial, a sequence of numbers, or a numerical index (topological index).[43]

The connectivity index, χ, introduced by Randić[44,45] and developed by Kier and Hall,[33] has been extensively applied in QSRR studies. There are several modifications of the original Randić connectivity index, but the most popular is the first-order connectivity index, $^1\chi$, calculated by means of the following equation:

$$^1\chi = \sum_{s=1}^{t} (D_i D_j)s^{-1/2} \tag{13}$$

where s stands for an edge in a graph, t is the total number of edges, D represents the values attributed to the adjacent atoms i and j, according to the difference between the number of valence electrons and the number of hydrogen atoms suppressed.

In his original work, Randić[44] observed satisfactory agreement between the empirical branching index of Kováts and the connectivity index. Soon, a good correlation was reported by Karger et al.[17] between R_M values obtained by both RP-TLC (thin-layer chromatography) and RP-HPLC of isomeric alcohols and the connectivity index (group contribution). Regression analysis done by Kaliszan and Foks[46] yielded a highly significant relationship ($r = 0.95$) between $^1\chi$ and R_M data determined by RP-TLC for a group of 20 pyrazine carbothioamides. It must be emphasized that the solutes studied contained various alkoxy, aryloxy, aminoalkoxy, and alkylamino substituents.

Analyzing the literature for GC retention data[47] for cyclic alcohols and esters (Figure 6), Kaliszan[48] found a strong linear correlation ($r = 0.95$) of the retention index determined on nonpolar phase SE-30 and connectivity index. However, the analogous equations derived for polar stationary-phase Carbowax® 20M and EGSSX® (Supelco, Inc., Bellefonte, Pa., U.S.) were of little statistical value (r was 0.59 and 0.62, respectively). Thus, in the case of a more structurally diverse group of solutes interacting with polar stationary phases, the connectivity index does not suffice to discriminate individual compounds. Whereas, on nonpolar phases, the nonspecific dispersive interactions determine retention, the more specific electrostatic and inductive interactions predominate in the case of polar phases and polar solutes.

Using connectivity indexes as the GC retention predictors was successfully applied in QSRR studies of various isomeric hydrocarbons.[49-53] Other congeneric groups of solutes were also studied. Kier and Hall[54] related retention indexes of separate groups of aliphatic alcohols, aliphatic ketones, aliphatic ethers, and aliphatic esters to various connectivity indexes. For each group considered separately, good correlations were obtained between retention indexes determined on a nonpolar phase, squalene, and the first-order connectivity numbers. However, when the data determined on a polar stationary phase were considered,

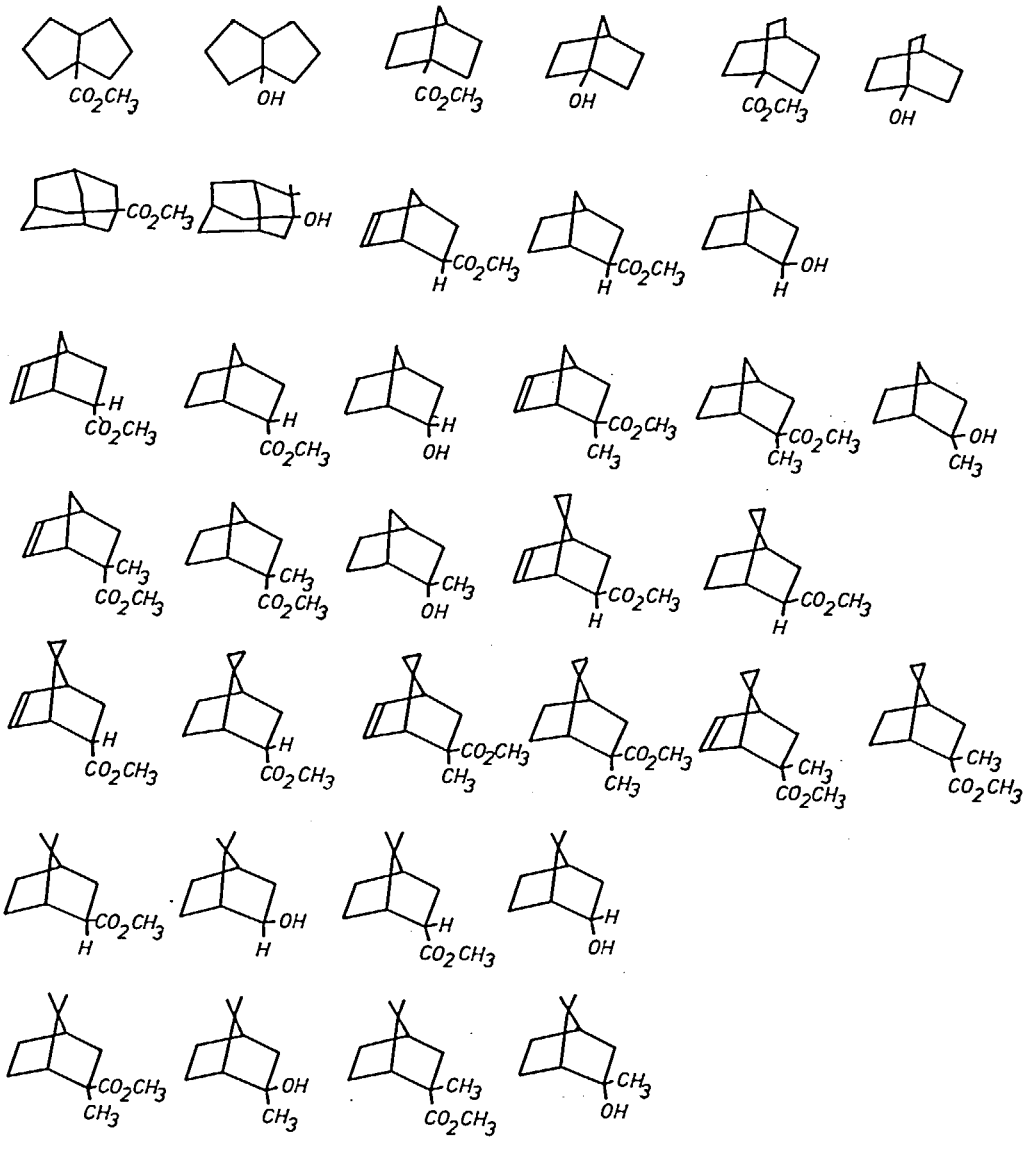


FIGURE 6. The group of methyl esters and alcohols considered in the retention index-connectivity index correlation analysis. (After Kaliszan, R., *Chromatographia*, 10, 529, 1977. With permission.)

the $^1\chi$ gave no satisfactory description of retention. To get higher correlation coefficients, different connectivity indexes were used in the two-parameter equations. The same was recently done by Sabljić[55] in the case of chlorinated benzenes. However, the two connectivity indexes used together in one regression equation are highly intercorrelated, which is especially evident for the $^1\chi$ and $^4\chi_{PC}$ parameters.

Similar reservations concern the multiparameter regression equations in which the connectivity indexes are applied along with a colinear parameter like molar volume.[32,56]

Sasaki et al.[57] introduced a novel substituent entropy constant representing the molecular connectivity index and correlated it with GC retention data for a congeneric group of solutes. Kaliszan[58] analyzed the relationship between connectivity indexes and ΔG, a GC-retention related parameter for a group of 39 methyl esters of saturated, mono-, di-, tri-, tetra-, penta-, and hexaunsaturated fatty acids. Free energies of solution in the stationary phase,

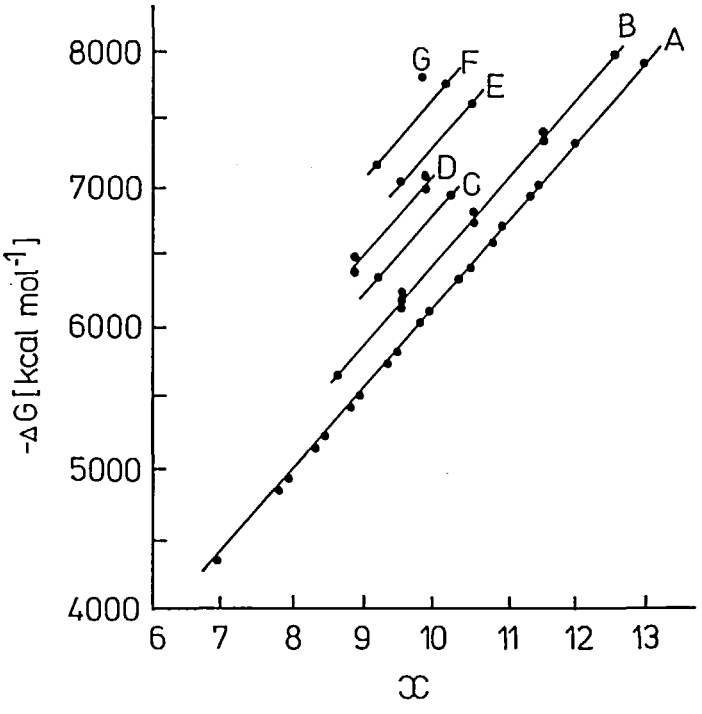


FIGURE 7. Partial molar free energies of solution in SILAR 5 CP as a function of the connectivity indexes for the methyl esters of saturated (A), monounsaturated (B), diunsaturated (C), triunsaturated (D), tetraunsaturated (E), pentaunsaturated (F), and hexaunsaturated (G), fatty acids. (After Kaliszan, R., *Chromatographia*, 12, 171, 1979. With permission.)

ΔG, were taken from the literature.[59] The ΔG values were precisely correlated to $^1\chi$ for each subgroup of solutes. The regression lines for individual subgroups were shifted in parallel (Figure 7).

In Figure 7, the discriminative power of $^1\chi$ towards isomers may be clearly seen. However, the connectivity index does not adequately reflect the retention differences caused by varying unsaturation. Thus, to describe retention of the solutes, a general equation was proposed:

$$-\Delta G = k_1 \cdot {}^1\chi + k_2 \cdot N + k_3 \qquad (14)$$

where k_1 to k_3 are constants and N is a function of the number and position of the double bonds in the acid chain; N may be considered as a polarity parameter.

The connectivity index was successfully applied for evaluation of the HPLC retention selectivity among homologous series. Both straight-phase[60] and RP systems[61] were studied.

The advantages and limitations of the connectivity index as a molecular descriptor for QSRR can be best observed for barbiturates. Barbiturates form a group of solutes differing in the structure of their hydrocarbon substituents in a pyrimidine ring. First correlations between retention data and the connectivity index for limited sets of barbiturates were published in 1978. Millership and Woolfson[51] analyzed GC data and Bonjean and Luu Duc[62] analyzed R_M values obtained by the RP-TLC technique. Later, a number of papers were published in this area.[56,63-68] Individual authors claimed that either the variously modified connectivity index or its combination with other connectivity indexes allowed precise prediction of a solute retention.

The most conclusive results for QSRR studies of barbiturates are those of the extensive

analyses performed by Bojarski and Ekiert.[56,65,68] According to the authors, the different modifications of the first-order connectivity index have no unambiguous advantages over the classical $^1\chi$, whereas it is more difficult to assign defined physical meaning to some of those modifications and transformations. The exception may be the modified first-order valence connectivity index applied by Stead et al.[66] to GC retention data.

The correlations with connectivity indexes are good for a closely related subseries of barbiturates, e.g., when simple saturated aliphatic substituents are present. The more diverse the structure of the solutes considered is, the lower the correlation coefficients independent of the chromatographic technique used for generating the retention data are. If the relationship log k′ vs. first-order valence index is considered,[65] one may note that when a subgroup of aliphatic substituents have a correlation coefficient that is r = 0.9920, then r drops to 0.9655 after inclusion of alkenyl substituents and to 0.9143 when *N*-methyl-*C*-5-disubstituted derivatives are additionally considered. For all the available derivatives, the respective correlation is r = 0.7426.

The last correlation coefficient realistically reflects the potency of the connectivity index as a molecular descriptor for QSRR studies of different solutes of a given congeneric class. Although the potency of χ as a parameter quantitatively reflecting molecular branching is unquestionable, its ability to differentiate a solute participation in complex chromatographic interactions should not be overestimated. The connectivity index is most probably a useful descriptor of nonspecific, dispersive interaction increments to retention.

Similar conclusions can be drawn from the multiple regression studies of Buydens et al.[69,70] These authors analyzed GC retention indexes determined on phases of different polarity for a large set of nonheterocyclic solutes. Multiparameter QSRR equations were obtained in which various connectivity indexes were present along with the other topological indexes, substituent electronic and hydrophobic constants, and quantum chemically calculated electronic indexes. In the case of nonpolar GC stationary phases, when the dispersion forces predominate in the intermolecular interactions, the chromatographic behavior of solutes can be explained, at least in part, by means of topological parameters. However, in the case of solutes of varying polarity, the significance of topological indexes for prediction of retention diminishes due to differences in inductive interactions with a stationary phase not accounted for by χ. With the polar phases and diverse sets of solutes, the χ parameters become of secondary importance for retention as the specific polar interactions become decisive.

For a number of topological indexes in use in chemistry,[43] some applications in QSRR studies found Wiener number, W, and Hosoya's index, Z. The connectivity index is generally highly intercorrelated with both W and Z indexes.[53,69] There is no published evidence proving the advantage of one of the topological indexes over another. The differences found by Sabljić[55] in his QSRR studies of GC retention indexes of chlorobenzenes may not be considred significant. The sequence as reported by Sabljić[55] would be: χ as the best, then W, and next J, another topological index proposed by Balaban.[71]

The so-called correction-structural number, STN, introduced by Dimov and Papazova,[72] may be calculated for several hydrocarbon classes by a correlation analysis of the influence of the different structural elements of the solutes on GC retention on a nonpolar phase. The theoretical retention index is predicted as the sum of STN and the physicochemical index, PCI, calculated on the basis of the vapor pressure and molecular volume of the solute and the corresponding *n*-alkanes.

2. *Parameters Related to the Specific Physicochemical Properties of the Solutes*

a. Dipole Moments and Electronic Substituent Constants

Following the early suggestions by Kováts, Gassiot-Matas and Firpo-Pamies[41] proposed a general equation for the calculation of the GC retention index, I:

$$I = a \cdot \chi + b \cdot \mu^2 + c \quad (15)$$

where a, b, and c are constants; χ is the connectivity index; and μ is the solute dipole moment. Equation 15 is similar to the previously discussed Equation 14 and may be considered as a special case of the general equation (Equation 5) with μ^2 as a solute polarity descriptor and χ as its dispersive descriptor. High correlations have been reported between the experimental retention indexes and those calculated from Equation 15 for several families of chemicals chromatographed on phases of different polarity. Earlier, some evidence of correlations between retention data and dipole moments was published by Ainshtein and Shulyatieva.[73]

Analyzing GC retention indexes of a large group of substituted phenols, Kaliszan and Höltje[42] used solute molar refractivity, MR, instead of χ as a dispersive descriptor. In the literature,[74] retention indexes were considered determined on a nonpolar phase dimethylpolysiloxane (SE-30) and two polar phases: 3-cyanopropylmethylpolysiloxane (OV-225) and polyneopentyl glycol adipate (NGA). For a limited set of 16 derivatives (experimental dipole moments were known for 20 solutes), the following relationships were found:

$$I\,(SE\text{-}30) = 66.67(\pm 14.42) \cdot \mu^2 + 25.34\,(\pm 5.22) \cdot MR + 57.33 \quad (16)$$

$$n = 16$$

$$r = 0.9679$$

$$s = 31$$

$$I\,(OV\text{-}225) = 164.72\,(\pm 36.61) \cdot \mu^2 + 24.48\,(\pm 12.90) \cdot MR + 408.33$$

$$n = 16$$

$$r = 0.9472$$

$$s = 77 \quad (17)$$

$$I\,(NGA) = 155.69\,(\pm 33.63) \cdot \mu^2 + 23.99\,(\pm 12.19) \cdot MR + 599.81$$

$$n = 16$$

$$r = 0.9477$$

$$s = 73 \quad (18)$$

where n is the number of solutes considered, r is the multiple correlation coefficient, and s is the standard deviation from the regression equation; the numbers in parentheses are the 95% confidence intervals as calculated by the *t*-test.

The correlations obtained in Equations 16 to 18 are highly significant statistically, but the predictive power of the equations is too low for analytical purposes. It should be noted that the increment of the dispersive interactions on all three phases employed is nearly constant, but the polarity term depends strongly on the characteristics of the individual stationary phase.

In order to find the relation between the polarity descriptor in Equation 5 and the electrostatically determined total dipole moment, the following argument was proposed.[8,42] If

one has retention indexes for a given set of compounds on a polar phase, I_p, and on a nonpolar phase, I_{NP}, then according to Equation 5 one can write:

$$I_P = a_P \cdot P + b_P \cdot D + c_P \tag{19}$$

$$I_{NP} = a_{NP} \cdot P + b_{NP} \cdot D + c_{NP} \tag{20}$$

where a, b, and c are constants, depending on the properties of the individual phases; P and D are solute polarity and dispersive descriptors, respectively. Taking P from Equation 20:

$$P = (I_{NP} - b_{NP} \cdot D - c_{NP})/a_P \tag{21}$$

Equation 19 can be rewritten as:

$$I_P = a_P(I_{NP} - b_{NP} \cdot D - c_{NP})/a_{NP} + b_P D + c_P \tag{22}$$

After rearrangement one gets:

$$I_P = \frac{a_P}{a_{NP}} \cdot I_{NP} - \left(\frac{a_P}{a_{NP}} \cdot b_{NP} - b_P\right) \cdot D + \text{const} \tag{23}$$

or

$$I_P = k_1 \cdot I_{NP} - k_2 \cdot D + k_3 \tag{24}$$

where k_1 to k_3 are constants.

The dispersive properties of solutes are assumed to be well characterized by the previously discussed additive molecular volume, topological indexes, etc. The relation[74] of the type expressed by Equation 24 was reported in the literature with MR as a dispersive descriptor. After recalculating the reported data[74] for 43 phenols, the following relationships were found:

$$\begin{aligned} &I\,(\text{OV-225}) = 1.95\ (\pm 0.16) \cdot I\,(\text{SE-30}) - 22.08\ (\pm 3.56) \cdot \text{MR} + 285.76 \\ &n = 43 \\ &r = 0.9691 \\ &s = 71 \end{aligned} \tag{25}$$

$$\begin{aligned} &I\,(\text{NGA}) = 1.81\ (\pm 0.20) \cdot I\,(\text{SE-30}) - 24.50\ (\pm 4.36) \cdot \text{MR} + 682.59 \\ &n = 43 \\ &r = 0.9472 \\ &s = 87 \end{aligned} \tag{26}$$

Equations 25 and 26 support the general validity of Equation 24.

Very recently, relationships of the type described by Equation 24 were found for complexes of dialkyldithiocarbamates with copper and nickel.[75] GC retention times measured on polar phases (NGA, OV-225) were related to analogous data obtained on a phase of low polarity (OV-101) with the help of an additional factor reflecting the size of the alkyl-substituent.

The constants a and b in Equations 19 and 20 may be related to polar and dispersive properties of stationary phases, respectively. Rearranging Equations 19 or 20, one gets:

$$I_P - b_P \cdot D = a_P \cdot P + c_P \quad (27)$$

or

$$I_{NP} - b_{NP} \cdot D = a_{NP} \cdot P + c_{NP} \quad (28)$$

Thus the term $I - b \cdot D$ would be related to solute polarity. Assuming D is reliably calculable, e.g., from MR, and having retention index, I, determined, one can get a quantity related to a solute chromatographic polarity. The quantity which must be known is the constant b. When $b_P = b_{NP}$, i.e., when the two phases, polar and nonpolar, have equal dispersive properties, then:

$$b_P = b_{NP} = k_2/(k_1 - 1) \quad (29)$$

where k_1 and k_2 are the coefficients of the regression equation (Equation 24) as described in Equations 25 and 26. For a first approximation, one can assume $b_P \approx b_{NP}$ for two phases of similar molecular weights. If the dispersive characteristics of the phases, b_P and b_{NP}, differ significantly, one of the two b values must be known. Then,

$$b_P = k_1 \cdot b_{NP} - k_2$$

or

$$b_{NP} = (k_2 + b_P)/k_1 \quad (30)$$

A possible way to obtain data related to the dispersive properties of a chosen standard phase would be to find the relation between the dispersive descriptors and retention indexes for a group of nonpolar compounds. The phase chosen should be as nonpolar as possible. Alternatively, different values of b calculated from Equation 30 can be compared with those obtained from Equation 5. In order to get a statistically significant relationship for an equation of the type of Equation 5, a certain number of retention indexes, polarity descriptors, and dispersive descriptors are required.

To evaluate the chromatographic polarity measure, $(I_P - b_P \cdot D)$ or $(I_{NP} - b_{NP} \cdot D)$ as expressed in Equations 27 and 28, for all the phenols studied, the electrostatically determined total solute dipole moment was assumed as the polarity descriptor. The numerical data of dipole moment determined in benzene solutions were known for 20 phenols. However, four of them have not fit Equations 16 to 18. Coefficients b in Equations 16 to 18 are very similar. Taking $b_{NP} = 25.34$ from Equation 16 and $k_1 = 1.95$ and $k_2 = 22.08$ from Equation 25, the $b_P = 27.3330$ can be calculated by Equation 30. Next, the chromatographic polarity parameter is calculated from Equation 27 $(I_P - b_P \cdot D)$ where D is represented by MR.

The chromatographic polarity parameter, as calculated by Equations 27 or 28, was correlated with quantum chemically calculated dipole moments for all 43 phenols considered. The dipole moments were calculated using the standard CNDO/2 procedure. In cases where more than one energetically favored conformer existed, the calculated dipole moment represented the arithmetic mean of the dipole moment values of the corresponding conformers. For the molecular geometries of the phenols, standard values were used.

The convincing correlation ($r = 0.92$) between the chromatographic polarity parameter and quantum chemically calculated dipole moment was found for 27 of 43 phenols considered. That observation provides additional evidence in support of the theoretical assumption that there is no identity of solute polarity and the total molecular dipole moment either electrostatically determined or quantum chemically calculated.

It seemed interesting whether the chromatographic polarity parameter could be of value for prediction behavior of chemicals in nonchromatographic systems. One such system is in a living organism. The chromatographic polarity parameter has actually been found for the description of the olfactory activity of a group of substituted phenols.[76]

In QSRR studies, polar properties of solutes were sometimes characterized by the well-known electronic substituent constants introduced by Hammett and Taft. In a series of papers, Fellous et al.[77,78] and Haken et al.[79,80] studied the Taft relationship, describing the logarithm of the GC retention time in terms of the polar constant of the substituent. For a homologous ester series, the correlations are reported as successfully established.[80]

Classical Hammett substituent constants for the *meta* and *para* positions were added together with the Taft constants for the *ortho* position for a series of 26 substituted phenols.[81] The thus-obtained polarity descriptor was found to be meaningful in multiparameter regression equations derived to predict retention of the solutes on polar GC phases. The question arises, however, as to what may be the physical meaning of a sum of different substituent constants.

Hammett sigma, σ, was applied by Jinno and Kawasaki[82] in studies of QSRR of phenols chromatographed on a C-18 HPLC column. Logarithms of capacity factors, log k′, were the dependent variables. Along with the Hammett constant, the hydrophobic substituent constant, π,[83] was applied as an independent variable. The σ-values were found to reflect differences in polarity among the set of solutes studied. The equation obtained has the form:

$$\log k' = k_1 \cdot \pi + k_2 \pi\sigma(1 - \pi) + k_3 \qquad (31)$$

where k_1 to k_3 are constants. In the composition of binary mobile-phase acetonitrile/water (65:35), the correlation coefficient of Equation 31 was $r = 0.954$. The authors[82] analyzed the relationship between the constants k_1 to k_3 and the volume fraction, X, of the organic modifier in the mobile phase. X ranged form 0.3 to 0.65 acetonitrile. The constant k_2 did not depend on X, whereas k_1 and k_3 were described by a two-parameter regression equation involving the X^2 and X terms. Five data points were used to perform multiregression analysis of k_1 to k_3 constants as functions of the mobile-phase composition. Thus, for the set of solutes chromatographed on the given RP column, one can predict the HPLC retention at any binary solvent composition, provided there is no chance correlation between the constants k_1 to k_3 in Equation 31 and the mobile-phase parameters.

Mokrosz and Ekiert[84] analyzed the effect of substituents in the phenyl ring of 5-arylidenebarbiturates and their *N,N*-dimethyl analogs on ability of the solutes to adsorb on silica gel. Apparent correlations were found between R_M values as determined by the TLC technique and the electronic substituent constants. The correlations were significant when the compounds studied were divided into subgroups, depending on the type and position of a substituent. Although the predictive potency of the relationship is too low for analytical purposes, the regularities found can be of use in discussing the TLC adsorption mechanism.

Buydens et al.[70,85] studied the importance of Hammett sigma, derived from the hydrolysis of aliphatic acids, for the prediction of GC retention on polar stationary phases. As could be expected, the electronic constant was the significant parameter in regression equations obtained. Generally, however, when the quantum chemical electronic descriptors are included in the regressions, they are preferred to the experimental σ-parameter.[70]

The electronic substituent constants have found limited application in QSRR. One reason for this is the lack of constants for many chemical moieties. The additivity of the sigmas may often be questioned. The effect of the chemical environment on electronic properties of the submolecular fragment considered is often prevailing. Most of the electronic constants were derived from the benzene system. Thus extrapolating them over heterocyclic and aliphatic systems can be dangerous.

In conclusion, one may summarize that generally the electronic substituent constants have no real advantage over dipole moments and quantum chemical indexes as the molecular polarity descriptors. Occasionally, electronic constants, used in addition to hydrophobic or dispersive parameters, may yield relationships comprising two or more related classes of solutes. This is observed when the separation process is performed on polar GC phases or when RP-HPLC is done on relatively polar solutes.

b. Quantum Chemical Indexes

Looking for a reliable measure of solute polarity, several authors turned their attention to quantum chemistry. For some time, standard computer programs have been commercially available that can be routinely used. Thus, at present, one need not be a professional quantum chemist to generate structural data of interest for QSRR studies.

To calculate molecular parameters of orbitals, the now classical Roothaan method is commonly applied consisting of the complete neglect of differential overlap (CNDO/2 method). This semiempirical approximate molecular orbital method, in which only the valence electrons are explicitly considered, is recognized as satisfactorily reflecting electronic properties of the solutes. In most cases, the calculations are done for the most stable conformation for molecules in the gaseous phase. The energetically favored conformations can be determined quantum chemically. For the calculation, lengths and angles of bonds can be taken from X-ray crystallography or the standard parameterization is assumed.

One of the first applications of quantum chemically calculated molecular descriptors in QSRR studies was specifically concerned with solute dipole moments.[42] As it was discussed in the preceding section of this article, the attempt to describe GC retention indexes of phenols in terms of MR and dipole moment have met with only limited success. As one could expect, the significance of the dipole moment term in regression equations (Equations 16 to 18) was higher in the case of polar stationary phases than in nonpolar ones.

In 1983, Buydens et al.[70] published the results of studies on the prediction of GC retention indexes for a group of solutes consisting of aliphatic ethers, esters, alcohols, ketones, and aldehydes. The authors used, in the multiple regression analysis, the following CNDO/2 calculated electronic parameters: magnitude of the dipole moment, DM; the sum of absolute values of the charges in a given molecule, QT; and the sum of absolute values of the charges of the atoms constituting the functional group and the atoms in α-position to this functional group, QA. As, in the dipole-dipole interaction, the energy of the process is proportional to the square of dipole moments; the square terms of the individual parameters were also included in the regression analysis.

To get meaningful QSRR, the subsets of mono- and bifunctional derivatives were considered separately.[70] Multiparameter regression equations involving quantum chemical indexes, along with several topological indexes and substituent constants, have shown the importance of electronic parameters for solute retention on polar stationary phases. Whereas for the nonpolar stationary phases the topological parameters of monofunctional molecules can explain a great part of the total retention variance, for the polar stationary phases a combination of topological and electronic parameters is necessary. When the quantum chemical indexes are included in the regression, they are preferred to the experimental Hammett sigma. The same authors[70] came to the conclusion that for the bifunctional molecules no parameter combination studied was really efficient enough to describe the specific interactions on the polar phases. As reported by the authors themselves, there is a significant intercorrelation among the structural parameters used simultaneously in individual regression equations. This makes it difficult to interpret the physical meaning of a particular parameter applied. However controversial the stepwise entry of the variables is, one can agree with the authors[70] that the parameters describing the local polarity (QA) are much more important than the total dipole moment, DM. This is especially evident when the retention indexes on polar stationary phases are considered.

CNDO/2 indexes were applied in QSRR studies of a series of *n*-alkenes by Garcia-Raso et al.[86] The authors analyzed GC retention indexes on squalane, previously used by Dubois et al.[87] in their topological analysis of alkenes. Very high correlations were observed between the Kováts indexes, I, and the quantum chemically calculated total energy, E_T, and binding energy, E_b. When the subgroup of *n*-alkenes was excluded, a three-parameter equation was derived for the remaining 62-*cis*- and *trans*-alkenes, predicting a retention index with a standard deviation as low as s = 4.66. Apart from E_T, the solute-stationary phase interaction factor, ΔE, and the energy of highest occupied molecular orbital, E_{HOMO}, were included in the equation. As the authors[86] emphasize, the three-parameter equation accurately represents the chromatographic behavior of *cis* and *trans* isomers of homologous alkenes.

Some explanation is required by the statement of the authors[86] that the two-parameter equations involving either E_T and ΔE or E_T and E_{HOMO} reduce the standard deviation of the equation I vs. E_T "slightly", but if they consider these two factors jointly (ΔE and E_{HOMO}), a large reduction in standard deviation results. The question arises whether the introduction of individual parameters into regression equations is statistically justified or if a chance correlation takes place. In any case, the quantum chemically calculated total energy (or binding energy) is the parameter of deciding importance for retention of this closely congeneric group of solutes of relatively low and nearly equal polarity. This is the case for retention on nonpolar stationary phases.

Another congeneric set of solutes studied by the same group of authors[88] forms seven homologous series of *n*-aliphatic esters. Excellent correlation was found between the CNDO/2 calculated total energy, E_T, and GC retention indexes determined on squalane and Carbowax® 1540 for an individual homologous series of esters. The well-known shift of the individual plots of I vs. E_T has been observed. Thus, considering the esters of the same E_T value, the most strongly retained were formates, then acetates, propionates, etc. The shifts of the plots for the respective homologous series were greater on polar Carbowax® 1540 phase than on the nonpolar squalane. The authors[88] attempted to find the selectivity parameters determining retention of esters with the same number of carbons, thus having very similar E_T values. They considered the orbital coefficients and energy of highest occupied molecular orbital. However, no two-parameter equation is given relating the retention index to total energy and the selectivity parameter proposed.

The more promising approach to the prediction of chromatographic selectivity based on molecular orbital calculations was reported by Nondek and Ponec.[89] Until now no application in QSRR studies has been found in the literature.

Analyzing the existing reports on the application of quantum chemical parameters in QSRR studies, one can conclude that there are still no quantities proposed that accurately characterize solute ability to participate in specific polar interactions with chromatographic phases. On the other hand, the total energy (or binding energy) seems to be a convenient descriptor of solute dispersive properties. Bearing in mind the complex nature of the specific chromatographic interactions as previously discussed, one realizes that it may be extremely difficult, if not impossible, to characterize a solute chromatographic polarity by means of a single parameter or a simple function of it. Nevertheless, quantum chemical calculations supply a large amount of information concerning the electronic structure of a solute, however approximate they may be. Undoubtedly, there is a chance that appropriate adaptation of quantum chemical indexes will yield a parameter comprising different solutes and stationary phases, as well as different chromatographic modes.

Recently, our group studied QSRR in a structurally diverse set of primary, secondary, and tertiary (heterocyclic) amines.[90] GC retention indexes were obtained on the methyl phenyl silicone (OV-101) phase of low polarity (Table 1).

Among the several CNDO/2 calculated electronic indexes, the total energy, E_T, was found to be of prevailing effect on retention. It describes 79% of the retention index variance

Table 1
KOVÁTS RETENTION INDEXES NORMALIZED TO THE TEMPERATURE 130°C AND STRUCTURAL PARAMETERS OF THE AMINES STUDIED

Solute	Retention indexes (I_{OV-101})	Total energy E_T (a.u.)	Polarity parameter (electrons)	Energy of HOMO (a.u.)	Dipole moment μ (D)	Molar refractivity (MR)
Allylamine	463	−38.180	0.3101	−0.4964	1.7229	18.926
n-Butylamine	553	−48.597	0.3110	−0.4843	1.8984	24.044
sec-Butylamine	471	−48.596	0.3413	−0.4847	1.8975	24.044
tert-Butylamine	501	−48.593	0.3600	−0.4895	1.8971	24.044
n-Pentylamine	635	−57.281	0.3113	−0.4767	1.9067	28.688
n-Propylamine	466	−39.717	0.3139	−0.4936	1.8930	19.400
iso-Pentylamine	615	−57.272	0.3145	−0.4793	1.8896	28.688
iso-Propylamine	469	−39.921	0.3439	−0.5048	1.8317	19.400
Diallylamine	660	−62.519	0.2833	−0.4685	1.7733	32.572
Di-*n*-propylamine	694	−65.964	0.2917	−0.4655	1.8426	33.520
Dietylamine	527	−48.602	0.2897	−0.4767	1.8613	24.323
Methyl-*n*-pentylamine	706	−65.963	0.2786	−0.4626	1.8929	33.520
Methyl-*n*-hexylamine	871	−74.583	0.2786	−0.4590	1.9108	38.164
Methyl-*n*-butylamine	630	−57.279	0.2786	−0.4670	1.8793	28.876
Di-*n*-butylamine	943	−83.242	0.2875	−0.4595	1.8501	42.808
Pyrazine	696	−54.621	0.1723	−0.4564	0.0048	21.482
Pyridine	692	−50.866	0.2338	−0.4707	2.1019	23.890
β-Picoline	841	−59.554	0.2198	−0.4652	2.1261	28.508
3-Chloropyridine	890	−66.356	0.2570	−0.4757	2.1976	26.349
Chloropyrazine	895	−70.038	0.3024	−0.4658	2.1565	28.757
2-Chloropyridine	870	−66.245	0.3120	−0.4737	3.6424	26.349
4-Cyanopyridine	955	−68.643	0.2471	−0.4809	0.9618	28.205

among the solutes. If the subseries of primary, secondary, and heterocyclic amines are analyzed separately, E_T is correlated to retention index at the same level as MR, but only for the primary and secondary amines. In the third case, i.e., for heterocylic amines differing significantly in structure, E_T is much better correlated with retention than MR. Thus the CNDO/2 calculated total energy seems to be the most reliable parameter to quantify the ability of a solute to participate in dispersive interactions with stationary phase.

Since about 20% of variance in retention data has not been explained by the total energy, attempts were undertaken to characterize a solute polarity increment to the retention. However, when the CNDO/2 calculated dipole moment was used along with E_T in a two-parameter regression equation, its significance level was only $p<0.5$. Looking for a more reliable measure of chromatographic polarity, we considered electron excess charges on individual atoms in a molecule. In effect, we proposed a submolecular polarity parameter, Δ. To determine Δ, electron densities on each atom in the molecule are calculated and two atoms are found with highest electron excess and deficiency. The difference of electron densities for the two atoms gives Δ (see Figure 8 and Table 1).

. The regression equation relating Kováts index, I, to E_T and Δ is as follows

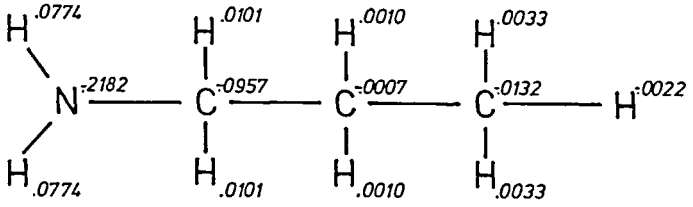
$$I = -11.66 \cdot E_T - 1016.80 \cdot \Delta + 301.88$$

$$n = 22$$

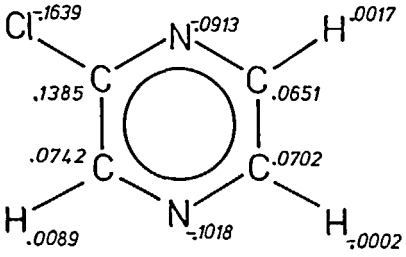
$$r = 0.93$$

$$s = 67.45 \qquad (32)$$

Equation 32 is significant at the $p<0.0001$ level; the terms E_T and Δ are significant at the



$$\Delta = 0{,}0957 - [-0{,}2182] = 0{,}3139$$



$$\Delta = 0{,}1385 - [-0{,}1639] = 0{,}3024$$

FIGURE 8. Electron excess charge densities and polarity parameter, Δ. (After Ośmiałowski, K., Halkiewicz, J., Radecki, A., and Kaliszan, R., *J. Chromatogr.*, 346, 53, 1985. With permission.)

$p<0.0001$ and $p<0.006$ levels, respectively. Like the previously discussed local polarity indexes proposed by Buydens et al.,[70] the parameter Δ differentiates solutes polarities better than dipole moment. The amines described by Equation 32 can hardly be considered as congeneric in respect to their polar properties. The lone electron pair of nitrogen atoms present in each compound makes comparable some feasible interactions with the stationary phase, e.g., charge-transfer interactions. Having the charge-transfer interactions in mind, we considered the energy of the highest occupied molecular orbital. The statistics of the three-parameter equation relating Kováts index to E_T, Δ, and E_{HOMO} has been good, but high intercorrelation between E_T and E_{HOMO} ($r = 0.77$) makes any interpretation at least controversial.

The approximate character of polarity parameter, Δ, is obvious. It better characterizes polar interactions with stationary phases than does dipole moment as, probably, the polar interactions in chromatography are actually the sum of contributions from several local intramolecular dipoles in closest contact with the phase. The Δ is as good an approximation of these interactions as the largest of the acting dipoles, prevailing over the others. In any case, the local polarity measure can be a subsequent step on our way to also accomodate the noncongeneric solutes in QSRR.

Although there are numerous reports dealing with quantitative relationships between liquid chromatographic distribution parameters and solute hydrophobic/hydrophilic partition coefficients, there are only a few publications on QSRR involving structural descriptors derived from a simple molecular formula. The additive dispersive descriptors used in QSRR studies of strictly congeneric families of solutes were discussed earlier.

Unlike the GC separation where the differences in solute-carrier gas interactions can be neglected, there are three main variables determining distribution of a solute between a

mobile and a stationary LC zone. At constant temperature of separation, the three variables are: chemical structure of the solute, physicochemical properties of the mobile phase, and physicochemical properties of the stationary phase.

In HPLC, solute distribution is easily quantified by means of retention parameters, usually the capacity factor. If one gets numerical measures of properties of the solutes, of the mobile phases, and of the stationary phases, one can attempt to derive a general relationship linking the appropriate quantities and retention parameters together. If the quantitative relationships between the retention data, as dependent variables, and the numerically expressed properties of the solutes, the mobile phases, and the stationary phases, as independent variables, are precise enough, one may use them to predict retention of a given solute at a given mobile-phase composition on a given stationary phase. Recently, we have attempted to find such a general relationship for a diverse set of benzene derivatives chromatographed in several RP-HPLC systems.[91]

As it is well known, at some range of a two-component mobile-phase composition, the logarithm of the HPLC capacity factor, $\log k'_{ij}$, for i-th solute determined on j-th stationary phase, depends linearly on the mole fraction, X, of one of the solvent components, as described by the Soczewiński-Wachtmeister relation:[10]

$$\log k'_{ij} = a_{ij} \cdot X + b_{ij} \tag{33}$$

where a_{ij} and b_{ij} are constants for a given solute, i, chromatographed on an individual stationary phase, j. In fact, the relationship between the logarithm of the RP-HPLC capacity factor and the volume fraction of an organic modifier in a binary mixture with water is more complex[92,93] (Figure 9). However, over limited ranges of binary composition, a linear relationship can often be used as a good approximation.

In our studies,[91] we observed linearity between log k′ of benzene derivatives and mole fraction, X, of water in a methanol/water solvent for four C-18 stationary phases of different hydrocarbon coverage. Thus, for a given stationary phase coverage, the constants a_{ij} and b_{ij} are some functions of the structure of the solutes. Using multiparameter regression analysis, we have found that the constants a_{ij} and b_{ij} are satisfactorily described by a two-parameter equation involving the quantum chemically calculated total energy of a solute, E_{Ti}, and its polarity parameter, Δ_i, previously discussed. The constants a_{ij} and b_{ij} determined for an individual solute, i, on the stationary phase, j, are

$$a_{ij} = \alpha_j \cdot E_{Ti} + \beta_j \cdot \Delta_i + \gamma_j \tag{34}$$

$$b_{ij} = \alpha'_j \cdot E_{Ti} + \beta'_j \cdot \Delta_i + \gamma'_j \tag{35}$$

where α_j, β_j, γ_j, α'_j, β'_j, and γ'_j are regression coefficients derived by the conventional least-squares method.

Having the a_{ij} and b_{ij} data for i compounds determined on j phases from i × j regression equations of the form of Equation 33, one can attempt to describe them in terms of E_{Ti} and Δ_i of i-th compound. If Equations 34 and 35 are statistically significant, then Equation 33 for the j-th phase may be rewritten as follows:

$$\log k'_{ij} = (\alpha_j \cdot E_{Ti} + \beta_j \cdot \Delta_i + \gamma_j) \cdot X + (\alpha'_j \cdot E_{Ti} + \beta'_j \cdot \Delta_i + \gamma'_j) \tag{36}$$

The phases under study differed in their C-18 coverage. A linearity among log k′ values determined for alkylbenzenes on hydrocarbonaceous stationary phases of different alkyl chain lengths (C-2, C-8, and C-18) was reported.[94] We expected a similar relationship in

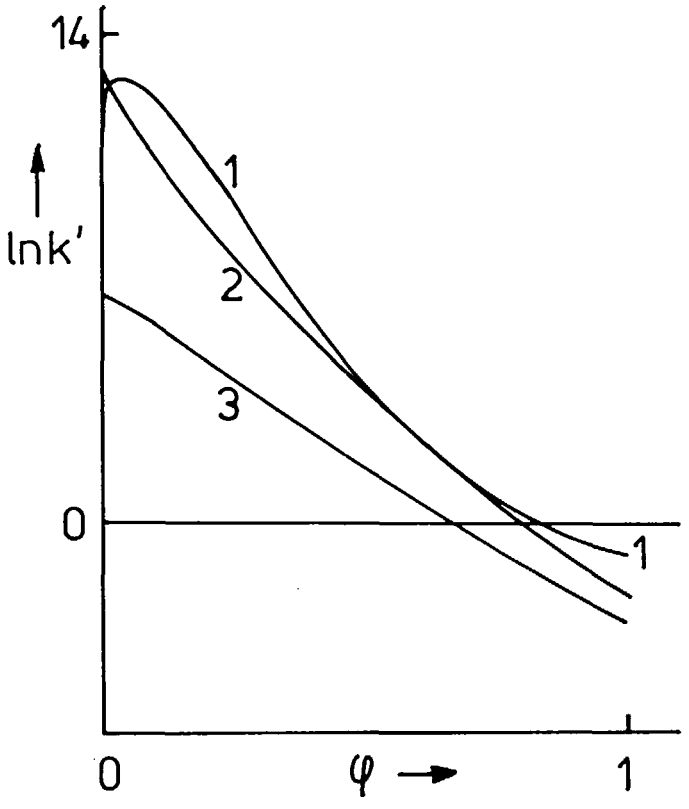


FIGURE 9. An example of experimentally observed relationship between logarithm of the RP-HPLC capacity factor, ln k', and volume fraction, ϕ, of methanol in methanol/water system. Solutes: 1 = naphthalene; 2 = benzophenone; 3 = *p*-chlorophenol. (After Schoenmakers, P. J., Billiet, H. A. H., and de Galan, L., *J. Chromatogr.*, 282, 107, 1983. With permission.)

respect to the stationary-phase surface coverage with octadecyl chains. Actually, for a given solute, i, chromatographed at a fixed mobile phase composition, $X_{constant}$, linearity has been found between log $k'_{ij,X}$ and C-18 surface coverage, C_j, for the first three of four phases studied:

$$\log k'_{ij,X} = A \cdot C_j + B \tag{37}$$

where A and B are regression coefficients. In such a situation, it seemed probable that coefficients a_{ij} and b_{ij} of Equation 33 (or coefficients α_j, β_j, γ_j, α'_j, β'_j, and γ'_j in Equations 34 and 35) depend not only on the solute structure described by individual E_{Ti} and Δ_i parameters, but also depend linearly (over a limited range) on stationary phase C-18 coverage, C_j. Thus the most general equation describing capacity factors in terms of solute structure, mobile-phase composition, and stationary-phase surface properties is

$$\log k'_{ij} = (\alpha \cdot E_{Ti} + \beta \cdot \Delta_i + \gamma \cdot C_j + \delta) \cdot X + (\alpha' \cdot E_{Ti} + \beta' \cdot \Delta_i + \gamma' \cdot C_j + \delta') \tag{38}$$

To derive the regression coefficients α, β, γ, δ, α', β', γ', and δ', the variable matrices of (i × j) × 4 dimensions are considered,

$$
\begin{array}{cccc}
a_{11} & E_{T1} & \Delta_1 & C_1 \\
a_{21} & E_{T2} & \Delta_2 & C_1 \\
a_{31} & E_{T3} & \Delta_3 & C_1 \\
\cdots & \cdots & \cdots & \cdots \\
\cdots & \cdots & \cdots & \cdots \\
a_{i1} & E_{Ti} & \Delta_i & C_1 \\
a_{12} & E_{T1} & \Delta_1 & C_2 \\
a_{22} & E_{T2} & \Delta_2 & C_2 \\
a_{32} & E_{T3} & \Delta_3 & C_2 \\
\cdots & \cdots & \cdots & \cdots \\
\cdots & \cdots & \cdots & \cdots \\
a_{i2} & E_{Ti} & \Delta_i & C_2 \\
\cdots & \cdots & \cdots & \cdots \\
\cdots & \cdots & \cdots & \cdots \\
a_{1j} & E_{Ti} & \Delta_i & C_j \\
\cdots & \cdots & \cdots & \cdots \\
\cdots & \cdots & \cdots & \cdots \\
a_{ij} & E_{Ti} & \Delta_i & C_j
\end{array} \tag{39}
$$

and analogously for b_{ij} coefficients.

Of the 14 benzene derivatives chromatographed at varying conditions, 2 have been ex cluded from correlation analysis after preliminary attempts. The two were toluene an chlorotoluene, highly hydrophobic nonpolar solutes. The compounds under study were eac chromatographed at four methanol/water mobile-phase compositions on four stationary phase of varying C-18 coverage. The capacity factors, k′, were calculated from the standard relatio $k' = (t_R - t_O)/t_O$, where t_R was retention time of the solute and t_O was determined for th unretained tracer, uracil, at specific conditions.

For each compound chromatographed on all four phases, the relation of the form o Equation 33 was derived yielding 48 a_{ij} and 48 b_{ij} coefficients (Table 2).

The studies of the relationship between log k′ determined for a given solute at any give mobile-phase composition and any given C-18 coverage of stationary phase have shown tha the linearity was observed at the C-18 surface concentration range 1.54×10^{-4} to 4.96×10^{-4} g of C-18 per gram. In such a situation, the phase of 6.6×10^{-4} g of C-18 per gra coverage has been excluded from further correlation analysis.

To derive coefficients a_{ij} and b_{ij} of Equation 33, as expressed in Equation 38, the variabl matrices of $(12 \times 3) \times 4$ dimensions (Equation 39) were considered. The final equatio thus obtained is

$$
\begin{aligned}
\log k'_{ij} = {} & [0.0454\ (\pm 0.0071) \cdot E_{Ti} + 2.6493\ (\pm 0.9187) \cdot \Delta_i \\
& - 0.1053\ (\pm 0.0672) \cdot C_j - 0.4946\ (\pm 0.5828)] \cdot X \\
& + [-0.0381\ (\pm 0.0039) \cdot E_{Ti} + 2.1659\ (\pm 0.4919) \cdot \Delta_i \\
& + 0.1696\ (\pm 0.0359) \cdot C_j + 1.2963\ (\pm 0.3120)]
\end{aligned} \tag{40}
$$

The statistics are, for the a_{ij} term, $n = 36$, $s = 0.2756$, and $r = 0.9251$; and for the b_{ij} term, $n = 36$, $s = 0.1476$, $r = 0.9715$. When the two hydrophobic solutes, toluene an chlorotoluene, were included, the statistics deteriorated: for the a_{ij} term, the correlatio coefficient was $r = 0.9035$, and for the b_{ij} term, its value was $r = 0.9285$.

Table 2
COEFFICIENTS a AND b OF THE EQUATION LOG k = aX + b AND CNDO/2 MO PARAMETERS USED IN CORRELATION ANALYSIS

Solute	Coverage 1.54×10^{-4} g (C_{18}/g) a	b	Coverage 3.18×10^{-4} g (C_{18}/g) a	b	Coverage 4.96×10^{-4} g (C_{18}/g) a	b	Coverage 6.6×10^{-4} g (C_{18}/g) a	b	Total energy (a.u.) E_T	Maximum excess charge difference Δ	Dipole moment (D) μ
Phenol	−2.6959	0.4536	−2.5581	0.6639	−2.8606	0.9593	−2.6078	0.9413	−65.5548	0.4328	1.7492
Acetophenone	−3.0864	0.9565	−3.0556	1.1786	−3.4881	1.5156	−2.5891	1.2605	−81.1756	0.5088	3.0417
Nitrobenzene	−2.7960	0.9358	−2.7800	1.1596	−3.5946	1.6078	−2.9937	1.5424	−94.8446	0.7774	5.0589
Methylbenzoate	−3.4745	1.3031	−3.5860	1.6051	−3.9130	1.9501	−3.6501	1.9751	−99.6184	0.6836	2.0376
p-Cresol	−3.0970	0.8482	−2.9067	1.0646	−3.3485	1.4121	−3.0797	1.4014	−74.2375	0.4265	1.7379
p-Ethylphenol	−3.3854	1.1933	−3.5349	1.5152	−3.7593	1.8154	−3.5988	1.8782	−82.6639	0.4275	2.3324
p-Propylphenol	−4.0343	1.6683	−4.1325	2.0059	−4.3633	2.3292	−4.2734	2.4389	−91.3479	0.4270	2.1472
p-sec-Butylphenol	−4.4055	1.9693	−4.6025	2.3567	−4.8630	2.7080	−4.7467	2.8292	−100.1758	0.4246	1.8457
Aniline	−2.4448	0.6024	−2.1299	0.5849	−2.5553	0.8762	−2.1672	0.7902	−59.5473	0.3737	1.5206
N-Methyl-aniline	−2.8347	0.9873	−2.6928	1.0879	−2.8933	1.3476	−2.8162	1.4047	−68.2305	0.3564	1.1504
p-Chloroaceto-phenone	−3.4547	1.3868	−3.8131	1.7990	−3.8413	2.0147	−3.8169	2.1259	−96.6241	0.5028	2.2894
3,4-Dichloro-acetophenone	−3.8477	1.8329	−4.3011	2.2945	−4.3579	2.5474	−4.3741	2.6970	−112.3142	0.4922	1.2809

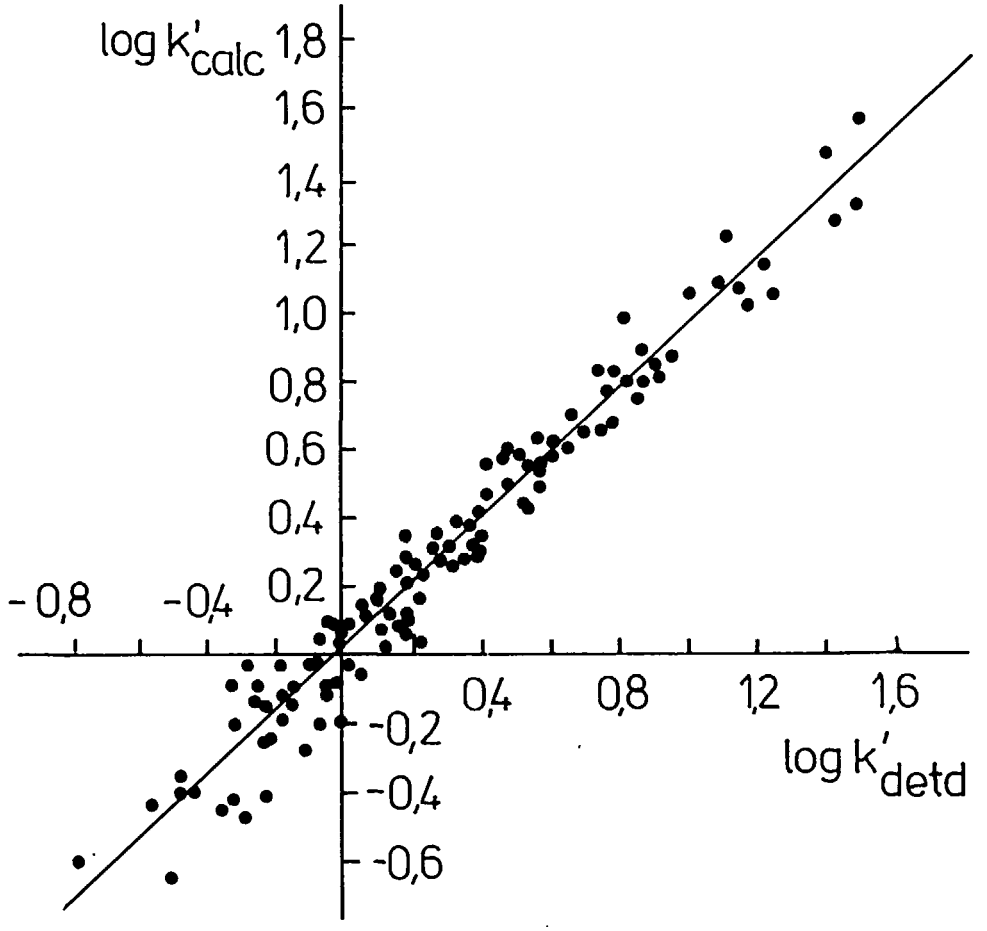


FIGURE 10. Relationship between the log k' determined experimentally and calculated by means of Equation 40. (After Kaliszan, R., Ośmiałowski, K., Tomellini, S. A., Hsu, S.-H., Fazio, S. D., and Hartwick, R. A., *J. Chromatogr.*, 352, 141, 1986. With permission.)

For the sake of comparison, a similar regression analysis was done after replacing the polarity parameter, Δ_i, with the CNDO/2 calculated dipole moment. The statistics obtained were significantly lower. For a_{ij} calculated in terms of E_{Ti}, M_i, and C_j, the correlation coefficient was r = 0.8710; for b_{ij}, the corresponding value was r = 0.9040.

The correlation between dipole moment, μ_i, and the polarity parameter, Δ_i, for 12 solutes is r = 0.7689; this is what precludes their being used together in one regression equation. For the same reason, energies of highest occupied and lowest empty molecular orbitals were not included in multiparameter regression equations.

In deriving Equation 40, the data determined on the phase of coverage 6.6×10^{-4} g of C-18 per gram were not included since for that phase a marked deviation from Equation 37 was observed. For all the remaining data, a good correlation was found between the retention data observed experimentally and those calculated by Equation 40 (Figure 10):

$$\log k'_{ij,\,detd} = 0.95 \log k'_{ij\,calc} + 0.01$$

$$n = 144$$

$$r = 0.9862 \qquad (41)$$

The benzene derivatives analyzed in our work cannot be considered congeneric from the point of view of their polarity. This becomes evident if one compares their dipole moments. The polarity parameter, Δ_i, comprises that diverse set of solutes in one relationship and has convincing advantages in that respect over the CNDO/2 calculated dipole moment. The Δ_i parameter has obvious limitations and a parameter better reflecting solute chromatographic polarity should be sought.

c. Molecular Shape Descriptors

The general equation derived by Scott[11] (Equation 1) describing the distribution coefficient of a solute between chromatographic phases comprises the constant, Φ, that incorporates the probability of the position of contact of the solute molecule with another molecule of a specific type. The constant Φ is decided by the size and geometry of the molecules considered.

To describe molecular shape in quantitative terms is a difficult task, even for relatively rigid molecules. Besides, the shape increment to retention is generally of minor importance in comparison to differences resulting from both polar and dispersive interactions. In the case of polar solutes, the molecular shape differences are, at least in part, incorporated in parameters describing the changes in solute polarity. Thus, the separation of shape factors for QSRR purposes may appear impossible.

The number of publications concerning QSRR and involving molecular shape parameters is quite limited. In fact, the single family of solutes for which some evidence is reported of the significance of shape parameters in QSRR equations is the group of PAHs. PAHs form a unique group of solutes of very low (and practically the same) polarity. As the compounds are actually rigid and coplanar, their shape may be represented by a two-dimensional formula. The group comprises a large variety of isomers for which dispersive properties are also very similar. Thus the differences in retention data experimentally observed may be considered as reflecting the shape variations of solutes.

No successful application in QSRR studies of steric substituent constants has been found. To be sure, Chumakov et al.[95] reported some linearity between logarithms of retention volumes, log V_R, and Taft, Hancock, and Palm steric constants for a small series of alkylpyridines. The retention data were determined by HPLC on alumina, silica gel, or alumina impregnated by cobalt chloride. A dioxane/isooctane mobile phase was employed. The authors claim that the best correlation is observed between log V_R and the Palm steric constant for the five solutes under study. However, in the figure included in their paper, the best correlation is actually observed between the log V_R and Taft E_s constant. The authors[95] conclude that steric hindrance determines adsorption in the case of alkylpyridines. This conclusion may be questioned since the series of compounds is closely congeneric and thus the constants assumed as being steric reflect dispersive properties as well.

In their fundamental works, Janini et al.[96] and Zielinski and Janini[97] noted that in GC the retention of PAH on liquid-crystal stationary phases depended on the shape of the solute molecule. In studies on structure-biological activity relationships of PAHs, Kaliszan et al.[98] introduced the shape parameter, η. The parameter is defined as the ratio of the longer to the shorter side of a rectangle having a minimum area, which can envelop the formula of a molecule (Figure 11).

The parameter, η, was next found to be of significance in two-parameter regression equations relating GC retention indexes determined on the nematic BMBT phase [*N,N-bis*(*p*-methoxybenzylidene)-α,α′-bi-*p*-toluidine] to both ω and the connectivity index.[99] The analogous data determined on a normal isotropic phase were satisfactorily described by a one-parameter equation involving the connectivity index. Based on that observation, a relationship was proposed which would allow one to calculate the shape parameter from retention indexes determined on both the nematic phase, I_N, and the isotropic phase, I_I:

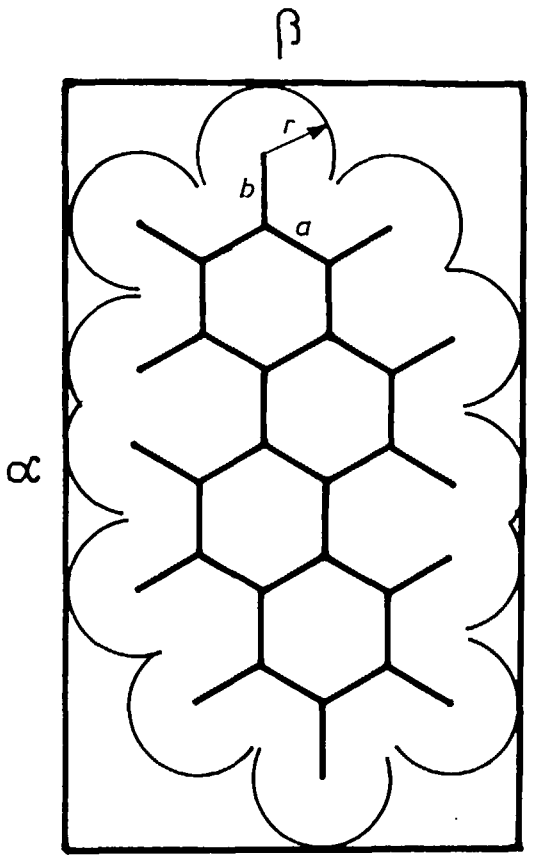


FIGURE 11. Determination of the shape parameter. $\eta = \alpha/\beta$ as exemplified by chrysene, where a and b denote approximate lengths of the C–C and C–H bonds (1.4 and 1.1 Å, respectively); r (= 1.2 Å) is the van der Waals radius of the hydrogen atom. (After Radecki, A., Lamparczyk, H., and Kaliszan, R., *Chromatographia*, 12, 595, 1979. With permission.)

$$\eta = k_1 \cdot I_N - k_2 \cdot I_I + k_3 \quad (42)$$

where k_1 to k_3 are regression coefficients.

Correlation analysis done for 14 coplanar PAHs yielded relationships in the form of Equation 42, with correlation coefficient r = 0.91. Looking retrospectively, however, I have to reconsider those results critically. There is quite a high intercorrelation between the two retention indexes applied for derivation of the regression equation. Thus the correlation found may be a chance correlation. This means that the relation holds true only as long as one remains inside the limited group of solutes used to derive the regression. Any other solute, even closely similar in structure, may not fit the equation.

In the case of nematic GC stationary phases and isomeric PAHs as solutes, the meaning of molecular shape for retention differences seems to be unquestionable. The question arises whether the shape parameter, η, adequately reflects structural differences of importance for retention. For closely related compounds, such as the 11 isomeric monomethylbenz [*a*] anthracenes analyzed by Lamparczyk,[100] the shape parameter is significant in two-parameter regression equations linking relative retention on nematic phases to the corresponding data determined on an isotropic phase. In this case, the intercorrelation between the variables is acceptable.

As the separation of PAHs has long been of special analytical interest, the shape parameter has also been employed in QSRR studies of retention data generated by HPLC. In their extensive publication, Wise et al.[101] used a modified shape parameter in considering the rectangle that envelopes the molecule and at the same time maximizes the length-to-breadth (L/B) ratio. The retention data were determined on RP C-18 columns and were presented as the logarithms of retention indexes calculated analogously to the GC Kováts indexes. In addition to the shape parameter, the connectivity index was used as a measure of the size of the molecules. It was found that both the L/B ratio and shape parameter were useful in predicting differences in a liquid chromatographic elution of an isomeric PAH, but they could not be used for comparison of PAHs of different molecular weights. Thus the Wise et al.[101] conclusion is in agreement with the results obtained later by Lamparczyk[100] for GC retention indexes of isomeric monomethylbenz [*a*] antharcenes.

Jinno and Kawasaki[102] considered logarithms of capacity factor, log k′, determined for 26 PAHs on various RP-HPLC columns (phenyl, ethyl, octyl, octadecyl). The log k′ values determined on individual phases were highly intercorrelated. For each phase considered, the authors obtained a high correlation ($r > 0.99$) between log k′ and the so-called correlation factor, F. The size factor F was calculated as F = (number of double bonds) + (number of primary and secondary carbon atoms) − 0.5 for a nonaromatic ring. The correlations between log k′ and L/B as the shape parameter were low. When the two structural descriptors, F and L/B, were used together in a multiregression analysis, the authors[102] found some improvement of correlation in relation to F alone, but only in the case of short chain phases, i.e., phenyl and ethyl phases.

The same authors analyzed 12 PAHs by a normal-phase HPLC mode on pure silica and aminosilica phases with *n*-hexane as the mobile phase.[103] The retention data (log k′) thus determined along with the literature data obtained on dimethylsilica, phenylsilica, octylsilica, and octadecylsilica phases were subjected to a QSRR analysis. The correlation between the capacity factors measured on various phases were high. The lowest was the intercorrelation between log k′ values as determined on the pure silica phase and those obtained in an octadecylsilica RP system ($r = 0.9474$). In such a situation, also similar were the statistics of the two-parameter equations relating log k′ to the size factor, F, and the shape parameter, L/B. The significance level of the shape parameter in regression equations was not given.

In conclusion, one can stress that at the present stage of development of QSRR studies the extension of the existing shape parameters beyond the coplanar positional isomers is not unequivocally justified. Conversely, the same holds true in the case of the chromatographic evaluation of the molecular shape of solutes. As the molecular shape importance for retention is much smaller than are the solute polarity and its dispersive properties, the later two characteristics must be precisely determined at first.

B. Substituent Contributions to Retention

One of the extrathermodynamic approaches to the analysis of physicochemical data is based on enthalpy-entropy compensation, which manifests itself in a linear dependence on the overall free energy changes of the corresponding enthalpy change for intrinsically similar physicochemical phenomena. The term "extrathermodynamic" denotes the fact that the approach lies outside the formal structure of thermodynamics, although it resembles that of thermodynamics in that detailed microscopic mechanisms do not need to be explicitly identified during use.

Free energy changes, ΔG, are related to enthalpy, ΔH, and entropy, ΔS, changes by the Gibbs equation:

$$\Delta G = \Delta H - T \cdot \Delta S \tag{43}$$

where T is temperature. For LFERs to be found between real and model systems, changes

either in entropy or enthalpy must be constant, or the enthalpy changes must be linearly related to entropy changes:[15]

$$\Delta H = \beta \cdot \Delta S + \Delta G_\beta \qquad (\text{at } T = \beta) \tag{44}$$

When enthalpy-entropy compensation is observed with a family of compounds in a particular chemical transformation, then the values of β and ΔG are invariant and β is called the compensation temperature.

Using the Gibbs relationship, Equation 43, one can rewrite Equation 44 in order to express the free energy change, ΔG_T, measured at a fixed temperature, T, for isoequilibrium processes as:

$$\Delta G_T = \Delta H \cdot \left(1 - \frac{T}{\beta}\right) + \frac{T \cdot \Delta G_\beta}{\beta} \tag{45}$$

Liquid chromatographic capacity factor, k′, is related to the thermodynamic equilibrium constant, K, for solute binding by $k' = \phi \cdot K$, where ϕ is the phase ratio of the column. The free energy change for the chromatographic process is expressed by the following equation:

$$\Delta G = -R\,T\,\ln K = -R\,T\,\ln(k'/\varphi) \tag{46}$$

where R is a gas constant. As shown by Melander et al.,[15] the substitution of Equation 46 into Equation 43 yields for the capacity factor:

$$\ln k' = -\frac{\Delta H}{R\,T} + \frac{\Delta S}{R} + \ln \varphi \tag{47}$$

If the mechanism of the process is invariant over the temperature range studied and the enthalpy is constant, a Van't Hoff plot of ln k′ against 1/T yields a straight line. From the slope of the line, the enthalpy change, ΔH, for a given solute can be assessed. Equations 45 and 46 can be combined to give:

$$\ln k'_T = -\frac{\Delta H}{R} \cdot (1/T - 1/\beta) - \frac{\Delta G_\beta}{R \cdot \beta} + \ln \varphi \tag{48}$$

where k'_T is the capacity factor at temperature T. According to Equation 48, plots of $\ln k'_T$ of various solutes measured at a given temperature, T, under different conditions against the corresponding enthalpy change are linear when the enthalpy-entropy compensation occurs (Figure 12).

In such a situation, the reversible binding of the solutes by a stationary phase involves essentially the same mechanism. To avoid statistical artifacts, it is recommended that the reference temperature T in Equation 48 be near the harmonic mean of the experimental temperatures used for the evaluation of the enthalpies by Equation 47.[15,105,106]

From the slope of compensation plot of $\ln k'_T$ vs. ΔH (Equation 48), the compensation temperature, β, may be obtained. If values of β for different RP-HPLC systems approach their 95% confidence limits, then the retention mechanism is assumed to be the same.

The compensation behavior was found for liquid chromatographic systems with popular alkyl silica stationary phases and aqueous/organic eluents.[14,15,107]

The enthalpy-entropy compensation found for different chromatographic systems substantiates the comparison of the chromatographically derived physicochemical data for com-

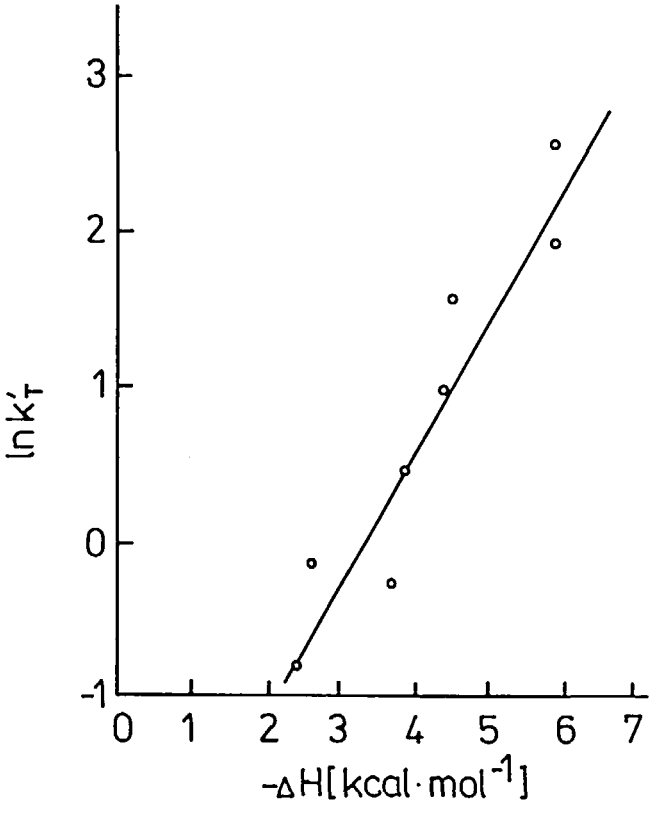


FIGURE 12. Enthalpy-entropy compensation plot for a group of aromatic acids chromatographed in different RP-HPLC systems. (After Melander, W., Campbell, D. E., and Horváth, Cs., *J. Chromatogr.*, 158, 213, 1978. With permission.)

pounds significantly differing in properties. From that point of view, of special interest is the LFER-based approach to chromatographic distribution, assuming that the free energy of the process comprises the independent contributions of the component functional groups.

Tomlinson et al.[14] determined functional group contributions towards RP-HPLC retention for sets of methyl-to-decyl unsubstituted and methyl-to-decyl substituted (*o*, *m*, *p*-OCH_3, -NO_2, -Cl) benzoates at conditions where enthalpy-entropy compensation was found. The authors defined group contribution, τ, as:

$$\tau = \log r_{ij} = \log (k_j' k_i') = \log [(t_{R_j} - t_{R_0})(t_{R_i} - t_{R_0})] \quad (49)$$

where k′ and t_R are the capacity ratios and retention times of solutes j and i, which differ by a functional group; r is the chromatographic selectivity coefficient; and t_{R_0} is the retention time of an unretarded tracer. The τ-values for different groups at different positions of substitution were found to depend linearly on mobile-phase composition as expressed by the mobile-phase surface tension. This is in agreement with the Horváth theory[13] of solvophobic chromatography. Thus the hypothetical τ-values at the 100% water mobile phase could be derived. These values were fairly well correlated ($r = 0.915$) to standard[34] *n*-octanol/water hydrophobic substituent constants obtained for a phenoxyacetic acid series. The authors[14] were also able to calculate the functional group contributions in free energy, enthalpy, and entropy terms.

When the enthalpy-entropy compensation is observed, the extrapolation of retention data in a simple binary system to a pure aqueous eluent offers the possibility of obtaining consistent, comparable data concerning capacity factors and group contributions. As proposed by Hafkenscheid and Tomlinson,[108] such theoretical capacity factors, together with the function of solute melting points (mp) and entropies of fusion, can be used to give estimates of aqueous solubilities according to the formula resembling the Yalkowsky-Valvani equation:[109]

$$-\log X_w = a\,k'_w + b \cdot \Delta S_f\,(T_m - 20) + c \qquad (50)$$

where X_w is the mole-fraction aqueous solubility; ΔS_f is the entropy of fusion; and T_m is the mp.

There are not many reports published in which enthalpy-entropy compensation have been analyzed. Normally, the LFERs are assumed to exist between the systems studied.

Since the early works by Martin,[1] followed by numerous publications by Green et al.,[3] a great many reports appeared in which substituent increments to retention were determined. The contributions of the substituents to retention have often been found constant and additive for a given chromatographic system. The best predictions of retention behavior have been obtained in the case of RPLC[7] or GC on nonpolar stationary phases.[110]

Chen and Horváth[6] presented a general approach to the evaluation of substituent contributions to chromatographic retention by using a multiple linear regression analysis and indicator variables. Retention data, κ_i, (log k′ from HPLC or R_M from TLC) of n congeners containing m-possible substituents were expressed by a set of linear equations:

$$\begin{aligned}
\kappa_1 &= \kappa_p + I_{11} \cdot \tau_1 + I_{12} \cdot \tau_2 + \ldots\ldots + I_{1m} \cdot \tau_m \\
\kappa_2 &= \kappa_p + I_{21} \cdot \tau_1 + I_{22} \cdot \tau_2 + \ldots\ldots + I_{2m} \cdot \tau_m \\
&\cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots \\
&\cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad \cdots \\
\kappa_m &= \kappa_p + I_{n1} \cdot \tau_1 + I_{n2} \cdot \tau_2 + \ldots\ldots + I_{nm} \cdot \tau_m
\end{aligned} \qquad (51)$$

where τ_{ji} is a substituent parameter that measures the change in chromatographic retention upon replacing a hydrogen atom in a substance, i, by substituent, j. The maximum number of substituent parameters is m for the set of congeners. The τ_{ji}-values for a given substituent in a certain position are the same in different compounds i, thus $\tau_{ji} = \tau_j$. A given substituent in different positions, however, may have different τ_j-values, depending on the particular molecular environment, and therefore the number of τ_j may be greater than the number of actual substituents. In the set of equations of Equation 51, κ_p is the retention value for the parent (unsubstituted) compound. The coefficients I_{ij} are the indicator variables, which are set to unity when a τ_j-value is assigned to a substituent in compound i, and to zero otherwise. For the parent compound, all indicator varaiables, I_{ij}, in Equation 51 are zero and for the congeners only those that correspond to a position without substituent. The matrix Equation 51 is solved by using a multiple regression analysis. For statistical reasons, the number of congeners, n, should exceed the number of τ_j-values, m.

In their publication, Chen and Horváth[6] analyzed the paper chromatographic retention data obtained by Kuchař et al.[111] for aromatic-aliphatic acids. The R_M values predicted on the basis of the statistically evaluated ΔR_M values have been consistent with the observed data. Similar analyses were done with a set of aromatic amines, acids, and amino acids chromatographed in RP-HPLC systems. In this instance, the number of retention increments, τ_j, was greater than that of the substituents. The authors[6] have also found that the τ_j-values

calculated from the corresponding κ_i-data obtained with three slightly different systems (octadecyl-silica stationary phases: Partisil, Spherisorb, and LiChrosorb; temperature 296 and 343 K) show satisfactory correlation. Thus the prediction of retention data from structural parameters is possible for different chromatographic conditions.

DiBussolo and Nes[112] assigned the contributions that individual molecular features made to the HPLC retention relative to cholesterol of serveral dozen sterols. The features examined included the presence and absence of various methyl and ethyl groups, double bonds and hydroxyl groups in the nucleus and side chain, the chirality at C_3 and C_{24}, the configuration about certain double bonds, and the length of the side chain and branching. The comparison of the observed retention data for a given sterol with those calculated by means of structural contributions can be used to gain structural information on a sterol from its chromatographic behavior.

If two series of solutes differ in a constant structural feature, then their retention behavior is similar. For example, Tacke et al.[113] observed close intercorrelations between R_M values determined by RP-TLC for a series of nifedipine-like drugs and a corresponding series of their Si-substituted derivatives.

The interrelationships among the individual chromatographic data deserve a separate review. This article concerns, rather, the relationships between retention data and solute structural parameters.

The substituent parameter most often used in QSRR studies is the Hansch π hydrophobic constant.[114] The π-value for a given substituent is defined as the logarithm of the ratio of the *n*-octanol-water partition coefficients of a substituted to an unsubstituted derivative. The standard π-values are derived from benzene compounds.[23] The important feature of π-constants is their additivity.

Close correlation between retention data and π-values is now well established, mainly due to the extensive TLC studies by Biagi et al.[115,116] and by others.[117,118] As one would expect, the correlations are very good for congeneric solutes if RP chromatographic systems are considered.

An example of a closely congeneric set of solutes is given in a paper by Jinno and Kawasaki.[119] Log k′ values of alkylbenzenes were correlated with the π-values of the alkyl-substituent when capacity factors were determined on either a C-8 or C-18 column at different concentrations of acetonitrile in the aqueous mobile phase.

When the solutes chromatographed differ structurally, the correlations between log k′ and π often deteriorate. Relationships between RP-HPLC capacity factor and π for different phenyl-substituted phenylcarbamoylmethyliminodiacetic acids divide the solutes into three groups whose membership is determined by the degree of *ortho* substitution.[120]

To get QSRR for the RP-HPLC-determined log k′, in the case of diverse benzene derivatives, a term related to substituent polarity must be added to π in a two-parameter equation.[121]

It should be noted here that in 1967 correlation was reported between Zahradnik β-constants and R_M values.[122] These β-constants are regarded as analogous to the π-parameter.

According to the general Collander equation, the partition coefficients measured in different solvent systems are linearly related to each other. Therefore, if the partition is the factor governing chromatographic separation, then the retention data should be related to the partition data determined by the standard equilibration methods.

In a study of the effect of ionization on the chromatographic behavior of some β-aryl-*n*-butyric acids, Kuchař et al.[123] have obtained equations relating R_M values to π-values with R_M data derived from a chromatographic system (A) where the acids would be ionized and another system (B) where they are not. In system A, the introduction of the Hammett electronic constant, σ, improves the correlation over the straight π vs. R_M, whereas the term is not needed for system B.

In studies on QSRR of *O*-alkyl-*O*-arylphenylphosphonothioates, Steurbaut et al.[124] analyzed GC relative retention times as compared with the unsubstituted compound, as well as

TLC R_M values determined in both a RP system and on polyamide layers. Some correlations with π-constants were found only for the RP-TLC system. On polyamide layers, the Hammett electronic constant was most significant in regression equations. In the case of GC data, the Taft steric substituent constant was the most important for retention on nonpolar phases, whereas the Hammett constant gave the highest correlations on a polar phase. Qualitatively, the results are as one would expect. However, the statistical value of the relationships reported is too low for any predictive purposes.

The correlations between partition chromatographic parameters and substituent π-values depend on the overall properties of the chromatographic system employed as well as on the deviations from the additivity of π-values in the case of an individual molecular environment in a given solute molecule. For example, Barbaro et al.[125] observed better correlation R_M vs. the sum of π for oligopeptides chromatographed on a silicone stationary phase when buffer/methanol solvent was used than for that obtained with buffer/acetone mobile phase. This finding seems to be in agreement with the Kuchař et al.[126] observation that lipophilicities of benzyloxyarylaliphatic acids are better described by the tabulated parameters π than by the experimental values obtained from partition TLC on silica gel impregnated with silicone oil with 50% acetone as the mobile phase.

Rittich et al.[127] have found some relationship between log k′, obtained on the C-18 chemically bound phase with a dioxan/water eluent, and Hansch π for a group of variously substituted phenols. However, their relationship holds true only when no more than 18 compounds of 26 studied are arbitrarily selected.

Side-chain hydrophobic constants, π_R, determined for 20 amino acids, were related to TLC and HPLC retention data.[128] Correlations were good when the side-chain contributions to chromatographically determined hydrophobicity were considered. The correlation of overall amino acid hydrophobicity with the retention coefficients was much lower, but still significant.

To facilitate the precise evaluation of solute-partitioning properties, Rekker[129] introduced the hydrophobic fragmental constant, f. Whereas Hansch π is based on differentiation in the solute group, the Rekker f reflects differentiation in the solute structure. Arguing that adding a fragmental constant of hydrogen, f_H, to the π-constant for any substituent gives the fragment constant, f_X, for that structure, Hansch and Leo[34] developed their own fragment method of hydrophobicity calculation. The thus-calculated hydrophobicity was correlated with TLC retention data for a series of crotonolactones.[130] Chromatography was carried out in this case in a straight-phase system consisting of cellulose sheets impregnated with formamide and cyclohexane as the mobile phase. Correlations between relative R_M values and the calculated hydrophobicity were $r = 0.877$ and $r = 0.919$, at low and high coverage of the stationary phase with formamide, respectively.

For nonpolar solutes, alkylbenzenes, excellent correlation of log k′ determined on a C-18 HPLC column with methanol/water (70:30) as the eluent was obtained with the Rekker constant.[19] The correlation was better than that obtained with an *n*-octanol/water partition coefficient. However, as the compounds studied were closely congeneric, equally good correlation was found by the same authors between log k′ and molecular connectivity indexes.

Analyzing the literature for TLC retention data for another set of congeners, Rekker[131] and Rekker and Kakoulidou[132] proposed R_M fragmentation similar to that applied for the partition coefficient.

III. RELATIONSHIPS BETWEEN LIQUID CHROMATOGRAPHIC RETENTION AND PARTITION COEFFICIENTS

Hydrophobic substituent or fragmental constants are derived with reference to the standard *n*-octanol/water partitioning system. The constants can be related to chromatographic data

determined in systems in which partition processes play an exclusive role. In such instances, the general Collander equation can be applied:

$$\log P_a = m \cdot \log P_b + n \quad (52)$$

where P_a and P_b are partition coefficients determined in the organic/water solvent systems a and b; m and n are constants that are characteristic of the two solvent systems employed. Assuming the extrathermodynamic LFER, the chromatographic parameters, R_M or log k′, can be linearly related to log P. However, as already observed by Collander, the fit implied in Equation 52 became poorer as the polarity differences between the organic solvents in the two aqueous partition systems became larger. Thus a good fit can be expected for the *n*-octanol/water vs. the pentanol/water system, while the combination of cyclohexane/water vs. *n*-octanol/water is typical of a poorly fitting Equation 52.

Keeping the above in mind, one can expect good correlations between the retention data and partition coefficients or substituent/fragmental hydrophobic constants if the chromatographic system is of a partitioning type and solute interactions with both phases are similar to those observed in the *n*-octanol/water system.

The Hansch approach to the studies of QSAR consists of the assumption that, to a first approximation, the free energy change in a standard biological response is a linear sum of individual energetic contributions, namely hydrophobic, electronic, and steric.

The change in the free energy of a biological response due to the hydrophobic nature of the drug can be represented by the logarithm of the *n*-octanol/water partition coefficient, log P, or quantities related to it. Although the choice of *n*-octanol as a compound reflecting the properties of the lipid components of the cell membrane has been questioned, the large amount of *n*-octanol/water partition data collected by the Hansch group has made *n*-octanol a common reference standard.

Measurement of log P by the conventional "shake flask" method is tedious. It is difficult to determine log P for compounds that are poorly soluble in water or that cannot be detected by conventional methods. The equilibration method is not applicable to surface-active and organometallic substances. It is difficult to determine reliable log P data for ionic substances, volatile compounds, and solutes for which association or dissociation processes are observed. Additional problems are caused by impurities, phase separation, and formation of emulsions.[133]

Instead of measuring log P values by equilibration methods, partition chromatographic data can be used. The advantages of the chromatographic methods of hydrophobicity determination have often been emphasized.[7-9] Recently, Harnisch et al.[133] summarized the information accumulated by reanalyzing the results of several historically important reports[134-144] concerning the RP-HPLC determination of hydrophobicity.

The advantages of the RP-TLC method are similar to those of RP-HPLC, but the former requires considerably less laboratory equipment and many solutes can be simultaneously stances containing impurities. Also, the substance mixtures can be analyzed. The method requires no quantitative determination and is applicable to volatile substances. The RP-HPLC method is highly reproducible. Its measuring range is practically of 1 to 8 log P units, but Tomlinson et al.[14] suggest that in systems where an organic modifier may be added to a mobile phase a distribution coefficient of 10^{-12} to 10^{12} is possible.

The advantages of the RP-TLC method are similar to those of RP-HPLC, but former requires considerably less laboratory equipment and many solutes can be simultaneously analyzed. However, accuracy in TLC demands $0.2 < R_f < 0.8$. This gives a range of R_M less than one and a half decades. In such a situation, it is often necessary to change the composition of the mobile phase in order to obtain reliable R_M values when one deals with a series of compounds of various polarities. Obviously, TLC is less reproducible and less sensitive than HPLC. As there may be a selective adsorption of mobile-phase components

in TLC, a quasi-gradient elution may be observed for less-retained solutes. The same is true for buffer components. In contrast, HPLC provides precise control of pH and ionic strength during the separation process. Temperature control is also conveniently attained.

The main disadvantages of chromatographic methods of hydrophobicity determination are that the methods are not applicable to organometallic substances and are applicable to only a limited extent to surface-active and ionic substances. Additionally, TLC is not applicable for volatile substances. Contrary to the direct method of partition coefficient determination, the chromatographic methods require a reference system.

Much effort has been directed to getting the HPLC systems to mimic the conventional *n*-octanol/water partition system. To obtain such an HPLC system, Mirrlees et al.[142] coated a silanized Kieselguhr support with water-saturated *n*-octanol and used *n*-octanol-saturated buffer as an eluent. At the same time, Henry et al.[136] prepared columns using bonded RP C-18 packing material with 1% loadings of *n*-octanol. The eluents were acetate buffers of pH 4.0 and 5.0 and a phosphate buffer of pH 6.5 presaturated with the stationary phase. Stable *n*-octanol-like columns were prepared by Miyake and Terada[143] by mixing *n*-octanol with hot octadecylsilica material. In a series of papers, Unger et al.,[144] Unger and Feuerman,[145] and Unger and Chiang,[146] described optimization of HPLC conditions for determining *n*-octanol-aqueous partition. Their method involves the use of the persililated octadecylsilica phase with a *n*-octanol-saturated buffer of adjusted ionic strength. In the case of lipophilic amines, Unger and Chiang[146] added *N,N*-dimethyloctylamine to the eluent to swamp out binding to residual silanol sites. With this procedure, the authors obtained data on apparent distribution coefficients of solutes at a given pH as well as partition coefficients for ionized and un-ionized species, together with ionization coefficients.

In the case of TLC, an octanol-like system was reported by Bird and Marshall,[147] who impregnated microcrystalline cellulose layers with *n*-octanol and used a *n*-octanol-saturated buffer as the solvent. For a group of penicillins, the plots of R_M vs. logarithm of the *n*-octanol/water partition coefficient were straight lines of a slope of close to unity when pH was below the pK_a of the solutes. In that context, it is interesting that recently Cserháti[148] reported that the lipophilic character of 3,5-dinitrobenzoic acid esters measured on unimpregnated cellulose layers correlated well with similar values determined on silica and alumina impregnated with paraffin oil.

There are some disadvantages of the *n*-octanol-like chromatographic systems. The most important is that the distribution coefficient range may not be wider than 10^{-2} to 10^{6} as it is in the "shake flask" methods.

Since chemically bonded RP materials were first employed[149,150] in the HPLC determination of partition data, many papers have been published in which log k′ vs. log P relationships were reported. For homologous or congeneric sets of solutes, high correlations were observed between standard *n*-octanol/water partition coefficients and capacity ratios determined on chemically bonded hydrocarbonaceous phases at a given mobile-phase composition. However, for more a diverse series of compounds, the linearity between log k′ and log P was often not found. Even for closely related barbiturates, a square term $(\log P)^2$ must be added along with log P to get satisfactory ($r = 0.95$) prediction of log k′.[64]

If one considers the relation between log k′ or R_M and log P as a special case of the Collander equation (Equation 52), then that relation should hold for all compounds analyzed in two-partitioning systems. However, marked discrepancies were noted, especially if the solutes studied differed significantly in polarity.

To reduce adsorption effects, the residual free silanol sites are silylated.[134,151] In spite of the special procedures involved, however, there always may be some amount of free silanol groups on the surface of chemically bonded material. The presence of these active silanol sites may greatly influence the retention of solutes, especially those which are ionized in the conditions applied. In special cases, a total exclusion of anionic solutes from pores of a RP matrix may even be observed, e.g., simple aromatic acids are excluded from ODS-

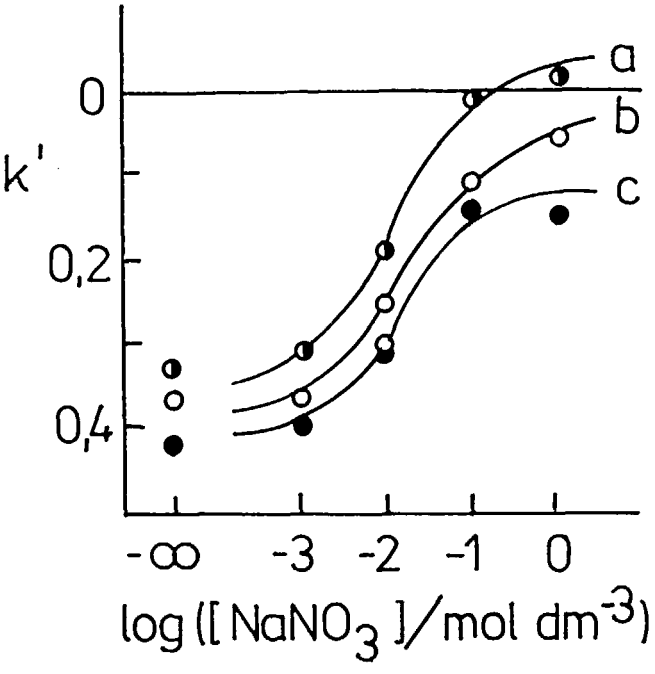


FIGURE 13. Dependence upon ionic strength of capacity factors, k', for (a) benzoic, (b) salicylic, and (c) sulfanilic acids eluted from octadecylsilica material.

Hypersil columns when eluted with water and water/ethanol mixtures.[152] For such excluded solutes, the capacity factors, k', become negative. To suppress or diminish the exclusion of ionic solutes, high ionic strength of the eluent (1 *M* $NaNO_3$) is required (Figure 13), which may influence the retention of other solutes studied.

Brent et al.,[151] who used a highly deactivated octadecyl-bonded silica column and a mobile phase of a pH about 7, observed exclusion (the compound was "unretained") of sulfacetamide.

The effects of ionization must be taken into consideration when interpreting chromatographic data. This may be difficult, especially when working with a higher organic modifier content in the mobile phase. In such instances, one is not able to predict ionization behavior of the solutes since the apparent pH gives no precise information.

The reference *n*-octanol/water partition coefficient refers to the ratio of the neutral, unionized compound in each phase. What one actually observes is the ratio of the concentration of all species (neutral and ionized) in the aqueous phase at a given pH and the concentration of a compound in the organic phase. The organic phase is assumed to contain only unionized species, which is never true as there may be some solution of an ionized compound in the hydrophobic phase as an ion pair with the buffer ions. Based on the work by Horváth et al.,[153] the method has been developed by Unger and Feuerman[145] to model bulk-phase partitioning for lipophilic acids, including ion-pair partitioning.

To reduce effects of ionization on RP-HPLC retention, various buffers are used for acidic and basic solutes. For example, Braumann et al.[154] used 0.5 *M* acetate buffer for acids, whereas Garst[156] and Garst and Wilson[155] employed 0.004 *M* trifluoroacetic acid for acidic solutes and 0.035 *M* triethylamine for bases. According to Wang and Lien,[157] for acidic and neutral solutes, phosphate buffer appears to give partition coefficients closer to the values obtained from the *n*-octanol/water system than acetate and bicarbonate buffers.

Specific interactions with mobile-phase components can be exploited in the case of particular solute series. Riley et al.,[158] based on extensive studies of ion-pair RP-HPLC, proposed a system using sodium dodecylsulfate as the pairing ion and methanol as the organic modifier

for the determination of indexes of hydrophobicity in a group of 1,3,5-*s*-triazines.

Although the dependence of log k′ or R_M on volume fraction, X, of an organic modifier in an aqueous binary mobile phase is more complex,[92,93] usually a linear relationship is assumed in QSRR studies:

$$\log k' = a \cdot X + b \tag{53}$$

where a and b are constants. Thus, for pure water as the eluent (X = 0), the log k'_w of an individual solute should be constant for a given stationary phase, independent of the organic solvent used in mixtures with water to derive Equation 53. However, the reported capacity coefficients, k'_w, obtained by extrapolation of Equation 53 to pure water depend on the nature of the organic modifier in the aqueous mobile phase. For a given set of solutes, log k'_w values derived from different solvent systems are more or less intercorrelated. For example, for a group of phenylurea herbicides, the relationship between the extrapolated capacity factors from the methanol/water system, log k'_w (CH_3OH), and those obtained in the acetonitrile/water system, log k'_w (CH_3CN), is[154]

$$\log k'_w (CH_3OH) = 1.435 \log k'_w (CH_3CN) + 0.279 \tag{54}$$

with correlation coefficient r = 0.939. The moderate correlation indicates that log k'_w indeed reflects basically the same molecular properties of the solute in both solvents, but these properties contribute differently to retention.

The extrapolated R_M values of acetophenone determined on the paraffin oil-impregnated plates with acetone/water, methanol/water, and dimethylformamide/water solvents differed significantly, but similar values of methylene group contribution were found for a series of *n*-alkylphenyl ketones.[158]

The extrapolated log k'_w[164] or R_M[161] values are commonly used for QSRR studies involving log P from the *n*-octanol/water system. The organic modifier most often used in HPLC is methanol, whereas in TLC both methanol and acetone are frequently employed due to numerous publications by the Biagi group.

In a recent paper, Biagi and co-workers[159] determined R_M values of a series of demorphin-related oligopeptides in two RP-TLC systems, the mobile phase being an aqueous buffer alone or mixed with various amounts of methanol or acetone. A nonpolar stationary phase was obtained by impregnating the silica gel layer with silica DC 200 (Applied Sciences Labs.). The following relationship between the R_M values extrapolated to pure water, R_M^w, for the methanol/water and acetone/water systems was reported:

$$R_M^w (CH_3OH) = 1.76 - 0.482 R_M^w (CH_3COCH_3) \tag{55}$$

For 23 solutes considered, the correlation coefficient was r = 0.987. The authors[159] suggest, however, that the R_M^w values extrapolated from the R_M data determined at low concentrations of the organic modifier in the mobile phase were very similar in both systems and close to the experimental R_M^w that was available for five compounds of the series. As for the more lipophilic solutes, higher volume fractions, X, of organic solvents are required; the linearity of the R_M vs. X relationship deteriorates due to the perturbations of the water structure.

In the RP-TLC systems introduced[160] and extensively studied by the Italian group, silicone oil has usually been the nonpolar stationary phase. In that system, specific adsorption interactions with solutes were shown by Kuchař et al.[163] This is probably the reason that the correlation between R_M^w and log k'_w for a set of 5-nitroimidazoles determined on a C-18 column by Guerra et al.[161] is low (r = 0.467). R_M^w is practically independent of log P

($r = 0.331$), whereas the correlation between log k'_w and log P for the same set of solutes is apparent ($r = 0.921$).

Since thin-layer plates precoated with nonpolar chemically bonded phases[165] became commercially available, they have often been employed for hydrophobicity determinations. Eight types of such plates were compared.[166] As reported, the advantage of the RP-18-coated plates (Merck) of unlimited use without added NaCl is partly offset by the shorter migration time observed with KC_{18}-end-capped-with C_2 plates (Whatman) in the often used 30 to 70% methanol range.

As pointed out by Braumann et al.,[154] the dependence of log k'_w or R_M^w on the nature of the organic modifier in the aqueous mobile phase reveals that log P and log k'_w are not completely interchangeable. These authors illustrated their finding by obtaining three separate plots of log P vs. log k'_w for phenylureas, phenoxycarbonic acids, and phenoxycarbonic acid methyl esters. For a group of *s*-triazines studied, they obtained no correlation ($r = 0.555$) between the two quantities discussed. The conclusion was that certain solutes or substituents behave differently in RPLC and in the true liquid-liquid partitioning system.

The solutes for which precise correlations are observed between log P and chromatographic data are structurally congeneric. The term "congeneric" means that the solutes of a series exhibit a consistent partition behavior towards solvent systems with dissimilar phase polarities. Congenerics are undoubtedly homologues of a given series. As noted by Koopmans and Rekker,[19] in many instances noncongenericity may be hidden in a series of compounds consisting of outwardly congeneric-looking structures. On the other hand, congenerics, with regard to the systems considered, may be a class of apparently diverse solutes.

Several authors assume that the extrapolated log k'_w or R_M^w values are based mainly on hydrophobic interactions, whereas the specific solute-mobile-phase interactions are reduced. Thus a relationship between the extrapolated chromatographic parameter and log P should concern different substances. Such a diverse group of solutes form mono- and disubstituted benzene derivatives (-OH, $-NH_2$, $-NO_2$, $-CH_3$, -Cl; altogether 49 compounds) analyzed by Nabil El Tayar et al.[167] The solutes were chromatographed on a C-18 column with a methanol/buffer (3-morpholinopropane sulfonic acid, 10^{-2} *M*) as the mobile phase and *N*-decylamine (0.2% v/v) as the masking agent. The correlation between *n*-octanol/water partition coefficients, log P, and the HPLC-extrapolated capacity factors, log k'_w, is quite high ($r = 0.982$); the correlation decreases ($r = 0.967$) when the capacity factor determined at 50% methanol in the mobile phase is used instead of the value extrapolated to pure water. When an indicator variable, m_w (hydration factor), is additionally introduced, the relation log $k'_w = f(\log P, m_w)$ is characterized by the correlation coefficient $r = 0.9998$.

Kuchař et al.[168] determined R_M and log k' values in different RP-TLC and -HPLC systems for alkoxy and phenylalkoxy arylacetic acids. It was found that when an aqueous mobile phase containing an organic solvent (50% acetone or 60% methanol) was used the changes in hydrophobicity corresponded to the changes in log P measured in the reference system *n*-octanol/water. Extrapolation of retention indexes to pure water was not advantageous and negatively influenced the calculation of hydrophobic substituent parameters from the corresponding retention indexes.

Similar conclusions were drawn by Jinno,[169,170] based on QSRR studies of log k' values determined by the micro-HPLC technique for alkylbenzenes. The data analyzed by the author were determined on C-2, C-8, and C-18 hydrocarbonaceous columns with acetonitrile/water mixtures as mobile phases.

As the result of extensive studies, Jinno and Kawasaki[171,172] reported two-parameter equations relating log k' values for separate sets of alkylbenzenes (plus PAHs), substituted benzenes (except phenols), and phenols to the *n*-octanol/water log P, along with a factor reflecting electronic properties of the solutes considered. The authors investigated a computer-assisted system for retention prediction at varying volume fractions of an organic

modifier in the mobile phase. For a given volume fraction, X_i, the respective relationship considered is

$$\log k_i' = a_i \cdot \log P + b_i \cdot F + c_i \tag{56}$$

where a_i, b_i, and c_i are constant for a given X_i, and F is a specific correlation factor proposed by Schabron et al.[173] The approach by Jinno and Kawasaki[171,172] consisted of assuming that the coefficients a, b, and c can be described as functions of the volume fraction of the organic solvent, X. For the methanol aqueous mobile phase (80 to 40% of methanol), the coefficients a, b, and c were found to depend on X as follows:

$$\begin{aligned} a &= -0.184 \cdot X^4 - 0.327 \cdot X^3 + 0.392 \\ n &= 5 \\ r &= 0.995 \end{aligned} \tag{57}$$

$$\begin{aligned} b &= 0.675 \cdot X^2 - 0.971 \cdot X + 0.431 \\ n &= 5 \\ r &= 0.985 \end{aligned} \tag{58}$$

$$\begin{aligned} c &= 0.738 \cdot X^4 - 1.701 \cdot X + 0.155 \\ n &= 5 \\ r &= 0.965 \end{aligned} \tag{59}$$

where n is the number of data points used to derive the above regression equations. Thus the general equation relating capacity factors, log k′, to the partition coefficient, log P, and the electronic factor, F, of a series of alkylbenzenes, and to the mobile phase composition, X, is

$$\begin{aligned} \log k' = {} & (-0.184\,X^4 - 0.327\,X^3 + 0.392) \cdot \log P \\ & + (0.675\,X^2 - 0.971\,X + 0.431)\,F \\ & + (0.738\,X^4 - 1.701\,X + 0.155) \end{aligned} \tag{60}$$

Assuming the correctness of Equation 60, a computer-assisted retention prediction may be obtained for related hydrocarbons. Objections may be raised, however, as to whether the complex relationships (Equations 57 to 59) are reliable since only five data points (or four for aqueous acetonitrile) were considered.

Hanai and Hubert[174] related the RP-HPLC log k′ to log P for 61 phenols. They also derived an equation relating the capacity factor changes to the concentration of acetonitrile in the aqueous mobile phase. The mean difference between the predicted and measured capacity factors was within 10%.

Hafkenscheid and Tomlinson[175] studied the correlations between the RP-HPLC capacity factor and log P for a set of 87 diverse organic compounds. The conclusion drawn by the authors was that the capacity factor did not reflect a purely hydrophobic solute property.

As is evident from the above discussion, the quantitative relationship between the chromatographic retention data and the *n*-octanol/water partition coefficients is more complex

than predicted by the general Collander equation. The problem is that the exact physicochemical nature is not known, either for partition coefficient or for the chromatographic processes. Both types of data are essentially experimental. There was some progress recently observed in elucidation of the retention mechanism. As far as log P is concerned, studies by Moriguchi and co-workers[176] are promising. These authors analyzed log P data for more than 200 truly diverse molecules. It was found by multiple regression analysis that log P value had a strong correlation (r = 0.95) with the surface area, net charge of each atom, volume of the molecule, ionization potential, and electron affinity. Certainly, it would be difficult, if not impossible, to design a chromatographic system equally sensitive to so many structural variables.

The relationships between retention data and log P have been studied extensively by medicinal chemists. The reason for this is that a *n*-octano/water partitioning system provides a single, continuous scale for measurement of hydrophobicity, and the respective log P data are collected for several thousand compounds. In fact, however, the criterion for usefulness of chromatographically derived data in medicinal chemistry is their ability to characterize the action of drugs and not their colinearity with the *n*-octanol/water partitioning data. On the contrary, it may happen that the particular chromatographic parameters better describe drug behavior in a living system. It may even be argued[177] that the ordering of hydrocarbon side chains facing an aqueous interface on one side and a polar core structure on the other side is very similar for both C-18 RPLC and a typical bilayer biomembrane system.

To unify the comparison of RP-HPLC data produced at different chromatographic conditions for compounds of varying polarity, Baker and Ma[178] and Baker[179] have introduced a retention index scale similar to that used in GC according to Kováts. The scale is based on the relative retention of a homologous series of C_3-C_{23} 2-keto alkanes.

The retention index, I, of a given solute is calculated from its observed capacity factor, k'_s, the capacity factor for a 2-keto alkane eluting just before the test compound, k'_N, and the capacity factor of the next higher homologous, k'_{N+1}:

$$I = 100 \frac{\log k'_s - \log k'_N}{\log k'_{N+1} - \log k'_N} + 100\,N \qquad (61)$$

The retention index of a given 2-keto alkane standard is by definition equal to 100 times the number of carbons in the formula. It was found that the retention index of a given compound remains nearly constant, although its retention time may vary significantly with changes in the composition of the eluent (Figure 14).

As expected, the index for congeneric sets of solutes correlates fairly well with log P. However, as noted by Brent et al.,[151] 2-keto alkane standards cannot account for all interactions with the column and the mobile phase that would be experienced by molecules bearing the full range of possible functional groups.

To unify chromatographic data for medicinal chemistry, several authors describe in detail specific procedures of hydrophobicity determinations.[133,151,155,162]

Interestingly, Rowland and Toon[180] and Rowland and co-workers[181] proposed an RP-HPLC-based index of hydrophobicity, R_Q, defined as follows:

$$R_Q = \log (1 - R_0/R_T) \qquad (62)$$

where R_0 and R_T are retention times of an unretained solute and the compound studied, respectively. Contrary to log k', the R_Q term gives a linear relation when plotted against acetonitrile concentration in the mobile phase for homologous barbiturates. As the authors themselves noted, the R_Q, by definition, cannot exceed zero. Yet, several of the extrapolated-

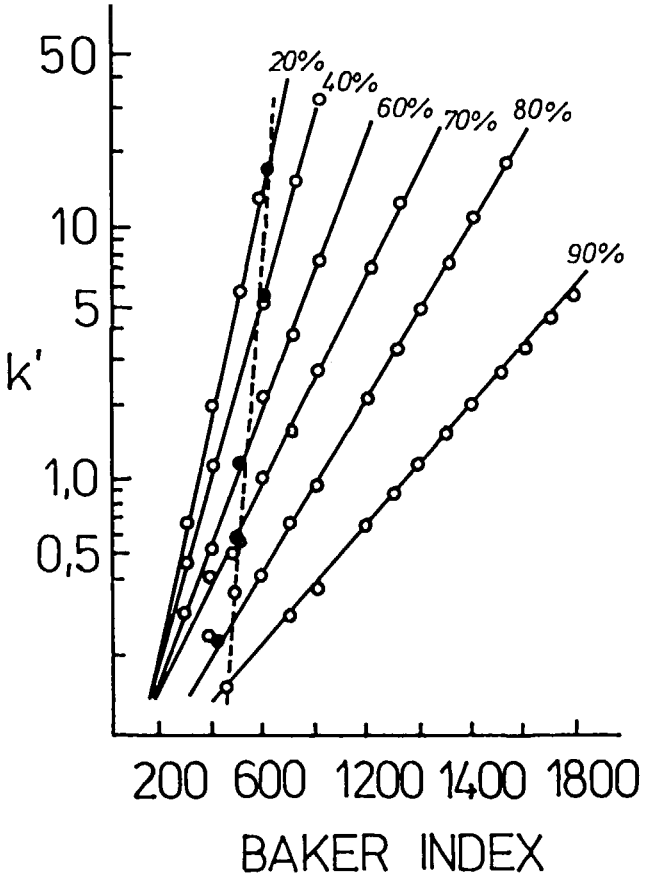


FIGURE 14. Effect of methanol content in aqueous solvent on capacity factor, k', for (○) 2-keto alkanes standards and (●) phenacetin. The Baker retention index is nearly independent of the eluent changes. (After Baker, J. K. and Ma, C.-Y., *J. Chromatogr.*, 169, 107, 1979. With permission.)

to-pure water R_Q^w values are numerically greater than zero. These values (?) form excellent correlations with log P.

The determination of partition coefficients of volatile substances is often difficult and time consuming. For this reason, Boček[182] developed a GC method using water and oleyl alcohol as the liquid stationary phases. The method is based on the assumption that the partition coefficient of a compound in the oleyl alcohol-water system is equal to the ratio of partition coefficients in the oleyl alcohol system (saturated with water) — nitrogen and water — nitrogen that can be determined by GC. The method is complicated as care must be taken to eliminate the influence of adsorption in order to determine true partition coefficients.

IV. CORRELATION OF RETENTION DATA WITH MOLECULAR PROPERTIES OF THE SOLUTES USING FACTOR ANALYSIS

Factor analysis is widely applied in chemistry[183] to determine the "intrinsic dimensionality" of certain experimentally determined chemical properties, i.e., the number of "fundamental factors" required to account for the variance.

The cross correlation between the independent variables considered in multiple regression analysis can be assessed by examination of the correlation matrix of the parameters. Next,

the manipulations can be performed on this matrix or on the variance-covariance matrix, including the dependent variable. Such a matrix may be transformed into another one having nonzero elements only on its diagonal. With each eigenvalue of the matrix thus obtained, an eigenvector is associated that is a linear combination of the original set of variables. The eigenvectors are exactly orthogonal. If a set of variables has substantial covariance, then most of the total variance will be accounted for by a number of eigenvectors equal to a fraction of the original number of variables. Thus a reduced set containing only the major eigenvectors or principal components may be used. Other manipulations of the eigenvectors may be performed to gain insight into the structure of a multidimensional data set.

Huber et al.[184,185] applied factor analysis to the study of the partition of steroids and pesticides in a ternary system by means of liquid-liquid chromatography. They found that three factors were sufficient to span the space, but they did not attempt to rotate the abstract factors into physically significant parameters. Nonetheless, the authors proved the value of factor analysis as a good retention prediction tool.

In 1966, Rohrschneider[186] postulated that the GC retention index of a solute i in a liquid phase α could be expressed as a linear sum of terms in product function form. Multiple correlation analysis of GC retention indexes using equations of this form was the subject of numerous publications.[187-190]

In studies on correlation of HPLC retention volumes of substituted carboranes with molecular properties using factor analysis, Kindsvater et al.[191] considered the following relationship:

$$F_v(i,\alpha) = \sum_j U(i,j) \cdot V(j,\alpha) \tag{63}$$

where $F_v(i,\alpha)$ represents the function of the chromatographic retention of solute i in solvent system α; U(i,j) represents the j-th solute parameter for the i-th solute; V(j,α) corresponds to the j-th solvent or stationary support factor for the α-th system, and j is a dummy variable equal to the number of important factors in the space. Kindsvater et al.[191] measured retention volumes for 15 solutes in 8 RP systems. Applying the factor analysis scheme to both the logarithms of the retention volume set and its transpose, it was found that three factors were sufficient to reproduce the data within experimental error. Once the number of factors needed to span the space is determined, the next step is to try to identify these abstract factors with physically significant parameters. The advantage that factor analysis has over regression analysis is that individual factors can be tested for possible identification with the abstract factors without simultaneously identifying all the other important factors of the space. Of the several parameters associated with the solutes tested, the effective carbon number, molecular weight, and GC retention time on a polar phase were apparently correlated with individual factors.

Laffort and co-workers[192] determined solubility factors for an impressive number of 240 solutes and 207 stationary phases in GC. In their approach, the solute factors, represented by Greek letters, and the stationary phases factors, represented by Latin letters, are involved in a linear equation of predictability of retention indexes, I, as follows:

$$I = \alpha \cdot A + \omega \cdot O + \epsilon \cdot E + \pi \cdot P + \beta \cdot B + 100 \tag{64}$$

The constant 100 in Equation 64 is used since all solute factors are referred to methane.

The solute factors are linear combinations of retention indexes measured on five selected stationary phases of different polarities. The factors of stationary phases are calculated from retention indexes of five standard solutes of different classes. Physicochemical meanings are given to the solute factors. Thus, α is proportional to molecular volume; ω is proportional

to the square of the dipole moment for simple molecules; ϵ is related to the ratio between molecular refraction and molecular volume; π is a proton donor factor; and β is a proton acceptor factor. Among the stationary phase factors, only the factor E is identified. It is supposed to reflect the "compactability" of the solvent, i.e., the relative absence of "holes".[192] Laffort and co-workers[192] and Laffort and Patte[193] related their solute factors to the Karger et al.[12] solubility parameters, partition coefficients, and biological activity (olfactory thresholds). Log P was described ($r = 0.95$) by a three-parameter equation involving α-, ω-, and ϵ-factors.

Buydens and Massart[69] and Buydens et al.[70] applied factor analysis in their studies on the correlation of GC retention indexes with topological and quantum chemical parameters. For diverse solutes chromatographed on phases of different polarities, two or three important factors together explained about 85 to 90% of the variance in retention indexes.

Recently, Cserháti et al.[194] evaluated RP-TLC retention data generated for seven 5,5-dialkyl-substituted barbiturates in 21 mobile-stationary phase systems. As the result of principal component analysis, it was found that >95% of the total variance could be explained on the basis of one hidden variable. As one could expect, the determinating factor in the grouping of compounds according to their chromatographic behavior is the number of carbons in the alkyl chains, i.e., solute hydrophobicity.

V. APPLICATION OF QSRR IN MEDICINAL CHEMISTRY

Since the first studies of QSAR in the 1960s, chromatography has been extensively exploited as a tool for the quantitation of the physicochemical properties of drugs. Until 1974, TLC and paper chromatography were applied. Later, HPLC and GC became increasingly popular.

The QSAR methods commonly applied are based on the assumption that the LFERs exist between the chemical and biological reactions. Hansch[195] attributed the free energy change in a standard biological response to hydrophobic, electronic, and steric contributions. If consistent numerical bioactivity data are known for a series of compounds, attempts can be undertaken to relate them quantitatively to a set of individual numerical molecular descriptors reflecting the hydrophobic, electronic, and steric properties of a given compound of importance for its activity. To quantify the physicochemical properties of importance for the biological activity of the compounds considered, various chromatographic techniques and procedures are applied.

Applications of chromatography in QSAR studies were reviewed in part previously.[7-9] Here, the available information is collected and briefly discussed, including the recent findings.

The earliest reported study relating chromatographic parameter (R_M from RP-TLC) to biological activity was that by Boyce and Milborrow.[5] The R_M values were obtained on a stationary phase impregnated with liquid paraffin using an acetone/water mobile phase. A parabolic relation was obtained when the molluscicidal activities of some *N-n*-alkyltritylamines were plotted against their R_M values. According to Hansch and Fujita,[114] the parabolic relationship between an index of hydrophobicity and bioactivity may be related to the probabilistic movement of a drug from an extracellular phase to its site of action.

Another quadratic relationship between the R_M values (extrapolated to pure water) and the logarithm of the reciprocal of the minimum lethal dose (MLD) in cats was found by Biagi[196] for some cardiac glycosides. In the same year, 1967, Biagi,[197] in a study of the lipid solubility and human serum binding of various penicillins, showed that for some of the penicillins the correlation between the RP R_M and human serum binding was greater than that obtained using Hansch π-values.

A quadratic relationship was found for a group of 4-(1-cyclo-pentyl-*n*-alkyl)-2,6-dinitrophenols between their substituent ΔR_M values and their fungicidal activities.[198]

In studies of the influence of the hydrophobic character on the activities of a series of penicillins and cephalosporins, Biagi et al.[199] found a linear relationship between R_M and the logarithm of the reciprocal of minimum antibacterial concentration against *Escherichia coli*. The negative sign for the slope coefficient in the correlation equation derived suggests that the activity increases with a decrease in the hydrophobic character of the penicillins. Activity of penicillins against *Streptococcus aureus* and *Trypanosoma pallidum*, as well as the activities of cephalosporins against all the three bacterial species studied, were related to R_M values by typical quadratic functions. The same was the case with rifamycins studied separately by the same authors.[200] A further study on the influence of the lipophilic character on the bioactivity of some oligosaccharide antibiotics has demonstrated the effectiveness of using R_M in this type of correlation.

In vitro hemolytic activity of a set of testosterone esters was described by two-parameter equations comprising R_M and $(R_M)^2$ terms.[115] R_M values were used corresponding to 54% concentrations of acetone or methanol in the mobile phase. The plates were impregnated with silicone oil. The QSAR equations obtained have been of a quality comparable to that obtained with Hansch π-values.

The effect of some testosterone esters in the capon's comb test was linearly related to the R_M values in another study by Biagi et al.[201] A linear relationship was also found between the percentage of binding of corticosteroids to serum albumin and R_M values.[202]

Dearden and Tomlinson[203] found a linear correlation between human buccal absorption data of some acetanilide drugs and their ΔR_M substituent constants derived from R_M measurements in TLC systems using either *n*-octanol or liquid paraffin as the stationary phase. The *n*-octanol/water solvent pair acts as a better model reference system than the liquid paraffin system. Similar differences in linear correlations were found by Tomlinson and Dearden[204] in studies on the protein binding of acetanilides to bovine serum albumin. Correlation similar to that[117] obtained with the *n*-octanol/water-TLC system was found when R_M was used as determined in a polyamide/acetone-water-dioxan (1:2:1) system.

Analgesic potencies of a group of *p*-substituted acetanilides in mice were correlated with substituent ΔR_M values.[205] A two-parameter equation involving ΔR_M and $(\Delta R_M)^2$ was of a higher statistical value when chromatography was carried out on *n*-octanol-impregnated plates as compared to the liquid paraffin stationary phase.

Biliary excretion of penicillins in the rat was related ($r = 0.84$) to the R_M data measured in a RP silicone/oil/water TLC system.[206]

For *N*-alkyl-substitued normeperidine homologues, the R_M values obtained on cellulose TLC plates with an ethanol/water (40:60) solvent system were linearly related to butyryl-cholinesterase-drug dissociation constants.[207] Linear relationships were not observed, however, with acetylcholinesterase.

Concentrations of a homologous series of *N,N'-bis* dichloroacetyl diamines inhibiting in vitro mitochondrial electron transport were related to R_M and $(R_M)^2$ by two-parameter regression equations.[208] R_M values on silicone oil phase were determined by extrapolating the data to 50% acetone in the mobile phase.

For a limited set of five thiolactam compounds, the lethal toxicities in mice were better correlated with R_M values than with *n*-octanol/water partition coefficients.[118]

Plá-Delfina et al.[209-211] found that for a group of barbituric acids studied, the gastric absorption rate constants were correlated ($r \approx 0.9$) with R_M values determined in several RP-TLC systems. Later, that research group found a hyperbolic relationship between the rat gut *in situ* absorption rate constants, k_a, and RP-TLC data for 18 antibacterial sulfonamides:[212]

$$k_a = [3.9396\ (1/R_f - 1)^{2.6364}]/[0.0719 + (1/R_f - 1)^{2.6364}] \quad (65)$$

The correlation was better than that observed when log k_a was related to R_M and $(R_M)^2$.

Chaudry and James,[213] using literature R_M values of some nandrolone esters, have related ($r = 0.84$) the anabolic activities of these compounds to the chromatographic parameter, R_M, determined in a straight-phase system.

Correlations between R_M values obtained from TLC and the bioactivity of steroids,[214] phenols,[215] naphtols and acetophenones,[216] and benzodiazepines[217] were reported by the Biagi group. With steroids, there is a parabolic relationship between the data for hemolytic activity and the membrane binding of the drugs with regard to their lipophilicity expressed by R_M values. On the other hand, introduction of the R_M^2 term does not improve the correlation between either the protein binding or the duration of action of testosterone esters and hydrophobicity.

Hemolytic activity, antibacterial activity against *S. aureus*, and acute toxicity to mice for a group of phenols have been shown to be linearly dependent on R_M values.[215] The data for phenols were later combined with the corresponding data for naphtols and acetophenones.[216] In spite of the fact that the R_M values for phenols, naphtols, and acetophenones were determined at different times, the linear relationships between either acute toxicity of hemolytic activity and R_M values for a group of nearly 60 compounds were satisfactory (for 5 halogenated acetophenones, an indicator variable was additionally used). The Biagi group preferred silicone oil as the stationary phase. They found the R_M values from the silicone system to be better correlated with bioactivity of benzodiazepines than those obtained on *n*-octanol-impregnated TLC plates.[217]

R_M data from the liquid paraffin/acetone-water system gave no significant correlation with antibacterial activity against *Mycobacterium tuberculosis* in a series of isonicotinic acid hydrazide derivatives.[218] Correlation improved when pK_a data were included, thus providing a correction for ionization to the R_M data. A similar situation has been observed in the case of pyrazine carbothioamide derivatives[219] and 2-cyanomethylbenzimidazole derivatives.[220] Here too, TLC data from the liquid paraffin/water system gave satisfactory correlation with bioactivity when used together with the spectroscopic data related to polarity.

A parabolic dependence of activity against eight bacterial species on R_M values determined on Kieselgel 60 silanisiert plates with acetone/water (60:40) as the mobile phase for a series of α, β-unsaturated γ-lactones was observed by Dal Pozzo et al.[221]

Direct partition TLC data on silica gel and a cellulose support impregnated with formamide were linearly correlated with the local-surface anesthetic activity of a series of 2-morpholinoethyl esters of 2-, 3-, and 4-alkoxycarbanilic acids.[222] The correlations were derived separately for *ortho-*, *meta-*, and *para*-substituted derivatives.

Hulshoff and Perrin[223] obtained a good correlation of the R_M values (extrapolated to pure water) with the bovine serum albumin-binding constants, antihemolytic activity, and the inhibition of Na^+K^+-activated adenosine triphosphatase activity for a series of phenothiazine derivatives. The biological activity data used were corrected for the state of ionization.

TLC partition data proved useful for QSAR studies with a series of rifamycins as inhibitors of viral RNA-directed DNA polymerase and mammalian α- and β-DNA polymerases.[224]

Partitioning into erythrocytes of potential antimalarial sulfonamaides was also related to R_M values obtained in TLC in the liquid paraffin/phosphate buffer of a pH 5 system.[225]

Ferguson and Denny,[226,227] based on R_M values from partition chromatography, concluded that for the tumor-active but mutagenic anilinoacridines separation of the two classes of bioactivity was possible by simple manipulation of the agent lipophilic-hydrophilic balance.

Fujii and co-workers[229] applied R_M values for the description of in vivo antistaphylococcal activity in mice of ω-amino acids and their L-histidine dipeptides[228] and carboxylic acids.

Maksay et al.[230] found a good correlation between pharmacokinetic constants characterizing oxazepam brain levels observed after the i.v. administration of prodrugs (oxazepam esters) and the chromatographic R_M values. They found that an increase in hydrophobicity

of the esters decreased oxazepam brain penetration. To explain this, they suggested that hydrolysis precedes brain penetration and hydrophobicity might primarily influence the hydrolysis rate. The amount of tissue storage, total excretion rates, and serum binding were also correlated with hydrophobicity.[231]

Areas under effect-time curves have been correlated with R_M values for some hypoglycemic sulfonamides.[232]

Higher potency of the positive intropy of substituted grayanotoxins was observed for compounds with lower R_M values, indicating hydrophilic properties of the test substances.[233]

The lipophilicity determined by RP-TLC showed a good correlation with the ability of some neuroleptics to enhance striatial dopamine release.[234]

Kuchař et al.[126] have found in studies of fibrinolysis and inhibition of denaturation of serum albumin by a large series of substituted benzyloxyarylaliphatic acids that the lipophilicities of the compounds were better described by Hansch π than by experimental TLC values obtained on silica gel impregnated with silicone oil.

Dadákova et al.[130] determined ΔR_M values for a series of crotonolactones. A stationary phase was a formamide-impregnated cellulose, and the development was carried out by cyclohexane. The correlation of the chromatographic parameters with in vitro antituberculotic activity has been rather low ($r = 0.74$).

R_M determined on a cellulose support was related to antitumor activity of derivatives of ansacrine.[235]

In 1975, Carlson et al.[135] correlated toxicity against *Daphnia magna* with the RP-HPLC capacity factor for a series of nine phenols. The correlation coefficient of a linear relationship ($r = 0.68$) was similar to that obtained with Hansch π ($r = 0.76$).

The ability of a short series of 1,3,5-triazine herbicides to inhibit the Hill reaction has been correlated with HPLC retention times determined on octadecylsilica columns with water/methanol (95:5) as the mobile phase.[236]

An extensive study on the application of HPLC data in correlation with the activity of sulfonamides against *E. coli* and inhibitory potencies of barbiturates on rat brain oxygen uptake and *Arbacia* egg cell division was described by Henry et al.[136]

Later, Riley et al.,[158] based on extensive studies on ion-pair RP-HPLC, proposed a system using sodium dodecylsulfate as the pairing ion and methanol as the organic modifier for the determination of indexes of hydrophobicity. They successfully applied their hydrophobicity indexes to describe the antibacterial activity of a group of 1,3,5-*s*-triazines.

Fungicidal activity of 12 to 13 mono- and disubstituted phenols was correlated with RP-HPLC data.[127] The log k′ values were corrected for ionization at the environmental pH. Nonetheless, the pK_a parameter was used along with log k′ in two-parameter regression equations.

Rabbit hypnotic dose, rat hypnotic dose, and concentration for 50% inhibition of rat brain were related to the RP-HPLC log k′ by means of a quadratic regression.[64] Correlations equivalent to those derived with *n*-octanol/water log P were obtained.

Unger proved the usefulness of his procedure[144,145] of chromatographic determination of hydrophobicity by deriving QSAR equations relating the literature bioactivity data to log k′ for a series of lipophilic amines.[146]

Baker et al.[177] claim the superiority of their HPLC retention index[178,179] over *n*-octanol/water partitioning data for the quantitative description of biological activity of propranolol and barbiturate analogs. However, in the case of the anti-inflammatory activity of the anthranilic acid derivatives, the same authors found a better description of pharmacological data when using the *n*-octanol/water partition data. The HPLC retention index was successfully applied in QSAR studies of 4-hydroxyquinoline-3-carboxylic acids as inhibitors of cell respiration.[237] The Baker index was also applied for prediction of the antihypertensive activity of quinazolinamine derivatives.[238]

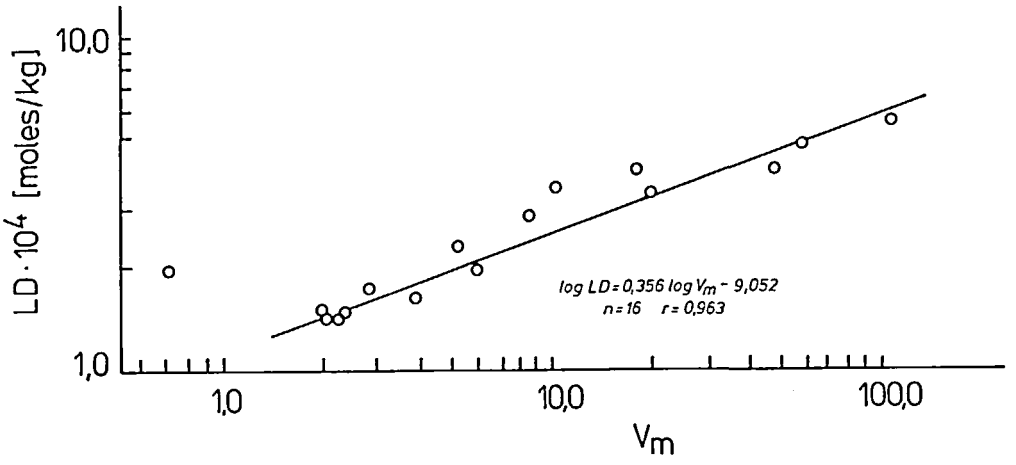


FIGURE 15. Correlation between bioactivity (LD) of a series of cardiac glycosides and their retention volumes, V_m. Compound s (G-strophantin) is not included in the correlation equation. (After Jinno, K., *J. Chromatogr.*, 264, 485, 1983. With permission.)

Davydov et al.[239] observed a relationship between toxicity (LD) and chromatographic behavior of 17 cardiac glycosides. Recently, Jinno[240] recalculated Davydov et al.[239] data using the logarithmic scale. The more convincing QSAR was obtained (Figure 15).

According to Braumann et al.,[154] log k′ determined on C-18 columns can be used to model the behavior of *s*-triazines in thylakoid membranes, whereas log P cannot.

Log k′ data determined on a highly deactivated octadecyl-bonded silica column with methanol/buffer mobile phase were used in QSAR studies concerning several types of bioactivity data of sulfonamides and barbiturates.[151] As far as the quality of QSAR equations was considered, the results obtained were indistinguishable from those produced by the HPLC retention index.

RP-HPLC data of ligands obtained on C-18 columns with buffer/methanol mobile phase were related to renal clearance of substituted phenylcarbamoylmethyliminodiacetic acids technetium-99m complexes. Moderate correlation (r = 0.82) was observed when mono- and *ortho*-substituted derivatives were excluded.[120]

Garst[156] reported better predictivity of the in vivo concentration process of several toxicants in fish when his procedure[155] of HPLC determination of hydrophobicity was applied as compared to log P. However, his extrapolation to pure water log k′ equally correlates the binding to bovine serum albumin by 34 chemicals as predicted by log P values.

Recently, Jinno[241] used capacity factors determined by RP-micro-HPLC on a C-18 phase with a methanol/water solvent as the molecular descriptor in QSAR studies of phenols. Several literature bioactivity data sets were related to log k′ with correlation coefficients ranging from 0.90 to 0.99.

At present, chromatography is routinely and perhaps mechanically used for the determination of data related more or less directly to the hydrophobicity of bioactive substances. The chromatographically generated information concerning electronic and steric properties of drugs is only used occasionally in QSAR studies.

The gas chromatographic measure of polarity[42] was applied in QSAR studies of the olfactory activity of phenols.[76] The activity, expressed by human detection thresholds, has been satisfactorily described by hydrophobicity parameters, and the introduction of no electronic data gives a statistically significant improvement. The chromatographic polarity measure proved to be useful as a correction factor for ionization to the hydrophobicity parameter.

Previously, Laffort and Patte[193] reported correlation (r = 079) between experimental

olfactory thresholds and the calculated ones based on the authors' gas chromatographic solubility factors.

Data concerning the induction of an enzyme *N*-demethylase by a group of PAHs have been correlated with the shape parameter calculated from GC retention data determined on nematic phases.[242] In the same work, the chromatographic shape parameter was used for a quantitative description of the mutagenicity of the compounds.

VI. CONCLUDING REMARKS

The attempts to describe quantitatively the observed physicochemical phenomena in terms of different quantitites related to molecular structure of the interacting species are characteristic of modern chemistry. The noncovalent interactions operating in chromatography are effective in universal physicochemical and, most probably, biological processes. Thus a deeper understanding of chromatographic phenomena at the molecular or submolecular level may provide information useful for other chemical and biological interphase interactions.

Chromatography is a unique model for studying dynamic processes. The method is able to generate great amounts of precise data. The separation process may be thermodynamically controlled, and the number of interacting species may be limited. Of the three main chromatographic variables, i.e., the stationary phase, the mobile phase, and the solute, two may be kept constant, and thus the resulting data may be interpreted in terms of the selected variable changes.

As discussed in the preceding sections, an increasing interest has been observed in studies of solute structure-retention relationsips. For congeneric groups of solutes, the relationships obtained are often of practical predictive value. It is still difficult, however, to accommodate diverse chemical structures in single QSRR equations.

To relate reactivities quantitatively to diverse molecular structures is a general problem in chemistry. The reason is the lack of structural descriptors comprising the information required. A search for such descriptors may be facilitated in the case of chromatography. A submolecular approach based on quantum chemical calculations seems to be particularly promising.

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